Effect of β-Glucan and Environmental Factors on the Physical and Chemical Properties of Wort and Beer

by

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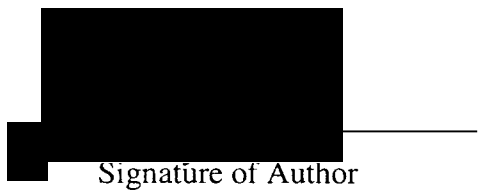

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
ASBC	American Society of Brewing Chemists
BAC	Brewers Association of Canada
BSA	bovine serum albumin
CFMF	crossflow membrane filtration
DDW	double-distilled de-ionized water
DE	diatomaceous earth
DMS	dimethyl sulfide
DMSO	dimethylsulfoxide
DP	degree of polymerization
EBC	European Brewery Convention
FA	foamability
FHU	formazin haze unit (EBC)
FIA	flow injection analysis
FS	foam stability
FTU	formazin turbidity unit (ASBC)
GHCl	guanidine hydrochloride
HPSEC	high performance size exclusion chromatography
IUPAC	International Union of Pure and Applied Chemistry
kDa	kilo Dalton
LTP	lipid transfer protein
MF	membrane filtration
M_n	number-average molecular weight
MW	molecular weight

M_w	weight-average molecular weight
M_{w0}	weight-average molecular weight of monomers
NaAc	sodium acetate
NIR	near infrared reflectance spectroscopy
NSP	non-starch polysaccharides
RMS	root mean square
SDS	sodium dodecyl sulfate
SEC	size exclusion chromatography
SG	specific gravity
UC	ultracentrifugation
α	an exponent of the Mark-Houwink equation
ε	porosity of particles in a filter bed
σ	shear stress
γ	surface tension
$\dot{\gamma}$	shear rate
η	viscosity
$\eta_{20°C}$	viscosity at 20°C
η_{BG}	viscosity of wort and beer containing β-glucans
η_{NB}	viscosity of wort and beer containing no β-glucans
ΔP	pressure difference across the filter bed
ΔP_c	corrected pressure difference across the filter bed
ΔP_{uc}	un-corrected pressure difference across the filter bed
η_{rel}	relative viscosity
η_{sp}	specific viscosity
$[\eta]$	intrinsic viscosity
°P	degrees Plato

A	area of the parallel planes
A_f	a frequency factor for the Arrhenius equation
A_{550}	absorbance at 550 nm
C	concentration of β-glucans in wort and beer
C^*	critical overlap (or entanglement) concentration
C_0	a constant
D	diameter
d	distance between the parallel planes
D_c	diameter of the capillaries for fluid flow
dq/dt	linear flow velocity (m^3/m^2hr)
dv/dt	flow rate (m^3/hr)
E	ethanol content of beer (% v/v)
E_a	activation energy (cal/mol)
F	force
$F_{>0.01}$	fraction (%) of β-glucan particles >0.01 μm in diameter
$F_{0.01\text{-}0.1}$	fraction (%) of β-glucan particles between 0.01-0.1 μm in diameter
F_i	filtration index (i.e., the slope of filtration curve)
F_m	fermentation
H	height
I_{580}	intensity of light (580 nm) scattered at 90° to the incident beam
K	constant of D'Arcy's law
K'	constant of the Kozeny-Carman equation
K_1	permeability coefficient of filter bed
k	constant of the Mark-Houwink equation
k'	Kraemer coefficient
k''	Huggins coefficient
L	length of the capillaries for fluid flow or thickness of the filter cake

m	consistency coefficient of the power law model for fluid flow
M	molecular weight in the Mark-Houwink equation
Mal	maltose concentration of wort (% w/w)
n	flow behaviour index of the power law model for fluid flow
P	pressure
P_m	pressure difference across the filter medium (cloth)
Q_{init}	initial flow rate of membrane filtration
r	specific resistance of the filter cake
R	universal gas constant
R_a	resistance of filter cloth per unit area and per unit viscosity
R_c	resistance of the filter cake
R_f	relative flux (%)
R_m	resistance of the filter cloth per unit area
S	shearing treatment of wort and beer
S_0	initial flow rate of filtration
S_s	specific surface
T	temperature
t	time
T_s	shearing temperature
U_s	speed of the upper plane for parallel laminar flow
U	unit of lichenase (β-glucanase) activity
v	volume of filtrate at time t
V_{max}	maximum volume of beer filtered through a membrane filter
w	weight of suspended solids in unit volume of liquid

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ABSTRACT

Behaviour of barley β-(1→3)(1→4)-D-glucans in wort and beer was examined to investigate their effect on brewing process and beer quality. Beta-glucans at 0-1000 mg/L increased wort and beer viscosity linearly with MW and concentration. The Arrhenius relationship governed the effect of temperature on wort viscosity. Beta-glucan viscosity in wort was lowered after shearing although shearing increased the apparent wort viscosity ($p<0.001$). Shearing decreased beer viscosity ($p<0.001$) but did not affect the β-glucan viscosity in beer ($p>0.05$). Shearing temperature did not affect the viscosities of wort or beer ($p>0.05$). Increasing pH raised wort viscosity ($p<0.001$) but did not affect beer viscosity ($p>0.05$). Maltose in wort and ethanol in beer enhanced the viscosity of β-glucan polymers ($p<0.001$). Beta-glucan-caused viscosity was found to be an indicator of potential technical problems. Beta-glucans had higher intrinsic viscosities (5°C) in beer than in wort ($p<0.001$). The critical overlap concentration (C*) for β-glucans (31-443 kDa) in beer and wort was found to vary in the range of 1.3-8.3 g/L.

Apparent particle size of β-glucans in wort and beer increased with their MW and concentration ($p<0.001$). Beta-glucans had greater particle size in beer (mainly 0.01-0.1 μm in diameter) than in wort (even distributed in <0.01 μm and >0.01 μm fractions) ($p<0.001$). Beta-glucan particles were larger at higher pHs of wort and beer ($p<0.001$). Maltose did not affect the β-glucan particle size distributions in wort ($p>0.05$). High ethanol concentrations increased the 0.01-0.1 μm fraction and decreased the >0.1 μm fraction of the 443 kDa β-glucan in beer ($p<0.001$). Shearing of wort and beer resulted in a decrease in the <0.01 μm fraction of β-glucans and an increase in 0.01-0.1 μm β-glucan fraction ($p<0.001$) but shearing temperature did not affect the particle size distribution ($p>0.05$).

Wort and beer turbidity increased with higher MWs and concentrations of β-glucans ($p<0.001$), shearing ($p<0.001$), lower maltose and ethanol levels and lower pHs ($p<0.001$). Shearing (20-76°C) at high temperatures resulted in lower wort turbidity values than low temperatures ($p<0.001$) but shearing temperature (0-10°C) did not affect beer turbidity ($p>0.05$).

The resistance of wort to diatomaceous earth (DE) filtration was termed filtration index (F_i) to characterize the filtration performance of wort samples. High MW β-glucans and shearing increased the filtration index ($p<0.001$). Higher concentrations of maltose and lower pHs also resulted in higher filtration index values ($p<0.001$). Membrane filterability (20°C) of wort correlated negatively to the DE filtration index ($r=0.60$, $n=152$, $p<0.001$).

Membrane filtration of beer showed that the maximum volume of filtrate (V_{max}) and the initial filtration rate (Q_{init}) were lowered by higher MWs and concentrations of β-glucans, as well as lower pHs ($p<0.001$). Shearing beer decreased both V_{max} and Q_{init} ($p<0.001$) while shearing temperature (0-10°C) had no effect ($p>0.05$). The addition of ethanol at 5-10% v/v decreased Q_{init} ($p<0.001$), but improved V_{max} ($p<0.001$). However, the relative V_{max} (compared to the β-glucan-free beer) was lowered by 5-10% v/v of ethanol ($p<0.001$). Cold storage of beer at 4°C for 2 weeks did not improve beer filterability ($p>0.05$).

Beta-glucans having MWs of 31-443 kDa (1000 mg/L) lowered surface tension of their aqueous solutions. Beer surface tension increased with high MWs of β-glucans ($p<0.01$) and lower ethanol concentrations ($p<0.001$) but was not affected by pH and β-glucan concentration ($p>0.05$). Shearing beer increased surface tension ($p<0.001$) while shearing temperature had no effect ($p>0.05$). Beer foamability was enhanced by elevated temperatures (0-10°C) and pHs (3.8-4.6) with and without 600 mg/L of 443 kDa β-glucan. The addition of 5-10% v/v ethanol or high MW β-glucans at high concentrations lowered beer foamability ($p<0.01$) and foam stability ($p<0.001$).

1 GENERAL INTRODUCTION

Beer is a carbonated alcoholic beverage fermented with yeast from raw materials including barley malt, adjunct, hop and water. Malting barley has been one of the hot topics in grain science and crop breeding due to the desirable agronomic, processing and nutritive properties. Desired properties of malting barley include high extract, low β-glucan content, rapid and even modification (i.e., degradation) of endosperm, low dimethyl sulfide (DMS) content in wort, slight dormancy, low husk content and husk adhesion, and appropriate (10.5-12%) protein content.

Despite the numerous types of beer brewed all over the world, the brewing process can be divided into several stages of malting, mashing and wort production, fermentation, maturation, filtration and packaging. The major unit operations of brewing are illustrated in Figure 1.1. Raw barley in storage is cleaned before malting which is a process of steeping, germination, and kilning/curing. Besides pale malt, specialty malts such as dark malt, roasted malt and crystal malt can be produced with alternate kilning/roasting regimes. The main purposes of malting are to develop/activate hydrolytic enzymes such as amylases, proteases and β-glucanases to achieve desired modification of those macromolecules during malting; to preserve the enzyme activities for mashing by optimized kilning; and to form particular aroma and colour.

During mashing (with or without adjuncts), controlled hydrolysis of starch, β-glucan and protein provides the brewers with sweet wort containing fermentable carbohydrates, alpha-amino nitrogen, and other yeast nutrients such as minerals and vitamins. After separation of spent grains, the sweet wort is boiled in the kettle to adjust wort specific gravity (SG, which indicates extract content) as well as to remove coagulatable protein which is one of the components responsible for beer haze. As hops are added at this stage, the extraction of hop oil and bitter substances mainly α-acids (as well as the isomerization of α-acids) is another important function of wort boiling. After boiling

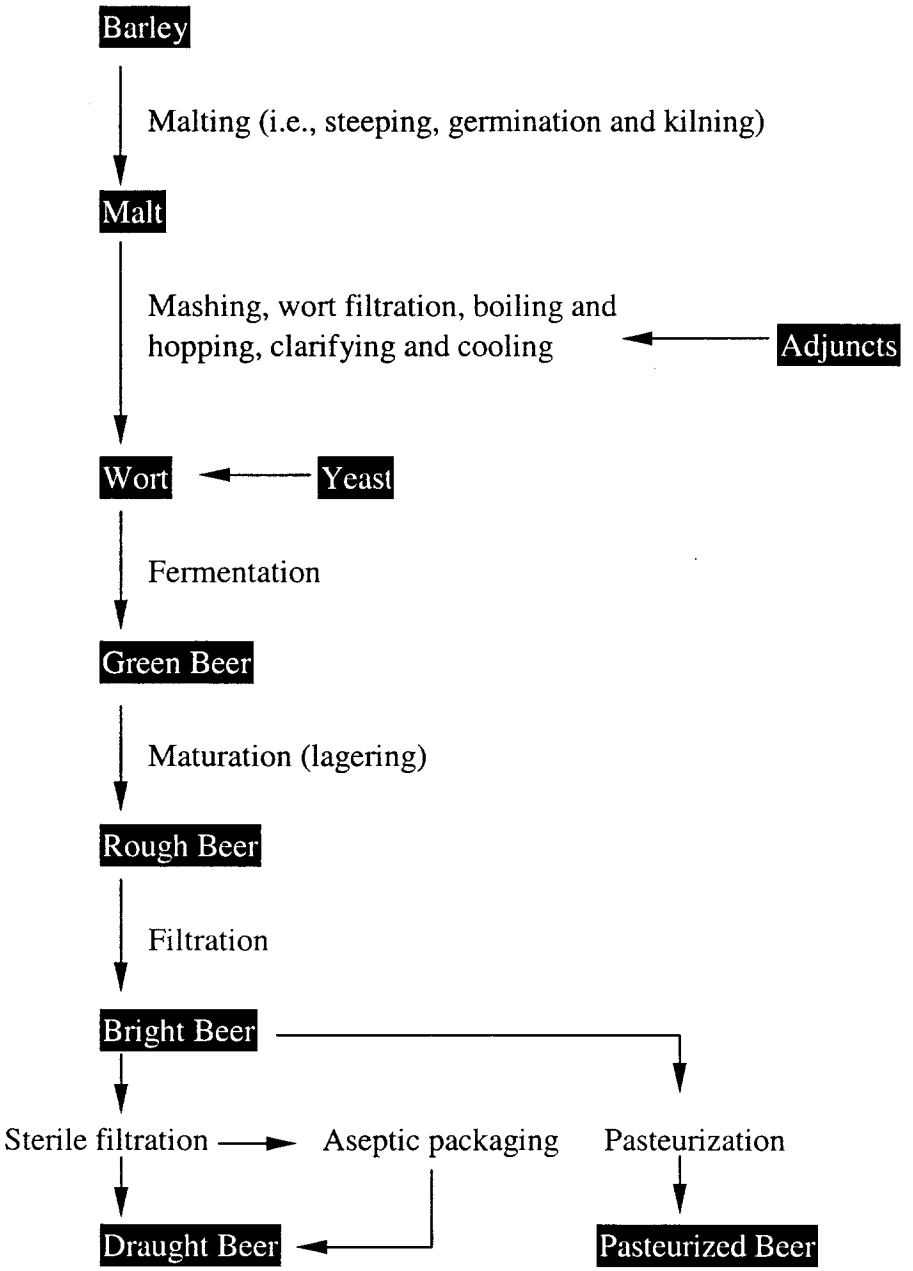

Figure 1.1 Simplified diagram of the brewing process.

for 60-90 minutes, the wort is then clarified in a whirlpool to separate the hot trub (i.e., coagulated proteins) and often cooled with plate heat exchangers to the fermentation temperature followed by aeration.

When the aerated wort is pitched with brewing yeast, fermentation starts and the fermentable sugars are converted into biomass, ethanol and other by-products such as carbon dioxide and compounds which contribute to beer flavor and aroma. Traditional beer fermentation is accomplished by primary fermentation (at 10-20°C for ca. 3-6 days; lower temperatures for lager and higher temperatures for ale) and maturation (i.e., 0°C to –1°C for ca. 10-15 days). After the rough beer is filtered, the bright beer is packaged, pasteurized, and then distributed to the market. Beer filtration is usually performed in filters using diatomaceous earth (DE) as a filter aid. DE filters remove the suspended colloidal particles and yeast cells to reach a specified clarity of beer. Besides the packaged, pasteurized beer, draught or un-pasteurized beer is also processed. Draught beer can be kegged, bottled or canned (Figure 1.1). In Canada, the production of un-packaged draught beer had reached 11.5% of the total sales in the year 2000 (BAC, 2000). Bright beer must be sterile filtered in order to remove spoilage microorganisms in the "draught" product. Sterile filtration of bright beer can be carried out with ceramic candle, sheet, or membrane filters before being aseptically packaged.

It has been reported that barley β-glucans [(1→3)(1→4)-β-D-glucans] and arabinoxylans can cause beer filtration difficulties and haze problems (Egi, 2002; Egi *et al.*, 2001; Jin *et al.*, 2001; Letters, 1995a; Nischwitz *et al.*, 1999; Oonsivilai, 2000; Oonsivilai *et al.*, 1999; 2000; Patelakis, 1999; Patelakis *et al.*, 1999; Siebert *et al.*, 1984). Beta-glucans, particularly after being sheared, resulted in higher turbidity in solutions or beer samples (Jin *et al.*, 2001; Letters, 1977; 1995a; Patelakis, 1999; Patelakis *et al.*, 1999). As a viscous polysaccharide which is abundant in barley endosperm cell walls, β-glucans may affect unit operations throughout the brewing process (Table 1.1). Also, other

Table 1.1 Technical difficulties related to β-glucans during malting and brewing

Process	Possible changes of β-glucan	Possible problems
Malting	Degradation	Under-modification of the endosperm cell walls
Mashing	Degradation	Low extract yield and high viscosity
Lautering	High viscosity and thick fines	Low extract yield; slow runoff and scheduling problems
Cooling	High viscosity and increased cold trub	Lowered heat exchange coefficient and fouling of cooler
Fermentation	Non-fermentable; High viscosity	Low attenuation; low heat transfer; and slow particle sedimentation
Lagering/Maturation	Aggregation and sedimentation	Slow clarification of beer (i.e., high filter loads)
Deep bed filtration	High viscosity and haze particles	Slow filtration rate and increased process cost
Membrane filtration	High viscosity and haze particles	Slow or even terminated filtration; increased cost; and risk of beer haze
Distribution of beer	Pseudo-haze and precipitates in packaged beer	Haze formation from β-glucans; product defect; and loss due to recall

biopolymers such as arabinoxylans and proteins can lead to processing difficulties similar to those caused by β-glucans. This thesis focused on the effect of barley β-glucans on the viscosity, turbidity, filtration performance, interfacial and foaming properties of wort and beer. The term "β-glucan(s)" is used throughout this thesis as an abbreviation of the barley (1→3)(1→4)-β-D-glucan(s).

Following a general introduction and a literature review, findings of the research are presented in seven chapters with regard to viscosity caused by β-glucans (Chapter 3); the apparent particle size of β-glucans (Chapter 4); effect of β-glucans on surface tension of wort and beer, and on the foaming properties of beer (Chapter 5); increased turbidity of wort and beer by β-glucans (Chapter 6); impaired wort filtration by β-glucans (Chapter 7); membrane filtration performance of beer containing β-glucans (Chapter 8); and the identification of technical difficulties related to β-glucans (Chapter 9). The thesis closes with an overall discussion and conclusions in Chapter 10. It is the ultimate goal of this thesis to improve understanding of the mechanisms of the occurrence of processing difficulties caused by β-glucans, and therefore, to aid in prevention and/or elimination of the problems during the brewing process.

2 INFLUENCE OF BARLEY β-GLUCANS ON BREWING: A REVIEW

This chapter reviews the physical and chemical properties of β-glucans, their preparation and determination methods, and the influence of these polymers on brewing process and beer quality. Beta-glucan polymers in barley endosperm cell walls have become one of the major concerns in the brewing industry during the past 50 years. These polymers are present in barley malt and are highly polydisperse (i.e., a broad distribution of molecular weight). The amount and molecular weight of β-glucans in barley influence malt quality, brewhouse extract yield, wort and beer apparent viscosities, wort separation, and filtration, particularly membrane filtration of beer. The tendency of β-glucan polymers to aggregate has an impact on both beer filtration and beer colloidal stability. The aggregation and precipitation of β-glucan polymers in wort and beer have been loosely termed "gel formation" when causing filtration difficulties by some researchers. Gel formation can be defined as a process where random or amorphous polymers build up ordered conformations. A typical gel is a continuous three-dimensional network of connected molecules or particles entrapping a large volume of a continuous liquid phase. In brewing, however, the β-glucan polymers at very low concentrations can only form gel-like aggregates and precipitates. Thus, the term precipitation instead of gel formation of β-glucans is used in this thesis. Understanding the mechanisms of β-glucan aggregation and precipitation will help brewers to adopt preventative and corrective operations that can minimize or eliminate the processing difficulties caused by β-glucans.

2.1 Introduction

Barley β-glucans have attracted research interest from brewing scientists because of their contribution to beer mouth feel (Forrest and Wainwright, 1977; Krauss, 1970; Luchsinger, 1967; Narziβ, 1973), and the lautering, filtration and stability problems they cause (Annemüller and Schnick, 1999; Bamforth, 1994; Barrett *et al.*, 1973; Crabb and Bathgate, 1973; Egi *et al.*, 2001; Erdal and Gjertsen, 1967; Gans and Denk, 1995;

Gjertsen, 1966; Jin *et al.*, 2001; Leedham *et al.*, 1975; Letters, 1969; 1995a; Lotz *et al.*, 1997; Meier *et al.*, 1995; Narziβ *et al.*, 1989a; Nischwitz *et al.*, 1999; Oonsivilai, 2000; Oonsivilai *et al.*, 1999; 2000; Palmer, 1972; Patelakis, 1999; Patelakis *et al.*, 1999; Siebert *et al.*, 1984; Wainwright, 1997; 1999). Both the structure and size of these β-glucans can affect the rate of endosperm modification during malting (Ahluwalia and Ellis, 1985; Anderson *et al.*, 1978; Narziβ *et al.*, 1989b). Therefore, the content and type of barley β-glucans have been promoted as indicators of the malting potential of barley varieties (Aastrup and Munck, 1985; Bendelow, 1975; Haselmore *et al.*, 1990; Martin and Bamforth, 1980; Narziβ *et al.*, 1989b). Development of new cultivars that exhibit less filtration problems are usually given a high research priority by barley breeding scientists (MacGregor, 1999; Powell *et al.*, 1989; Ullrich *et al.*, 1996).

Preece (Preece, 1948; Preece and MacKenzie, 1952a; 1952b) and Meredith *et al.* (1951) pioneered the very early work on the nature of β-glucan and β-glucanase in brewing. Gjertsen (1966) first reported the composition of a gelatinous precipitate that caused filtration problems for strong beers (i.e., beers containing high ethanol content). The precipitate was characterized as (1→3),(1→4)-β-glucan (Gjertsen, 1966). It was believed that these polymers were derived from barley endosperm cell walls and were reported to cause filtration problems (Gjertsen, 1966; Letters, 1969). Such β-glucan problems are now often associated with under-modified malt, malt with low β-glucanase activities, or the use of high mashing and/or sparging temperature (Wainwright, 1999; Whitear, 1997).

Beta-glucan polymers have a major influence on wort and beer apparent viscosities (Adamic, 1977; Charalambous, 1981), which affect filtration performance. The high viscosity of β-glucan solutions and their tendency to precipitate lead to wort and beer filtration problems and colloidal instability of packaged beer (Annemüller and Schnick, 1999; Barrett *et al.*, 1973; Crabb and Bathgate, 1973; Drost *et al.*, 1987; Egi, 2002; Egi *et al.*, 2001; Gans and Denk, 1995; Jin *et al.*, 2001; Leedham *et al.*, 1975; Lotz *et al.*, 1997; Meier *et al.*, 1995; Patelakis, 1999; Oonsivilai, 2000; Sudarmana *et al.*, 1996). Beer or

wort hazes derived from malt usually contain high levels of β-glucans in addition to proteins, polyphenols and pentosans (particularly arabinoxylans) (Coote and Kirsop, 1976; Gjertsen, 1966; Igarashi and Amaha, 1969; Jackson and Bamforth, 1983; Letters, 1977; Moll, 1987; Takayanagi *et al.*, 1969; Whitear *et al.*, 1983). In some cases, β-glucans agglomerate in the packaged beer to increase turbidity (Gjertsen, 1966; Whitear *et al.*, 1983). Early in the 1960s, it had been recognized that a low β-glucan level is required to minimize the risk of haze in strong beers (Erdal and Gjertsen, 1967).

Beta-glucan polymers also reduce brewhouse extract yields (Bamforth, 1994; Edmunds *et al.*, 1994; Evans *et al.*, 1998; Haselmore *et al.*, 1990; Henry, 1986; Munck, 1987) by impeding access of amylases to starches during mashing and lowering wort filtration efficiency (Bamforth, 1994). Because the endosperm cell walls enclose the starch and protein reserves of the barley kernels, poor breakdown of the β-glucans in the cell walls will reduce extract yield during wort preparation. Unconverted starch and proteins can be found in the spent grains after mashing and lautering (Bathgate and Palmer, 1973; 1975; Bathgate *et al.*, 1974; Palmer, 1972). Such residuals have been reported to form a layer of fines, which retards the flow of wort through the mash bed (Bathgate and Palmer, 1975).

The development and applications of some technologies in brewing have made β-glucan-related problems more common (Narziβ, 1992). For example, the high ethanol content of dry and ice beers, high gravity brewing, high shear rate (caused by pumping and centrifugation), and turbulence all accelerate the formation of β-glucan gels and the clogging of membrane filters. There have been continuing research efforts to elucidate the fine structural characteristics of β-glucans and their structure-property relationship (Izydorczyk *et al.*, 1998a). This chapter focuses on our understanding of the physical and chemical properties of barley β-glucans and their influence on malting and brewing processes. Various approaches to the solution of β-glucan-caused problems are also discussed.

2.2 Localization and Content of Barley β-Glucans

Beta-glucans and arabinoxylans are the principal constituents in the barley endosperm cell walls. The structure of a barley kernel is shown in Figure 2.1. The inset illustrates the endosperm cell walls. The barley endosperm cell walls contain about 20% arabinoxylans and 70% β-glucans; in contrast, the barley aleurone cell walls contain about 65-67% arabinoxylans and 26-29% β-glucans (Palmer, 1989). Structural proteins (about 1-6% in isolated endosperm cell walls) are also important to the integrity of the endosperm cell walls (Palmer, 1989). Barley β-glucan is bound to the cell wall through protein-polysaccharide linkages (Bamforth and Martin, 1983; Baxter and Wainwright, 1979; Forrest and Wainwright, 1977) or through phenol-ester linkages formed among protein, ferulic acid and polysaccharide molecules (Selvendran, 1983).

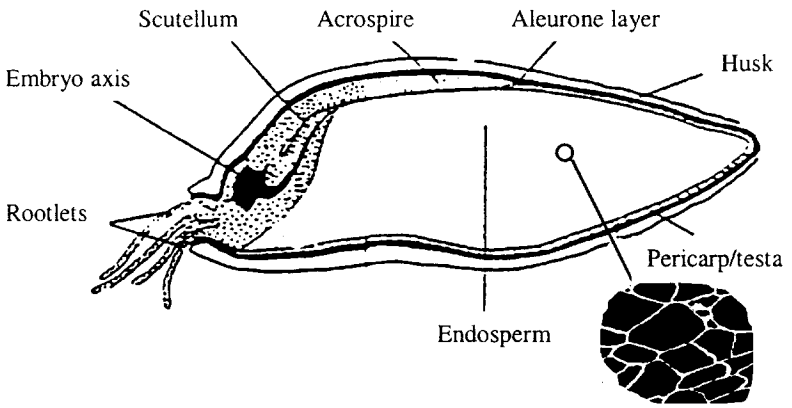

Figure 2.1 Diagram of a germinated barley kernel.

Low β-glucan barley mutants have thinner endosperm cell walls, faster modification during germination and lower extract viscosity compared to their parent varieties (Aastrup *et al.*, 1985). Low-density grains (i.e., low weight per unit volume) that have mealy characteristics (such as being crushed readily during milling) and faster modification rates contain less β-glucans than the high-density grains (Aastrup *et al.*,

1985; Drost *et al.*, 1987). However, it has been argued that the different malting rates of barley varieties rarely reflect differences in cell wall thickness (Palmer, 1989). In other words, the number of small endosperm cells has more impact on endosperm modification than the thickness of large endosperm cell walls (Wischmann and Schildbach, 1987).

Genetic variations influence the β-glucan content of barley (Åman, 1986; Bourne and Wheeler, 1984; Gill *et al.*, 1982; Lehtonen and Aikasalo, 1987). Investigations have confirmed that differences in β-glucan content exist and vary among malting barley cultivars, growing conditions and years (Edney *et al.*, 1998; Özkara *et al.*, 1998; Pérez-Vendrell *et al.*, 1996). Barleys grown under drought or stressful conditions contain increased level of soluble β-glucans (Coles, 1979; Kendall, 1994). Judged by the extract viscosity, β-glucan content of the North American barleys is more influenced by growing conditions than cultivar (Hwang and Lorenz, 1986). Heat stress, (i.e., several days of high temperature during mid-grain-filling) affects both barley yield and malting quality. Compared to a control sample, the heat-stressed grains accumulated more protein, less starch and less β-glucan. However, β-glucanase levels were also reduced by the heat stress (Wallwork *et al.*, 1995). Literature reports of β-glucan contents in barley, wort and beer are listed in Table 2.1. Among the collected data, the majority of the papers reported a barley β-glucan level of 2-6% w/w, while about one-third reported β-glucan contents <2% w/w and one-sixth reported β-glucan levels >6% w/w, respectively. Malt β-glucan content for most reports was <1% w/w. Beta-glucan levels in wort and congress wort were reported to vary from ≈0 to 1290 mg/L although 100-300 mg/L might be perceived normal in practice. Beer β-glucan level varied from 0 to 1152 mg/L. However, unusually high levels of β-glucan in pilot beers (2100-3950 mg/L) were reported by Leedham *et al.* (1995).

Table 2.1 Literature reported β-glucan content in brewing

Sample	Beta-glucan (%)	Reference
Barley	0.14-6.36	Aastrup, 1979a
Barley	0.16-8.29	Aastrup, 1979b
Barley	2.50-5.64	Anderson *et al.*, 1978
Barley	3.5-5.3	Bamforth, 1983
Barley	1.3-2.5	Bathgate *et al.*, 1974
Barley (nonwaxy)	5.40-5.79	Beer *et al.*, 1997
Barley (waxy)	6.15-8.07	Beer *et al.*, 1997
Barley	1.6-7.4	Bendelow, 1975
Barley	1.52-3.56	Bourne and pierce, 1970
Barley	3.03-4.80	Bourne and Wheeler, 1984
Barley	2.23-4.43	Carr *et al.*, 1990
Barley	2.52-5.18	Edney *et al.*, 1998
Barley	0.95-1.12	Fleming *et al.*, 1974
Barley	2.4-3.6	Forrest and Wainwright, 1977
Barley	2.7-5.2	Gill *et al.*, 1982
Barley	3.70-3.99	Han and Schwarz, 1996
Barley	3.4 –5.7	Henry, 1986
Barley	4.03-5.26	Henry, 1985
Barley	0.43-1.66	Jørgegnsen, 1988
Barley	3.9-5.2	Jørgegnsen and Aastrup, 1986
Barley	3.4-8.9	Knuckles and Chiu, 1999
Barley	0.22-5.27	Manzanares and Sendra, 1996
Barley	1.25-8.62	Martin and Bamforth, 1981
Barley	3.80-4.81	McCleary and Glennie-Holmes, 1985
Barley	6.3-7.9	Oscarsson *et al.*, 1996
Barley (waxy)	5.4-5.8	Oscarsson *et al.*, 1996
Barley	3.80-4.88	Özkara *et al.*, 1998
Barley	3.7-4.4	Palmer, 1975
Barley	1.9-5.8	Pérez-Vendrell *et al.*, 1996
Barley	0.44	Preece and MacKenzie, 1952a
Barley	4.5-8.2	Prentice *et al.*, 1980
Barley	2.04-3.02	Scott, 1972
Barley	0.83-5.78	Smith *et al.*, 1980
Barley	0.58-1.02	Sparrow and Meredith, 1969
Barley	2.4-5.1	Suortti, 1993
Barley	0.9 (As NSP[1])	Voragen *et al.*, 1987
Barley	0.35-2.77	Wood *et al.*, 1977

Table 2.1 Literature reported β-glucan content in brewing (Continued)

ASBC[2)] Check malt	53-169 mg/L[2)]	ASBC, 2001
ASBC Check malt	70-194 mg/L[3)]	ASBC, 1997a
ASBC Check malt	127-239 mg/L[3)]	ASBC, 1996b
Malt	0.45-0.75	Anderson *et al.*, 1989
Malt	0.05-1.14	Edney *et al.*, 1998
Malt	0.50-0.57	Han and Schwarz, 1996
Malt	<0.2-0.7	Home *et al.*, 1993
Malt	0.3-1.0	Jørgegnsen and Aastrup, 1986
Malt	0.65	McCleary and Glennie-Holmes, 1985
Malt	0.18-0.49	Narziβ *et al.*, 1989a
Malt	0.8-3.8	Suortti, 1993
Malt	0.2 (As NSP)	Voragen *et al.*, 1987
Congress wort	305, 655 mg/L	Aastrup and Erdal, 1987
Congress wort	14-438 mg/L	Edney *et al.*, 1998
Congress wort	28-420 mg/L	Edney and Tipples, 1997
Congress wort	110-770 mg/L	Sjöholm *et al.*, 1994
Congress wort	117 & 258 mg/L[4)]	Smart *et al.*, 1993
Wort (15.7°P)	0-1290 mg/L	Aastrup and Erdal, 1987
Wort	312-323 mg/L	Haikara and Home, 1991
Wort	190-746mg/L	Jørgegnsen and Aastrup, 1986
Wort	260-940 mg/L	Whitear *et al.*, 1983
Beer	12-296 mg/L	Edney *et al.*, 1998
Beer	308 mg/L	Gans and Denk, 1995
Beer	330-580 mg/L	Han and Schwarz, 1996
Beer	150-730 mg/L	Henry and Blakeney, 1988
Beer	70-420 mg/L	Home *et al.*, 1993
Beer (lab brewing)	165-1152 mg/L	Letters *et al.*, 1985
Beer	360-381 mg/L	Lusk *et al.*, 2001
Beer (pilot brewing)	0.19-318 mg/L	Stewart *et al.*, 1998

[1)]: NSP = non-starch polysaccharides; [2)]: ASBC = American Society of Brewing Chemists; Mean values of 4 sample pairs determined by 44 laboratories; [3)]: Mean values of 4 sample pairs determined by 49 laboratories; [4)]: Commercial malts, mean values of n = 64 and 56, respectively.

2.3 Structural Properties of Barley β-Glucans

Barley β-glucans were first identified as having unbranched chains of β-D-glucopyranose residues with equal proportions of β-(1→3)- and β-(1→4)-linkages (Aspinall and Telfer, 1954). Later work proposed different ratios of β-(1→4)- to β-(1→3)-linkages in the range of 2.3~6.6:1 (Clarke and Stone, 1966; Igarashi and Amaha, 1969; Izydorczyk *et al.*, 1998c; Luchsinger *et al.*, 1965; Parrish *et al.*, 1960; Peat *et al.*, 1957; Perlin and Suzuki, 1962; Saulnier *et al.*, 1994; Stone and Clarke, 1992; Wood *et al.*, 1991a). A diagram of the β-glucan structure is shown in Figure 2.2. More than 90% by weight of the 40°C water-soluble barley β-glucan fraction can be represented as a copolymer of cellulotriosyl and cellulotetraosyl units (i.e., three and four glucose residuals joined by β-1,4-linkages, respectively) connected by (1→3)-β-linkages, while the remaining portion of this fraction consists of longer blocks of up to 20 consecutive (1→4)-β-glucosyl residues (Edney *et al.*, 1991; Izawa *et al.*, 1993; Izydorczyk *et al.*, 1998c; Luchsinger *et al.*, 1965; Woodward *et al.*, 1983a). The ratio of tri- to tetra-saccharides is slightly higher in β-glucans extracted at 65°C than in those extracted at 40°C (Izydorczyk *et al.*, 1998b). The lower solubility of 65°C water-soluble β-glucans is associated with their higher molecular weight (MW) and higher level of β-(1→4)-linkages (Izydorczyk *et al.*, 1998b). Blocks of adjacent (1→4)-linkages in β-glucan promote interchain aggregation that is formed through hydrogen bonding along the cellulose-like regions, making the polymers less soluble in water (Izawa *et al.*, 1993; Izydorczyk *et al.*, 1998b; Woodward *et al.*, 1988). Buliga *et al.* (1986) suggested that the (1→3)-linkages in the β-glucans give the molecular chains increased flexibility as compared to cellulose chains, which are β-(1→4)-linked only. Computer simulation has shown that the β-glucan chains exist as rather extended random coils in solution (Buliga *et al.*, 1986). It is unknown whether the minor differences in the percentage of β-(1→4)-linkages affects rheological and other properties of these solutions (Wood *et al.*, 1991b), although a theoretical model has suggested it does (Buliga *et al.*, 1986).

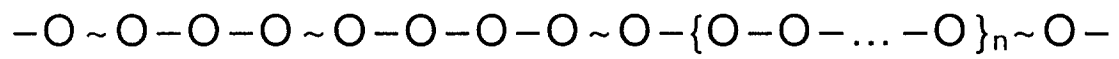

Figure 2.2 An illustration of the β-glucan structure. Note: ~ represents β-(1→3)-linkages; – indicates β-(1→4)-linkages. The fragment in parentheses indicates contiguous β-(1→4)-linked glucose units rather than a repeating unit. The number of glucose units in this fragment (n) can be up to 20.

The presence of adjacent β-(1→3)-linkages in barley β-glucan was reported by several early workers (Bathgate *et al.*, 1974; Clarke and Stone, 1966; Fleming and Kawakami, 1977; Fleming and Manners, 1966; Igarashi, 1967; Moscatelli *et al.*, 1961). However, no evidence for consecutive β-(1→3)-linkages has been found in recent reports (Edney *et al.*, 1991; Vårum and Smidsrød, 1988; Wood *et al.*, 1994). The early controversial finding was probably caused by mis-identification of the derived products for analysis (Wood *et al.*, 1991a; Woodward and Fincher, 1983; Woodward *et al.*, 1983b). Barley endosperm cell walls also contain a small amount of β-(1→3)-glucans (Fulcher *et al.*, 1977). These polymers exist in the form of small discrete deposits throughout the endosperm but in some cultivars, large deposits have been found in the outer most cell walls of the endosperm (Fulcher *et al.*, 1977; MacGregor *et al.*, 1989). The function and influence of β-(1→3)-glucans on malt modification is still not clear.

2.4 Molecular Weight Distribution of β-Glucans

The β-glucan level alone does not allow prediction of processing difficulties because its MW distribution is also important (Sarx and Rath, 1995). Literature reports of β-glucan molecular weights in barley, malt, wort and beer are summarized in Table 2.2. The range of β-glucan MW in beer has been observed to range from 1 to 10,000 kDa (Izawa *et al.*,

Table 2.2 Reported β-glucan MWs in the literature

Sample	Method	MW (kDa)	Reference
Barley	SEC [1)]	>300	Ahluwalia and Ellis, 1984
Nonwaxy barley	HPSEC [2)]	1,097-1,937	Beer *et al.*, 1997
Barley	SEC	800-1,000	Fincher, 1975
Barley	SEC	800-4,000	Forrest and Wainwright, 1977
Barley	HPSEC	9-600	Gómez *et al.*, 1997a
Barley	HPSEC	400 and 900	Izydorczyk *et al.*, 1998c
Barley	HPSEC	270-1,150	Knuckles and Chiu, 1999
Barley	SEC	185-1,700	Suortti, 1993
Oat aleurone	HPSEC	71	Vårum *et al.*, 1991
Barley	SEC	2,140	Wood *et al.*, 1991a
Oat bran	SEC	3,000	Wood *et al.*, 1991a
Barley	UC [3)]	290	Woodward *et al.*, 1983a
Barley	UC	150	Woodward *et al.*, 1988
Malt	SEC	800	Forrest and Wainwright, 1977
Malt	SEC	1,220	Wood *et al.*, 1991a
Beer	UC	70	Gjertsen, 1966
Beer	SEC	1 - 10,000	Izawa *et al.*, 1990
Beer	SEC	55-760	Izawa *et al.*, 1993

[1)]: SEC = size exclusion chromatography;
[2)]: HPSEC = high performance size exclusion chromatography;
[3)]: UC = ultracentrifugation.

1990). High MW β-glucans can precipitate easily after freezing-and-thawing treatment, whereas the fractions with MWs lower than 200 kDa do not precipitate even after repeated freezing and thawing (Izawa *et al.*, 1990). A correlation between beer β-glucan concentration and filterability V_{max} (a maximum volume of beer which can be filtered through a membrane filter) has been reported (Nischwitz *et al.*, 1999). When both the level and the MW of β-glucans were examined for their effect on V_{max}, it was found that both factors are important in determining beer filterability (Nischwitz *et al.*, 1999).

The size of β-glucans as measured by size exclusion chromatography (SEC) can be calibrated with standards of β-glucans, lichenans, pullulans or dextrans. In each case the regression coefficients (log of MW vs. retention volume) differ from one another (Wood *et al.*, 1991a). It has been argued that calibration with dextrans or α-glucans in SEC methods may yield incorrect MW values for β-glucans due to their difference in solution behavior (Grimm and Krüger, 1994). However, a "universal calibration" for SEC has been investigated for β-glucans, alginates and pullulans, that have the same linear relationship between hydrodynamic volume ($[\eta]\times MW$ where $[\eta]$ is intrinsic viscosity of a polymer) and elution volume for all three polysaccharides (Vårum *et al.*, 1991). SEC generally leads to over-estimation of MW for asymmetrical polysaccharides such as β-glucans while the measurement of MW by sedimentation equilibrium ultracentrifugation is believed more reliable (Bamforth, 1994; Fleet and Manners, 1975; Grimm and Krüger, 1994; MacGregor and Fincher, 1993; Yalpani, 1988). The latter technique is an absolute method but only a weight average molecular weight can be obtained. As an absolute method, static light scattering has also been used for β-glucan MW determination (Grimm and Krüger, 1994).

2.5 Extraction and Preparation of Barley β-Glucans

Different procedures for extracting β-glucans have been reported in the literature. Only common methods are briefly discussed here. To prepare barley β-glucans, endosperm cell

walls are usually isolated for further extractions. The isolated endosperm cell walls are then often dried after preparation (Fincher, 1975; Palmer, 1975). However, drying barley endosperm cell walls can reduce their solubility (Palmer and Bathgate, 1976). About 60% to 80% of β-glucans are soluble in hot water (65°C) from high and low-grade malting barleys (Ahluwalia and Ellis, 1985). Wood *et al.* (1991a) reported on an extraction procedure involving pre-treatment with ethanol before extracting β-glucan from ground barley samples. The final extract can be stored frozen if not immediately analyzed (Wood *et al.*, 1991a). Barley samples can also be extracted with a hot mixture of 2-propanol and petroleum ether to remove lipophilic compounds and inactivate endogenous enzymes (Westerlund *et al.*, 1993; Wikström *et al.*, 1994). Starches can be removed from the residues by incubation with a thermostable α-amylase in water at 96°C for 2 hours. Proteins in the water extract can be digested with pancreatine at 40°C for 3 hours and the soluble crude β-glucans are precipitated in 60% ethanol, further purified by precipitation in 20% (w/v) aqueous ammonium sulfate and then by dialysis and centrifugation (Westerlund *et al.*, 1993; Wikström *et al.*, 1994). The Institute of Brewing (IOB) Analysis Committee recommended a similar but simpler procedure to prepare crude barley β-glucans as a substrate for β-glucanase assays (IOB, 1985). In any case, β-glucan yield is normally higher than 50% recovery of the total barley β-glucan (Beer *et al.*, 1997; Bhatty, 1995; Forrest and Wainwright, 1977). Extraction of barley β-glucans can be increased with the use of higher temperature but not with higher pH (Temelli, 1997). When hot water (90°C) containing a thermostable α-amylase is used to extract β-glucan, addition of dimethyl-sulfoxide (DMSO) increases the β-glucan yield; and NaOH together with $NaBH_4$ gives an even higher recovery (Beer *et al.*, 1997).

Addition of ethanol to a final concentration of 80% (v/v) can precipitate starch and non-starch polysaccharides from sweet and hopped worts (Viëtor *et al.*, 1993). After dissolution and a second precipitation with 80% ethanol, the precipitate can be re-dissolved in water and stored frozen (Viëtor *et al.*, 1993). Preparative SEC can be employed to further fractionate the isolated β-glucans (Vårum *et al.*, 1991). Generally,

different extraction methods will isolate β-glucans with distinct MWs (Table 2.2). This may partly explain literature reports of the varying molecular sizes and properties of β-glucans. Freezing and thawing leads to β-glucan precipitation (Casey and Ingledew, 1985; Haas and Fleischman, 1964; Izawa *et al.*, 1990; Letters, 1977; Takayanagi *et al.*, 1969; Tanaka and Sakuma, 1999), although it is not clear if this procedure alters the properties of the isolated β-glucans. As an alternative method, 100 mg/L sodium azide can be used as a microbial stabilizer at room temperatures in order to avoid using frozen storage of the samples.

2.6 Analysis of β-Glucans in Wort and Beer

Several methods have been developed for determination of β-glucan content in malting and brewing. Preece and MacKenzie (1952a; 1952b) found that ammonium sulfate precipitation of β-glucans can be used to separate them from arabinoxylans. With 30% aqueous ammonium sulfate, the precipitate contained 96% glucose, 1.5% xylose and 2.5% arabinose. At higher concentrations of ammonium sulfate, more arabinoxylans were precipitated along with β-glucan polymers (Preece and MacKenzie, 1952a; 1952b). This method was used to determine the precipitatable β-glucans for about 30 years until the enzymatic and the fluorimetric procedures were developed in the early 1980s. Recent analytical procedures developed include enzymatic methods (Åman and Graham, 1987; Anderson *et al.*, 1978; Bamforth, 1983; Carr *et al.*, 1990; EBC, 1987; Henry, 1984; Henry and Blakeney, 1986; 1988; Martin and Bamforth, 1981; McCleary and Codd, 1991; McCleary and Glennie-Holmes, 1985; McCleary and Mugford, 1997; McCleary and Nurthenm, 1986; Prentice *et al.*, 1980), Calcofluor-fluorimetric methods (ASBC, 1992; EBC, 1987; Izawa *et al.*, 1994; 1995; 1996a; 1996b; Jensen and Aastrup, 1981; Jørgegnsen, 1988; Jørgegnsen and Aastrup, 1986; Jørgegnsen *et al.*, 1985; 1987; Manzanares *et al.*, 1991; 1993; Manzanares and Sendra, 1996; Nischwitz *et al.*, 1999), a spectroscopic method with Congo red binding (Anderson, 1990; Li *et al.*, 1997; Yin *et al.*, 1994), viscometric assays (Bendelow, 1975; Doehlert *et al.*, 1997; Greenberg and

Whitmore, 1974) and Near Infrared Reflectance (NIR) spectroscopy techniques (Czuchajowska *et al.*, 1992; Sjöholm *et al.*, 1993; Stuart and Edmunds, 1994; Szczodrak *et al.*, 1992). Two of these methods have been adopted as official methods for analysis of β-glucan in barley, malt, wort and beer by ASBC (1992) and European Brewery Convention (EBC, 1987). Both enzymatic and fluorimetric methods have been included in Analytica-EBC since 1989 (EBC, 1987) while the Calcofluor method has been included in the *ASBC Method of Analysis* (ASBC, 1992). Recently, an enzymatic method has been adopted by AOAC International (McCleary and Mugford, 1997).

An enzymatic assay of β-glucan polymers was first attempted by Anderson *et al.* (Anderson *et al.*, 1978). Enzymatic quantification of the polymer in wort and beer samples involves precipitation of the β-glucans with ammonium sulfate followed by a wash with aqueous ethanol to remove free sugars. The β-glucan polymers are then de-polymerized with lichenase (a β-glucan degrading enzyme) and the resulting β-gluco-oligosaccharides are hydrolyzed to glucose with β-D-glucosidase. Finally, the released glucose is determined with the glucose oxidase-peroxidase assay (McCleary and Nurthenm, 1986). However, when beer samples containing low levels of β-glucan (<100 mg/L) are used, incomplete precipitation of the high MW β-glucans by ammonium sulfate may lead to under-estimation of β-glucan content (EBC, 1989).

Calcofluor dye is readily soluble in water (Conn, 1977) and has been used for staining of cellulose (Maeda and Ishida, 1967; Wood, 1980; Wood and Fincher, 1978) and fungal cell walls (Harrington and Raper, 1968). The optical brightener *Calcofluor White M2R New*, also called *Fluorescent Brightener 28* or *Tinopal LPM*, is a fluorescent dye used commercially as a whitening agent. As a useful fluorochrome for microscopic studies (Hughes and McCully, 1975; Wood and Fincher, 1978), its major active ingredient is the di-sodium salt of 4,4'-bis{{4-anilino-6-[bis(2-hydroxyethyl)amino]-δ-triazin-2-yl}amino}-2,2'-stilbenedi-sulfonic acid (Figure 2.3). This dye has proved to be a specific stain for β-glucans (Harrington and Raper, 1968; Maeda and Ishida, 1967). Particularly

intense Calcofluor fluorescence has been noted when staining barley endosperm cell walls (Fulcher *et al.*, 1977; Hughes and McCully, 1975). Beta-glucans are the major component of these walls.

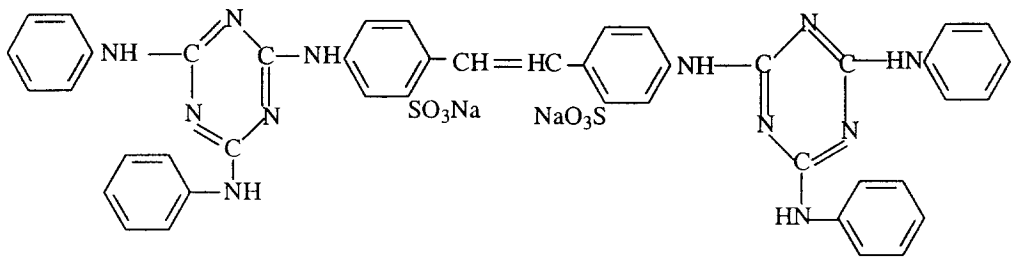

Figure 2.3 Structure of Calcofluor.

Jensen and Aastrup (1981) pioneered the use of Calcofluor in a fluorimetric procedure for measuring β-glucans. The method is based on the specific adsorption of Calcofluor dye to β-glucans (Harrington and Raper, 1968; Hughes and McCully, 1975; Maeda and Ishida, 1967; Wood, 1980; 1981; Wood and Fincher, 1978). This technique was later modified and automated into a flow injection analysis (FIA) system (ASBC, 1992; EBC, 1987; Izawa *et al.*, 1994; 1995; Jørgegnsen, 1988; Jørgegnsen and Aastrup, 1986; Jørgegnsen *et al.*, 1985; 1987; Manzanares *et al.*, 1991; Mekis *et al.*, 1987; Nischwitz *et al.*, 1996; Sendra *et al.*, 1989; Ullrich *et al.*, 1991). Many factors influence the β-glucan determination with Calcofluor. These factors include: (1) type and concentration of Calcofluor dye, (2) pH and ionic strength of buffer, (3) Calcofluor photo-decomposition, (4) excitation and emission assay wavelengths, (5) MW of β-glucan standard, (6) the presence of maltose in wort, (7) darkness of sample color and (8) instrumentation used (ASBC, 1992; Izawa *et al.*, 1994; 1995; Manzanares *et al.*, 1991; 1993; Nischwitz *et al.*, 1996). Of course the reaction between Calcofluor and β-glucan can also be used in manual analytical procedures by mixing the dye with sample, and subsequently measuring the fluorescence intensity with a spectrofluorimeter (Jørgegnsen, 1988).

Calcofluor probably underestimates β-glucans concentrations by failing to detect β-glucans below 10 kDa (Jørgegnsen, 1988; Manzanares *et al.*, 1991; Ullrich *et al.*, 1991). Only β-glucan molecules of MWs higher than 200 kDa bind "fully" with Calcofluor and can be "fully" detected (Manzanares *et al.*, 1991). By increasing ionic strength of the reaction buffer, the detection limit for MW can be lowered to 55 kDa, however, β-glucan molecules <10 kDa could still not be detected (Manzanares *et al.*, 1991; Gómez *et al.*, 2000). Not surprisingly, the MW of the reference β-glucans has been proved to influence the fluorescence response of Calcofluor. A reduction in fluorescence intensity has been noted with standard β-glucans of 82 kDa or less (Nischwitz *et al.*, 1996). The 123 kDa β-glucan polymer gives much higher fluorescence intensity than the 40 kDa β-glucan (So, 1999, personal communication). The response of the β-glucan-Calcofluor reaction was independent of MW in the range of 185-1700 kDa (Suortti, 1993). When the β-glucan content is monitored by the Calcofluor reaction during degradation by β-glucanase, the apparent β-glucan content increased in the early stage of hydrolysis of high MW β-glucans (Gómez *et al.*, 1997a; Navarro *et al.*, 1995). This trend was not observed with hydrolysis of low MW β-glucans. It is unlikely that β-glucanase either increases the β-glucan content or causes any rapid dissociation of β-glucans (enhancing fluorescence intensity) as suggested by Gómez *et al.* (1997a). The apparent increase in β-glucan levels is most likely caused by the altered response of Calcofluor dye with the changing β-glucan MWs. For β-glucan polymers of a very high MW such as the 573 kDa used by Gómez *et al.* (1997a), the polymer might be attacked by β-glucanase and split into two intermediate MW molecules. The total fluorescence intensity would be higher than the fluorescence intensity resulting from Calcofluor attachment to the single high MW β-glucan polymer.

With the fluorimetric method, β-glucan present in barley and malt must be solubilized prior to analysis (EBC, 1989). To release β-glucan bound to proteins in barley, either hydrazine (Forrest and Wainwright, 1977; Martin and Bamforth, 1981) or perchloric acid

(Ahluwalia and Ellis, 1984) can be incorporated into the extraction system. The EBC method applies 75 m*M* H_2SO_4 to extract β-glucan from barley samples (EBC, 1987). An ASBC collaborative trial since 1994 failed to find an acceptable repeatable and reproducible Calcofluor-fluorimetric method (ASBC, 1996a; 1997b). The Calcofluor method also gives lower results than other analysis methods (ASBC, 1997b). However, Jørgensen (1988) reported higher β-glucan results from a Calcofluor-fluorimetric method than an enzymatic method.

The β-glucan content of dark beer samples can not be accurately determined by the ordinary Calcofluor FIA. When an SEC column is incorporated with FIA to measure β-glucan content, the background level can be much lower than with the ordinary FIA (Suortti, 1993). High performance SEC (HPSEC)-FIA (i.e., post-column FIA) techniques determine both the MW distribution and concentration of β-glucan polymers (Manzanares *et al.*, 1993). No difference was observed between the results for dark beer samples by the post-column FIA and the enzymatic methods (Izawa *et al.*, 1996b).

Spectroscopic analysis of β-glucans with Congo red has been reported (Anderson, 1990; Li *et al.*, 1997; Yin *et al.*, 1994). Congo red (Figure 2.4) is a direct cotton dye, which is soluble in water, ethanol and glycol. The detection by Congo red apparently depends on hydrogen bonding with β-glucans. The most rapid and convenient procedure of barley β-glucan determination is probably NIR spectroscopy (Czuchajowska *et al.*, 1992; Sjöholm *et al.*, 1993; Stuart and Edmunds, 1994; Szczodrak *et al.*, 1992) although considerable time is required for calibration of this method.

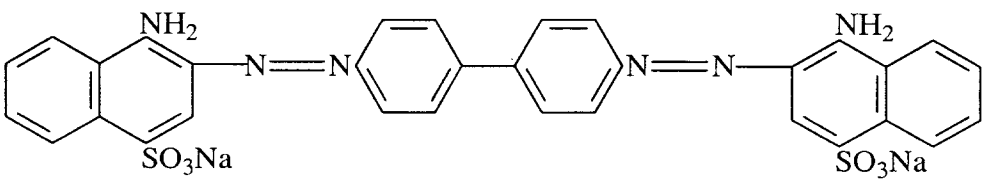

Figure 2.4 Structure of Congo red.

2.7 Beta-glucan Precipitation in Brewing

In the last 40 years, β-glucans have been shown to be the primary component of the precipitate formed by freezing and thawing of beer (Casey and Ingledew, 1985; Haas and Fleischman, 1964; Izawa *et al.*, 1990; Letters, 1977; Skinner *et al.*, 1993; Takayanagi *et al.*, 1969; Tanaka and Sakuma, 1999). Beer β-glucan concentration (in the range of 40-90 mg/L) correlated with the amount of the frozen beer precipitate with an r=0.96 (n=6; Tanaka and Sakuma, 1999). The degree of polymer aggregation (M_w/M_{w0}) can be calculated from the particle weights (M_w) in a given solvent and from that of the monomer (M_{w0}) (Grimm *et al.*, 1995a). The M_w/M_{w0} ratio of β-glucans varies from 4-5 for oat aleurone β-glucans (Vårum *et al.*, 1992) to 17-70 barley β-glucans isolated from beer (Grimm *et al.*, 1995a). Strangely, there has been a report on both gelling and non-gelling forms of β-glucans (Letters *et al.*, 1985). Bamforth (1994) has suggested that low MW β-glucans (<10 kDa) do not cause processing problems while high MW β-glucans precipitate in beer (Forrest and Wainwright, 1977; Izawa *et al.*, 1993; Letters, 1977). Izawa *et al.* (1990) reported that even β-glucans <200 kDa did not precipitate in frozen beer. However, some researchers have argued that small β-glucan molecules (i.e., dialyzed β-glucans) may associate via hydrogen bonding and cause processing problems (Hinchliffe and Box, 1985; Letters, 1977; 1995a).

Pilot brewing has shown that the formation of β-glucan particles in beer is influenced by malt quality, the extent and method of mash agitation, and grist fineness (Leedham *et al.*, 1975). Shearing has a substantial effect on both the viscosity and filtration performance (Narziβ, 1993; Patelakis, 1999; Patelakis *et al.*, 1999). Also, the haze level of β-glucan solutions can be enhanced by a turbulent shear of a 0.5% w/w β-glucan solution especially in the presence of 6% v/w ethanol (Patelakis, 1999; Patelakis *et al.*, 1999). The aggregation of β-glucan polymers in aqueous solutions depends on the solvent composition and temperature (Grimm *et al.*, 1995a, Patelakis, 1999; Patelakis *et al.*, 1999). The presence of low MW hydrophilic molecules, such as sucrose and maltose, can

lower water activity in aqueous glycan solutions. Yalpani (1988) has proposed that lowered water activity due to high sugar content may induce interchain binding.

Several factors may influence polysaccharide precipitation in beer. Polymer size, ethanol concentration, polysaccharide concentration, mechanical shear, and freezing and thawing can be responsible for the precipitation of β-glucans in beer (Letters, 1995a). Ethanol promotes association of β-glucan molecules due to a change in the dielectric constants of the medium (Letters, 1995a). Ethanol concentrations up to 20% v/v cause β-glucan precipitation while at higher ethanol concentrations, α-glucans will be precipitated as well. The "gel potential" of samples (i.e., tendency of β-glucans to precipitate) was found to be proportional to the high MW β-glucan concentration (Byrne and Letters, 1992). Freezing and thawing of beer also accelerates β-glucan precipitation presumably due to an increase in the concentrations of β-glucan and ethanol (Letters, 1995a). The lowered water activity during freezing also favors the association of β-glucan molecules. The structure of water also plays a critical role in the interaction and stability of polysaccharides in solution, and precipitation may be affected by agents that alter the extent of hydrogen bonding. Heating as well as structure breakers such as urea have a destabilizing effect on ordered polysaccharide assemblies (Yalpani, 1988). The precipitation of β-glucans in beer can be enhanced by centrifugal pumping and disc centrifugation (Letters, 1977) where high shear rates are generated. However, precipitation of β-glucans can be reduced by keeping mash velocity below 1.5 m/s and using wide throat low shear pumps for mash conveyance (Andrews, 1996). The amount of β-glucan precipitate increases with beer β-glucan concentration, storage time and concentration of ethanol (Gjertsen, 1966; Grimm and Krüger, 1994). Thus, higher concentrations of β-glucans may give rise to larger precipitated particles (Grimm and Krüger, 1994). Gjertsen (1966) noted that under-modified malt produced β-glucan precipitate in beer after lagering at 0°C for 2 months whereas a beer made from well-modified malt did not. There exists one confusing report that the apparent MWs of β-glucans (i.e., haze particle sizes) measured by light scattering was higher at 70°C than

that at 25°C (Gómez *et al.*, 1997a). Other workers have stated that heating to pasteurization temperatures can solubilize β-glucan hazes (Annemüller and Schnick, 1999; Esser, 1996; Letters, 1995a; Sudarmana *et al.*, 1996).

Malt β-glucanase level and mashing temperature(s) also affect association and precipitation of these polymers. Beta-glucans dissolved during mashing up to 65°C have a higher potential to precipitate than those extracted above 65°C (Letters *et al.*, 1985). When 40°C and 65°C extracted β-glucans are hydrolyzed with a commercial endo-β-glucanase, limited degradation facilitates the β-glucan precipitation after a freezing-and-thawing treatment (Izydorczyk *et al.*, 1998b; Palmer, 1989). Thus, insufficient enzymatic action during malting and mashing may cause incomplete breakdown of β-glucans and result in products having a high tendency to associate and precipitate (Palmer, 1989). Beta-glucanases may also catalyze the transfer of glucosyl residues between oligosaccharides and form insoluble products that become nuclei of white flake-like precipitates in frozen beer (Yamashita *et al.*, 1987). As a de-stabilizer of hydrogen bonding, urea has been shown to distinguish the aggregated high MW β-glucans that urea can break up from those small ones that remain upon treatment (Letters, 1977; Hinchliffe and Box, 1985). These observations are evidence that hydrogen bonding is involved in the β-glucan aggregation process (Grimm and Krüger, 1994).

Until the 1920s, the micelle theory of polymer structure served as a model to explain the loose agglomeration of molecules (Fava, 1980). Vårum *et al.* (1992) postulated a micelle-like structure from their light scattering data of oat aleurone β-glucan solutions. Later, Grimm and Krüger (1994) found that when the particle weight of β-glucan grew by a factor of about 1,000, the radius of gyration increased only slightly (about 5 folds). They explained this with the fringed micelle model where β-glucan chains aggregate side-to-side by hydrogen bonding (Grimm and Krüger, 1994) as illustrated in Figure 2.5. The lateral alignment of the β-glucan molecules are envisaged to form a fairly rigid "stem" that forms the inner region of the micelle (Figure 2.5). In the outer region of the micelles,

the chain sections keep their flexibility when the "stem" becomes stiffened (Grimm and Krüger, 1994). Kunze (1996) championed this concept in *Technology Brewing and Malting* while Linemann and Krüger (1997; 1998a) further explained this model while discussing β-glucan association. In brewing, the fringed micelle model can explain the entanglement of β-glucan molecules in semi-dilute and concentrated solutions (i.e., higher than the critical overlap concentration C*) and therefore helps to explain the aggregation and precipitation of β-glucans in solutions or beer samples.

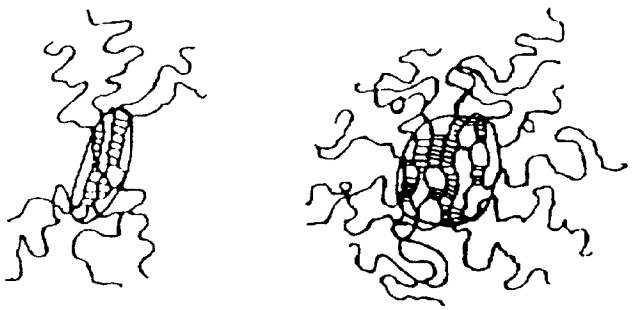

Figure 2.5 Micelle-like aggregates of β-glucan (Grimm *et al.*, 1995a; with permission).

Aside from the fringed micelle theory, β-glucans have been hypothesized to form coiled helices (Letters, 1995a). Beta-glucan molecules are highly asymmetric, with axial ratios of approximately 100 in 40°C water extracted samples; therefore, they exist in solution as extended worm-like chains (Woodward *et al.*, 1983a). Computer modeling suggested that β-glucans exist in a rather extended form, as random coil in solution (Buliga *et al.*, 1986). This was later examined by Vårum *et al.* (1991). Similar to the gel formation mechanism of carrageenans (Rees, 1972), a model was proposed that the random coiled β-glucan molecules form lined-up strands and hydrogen bonded helices (Letters, 1977). Letters (1995b) further modified this model (Figure 2.6) in a way similar to that proposed by Rees (1972) and Morris *et al.* (1980). Random coil aggregation is probably a more

helpful model to understand the interactions among β-glucan molecules when gel formation is observed in concentrated β-glucan solutions. The micelle-like aggregation model on the other hand is more useful to explain the increased particle size of β-glucans.

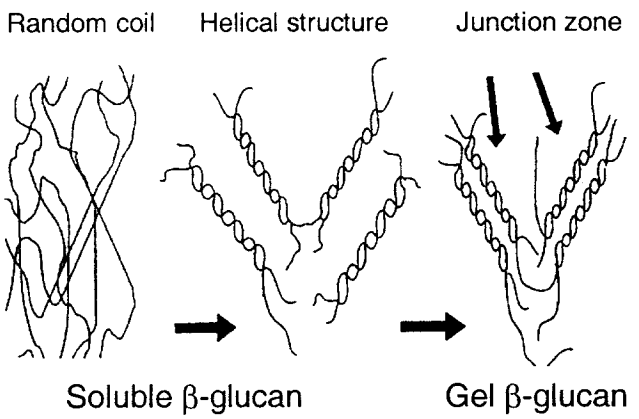

Figure 2.6 Gel formation from β-glucan helices (Letters, 1995b; with permission).

To summarize, the association of β-glucans is thermally reversible, sugar and urea sensitive and shear-enhanced. The hydrogen bonding in the above models is relatively weak and is readily disrupted by structure breakers or high temperatures. Both the fringed micelle and random coil models explain the inhibition of β-glucan association by urea, maltose and heating such as pasteurization. Further studies on the molecular characteristics and structure-property relationships of β-glucans will help us to understand the association and precipitation of β-glucans, and hopefully prevent filtration problems caused by aggregated β-glucans in beer.

2.8 Rheological Properties of β-Glucans

Beta-glucans are linear polymeric molecules. In aqueous solutions, they flex and gyrate, and sweep out a large space. Such molecules in a fluid frequently collide with one

another and cause friction. Viscosity is the measurement of the internal friction and is a characteristic of flow resistance for a fluid. This friction can be easily understood when movement is made between two layers of fluid. The greater the friction (i.e., the higher the viscosity), the greater the amount of force required to cause the movement. Such a force required for the movement is called the "shear stress". The velocity gradient between the two layers as they move by each other is termed the "shear rate". The force per unit area required to produce the shearing is referred to as "shear stress". Numerically, shear stress is the multiplication of viscosity and shear rate. According to Newton, viscosity at a given temperature is independent of the shear rate. Liquids such as water and beer behaving in this way are referred to as *Newtonian fluids*. However, many fluids do not follow Newton's assumptions and are called *non-Newtonian fluids*. Viscosity of a non-Newtonian fluid is called "apparent viscosity" because it is shear-dependent. A typical non-Newtonian fluid can be plastic, pseudoplastic, dilatant, thixotropic or rheopectic. When a certain amount of force must be applied before any flow is induced, the fluid is plastic and the required force is a "yield" stress. If a fluid displays a decreased viscosity with an increasing shear rate (i.e., shear-thinning), it is defined as a pseudoplastic fluid. Otherwise it is a dilatant (shear-thickening) fluid. A thixotropic fluid displays a decrease in viscosity with time at a constant shear rate. Its opposite behavior is rheopectic. For Newtonian flow:

$$\eta = \sigma / \dot{\gamma} \qquad (2\text{–}1)$$

where η is viscosity (Pa·s), σ is shear stress (Pa), and $\dot{\gamma}$ is shear rate (s^{-1}). For shear-thinning or shear-thickening fluids, the power law decides the flow behavior:

$$\sigma = m\ \dot{\gamma}^{n} \qquad (2\text{–}2)$$

where m is the consistency coefficient and n is the flow behavior index. When $n<1$, the fluid behaves as a shear-thinning fluid; when $n>1$, it displays shear-thickening; if $n=1$, the fluid is Newtonian. Generally, high β-glucan contents in malting barley correspond with high wort viscosity (Bendelow, 1975; Greenberg and Whitmore, 1974; Jørgensen and Aastrup, 1986). Since the high MW fractions have a greater effect on viscosity, estimation of the total β-glucan concentration allows limited conclusive information to be

gained. This may also partly explain why estimations of the total β-glucan content sometimes do not correlate very well with the viscosity results (Schur *et al.*, 1974).

An important parameter in relation to a polymer viscosity is its intrinsic viscosity, which is defined as the limiting viscosity value at zero concentration. According to the Mark-Houwink law, the intrinsic viscosity of a polymer is dependent on the shape and deformability of the molecules:

$$[\eta] = K M^{\alpha} \qquad (2\text{–}3)$$

where $[\eta]$ is intrinsic viscosity (dL/g), M is molecular weight, K and α are constants. The intrinsic viscosity of a 327 kDa barley β-glucan has been reported to vary from 4.64-8.62 dL/g under different conditions of model worts (Oonsivilai *et al.*, 2000). Reflecting the molecular size, intrinsic viscosity of barley β-glucan preparations decreased from 4.6-5.2 dL/g to 0.28-1.77 dL/g after enzymatic degradation (Gómez *et al.*, 1997a). As discussed above, β-glucan molecules have been proposed to exist as random coils in aqueous solutions (Buliga *et al.*, 1986; Letters, 1977; 1995b; Woodward *et al.*, 1983a). The value of the exponent α in the Mark-Houwink equation (Eq. 2–3) for β-glucans has been determined as 0.75, which suggests a random coil conformation (Vårum *et al.*, 1991). The 65°C water-soluble barley β-glucan has an intrinsic viscosity of 4.04 dL/g (Woodward *et al.*, 1988). The axial ratio of this β-glucan is about 70 (Woodward *et al.*, 1988). This magnitude of axial ratio suggests a rod-like structure according to Cantor and Schimmel (Cantor and Schimmel, 1980; Woodward *et al.*, 1988). However, it is not clear if the structure is truly rod-like or a coiled-coil conformation.

By measuring the apparent viscosities of an oat bran β-glucan solution (0.375% w/w) at 20, 40, 60 and 80°C, the Arrhenius relationship indicates activation energies of 42 and 17 kJ/mol sheared at 5.81 and 581 s^{-1}, respectively (Wikström *et al.*, 1994). The flow index value for β-glucans has indicated shear dependence of β-glucan viscosity (Autio *et al.*, 1992; Bhatty, 1995; Gómez *et al.*, 1997b; Wood *et al.*, 1989). The apparent viscosity of a rye extract (Härkönen *et al.*, 1997) correlated well with arabinoxylan content (r=0.98) but

poorly with β-glucan content (r=0.67). This was explained by less Newtonian behavior (shear thinning) of β-glucan and the more pronounced Newtonian behavior of arabinoxylan solutions (Härkönen *et al.*, 1997). When the relationship between β-glucan concentration and the relative viscosity of its suspension was examined, an overlap concentration (C*) had been estimated to be 0.42 g/L (Linemann and Krüger, 1997). Lately, the C* value of a 327 kDa barley β-glucan has been reported to be 2.1-6.5 g/L in different buffers (Oonsivilai, 2000; Oonsivilai *et al.*, 1999). Such a "critical concentration" indicates the transition of β-glucan solutions between dilute (C<C*) and semi-dilute and marks the onset of significant molecular overlap and inter-penetration (Morris *et al.*, 1981). Intrinsic viscosities can be useful in deriving the overlap or entanglement concentration (C*) of a polymer in a given solvent system. The concentration at which the reduced concentration (C[η]) equals to 1 has been taken as C* of a polymer (Kasaai *et al.*, 2000). The values of C*[η] for wheat arabinoxylans have been found to be 1.2-1.3 (Izydorczyk and Biliaderis, 1992a). Goodwin and Hughes (2000) have derived the following relationship in theory:

$$C^* = 1.08 / [\eta] \qquad (2\text{-}4)$$

Several factors may alter the flow behavior of β-glucan solutions. The attachment of proteins to β-glucan molecules causes more pronounced shear-thinning behavior of the β-glucan solutions (Autio *et al.*, 1992; Vårum and Smidsrød, 1988). Protease treatment resulted in lower consistency coefficients and higher flow indices (Autio *et al.*, 1992). Sucrose also increases the apparent viscosity of oat β-glucan solutions even at low concentrations and low shear rates (Autio *et al.*, 1987). Among factors evaluated (i.e., ethanol, pH, maltose, yeast protein, freeze-dried beer and the following ions: Ca^{2+}, Mg^{2+}, Na^{+}, Zn^{2+}, SO_4^{2-}, PO_4^{2-}), only ethanol, pH and maltose had significant effects ($p < 0.05$) on the viscosity of a 0.5% w/w β-glucan suspension (Patelakis, 1999; Patelakis *et al.*, 1999).

Mechanical shearing during brewing has an influence on the flow behavior and the gelling properties of β-glucan polymers. After a survey on the shear rates and shear history during brewing, a shear rate of 400 s^{-1} has been suggested as an "average" rate to be used to simulate that occurring in commercial beer production (Oonsivilai, 2000; Patelakis, 1999; Patelakis *et al.*, 1999). Shear rates higher than 1200 s^{-1} have been observed at the stage of pumping from the whirlpool to the wort cooler and from the wort cooler to the fermenters (Oonsivilai, 2000; Patelakis, 1999). Pumping hot wort gently is necessary to avoid an impaired beer quality (Denk, 1996). For mash transfer, pipework should be designed to minimize the length and number of bends, with the possible largest bend radii and lower velocities than 1.5 m/s (Andrews, 1996). Mash transfer should be done at low speeds with wide throat pumps and operated at the optimum region of their performance curves to minimize shear within the pump casing (Andrews, 1996). In the brewery operations, the shear caused by conveying is affected by the design of straight pipeline and infeed flow, bends, manifolds and T-pipes, valve bodies, wort boiling kettle, whirlpool, centrifugal pumps, and other mixing equipment (Denk, 1996). An optimum shear during wort boiling exists with a power uptake around 5-8 w/hL (Reed, 1991; Reed and Jordan, 1991). Because shearing enhances aggregation and precipitation of β-glucans (Letters *et al.*, 1985; Narziβ, 1993; Patelakis, 1999; Patelakis *et al.*, 1999), pumping of fermenting wort, centrifuging of beer, and cross-flow membrane filtration that employs high rate of tangential flow will certainly impair beer filtration rate particularly during membrane filtration.

2.9 Prediction of Problems Caused by β-Glucans

The total β-glucan content alone cannot always predict the performance of beer filtration properly (Narziβ *et al.*, 1989a; Stewart *et al.*, 1998; Sudarmana *et al.*, 1996). Predictive measurements of wort or beer properties have become one of the major goals in barley β-glucan research. Since the 1970s, membrane filtration test has been adopted by many researchers to predict the filtration behavior of beer samples (Annemüller and Schnick,

1999; Back, 1997; Egi, 2002; Egi *et al.*, 2001; Esser, 1972; Esser and Schildbach, 1972; Eyben and Duthoy, 1979; Gans and Denk, 1995; Jin *et al.*, 2001; Meier *et al.*, 1995; Oonsivilai, 2000; Oonsivilai *et al.*, 1999; Patelakis, 1999; Patelakis *et al.*, 1999; Reed *et al.*, 1983; Reid *et al.*, 1990; Siebert *et al.*, 1984; Stewart *et al.*, 1998; 2000; Sudarmana *et al.*, 1996). For the membrane filtration test of rough beer, pre-filtration with a 0.65 μm membrane is necessary to avoid the early clogging of the membrane by yeast deposits (Siebert *et al.*, 1984). As any filtration shears the sample (thus potentially affecting β-glucan colloidal status), one might argue that mild lab centrifugation would be preferable to this 0.65μm pre-filtration step. Since rough beer filterability correlated very well with that for bright beer, it would be appropriate to perform the membrane filtration test at the end of maturation to predict filtration problems (Sudarmana *et al.*, 1996) before it becomes too late for corrective actions.

Both concentration and MW of β-glucans affect viscosities of wort and beer. Congress wort viscosity rather than β-glucan content was proposed as a simple prediction of the β-glucan related problems by early workers (Erdal and Gjertsen, 1967). Only high MW β-glucan levels in wort and beer correlate well to viscosities of wort and beer as well as beer filterability. However, as previously noted, total β-glucan levels in wort and beer, have poor correlation to beer filterability (Narziβ *et al.*, 1989a; Stewart *et al.*, 1998; Sudarmana *et al.*, 1996). Based on theory that hydrogen-bonding is involved in association of β-glucan molecules, urea at 3% has been used to identify the non-dialyzable (i.e., high MW and aggregated) β-glucan fractions in beer samples (Hinchliffe and Box, 1985; Letters, 1977). Similar β-glucan levels are found in the presence of urea while different non-dialyzable β-glucan levels are detected in different samples. This indicates the existence of aggregated high MW β-glucans that correlate to beer filterability (Hinchliffe and Box, 1985).

The formation of a viscous gel indicates problematic β-glucans when an aqueous solution (10% w/v) of a fluorescent brightener (i.e., Calcofluor) is added dropwise to 0.5 mL β-

glucan solutions or wort and beer samples (Letters, 1995a). Shearing beer samples provides another way of predicting the potential problem of beer (Letters *et al.*, 1985). A turbulent shear of a 0.5% w/w β-glucan solution enhanced the haze formation as well (Patelakis, 1999; Patelakis *et al.*, 1999). However, it has been argued that the "gel potential" proposed by Letters *et al.* (1985) or "gel concentration" proposed by Krüger *et al.* (1989) can only predict the problem that will occur in a brewery when the specific brewing procedures and parameters are used (Wainwright, 1990). The tendency of β-glucan precipitation can also be indicated by viscosity reduction after lichenase treatment (Grimm *et al.*, 1995b). Large viscosity reductions represent high concentrations of degradable β-glucans and indicate a high risk of β-glucan precipitation (Grimm *et al.*, 1995b).

Upstream prediction methods of problem β-glucan levels prior to fermentation would be preferred by brewers. However, high deviations of membrane filterability of production wort make judgement difficult (Siebert *et al.*, 1984). Adjustment of wort pH value to that of beer (i.e., lowering pH) and ethanol content to the levels in beer followed by equilibrating for about 18 hours improves the reliability of the membrane filtration prediction (Siebert *et al.*, 1984). An earlier detection method for problematic β-glucans has recently been proposed by Narziβ and Wagner (Narziβ, 1993; Wagner, 1999). Similar to measuring the fine/coarse extract difference, the fine/coarse β-glucan difference between the worts prepared from fine and coarse ground malt may indicate the extractable level of high MW β-glucan fraction (greater in under-modified or unevenly modified malt) (Narziβ, 1993; Wagner, 1999).

It is difficult to accurately predict beer filterability as it depends on the malt used, the mashing and brewing conditions applied and the yeast employed (Wainwright, 1997). When barley adjuncts are used, with or without exogeneous β-glucanases, the effect of malt high MW β-glucans on beer filterability becomes less apparent. However, if filtration becomes difficult, β-glucanases can be used to degrade the β-glucan polymers

(Forage and Letters, 1986). However, most commercial β-glucanase preparations are contaminated with protease activities, which may degrade beer proteins and affect beer foaming properties. Fining with isinglass at 10-20 ppm for 5 minutes to 40 hours also improved beer membrane filtration performance (Sudarmana *et al.*, 1996). Heat dissociation of the aggregated β-glucans is another immediate means to eliminate the filtration problems caused by β-glucan in the packaging plant. Heating beer to 70°C or flash heating beer to 76-80°C solubilizes β-glucan aggregates (Annemüller and Schnick, 1999; Esser, 1996). The filtration will be easier upon cooling down the beer. These are in agreement with the dissociation of β-glucan precipitates by heating (Letters, 1995a). It must be borne in mind, however, (1) that heat treatment has a potential to affect the beer flavor stability and (2) may only be a temporary solution.

2.10 Research Needs and Thesis Objectives

There have been many advances in understanding the basic structure, histochemical localization, analytical methods, and physiochemical properties of β-glucans in the past 50 years. It is evident that the high viscosity and molecular aggregation of these polymers lead to predictable problems in beer filtration and beer stability. However, there has been no sophisticated approach to alerting the maltsters and brewers with β-glucan related problems. An early upstream prediction of the presence of problematic β-glucans reserves a high research priority. The β-glucan structure-property relationship, the exact course of molecular aggregation, and β-glucan behaviour in brewing still remain to be elucidated. Shear-induced β-glucan haze formation and related technological difficulties are emerging issues of these problematic polymers. Examining the rheological properties of β-glucan precipitates will certainly help us to understand the mechanism(s) of and solutions to brewing problems caused by β-glucans. Interestingly, barley β-glucans as large saccharides have been found to show no foam-enhancing or foam-stabilizing effects in beer (Lusk *et al.*, 2001). However, β-glucans have not been examined to determine if they influence the surface tension of beer.

The major goal of this study was to investigate the effect of barley β-glucans on the brewing process and beer quality, and to seek techniques to predict such processing difficulties. In order to reach such a research goal, four objectives were proposed based on the current understanding of this problematic polymer as discussed previously. The first objective was to determine the effect of shear on the particle size distribution of β-glucan polymers in wort and beer and its contribution to wort and beer viscosities and beer haze. The hypothesis examined was that, "shearing of β-glucan solutions or wort and beer samples will cause an aggregation of β-glucan molecules resulting in an increase in β-glucan particle size, haze and apparent viscosity". A second objective was to test the hypothesis that wort and beer filterability is affected by β-glucan MW and concentration. The third objective was to develop a rapid, simple and upstream method for the detection of problematic β-glucan polymers. This would provide a sound judgement of malt/wort quality regarding the potential β-glucan problems and could be used to develop preventative and corrective techniques for eliminating such problems in final beer. The last objective was to examine whether and how β-glucans affect the interfacial and foaming properties of wort and beer.

3 EFFECT OF β-GLUCANS ON THE VISCOSITIES OF WORT AND BEER

This chapter reports the influence of MW and concentration of β-glucans on wort and beer viscosities. Environmental conditions such as pH, maltose level in wort, ethanol content of beer, shearing and shearing temperatures were also examined for their effects on the viscosities of wort and beer.

3.1 Introduction

Fluids can be characterized by their viscosity, which represents their resistance to flow. The concepts of shear rate and viscosity can be explained by a parallel laminar flow illustrated in Figure 3.1. One can consider a fluid between two parallel planes of an area (A) and separated by a given distance (d). When a force (F) is applied to move the upper plane at a given speed (U_s), the following relationships govern the flow field:

$$\begin{aligned} \dot{\gamma} &= U_s / d \\ &= (F / A) / \eta \\ &= \sigma / \eta \end{aligned} \qquad (3\text{–}1)$$

where $\dot{\gamma}$ is the shear rate. The force applied per unit area is termed shear stress (σ) and η is defined as the apparent viscosity of the fluid. Fluids that have viscosities independent of shear rates are Newtonian fluids. If the viscosity of a fluid is shear-dependent, it is termed a non-Newtonian fluid. However, wort and beer are often considered to be Newtonian fluids.

The viscosity of wort and beer have several influences on the brewing process and beer quality. Beer viscosity contributes to its body (Hough *et al.*, 1982b). A high beer viscosity can retard the drainage of liquid from foam bubble walls and lead to better head retention of beer foams (Lusk *et al.*, 1995). However, high viscosities of wort and beer

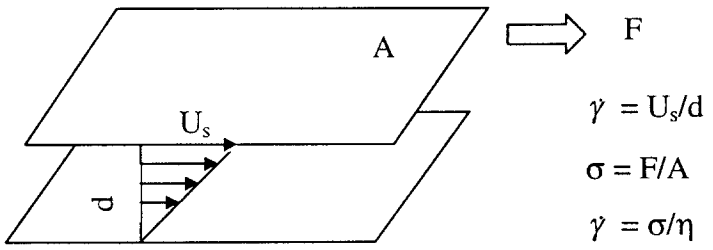

Figure 3.1 Slit flow of viscous fluids between two parallel planes.

can lower the efficiency of many unit operations including mixing and stirring of mashes, pumping, wort separation from spent grains, wort boiling, cooling of wort, "mixing" of beer by the convective current in fermenters, beer clarification (i.e., sedimentation of yeast cells and colloidal particles), as well as beer filtration (Table 1.1). Wort viscosity has been reported to vary between 1.59-5.16 mPa·s for specific gravity (SG) 1.030-1.100 while beer viscosity can vary between 1.45-1.96 mPa·s at 20°C (Hough *et al.*, 1982a; Oonsivilai, 2000). However, wort is usually processed at temperatures of 50 to 100°C while beer is fermented and filtered at 20 to -1°C. Thus, the viscosity of hot wort is often lower than that of beer (although wort contains high levels of fermentable sugars). The unit operation in the brewhouse most affected by high viscosities is wort separation with lauter tuns or mash filters. Slow wort separation can result in longer equipment residence times and make it difficult to complete scheduled brews. Also, high beer viscosity values can cause serious processing difficulties in filtration.

Factors influencing the viscosities of wort and beer include temperature, pH, and levels of maltose, ethanol and macromolecules such as proteins and polysaccharides. The purpose of this study was to determine how β-glucan MW and concentration affect the viscosities of wort and beer, under various conditions of shearing, temperatures, and wort and beer composition.

3.2 Materials and Methods

3.2.1 Materials

Barley β-glucans purchased from Megazyme International Ireland Ltd. (Bray, IRL) had molecular weights of 31 kDa (Lot No. 41101), 137 kDa (Lot No. 90401), 250 kDa (Lot 60501) 327kDa (Lot No. 40301) and 443 kDa (Lot No. 90501). All β-glucan samples containing various amounts of moisture were dried at 50°C under a vacuum of 760 mmHg for 6 hours and constant weights were reached. Dried β-glucans were stored dry at room temperature (20°C). Purified lichenase (EC 3.2.1.73) from *Bacillus subtilis* was also purchased from Megazyme (Bray, IRL). Lichenase is an endo-(1→3)(1→4)-β-glucanase, which specifically cleaves the β-1,4-linkage of the O-3-substituted glucose units (i.e., $-^{3}G_{1}—^{4}G_{1}-$) (McCleary and Glennie-Holmes, 1985; Müller *et al.*, 1998; Wood *et al.*, 1991b). It has the identical specificity as barley malt (1→3)(1→4)-β-D-glucan 4-glucanohydrolase (Müller *et al.*, 1998). The name of lichenase is used throughout this thesis to distinguish this enzyme from other types and other sources of β-glucanases. A commercial β-glucanase prepared from *B. subtilis*, Filtrase B^{TM}, was kindly provided by Gist-Brocades France S.A. (DSM Food Specialties, Seclin, FRA).

A low β-glucan pale malt (*cultivar* Harrington, crop year of 1998, containing β-glucan at 100 mg/L in Congress wort, which was prepared by following the EBC mashing procedure) was commercially prepared at Canada Malting Co. Ltd. (Calgary, AB). Malt samples were stored in airtight containers at 4°C until used (up to 12 months).

Congo red (Lot 49H2608) was purchased from Sigma Chemical Co. (St. Louis, MO) while diatomaceous earth (DE, product number D3877) was purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON).

"AcetatePlus" (supported, plain) membranes (25 mm in diameter) with a nominal pore size of 0.45 μm (Cat. No. A04SP02500, Material No. 1215635, Batch No. 77050, 088439, 087551 and 093062) were purchased from Osmonics Inc. (Minneapolis, MN).

3.2.2 Preparation of β-Glucan-free Wort

A "low β-glucan" Harrington pale malt (i.e., the Congress wort of this malt contained 100 mg/L β-glucan) was used to prepare wort. An Osterizer 8 Food Processor Accessory (Sunbeam Corporation Canada Ltd., Missassauga, ON) set at "Chop" was used to grind 125 g of malt for 20 s.

Mashing was carried out in a 20.5 L stainless steel container (D=30.5 cm, H=28 cm) heated on an adjustable 1 kW hot plate. The mash was gently stirred manually every 5 minutes. The mashing procedure was modified from the EBC Congress mashing method (ASBC, 1992). The mashing process started with mixing 3.0 kg of ground malt with 9 L of double distilled and de-ionized water (DDW) at 47°C leading to a mash temperature at 45°C. The start pH value of the natural mash was 5.4 at 45°C. The mash was kept at 45±1°C for 30 minutes followed by heating at 1°C/min for 25 minutes up to 70°C. The mash was further kept at 70±1°C for 60 minutes for saccharification. An iodine test with 0.02 *N* I_2 solution showed no iodine reaction of the liquid phase of the mash after saccharification for 10 minutes. The mash temperature was then brought to 76°C to separate wort from the spent grains by laboratory lautering. The small-scale lautering was performed using a ceramic flat-bottomed Büchner filter (D=24 cm, H=10 cm) with one layer of Whatman No. 2 filter paper. Lautering was accelerated by application of 500 mm Hg of vacuum. The resulting clarified wort was collected and termed the "first wort" (15°P, measured by a 8-16°P hydrometer, Cat. No. R-165-000, Rascher & Betzold, Inc., Chicago, IL). Sparging water (distilled water at 76°C, 3 L and 2 L, respectively) was used to collect aliquots of second and third worts (of 7°P and 3.5°P, respectively). All worts were mixed and boiled for 1 hour to allow 25% v/v of evaporation. The hot wort was

filtered through Whatman No. 1 filter paper to remove the hot trub. About 9 L of high gravity (16.9°P) wort was collected. This wort was further treated with a commercial β-glucanase to reduce its β-glucan to an undetectable level.

A β-glucanase preparation (Filtrase B^{TM}, DSM Food Specialties, Seclin, FRA) at 0.1 g/L wort was added to hydrolyze β-glucan at natural wort pH (5.4) and at 48°C for 3 hours. Beta-glucan in the treated wort was undetectable by Congo red assay. This β-glucan-free wort was dispersed into 1000 mL Erlenmeyer flasks and covered with 4 layers of aluminum foil. The wort was autoclaved at 121°C for 15 minutes in order to inactivate the remaining β-glucanase. The wort was then filtered through an "M" Kimax Büchner funnel (Fisher Scientific Co. Ltd., Nepean, ON) with 1.0 g/100 mL of diatomaceous earth (Sigma-Aldrich Canada Ltd., Oakville, ON) as a filter aid to remove coagulated and suspended particles. SG of the wort was 1.0695 (i.e., 16.9°P) at 20°C. The clarified wort was preserved with 100 mg/L of NaN_3 at room temperature and stored until used (up to 16 weeks). This wort was found to be stable (i.e., no microbial growth or haze development) during the storage.

3.2.3 Preparation of Wort Samples at Various β-Glucan Levels

The commercial "Megazyme" barley β-glucans of various MWs (i.e., 31, 137, 250, 327 and 443 kDa) were dissolved in DDW with 100 mg/L of NaN_3 to make 0.500 g/100 mL stock solutions. The pH value of the high SG wort was 5.28 and was adjusted to pH 5.40 by adding 0.5 mL of 1 *N* NaOH per liter of wort. An adequate amount of β-glucan stock solution was mixed with the pH 5.4, β-glucan-free wort (16.9°P), and DDW (pH was adjusted to 5.4) to prepare β-glucan solutions at 0, 50, 100, 200, 400, 600, 800 and 1000 mg/L in 12.0°P wort at pH 5.4. Duplicate wort samples (at five MWs and seven concentrations) were sheared (described in section 3.2.6). Sheared and unsheared wort samples were then examined for their viscosities and other properties (i.e., filtration

performance, haze level, β-glucan particle size distribution, wort surface tension and foaming properties).

To investigate the effect of β-glucans at various pH values and maltose levels, the 16.9°P, β-glucan-free wort and a 443 kDa (the highest MW available) β-glucan solution (0.500 g/100 mL) were used to prepare 8°P wort containing 600 mg/L of β-glucan. Maltose (Sigma Chemical Co.) containing 5.4% moisture was incorporated (at 4.0% and 10.0% w/w of dry matter) into the 8°P wort to prepare 12°P and 18°P worts, which were only different in maltose content from the 8°P wort. The maltose content of the 8°P wort was 6.1% w/w determined with Fehling's solutions (ASBC, 1992). Therefore, the 8°P, 12°P and 18°P worts contained 6.1%, 10.1% and 16.1% w/w of maltose, respectively. Each wort sample was divided into three equal portions and the pH value was adjusted from 4.95 to 4.0 (with 5.40 mL of 1 *N* HCl per liter of wort), 5.4 (with 0.16 mL of 1 *N* NaOH per liter of wort) and 6.8 (with 9.60 mL of 1 *N* NaOH per liter of wort), respectively. The ionic strengths of worts differed between 4.2-9.4 m*M*, due to pH adjustment. This minor difference in ionic strength was assumed not to affect experimental results. These worts were examined under a three factor, three level experimental design (Figure 3.2) to investigate the effects of pH, maltose content and shearing temperature. Adequate amounts of 1 *M* sodium azide solution were incorporated to adjust the final concentration of NaN_3 to 100 mg/L. Measurements for each sample were completed within 5 days after sample preparation although no microbial growth was noted for at least 6 months.

3.2.4 Preparation of β-Glucan-free Beer

A commercial lager beer (Labatt Blue™, product code E10H11C, UBC 062067351013, ethanol content 5% v/v, Oland Breweries Ltd., Halifax, NS) was purchased locally and used as the beer base where β-glucan was hydrolyzed. The real extract was determined to be 3.3% with the ASBC method (ASBC, 1992). Beer was first degassed by filtering

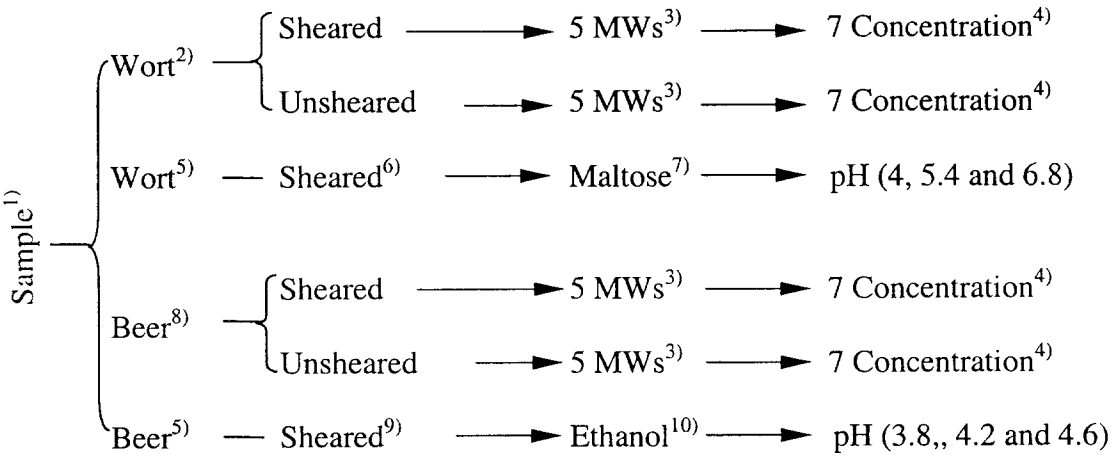

Figure 3.2 Experimental design and sample preparation.

[1]: Experiments were carried out in duplicate; [2]: Tested at pH = 5.4, SG = 12.0°P and T = 5.0, 20.0, 48.0 and 76.0°C; sheared at 20.0°C; [3]: 31, 137, 250, 327 & 443kDa; [4]: 0, 50, 100, 200, 400, 600, 800, 1000 mg/L; [5]: Containing 443 kDa β-glucan at 600 mg/L; [6]: Sheared at 20.0, 48.0 and 76.0°C; [7]: Worts (12°P and 18°P) were prepared from 8°P wort (containing 6.1% w/w of maltose) by supplementing maltose to 10.1 and 16.1% w/w, respectively; [8]: Samples were tested at pH = 4.2, Ethanol = 5.0 % v/v and T = 5.0°C; sheared at 5°C; [9]: Sheared at 0, 5.0 and 10.0°C; [10]: Ethanol concentrations at 0, 5.0 and 10.0% v/v.

through Whatman No. 1 filter paper under a vacuum of 20 mm Hg. The degassed beer (25 L) was then boiled for two hours to remove ethanol and other volatile components (which resulted in 55 g of wet precipitate). The concentrated beer was cooled and kept at 50°C followed by adding lichenase at 0.1 U/mL (Megazyme, Bray, IRL) to hydrolyze β-glucan for 30 minutes until the residual β-glucans were undetectable by Congo red dye. One lichenase activity unit (U) was defined as the amount of enzyme required to release 1 μmol of glucose per minute from a barley β-glucan substrate at 10 mg/mL (McCleary and Mugford, 1997). The enzyme was then inactivated by autoclaving in Erlenmeyer flasks covered with 4 layers of aluminum foil at 121°C for 15 minutes. The concentrate having an extract content of 7.14% w/w was kept at room temperature until used (up to 2 months). There were no stability problems noted during the storage.

3.2.5 Preparation of Beer Samples at Various β-Glucan Levels

Beta-glucans (31, 137, 250, 327 and 443 kDa) were dissolved in DDW at a concentration of 0.300 g/100mL. Degassed "beer" samples were prepared by mixing adequate amounts of the β-glucan stock, beer concentrate, anhydrous ethanol and DDW. To investigate the effect of MW and concentration of β-glucans on beer viscosity, β-glucans (MWs at 31, 137, 250, 327 and 443 kDa) were included at concentrations of 0, 50, 100, 200, 400, 600, 800 and 1000 mg/L in beer, which contained 5.0% v/v of ethanol and 3.3% w/w of real extract at pH 4.2. Beer samples were also subjected to shearing and their viscometric properties examined.

Beer samples containing 600 mg/L of 443 kDa β-glucan were prepared to investigate the effect of pH (3.8, 4.2 and 5.4) and ethanol (0, 5 and 10 % v/v) on beer viscosity and other properties discussed in the following chapters. These samples were also subjected to shearing at different temperatures (0, 5 and 10°C). Sodium azide was not used as a preservative and fresh samples were stored at 5°C and analyzed within 3 days (samples were stable until used).

3.2.6 Shearing Test of Wort and Beer Samples

A Lourdes blender (Model MM-1B, Lourdes Instrument Corp., Brooklyn, NJ) was employed to shear wort and beer samples. A custom-made flat paddle (13×30 mm in breadth×height) rotated inside a 25 mL "clover-leaf shaped" cup. The cup fit the blending paddle device tightly limiting the contact of samples with air during shearing. To minimize the interference of wort/beer oxidation, headspace of the cup was flushed with compressed N_2 for 10 s prior to shearing. Samples after shearing were transferred into a 50 mL plastic centrifuge tube and flushed with N_2 for 10 seconds followed by sealing with caps. In a preliminary shearing experiment, 10.0 mL of a 1000 mg/L solution of 443 kDa β-glucan dissolved in 100 m*M* acetate buffer (pH 4.0, containing 5.0 % v/v of

ethanol) was sheared at speed settings of "30", "60" and "90" for 10, 35 and 60 seconds. As the highest turbidity was observed when the samples were sheared at a speed setting of "60" for 35 seconds (Figure A.1 in Appendix 1), shearing tests thereafter were carried out under these conditions.

Under the above shearing conditions, the power consumption of water (10 mL) was measured with a transducer. An average shear rate of the shearing process was calculated from the power dissipation of the liquid (Cleasby, 1984; Patelakis, 1999). The shear rate was found to be $1.3\pm0.2 \times 10^4$ s^{-1} (n=3). This is only a first approximation of the shear rate since the volume of sample was very small and the power consumption was not an accurate measurement.

3.2.7 Determination of β-Glucan Concentration with Congo Red Dye

Determination of β-glucan content with Congo red dye was modified from the method of Li *et al.* (1997). Beta-glucan samples (100 μL) were mixed with 3.0 mL of 100 mg/L Congo red dissolved in 0.1*M* (pH 9.0) glycine-NaOH buffer. Absorbance at 550 nm was measured with an HP8453 spectrophotometer (Hewlett Packard GmbH, Waldbronn, DEU) and DDW (100 μL) was mixed with 3.0 mL of 100 mg/L Congo red as a blank. Beta-glucan concentrations in the range of 0–1000 mg/L at intervals of 100 mg/L were used to prepare calibration curves.

3.2.8 Determination of Wort and Beer Viscosities

Viscosities of wort and beer were measured with a Bohlin VOR controlled-rate rheometer (Bohlin Instruments Inc., Cranbury, NJ) by using a C-14 coaxial cylinder geometry and a 4.00 g·cm torsion bar. Other conditions were set as follows: shear rates of 91.9, 184, 367, 581, 731 and 921 s^{-1}; an initial equilibrium time of 30 s; autozero "On" and continuous rate option "On"; an autozero delay time of 3 s; a delay time of 10 s and an integration

time of 10 s. Measurement temperature was 20°C for wort and 5°C for beer unless otherwise specified. Shear stress values were measured and the viscosity was obtained from the slope of plotting shear stress versus shear rate ($r^2 \geq 0.99$). The rheometer was calibrated with DDW.

3.2.9 Experimental Design

Experiments were conducted according to the designs depicted in Figure 3.2. Worts (12°P, pH 5.4) containing β-glucans of different MWs (31-443 kDa) at various concentrations (0-1000 mg/L) were examined for their viscosities at 5, 20, 48, and 76°C. The above temperature range was selected to reflect the temperatures during mashing, wort separation, fermentation and maturation. These worts were also sheared at 20°C to simulate the shear effect they may experience during brewing; and their viscosities at 20°C ($\eta_{20°C}$) were measured. A second series of worts were prepared to investigate the effect of β-glucan (443 kDa at 600 mg/L), shearing temperature (20, 48 and 76°C), wort pH (4.0, 5.4 and 6.8), and maltose content (6.1, 10.1 and 16.1% w/w) on wort viscosity ($\eta_{20°C}$). Viscosities of sheared and unsheared beers containing 31-443 kDa β-glucans at 0-1000 mg/L were determined at 5°C. In contrast to β-glucan-free beers (containing 0, 5, and 10% v/v of ethanol at pH 3.8, 4.2 and 4.6), beer samples containing 600 mg/L of 443 kDa β-glucan were sheared at 0, 5, and 10°C and their viscosities were measured at 5°C (to compare the effect of shearing temperature).

The above samples were also examined for the particle size distribution of β-glucans, turbidity, surface tension, foaming properties, wort filtration and beer membrane filtration performance (see later). Duplicate experiments were carried out and mean values and standard deviations were calculated. The amount of replication must be at least two to give ten or more degrees of freedom (Clarke, 1994). However, if there is a large number of treatments, the amount of replication can be a minimum of two (Clarke,

1994). Experimental results were analyzed with SYSTAT version 5.05 for Windows (SPSS Inc., Chicago, IL).

3.3 Results and Discussion

To investigate the effect of β-glucans on wort and beer viscosities, barley β-glucans having MWs of 31, 137, 250, 327 and 443 kDa were added to wort and beer in the range of 0-1000 mg/mL. Wort viscosity was measured at temperatures of 5, 20, 48 and 76°C. The effects of shearing temperature, pH and maltose content on wort viscosity with and without the addition of β-glucan (443 kDa at 600 mg/mL) were examined. Viscosities of sheared and unsheared beer samples were determined at 5°C. The influence of β-glucan (443 kDa at 600 mg/mL), shearing temperature, pH and ethanol content on beer viscosity was studied as well. The intrinsic viscosity of β-glucans (31-443 kDa) was determined and used to compare their molecular properties in wort and beer. The wort and beer samples studied were found to be Newtonian over the range of shear rates between 91.9-921 s^{-1}. Previously, Oonsivilai (2000) had reported possible shear thickening in commercial worts below 100 s^{-1} at 5 and 25°C.

3.3.1 Effect of MW and Concentration of β-Glucans and Temperature on Wort Viscosity

The base wort used in this study was 12°P unhopped wort containing no β-glucan. The viscosity of this wort at 20°C was 1.521 mPa·s. Barley β-glucans (31-443 kDa) added to this wort up to 1000 mg/L increased its viscosity to 1.555-2.185 mPa·s as shown in Figure 3.3. Wort viscosity increased with MW and concentration ($p<0.001$) with the following relationship ($R^2=0.782$; $n=80$; $p<0.001$):

$$\eta = 1.443 + 3.999\times10^{-4}\,MW + 3.622\times10^{-4}\,C \qquad (3\text{-}2)$$

where MW is the molecular weight (kDa) and C is the concentration of β-glucans (mg/L). However, the low MW β-glucan (31 kDa) showed limited viscosity increase up

to 1000 mg/L (Figure 3.3). When β-glucans of 137 to 443 kDa were regressed individually, the wort viscosity increased with their concentration linearly (r^2>0.99) as shown in Figure 3.3. When the lowest MW viscosity values at 31 kDa were excluded and the remaining viscosity values were analyzed, a better correlation was found (R^2=0.907; n=64; p<0.001):

$$\eta = 1.445 + 2.738\times10^{-4}\,MW + 4.411\times10^{-4}\,C \qquad (3\text{-}3)$$

Higher viscosities are detrimental for many unit operations such as pumping and filtration. Thus, brewers desire a minimal level of β-glucans, resulting in lower viscosities of wort and beer. Although other components such as proteins and maltose in wort contribute to viscosity, such components cannot be reduced because their concentrations have influence on the physical and sensory properties of beer. Thus, the most effective way to obtain a low wort viscosity is to reduce the level of β-glucans (and perhaps pentosans as well).

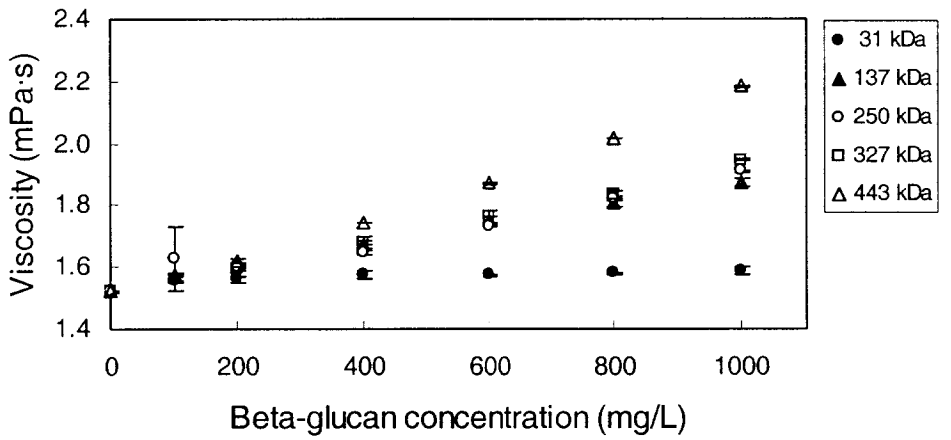

Figure 3.3 Effect of MW and concentration of β-glucans on the viscosity (20°C) of 12°P wort. Values are given as means ± one standard deviation (S.D.) of duplicate experiments.

Beta-glucans at various concentrations and MWs behaved similarly with respect to wort viscosity (Figure 3.3). At a given concentration, the greater the MW of β-glucan, the higher the wort viscosity. The influence of β-glucan molecular weight (at 1000 mg/L) on wort viscosity ($p<0.001$) is shown in Figure 3.4. A linear correlation existed between the MWs and the viscosity of wort ($r^2=0.903$; $n=10$; $p<0.001$):

$$\eta = 1.6025 + 1.3\times10^{-3}\ MW \qquad (3\text{-}4)$$

Results in Figure 3.4, however, appeared to have a sigmoidal trend. The low MW (31 kDa) β-glucan had less impact on the wort viscosity even at 1000 mg/L (1.589 mPa·s compared to 1.521 mPa·s of the same wort containing no β-glucans). The viscosity of worts containing high MW (137-443 kDa) β-glucans at 1000 mg/L increased in an exponential relationship with MW ($R^2=0.965$; $n=8$; $p<0.001$):

$$\eta = 1.1885\ MW^{0.08721} \qquad (3\text{-}5)$$

This relationship is similar to the Mark-Houwink equation, which describes the relationship between the intrinsic viscosity and MW of polymers (Eq. 2-3).

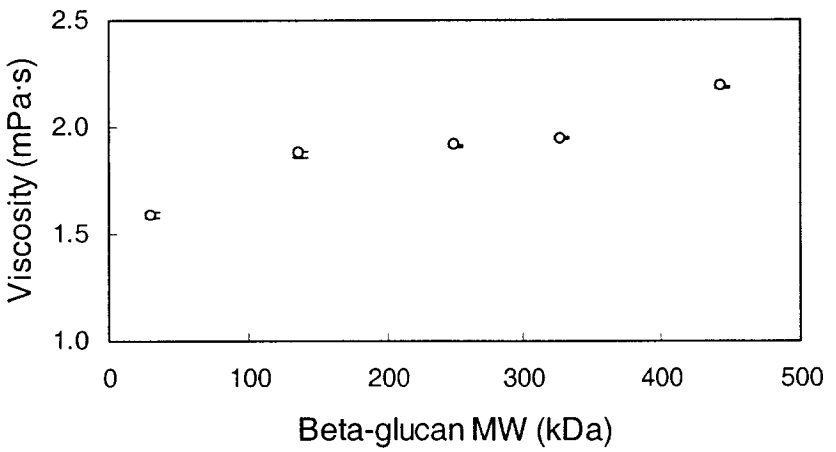

Figure 3.4 Effect of MW of β-glucans at 1000 mg/L on $\eta_{20°C}$ of 12°P wort. Values are given as means ± S.D. of duplicate experiments.

As expected, wort viscosities decreased at higher temperatures (Figures 3.3 and 3.5) for β-glucan MWs and concentrations studied. This is in agreement with the effect of crude β-glucan on wort viscosity as noted by Barrett *et al.* (1973). In this study, the temperatures were set at 48°C to reflect the mashing-in temperature; 76°C to reflect the lautering (wort filtration) temperature; 20°C for that of ale fermentation and 5°C to compare wort viscosity with that of beer at 5°C. In this temperature range, the effects of MW and concentration of β-glucans on wort viscosity were temperature-dependent. The multiple linear regression was used to model the changes in wort viscosity (R^2=0.981; n=320; p<0.001):

$$\eta = 3.050 + 3.3\times10^{-4}\,MW + 1.3\times10^{-4}\,C - 0.666\,T + 1.12\times10^{-6}\,MW\times C + 4.7\times10^{-4}\,T^2 - 1.0\times10^{-5}\,MW\times T \quad (3\text{-}6)$$

where T is the temperature (°C). It is noteworthy that Eq. 3-6 is only a simplified approximation because the effects of MW and temperature on viscosity could possibly be non-linear at higher concentrations. In theory, the solution viscosity as a function of temperature can be described by the Arrhenius relationship (Steffe, 1996):

$$\eta = A_f e^{Ea/RT} \quad (3\text{-}7)$$

where A_f is a frequency factor; E_a is the activation energy (however, there is a negative sign when reaction rate constants are analyzed); R is the universal gas constant; and T is absolute temperature (K). The Arrhenius model was fit to the viscosity data for β-glucans at 1000 mg/L (Figure 3.6). The determination coefficient of this fit for β-glucans (31-443 kDa; n=8) were all >0.99. The E_a value was further calculated from the slope of the Arrhenius plots (Table 3.1) as E_a was affected by β-glucan MW (p<0.001). Beta-glucans having MWs of 137, 250 and 327 kDa had similar E_a values of about 4 kcal/mol, whereas the 443 kDa β-glucan had a slightly higher E_a (4.2 kcal/mol). The 31 kDa β-glucan had a lower E_a at 3.7 kcal/mol. This suggests that β-glucan solutions with a higher MW have greater viscosity dependencies on temperature. Literature reported E_a values for 0.6-1.0% oat β-glucans were 2.4-4.6 kcal/mol (Autio *et al.*, 1987) in general agreement with the results in Table 3.1.

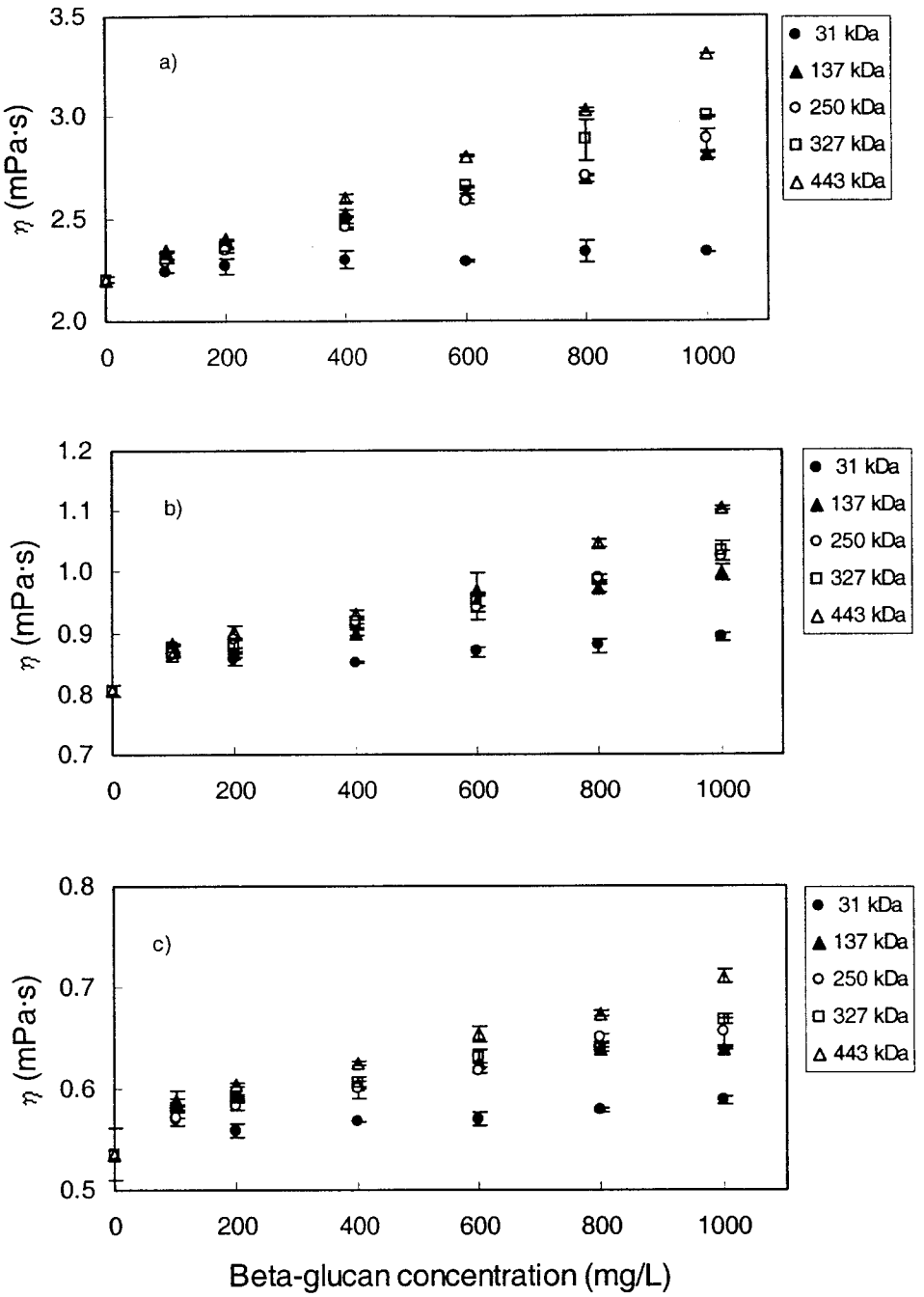

Figure 3.5 Effect of β-glucan MW and concentration on 12°P wort viscosity at (a) 5°C; (b) 48°C and (c) 76°C. Values are given as means ± S.D. of duplicate experiments.

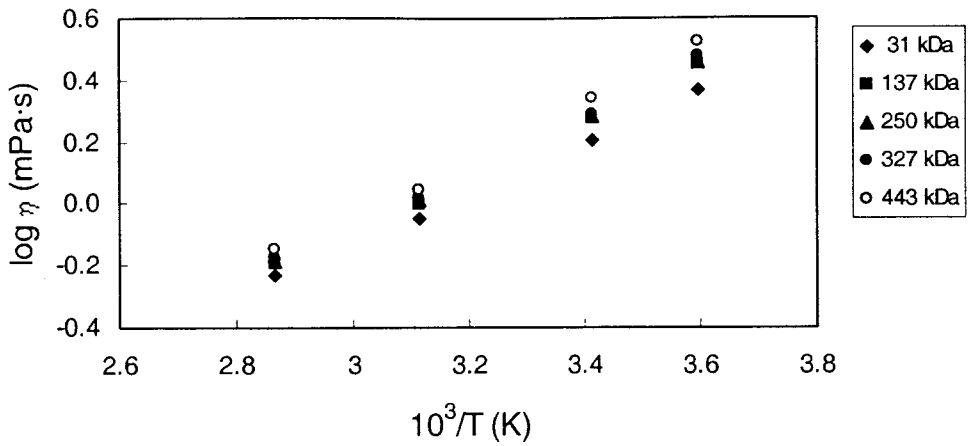

Figure 3.6 Arrhenius plots of β-glucans (1000 mg/L) in 12°P wort. Values are given as means ± S.D. of duplicate experiments.

Table 3.1 Arrhenius activation energy (E_a) of β-glucans in 12°P wort [a)]

β-Glucan MW (kDa)	31	137	250	327	443
E_a (cal/mol)	3746±15	4038±19	4036±84	4088±30	4220±16

[a)]: Values are given as means ± S.D. of duplicate experiments.

The above models of wort viscosity are somewhat empirical as the constants were influenced by solvent conditions (i.e., wort composition). To compare the contributions of MW and concentration of β-glucans to wort viscosity, specific viscosity (η_{sp}) was used to cancel the effect of changes in solvent conditions. The η_{sp} of a solution is defined as:

$$\eta_{sp} = (\eta_{solution} - \eta_{solvent})/\eta_{solvent} = \eta_{rel} - 1 \quad (3\text{-}8)$$

where η_{rel} is the relative viscosity. Specific viscosity of wort samples was calculated by using the viscosity of β-glucan-free wort as a reference:

$$\eta_{sp} = (\eta_{BG} - \eta_{NB})/\eta_{NB} \quad (3\text{-}9)$$

where η_{BG} is the viscosity of wort containing β-glucan and η_{NB} is the viscosity of wort containing no β-glucan, i.e., the β-glucan-free wort was treated as a solvent for β-glucan polymers. The specific wort viscosities are shown in Figure 3.7. The η_{sp} of β-glucans

increased with MW and concentration, but to a lesser extent at higher temperatures ($p<0.001$). The following expression could be used to describe the changes in β-glucan η_{sp} with concentration, molecular weight and temperature ($R^2=0.758$; n=320; $p<0.001$):

$$\eta_{sp} = 2.142\times10^{-4}\,C + 1.893\times10^{-4}\,MW - 2.317\times10^{-4}\,T \qquad (3\text{-}10)$$

The relationship between η_{sp} and the concentration of each β-glucan was essentially linear up to 1000 mg/L (Figure 3.7). It has been reported that higher concentrations such as 0.4-2.4% w/v of crude barley β-glucans showed non-linear increases in their η_{sp} (Bhatty *et al.*, 1991; Scott, 1972). The effect of MW on β-glucan η_{sp} was similar to its effect on wort viscosity discussed earlier, i.e., higher MWs of β-glucans led to greater specific viscosities ($p<0.001$). When temperature was lowered from 76°C to 5°C, β-glucan η_{sp} increased ($p<0.001$) about 1.5-2 fold (Figure 3.7) whereas the wort viscosity increased 4-5 fold (Figures 3.3 and 3.4). This implies that other contributors to wort viscosity (e.g., water and proteins) were also sensitive to temperature. The η_{sp} of β-glucans of 31-443 kDa at 1000 mg/L in the range of 5-76°C varied from 0.0448 to 0.5018 (Figure 3.7). Similarly, a low β-glucan level at 200 mg/L (31-443 kDa) accounted for an increase of 3-12% in the viscosity of the 12°P β-glucan-free wort.

To compare the contributions of β-glucan and other wort components to wort viscosity, the viscosity caused by β-glucans was calculated from the difference between viscosities of β-glucan worts and their controls. The value of such a "differential viscosity" relative to viscosity of water was further calculated (Figure 3.8; Table 3.2). The data agree with the trend shown in Figure 3.7 (i.e., the viscosities caused by β-glucans increased at higher MWs and concentrations, but decreased with increasing temperatures). By using water as a reference solvent, the β-glucans (31-443 kDa) at 200 mg/L only increased viscosity by less than 20% of water viscosity (Figure 3.8). At a high concentration (1000 mg/L), high MW β-glucans increased the viscosity values by 30-70% of water viscosity. Most of the

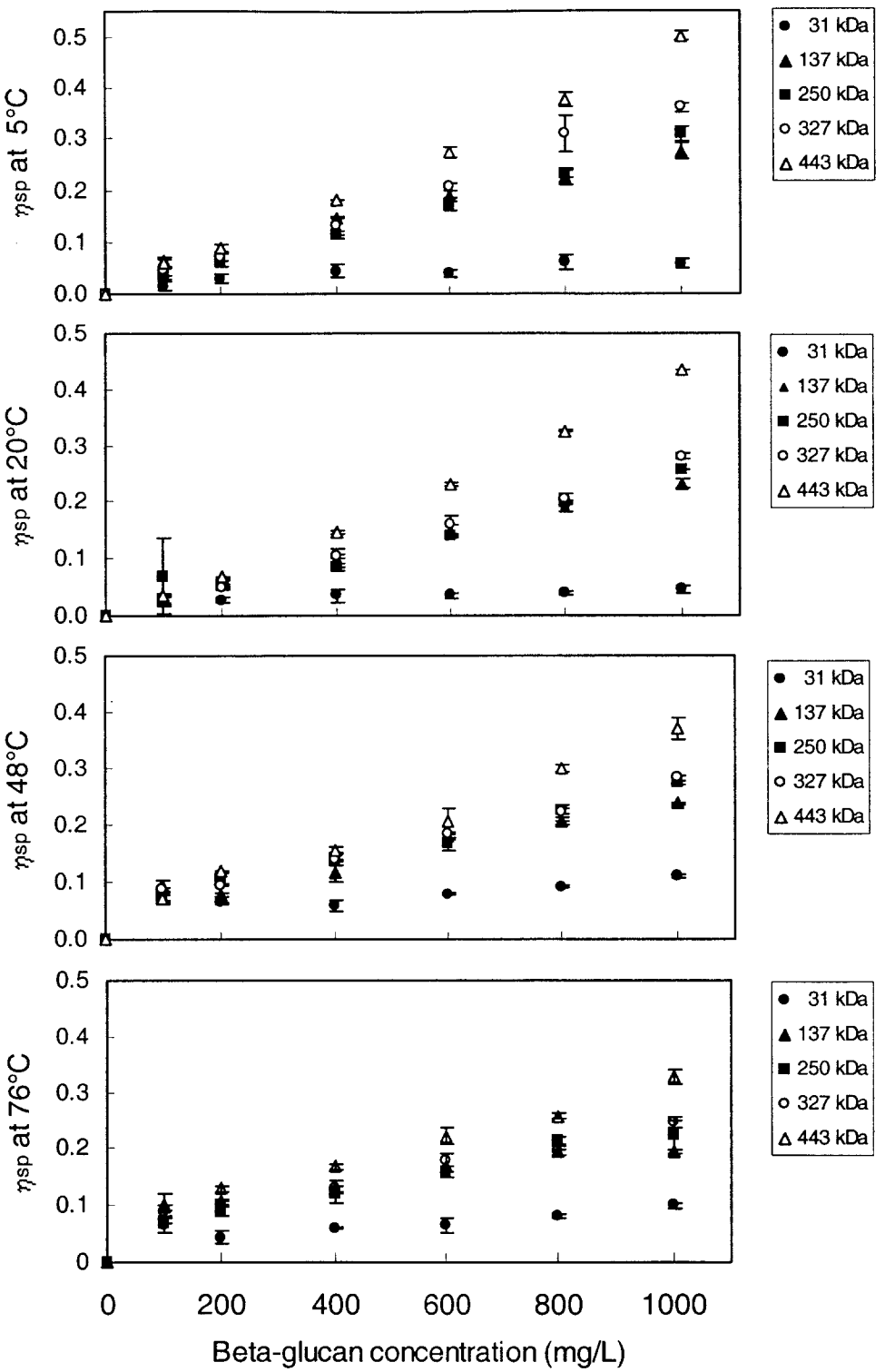

Figure 3.7 Effect of MW and concentration on the η_{sp} of β-glucans in 12°P wort at 5-76°C. Values are given as means ± S.D. of duplicate experiments.

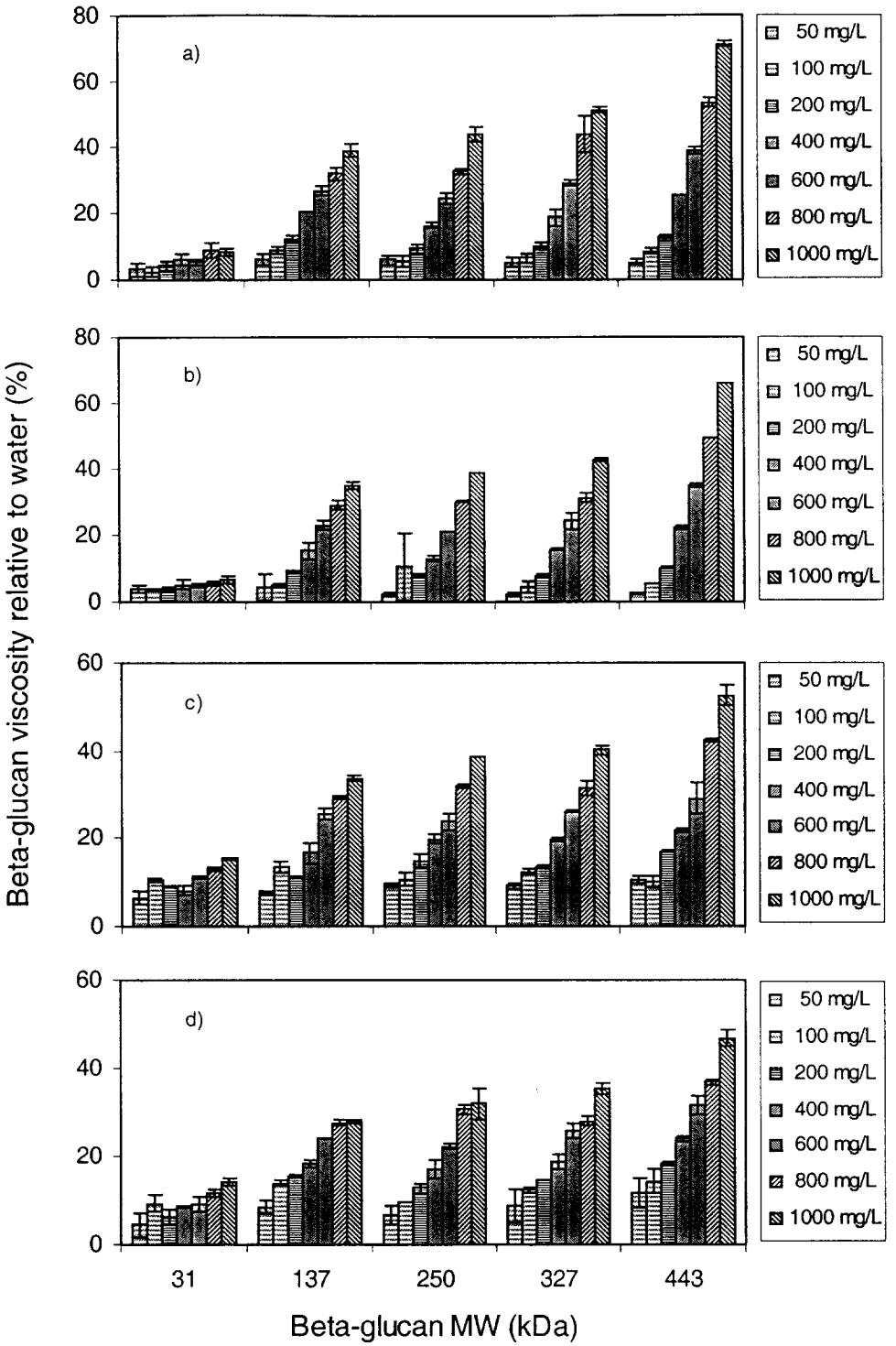

Figure 3.8 Viscosities caused by β-glucans relative to water viscosities at (a) 5°C; (b) 20°C; (c) 48°C and (d) 76°C. Values are given as means ± S.D. of duplicate experiments.

Table 3.2 Viscosity of the β-glucan-free wort relative to water [a)]

Temperature (°C)	5	20	48	76
Wort viscosity relative to water (%)	42±1	52±0	73±3	115±0

[a)]: Values are given as means ± S.D. of duplicate experiments.

β-glucan viscosities relative to water viscosity were lower than the specific viscosity of the β-glucan-free wort. This further suggests that β-glucan polymers are lesser contributors to the wort viscosity compared to other wort components as a whole. It is interesting that the specific viscosity of wort containing no β-glucans increased at higher temperatures (Table 3.2) although the wort viscosity decreased (Figures 3.3 and 3.5). The decrease of viscosity of the β-glucan-free wort at high temperatures was therefore caused by decreased solvent viscosity. The major components of 12°P wort are maltose and proteins. The increased specific viscosity of the β-glucan-free wort may be due to heat enhanced protein-protein hydrophobic interactions. It is hypothesized that the interactions among β-glucan molecules were presumably dominated by hydrogen bonding, which is weaker at higher temperatures.

A concept of "glucan viscosity" (i.e., β-glucan viscosity) was used by He *et al.* (1996) to evaluate the potential risk caused by β-glucans:

$$\text{"Glucan viscosity"}\ (\text{mPa·s/g/L}) = (\eta_{BG}-\eta_{NB})/C \qquad (3\text{-}11)$$

where η_{NB} is the viscosity of wort after treated with lichenase (i.e., viscosity of the β-glucan-free wort) and C is the β-glucan content (g/L) before lichenase treatment. Examination of the viscosities of wort samples with and without β-glucans showed that the "β-glucan viscosity" increased slightly with an increase in MW, but decreased at higher concentrations (Figure 3.9). In this thesis, the "β-glucan viscosity" did not prove to be a good tool in predicting potential problems caused by high MW β-glucans at high concentrations. It was also not useful to divide the differential viscosity ($\eta_{BG}-\eta_{NB}$) by β-glucan concentration. Although a relative parameter, the β-glucan specific viscosity (η_{sp}),

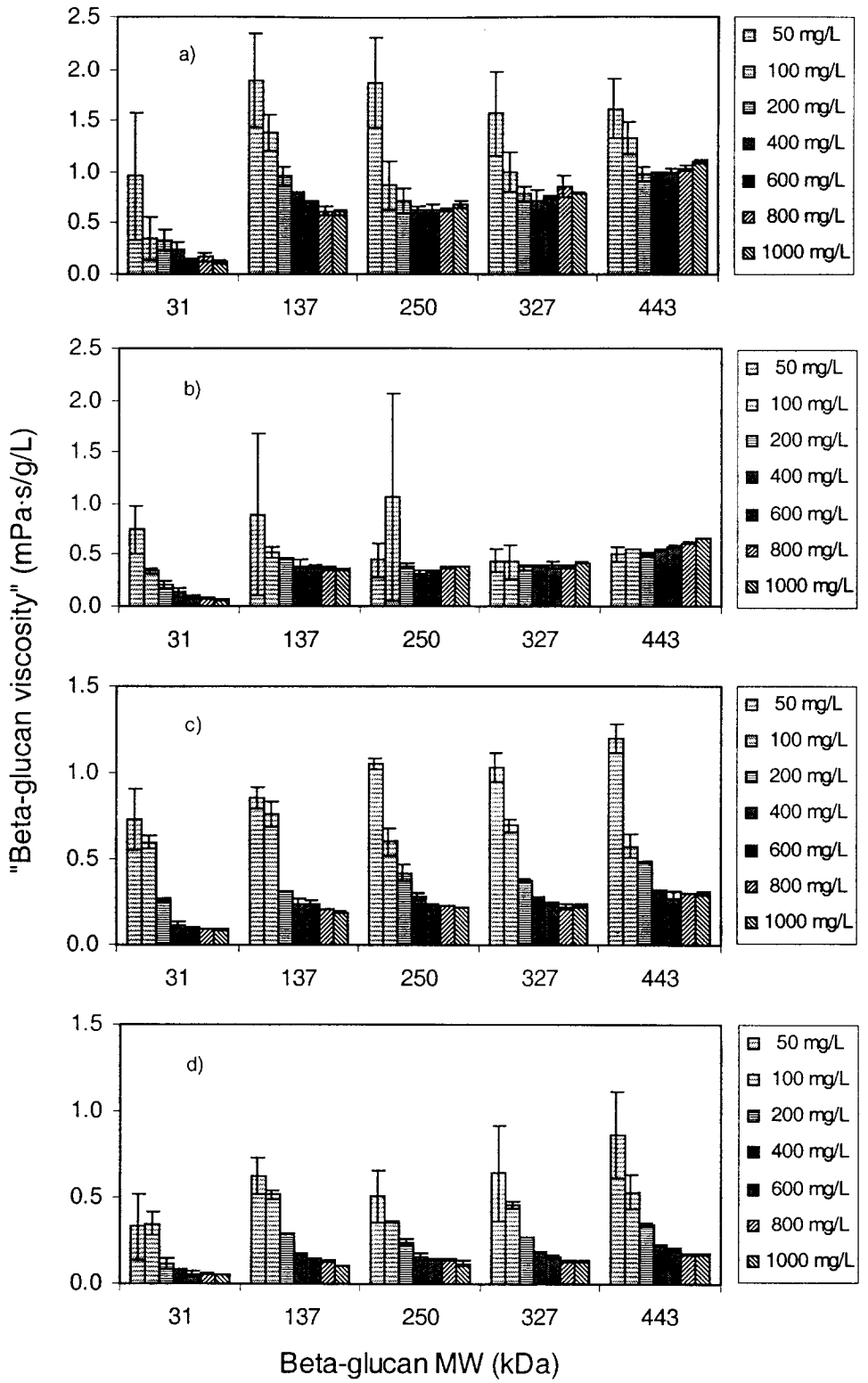

Figure 3.9 "Beta-glucan viscosity" of wort samples at (a) 5°C; (b) 20°C; (c) 48°C and (d) 76°C. Values are given as means ± S.D. of duplicate experiments.

appeared to be a better index to evaluate the contribution of β-glucans to wort viscosity. Also, large values of viscosity due to degradable β-glucans represent a high risk of β-glucan precipitation in beer (Grimm *et al.*, 1995b).

3.3.2 Effect of Shearing at 20°C on Wort Viscosity ($\eta_{20°C}$)

Shearing has been reported to enhance "gel formation" (precipitation) of β-glucans (Letters *et al.*, 1985; Narziβ, 1993; Patelakis, 1999; Patelakis *et al.*, 1999). Shearing of 0.5% w/w β-glucan solutions also significantly ($p<0.05$) retarded 0.45 μm membrane filtration (Patelakis, 1999). Agitation during wort boiling has also been reported to increase the "β-glucan viscosity" (He *et al.*, 1996). To determine if and how shearing would affect the viscosity of wort containing β-glucans, samples were sheared at 20°C and their viscosities determined at 20°C. The viscosities of sheared wort increased compared to the unsheared wort at the same temperature ($p<0.001$; Figure 3.10a). Viscosity of the β-glucan-free wort also increased from 1.521 mPa·s to 2.161 mPa·s after shearing. The η_{sp} of β-glucans at various concentrations are illustrated in Figure 3.10b. Compared to the η_{sp} (20°C) of unsheared samples (Figure 3.7), the β-glucan η_{sp} (i.e., the viscosity caused by β-glucans relative to the wort "solvent") decreased after being sheared ($p<0.001$). For β-glucan-free wort, the viscosity was thus caused by all wort components other than β-glucans. As shearing can cause unfolding and denaturation of protein molecules, the unfolded proteins may have swept out a greater space in the aqueous solution resulting in a higher viscosity. More hydrophobic groups on the protein molecules could have been exposed after shearing and enhanced protein-protein interactions with increased solution viscosity. The decrease in β-glucan-caused viscosity after shearing may be due to association of β-glucan molecules to form larger particles (discussed in Chapter 4) resulting in a lower solution concentration of the particles and therefore lower viscosity.

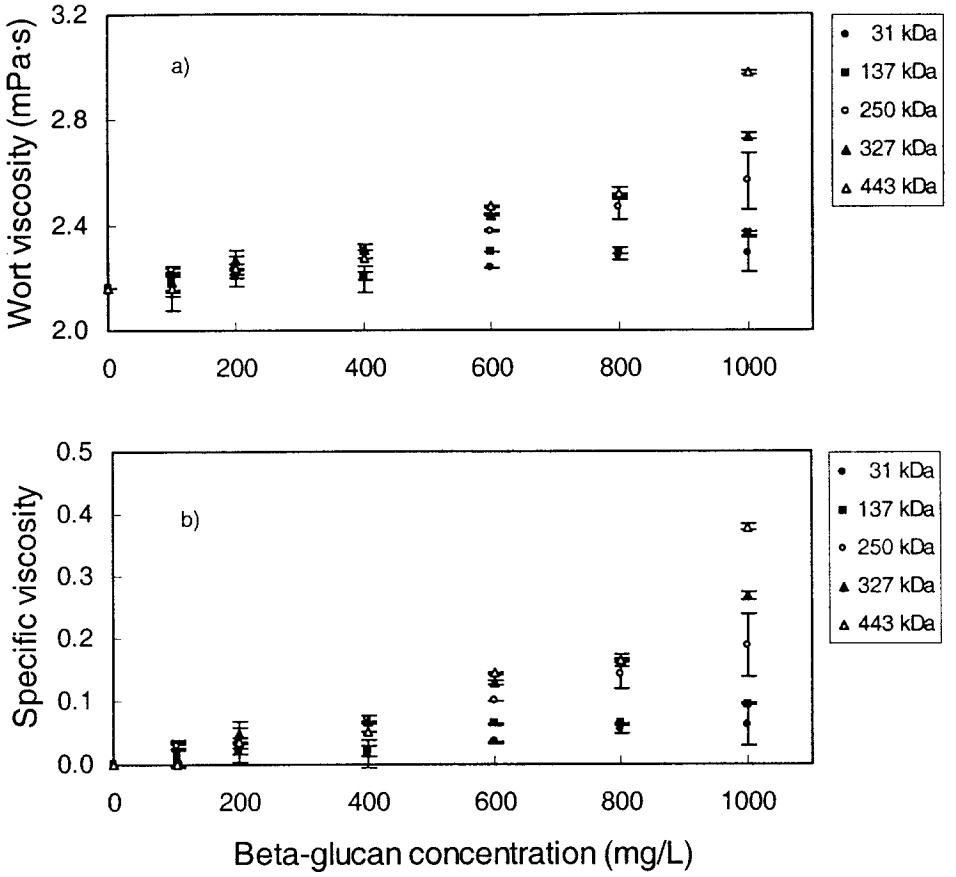

Figure 3.10 Viscosities at 20°C (a) and specific viscosities at 20°C (b) of wort samples sheared at 20°C. Values are given as means ± S.D. of duplicate experiments.

3.3.3 Effect of pH, Maltose Level and Shearing Temperature on Wort Viscosity

A 16.9°P wort was used to prepare an 8°P wort containing 0 and 600 mg/L of 443 kDa β-glucan. This 8°P wort had 6.1% w/w of maltose. Maltose was incorporated into this wort to make 12°P and 18°P worts containing maltose at 10.1% and 16.1% w/w, respectively. These wort samples prepared with or without β-glucan were only different in their maltose and β-glucan levels. At pHs of 4.0, 5.4 and 6.8 (the variation of ionic strength was 4.2-9.4 m*M* and was assumed not to affect viscosity, section 3.2.3 on page 41), wort

samples were sheared at 20, 48 and 76°C prior to viscosity measurement at 20°C. Results are shown in Figure 3.11. The presence of β-glucan (443 kDa at 600 mg/L) increased wort viscosity ($p<0.001$). In addition to the maltose and pH levels, shearing temperature also affected viscosities of the wort samples ($p<0.001$) containing 600 mg/L of 443 kDa β-glucan. Shearing worts at 20°C resulted in lower viscosities than at 48°C and 76°C ($p<0.001$). The viscosity of sheared worts containing 600 mg/L of 443 kDa β-glucan could be expressed by the following relationship ($R^2=0.978$; $n=108$; $p<0.001$):

$$\eta_{20°C} = 0.9960 + 0.001\ T_s + 0.061\ Mal + 0.018\ pH \qquad (3\text{-}12)$$

where $\eta_{20°C}$ is wort viscosity at 20°C (mPa·s); T_s is the shearing temperature (°C) and Mal is the maltose concentration (% w/w) of wort. For a real-world wort sample, however, the level of β-glucans should be considered as well. The data of all the sheared worts, with and without β-glucans, could be modeled by the following relationship ($R^2=0.976$; $n=108$; $p<0.001$):

$$\eta_{20°C} = 0.878 + 3.751\times10^{-4}\ C + 7.564\times10^{-4}\ T_s + 0.059\ Mal \qquad (3\text{-}13)$$

Standard coefficients of the regression indicated that maltose concentration was the most important factor, followed by β-glucan concentration and shearing temperature. It is not surprising that the wort viscosity was primarily controlled by the maltose level because maltose is a major component in brewers' wort and was varied over a large range (6.1-16.1% w/w) in this study. It was found that maltose dissolved in water (20°C) at 6.1%, 10.1% and 16.1% w/w had viscosities of 1.2516±0.0015, 1.4198±0.0020 and 1.756±0.015 mPa·s (n=2), respectively. Since purified β-glucans are neutral, their viscosities were not affected by the change in pH. For example, it was reported in previous work that viscosity of an oat β-glucan solution was not affected by pH in the range of 2-10 (Dawkins and Nnanna, 1995).

Generally, wort viscosity is proportional to its specific gravity (i.e., extract content) (Andrews and Wilkinson, 1996; Nielsen *et al.*, 1995). The β-glucan-free wort showed an exponential increase in viscosity at 20°C in the range of 2-24°P (Figure A.2 in Appendix 2). High concentrations of maltose (6-15% w/v) increased the viscosity of 0.1-0.5%

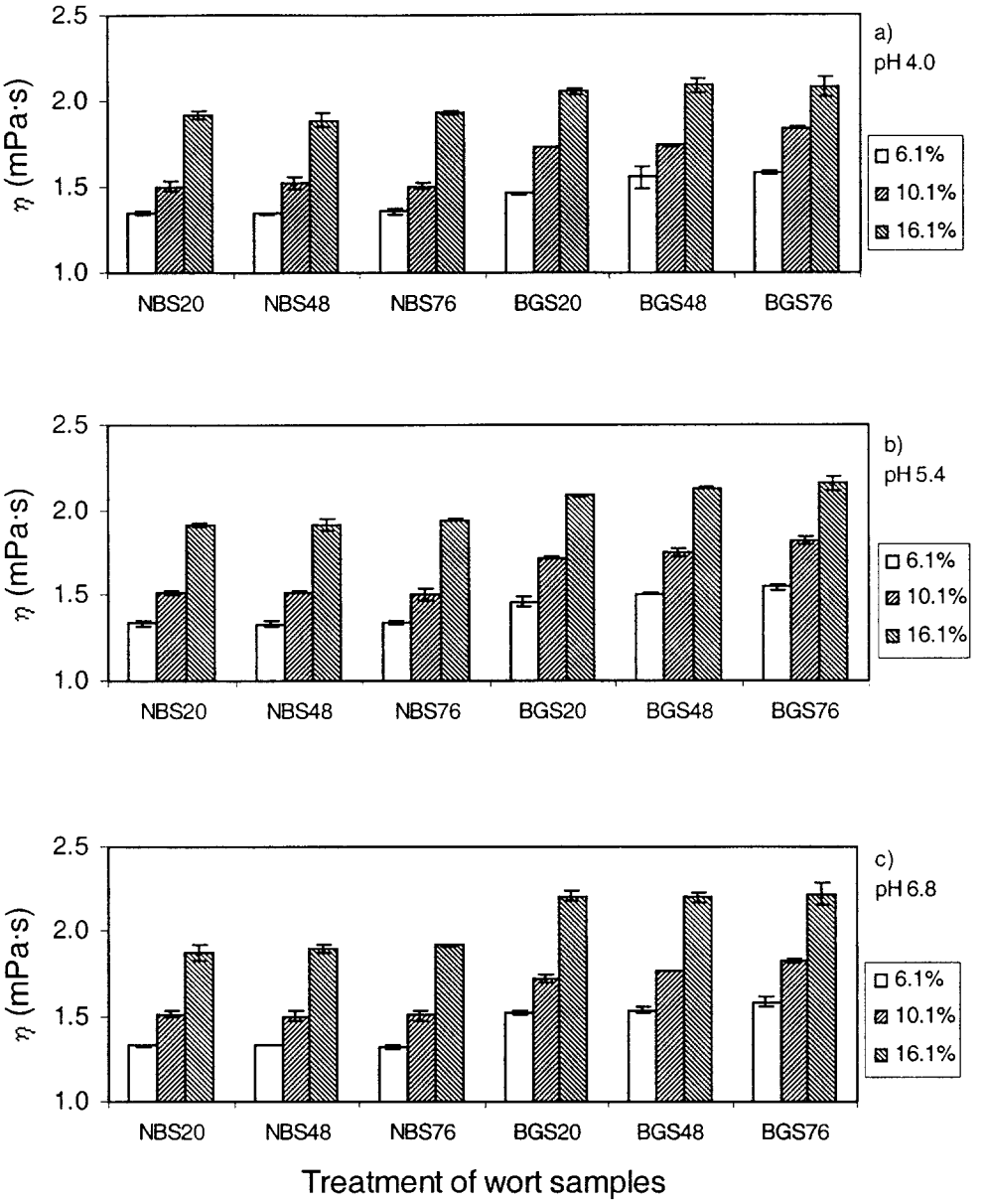

Figure 3.11 Effect of β-glucan (443 kDa at 600 mg/L), maltose content (6.1%, 10.1% and 16.1% w/w), and shearing temperature on the viscosities ($\eta_{20°C}$) of worts at pH 4.0-6.8. NB = no β-glucan; S20 = sheared at 20°C; S48 = sheared at 48°C; S76 = sheared at 76°C; BG = wort containing 443 kDa β-glucan at 600 mg/L. Values are given as means ± S.D. of duplicate experiments.

barley β-glucans in artificial solvent systems (Grimm and Krüger, 1994; Patelakis, 1999). Interestingly, the viscosity was reported to decrease when maltose increased from 2% to 5% (Grimm and Krüger, 1994). The presence of maltose not only increases the solution viscosity, but also affects the dispersion/aggregation state of β-glucan polymers (Grimm and Krüger, 1994; Letters, 1977). However, the literature is contradictory as to how the aggregation of β-glucan polymers is affected by the maltose level. The addition of 7-14% w/v maltose inhibited the precipitation of β-glucans by 20% v/v of ethanol although 3.3% w/v maltose did not (Letters, 1977). Increasing maltose from 6% w/v to 10% w/v increased the intrinsic viscosity of β-glucan solutions as well as the apparent MW (i.e., the degree of aggregation) of β-glucans (Grimm and Krüger, 1994; Grimm *et al.*, 1995a). High concentrations of maltose in water should increase the solution viscosity. To examine if maltose affected the wort viscosity caused by β-glucans, the specific viscosities of the samples were further compared. Results are displayed in Figure 3.12.

Shearing lowered the specific viscosities of wort samples ($p<0.001$) in the range of pH 4.0-6.8 and maltose at 6.1-16.1% w/w (Figure 3.12). This is in agreement with the results in Figure 3.10b. An analysis of variance (ANOVA) of the η_{sp} for sheared worts indicated that η_{sp} values at 20°C (i.e., wort viscosity due to 443 kDa β-glucan at 600 mg/L) increased with pH and maltose levels, as well as shearing temperature ($p<0.001$). Shearing at 20°C reduced the β-glucan η_{sp} more than shearing worts at 48°C and 76°C ($p<0.001$). The decreased β-glucan η_{sp} is hypothesized to be a result of the altered β-glucan particle size distribution in sheared wort (Figure 4.5, Chapter 4). As discussed later, shearing at 20°C increased the percentage of fractions smaller than 0.01 μm compared to worts sheared at 48°C and 76°C ($p<0.001$; Figure 4.5).

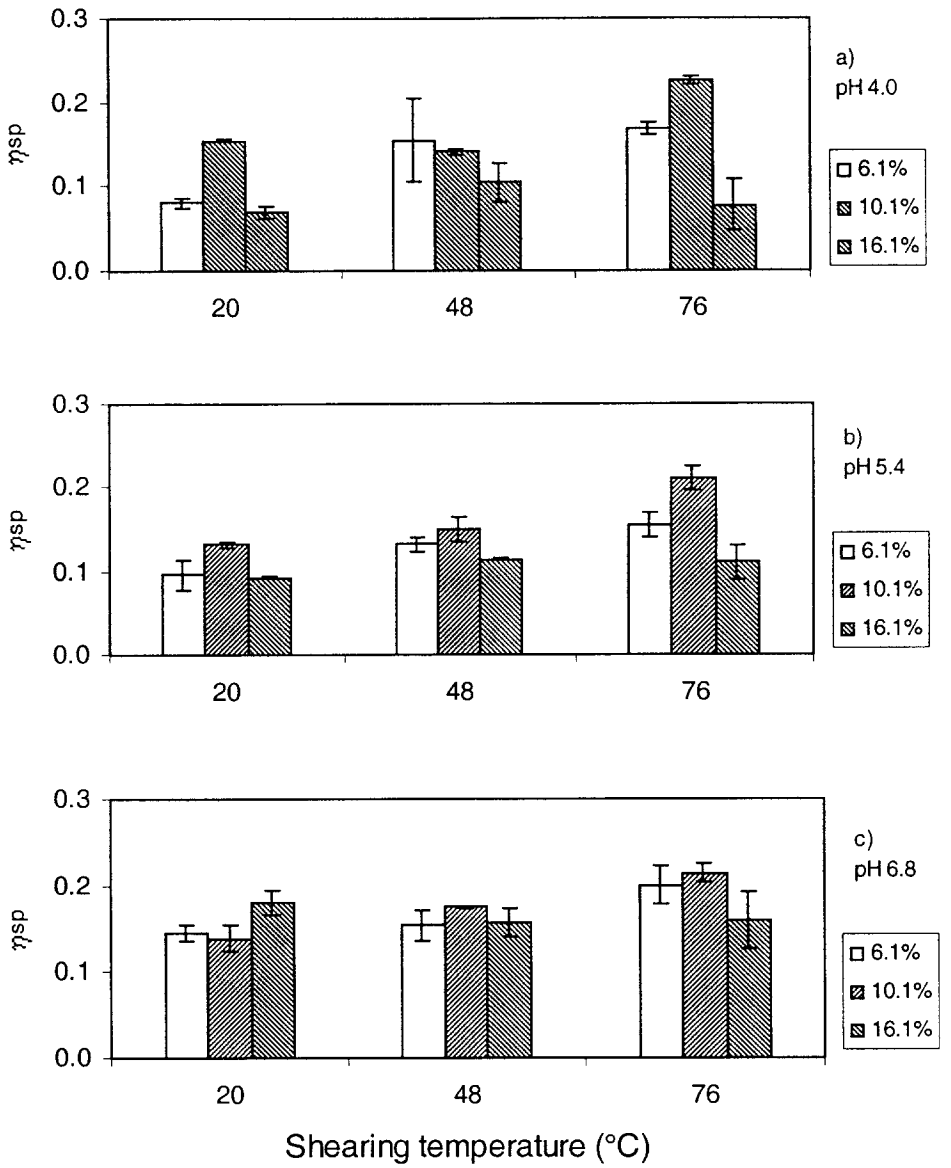

Figure 3.12 Effect of maltose level and shearing temperature on the specific viscosity (20°C) of 443 kDa β-glucan (600 mg/L) in wort samples at pH 4.0-6.8. Values are given as means ± S.D. of duplicate experiments.

3.3.4 Effect of β-Glucan MW and Concentration on Beer Viscosity

The effect of β-glucans at various MWs and concentrations on beer viscosity at 5°C was examined (Figure 3.13a). As could be expected (similar to wort), beer viscosity increased linearly with increased MW and concentration of β-glucans ($p<0.001$). The following relationship governed the beer viscosity (5°C) in the presence of β-glucans ($R^2=0.989$; $n=80$; $p<0.001$):

$$\eta = 2.106 + 8.041\times10^{-7}\,MW^2 + 2.518\,C^2 + 4.714\times10^{-6}\,MW\times C - 6.1\times10^{-4}\,MW - 1.581\times10^{-4}\,C \quad (3\text{-}14)$$

Also, the viscosity due to β-glucan (at a given MW and concentration) was higher in beer than in wort ($p<0.001$; Figures 3.5a and 3.13a). When the specific viscosities were examined by using the β-glucan-free beer (i.e., the control) as a solvent, they could be explained by MW and concentration of β-glucans ($R^2=0.991$; $n=80$; $p<0.001$):

$$\eta_{sp} = 2.215\,MW\times C + 9.729\times10^{-8}\,C^2 \quad (3\text{-}15)$$

Specific viscosity is useful in examining the contribution of β-glucans to beer viscosity. When the concentration of β-glucans was low (i.e., ≤200 mg/L), the viscosity caused by β-glucans accounted for less than 20% of that of β-glucan-free beer (Figure 3.13b). Even at a concentration as high as 800 mg/L, β-glucans only increased beer viscosity by <80%. This indicates that viscosity caused by all other components was higher than that by β-glucans alone. However, it is unknown whether β-glucan contributed more than any other individual component of the beer sample.

Specific viscosity of β-glucans in beer was approximately twice as high as that in wort (Figures 3.7a and 3.13b). The results suggest that beer (containing 3.3% w/w of real extract and 5.0% v/v of ethanol, pH 4.2) is a better solvent for barley β-glucans than wort (12% w/w of extract of which 9.2% w/w was maltose, pH 5.4). In good solvents, the solvent-polymer interactions are preferred over intrachain and interchain interactions,

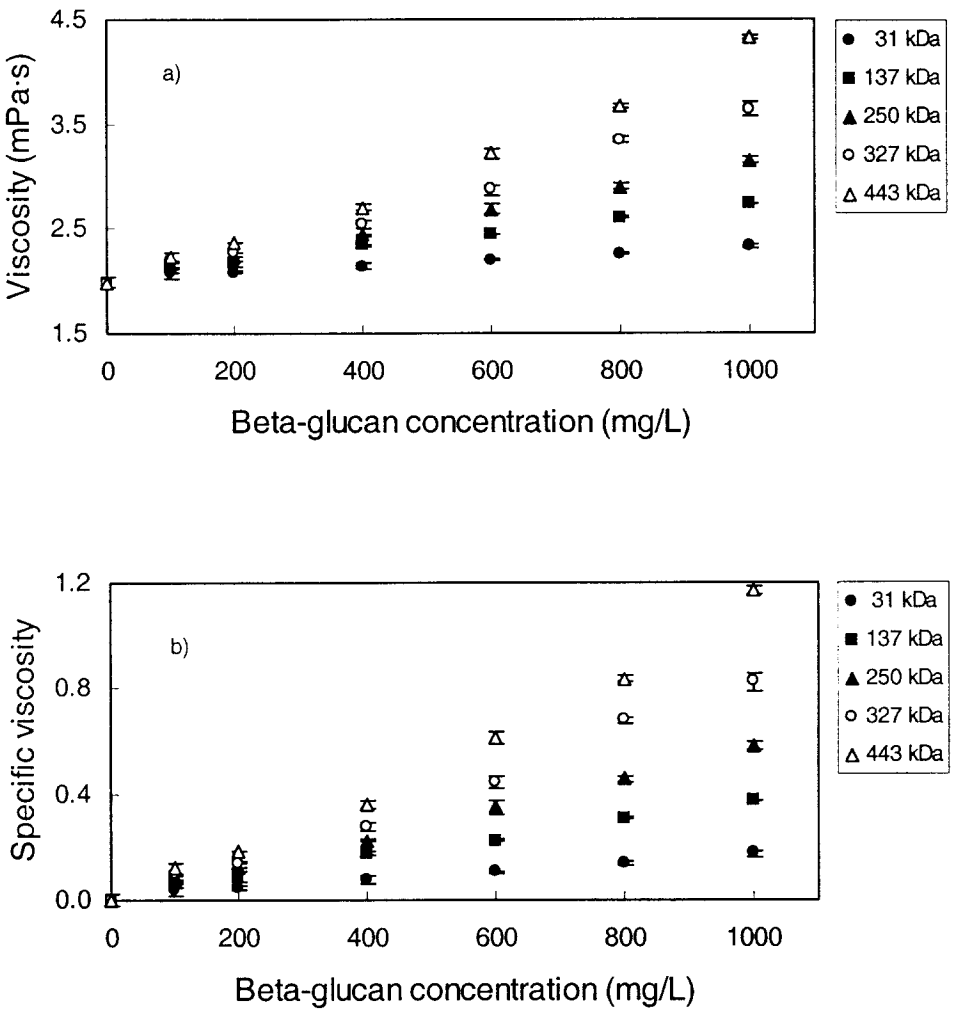

Figure 3.13 Effect of MW and concentration of β-glucans on (a) beer viscosity and (b) specific viscosity of β-glucans in beer (5°C). Values are given as means ± S.D. of duplicate experiments.

whereas in poor solvents the latter are favoured (Harding, 1998). Polymer molecules occupy excluded volume in good solvents (Harding, 1998) resulting in higher viscosity. However, the comparison of η_{sp} values of β-glucans in wort and beer were based on different solvent systems (i.e., wort and beer containing no β-glucan). A common reference system such as water is more useful to compare the viscosity behaviour of β-glucans as well as their solvents.

Beta-glucan-caused viscosity relative to water is therefore used to compare the contributions of both β-glucans and their "solvent" to the viscosities of the wort and beer (Figures 3.8a and 3.14). Although β-glucans exhibited higher viscosities in beer than in wort when water was used as a reference (Figures 3.8a and 3.14), they played a less important role in beer than in wort at 5°C, because the non-β-glucan components of beer were more viscous (130% relative to water viscosity) than that of wort components which was 42% of water viscosity ($p<0.001$).

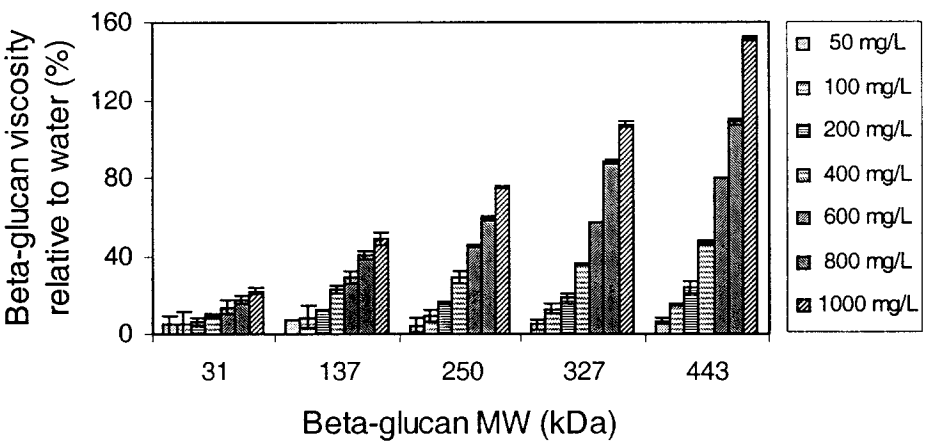

Figure 3.14 Comparison of beer viscosities caused by β-glucans relative to water viscosity at 5°C. The non-β-glucan components had 130% the viscosity of water. Values are given as means ± S.D. of duplicate experiments.

3.3.5 Effect of β-Glucan, Shearing, Shearing Temperature, pH and Ethanol Content on Beer Viscosity

Beer samples were sheared and their viscosities were examined at various β-glucan MWs and concentrations, pHs and ethanol levels. Shearing tests were conducted at different temperatures. Results are reported and discussed in this section.

3.3.5.1 Effect of Shearing at Various β-Glucan Concentrations on Beer Viscosity

When beer samples containing 0-1000 mg/L β-glucans (31-443 kDa) were sheared at 5°C, the viscosity declined significantly compared to the unsheared beers ($p<0.001$; Figure 3.15a). This disagreed with the effect of shearing wort at 20°C on the wort $\eta_{20°C}$ (wort viscosity increased after shearing; Figure 3.10a). For both sheared and unsheared beer samples, a significant relationship ($R^2=0.703$; $n=160$; $p<0.001$) was found:

$$\eta = 2.028 + 1.5\times10^{-4}\,C + 4.443\times10^{-6}\,S\times MW\times C - 1.3\times10^{-4}\,MW - 1.2\times10^{-4}\,S\times MW - 1.1\times10^{-4}\,S\times C \quad (3\text{-}16)$$

where S=1 for sheared beers and S=0 for unsheared beer samples. Further examination of the specific viscosities of β-glucans (β-glucan-free beer as a solvent; Figure 3.15b) found that the specific viscosity at 5°C was affected by MW and concentration of β-glucans ($p<0.001$), but not affected by shearing ($p>0.05$). It is worthy to note that shearing of wort at 20°C resulted in lower η_{sp} of wort at 20°C ($p<0.001$; Figure 3.10b). The different effects of shearing on β-glucan viscosities in wort and beer were due to the difference in "solvent" conditions (i.e., sugar and extract content, ethanol and pH levels).

3.3.5.2 Effect of Shearing Temperature, Ethanol and pH Levels on Beer Viscosity

The rheological properties of the unsheared beer samples were examined at various pHs, temperatures, and ethanol contents for beer with or without β-glucan (Figure 3.16).

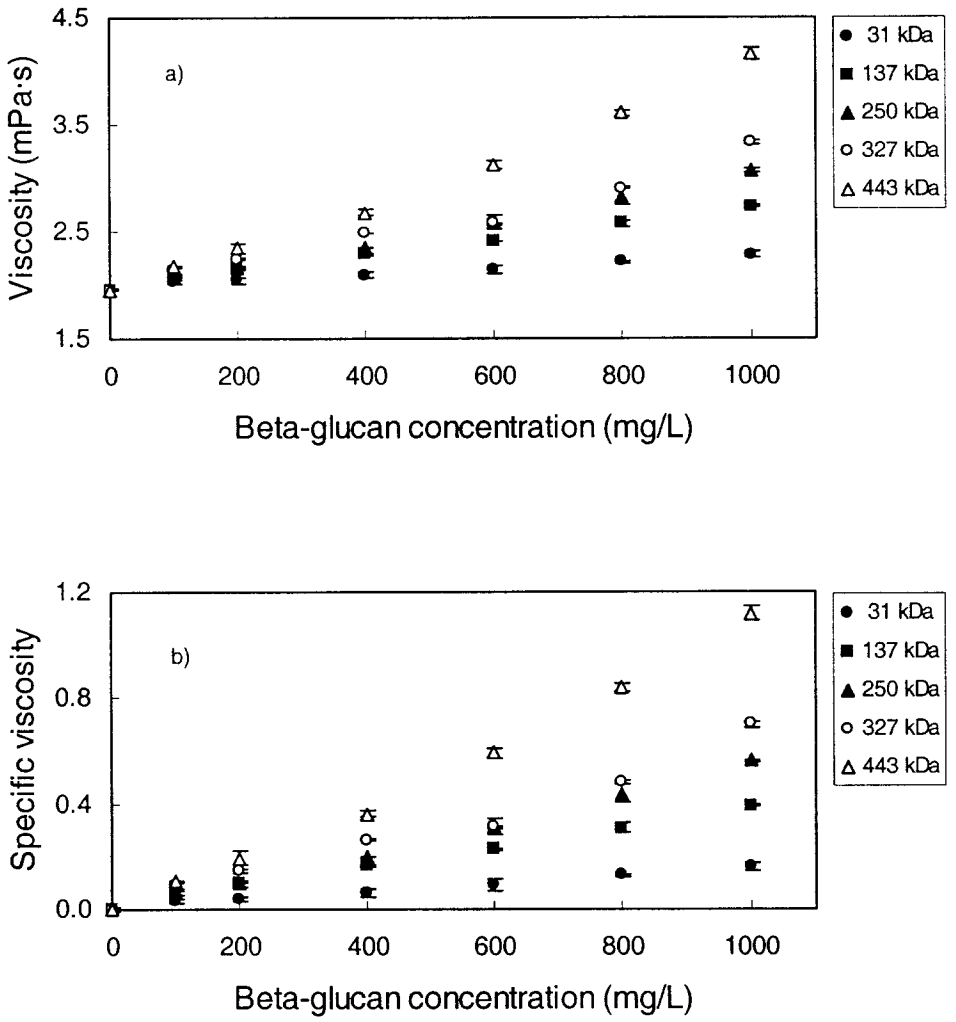

Figure 3.15 Effect of MW and concentration of β-glucans on (a) viscosity and (b) specific viscosity of beers sheared at 5°C. Values are given as means ± S.D. of duplicate experiments.

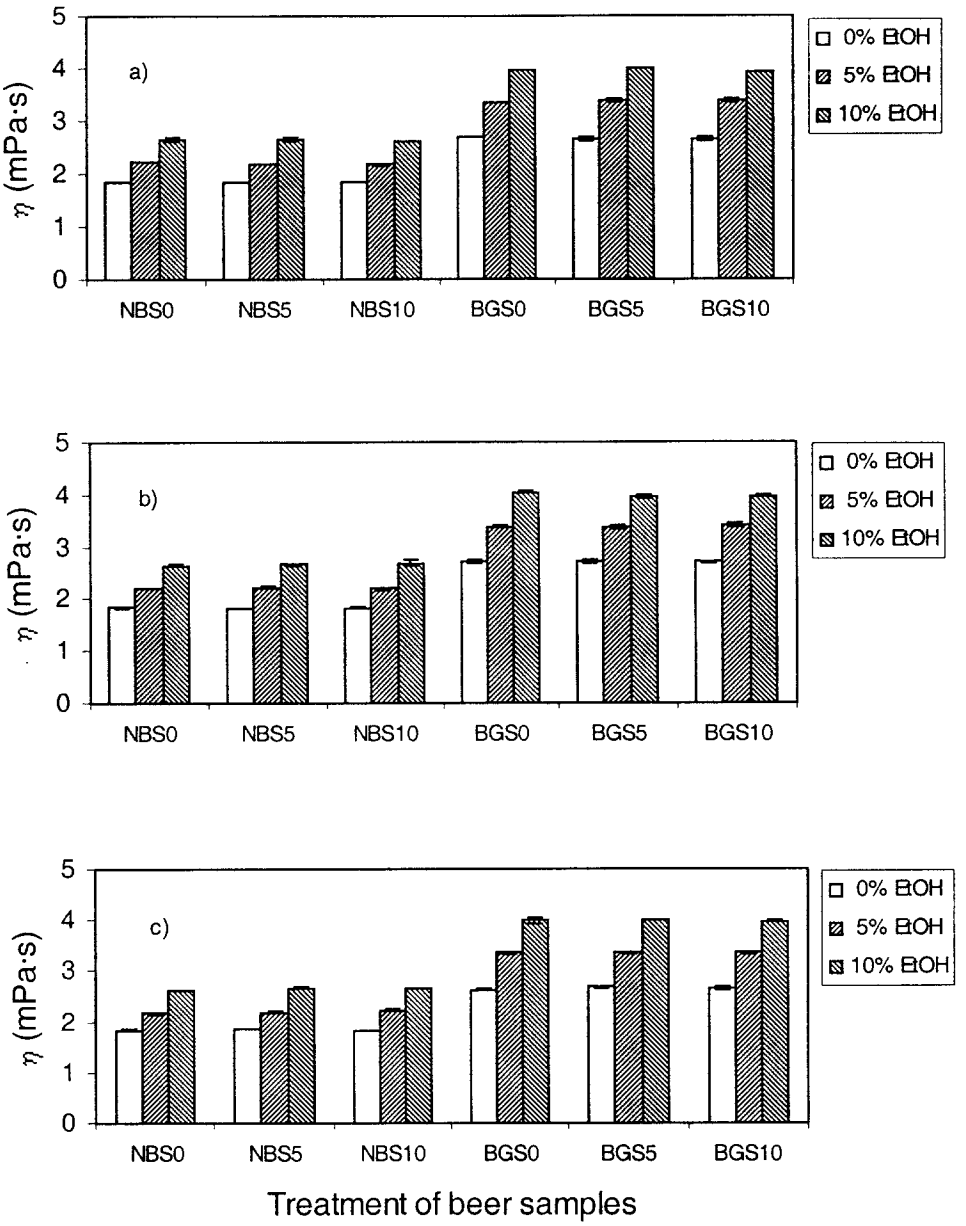

Figure 3.16 Effect of β-glucan (443 kDa at 600 mg/L), ethanol content, and shearing temperature on the viscosities ($\eta_{5°C}$) of beer at (a) pH 3.8; (b) pH 4.2 and (c) pH 4.6. NB = no β-glucan; S0 = sheared at 0°C; S5 = sheared at 5°C; S10 = sheared at 10°C; BG = beer containing 443 kDa β-glucan at 600 mg/L. Values are given as means ± S.D. of duplicate experiments.

The presence of 443 kDa β-glucan at 600 mg/L increased beer viscosity ($p<0.001$) for all samples. Beer pH in the range of 3.8-4.6 had no significant influence on viscosity ($p>0.05$; Figure 3.16). It is noteworthy that the preparations of barley β-glucans contained various amounts of proteins (0.72-9.35%; Table A.1 in Appendix 3). In the literature, purified oat aleurone β-glucans containing 4.0-8.0% protein showed an average of five charged groups in each β-glucan molecule with pKa values 4-4.5, and four groups with pKa values of 5-7.5 (Vårum and Smidsrød, 1988). If the proteins are attached to the β-glucan molecules, a broad change in pH should affect the viscosity of β-glucan solutions. However, pH between 3.8 and 4.6 did not affect the viscosity of β-glucans and other components such as proteins in beer. Since the 443 kDa β-glucan used in this study contained a small amount of proteins (3.34±0.28%; Table A.1), it is not surprising that beer viscosity was not affected by pH in a narrow range of 3.8-4.6. This pH range was selected for the samples to reflect the normal pH of beers (Hough *et al.*, 1982a). It has been reported that the viscosities of model beer samples containing 0.5% w/w β-glucan (327 kDa) were different at pH values of 3.6 and 5.2 ($p=0.05$; Patelakis, 1999) probably due to the effect by protein when such a high concentration of β-glucan was added. In theory, however, the β-glucan molecules are neutral in charge and their viscosities are not influenced by pH.

There was no significant difference ($p>0.05$) in the viscosities among samples sheared at 0, 5 and 10°C (Figure 3.16) since the temperature range was narrow. The increase in beer viscosity caused by ethanol was significant for all samples ($p<0.001$). The ethanol content (0-10% v/v) reflected the levels from non-alcohol beer to strong beer or high gravity beer before dilution. Ethanol in beer as an organic solvent is able to lower dielectric constant of the medium (Speers, 1991). The decreased dielectric constant causes a lower solubility of proteins. Addition of miscible solvents such as ethanol may also reduce the interactions between β-glucan and water molecules and increase the interactions among β-glucan molecules leading to higher solution viscosities. It was noted that ethanol (5°C) has a viscosity of 2.003±0.033 mPa·s which is higher than water

viscosity (1.516 mPa·s). Ethanol at 5% and 10% v/v in water had viscosities of 1.841±0.040 mPa·s and 2.176±0.018 mPa·s, respectively. The interaction between ethanol (10% v/v) and water molecules resulted in a higher viscosity than that of either water or ethanol. The multiple linear regression suggested that only β-glucan and ethanol content determined the beer viscosity at 5°C while pH and shearing temperature did not (R^2=0.957; n=108; p<0.001):

$$\eta_{5°C} = 1.709 + 0.002\ C + 0.105\ E \quad (3\text{-}17)$$

where E is the ethanol concentration (% v/v). Ethanol at 5% and 10% also increased the viscosity of beer containing no β-glucan (Figure 3.16). It is therefore necessary to compare the specific viscosities of β-glucan (443 kDa) using the β-glucan-free beer as a solvent (Figure 3.17). Ethanol not only increased the viscosity of the solvent (i.e., beer without β-glucan), but also enhanced the viscosity exhibited by β-glucan molecules (p<0.001). Interestingly, 5% v/v of ethanol caused a higher β-glucan viscosity than 10% v/v of ethanol. This might be one of the causes of slower beer membrane filtration at 5% v/v of ethanol (to be discussed in Chapter 8). Shearing of beer samples lowered the viscosity caused by β-glucan (p<0.001) but shearing temperature had an insignificant effect (p>0.05). Beer pH in the range of 3.8-4.6 showed no influence on the viscosities caused by 443 kDa β-glucan at 600 mg/L. This may be because the 443 kDa β-glucan contained a limited amount (3.34%, Table A.1) of charged proteins and/or because the pH range examined was narrow.

3.3.6 Intrinsic Viscosity of β-Glucans in Wort and Beer

When β-glucans are dissolved in wort or beer, they increase the viscosity of the system. As noted earlier in Eq. 3-8, specific viscosity can be expressed as:

$$\eta_{sp} = (\eta_{solution} - \eta_{solvent})/\eta_{solvent} = \eta_{rel} - 1 \quad (3\text{-}8)$$

The η_{sp} per unit concentration of β-glucans is called reduced viscosity (η_{red}):

$$\eta_{red} = \eta_{sp} / C \quad (3\text{-}18)$$

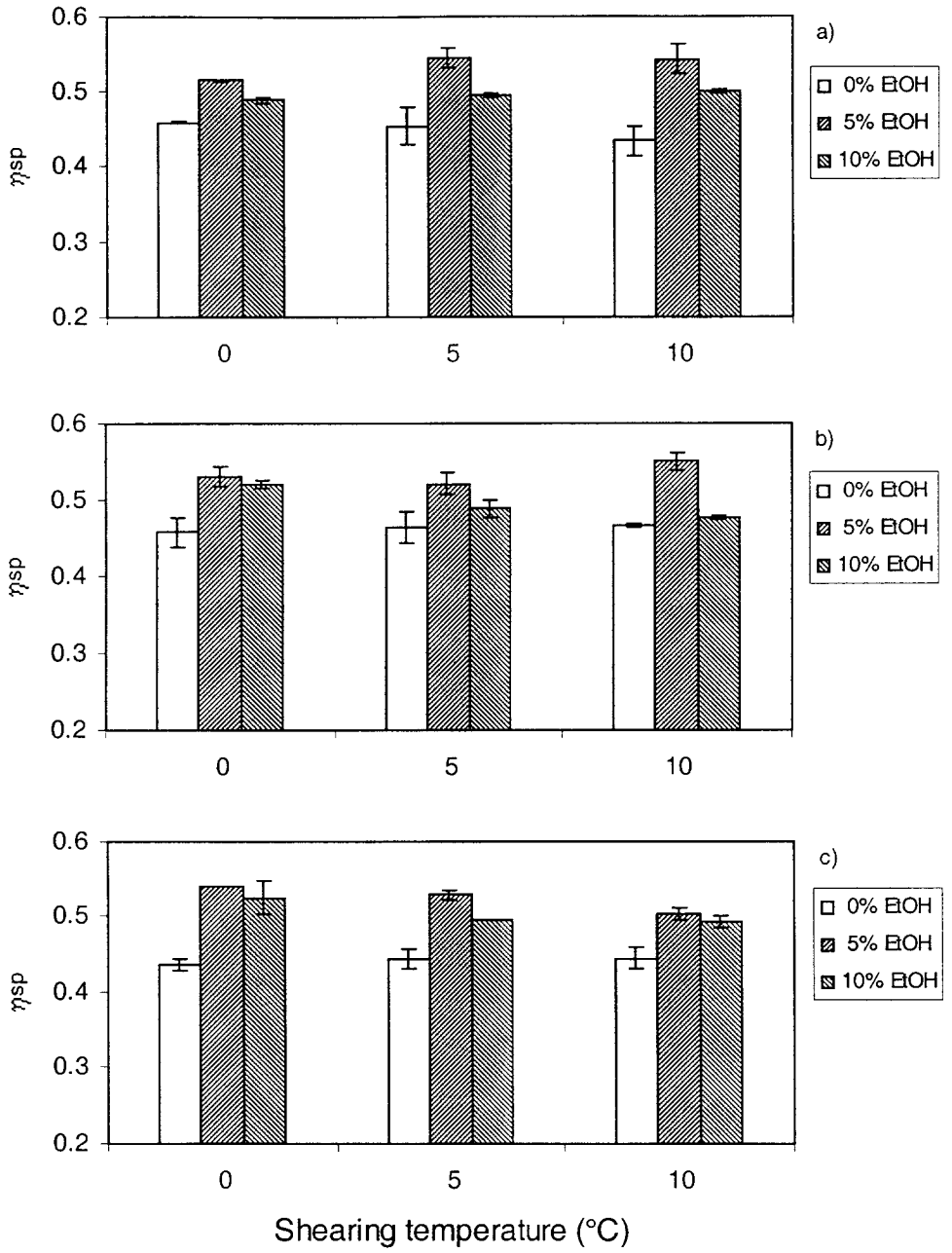

Figure 3.17 Effect of shearing temperature and ethanol content on specific viscosity (5°C) of 443 kDa β-glucan (600 mg/L) at (a) pH 3.8; (b) pH 4.2 and (c) pH 4.6. Values are given as means ± S.D. of duplicate experiments.

Thus a straight line can be obtained by plotting η_{red} versus C. This line has a Y-intercept, which is called intrinsic viscosity. This approach of generating intrinsic viscosity was first given by Kraemer (1938):

$$(\ln \eta_{rel}) / C = [\eta] - k' [\eta]^2 C \quad (3\text{-}19)$$

and further used by Huggins (1942) in the following equation:

$$\eta_{sp} / C = [\eta] + k'' [\eta]^2 C \quad (3\text{-}20)$$

where $[\eta]$ is the intrinsic viscosity (dL/g); k' is the Kraemer coefficient and k'' is the Huggins coefficient. The intrinsic viscosity is defined as the "limiting viscosity number" by the International Union of Pure and Applied Chemistry (IUPAC) in 1952 (IUPAC, 1952). The relative viscosity and specific viscosity were also re-named as "viscosity ratio" whereas the reduced viscosity was termed "viscosity number" by the IUPAC (1952). Only a few brewing researchers have adopted these recommended terms in the past 50 years. For example, Linemann and Krüger (1998b), and Izydorczyk *et al.* (1998a; 1998b) used limiting viscosity number to describe $[\eta]$. However, the term intrinsic viscosity has been recommended by the Society of Rheology (Dealy, 1995) and preferred by most authors (Egi, 2002; Gómez *et al.*, 1997a; Grimm *et al.*, 1995a; Izydorczyk and Biliaderis, 1992a; 1992b; Izydorczyk *et al.*, 1998c; Oonsivilai, 2000; Oonsivilai *et al.*, 2000; Vårum *et al.*, 1991; Woodward *et al.*, 1988). The usage of intrinsic viscosity can also be found in many recent textbooks (Goodwin and Hughes, 2000; Lapasin and Pricl, 1995; Steffe, 1996), and is mandated by the Journal of Rheology (Dealy, 1995). The term of intrinsic viscosity is also used in this thesis.

The intrinsic viscosities of β-glucans in wort (12°P, containing 9.2% maltose, pH 5.4) and beer (3.3% w/w of real extract, 5.0% v/v of ethanol, pH 4.2) at 5°C were determined by using both Kraemer's and Huggins' equations. Mean values are reported in Figure 3.18. The intrinsic viscosity is a characteristic of a polymer in a given solvent system. Literature reports on β-glucan intrinsic viscosities are summarized in Table 3.3. Caution must be taken when these data are compared because both β-glucan molecular size and solvent system varied. Unfortunately, few reports have studied β-glucans in solvent

systems which can mimic the wort or beer compositions or brewing conditions. It is more valuable to determine [η] in real wort and beer in order to study the behaviour of β-glucans in brewing. Results in Figure 3.18 can be described by the Mark-Houwink relationship (Eq. 2-3):

$$[\eta] = K M^{\alpha} \quad (2\text{-}3)$$

where K and α are constants; M is the molecular weight of the polymer. For the 31-443 kDa β-glucans in wort, the intrinsic viscosity was proportional to its weight average MW (M_w) used (R^2=0.927; n=10; p<0.001):

$$[\eta] = 0.467\ M_w^{0.371} \quad (3\text{-}21)$$

For β-glucans in beer, the relationship between [η] and MW was (R^2=0.982; n=10; p<0.001):

$$[\eta] = 0.465\ M_w^{0.466} \quad (3\text{-}22)$$

The K and α values are functions of both polymer characteristics and solvent systems, and only valid for the particular polymer in a given solvent system. These two parameters

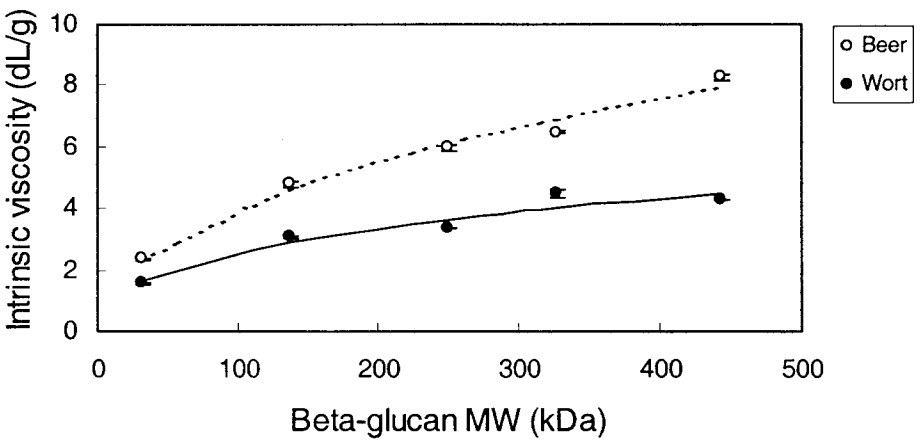

Figure 3.18 Effect of β-glucan MW on the [η] in wort and beer at 5°C. Lines indicate the best fit of the Mark-Houwink equation to the data. Values are given as means ± S.D. of duplicate experiments.

Table 3.3 Literature reported intrinsic viscosity of barley β-glucans

Beta-glucan	Solvent	[η] (dL/g)	Source
Barley, 114, 228 and 374 kDa (Megazyme)	0.05*M* Na_2SO_4 + 0.01*M* EDTA, 20°C	2.2, 4.0 and 6.2	Christinsen *et al.*, 2001
Barley, M_w = 231-573 kDa	Water, 25°C	2.7-5.2	Gomez *et al.*, 1997a
Beer, M_w 2920-5400 kDa	2*M* GHCl, 90% DMSO, and water; 20°C [a)]	1.4, 1.8, 2.2	Grimm *et al.*, 1995a
Barley (MW unknown)	Aqueous, 20°C	0.7-5.6	Izydorczyk *et al.*, 1998a
Barley, 31-258 kDa	0.1 M acetate buffer (pH 4.1, 5% v/v of ethanol, 0.5% w/v maltose), 0-5°C	18.7-31.0	Oonsivilai, 2000
Barley, 160 and 290 kDa	0.15*M* NaCl, 25°C	4.26-6.90	Woodward *et al.*, 1983a
Barley, 65°C water soluble, 150 kDa	50 m*M* NaAc buffer, pH 5, 25°C	4.04	Woodward *et al.*, 1988
Beer, 5400 kDa	Aqueous, 20°C	2.19	Linemann and Krüger, 1998a
Wort (MW unknown)	Wort, 20°C	0.81-3.43	Linemann and Krüger, 1998b

[a)]: GHCl = guanidine hydrochloride; DMSO = dimethylsulfoxide; NaAc = sodium acetate.

are related to the local stiffness of the polymer chains and polymer-solvent interactions. The exponent α varies from 0.8 in a good solvent to 0.5 in poor solvents for coil-like polymers and can be as high as 1.8 for rod-like polymers (Doublier and Cuvelier, 1996). Thus, purified β-glucans (31-443 kDa) exhibited coil-like conformations in wort and beer, which are poor solvents for β-glucans. Polymers tend to reduce their contact with the solvent and adopt more compact conformations in poor solvents (Harding, 1998).

The intrinsic viscosity values are indirect measures of the polymer dimensions. They can be used to determine polymer MWs. Intrinsic viscosities can also be useful in deriving the critical overlap or entanglement concentration (C*) of a polymer in a given solvent system. The reciprocal of [η] has been taken as C* (Kasaai *et al.*, 2000). Goodwin and Hughes (2000) have suggested the relationship in theory:

$$C^* = 1.08 / [\eta] \qquad (2\text{-}4)$$

The value of C* for β-glucans (31-443 kDa) derived from Eq. 2-4 varied from 2.5 to 8.3 g/L in wort and from 1.3 to 4.7 g/L in beer, respectively (Figure 3.19). The relationship between M_w and C* followed the power law, and can be described by empirical models for β-glucans in wort (Eq. 3-23) and beer (Eq. 3-24), respectively:

$$C^* = 39.173\ M_w^{-0.4646} \qquad (R^2=0.960;\ n=10;\ p<0.001) \qquad (3\text{-}23)$$

$$C^* = 22.846\ M_w^{-0.4613} \qquad (R^2=0.993;\ n=10;\ p<0.001) \qquad (3\text{-}24)$$

Barley β-glucans (31-327 kDa) have been found to have lower C* values (0.53-3.11 g/L) in 0.1*M* acetate buffer (pH 4.1) containing 5% v/v of ethanol and 0.5% w/v maltose (Oonsivilai, 2000; Oonsivilai *et al.*, 2000) using the approach of Linemann and Krüger (1997). A β-glucan sample isolated from beer (175 kDa) was reported to have a C* value of 0.42 g/L in water at 1.5°C (Linemann and Krüger, 1997). The difference in reported C* values here versus literature reports is believed to be a result of different solvent systems used. Apparently, β-glucan molecules more easily overlap or entangle one another at lower concentrations in water or acetate buffers than under real beer and wort conditions.

The derivation of C* by using "1/log η_{rel}" vs. C (Linemann and Krüger, 1997) or "log η_{sp}" vs. C (Morris *et al.*, 1981) was also attempted. However, the plots only showed curves rather than broken lines because the β-glucan concentration was low and the concentration range was narrow. The non-linearity of the plots was hypothesized to be due to the polydispersity of the β-glucans.

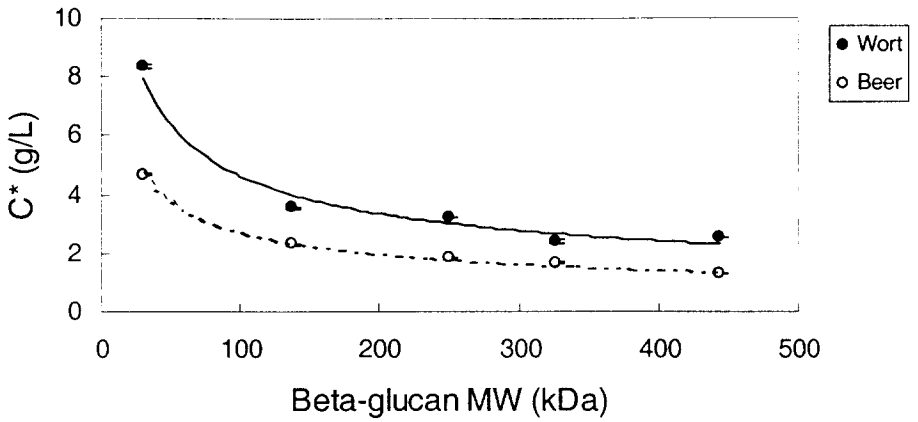

Figure 3.19 Effect of MW of β-glucans on their C* values in wort and beer. Lines indicate the best fit of the empirical model to the data. Values are given as means ± S.D. of duplicate experiments.

3.4 Conclusions

While other components in wort and beer contributed to their viscosities, β-glucans (31-443 kDa) increased solution viscosities. However, β-glucans did not "dominate" (compared to the viscosities caused by all other solutes) as long as the β-glucan concentration was lower than 800 mg/L. In the range of β-glucan concentrations studied (0-1000 mg/L), viscosity increased linearly with the MW and concentration of β-glucans. Arrhenius relationship governed the effect of temperature on wort viscosity. Shearing at a fixed rate (≈13,000 s^{-1}) for a given period of time (35 s) increased the wort viscosity but decreased beer viscosity ($p<0.001$). However, the β-glucan viscosity was lowered after shearing for wort ($p<0.001$) but not for beer ($p>0.05$). Shearing wort at 20°C influenced β-glucan viscosity more than shearing at 48°C and 76°C ($p<0.001$). Shearing temperature (0, 5 and 10°C) showed no significant effect on beer viscosity ($p>0.05$).

Wort viscosity was higher at high pHs in the range of pH 4.0-6.8 ($p<0.001$). The wort viscosity due to β-glucans was higher at pH 6.8 as well ($p<0.001$). However, beer pH in the range of 3.8-4.6 did not affect viscosities ($p>0.05$). Increasing the concentration of maltose in wort and ethanol in beer enhanced the viscosity of β-glucan polymers ($p<0.001$). At the same temperature (5°C), β-glucans had higher intrinsic viscosities in beer than in wort ($p<0.001$). Mark-Houwink equation was found to govern the influence of MW on the intrinsic viscosity of β-glucans. The critical overlap concentrations (C*) of β-glucans were higher in beer than in wort ($p<0.001$).

4 EFFECT OF MW, CONCENTRATION AND ENVIRONMENTAL CONDITIONS ON THE DISTRIBUTION OF β-GLUCAN PARTICLE SIZE IN WORT AND BEER

Membranes with nominal pore sizes of 0.45 μm, 0.1 μm and 0.01 μm in diameter were employed to evaluate the apparent particle size of β-glucans in wort and beer. The results are termed as apparent particle size because the ratings of the non-spherical pores of membranes are nominal and only the equivalent diameter of the non-spherical β-glucan particles was determined. Commercial β-glucans (31-443 kDa in MW) were used to prepare samples containing 0-1000 mg/L of β-glucans in 12°P wort at pH 5.4 and in degassed beer (pH 4.2; containing 3.3% w/w of real extract and 5.0% v/v of ethanol). Sheared and unsheared samples were examined to determine the effect of MW and concentration of β-glucans on their apparent particle size distribution. The influences of several other factors including pH of wort and beer, maltose concentration in wort, ethanol content of beer and shearing temperature on the β-glucan particle size were also investigated. It was found that β-glucans in wort had smaller apparent particle sizes and broader size distributions than in beer.

4.1 Introduction

Beta-glucans in beer are primarily derived from barley malt and barley adjuncts. These polymers together with arabinoxylans increase the viscosities of wort and beer, retard wort and beer filtration and result in higher turbidity of the packaged beer. Most malting barleys contain 2-6% β-glucans (Table 2.1). These β-glucans are degraded during malting and mashing. The β-glucan content of malt is normally lower than 1%. Beta-glucan levels in wort and beer can vary from trace levels to up to 1200 mg/L in the literature although 100-300 mg/L is commonly found in most beers. It is not surprising that β-glucans in beer have a very broad distribution of MW (1-54,090 kDa) since the polymers are degraded by β-glucanases and can also exhibit much higher apparent MWs

(Linemann and Krüger, 1998a) due to the association of molecules. Barley β-glucan molecules are linear, which consist of β-D-glucopyranose units linked by β-D-(1→3)- and β-D-(1→4)-glucosidic bonds (Aspinall and Telfer, 1954). The ratio of β-D-(1→3)- to β-D-(1→4)-linkages have been reported to be 1:2.3 to 1:6.6 in the literature (Clarke and Stone, 1966; Igarashi and Amaha, 1969; Izydorczyk *et al.*, 1998c; Luchsinger *et al.*, 1965; Parrishi *et al.*, 1960; Peat *et al.*, 1957; Perlin and Suzuki, 1962; Saulnier *et al.*, 1994; Stone and Clarke, 1992; Wood *et al.*, 1991a). Beta-glucan molecules have also been characterized as being β-(1→3)-linked cellulotriosyl and cellulotetraosyl units whereas long blocks of up to 20 consecutive β-(1→4)-glucosyl residues have also been found (Edney *et al.*, 1991; Izawa *et al.*, 1993; Izydorczyk *et al.*, 1998c; Luchsinger *et al.*, 1965; Woodward *et al.*, 1983a). These β-(1→4)-linked cellulose-like regions in β-glucans tend to form junction zones and to aggregate (Izawa *et al.*, 1993; Izydorczyk *et al.*, 1998b; Letters, 1995b; Woodward *et al.*, 1988). Aggregated β-glucan molecules in beer may precipitate and lead to filtration difficulties and haze defects in packaged beer. Such a problem has been described as "gel formation" of beer β-glucans (Grimm and Krüger, 1994; Letters, 1977; 1995a; Linemann and Krüger, 1997). However, the gel-like precipitation of β-glucans during fermentation and maturation is probably advantageous because they can sediment to the bottom of fermenters and then be easily removed before causing further processing difficulties.

High MW β-glucans slow down the filtration process due to their high viscosity and physical clogging of the filter medium. Beers brewed from malts with same specifications or even the identical batch may not always encounter filtration and clarity problems. Arguments can thus arise with regard to whether β-glucans are responsible or not. Recent studies have used controlling the extent of malt endosperm modification to obtain different β-glucan levels of malt (Evans *et al.*, 1999; Nischwitz *et al.*, 1999). Correlation between β-glucan level and beer filtration efficiency has been reported by

Nischwitz *et al.* (1999). However, β-glucan polymers were not the sole variable in those experiments and caution has to be exercised when interpreting the results.

Light scattering has been employed in determination of the size of particles in beer as well as model solutions (Gans and Denk, 1995; Gómez *et al.*, 1997a; Grimm *et al.*, 1995a; Linemann and Krüger, 1998a). In aqueous solutions of β-glucans, light scattering is a good means to monitor the size distribution of β-glucan particles. However, confusing results of β-glucan particle size determined by light scattering have been reported by Gómez *et al.* (1997a). The root-mean-squared (RMS) radius of a commercial 231 kDa β-glucan sample has been reported as 362 nm and 226 nm in the filtrate through 0.22 μm and 0.022 μm membranes, respectively (Gómez *et al.*, 1997a). Also, light scattering cannot distinguish the particles of β-glucans from other haze particles in beer such as protein-polyphenol complexes. In this thesis, filtration tests using 0.45 μm, 0.1 μm and 0.01 μm membranes (nominal pore diameters) were used to categorize β-glucan particles in wort and beer. The purpose was to investigate if the brewing conditions including pH, maltose level in wort, ethanol content in beer as well as shearing influence the apparent particle size of β-glucans.

4.2 Materials and Methods

Materials used in this study have been described in section 3.2.1. A β-glucan-free wort was prepared from a pale malt as described in section 3.2.2. Barley β-glucans purchased from Megazyme (Bray, IRL) had MWs of 31, 137, 250, 327 and 443 kDa. These β-glucan polymers were used to prepare wort samples with β-glucan concentrations in the range of 0-1000 mg/L (section 3.2.3). A commercial lager beer (Labatt Blue, product code E10H11C, UBC 062067351013, Oland Breweries Ltd., Halifax, NS) was used to prepare a β-glucan-free beer base (section 3.2.4). This β-glucan-free beer base was used to prepare beer samples varying in β-glucan type (31-443 kDa), concentration (0-1000

mg/L), pH (3.8-4.6), and ethanol content (0-10% v/v) as described in section 3.2.5. Shearing tests of wort and beer were conducted with a blender (Model MM-1B, Lourdes Instrument Corp., Brooklyn, NJ) at a speed setting of "60" for 35 seconds, equivalent to a shear rate of $1.3\pm0.2\times10^4$ s^{-1} (section 3.2.6). Beta-glucan concentration of samples was determined with the Congo red assay (section 3.2.7) modified from the method of Li *et al.* (1997).

Determination of the apparent particle size of β-glucans was carried out by membrane filtration tests at ambient temperature (20°C). All membranes were purchased from Osmonics Inc. (Minneapolis, MN). Samples of wort and beer (10 mL) were filtered through 0.45 μm "AcetatePlus" membranes (Cat. No. A04SP02500, Material No. 1215635, Batch No. 77050, 088439, 087551 and 093062) at 7 kPa (1 psi). A small portion of the collected 0.45 μm membrane filtrate (1-1.5 mL) was further filtered through 0.1 μm "Poretics" polycarbonate membranes (Cat. No. K01CP02500, Material No. 1215606, Batch No. 011111, 114216, and 139709) under a pressure of 140 kPa (20 psi) and 0.01 μm "Poretics" polycarbonate membranes (Cat. No. KN1CP02500, Material No. 1215321, Batch No. 090428, 101018 and 142587) under 350 kPa (50 psi). Samples were analyzed for β-glucan concentration with the Congo red assay (section 3.2.7) before and after the membrane filtrations. The apparent β-glucan particle size was categorized into fractions of >0.45 μm, 0.1-0.45 μm, 0.01-0.1 μm and <0.01 μm in diameter. The distribution of β-glucan particle size was expressed as the percentage of β-glucan in each fraction relative to the total β-glucan prior to membrane filtration.

The experimental design of this study is described in section 3.2.9 (Figure 3.2). Duplicate experiments were carried out and mean values and standard deviations were reported. Linear regression and analysis of variance (ANOVA) of the results were done with SYSTAT version 5.05 (SPSS Inc., Chicago, IL).

4.3 Results and Discussion

Beta-glucan particles were categorized into fractions of >0.45 μm, 0.1-0.45 μm, 0.01-0.1 μm and <0.01 μm (expressed as % of the total β-glucan before filtration). Congo red dye was used to determine the concentration of β-glucans in each fraction. It was found that the >0.45 μm fraction in wort and beer was very low (i.e., undetectable by the Congo red dye) and thus the particle size distribution of β-glucans was expressed as three fractions: >0.1 μm, 0.01-0.1 μm and <0.01 μm in diameter. The effect of MW and concentration of β-glucans on their particle size in wort and beer is discussed in sections 4.3.1 and 4.3.2, respectively. The influence of pH, maltose content of wort and shearing temperature on wort β-glucan particle size distribution is discussed in section 4.3.3. Lastly, the results of the β-glucan particle size analysis in beer under various conditions are discussed in section 4.3.4.

4.3.1 Effect of MW and Concentration of β-Glucans and Shearing on the Particle Size Distribution in Wort

Wort samples containing 31-443 kDa β-glucans at 50-1000 mg/L were examined to categorize the apparent β-glucan particle size. These samples were also sheared at 20°C followed by categorization of β-glucan particles at 20°C. Results of the particle size distribution of β-glucans in unsheared wort are shown in Figure 4.1. The majority (more than 80%) of the low MW particles (31 kDa) were smaller than 0.01 μm in diameter at concentrations between 50-1000 mg/L (Figure 4.1a). With high MW β-glucans (137-443 kDa), 40-90% of the particles were >0.01 μm in diameter.

These results differ from the literature reports on the particle size of β-glucans in water. Commercial and "lab-made" barley β-glucans (9-573 kDa) in water showed particle sizes of 24.4-86.6 nm (i.e., 0.02-0.09 μm) in diameter measured by light scattering (Gómez *et*

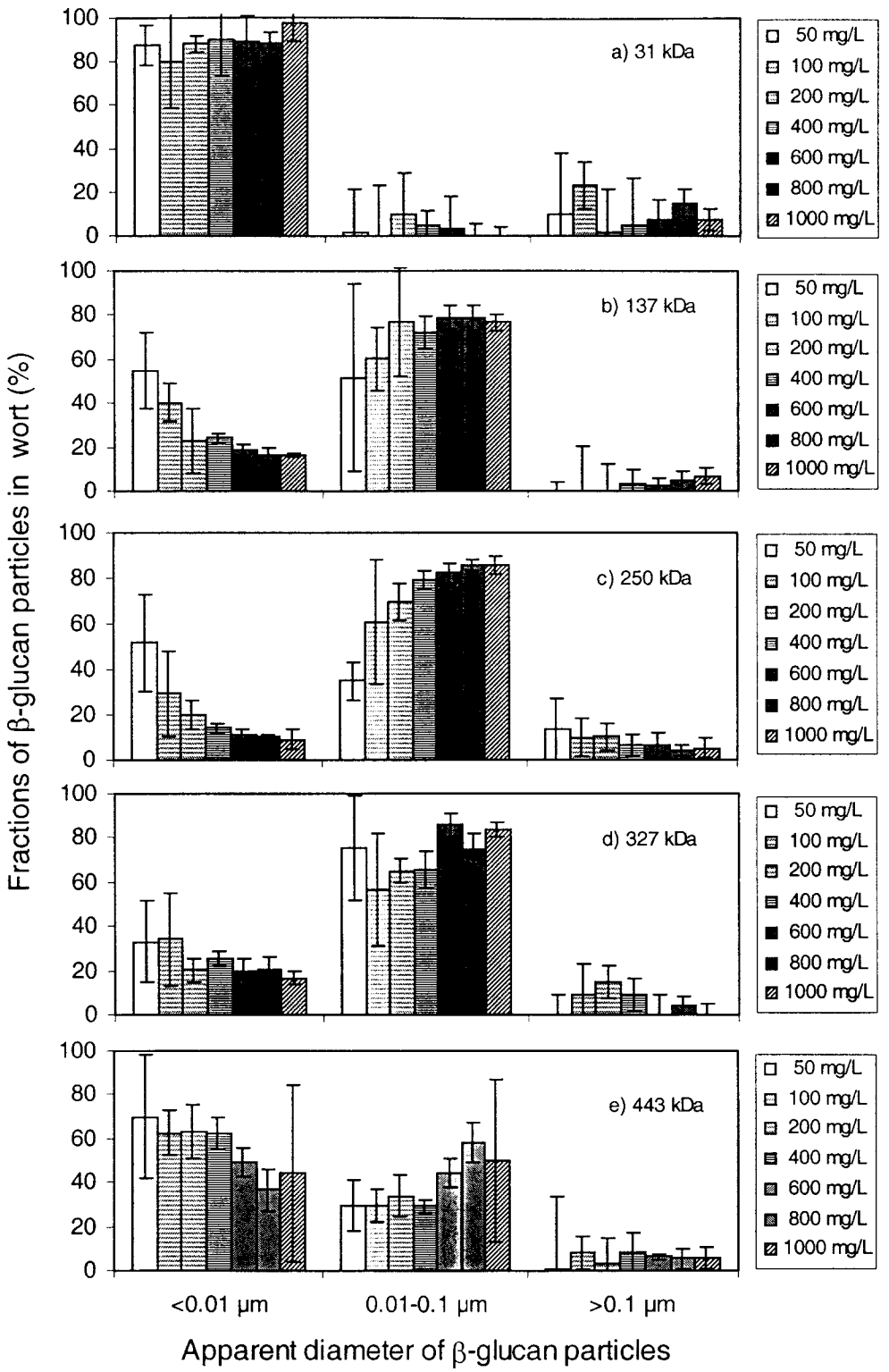

Figure 4.1 Effect of MW and concentration on the particle size distribution of β-glucans in 12°P wort (20°C). Values are given as means ± one standard deviation (S.D.) of duplicate experiments.

al., 1997a). The samples used by Gómez *et al.* were pre-filtered through 0.45 μm membranes before fractionation by size exclusion chromatography (Gómez *et al.*, 1997a). Barley β-glucans (160 and 290 kDa) were 0.03-0.04 μm in diameter in 0.15*M* NaCl (Woodward *et al.*, 1983a) while β-glucans (133-492 kDa in 0.1*M* $NaNO_3$, filtered through 0.4 μm membranes) were 0.04-0.08 μm in diameter detected by SEC and light scattering (Christensen *et al.*, 2001). Beta-glucan particles in this study had broad size distributions in wort. Both MW and concentration of β-glucans affected the size distributions ($p<0.001$; Figure 4.1). The 0.01-0.1 μm fraction increased at higher MW and concentration levels ($p<0.001$; Figure 4.1). The >0.1 μm fraction of β-glucans, however, was not significantly affected by MW or concentration ($p>0.05$; Figure 4.1). The increase in apparent particle size of β-glucans at higher concentrations cannot simply be explained by the entanglement of the molecules. According to the entanglement theory, molecules of a polymer in solvent will not overlap one another until their concentration reaches a critical value C* (Goodwin and Hughes, 2000; Graessley, 1974; Kasaai *et al.*, 2000; Linemann and Krüger, 1997). Beta-glucans (31-443 kDa) were found to have C* values of 2.5-8.3 g/L in 12°P wort (Figure 3.18). The magnitude of these values suggests that β-glucan molecules at 50-1000 mg/L in the wort samples were not entangled with one another. Thus, the greater particle size of β-glucans at higher concentrations was hypothesized to be caused by the association of some individual molecules to form micelle-like structures (Grimm and Krüger, 1994). The >0.01 μm fraction of β-glucans can be accounted for by their MW and concentration ($R^2=0.811$; $n=80$; $p<0.001$):

$$F_{>0.01} = 0.1648\ MW + 0.0629\ C - 1.2\times10^{-4}\ MW\times C \qquad (4\text{-}1)$$

where $F_{>0.01}$ is the fraction of particles >0.01 μm in diameter as a percentage of the total β-glucan in wort (%); MW is molecular weight in kDa; and C is the β-glucan concentration (mg/L).

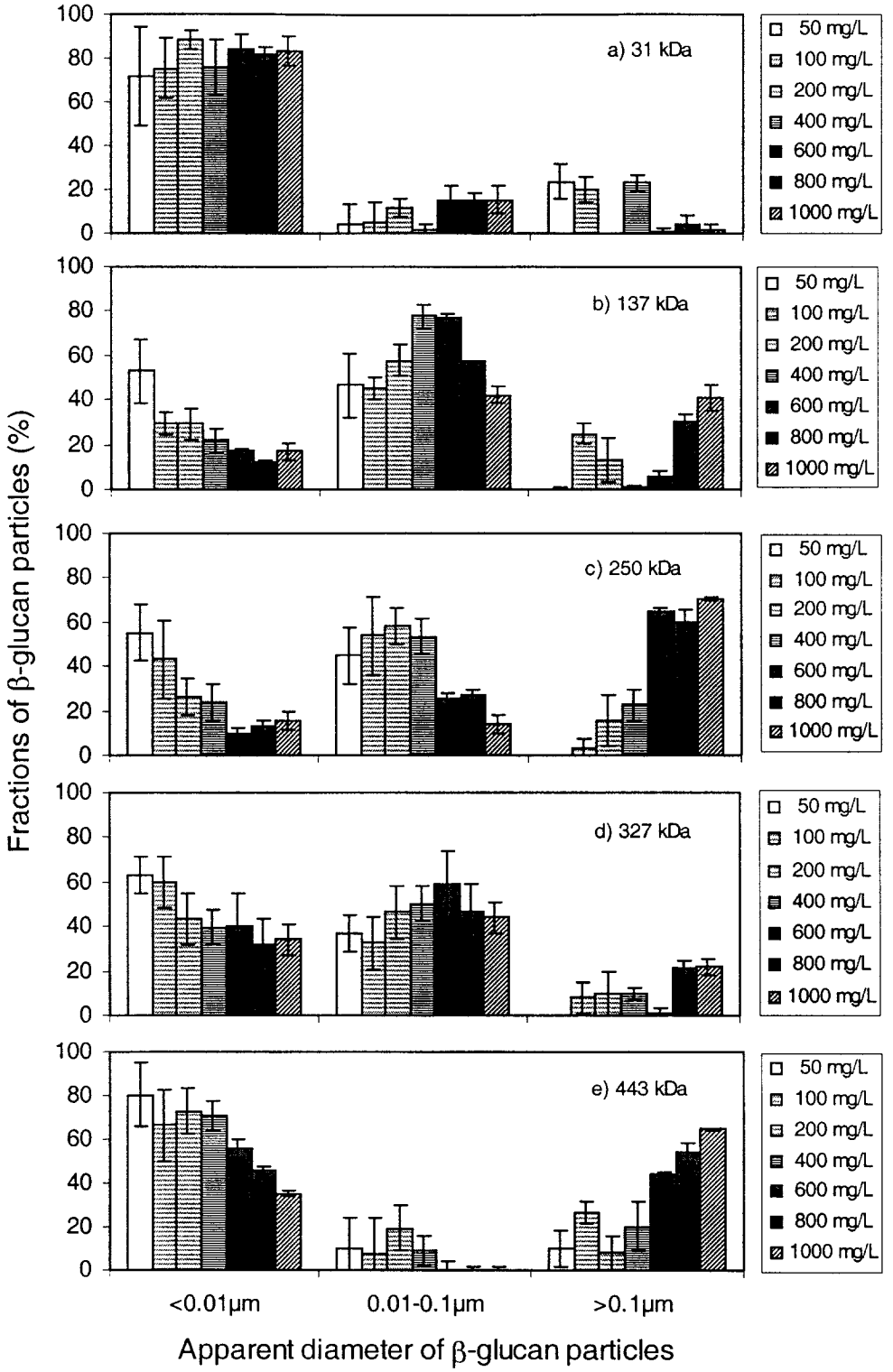

Figure 4.2 Effect of MW and concentration on the particle size distribution of β-glucans in sheared 12°P wort (20°C). Values are given as means ± S.D. of duplicate experiments.

In the sheared wort samples, β-glucan particle size in the >0.1 μm category increased with MW and concentration ($p<0.001$; Figure 4.2). However, the 0.01-0.1 μm fraction first increased with high concentrations and then decreased ($p<0.001$). The 31 kDa β-glucan had a decreased fraction of <0.01 μm compared to the unsheared wort ($p<0.001$) although 75-90% of the β-glucan molecules were still <0.01 μm. Only 10-70% of the high MW β-glucans (137-443 kDa) were <0.01 μm in sheared wort. The >0.1 μm fraction of 137-443 kDa β-glucans was higher in the sheared wort than in the unsheared wort ($p<0.001$). Shearing of wort resulted in a shift of the apparent size from <0.01 μm to >0.1 μm ($p<0.001$). Shearing may have forced β-glucan molecules to adopt an extended conformation, making it favorable for the extended polymers (after shearing) to associate by hydrogen bonding.

4.3.2 Effect of MW and Concentration of β-Glucans and Shearing on the Particle Size Distribution in Beer

Categorization of β-glucan particles by membrane filtration was carried out at 20°C for both unsheared beer and beer sheared at 5°C. The majority (60-100%) of the β-glucan particles were retained on the 0.01 μm membrane in the unsheared beer whereas 45-100% of the β-glucans had an apparent particle size of 0.01-0.1 μm in diameter (Figure 4.3). The amount of β-glucans between 0.01-0.1 μm in diameter was increased by both MW and concentration of β-glucans ($p<0.001$). The proportion of β-glucans >0.1 μm was related to MW ($p<0.001$) but not concentration ($p>0.05$). It was found that β-glucans in beer had greater particle size than in wort ($p<0.001$). This may help explain their higher viscosities in beer (discussed in Chapter 3) because, in shear, bigger particles swept a larger volume thus resulting in higher viscosities.

Shearing beer samples at 5°C also affected the size distribution (20°C) of β-glucan particles (Figure 4.4). The β-glucan fraction retained by 0.01 μm membranes increased

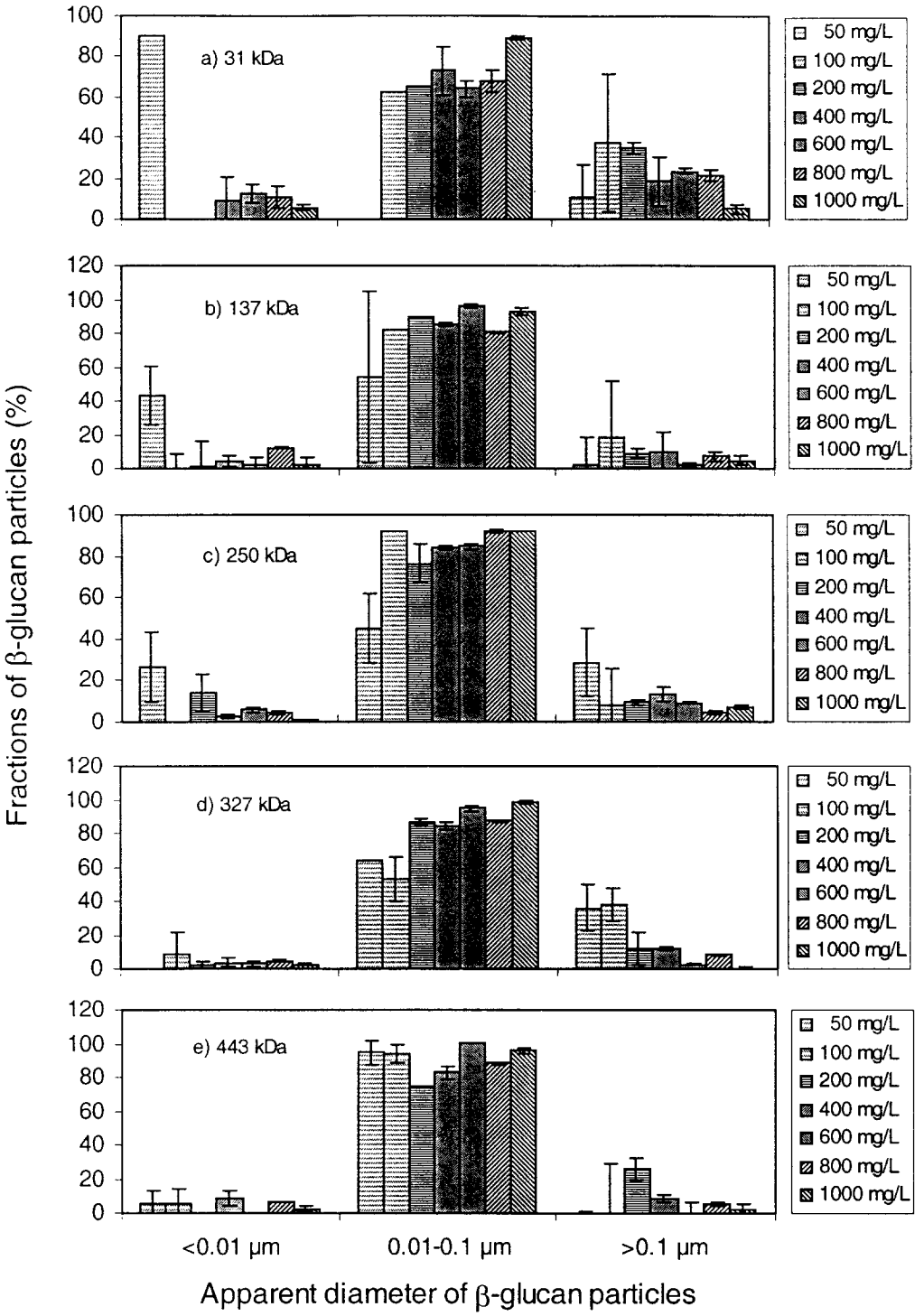

Figure 4.3 Effect of MW and concentration of β-glucans on the particle size distribution in the unsheared beer (20°C). Values are given as means ± S.D. of duplicate experiments.

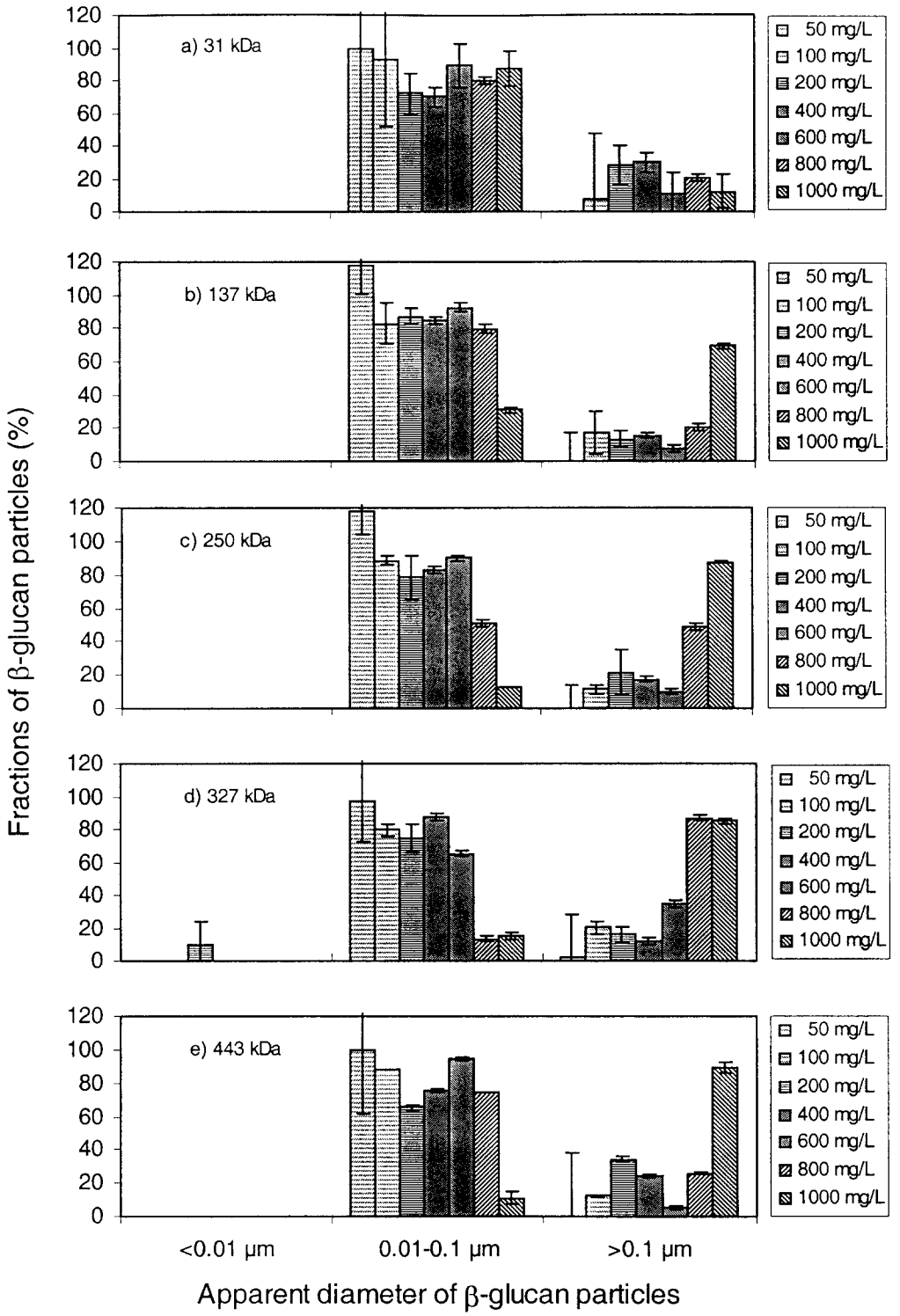

Figure 4.4 Effect of MW and concentration of β-glucans on the particle size distribution (20°C) in sheared beer. Values are given as means ± S.D. of duplicate experiments.

with higher MW and concentration ($p<0.001$), and was enhanced by shearing ($p<0.001$). The multiple linear regression ($R^2=0.863$; n=160; $p<0.001$) indicated that the >0.01 μm fraction in beer was determined by MW, concentration and shearing:

$$F_{>0.01} = 0.167\ MW + 0.057\ C + 35.613\ S \quad (4\text{-}2)$$

where S=0 for unsheared beer and S=1 for sheared beer. The proportion of β-glucans >0.01 μm in diameter can be described by the following relationship for wort and beer samples ($R^2=0.907$; n=320; $p<0.001$):

$$F_{>0.01} = 0.092\ MW + 0.036\ C + 12.272\ S + 52.118\ F_m \quad (4\text{-}3)$$

where F_m is fermentation ($F_m=0$ for wort and $F_m=1$ for beer). The standard coefficients showed that fermentation (i.e., the difference between wort and beer) had the most important effect on the proportion of >0.01 μm β-glucan particles, whereas shearing had a lesser but still significant effect.

4.3.3 Effect of Maltose Concentration, pH and Shearing Temperature on the Particle Size Distribution of 443 kDa β-Glucan in Wort

Wort samples at 20°C containing 443 kDa β-glucan at 600 mg/L were examined for the apparent diameter of β-glucan particles under various conditions (pH 4.0-6.8, maltose content 6.1-16.1% w/w, unsheared wort at 20°C versus wort sheared at 20°C, 48°C and 76°C). The experimental design is depicted in Figure 3.2. Approximately 10-70% of the β-glucan particles were <0.01 μm in diameter (Figure 4.5). A stepwise regression indicated that both 0.01-0.1 μm and >0.1 μm fractions increased at higher pH values ($p<0.001$). Interestingly, shearing and shearing temperature were not found to affect the size distribution of the 443 kDa β-glucan in wort ($p>0.05$). The distributions of β-glucan particle size in the unsheared wort (20°C) and wort sheared at 20°C were not affected by maltose level ($p>0.05$). However, increasing maltose level in worts which were sheared at 48°C and 76°C lowered the fraction of β-glucan in the category of 0.01-0.1 μm and increased the proportion of <0.01 μm compared to shearing at 20°C ($p<0.001$). This is not in agreement with a previous report by Grimm *et al.* (1995a). A 175 kDa β-glucan

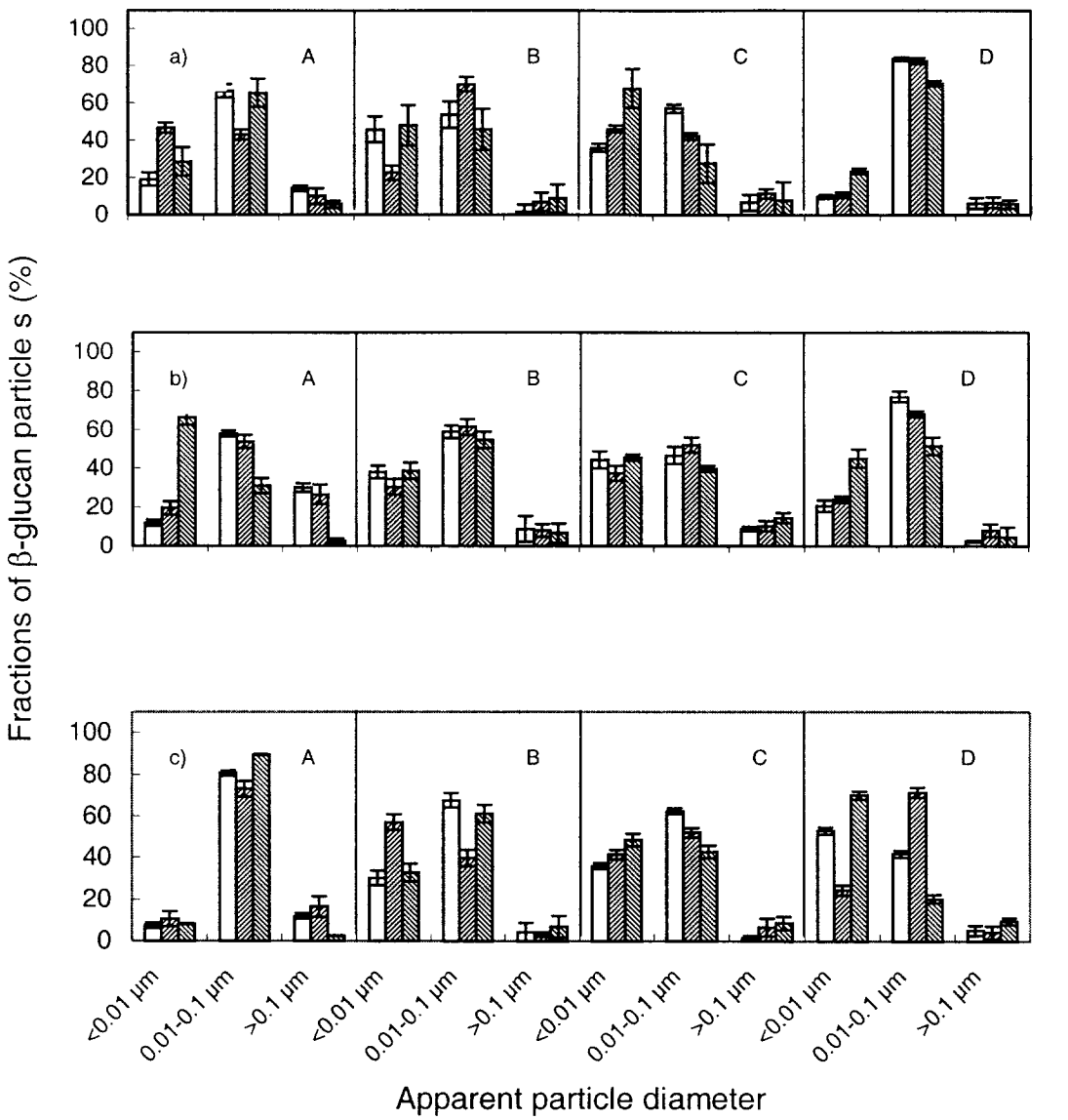

Figure 4.5 Effect of maltose level and shearing on the size distribution of 443 kDa β-glucan particles (600 mg/L) at (a) pH 4.0; (b) pH 5.4 and (c) pH 6.8. A = unsheared (20°C); B = sheared at 20°C; C = sheared at 48°C and D = sheared at 76°C. Values are given as means ± S.D. of duplicate experiments.

isolated from beer showed decreased and then increased particle size in water when maltose was increased from 2% w/v to 10% w/v (Grimm *et al.*, 1995a). The radius of gyration of the 175 kDa β-glucan at 1000 mg/L decreased from 209 nm to 126 nm when the maltose level was increased from 2% w/v to 6% w/v. However, the radius increased from 126 nm to 247 nm when maltose level was further raised to 10% w/v (Grimm *et al.*, 1995a). Linemann and Krüger (1998a) reported that 5% maltose increased the apparent MW of a barley β-glucan in water from 714 kDa to 2,900 kDa but decreased the apparent MW of a beer β-glucan from 5,400 kDa in water to 2,870 kDa. Those β-glucan samples were sheared with a homogenizer and stored at –4°C to precipitate the β-glucans from beer which were concentrated by 0.2 μm membrane filtration (Linemann and Krüger, 1998a). The difference in solvent conditions as well as sample history is responsible for the contradictory effects of maltose level on β-glucan particle size.

4.3.4 Effect of pH, Ethanol Content and Shearing Temperature on the Particle Size Distribution of 443 kDa β-Glucan in Beer

The 443 kDa β-glucan at 600 mg/L in beer was used to examine the effect of processing conditions on the β-glucan particle size distribution. More than 77% of the β-glucan molecules were 0.01-0.1 μm in diameter (Figure 4.6). Both 0.01-0.1 μm and >0.1 μm fractions increased at higher pH values in the range of 3.8-4.6 ($p<0.001$). It was unexpected to observe the influence of pH on the particle size of such a theoretically uncharged polysaccharide. However, even these commercially purified β-glucans contained 0.72-9.35% proteins (Table A.1). The 443 kDa β-glucan contained 3.34% proteins (Table A.1). It can be assumed that the small amount of protein was attached to the β-glucan molecules since proteins and β-glucans are cross-linked in the barley endosperm cell walls (Forrest and Wainwright, 1977; Izydorczyk and MacGregor, 2000). Thus, the changes in pH may have affected the interactions among β-glucan molecules through the altered electrostatic repulsion between the attached proteinaceous moieties.

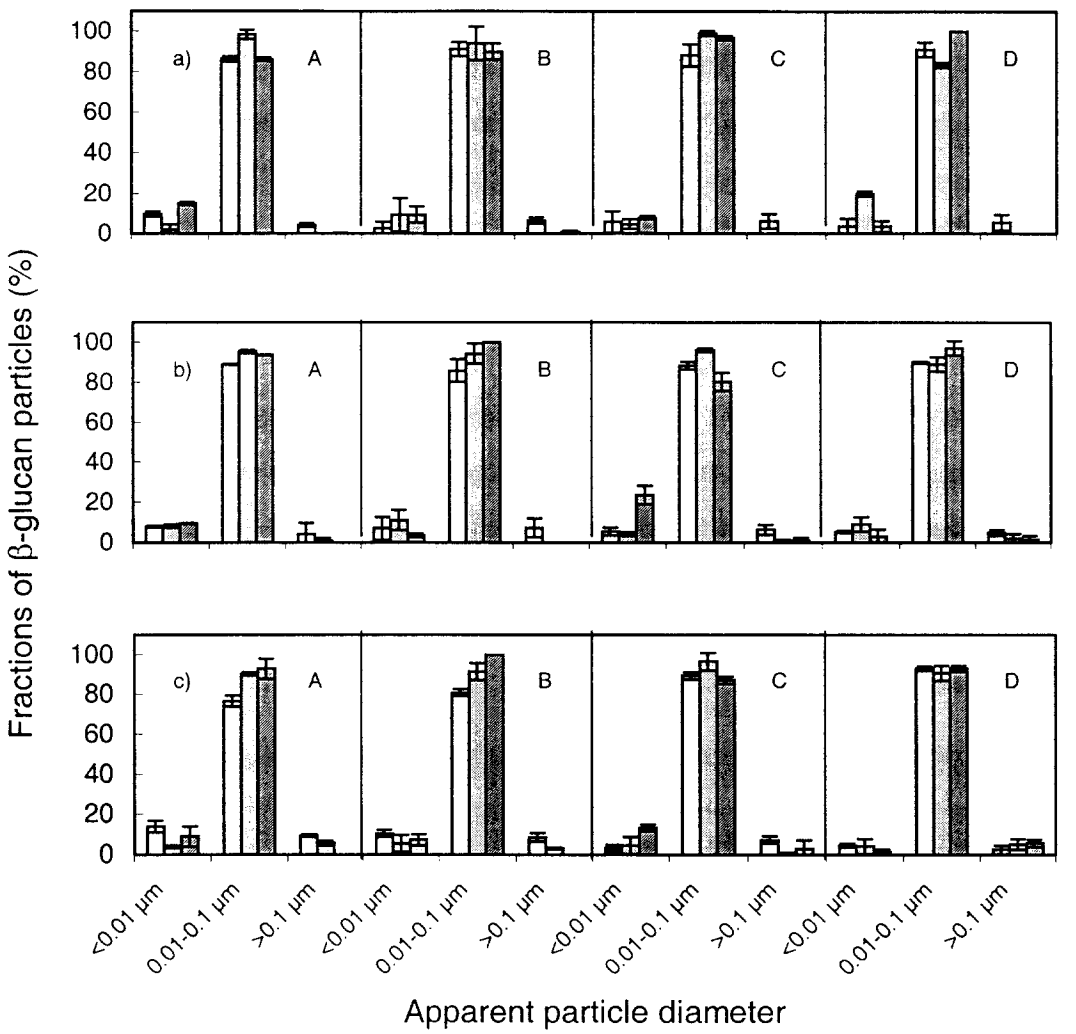

Figure 4.6 Effect of ethanol content and shearing on the particle size distribution of 443 kDa β-glucan (600 mg/L) in beer at (a) pH 3.8; (b) pH 4.2 and (c) pH 4.6. A = unsheared (5°C); B = sheared at 0°C; C = sheared at 5°C and D = sheared at 10°C. Values are given as means ± S.D. of duplicate experiments.

Shearing beer increased the apparent particle size of the 443 kDa β-glucan at 600 mg/L ($p<0.001$) although shearing temperature did not show any influence on particle size between 0-10°C ($p>0.05$). Increasing beer ethanol concentration increased the proportion of β-glucans in 0.01-0.1 μm but decreased the >0.1 μm fraction ($p<0.001$; Figure 4.6). Thus, the total amount of β-glucans retained by the 0.01 μm membranes was not influenced by ethanol concentration ($p>0.05$). The 0.01-0.1 μm fraction (%) of the 443 kDa β-glucan molecules (600 mg/L) can be described by ethanol (E, % v/v) and pH levels ($R^2=0.990$; $n=54$; $p<0.001$):

$$F_{0.01\text{-}0.1} = 0.737\ E + 20.708\ pH \qquad (4\text{-}4)$$

There was a report on a dramatic increase in the apparent M_w of a barley β-glucan (approximately 1000 mg/L) from 714 kDa in water to 45,360 kDa in water containing 5% v/v of ethanol (Linemann and Krüger, 1998a) as determined by light scattering. This indirectly supports the finding of this study that ethanol increased the apparent particle size of barley β-glucans in beer.

A glucosyl residue is assumed to have a dimension of 0.5 nm in length (Woodward *et al.*, 1983a). Thus, the "length" of β-glucan molecules can be estimated from their degree of polymerization (DP). The 31 kDa β-glucan molecules would be 0.1 μm long in an extended form, the high MW β-glucans (137-443 kDa) would be 0.4-1.4 μm in length, which represents the "diameter" of the hydrodynamic space they sweep over. However, the apparent particle size determined by membrane filtration tests was actually much smaller. For all the samples studied, the majority (86-88%) of the β-glucan molecules were <0.1 μm in wort and beer (Table 4.1). Thus, β-glucan molecules in wort and beer are compact in shape (e.g., coils) instead of extended linear molecules. The grand mean values of 106 wort samples and 106 beer samples tested in duplicate showed that β-glucans in beer had greater apparent particle size than in wort (Table 4.1). This can help explain the higher viscosities of β-glucans in beer than in wort (Chapter 3).

Table 4.1 Mean values of the apparent particle diameter of β-glucans (n=212)

Samples	Mean proportion of β-glucans (%)		
	<0.01 μm	0.01-0.1 μm	>0.1 μm
Wort	41.3±23.6	46.5±25.8	12.2±14.1
Beer	4.5±10.8	82.0±20.9	13.5±19.0

4.4 Conclusions

The effects of MW, concentration and processing conditions on the β-glucan particle fraction >0.01 μm in diameter are summarized in Table 4.2. Apparent particle size of β-glucans in wort and beer increased with their MW and concentration. Solvent conditions affected the distribution of β-glucan particles, i.e., β-glucans had greater size in beer than in wort. Beta-glucans exhibited greater particle size at higher pHs of wort and beer. Maltose showed no significant influence ($p>0.05$) on β-glucan particle size distributions in the unsheared wort. Ethanol at higher levels increased the proportion of the 0.01-0.1 μm fraction and decreased the amount of >0.1 μm fraction for the 443 kDa β-glucan in beer. Shearing of wort and beer resulted in a decrease in the <0.01 μm fraction of β-glucans and an increase in 0.01-0.1 μm (diameter) β-glucan fraction ($p<0.001$). However, shearing temperature did not show any significant effect on β-glucan particle size distribution ($p>0.05$). The fraction of β-glucans >0.1 μm accounted for only approximately 12-14% as a grand mean. This implies that purified β-glucans (31-443 kDa) in beer are not likely to plug the pores of 0.45 μm membranes directly in the early stage of membrane filtration because only the particles >0.45 μm in beer can retard the filterability by clogging (Ilberg *et al.*, 1995). When the pores are plugged by proteins and/or protein-polyphenol complexes, the effective pore size decreases and the membrane clogging by these β-glucan polymers can be observed.

Table 4.2 Effect of process conditions on the percentage of β-glucan particles >0.01 μm

Sample	Factor	Level	Response [a)]
Wort [b)]	Shearing	Unsheared / sheared	↑***
	MW of β-glucan	31-443 kDa	↑***
	β-Glucan level	50-1000 mg/L	↑***
Wort	Shearing temperature	20, 48, 76°C	NS
	pH	4.0, 5.4, 6.8	↑***
	Maltose	6.1, 10.1, 16.1% w/w	NS (20°C); ↓*** (48 &76°C)
Beer [c)]	Shearing	Unsheared / sheared	↑***
	MW of β-glucan	31-443 kDa	↑***
	β-Glucan level	50-1000 mg/L	↑***
Beer	Shearing temperature	0, 5, 10°C	NS
	pH	3.8, 4.2, 4.6	↑***
	Ethanol	0, 5, 10% v/v	NS (0.01-0.1 μm↑***; >0.1 μm ↓***)

[a)]: Effect of increasing the level of a factor; [b)]: Wort (12°P) at pH 5.4; [c)]: Beer at pH 4.2, containing 3.3% w/w of real extract and 5.0% v/v of ethanol; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; NS = not significant ($p>0.05$).

5 EFFECT OF β-GLUCANS AND PROCESSING CONDITIONS ON THE INTERFACIAL AND FOAMING PROPERTIES OF WORT AND BEER

In this study, the effects of β-glucans and various environmental conditions on wort and beer surface tension, beer foamability and foam stability were investigated. Beta-glucans of molecular weights of 31, 137, 250, 327 and 443 kDa and concentrations of 100-1000 mg/L were found to lower the surface tension of wort, but had no effect on beer. Shearing caused surface tension to decrease in wort but increase in beer. Shearing temperature had no effect on the surface tension of wort and beer. An increase in maltose level and acidification of wort decreased surface tension. Ethanol at concentrations of 5-10% v/v lowered beer surface tension but had a negative effect on foamability and foam stability. Large concentrations of high MW β-glucans (e.g., 600 mg/L of a 443 kDa β-glucan) were detrimental to beer foaming properties. Higher beer pHs improved foamability but lowered foam stability although pH did not affect beer surface tension.

5.1 Introduction

Beer foam directly influences its sensory quality. Good foam quality is usually related to fine, white foam with head retention values greater than 2-3 minutes. Foams are generated by the dispersion of CO_2 in beer to form spherical bubbles. The stability of foams depends on the rate of liquid drainage between the bubbles and the rupture of the bubble walls. Beer composition plays an important role in both foamability and foam stability because sufficient foam stabilizing agents (e.g., surface active proteins and gums) positively affect foam formation and stability while foam-destabilizing compounds such as lipids are detrimental to the foam head.

In food science, bubbling, whipping/beating, and shaking have been used to generate protein-stabilized foams (Halling, 1981). Bubbling gas through a porous sparger in a liquid gives more uniform bubble sizes and allows easy monitoring of the progress of

beer foaming. Whipping or beating can be carried out with a variety of devices that vigorously agitate a liquid and its interface with a bulk gas phase. Shaking has been suggested as a quick, on spot check of bright beer to identify severe foam defects (Bamforth, 1999a). In addition, pouring beer can produce foam with the properties which most likely resemble those perceived by a consumer. The pouring test has been automated to determine foam stability (Melm *et al.*, 1995). Without being whipped or poured, beer foams can still occur due to the dissolved CO_2 (0.2-0.3% w/w; equivalent to 1-1.5 volumes of gas) in beer (0.1 MPa). When the pressure is released (i.e., when a bottle is opened), CO_2 becomes supersaturated and bubbles form in small gas pockets present at the bottle wall. As bubbles rise, they continue to grow until forming a creamy layer known as the foam head. The presence of nucleation sites in beer (e.g., haze particles), on the wall of the glass (e.g., scratches or hydrophobic surfaces), or gas pockets all facilitate the continuous and gradual release of CO_2.

The presence of foaming agents is necessary for good foam stability in beer. Consequently, pure liquids can not produce stable foams. In the bulk phase of a pure liquid, such as water, all the molecules are under attractive forces. In contrast, a molecule at the surface of this liquid is in an unbalanced field. Depending on the solvent and solutes present in the system, either negative adsorption or positive adsorption of surface active agents at the phase boundary can be observed. In negative adsorption, molecules, such as mineral salts are eliminated from the surface of the solution. When positive adsorption occurs, polymer surfactants are preferentially adsorbed at the interface. In the case of a gas-liquid interface, the molecules of the liquid situated at the interface are drawn inwards by free surface energy. This interfacial energy corresponds to work per unit of surface area (J/m^2) and a force per unit of length (N/m or dyn/cm). The forces minimizing the free energy are called interfacial tension (γ), which is numerically equivalent to the interfacial free energy (Walstra, 1996). At an air-liquid interface, the term interfacial tension is used interchangeable with surface tension. Beer proteins together with iso-α-acids can lower the surface tension of water, for instance, from 72.75

dyn/cm (20°C) to 46.7-50.9 dyn/cm (Maeda *et al.*, 1991). Proteins possess both hydrophobic and hydrophilic side chains, therefore, are well adsorbed in both the hydrophobic and the hydrophilic sides of the interface, lowering interfacial tension. The capacity of proteins and polysaccharides for interfacial adsorption depends on the flexibility of the polymeric chain and its unfolding potential (Linden and Lorient, 1999).

Beer contains many surface-active compounds, which compete with one another for spaces in the bubble walls. Some compounds stabilize beer foam whereas others are detrimental to foam stability. In the past, research has focused on beer proteins or polypeptides. Three classes of beer foaming proteins have been studied: protein Z, lipid transfer protein, and lipid binding protein. Protein Z (a 40 kDa albumin) is derived from barley endosperm and survives the malting and brewing stages. Hejgaard (1977) first implicated the foaming potential of protein Z. The dominant form of protein Z in barley is protein Z4. Although it has been claimed that beer foaming performance is not affected by the absence of protein Z4 (Gibson *et al.*, 1996; Hollemans and Tonies, 1989), recent investigations have shown a positive correlation between the level of protein Z4 and beer foam stability (Evans *et al.*, 1995; 1999).

Another barley-derived protein implicated in beer foam is Lipid Transfer Protein 1 (LTP1). The LTP1 derived foam must be stabilized by another high MW fraction (believed to be primarily carbohydrates) (Sørensen *et al.*, 1993). It was later claimed by Lusk *et al.* (1995) that LTP1 (10 kDa) is the major component of beer foam, which gives the best foam stability among the foaming proteins. In addition, its level in wort is not affected by malting or mashing conditions (Lusk *et al.*, 1995). Lipid binding proteins have also been reported to improve beer foam stability (Clark *et al.*, 1994; Dickie *et al.*, 2001). It is not clear, however, if these lipid-binding proteins are related to the LTP1. To complicate the issue, Evans *et al.* (1999) have argued that beer foam stability depends on the level of protein Z4 rather than the LTP1 level.

To explain these controversial findings, it has been hypothesized that hydrophobicity of these proteins is more important than size for foam stability (Ishibashi *et al.*, 1996; Kauffman *et al.*, 1994; Nakai, 1983; Onishi and Proudlove, 1994; Slack and Bamforth, 1983). Additional studies on the interactions of foaming proteins among themselves or with other beer components, such as polysaccharides may help clarify their importance in foaming. Also, the foamability and foam stability of beer is rarely a result of any single protein fraction because polypeptides in beer have a very broad distribution in size due to extensive proteolysis during malting and mashing.

Hop bitter substances particularly iso-α-acids (isohumulones) can also enhance foamability and foam stability (Asano and Hashimoto, 1976; Bishop *et al.*, 1974; Roberts, 1976; Yokoi *et al.*, 1994). Divalent metal ions can cross-link iso-α-acids that are associated with foam polypeptides (Archibald *et al.*, 1973; Bishop *et al.*, 1974; Simpson and Hughes, 1994). Other factors influencing foam stability include pH, melanoidin type and content, ethanol and lipid levels (Bamforth, 2000; Brierley *et al.*, 1996; Furukubo *et al.*, 1993; Lusk *et al.*, 1995; Melm *et al.*, 1995; Taylor, 1990).

Protein-stabilized foams are very sensitive to destabilization by competitive adsorption and spreading of lipids (Walstra, 1996). Lipids are considered to be the principle head-destabilizing component in beer. The majority of these lipids originate from malt and adjuncts. Hop oils are another source of beer lipids, but occur at much lower levels. Lipid binding proteins have proven to reverse the foam destabilization caused by lipids (Clark *et al.*, 1994).

Polysaccharides recovered from beer are able to form foams even in the absence of proteins (Lusk *et al.*, 1995). Polysaccharides also exhibit moderately independent foam stability, although they are considered more important in lowering drainage from bubble walls (Lusk *et al.*, 1995) due to an increased viscosity. Both β-glucans and pentosans have been reported to affect foaming properties in model systems. Drainage of whey

protein foams was retarded by the addition of 1% crude barley β-glucans (Burkus and Temelli, 2000). Addition of wheat arabinoxylans reduced the foam capacity of bovine serum albumin (BSA) solutions but improved foam stability upon heating (Izydorczyk and Biliaderis, 1992a; 1992b). Studies have shown that the levels of malt protein Z4, wort β-glucans, wort viscosity, beer proteins, beer β-glucans and arabinoxylans are positively correlated with beer foam stability (Evans *et al.*, 1999; Nischwitz *et al.*, 1999). Since the content of these components all decrease with extended degradation during malting and mashing, it is difficult to judge if the increased foam stability is due to the high levels of proteins and/or polysaccharides. Beta-glucan levels of beer at 0, 183, 373 and 551 mg/L showed no difference in beer foam adhesion to glass (Furukubo *et al.*, 1993) although foamability and foam stability were not examined. No foam-enhancing or foam-stabilizing effects of β-glucans were found by either removal of β-glucans from beer by β-glucanase (from 381 mg/L to 19 mg/L) or by the addition of commercial β-glucans to model beer system (130-145 mg/L) (Lusk *et al.*, 2001).

The purpose of this study was to examine the effects of the addition of barley β-glucans on the surface tension of wort and beer, and on beer foamability and foam stability. The surface tension of samples was studied because equilibrium surface tension of low MW surfactants has been reported to correlate well with their foaming ability (Kitabatake and Doi, 1988), but still needs to be confirmed for beer foaming.

5.2 Materials and Methods

Materials used in this study are described in section 3.2.1. Beta-glucan-free, unhopped wort was prepared from pale malt as described in section 3.2.2. Barley β-glucans purchased from Megazyme (Bray, IRL) had MWs of 31, 137, 250, 327 and 443 kDa. These β-glucans were used to prepare wort samples with β-glucan concentrations in the range of 0-1000 mg/L (section 3.2.3). A commercial lager beer (Labatt Blue, product code E10H11C, UBC 062067351013, Oland Breweries Ltd., Halifax, NS) was used to

prepare a β-glucan-free beer base (section 3.2.4). This β-glucan-free beer base was used to prepare beer samples at various β-glucan concentrations (0-1000 mg/L of 31-443 kDa), pHs (3.8-4.6), and ethanol contents (0-10% v/v) as described in section 3.2.5. Beta-glucan concentration was determined using the Congo red assay described in section 3.2.7.

A Type K8600E Du Noüy Interfacial–Tensiometer (Optisch-Mechanische Werkstatten, Hamburg, DEU) was used to determine the surface tension of wort and beer. Double distilled and de-ionized water (DDW) at 20.0°C (72.75 dyn/cm) was used to calibrate the tensiometer. A mean value of four measurements of water surface tension (20.0°C) was 70.06 dyn/cm. Thus, the measured values for sample surface tension were corrected by a factor of 1.0312.

To determine the foaming properties of beer samples, 10.0 mL of degassed beer sample was sheared at 5°C (unless otherwise specified), with a blender (Model MM-1B, Lourdes Instrument Corp., Brooklyn, NJ) at a speed setting of "60" for 35 seconds. The shear rate of this process was estimated to be $1.3\pm0.2 \times 10^4$ s^{-1} (section 3.2.6). The sheared sample was transferred into a 25-mL volumetric cylinder and kept in a water bath at the same temperature. The total (foam and liquid) volume and the volume of liquid were recorded immediately. The volumes were examined again after 10 minutes. The initial volume of foam was defined as foamability. The volume of foam remaining after 10 minutes was defined as foam stability.

An experimental design is depicted in Table 5.1. Duplicate experiments were carried out and mean values and standard deviations were reported. Linear regressions and analysis of variance (ANOVA) of the data were done using SYSTAT version 5.05 (SPSS Inc., Chicago, IL).

Table 5.1 Experimental design for the studies on interfacial and foaming properties

Sample	Factor	Level	Measurement
Wort [a)]	Shearing	Sheared/unsheared	Surface tension
	MW of β-glucan	31-443 kDa	
	Concentration of β-glucan	50-1000 mg/L	
Wort	β-Glucan (443 kDa)	0, 600 mg/L	
	Shearing temperature	20, 48, 76°C	
	pH	4.0, 5.4, 6.8	
	Maltose	6.1, 10.1, 16.1% w/w	
Water	β-Glucans (1000 mg/L)	31-443 kDa	
Beer [b)]	Shearing	Sheared/unsheared	Surface tension Foamability Foam stability
	MW of β-glucan	31-443 kDa	
	Concentration of β-glucan	50-1000 mg/L	
Beer	β-Glucan (443 kDa)	0, 600 mg/L	
	Shearing temperature	0, 5, 10°C	
	pH	3.8, 4.2, 4.6	
	Ethanol	0, 5, 10% v/v	
Beer [b)]	β-Glucan (443 kDa)	200-1000 mg/L	β-Glucan level in collapsed foam

[a)]: Wort (12°P) at pH 5.4;
[b)]: Beer at pH 4.2 containing 3.3% real extract and 5.0% v/v of ethanol.

5.3 Results and Discussion

The effects of MW, β-glucan concentration, pH, maltose, ethanol, shearing and shearing temperature on the surface tension of wort and beer were investigated. Beer foamability and foam stability were also examined to determine if β-glucans affect beer foaming properties.

5.3.1 Effect of MW and Concentration of β-Glucans and Shearing on Surface Tension of Wort and Beer

Wort (12°P at pH 5.4) and beer (3.3% w/w of real extract with 5.0% v/v of ethanol at pH 4.2) contain proteins, which are responsible for lowering surface tension and foaming properties. The surface tension of water at 20°C is 72.75 dyn/cm. For the β-glucan-free wort, the surface tension was 57.5 dyn/cm (Figure 5.1a). Such a decrease in wort surface tension was hypothesized to be due to the presence of proteins and polypeptides. An ANOVA also found that β-glucans decreased surface tension of wort at high concentrations ($p<0.05$). Beta-glucans (31-443 kDa) at a concentration of 1000 mg/L decreased surface tension by 1-1.5 dyn/cm relative to the β-glucan-free wort over the entire MW range. At a concentration of 50 mg/L in a MW range of 137-443 kDa, β-glucans did not lower wort surface tension (Figure 5.1a). Molecular weight of the β-glucans between 137-443 kDa was not important in lowering surface tension ($p>0.05$). Thus, it was hypothesized that there was a "threshold" MW for β-glucans in lowering wort surface tension. The 31 kDa β-glucan at a concentration of 50 mg/L caused a reduction in surface tension. However, above this MW threshold, β-glucans (137-443 kDa) at 50 mg/L in contrast did not lower wort surface tension. At β-glucan concentrations ≥100 mg/L, wort surface tension decreased by approximately 1 dyn/cm, and remained unchanged at higher concentrations. It is hypothesized that the interfacial space was saturated at β-glucan concentrations ≥100 mg/L. The majority of the interfacial surface was believed to be occupied by non-β-glucan compounds such as proteins, which reduced the surface tension of water from 72.25 dyn/cm to 57.5 dyn/cm for the unhopped wort (Figure 5.1a).

After shearing wort at 20°C, surface tension of the β-glucan-free wort measured at 20°C decreased to 50.8 dyn/cm (Figure 5.1b). The shear force at 20°C may have enhanced unfolding of wort proteins, leading to an increased positive adsorption at the interface and

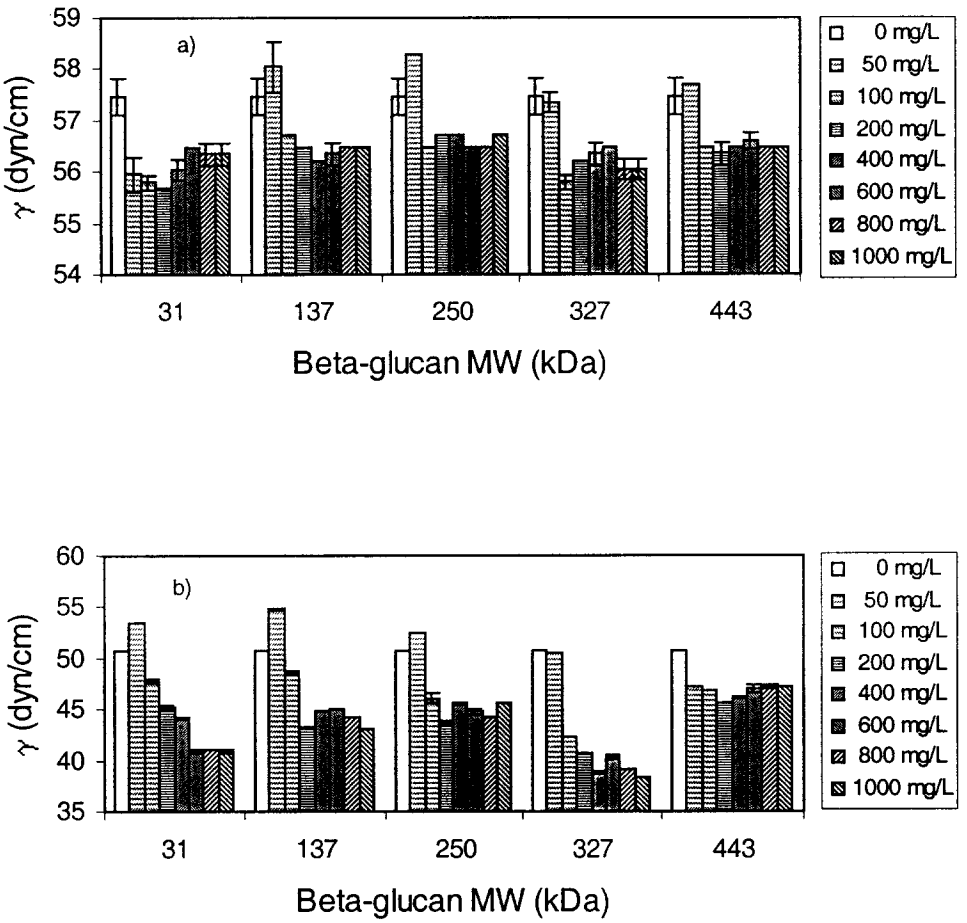

Figure 5.1 Effect of MW and concentration of β-glucans on surface tension at 20°C of (a) unsheared unhopped wort and (b) wort sheared at 20°C. Values are given as means ± one standard deviation (S.D.) of duplicate experiments.

a decreased surface free energy. Similar to the unsheared wort samples, 50 mg/L of 31-327 kDa β-glucans did not lower surface tension and in some cases increased wort surface tension. Increasing β-glucan concentrations to ≥100 mg/L lowered wort surface tension ($p<0.001$). An ANOVA showed no significant effect of the β-glucan MW on wort surface tension ($p>0.05$; Figure 5.1b). The following relationship was predicted by using multiple linear regression ($R^2=0.875$; $n=160$; $p<0.001$):

$$\gamma = 56.5 + 2.0\times10^{-5}\ S\times MW\times C - 6.1907\ S - 0.0109\ S\times MW - 0.01147\ S\times C \quad (5\text{-}1)$$

where γ is the surface tension (dyn/cm); C is the β-glucan concentration (mg/L); and S is shearing (S=0 for unsheared wort and S=1 for sheared wort). Since shearing wort can lower surface tension, foaming of wort during boiling will be facilitated by agitation. This will enhance the denaturation and aggregation of proteins to give better formation of hot trub and beer stability. However, the enhanced denaturation can also result in loss of foaming proteins in final beer product. It is still poorly understood if shearing of wort is detrimental to the foaming properties of packaged beer.

The surface tension of degassed beer (pH 4.2, containing 3.3% w/w of real extract, 5.0% v/v of ethanol and 0-1000 mg/L of 31-443 kDa β-glucans) was measured at 20°C (Figure 5.2a). The β-glucan-free beer had surface tension of 48.2 dyn/cm, which was much lower than that of 12°P wort (57.5 dyn/cm). At a β-glucan concentration of 50 mg/L, results showed only a minute reduction in surface tension compared to the β-glucan-free beer. An ANOVA showed that adding β-glucans at a concentration of 50 mg/L lowered beer surface tension ($p<0.001$), but MW showed no significant effect ($p>0.05$). Above a concentration of 50 mg/L, β-glucans displayed a "parabolic" trend between surface tension and concentration (Figure 5.2a). Surface tension was found lowest between β-glucan concentrations of 100-400 mg/L, then increased at high concentrations of 600-1000 mg/L. It is hypothesized that β-glucan molecules at very high concentrations have replaced some of the more surface active proteins at interface through competitive exclusion.

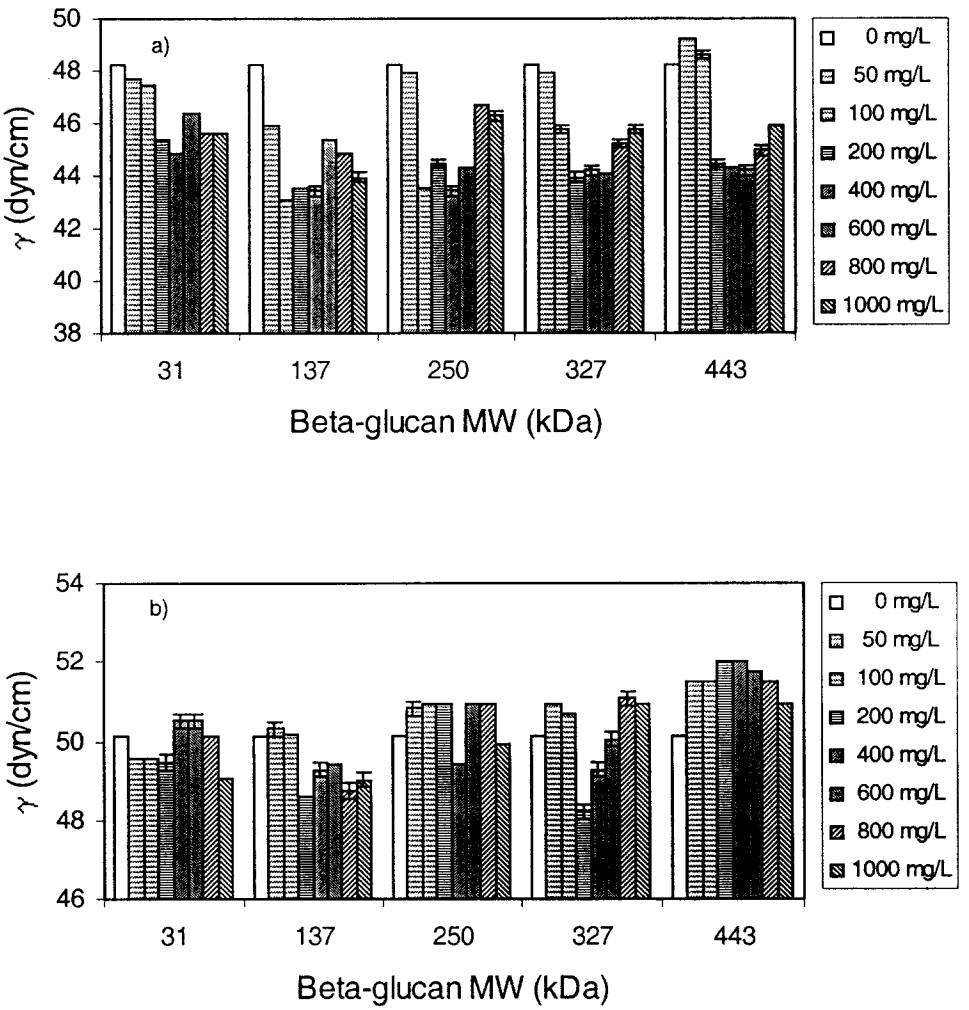

Figure 5.2 Effect of MW and concentration of β-glucans on surface tension at 20°C of (a) unsheared beer and (b) beer sheared at 5°C. Values are given as means ± S.D. of duplicate experiments.

Shearing beer samples at 5°C resulted in higher surface tension compared to the unsheared samples ($p<0.001$; Figure 5.2b). With the sheared beer, greater β-glucan MWs led to higher surface tension values ($p<0.001$). Increasing the concentration of β-glucans in sheared beer showed no clear trend in surface tension ($p>0.05$; Figure 5.2b). This is in contrast to the decreased surface tension found after shearing the wort samples, presumably due to the difference between wort and beer compositions. The increased beer surface tension after shearing implied lower foaming capacities of beer samples. The multiple linear regression indicated that both MW ($p<0.01$) and shearing ($p<0.001$) increased beer surface tension ($R^2=0.823$; $n=160$) whereas β-glucan concentration had no effect ($p>0.05$). The regression is given as follows:

$$\gamma = 45.8 + 4.3945\ S - 6.71\times10^{-3}\ MW + 2.0\times10^{-5}\ MW^2 + 4.9430\times10\text{-}6\ S\times MW\times C - 4.6343\times10^{-6}\ MW\times C \quad (5\text{-}2)$$

where MW is the molecular weight of β-glucans (kDa). This relationship suggested that shearing history of the beer and the presence of high MW β-glucans (even at concentrations as low as 50 mg/L) will increase surface free energy, which is unfavorable for foaming.

The surface activity of polymers is determined by both the polymer structure and the solvent conditions. When shearing was introduced, it decreased wort surface tension but increased beer surface tension (Figures 5.1b and 5.2b). Beta-glucan concentrations at ≥100 mg/L were found to lower the surface tension of sheared and unsheared worts and unsheared beer. After shearing, however, high MW β-glucans increased beer surface tension. This was hypothesized to be due to the replacement of the surface active proteins at interface by β-glucan molecules during/after shearing at 5°C. Proteins in the β-glucan-free beer lowered water surface tension from 72.75 dyn/cm to 48.2 dyn/cm (Figure 5.2a), whereas β-glucans at 1000 mg/L only lowered water surface tension to 66-68 dyn/cm (Figure 5.3). In comparison, wheat arabinoxylans at various concentrations also reduced surface tension of water. Surface tension was lowered to 70.0 dyn/cm by 0.1% w/v arabinoxylans at 25°C (Izydorczyk *et al.*, 1991). At the same concentration, barley β-

glucans were more surface active than wheat arabinoxylans. Because sheared beer samples exhibited higher surface tension, contradictory results in regard with the effect of β-glucans on beer foam are expected if samples were exposed to different shear histories.

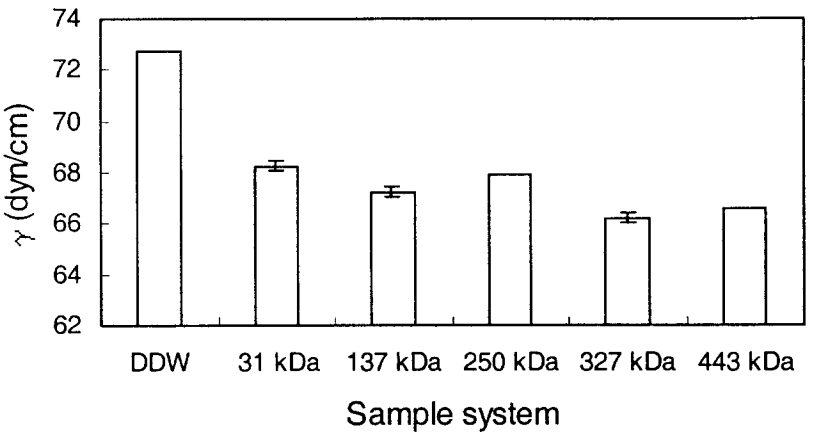

Figure 5.3 Effect of β-glucans at 1000 mg/L on the surface tension of water (20°C). DDW = double distilled and de-ionized water. Values are given as means ± S.D. of duplicate experiments.

5.3.2 Effect of pH, Temperature, β-Glucan (443 kDa at 600 mg/L), Maltose and Ethanol Levels on the Surface Tension of Wort and Beer

The influence of 443 kDa β-glucan at a concentration of 600 mg/L on surface tension of wort (6.1-16.1% w/w of maltose) was investigated at pH 4.0-6.8, unsheared or sheared at 20, 48 and 76°C (Figure 5.4). For the unsheared wort (samples denoted as NBNS and BGNS in Figure 5.4), surface tension decreased at lower pHs and higher maltose concentrations ($p<0.01$). An early study found that surface tension of hopped wort decreased from 41.2 to 39.1 dyn/cm (25°C) when pH of wort was adjusted from 5.7 to 4.4 (Bermann, 1948). The effect of 443 kDa β-glucan (highest MW available) at a

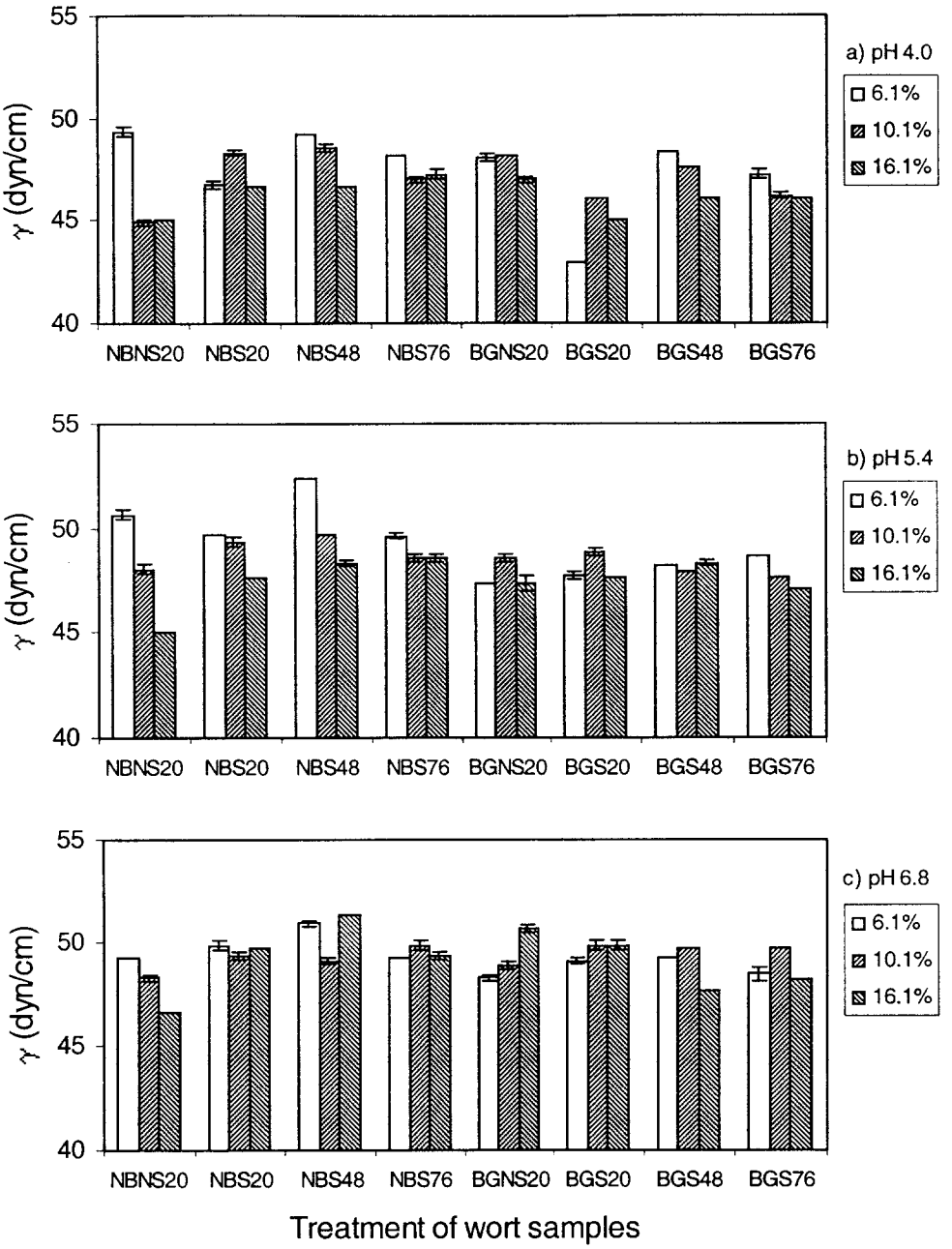

Figure 5.4 Effect of β-glucan, pH, maltose level and shearing temperature on wort surface tension at 20°C. NB = no β-glucan; NS20 = unsheared (20°C); S20 = sheared at 20°C; S48 = sheared at 48°C; S76 = sheared at 76°C; BG = 600 mg/L of 443 kDa β-glucan. Values are given as means ± S.D. of duplicate experiments.

concentration of 600 mg/L on the surface tension was insignificant ($p>0.05$) since the effect was dependent on the maltose level. In the 8°P wort (containing 6.1% maltose), the addition of β-glucan (443 kDa at 600 mg/L) decreased wort surface tension ($p<0.001$; Figure 5.4). When the wort contained higher maltose levels (10.1 and 16.1% w/w, but levels of other constituents were not changed), the addition of β-glucan (443 kDa at 600 mg/L) increased wort surface tension ($p<0.001$; Figure 5.4). It is inferred that surface tension decreases after fermentation due to the lowering of pH and the production of ethanol, which function to reduce surface tension, although the exhaustion of maltose by yeast tends to increase the surface tension.

Wort surface tension was compared after shearing wort at 20, 48 and 76°C. Surface tension was found to decrease at lower pH (pHs 4.0 and 5.4) and higher maltose concentrations (10.1 and 16.1% w/w) for both wort containing no β-glucan and 443 kDa β-glucan at 600 mg/L (Figure 5.4). Shearing at 20-76°C increased surface tension of the β-glucan-free wort ($p<0.001$) but decreased surface tension of the wort samples containing the 443 kDa β-glucan at 600 mg/L ($p<0.001$). Shearing temperature was found not to affect the overall trend of surface tension ($p>0.05$; Figure 5.4).

The surface tension of beer samples was also examined under various conditions of pH (3.8-4.6), ethanol (0-10.0% v/v), shearing (unsheared or sheared), shearing temperature (0, 5, and 10°C), and the 443 kDa β-glucan concentration (0 and 600 mg/L). With the unsheared beer samples, it was found that surface tension of beer was affected by 600 mg/L of the 443 kDa β-glucan ($p<0.05$) and ethanol ($p<0.001$) ($R^2=0.917$; $n=72$):

$$\gamma = 53.175 - 6.3\times10^{-4}\, C - 0.3925\, E \qquad (5\text{-}3)$$

where C is the 443 kDa β-glucan concentration (mg/L). For the sheared beer samples, only higher ethanol contents decreased surface tension ($p<0.001$; Figure 5.5). Shearing temperature, pH (3.8-4.6) and the presence of the 443 kDa β-glucan at 600 mg/L did not affect surface tension of the sheared beers ($p>0.05$; Figure 5.5).

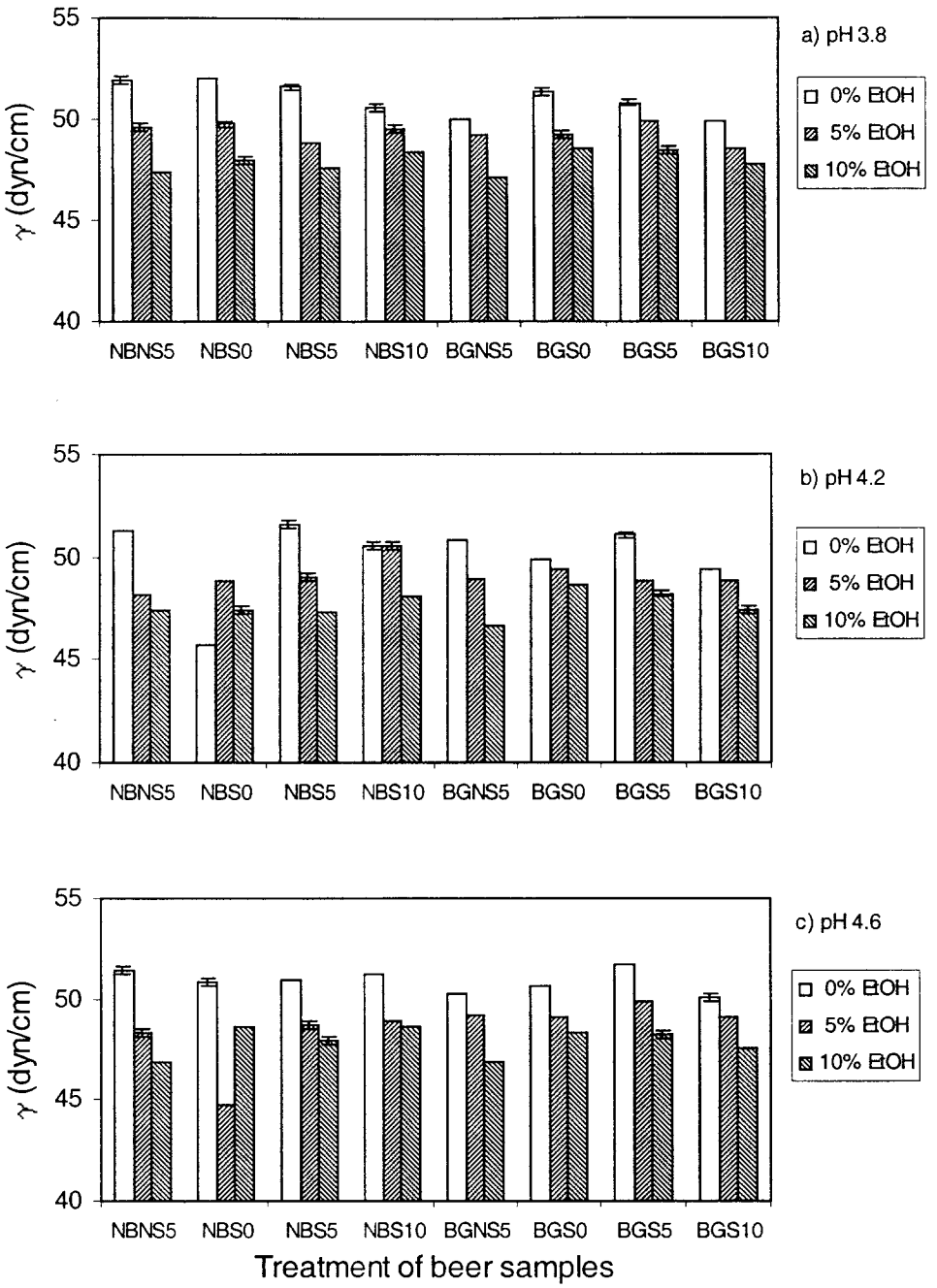

Figure 5.5 Effect of β-glucan (443 kDa at 600 mg/L), pH, ethanol content and shearing temperature on beer surface tension. NB = no β-glucans; NS5 = unsheared (sample was kept at 5°C before measuring surface tension at 20°C); S0 = sheared at 0°C; S5 = sheared at 5°C; S10 = sheared at 10°C; BG = 600 mg/L of 443 kDa β-glucan. Values are given as means ± S.D. of duplicate experiments.

5.3.3 Effect of MW and Concentration of β-Glucans on Beer Foamability and Foam Stability

Beer samples (pH 4.2, containing 3.3% w/w of real extract, 5.0% v/v of ethanol and 31-443 kDa β-glucans at concentrations of 0-1000 mg/L) were also sheared to generate foam at 5°C and to examine the foam stability at 5°C unless otherwise specified. The total volume of foam generated from 10.0 mL beer was defined as foamability, whereas the foam volume remaining after 10 minutes was defined as foam stability. Foamability was affected by both β-glucan MW ($p<0.01$) and concentration ($p<0.001$; Figure 5.6).

Relative to the foamability of β-glucan-free beer, low concentrations of β-glucans (50-200 mg/L) resulted in higher foamability whereas higher β-glucan concentrations (>400 mg/L) decreased foamability ($p<0.001$; Figure 5.6). Higher MWs of β-glucans were detrimental to beer foam capacity ($p<0.01$; Figure 5.6). These results are in agreement with the influence of β-glucans on beer surface tension as discussed above (Figure 5.2). High MW β-glucans at high concentrations led to higher surface tension of sheared beer (Figure 5.2b). A similar trend was also found for the foam stability (Figure 5.7). The presence of β-glucans (31, 137, 250 and 327 kDa) at low concentrations (50-200 mg/L) were found to be beneficial for foam stability ($p<0.001$) compared to that of the β-glucan-free beer. Foam stability was found to decrease at higher concentrations and higher MWs of β-glucans ($p<0.001$). It can be hypothesized that β-glucans increased the viscosity at the bubble walls (which should increase foam stability), but also replaced some of the more surface active foaming proteins at the interface leading to lower foam stability. At low concentrations, β-glucans presumably increased bulk viscosity and retarded drainage (Figure 5.7). As a result, foam stability was enhanced. At higher concentrations, bulk viscosity is higher, therefore greater foam stability is expected. However, it is hypothesized that β-glucans at high concentrations may replace foaming proteins at the interface. Since β-glucans have lower surface activity than other beer

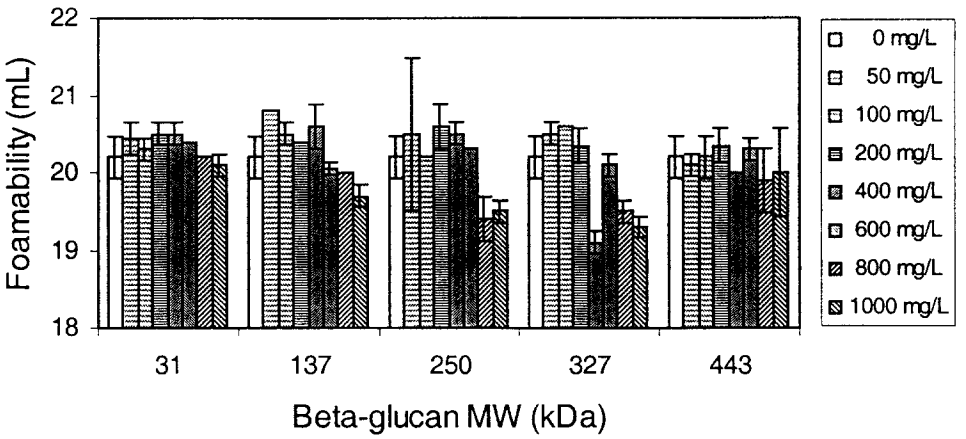

Figure 5.6 Effect of MW and concentration of β-glucans on beer foamability. Values are given as means ± S.D. of duplicate experiments.

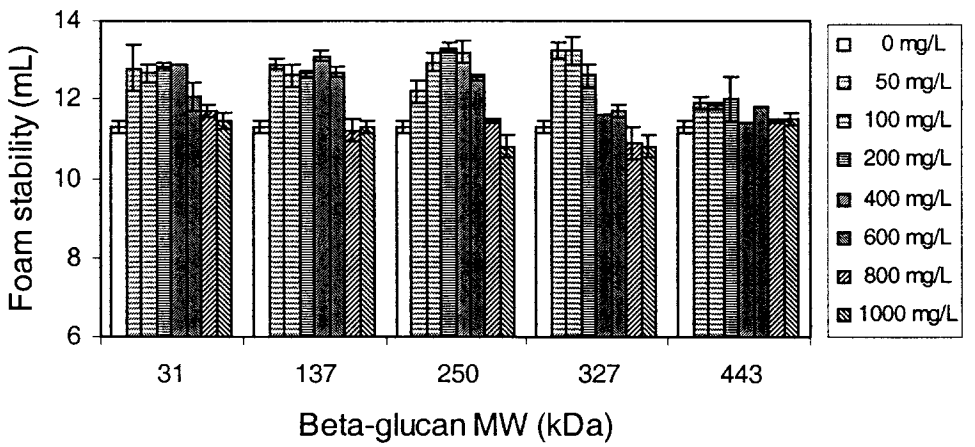

Figure 5.7 Effect of MW and concentration of β-glucans on beer foam stability. Values are given as means ± S.D. of duplicate experiments.

components (Figures 5.2 and 5.3), the presence of β-glucan at the bubble wall interface is hypothesized to enhance the rupturing of the bubbles. The overall trend seen in this study for high concentrations of high MW β-glucans was found to cause a decrease in foam stability.

5.3.4 Effect of β-Glucan (443 kDa at 600 mg/L), pH, Ethanol Content and Shearing Temperature on Beer Foamability and Foam Stability

Beer samples were compared under various conditions to examine the influence of pH, ethanol, 443 kDa β-glucan, and temperature on foamability and foam stability. The addition of 443 kDa β-glucan at 600 mg/L to beer lowered foamability for all samples ($p<0.001$; Figure 5.8). This is in agreement with the results in Figure 5.6 since high concentrations of high MW β-glucans decreased beer foamability. Similarly, wheat arabinoxylans were reported to reduce foamability of proteins as well (Izydorczyk and Biliaderis, 1992a; 1992b). Considering that low levels of low MW β-glucans may enhance foaming (Figure 5.6), it can only be suggested here that high MW β-glucans (443 kDa) at high concentrations (600 mg/L) were detrimental to foaming capacity of beer. Foaming at relatively higher temperatures in a narrow range of 0-10°C resulted in better foamability ($p<0.001$). The enhanced foaming capacity is hypothesized to be due to greater gas pressures in the interior of the bubbles, which resulted in an increase in foam volume although the number of foam bubbles was not examined. According to the gas law, the volume of a bubble would increase by 4% when the temperature is raised from 0°C to 10°C. Foamability of beer was greater at higher pH values in the range of 3.8-4.6 studied ($p<0.001$; Figure 5.8). At a pH near pI of beer proteins (i.e., pH 5, Sørensen and Ottesen, 1978), the lack of repulsive interactions of foam protein molecules favours the protein-protein interactions leading to enhanced foam capacity. Ethanol in beer increased viscosity (Chapter 3) and decreased surface tension (Figure 5.5). Presence of a small amount (1-3% v/v) of ethanol favoured the formation and stability of foam (Archibald *et al.*, 1973; Bamforth, 1985b; Brierley *et al.*, 1996). However, foamability was lower for

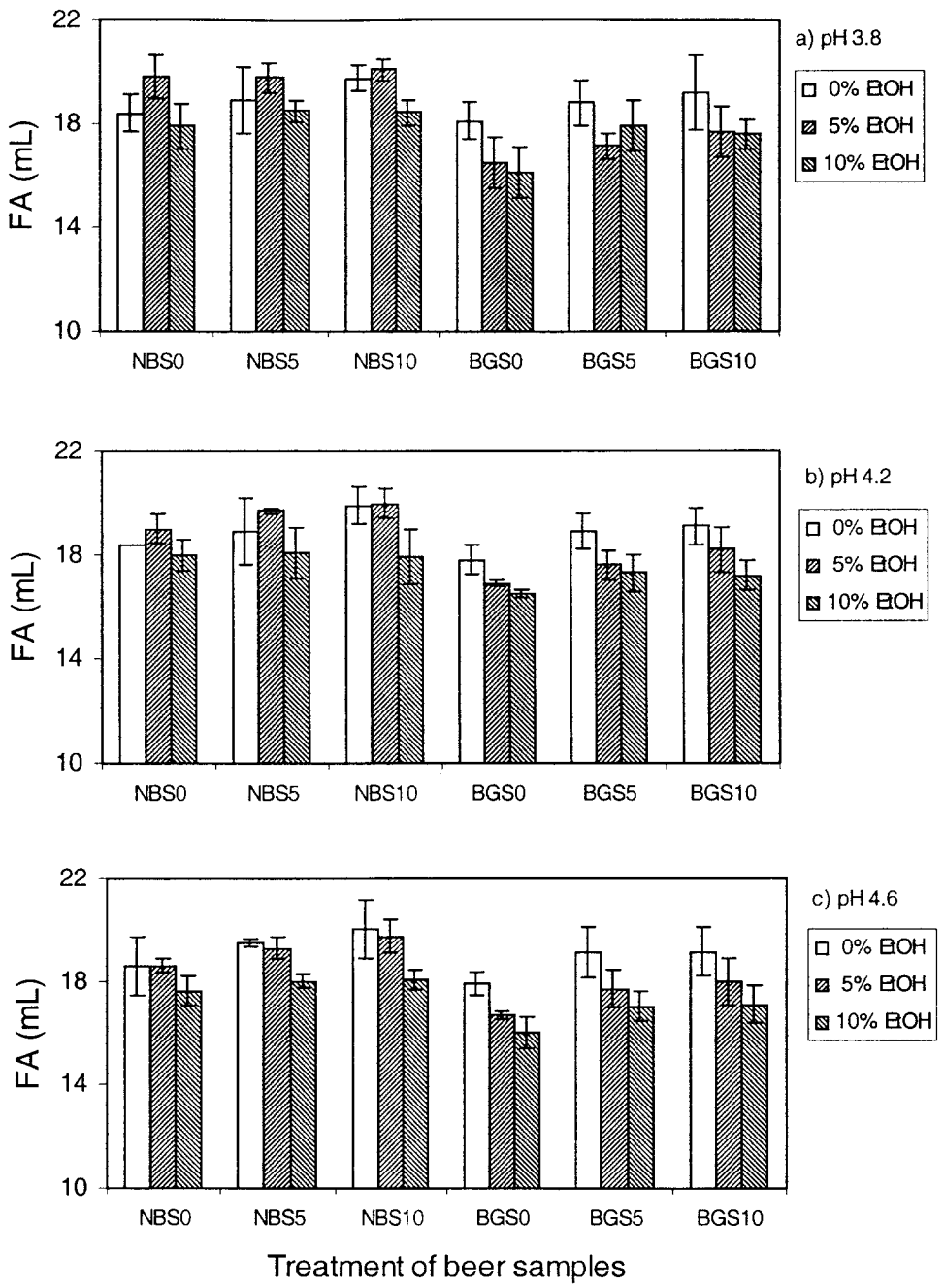

Figure 5.8 Effect of β-glucan, pH, ethanol content and temperature on beer foamability. Sample codes are identical to that given in Figure 5.5. Values are given as means ± S.D. of duplicate experiments.

beer samples containing higher levels of ethanol ($p<0.001$; Figure 5.8). At an increased concentration of >3% v/v, ethanol squeezes in between the foaming protein molecules as a competitive low MW surfactant leading to lower foam head (Bamforth, 2000; Brierley *et al.*, 1996).

Foamability under the conditions discussed above can be described by the following model ($R^2=0.991$; $n=108$; $p<0.001$):

$$FA = 0.1370\ T + 4.4224\ pH - 0.0017\ C - 0.1086\ E \quad (5\text{-}4)$$

where FA is beer foamability (mL) and T is the temperature at which beer foamed.

Foam stability was also studied under various conditions (Figure 5.9). Temperature in the range of 0-10°C did not affect foam stability ($p>0.05$). Foam stability was influenced by ethanol, β-glucan and pH ($R^2=0.981$; $n=108$; $p<0.001$):

$$FS = 3.0515\ pH - 0.3406\ E - 0.00202\ C \quad (5\text{-}5)$$

where FS is beer foam stability (mL). Increasing beer ethanol concentration was harmful to foam stability ($p<0.001$). It is hypothesized that ethanol molecules compete with foaming proteins for the interfacial space and destabilize bubble walls (Bamforth, 2000; Brierley *et al.*, 1996). Ethanol at concentrations of 1-3% v/v have been reported to favour foam formation (Archibald *et al.*, 1973; Brierley *et al.*, 1996). However paradoxically, ethanol (at even low concentrations), has been found to destabilize foam (Brierley *et al.*, 1996).

In the range of pH 3.8-4.6, foam was found to be more stable at higher pH values ($p<0.001$; Figure 5.9). Beer foam is formed and stabilized primarily by proteins. Protein-stabilized foam is most stable near the isoelectric point (pI) of proteins (Halling, 1981). The majority of beer proteins including protein Z have a pI of 5 (Sørensen and Ottesen, 1978). Thus, the foam was more stable at pH 4.6 because it was closer to the pI of beer foaming proteins. However, beer pH close to 4.0 has been reported to provide better foam stability (Furukubo *et al.*, 1993; Melm *et al.*, 1995; Taylor, 1990).

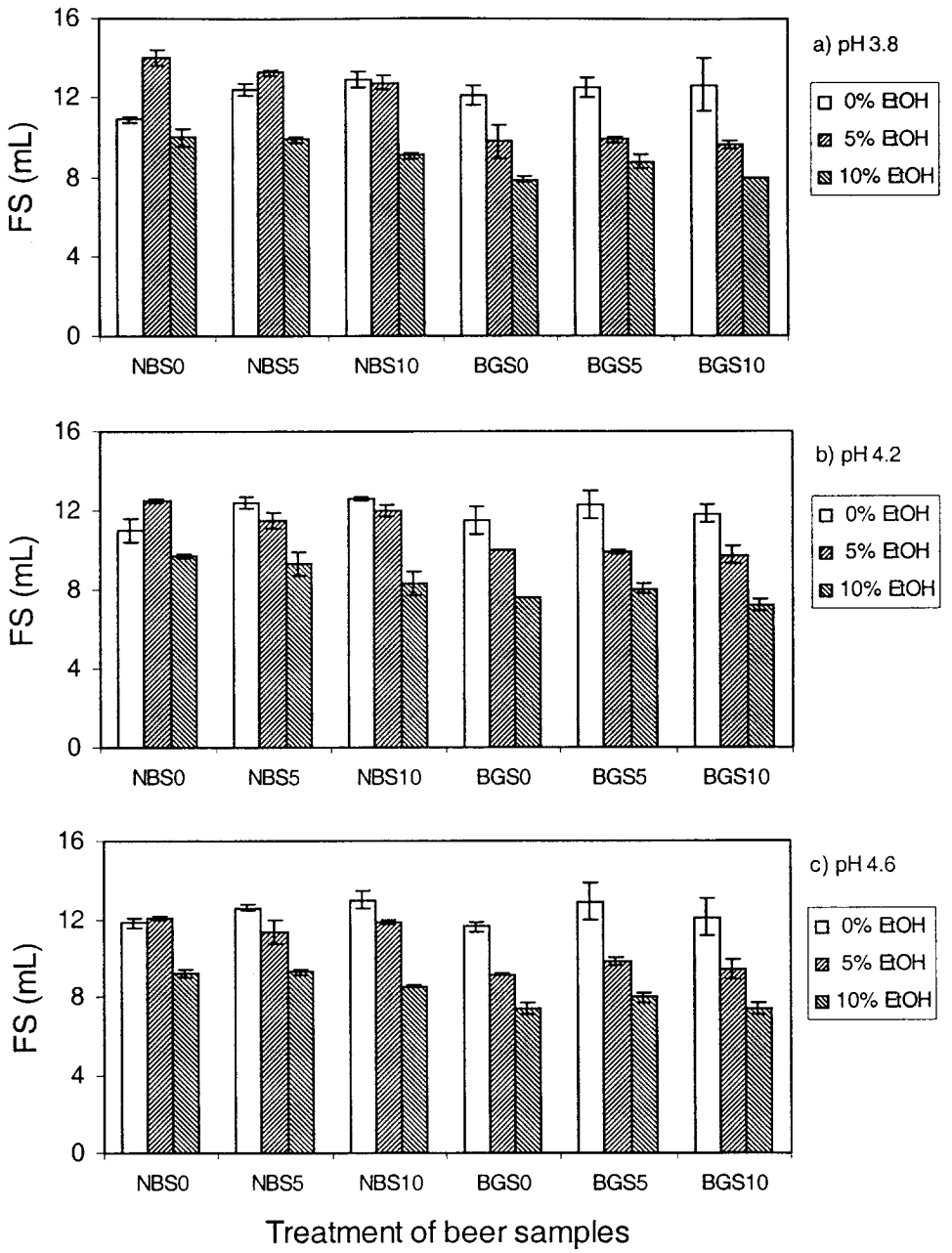

Figure 5.9 Effect of β-glucan, pH, ethanol content and temperature on beer foam stability. Sample codes are same as that in Figure 5.5. Values are given as means ± S.D. of duplicate experiments.

The presence of 443 kDa β-glucan at 600 mg/L decreased foam stability of beer ($p<0.001$). In theory, polysaccharides can retard drainage because they increase bulk viscosity of lamella or liquid (Linden and Lorient, 1999). Palate fullness and foam stability were also thought to be favoured by the presence of β-glucans in beer (Forrest and Wainwright, 1977; Krauss, 1970; Narziβ, 1973). It was also thought that beer products fermented from high β-glucan worts had a mellow mouth feel and a good head retention (Luchsinger, 1967). Bamforth concluded that there is no evidence from early literature to support the benefiting effect of β-glucans to beer foam although polysaccharides are foam-beneficent (Bamforth, 1985b; 1999a). Although malt β-glucan level was reported to be correlated positively with beer foam (Evans *et al.*, 1999; Nischwitz *et al.*, 1999), the methodology has been criticized because other foam-enhancing factors were present as well (Lusk *et al.*, 2001). Beta-glucans in a commercial beer were found to be ≤40-80 kDa in MW and not foam enhancing nor foam stabilizing at 381 mg/L (Lusk *et al.*, 2001). Beta-glucan at a very high concentration (1% w/w) did enhance foam stability of whey proteins (Burkus and Temelli, 2000). However, the concentration of β-glucans in beer is usually much lower (Table 2.1). This study shows that β-glucans at low concentrations (e.g., ≤400 mg/L) favours foaming capacity and foam stability (Figures 5.6 and 5.7). However, β-glucans are not true surfactants and do not interact with the hydrophobic phase of the emulsion/foam systems (Temelli, 1997). Increasing the concentration (e.g., ≥600 mg/L) or MW of β-glucans will lower the foamability and foam stability. This may partly explain why contradictory results have been observed by early researchers.

To elucidate the decreased foamability and foam stability by β-glucans, an experiment was carried out to monitor possible changes of β-glucan concentration in the liquid from collapsed beer foam. Beer samples (pH 4.2, 3.3% w/w of real extract and 5.0% v/v of ethanol) containing 200-1000 mg/L of 443 kDa β-glucan were sheared at 5°C and transferred into a jacketed column. Liquid collapsed from foam was collected over 16 min at intervals of 1-2 minutes. Beta-glucan levels were analyzed by reading absorbance

at 550 nm with Congo red (Figure 5.10). The values of A_{550} at 0 minute were obtained before the foaming test of beer samples. The data at 200 mg/L showed no change of β-glucan concentration in the drainage over time ($p>0.05$). At higher concentrations, β-glucan level was higher in the foam fractions which collapsed early and lower in liquid which collapsed later ($p<0.001$). The following model described the changes of β-glucan level in the fractions of 443 kDa β-glucan ($R^2=0.976$; $n=120$; $p<0.001$):

$$A_{550} = 0.06687 + 7.1\times10^{-4}\, C - 1.62\times10^{-3}\, t \qquad (5\text{-}6)$$

where A_{550} is the absorbance at 550 nm representing β-glucan level; C is β-glucan concentration (mg/L) at time zero; and t is time elapsed (min). This indicated a potential for negative adsorption of β-glucan molecules in beer. Similar results can be found in beer containing low MW β-glucans at 360 mg/L from which defoamed beer (i.e., the remaining 2000 mL of liquid after about 800 mL of foam fractions had been removed from 2840 mL of beer in a foam tower) had 398 mg/L of β-glucans (Lusk *et al.*, 2001). It has been reported that proteins and iso-α-acids in beer can concentrate in beer foam (Dale *et al.*, 1993). While no reports of partitioning of β-glucan molecules in beer foam have been found, in the present study, high MW β-glucans at high levels were found to partition into less stable foam fractions.

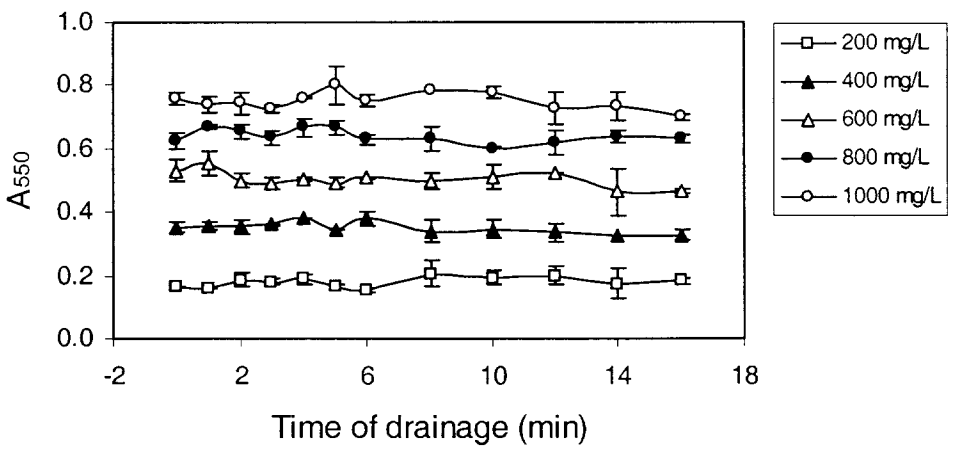

Figure 5.10 Changes in concentration of the 443 kDa β-glucan in drainage collapsed from beer foam. Values are given as means ± S.D. of duplicate experiments.

5.3.5 Correlation between Surface Tension and Foaming Properties of Beer

Results of beer surface tension and foam quality were analyzed by the Pearson correlation test. Mean values of duplicate surface tension measurements were correlated with foamability and foam stability. Beer surface tension correlated negatively with beer foamability ($r=-0.438$; $n=54$; $p<0.01$) as shown in Figure 5.11(a). A lower surface tension promoted beer foaming but did not significantly affect foam stability ($r=-0.09$; $p>0.05$; Figure 5.11b). As could be expected, foam stability correlated well with foaming capacity ($r=0.825$; $n=54$; $p<0.001$; Figure 5.12). Caution must be exercised while interpreting the foaming properties because the foam was generated by agitation and may or may not reproduce the foaming characteristics of beer being poured into a glass. It was also assumed that the data was homoscedastic.

5.4 Conclusions

Beta-glucans having MWs of 31-443 kDa (1000 mg/L) lowered surface tension of their aqueous solutions. The effect of β-glucan MW, concentration, and shearing on surface tension in wort differed from that in beer. Statistical analyses of experimental results are summarized in Table 5.2. It can be concluded that β-glucans at high concentrations lowered wort surface tension ($p<0.05$) regardless of their molecular weights ($p>0.05$). Surface tension of wort decreased at higher maltose concentrations and lower pH values ($p<0.001$). Beer surface tension, however, only decreased at higher ethanol concentration ($p<0.001$). The presence of high MW β-glucans increased beer surface tension ($p<0.01$). Beer pH values and β-glucan concentration showed no significant influence on surface tension ($p>0.05$). Shearing of samples lowered wort surface tension ($p<0.001$) but increased beer surface tension ($p<0.001$). Shearing temperature did not affect surface tension of wort or beer ($p>0.05$).

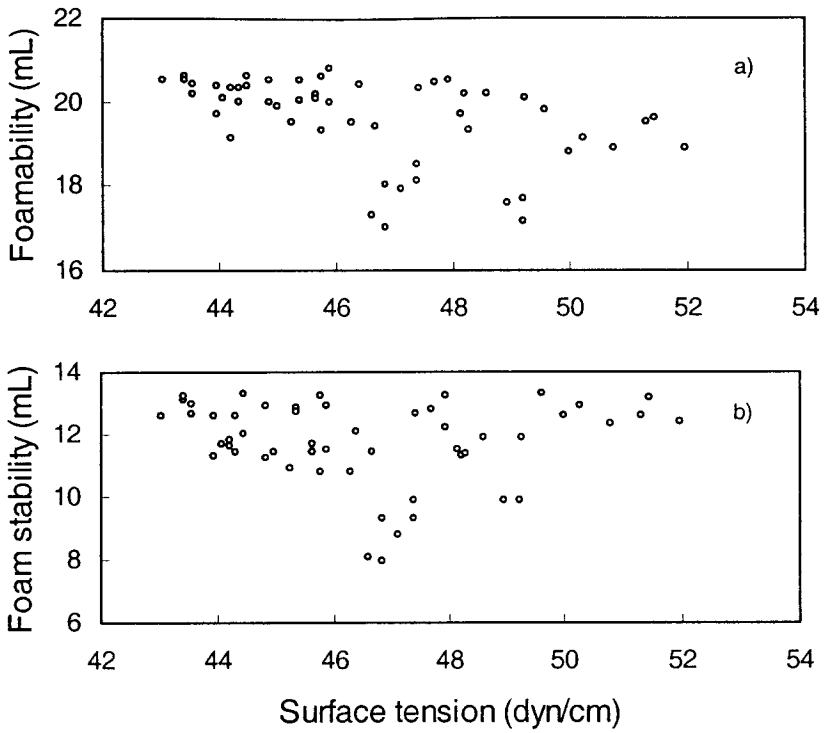

Figure 5.11 Correlation of surface tension with beer foamability and foam stability.

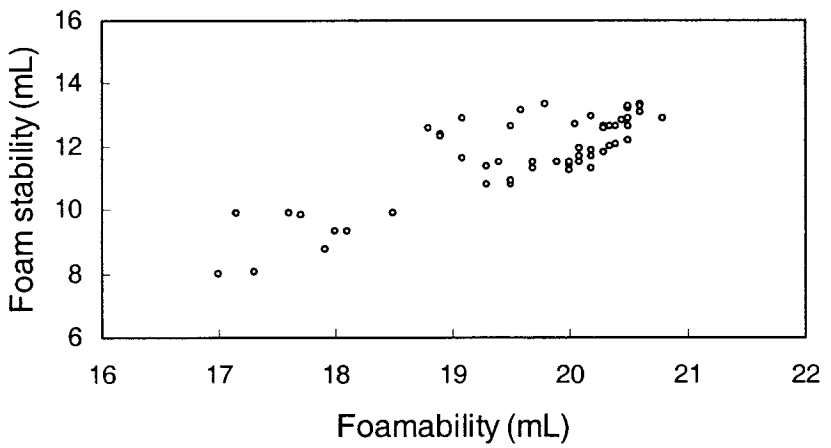

Figure 5.12 Correlation between beer foamability and foam stability.

Foamability of beer was found to be enhanced by elevated temperature (0-10°C) and pH (3.8-4.6) with and without the 443 kDa β-glucan at 600 mg/L ($p<0.001$). The presence of 5-10% v/v of ethanol or high MW β-glucans at high concentrations lowered beer foaming capacity ($p<0.001$). Higher pH values in the range of 3.8-4.6 favoured beer foamability and foam stability ($p<0.001$). Ethanol and β-glucans in beer lowered foamability and foam stability ($p<0.001$) while foaming temperature between 0-10°C had no significant effect ($p>0.05$). By monitoring the concentration of the 443 kDa β-glucan in collapsed foam fractions, negative adsorption was found because β-glucan molecules concentrated in the less stable foam fractions. This implies that beer foam fractions with higher β-glucan levels were less stable. Low values of surface tension were found to be favorable to beer foamability. Better foam stability also corresponded to greater foaming capacity.

Table 5.2 Summary of statistical analyses of the experimental results

Sample	Factor	Level	Response to high levels		
			Surface tension	Foam-ability	Foam stability
Wort [a)]	Shearing	Sheared/unsheared	↓***		
	MW of β-glucan	31-443 kDa	NS		
	β-Glucan level	50-1000 mg/L	↓*		
Wort	β-Glucan (443 kDa)	0, 600 mg/L	↓***		
	Shearing T	20, 48, 76°C	NS		
	pH	4.0, 5.4, 6.8	↑***		
	Maltose	6.1, 10.1, 16.1% w/w	↓***		
Beer [b)]	Shearing	Sheared/unsheared	↑***		
	MW of β-glucan	31-443 kDa	↑**	↓**	↓***
	β-Glucan level	50-1000 mg/L	↓↑ NS	↓***	↓***
Beer	β-Glucan (443 kDa)	0, 600 mg/L	↓*	↓**	↓***
	Shearing T	0, 5, 10°C	NS	↑***	NS
	pH	3.8, 4.2, 4.6	NS	↑***	↑***
	Ethanol	0, 5, 10% v/v	↓***	↓**	↓***

NB: [a)]: Wort (12°P) at pH 5.4; [b)]: Beer at pH 4.2 containing 3.3% w/w of real extract and 5.0% v/v of ethanol; [c)]: The symbol ↑ represents an increased response to a higher level of the treatment; ↓ indicates a lower response to a higher treatment level; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; NS = not significant ($p>0.05$).

6. EFFECT OF β-GLUCANS, SHEARING AND ENVIRONMENTAL FACTORS ON THE TURBIDITY OF WORT AND BEER

The intensity of scattered light at 90° to the incident beam was used to determine the turbidity of both wort and beer samples. Beta-glucan induced turbidity in wort and beer were investigated at various MWs and concentrations. The influences of wort and beer pH, maltose and ethanol levels, shearing and shearing temperature on turbidity were also examined. As well, changes in turbidity levels of wort and beer after a 0.45 μm membrane filtration were compared. Finally, beer samples stored at 4°C were also examined to determine if β-glucan induced turbidity could be lowered during the maturation process.

6.1 Introduction

The first positive assessment of beer quality is of the creamy foam above a "brilliant" beer. The clarity of beer is usually measured by light scattering of beer at a certain wavelength. The majority of the instruments for haze determination are designed to measure turbidity with light scattered at 90° to the incident light at 450-860 nm (Mundy and Boley, 1999). Although a few polymers are available to calibrate the turbidity, formazin has been used by the ASBC and the EBC (ASBC, 1992; EBC, 1987). However, two brewing organizations have defined the formazin turbidity by different scales. One EBC Formazin Haze Unit (FHU) is equivalent to 69 ASBC Formazin Turbidity Units (FTU). The ASBC turbidity scale was used in this study. The clarity of beer is categorized into five levels at different FTU ranges: brilliant (<35 FTU), almost brilliant (35-69 FTU), slightly hazy (69-138 FTU), hazy (138-276 FTU) and very hazy (>276 FTU) (EBC, 1987; Hough *et al.*, 1982a). A visual haze threshold has been recently found to be 0.38-0.82 nephelos turbidity units (equivalent to 6.6-14.1 FTU) determined by 90° scattering of white light (Siebert, 2000). Turbidity of beer can be caused by microbial cells (i.e., biological haze) or colloidal particles and mineral crystals. The biological

stability of beer can be achieved by pasteurization or sterile filtration. In order to achieve colloidal stability, non-biological hazes (and their precursors) must be removed before packaging to prevent haze formation after packaging of beer. The removal of haze precursors (i.e., haze-active proteins and haze-active polyphenols) is one of the techniques preventing the development of protein-polyphenol hazes in packaged beer.

Hazes present at both warm and cold beers are referred to as permanent hazes whereas those that only appear in beer at refrigerated temperatures are termed chill hazes. The colloidal haze particles are primarily developed from polymerization of proteins and polyphenols which have been recently reviewed by Bamforth and Siebert (Bamforth, 1999b; Siebert, 1999; Siebert and Lynn, 2001). Malt-derived hazes usually contain high levels of β-glucans in addition to proteins, polyphenols and pentosans (Coote and Kirsop, 1976; Gjertsen, 1966; Igarashi and Amaha, 1969; Jackson and Bamforth, 1983; Letters, 1977; Moll, 1987; Skinner *et al.*, 1993; Takayanagi *et al.*, 1969; Whitear *et al.*, 1983). Gelatinous particles of barley β-glucans dispersed in beer can also instantly clog the beer filters (Leedham *et al.*, 1975). Under certain circumstances such as high pressure or elevated filtration temperature, these polymers can force their way through the filter and aggregate slowly in the packaged beer leading to a particulate haze (Gjertsen, 1966; Whitear *et al.*, 1983). Since the 1960s it has been recognized that a low β-glucan level is necessary to avoid the risk of precipitates in packaged beer (Erdal and Gjertsen, 1967).

Even though β-glucans may not always form gelatinous precipitates in beer, they can still cause haze problems. Particles smaller than 0.1 μm scatter more light at 90° to the incident and result in pseudo- or invisible hazes (Bamforth, 1985a; 1999b; Jackson and Bamforth, 1983; Letters, 1995a; Vårum and Smidsrød, 1988). When added to beer, β-glucans having MWs of 31-443 kDa are primarily 0.01-0.1 μm in apparent diameter (Figure 4.3). The purpose of this study was to investigate how β-glucan MWs and concentrations affect the turbidity of wort and beer. The effects of shearing, shearing

temperature, pH, maltose content of the wort and ethanol content of beer on turbidity were also examined.

6.2 Materials and Methods

Details of the materials used in this study are described in section 3.2.1. Beta-glucan-free wort was prepared from pale malt as described in section 3.2.2. Barley β-glucans purchased from Megazyme International Ireland Ltd. (Bray, IRL) with molecular weights of 31-443 kDa and were used to prepare wort samples with β-glucan concentrations in the range of 0-1000 mg/L (section 3.2.3). A commercial lager beer (Labatt Blue, 5% v/v of alcohol, product code E10H11C, UBC 062067351013, Oland Breweries Ltd., Halifax, NS) was used to prepare a beer base free of β-glucans (section 3.2.4). This β-glucan-free beer base was used to prepare beer samples at various β-glucan concentrations (0-1000 mg/L), pHs (3.8-4.6), and ethanol concentrations (0-10% v/v) as described in section 3.2.5 (Figure 3.2). Shearing of wort and beer was carried out with the procedure described in section 3.2.6. Beta-glucan concentration of samples was determined with the Congo red binding assay described in section 3.2.7.

Turbidity of wort and beer was determined by 90° light scattering of the samples at 580 nm. This technique was based on and modified from the official ASBC method (1992). Three milliliters of sample was placed in a 10×10 mm four-sided clear styrene cuvette (Fisher Scientific Co. Ltd., Nepean, ON). The intensity of the 90° scattered light was then measured with an LS 50 spectrophotofluorimeter (Perkin-Elmer Ltd., Beaconsfield, Buckinghamshire, GBR) at 580 nm. The reading of scattered light at a 90° angle (I_{580}, arbitrary unit) was then calibrated with formazin turbidity standards and expressed as formazin turbidity units (FTU; ASBC, 1992). Double distilled and de-ionized water (DDW) was filtered through a 0.45 μm "Acetateplus" plain membrane (Cat. No. A04sp02500, Batch No. 088439; Osmonics Inc., Minneapolis, MN) before being used to prepare the formazin haze standards. A mixture of 10.0 mL of 1% hydrazine sulfate

(Sigma Chemical Co., St. Louis, MO) and 10.0 mL of 10% hexamethylenetetramine (Sigma Chemical Co.) was stirred for 24 hours at room temperature to form stable haze particles. This colloidal dispersion had a haze level of 69,000 FTU and was diluted to 6,900 FTUs. One mL of the 6,900 FTU suspension was then diluted to 50.0 mL with DDW to make a 138 FTU standard (equivalent to 2 EBC haze units). Standards of 0, 13.8, 27.6, 41.4, 55.2, 69.0, 82.8, 96.6, 110.4, 124.2 and 138.0 FTU were further prepared to obtain a calibration curve of the intensity of the scattered light at 580 nm (I_{580}) versus FTU ($r^2 \geq 0.99$). An example of the calibrating light scattering with formazin standards in the range of 0-138 FTU is shown in Figure 6.1.

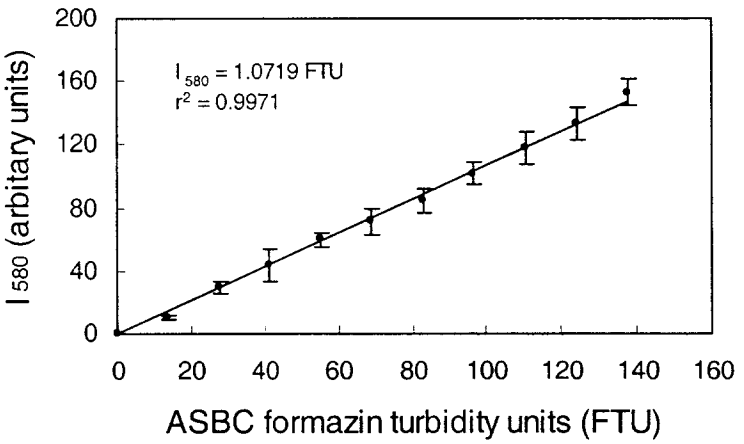

Figure 6.1 Intensity of 90° scattered light at 580 nm of the ASBC Formazin Turbidity standards. Values are given as means ± one standard deviation (S.D.) of duplicate experiments.

The experimental design of this study is shown in Table 6.1. Duplicate experiments were carried out and mean values and standard deviations were reported. Linear regressions and analysis of variance (ANOVA) of the data were executed with SYSTAT version 5.05 (SPSS Inc., Chicago, IL).

Table 6.1 Experimental design for the studies on the turbidity of wort and beer

Sample	Factor	Level
Wort [a)]	Shearing	Sheared / unsheared
	MW of β-glucan	31-443 kDa
	Concentration of β-glucan	50-1000 mg/L
Wort	β-Glucan (443 kDa)	0, 600 mg/L
	Shearing temperature	20, 48, 76°C
	pH	4.0, 5.4, 6.8
	Maltose	6.1, 10.1, 16.1% w/w
Beer [b)]	Shearing	Sheared / unsheared
	MW of β-glucan	31-443 kDa
	Concentration of β-glucan	50-1000 mg/L
Beer	β-Glucan (443 kDa)	0, 600 mg/L
	Shearing temperature	0, 5, 10°C
	pH	3.8, 4.2, 4.6
	Ethanol	0, 5, 10% v/v

[a)]: Wort (12°P) at pH 5.4; [b)]: Beer at pH 4.2 containing 3.3% w/w of real extract and 5.0% v/v of ethanol.

6.3 Results and Discussion

Effects of β-glucans MWs and concentrations, shearing, pH, maltose and ethanol contents on the turbidity of wort and beer are investigated. Changes of beer turbidity after 0.45 μm membrane filtration and "lagering" at 4°C were also examined.

6.3.1. Effect of Shearing, MW and Concentration of β-Glucans on Wort Turbidity

The β-glucan-free wort (12°P, pH 5.4) initially had a very low turbidity level at 22.2 FTU (Figure 6.2a), indicating brilliant clarity. The addition of β-glucans increased wort turbidity at higher MWs and concentrations ($p<0.001$). The increase in turbidity was

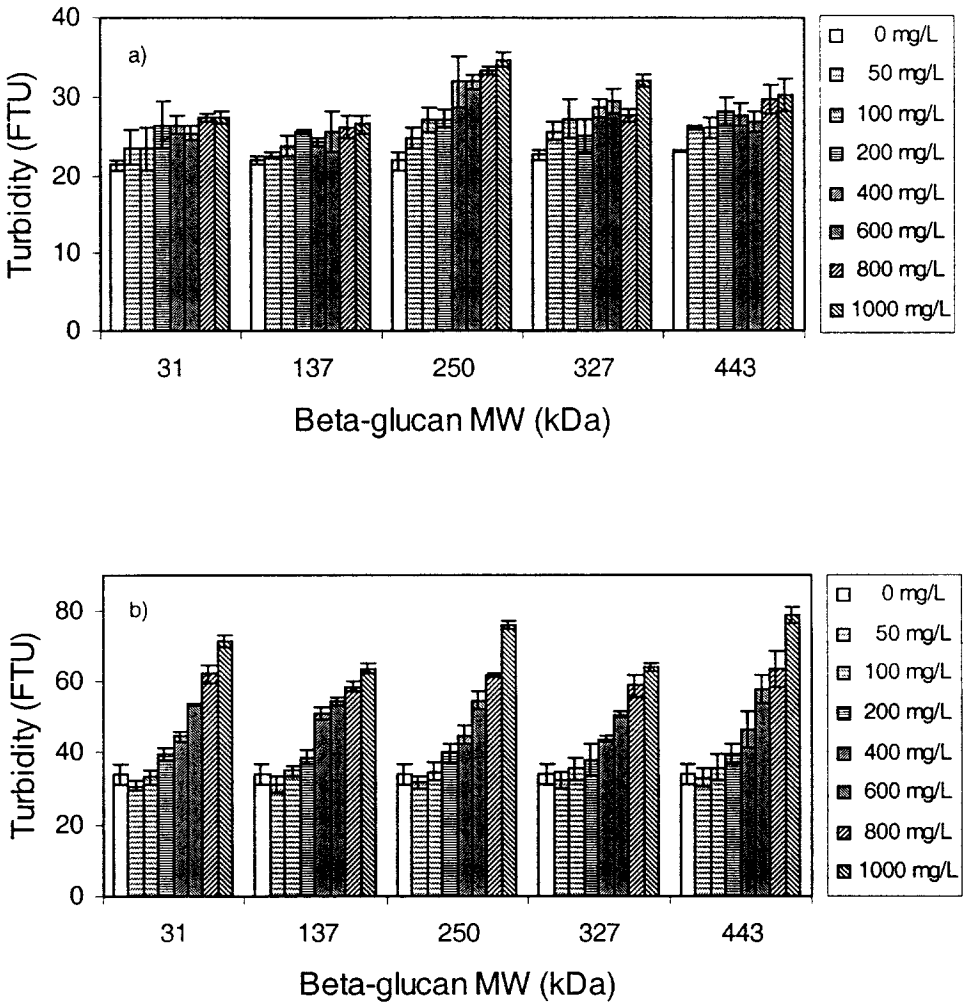

Figure 6.2 Effect of MW and concentration of β-glucans on the turbidity of (a) unsheared and (b) sheared wort at 20°C. Values are given as means ± S.D. of duplicate experiments.

linearly proportional to the increase in MW and concentration (R^2=0.851; n=80; p<0.001). Beta-glucans at higher MWs and concentrations have larger apparent sizes (Figure 4.1; Grimm and Krüger, 1994), which scatter more light at 90°. However, wort samples were all brilliant since the turbidity values were lower than 35 FTU (Figure 6.2a). After being sheared at 20°C, wort samples exhibited higher turbidity values (p<0.001; Figure 6.2b). The β-glucan-free wort had a turbidity of 33.8 FTU after shearing in contrast to 22.2 FTU for the unsheared sample. The turbidity of wort was found to increase with MW and concentration of β-glucans (p<0.001). The wort turbidity level can be described by the following model (R^2=0.954; n=160; p<0.001):

$$FTU = 23.46 + 3.02\times10^{-3}\,MW + 4.79\times10^{-3}\,C + 7.251\,S + 0.0318\,S\times C + 6.236\times10^{-6}\,MW\times C \qquad (6\text{-}1)$$

where FTU is the Formazin Turbidity Unit; MW is the molecular weight (kDa) of β-glucans; C is the concentration of β-glucans (mg/L); and S is shearing (S=0 for unsheared wort and S=1 for sheared wort). The increased turbidity by shearing can be explained by the greater particle size of β-glucans after shearing (Figure 4.2). Turbidity of β-glucan suspensions in buffer was also enhanced after shearing (Figure A.1 in Appendix 1; Patelakis, 1999; Patelakis *et al.*, 1999).

6.3.2 Effect of β-Glucan (443 kDa at 600 mg/L), Maltose Level, pH and Shearing Temperature on Wort Turbidity

Control (unsheared) wort samples containing 0 and 600 mg/L of a 443 kDa β-glucan at pH 4.0-6.8 and 6.1-16.1% w/w of maltose as well as sheared samples at 20, 48 and 76°C were measured for their turbidity at 20°C. The presence of 600 mg/L of 443 kDa β-glucan increased wort turbidity (p<0.001; Figure 6.3). This supports the results of the previous section (Figure 6.2). Turbidity values decreased at higher maltose levels and pH values (p<0.001). Higher maltose concentrations may have inhibited the interchain association of the β-glucan and protein-polyphenol molecules. In the literature, sucrose at 200 mg/L caused a decrease of turbidity formed by protein and polyphenol at pH 4.2

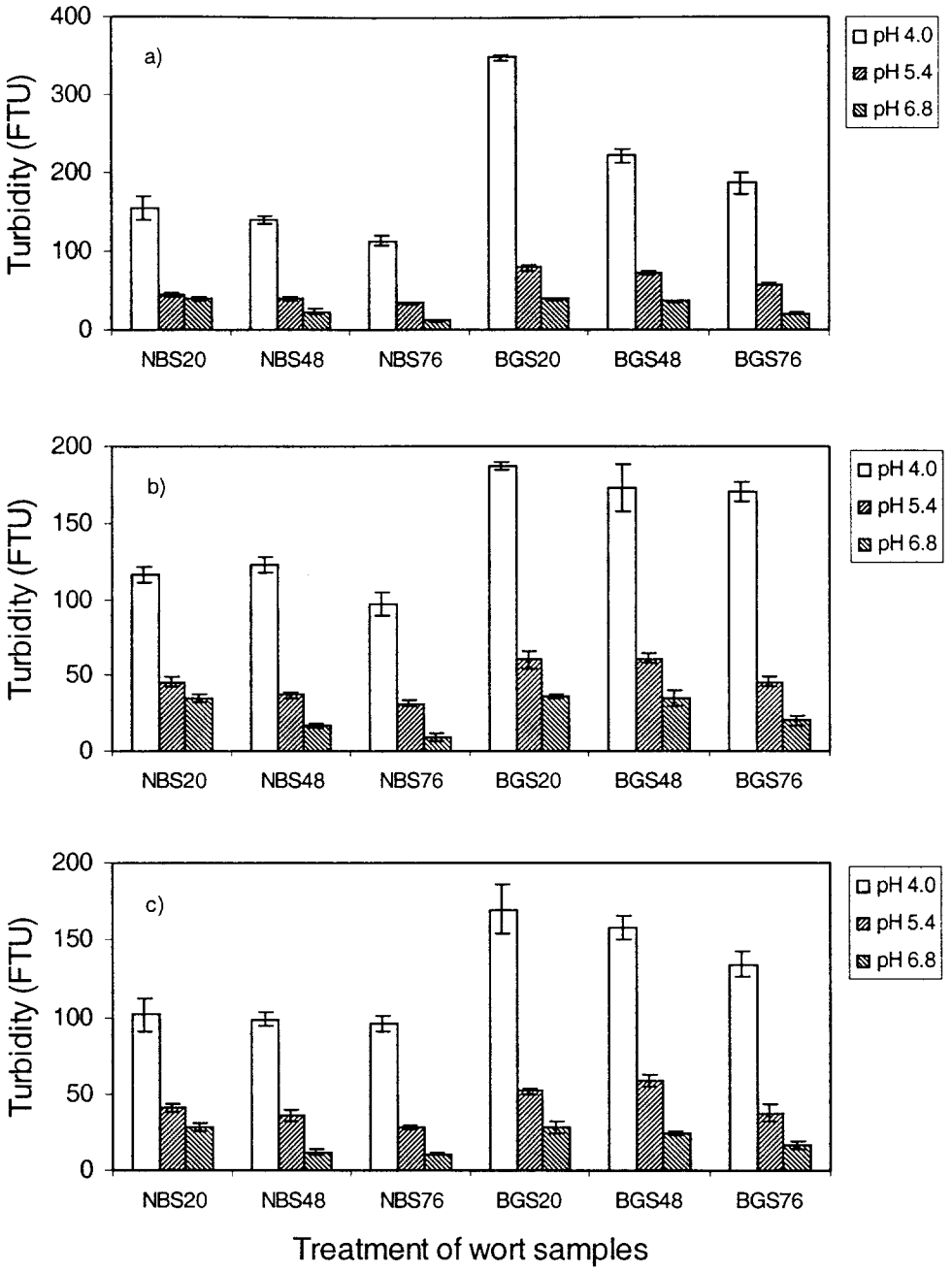

Figure 6.3 Effect of β-glucan (443 kDa at 600 mg/L), pH and shearing temperature on the turbidity of wort (20°C) at maltose levels of (a) 6.1%; (b) 10.1% and (c) 16.1% w/w. NB = no β-glucan; S20 = sheared at 20°C; S48 = sheared at 48°C; S76 = sheared at 76°C; BG = β-glucan (443 kDa at 600 mg/L). Values are given as means ± S.D. of duplicate experiments.

(Siebert *et al.*, 1996). It was also found that maltose at high concentration lowered the fraction of >0.01 μm β-glucan particles after shearing at 48°C and 76°C (Table 4.2). The observed higher turbidity at lower pH ($p<0.001$) was hypothesized to be caused by proteins. The highest turbidity level produced by protein and polyphenol was achieved at pH 4.1-4.2 in model system (Siebert *et al.*, 1996). One could agree that the effect of pH on wort turbidity was related to the electrostatic properties of proteins in wort and beer. Although the isoelectric point (pI) of beer proteins varied from 4-10, the majority of them have pI values around 5 (Sørensen and Ottesen, 1978). Most of the proteins in wort samples would have more net positive charges at pH 4.0 than at pH 5.4 (net negative charges), and more negative charges at pH 6.8 than at pH 5.4. Shearing of wort may have possibly enhanced the unfolding of protein molecules and exposure of the charged groups. At pH 4 (lower than pI), net positive charge of proteins increases the intermolecular electrostatic repulsion. At pH 5.4 which is close to pI of beer proteins, protein molecules possess less net charges and thus have less repulsive forces. At wort pHs above pI (e.g., 6.8), proteins are more negatively charged. Notably, the β-glucan-free wort had an increased turbidity after shearing, suggesting a protein contribution to haze.

Shearing temperature affected the turbidity of wort measured at 20°C ($p<0.001$). Shearing wort at higher temperatures decreased the turbidity of worts at all pHs and maltose concentrations (Figure 6.3). This was partly because shearing at higher temperature resulted in smaller apparent particle size of the 443 kDa β-glucan (Figure 4.5 and Table 4.2). A linear model was found to describe the changes in turbidity of sheared worts ($R^2=0.772$; $n=108$; $p<0.001$):

$$\begin{aligned} FTU = {} & 419.5 + 0.292\,C - 13.291\,Mal + 0.0453\,T_s{\times}Mal + 0.156\,T_s{\times}pH \\ & + 1.781\,Mal{\times}pH - 1.764\,S{\times}T_s - 56.830\,pH - 3.460{\times}10^{-3}\,C{\times}Mal \end{aligned} \qquad (6\text{-}2)$$

where T_s is the shearing temperature (°C). Wort turbidity decreased after shearing at higher temperatures ($p<0.001$). When sheared at a low temperature (20°C), the interchain molecular interactions may have been promoted leading to higher turbidity values. This was evidenced by the lower reactivity of the β-glucan polymers with the Congo red probe

when the samples were sheared at low temperature (20°C). When sheared at high temperatures such as 76°C (which exposed more Congo red reactive groups), the β-glucan molecules may be forced into extended conformation because of the breakup of hydrogen bonding at high temperature, resulting in lower turbidity (Figure 6.3).

It is noteworthy to mention that wort samples at pH 6.8 were all brilliant in clarity (Figure 6.3) despite the effects of the physical conditions discussed above. Worts at pH 5.4 (a "normal" value for production wort), were brilliant whereas wort containing 600 mg/L of 443 kDa β-glucan became slightly hazy after being sheared at 20°C. Wort samples at pH 4.0 had much higher turbidity than at pH 5.4 and 6.8. At pH 4.0, the β-glucan-free worts were slightly hazy while worts containing 600 mg/L of 443 kDa β-glucan became hazy with turbidity as high as 350 FTU (Figure 6.3). The hazes formed in the β-glucan-free worts were probably mainly proteins whereas the hazes in worts containing 443 kDa β-glucan were derived from both proteins and β-glucans.

6.3.3 Effect of Shearing, MW and Concentration of β-Glucans on Beer Turbidity

Similar to the turbidity of wort, the β-glucan-free beer was brilliant (20 FTU). The addition of 31-443 kDa β-glucans increased beer turbidity ($p<0.001$; Figure 6.4a). Turbidity significantly increased with both MW and concentration of β-glucans ($p<0.001$). The turbidity level of beer was linearly proportional to both β-glucan MW and concentration ($r^2=0.890$; $n=80$). Although β-glucans in beer resulted in higher turbidity, the samples were still in a satisfactory range of clarity (brilliant and almost brilliant as practiced by the brewing industry). After sheared at 5°C, turbidity increased significantly ($p<0.001$). Turbidity of the β-glucan-free beer was increased from 20 to 59 FTU after shearing. Beer samples containing β-glucans became slightly hazy after shearing. Macromolecules such as proteins and β-glucans in beer were presumably able to "unfold" during shearing and then capable of aggregating into bigger particles. Shearing also

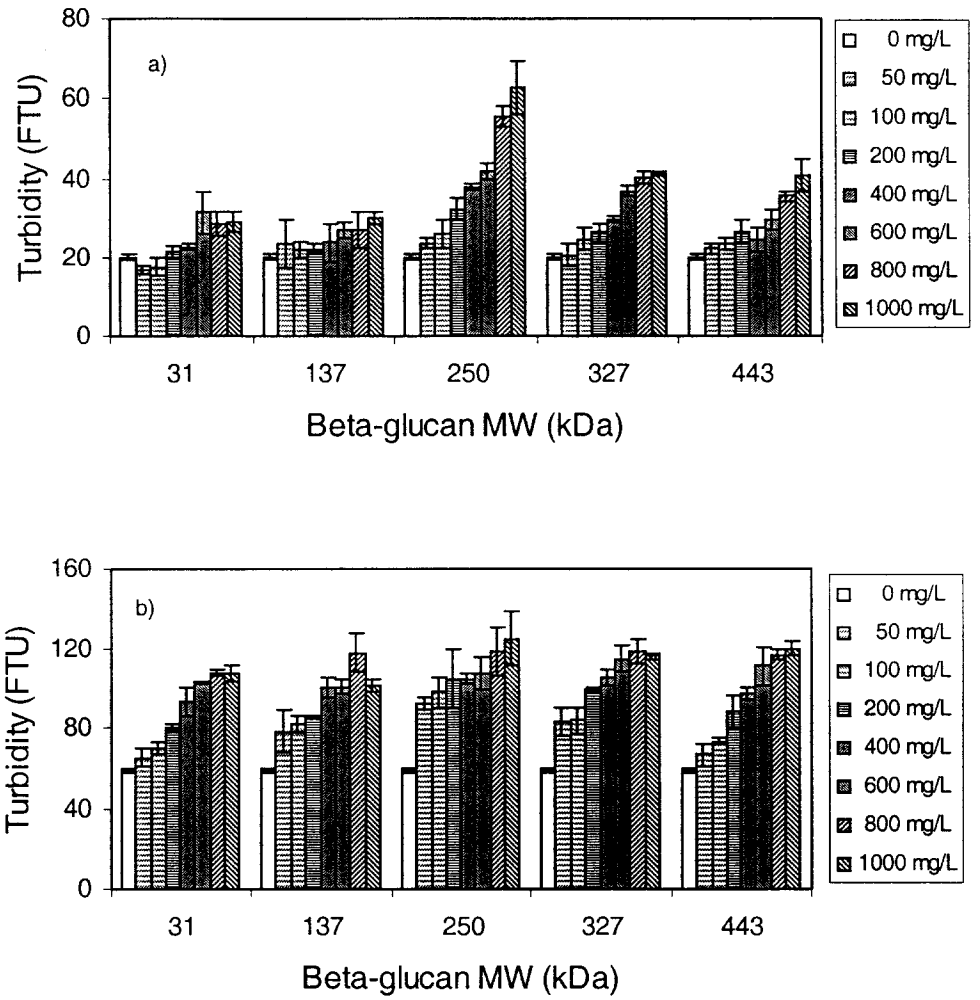

Figure 6.4 Effect of MW and concentration of β-glucans on the turbidity of beer (a) unsheared and (b) sheared at 5°C. Values are given as means ± S.D. of duplicate experiments.

increased the apparent particle size of the 443 kDa β-glucan molecules in beer (Figure 4.6; section 4.3.4). Turbidity values of the sheared beer samples increased with higher MWs and concentrations of β-glucans ($p<0.001$). The increase of beer turbidity by molecular weight, concentration and shearing can be described by a linear model ($R^2=0.987$; $n=160$; $p<0.001$):

$$FTU = 0.142\ MW + 0.0632\ C + 55.58\ S + 0.0254\ S{\times}C - 2.5{\times}10^{-4}\ MW^2 - 4.0{\times}10^{-5}\ C^2 \quad (6\text{-}3)$$

The coefficient of shearing, i.e., the shearing term for beer samples, was three fold greater than that for wort samples (Eq. 6-1), indicating that shearing had a greater impact on beer turbidity than wort turbidity. Shearing is recommended to be minimized during the brewing process in order to reduce the risk of increased beer turbidity.

6.3.4 Effect of β-Glucan (443 kDa at 600 mg/L), pH, Ethanol Content and Shearing Temperature on Beer Turbidity

The presence of 600 mg/L of 443 kDa β-glucan increased beer turbidity because this colloid had an apparent particle size of 0.01-0.1 μm (Figure 4.6) which is able to effectively scatter light. This result supports the findings presented in Figure 6.4. At higher pH values between 3.8-4.6, beer samples were more turbid ($p<0.001$). In theory, pure β-glucan polymers are neutral in charge and their intermolecular interactions are not affected by pH. However, the commercial β-glucans contained a small amount of protein (0.72-9.35%; Table A.1) although it is unclear how do β-glucans interact with proteins. As the majority of beer proteins have pI values around pH 5 (Sørensen and Ottesen, 1978), higher pHs (i.e., 4.6) led to fewer net charges of proteins and therefore stronger interactions among beer protein molecules. The addition of ethanol at 5% v/v decreased the turbidity ($p<0.001$; Figures 6.5a and b). However, the addition of 10% v/v of ethanol led to higher turbidity levels than 5% v/v ethanol ($p<0.001$; Figure 6.5c). This finding is similar with the effect of ethanol on protein-polyphenol haze formation in model system

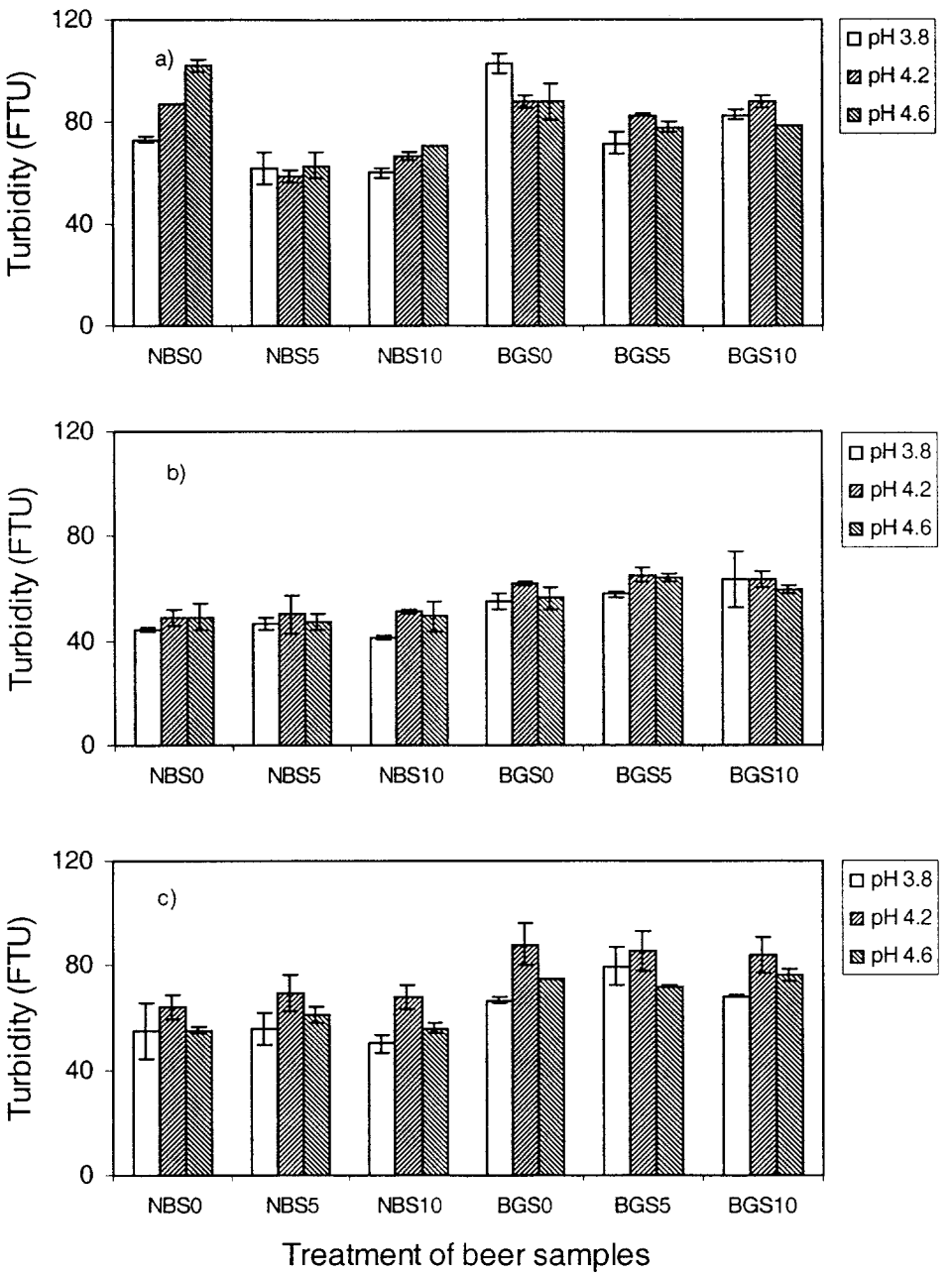

Figure 6.5 Effect of β-glucan (443 kDa at 600 mg/L), pH and shearing temperature on turbidity of beer containing ethanol at (a) 0%; (b) 5.0 % and (c) 10.0% v/v. Note: NB = no β-glucan; S0 = sheared at 0°C; S5 = sheared at 5°C; S10 = sheared at 10°C; BG = 600 mg/L of 443 kDa β-glucan. Values are given as means ± S.D. of duplicate experiments.

by Siebert *et al.* (1996). The decrease of turbidity caused by 5% v/v of ethanol was hypothesized to be due to weaker hydrophobic interactions between protein molecules caused by ethanol (Siebert *et al.*, 1996). The increased turbidity at high ethanol levels, however, was because ethanol can lower the dielectric constant of the medium leading to lower solubility of proteins. Among the sheared beers, turbidity can be described by the pH, ethanol and β-glucan concentration (R^2=0.965; n=108; p<0.001):

$$FTU = 0.0253\ C + 15.088\ pH - 0.868\ E \quad (6\text{-}4)$$

where E is the ethanol content of beer (% v/v). Shearing temperature did not affect beer turbidity (p>0.05) over this narrow temperature range.

6.3.5 Clarifying Beer with 0.45 μm Membrane Filtration

Sheared beer samples (10 mL) were filtered through 0.45 μm membranes in order to examine the decrease in turbidity after membrane filtration (Figure 6.6). The 0.45 μm membrane filtrates had lower turbidity (p<0.001) compared to the sheared beer (Figure 6.4b). The reduction in turbidity was mainly due to the removal of hazes of non-β-glucan components (Figures 6.4b and 6.6). The turbidity of the β-glucan-free beer dropped from 59 FTU to 13 FTU whereas the effects of MW and concentration of β-glucans on turbidity were similar to that of un-filtered beer (Figure 6.4b; Eq. 6-4). The turbidity of sheared beer can be described by the following relationship (R^2=0.921; n=480; p<0.001):

$$FTU = 72.2801 + 0.02193\ MW + 0.03245\ C - 72.42\ MF \quad (6\text{-}5)$$

where MF is 0.45 μm membrane filtration (MF=0 for un-filtered beers and MF=1 for filtered samples). It is assumed that the 0.45 μm membrane filtration did not remove the β-glucan turbidity completely although the hazes of non-β-glucans (presumably proteins) were reduced remarkably after the filtration of 10 mL of beer. It is notable that filtrate of beers containing the 443 kDa β-glucan had lower turbidity than 250 kDa and 327 kDa β-glucan (Figure 6.6) because the 443 kDa had larger particle sizes (Figure 4.4 and Table 4.2). The larger β-glucan particles were easily retained by the 0.45 μm membranes.

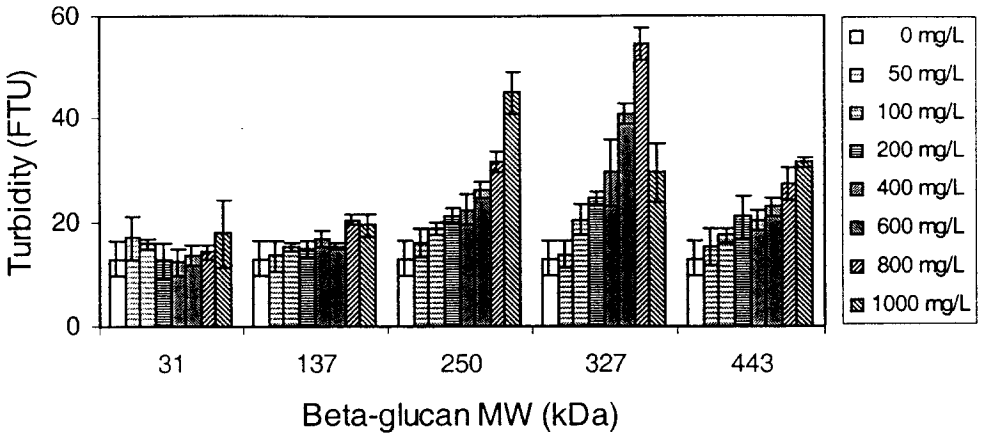

Figure 6.6 Turbidity of sheared beer samples after 0.45 μm membrane filtration. Values are given as means ± S.D. of duplicate experiments.

proteins) were reduced remarkably after the filtration of 10 mL of beer. It is notable that filtrate of beers containing the 443 kDa β-glucan had lower turbidity than 250 kDa and 327 kDa β-glucan (Figure 6.6) because the 443 kDa had larger particle sizes (Figure 4.4 and Table 4.2). The larger β-glucan particles were perhaps more easily retained by the 0.45 μm membranes.

6.3.6 Changes of Beer Clarity during Cold Storage

The sheared beer samples were stored at 4°C for two weeks to simulate the clarification of beer during lagering or maturation process. The turbidity of the β-glucan-free beer decreased from 59 FTU to 24 FTU during storage (Figure 6.7). The turbidity decreased at low β-glucan MWs of 31 and 137 kDa and low concentrations of 50-200 mg/L. However, the turbidity increased for beers containing 250-443 kDa β-glucans at 400-1000 mg/L after cold storage, leading to higher haze levels (Figure 6.7). A multiple linear regression

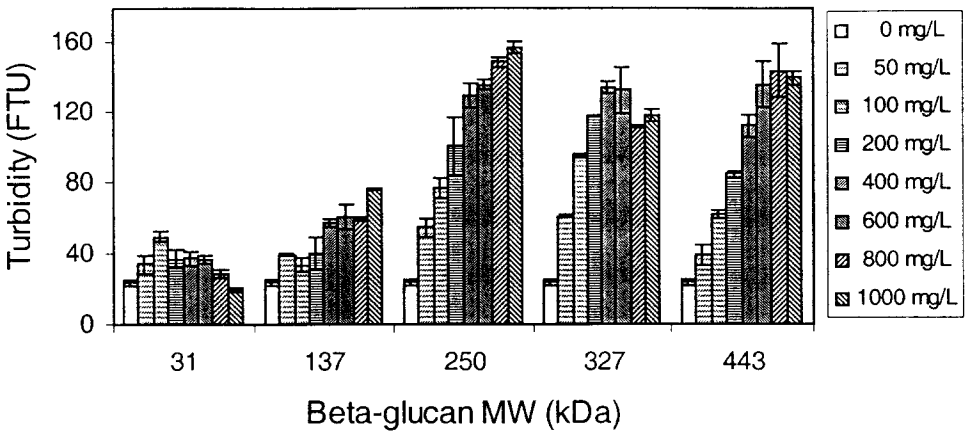

Figure 6.7 Turbidity of sheared beer samples after storage at 4°C for 2 weeks. Values are given as means ± S.D. of duplicate experiments.

indicated that the beer turbidity after cold storage was proportional to the levels of β-glucan MWs and concentrations with quadratic properties (Eq. 6-6). The interactions between lagering and the β-glucan MW and concentration may in part explain the observed changes in beer turbidity after the cold storage (R^2=0.960; n=160; p<0.001):

$$FTU = 0.434\ MW + 0.218\ C + 4.1\times10^{-4}\ L_g\times MW\times C - 6.8\times10^{-4}\ MW^2$$
$$-1.3\times10^{-4}\ C^2 - 0.0611\ L_g\times MW - 0.0955\ L_g\times C - 1.3\times10^{-4}\ MW\times C \quad (6\text{-}6)$$

where L_g represents the simulated lagering (i.e., cold storage). However, the above finding was unexpected because β-glucans after shearing had increased particle size at higher MWs and concentrations (Figure 4.4). Larger particles are expected to sediment faster and more readily than smaller ones, resulting in lower turbidity although their size was in the colloidal range. Beers containing no β-glucan, low MW β-glucans or low concentrations of high MW β-glucans were found to have reduced turbidity after storage. It was hypothesized that precipitation of proteins and/or other polymers caused the partial removal of β-glucans (Figure 6.8). It is also hypothesized that some of the β-glucan particles were adsorbed by or bound to the settling proteins because proteins have been

found to "wrap" or enclose β-glucan haze particles (Jackson and Bamforth, 1983). This binding/adsorption may have removed some of the β-glucans from beer (i.e., it settled to the bottom). Since only a given amount of proteins settled, the removed β-glucan was limited to a certain level. The remaining β-glucans are hypothesized to suspend well in beer because of their small size (0.01-0.1 μm). The increased turbidity of beer at higher MWs and concentrations may be caused by aggregation of the particles because β-glucans tend to precipitate during cold storage (Jackson and Bamforth, 1983). In work not reported here, it was observed that the 31-443 kDa β-glucans at 0.5% w/v suspended in DDW did not precipitate at 4°C within 2 months. After storage at 4°C for 4 months, the 0.5% w/v high MW (137-443 kDa) β-glucans exhibited coagulation but the 31 kDa β-glucan did not.

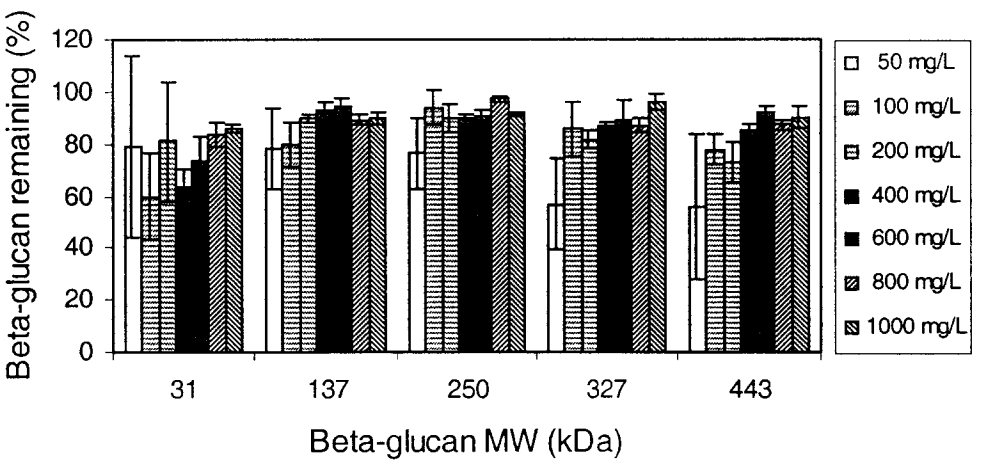

Figure 6.8 The percentage of β-glucans remaining in suspension of the sheared beer after storage at 4°C for 2 weeks. Values are given as means ± S.D. of duplicate experiments.

6.4 Conclusions

The experiments were carried out with clear wort and beer, which had very low turbidity levels (20-22 FTU). The addition of β-glucans increased turbidity of both wort and beer at higher MWs and concentrations ($p<0.001$) although samples still remained brilliant in clarity. Shearing increased turbidity of both wort and beer, especially those containing high MW β-glucans at higher concentrations ($p<0.001$). The increased turbidity is hypothesized to be due to larger particle sizes. Changes in the number of particles need to be further investigated.

The turbidity of wort decreased at higher maltose levels and higher pH values ($p<0.001$). Shearing wort at higher temperature decreased the turbidity of samples ($p<0.001$) compared to lower temperatures. The hazes formed in the β-glucan-free worts were believed to be caused by proteins, whereas the increased hazes in worts containing the 443 kDa β-glucan were believed to be derived from both proteins and β-glucans.

Beer turbidity was increased by β-glucans, shearing, and higher pH values ($p<0.001$), but was not affected by shearing temperature ($p>0.05$). The addition of ethanol decreased beer turbidity ($p<0.001$). After filtration through 0.45 μm membranes, beer samples were clarified due to removal of the non-β-glucan haze particles. However, filtration with the 0.45 μm membranes removed very small amounts of the hazes caused by β-glucans.

After lagering, turbidity of the sheared beers containing low MW β-glucans (31 and 137 kDa) or low concentrations (50-200 mg/L) of β-glucans decreased ($p<0.001$). However, turbidity of beer containing high MW (250, 327 and 443 kDa) β-glucans at concentrations higher than 400 mg/L increased ($p<0.001$). In conclusion, hazes caused by high MW β-glucans at high concentrations can not be thoroughly removed by 0.45 μm membrane filtration or lagering at 4°C for 2 weeks by gravitational sedimentation.

7. EFFECT OF β-GLUCANS, SHEARING AND ENVIRONMENTAL FACTORS ON WORT FILTRATION PERFORMANCE

Clear wort is critical for fermentation and beer clarification. The equipment used for wort separation is primarily a lauter tun, or mash filter, or alternatively a strainmaster. Although designs vary, all such equipment uses the solids in the mash (i.e., spent grains) as a filter bed, with a controlled differential pressure as a driving force for the wort flow. In this study, a model filter bed was used to investigate the effect of β-glucans and other environmental conditions on "lautering" at 76°C. Membrane filtration of wort was also performed at 20°C as an alternative test of wort filterability.

7.1 Introduction

Beta-glucans in barley endosperm cell walls influence the malting and brewing process in several ways (Table 1.1). The cell walls of the starchy endosperm enclose the starch and protein reserves of the grain. Incomplete breakdown of β-glucans in these walls will reduce extract yield in the brewhouse by leaving unconverted extractable starch and proteins in the spent grain after mashing and lautering (Bathgate and Palmer, 1973; Bathgate *et al.*, 1974). Strong negative correlations have been found between wort β-glucan content and hot water fine extract, and between wort β-glucan content and the extent of malt modification (Haselmore *et al.*, 1990; Munck, 1987). Intact endosperm cells in the spent grain of even well-modified malt have been observed (Bathgate and Palmer, 1975; Palmer, 1972). These researchers reported that the native β-glucan network was responsible for the failure of the extraction of the starch and proteins inside intact cells. The coating of β-glucans together with proteins on the surface of starch granules may explain the loss of extract in poorly modified malts.

Insoluble β-glucans decrease extract yield significantly (Bamforth, 1994; Edmunds *et al.*, 1994; Evans *et al.*, 1998) by impeding enzyme access and reducing lautering

performance (Bamforth, 1994). Beta-glucans dissolved in the mash can also retard wort separation because the high viscosity of solutions of β-glucans. Their tendency to precipitate from solution lowers filtration rate (Barrett *et al.*, 1973; Crabb and Bathgate, 1973; Lotz *et al.*, 1997). The purpose of this study was to determine how the MW and concentration of dissolved β-glucans affect the performance of wort filtration. The influences of shearing, wort pH and maltose content on wort filtration were also investigated. A model "lautering" test at 76°C using diatomaceous earth (DE) as "mash bed" and a 0.45 μm membrane filtration test at 20°C were also employed in this study.

7.2 Materials and Methods

Materials and methods which have been discussed in previous chapters are briefly mentioned in section 7.2.1. Methods used for wort DE filtration and membrane filtration tests are described in sections 7.2.2 and 7.2.3, respectively.

7.2.1 General Materials and Methods

Beta-glucan-free wort was prepared from pale malt as described in section 3.2.2. Barley β-glucans (31, 137, 250, 327 and 443 kDa) were purchased from Megazyme International Ireland Ltd. (Bray, IRL). The β-glucans were used to prepare wort samples at β-glucan concentrations of 0-1000 mg/L as described in section 3.2.3. A commercial lager beer (Labatt Blue, product code E10H11C, universal bar code 062067351013, Oland Breweries Ltd., Halifax, NS) was used to prepare a β-glucan-free beer base as described in section 3.2.4. This beer base was used to prepare beer samples at various β-glucan concentrations (0-1000 mg/L), pHs (3.8-4.6), and ethanol contents (0-10% v/v) as described in section 3.2.5. Beta-glucan concentrations were determined with the Congo red binding assay described in section 3.2.7.

7.2.2 Determination of Wort Filtration Performance

A micro-scale wort filtration device was built to conduct a laboratory "lautering" test of wort. Because rather than mash was tested, diatomaceous earth (DE) was added to simulate a filter bed. When the resistance of filter bed and medium (i.e., the filter cloth) is known, any changes in wort filtration rate would be caused by the resistance of wort. The DE filtration results were examined with a linear regression model derived later.

7.2.2.1 Wort Filtration device and operation

To investigate the influence of β-glucan particle size (molecular weights) and concentrations on wort filtration performance of clarified wort, a constant pressure filtration device was developed in this laboratory (Figure 7.1) with a 19/38 Pyrex glass condenser (Fisher Scientific Co. Ltd., Nepean, ON) and employed throughout this study. This device had an inner diameter of 10.4 mm and a filter area of 84.9 mm^2. Diatomaceous earth (product number D3877, Sigma-Aldrich Canada Ltd., Oakville, ON) was sieved through a 200 mesh/in^2 Tyler screen equivalent to a U.S. series No. 200 (W.S. Tyler Company of Canada Ltd., St. Catharines, ON). This fraction of DE powder was washed twice with double distilled and de-ionized water (DDW) and filtered with Whatman No. 1 filter paper under vacuum (760 mm Hg). The wet DE was then dried at 120°C overnight, cooled and stored in an airtight container. This washed fine grain DE powder was used to form a filtration cake in the filtration device. The filtration device was equilibrated at 76°C (the filtration temperature) for at least 15 minutes (Figure 7.1). This device was charged with test wort to prevent an air plug in the flow channel. A total of 0.623 g of DE (capable of forming a 20 mm deep filter bed) was weighed into a 50-mL plastic centrifuge tube and mixed with 25.0 mL of test wort. This tube was equilibrated in a 76°C water bath for 15 minutes. This tube was inverted 5 times, followed by the prompt transfer of the wort-DE mixture into the filtering device. After the stopper (with a constant pressure tube) was placed into position (Figure 7.1), a constant pressure

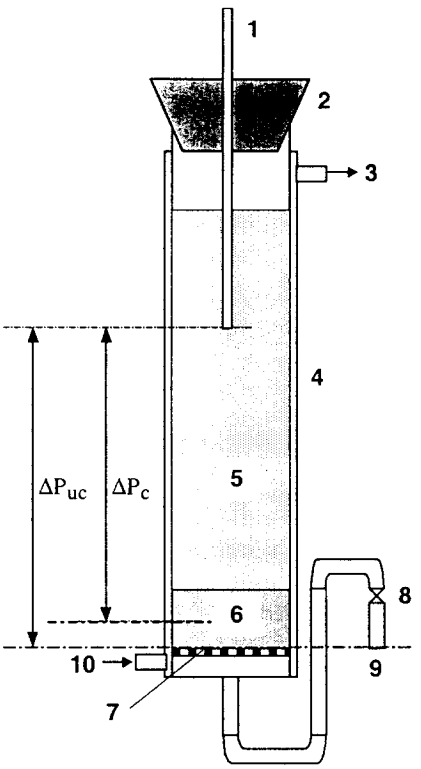

Figure 7.1 Laboratory device used for wort filtration test where 1 - constant pressure tube; 2 - stopper; 3 - outlet of circulating water; 4 - jacket in connection with a water bath at 76°C; 5 - wort sample; 6 - filter bed of DE on a supported layer of nylon cloth (7); 8 - cock; 9 - outlet of run off wort and 10 - inlet of circulating water. ΔP_{uc} is the uncorrected pressure drop across the filter bed and ΔP_c is the corrected pressure drop through the filter bed.

differential was established and filtration was started immediately. This filtration was conducted under a constant pressure of 150 mm H_2O, equivalent to 1470 Pa. Wort was collected in a 10-mL graduated cylinder and the filtrate volume was recorded over time. The test was stopped after approximately 10 mL of wort had been collected. Experiments were carried out in duplicate.

The filter cloth was cleaned with 200 ppm chlorine in 0.5*N* NaOH at 76°C for 10 minutes followed by rinsing with DDW five times. The chlorine wash solution was prepared by adding 4.0 mL of commercial bleach (containing 5.25 % sodium hypochlorite) to 1 L of 0.5 *N* NaOH (Cheryan, 1998). This cleaning was carried out before the initial filtration and every ten runs thereafter.

7.2.2.2 Modeling wort filtration data

The process of wort filtration discussed above is essentially a cake filtration where a filter bed forms during the operation. The filtration rate can be described by D'Arcy's law for the flow rate through sand beds (Eq. 7-1) or Poiseuille's law (Eq. 7-2) for circular capillary tubes (D'Arcy, 1856; Poiseuille, 1842):

$$dq/dt = K P / L \quad (7\text{-}1)$$

$$dq/dt = D_c^2 g P / 32 \eta L \quad (7\text{-}2)$$

where dq/dt is the linear flow velocity (m^3/m^2hr); K is a constant dependent on the properties of the filter bed; P is the pressure difference across the filter bed; L is the thickness of the filter cake and is considered to be the length of the capillaries; D_c is the diameter of the capillary tubes; g is the acceleration due to gravity; and η is the viscosity of the filtered liquid (i.e., separated wort). These two equations both describe the filtration rate. It has been suggested that viscosity should be included in D'Arcy's law (Carman, 1938; Purchas, 1971):

$$dq/dt = K_1 P / \eta L \quad (7\text{-}3)$$

where K_1 is termed as the permeability coefficient. This coefficient is dependent on the specific surface (S_s) and the porosity (ε) of the particles which form the filter bed (Carman, 1937; Kozeny, 1927) and is described by:

$$K_1 = g \varepsilon^3 / K' S_s^2 (1-\varepsilon)^2 \quad (7\text{-}4)$$

Therefore,

$$dq/dt = [g \varepsilon^3 / K' S_s^2 (1-\varepsilon)^2] P / \eta L. \quad (7\text{-}5)$$

This is known as the Kozeny-Carman equation where K′ is a constant. These filtration models are all related to viscous flow conditions where the only source of pressure drop is from frictional drag (Purchas, 1971). In practical use of the filtration equations, the specific resistance (r) is used as a characteristic parameter of the filter bed (Carman, 1938):

$$r = 1 / K_1$$

$$r = K' S_s^2 (1-\varepsilon)^2 / g \varepsilon^3. \quad (7\text{-}6)$$

Therefore, Eq. 7-3 becomes:

$$dq/dt = P / r \eta L \quad (7\text{-}7)$$

or $$dv/dt = P A / r \eta L \quad (7\text{-}8)$$

where dv/dt is the volume rate of flow (m^3/hr) and A is the filter area. The specific resistance represents the pressure required to obtain a unit flow rate of wort of unit viscosity through unit cube of the cake (Carman, 1938). The term "rηL" represents the pressure required to cause a unit flow rate of wort with a particular viscosity through the mash bed (i.e., the DE bed in this study). Thus, the applied pressure difference P across the cake determines the filtration rate through the mash bed.

For the particular filtration device, the pressure drop required to overcome the resistance of the filter medium (i.e., filter cloth) also needs to be considered. Hence, the wort filtration rate is expressed as the ratio of the driving force over the resistance:

$$dq/dt = (P + P_m) / (R_c + R_m) \quad (7\text{-}9)$$

where P_m is the pressure difference across the filter cloth; R_c is the resistance of the cake and R_m is the resistance of the filter medium (i.e., cloth). The overall pressure difference ($P + P_m$) in the filtration test can be termed an uncorrected pressure drop ΔP_{uc} (Figure 7.1). Considering the fact that the hydrostatic pressure differs at the top of the filter bed and the bottom of the filter cloth (i.e., the same level as the outlet of wort), an average value was taken as a corrected pressure drop ΔP_c for the filtration calculations. Therefore:

$$dq/dt = \Delta P_c / (R_c + R_m). \quad (7\text{-}10)$$

According to the definition of the specific resistance,

$$R_c = \eta\, r\, L \qquad (7\text{-}11)$$

and $$R_m = \eta\, R_a \qquad (7\text{-}12)$$

where R_a is the resistance per unit filter area for wort of unit viscosity (Carman, 1938). The thickness of the cake formed during filtration is assumed to be proportional to the volume of wort collected:

$$L = w\, v / A \qquad (7\text{-}13)$$

where W is the weight of suspended solids in the suspension and v is the volume of wort collected at time t. Therefore, Eq. 7-10 becomes:

$$dq/dt = \Delta P_c / (\eta\, r\, L + \eta\, R_a) \qquad (7\text{-}14)$$

$$dv/dt = \Delta P_c\, A / (\eta\, r\, L + \eta\, R_a)$$

$$dv/dt = \Delta P_c\, A^2 / \eta\, (r\, w\, v + R_a\, A) \qquad (7\text{-}15)$$

This rate equation governs the cake filtration process. The rate of wort filtration is directly proportional to the pressure applied and the filter area. It is inversely proportional to wort viscosity, the solid content and the specific resistance of the filter bed.

When the DE filtration experiments are carried out under a constant pressure, rearrangement and integration of Eq. 7-15 gives:

$$dv/dt = (\Delta P_c\, A^2 / \eta\, r\, w\, v)\, [1 / (v + R_a\, A / r\, w)]$$

$$(v + R_a\, A / r\, w)\, dv = (\Delta P_c\, A^2 / \eta\, r\, w)\, dt$$

$$t / v = (\eta\, r\, w / 2\, \Delta P_c\, A^2)\, v + \eta\, R_a / \Delta P_c\, A \qquad (7\text{-}16)$$

This equation was described by Carman (1938) and can be used to derive the two filtration constants r and R_a (Brown *et al.*, 1950; Foust *et al.*, 1960; Miller *et al.*, 1973). It has also been used to evaluate beer filtration performance by Oehmichen and Krabbe (1956). An empirical version of this relationship was described as the cake law by Hermans and Bredée (1936):

$$t / v = F_i\, v + C_0 \qquad (7\text{-}17)$$

where F_i is the slope of the filtration curve and is equivalent to $\eta rw/2\Delta P_c A^2$, and C_0 is a constant equivalent to $\eta R_a/\Delta P_c A$. Lacking a better term, the F_i is referred to as "filtration index" to characterize the DE filtration performance of wort samples in this thesis. When

the ratio of DE to wort is given and the pressure difference and filter area are constant, a change in F_i represents the variation in viscosity and specific resistance of the wort samples. Wort filtration results for 12°P worts containing 50 and 600 mg/L of a 443 kDa β-glucan are shown in Figure 7.2. The data was transformed using Eq. 7-17 to derive the filtration index (F_i) and constant C_0 in Figure 7.3.

7.2.3 Membrane Filtration Test of Wort

The membrane filtration test used in this study was based on the method by Patelakis (1999) with the following modifications: (1) "AcetatePlus" (supported, plain) membranes with a nominal pore size of 0.45 μm (Cat. No. A04SP02500, Material No. 1215635, Batch No. 77050; Osmonics Inc., Minneapolis, MN) were used with a 25 mm Swinnex filter holder (Millipore Inc., Bedford, MA); (2) 7 kPa instead of 35 kPa was applied; (3) 10 mL of wort was applied and (4) filtration temperature was controlled at 20°C. The filtration rates of wort samples were found to be constant over the filtration time. For a universal comparison of the filtration rate, the flux or filtration rate (m^3/m^2hr) of each sample was normalized with that of DDW and termed as relative flux (%). The maximum volume of wort which can be filtered through a membrane filter is termed as V_{max} and the initial filtration rate is termed as Q_{init} (Siebert *et al.*, 1984). The V_{max} and Q_{init} values were derived with the method used by Patelakis (1999).

7.2.4 Experimental Design

The experimental design for this study is illustrated in Table 7.1. Duplicate experiments were carried out and mean values and standard deviations were reported. Regressions and analysis of variance (ANOVA) of the data were done using SYSTAT version 5.05 (SPSS Inc., Chicago, IL).

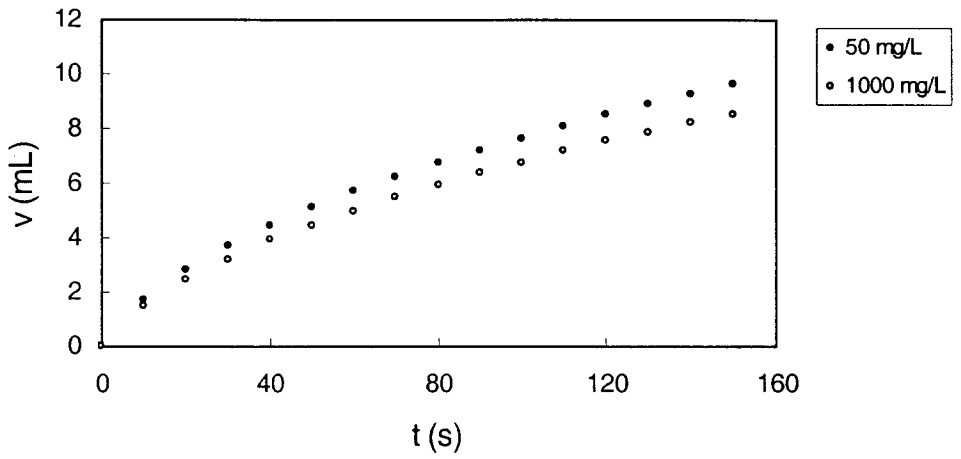

Figure 7.2 DE filtration at 76°C for wort containing 50 and 1000 mg/L of 443 kDa β-glucan.

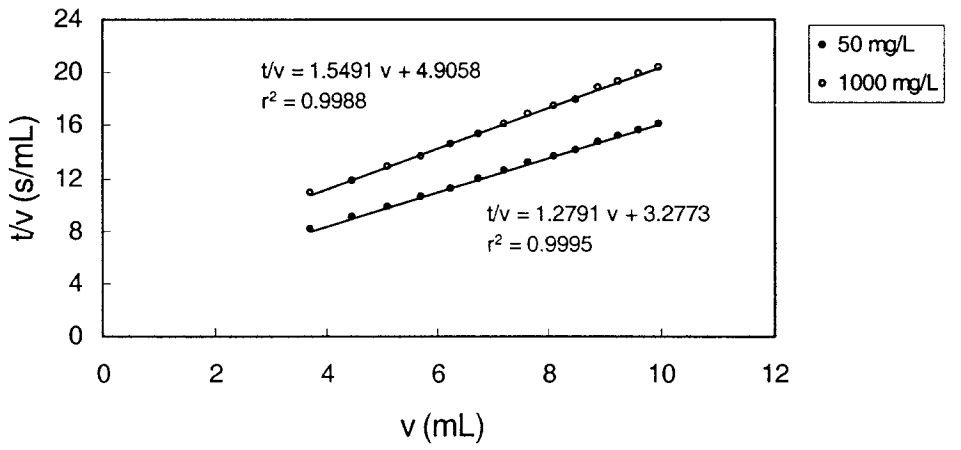

Figure 7.3 Analysis of wort DE filtration data from Figure 7.2 with Eq. 7-17. Lines indicate the best fit of the filtration model.

Table 7.1 Experimental design for the studies on wort filtration performance

Sample	Factor	Level	Test
Wort [a)]	Shearing	Unsheared / sheared	Diatomaceous filtration; Membrane filtration
	MW of β-glucan	31, 137, 250, 327 and 443 kDa	
	Concentration of β-glucan	50-1000 mg/L	
Wort	Shearing	Unsheared / sheared	
	β-Glucan (443 kDa)	0, 600 mg/L	
	Shearing temperature	20, 48, 76°C	
	pH	4.0, 5.4, 6.8	
	Maltose	6.1, 10.1, 16.1% w/w [b)]	

[a)]: Wort (12°P) at pH 5.4; b): Wort concentration was 8, 12 and 18°P, respectively.

7.3 Results and Discussion

Results of the DE filtration and membrane filtration of wort samples are discussed in this section. The filtration index (i.e., the F_i value in Eq. 7-17) was used to evaluate the DE filtration performance of wort at 76°C where a controlled "mash bed" was simulated with DE granules. A larger filtration index reflects greater resistance of wort to filtration. A relative flux of membrane filtration at 20°C was also examined as an indicator of the wort filterability.

7.3.1 Effect of Shearing, MW and Concentration of β-Glucans on Wort Filtration Performance at 76°C

The simulated lautering test was conducted at 76°C using DE as "mash bed" so that filtration performance of the wort could be studied. The ratio of DE to wort and the filter area are given and the test was carried out under a constant pressure. Changes in slope of the filtration curve (i.e., F_i value) represent the variations of viscosity and the specific resistance by wort. The presence of β-glucans at low concentrations caused higher

filtration index values ($p<0.001$). The filtration index was increased by both MW and concentration of β-glucans ($p<0.001$; Figure 7.4a). The increased resistance by β-glucans was due to the higher viscosity caused by higher β-glucan MWs and concentrations (Figure 3.5c). According to Poiseuille's law (Eq. 7-2), a higher viscosity of the filtrate leads to a lower flow rate during filtration, but only considers the influence of the filtrate on filtration. It is hypothesized that higher deposition of the suspended solids (mash bed formed from the spent grains) also retards filtration as indicated by Eq. 7-10. DE powder was used as "mash bed" and its effect on filtration was controlled. In a brewhouse, however, the permeability of a mash bed is determined by the malt modification and the presence of an adjunct. It is not surprising that β-glucan levels were not always found to be correlated with wort filtration and lautering performance (Eyben and Duthoy, 1979; Muts *et al.*, 1984; Sarx and Rath, 1995). When the resistance of the spent grains dominates, the viscosity and turbidity of wort at lautering temperature (i.e., 76°C) become less important in determining the filtration rate. Steely, under-modified grains of malt were found to be detrimental to the permeability of the spent grains leading to slower wort filtration in the laboratory (Jin and Wu, 1995). Besides proteins, un-degraded starch granules, β-glucans and pentosans also form a layer of gelatinous fines, which retard the lautering process (Bathgate and Palmer, 1975). A group of (so called) proteins causing problems in malting and mashing have been termed "gel proteins" by Graveland *et al.* (1979) since these proteins form a gelatinous layer in sodium dodecyl sulfate (SDS) solutions. Gel proteins consist of low (~40 kDa) and high (~1000 kDa) MW fractions linked by disulfide bonds (Baxter and Wainwright, 1979; Muts *et al.*, 1984; Muts and Pesman, 1986). However, gel proteins consisted of mainly (65-79%) carbohydrates (Muts and Pesman, 1986). Thus, only when the mash bed is well controlled, can the effect of wort compositions on lautering be studied.

For both sheared and unsheared worts, the filtration index can be predicted by the MW and concentration of β-glucans using multiple linear regression ($R^2=0.955$; $n=160$; $p<0.001$):

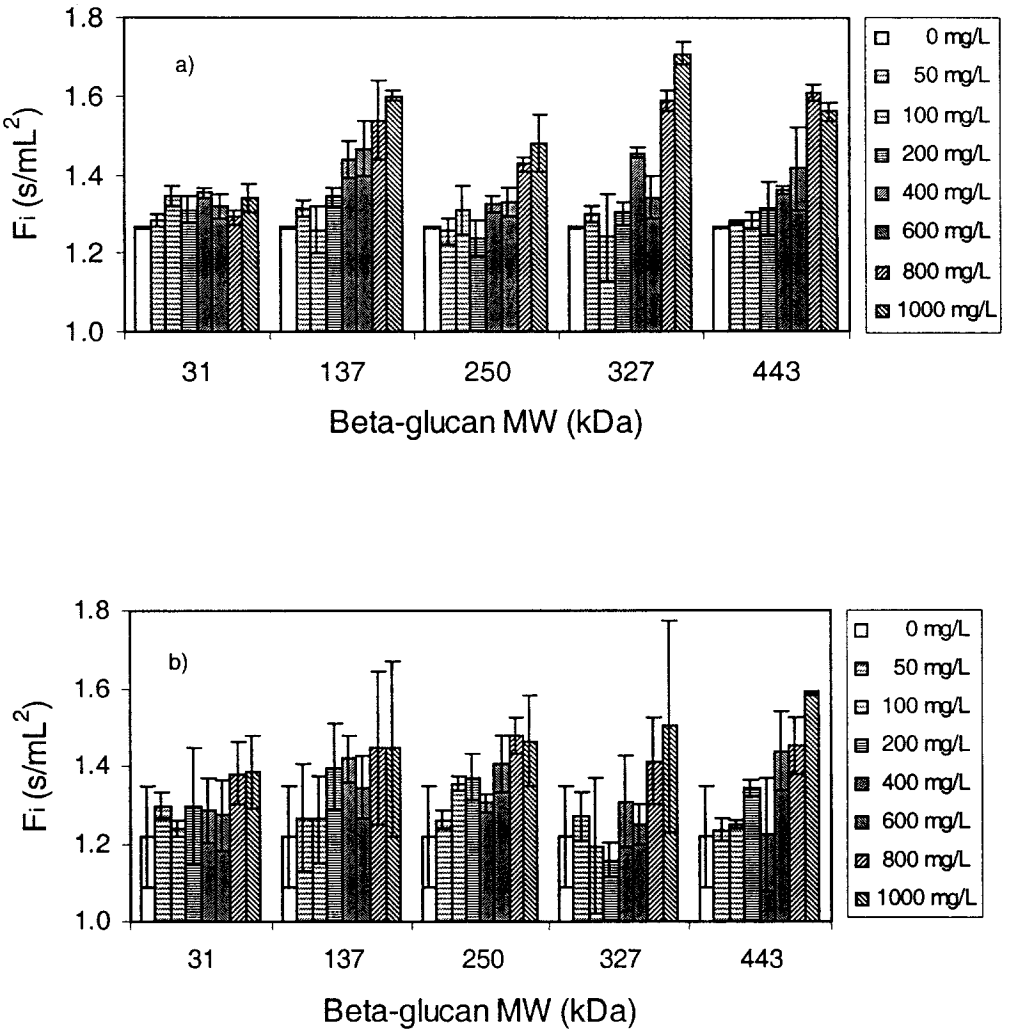

Figure 7.4 Effect of shearing, MW and concentration of β-glucans on the filtration index of (a) unsheared wort and (b) wort sheared at 20°C. Values are given as means ± one standard deviation (S.D.) of duplicate experiments.

$$F_i = 5.45\times10^{-3}\,MW + 2.32\times10^{-3}\,C + 0.997\,S + 4.36\times10^{-6}\,S\times MW\times C - 5.36\times10^{-6}\,MW^2 - 7.81\times10^{-7}\,C^2 - 3.25\times10^{-3}\,S\times MW - 1.44\times10^{-3}\,S\times C - 3.68\times10^{-6}\,MW\times C \quad (7\text{-}18)$$

where F_i is the filtration index (s/mL^2); MW is the molecular weight (kDa); C is the concentration of β-glucans in wort (mg/L); and S is shearing treatment (S=0 and S=1 for unsheared and sheared worts, respectively). Shearing wort at 20°C caused slower filtration at 76°C ($p<0.001$) compared to the unsheared samples. Shearing at 20°C was found to increase the wort viscosity and apparent particle size of β-glucans (Figures 3.10a and 4.2). Therefore, wort filtration performance at 76°C was impaired by the shearing treatment ($p<0.001$; Figure 7.4).

7.3.2 Effect of β-Glucan (443 kDa at 600 mg/L), pH, Maltose Level and Shearing Temperature on Wort Filtration Performance at 76°C

The filtration performance of 8-18°P wort samples (pH 4.0-6.8) containing 6.1-16.1% w/w of maltose, 0 and 600 mg/L of the 443 kDa β-glucan were examined after shearing at different temperatures (Figure 7.5). Maltose at higher concentrations increased wort viscosity (Figure 3.11), and the filtration index also increased with high maltose concentrations ($p<0.001$). Wort filtration was slower at lower pH values ($p<0.001$). At lower pHs, the viscosity of wort was lower (Figure 3.11), but the wort turbidity was much higher (Figure 6.3). The higher turbidity at lower pH values was hypothesized to be responsible for the increased filtration index observed in this study (although the turbidity was measured at 20°C and filtration was performed at 76°C). The higher turbidity was hypothesized to be formed by non-β-glucan components, particularly proteins of wort (Figure 6.3). At higher temperatures (76°C), hydrophobic interactions of proteins are enhanced and may result in higher filtration resistance. The addition of 600 mg/L of the 443 kDa β-glucan increased filtration index ($p<0.001$), which could be described with the following multiple linear regression ($R^2=0.992$; $n=108$; $p<0.001$):

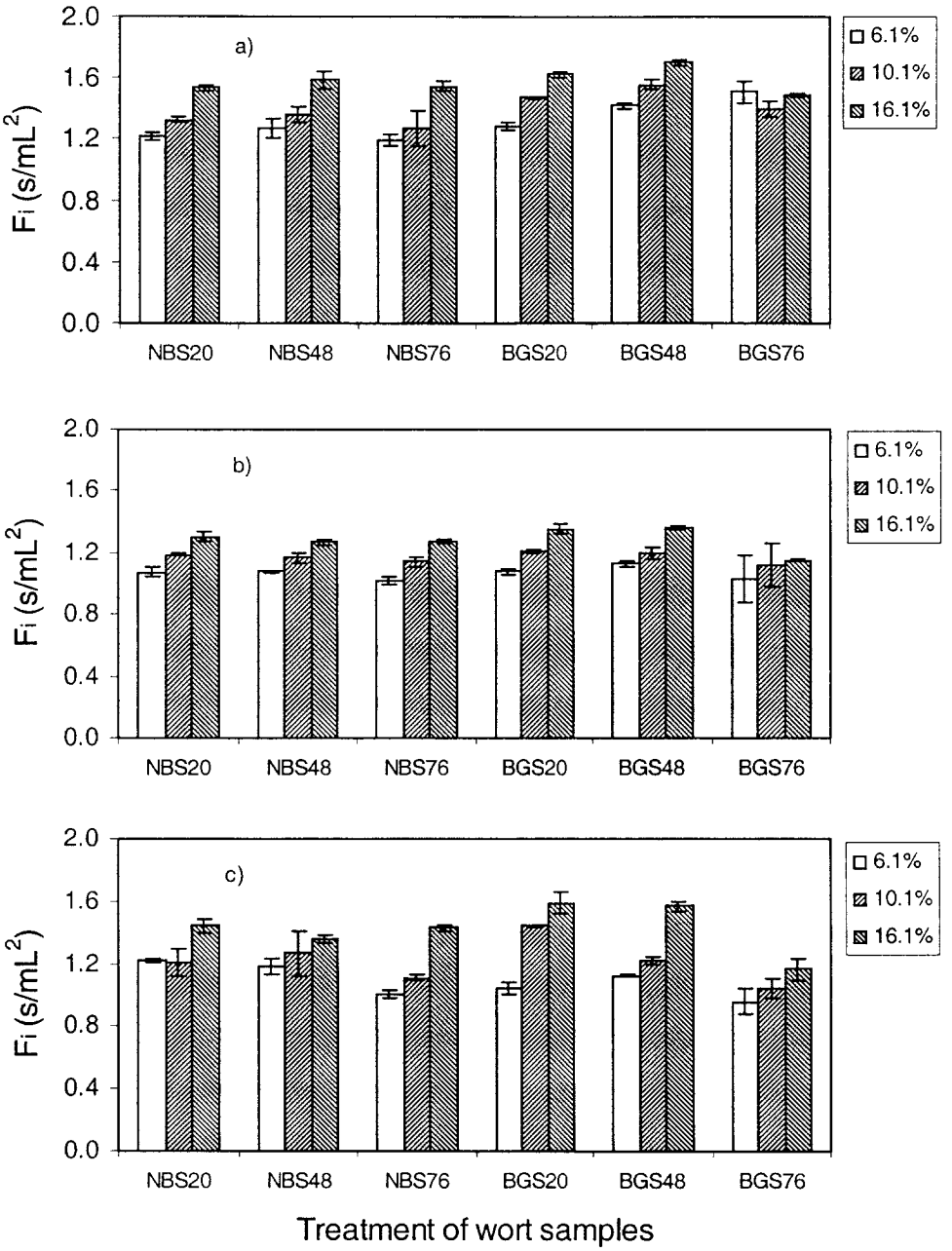

Figure 7.5 Effect of β-glucan, maltose and shearing on the filtration index of wort samples at (a) pH 4.0; (b) pH 5.4 and (c) pH 6.8. NB = no β-glucan; S20 = sheared at 20°C; S48 = sheared at 48°C; S76 = sheared at 76°C; BG = wort containing 600 mg/L of the 443 kDa β-glucan. Values are given as means ± S.D. of duplicate experiments.

$$F_i = 8.3\times10^{-4}\,C + 8.33\times10^{-2}\,Mal + 8.03\times10^{-2}\,pH + 2.46\times10^{-2}\,T_s - 1.1\times10^{-4}\,T_s^2 - 1.77\times10^{-3}\,Mal^2 - 2.98\times10^{-6}\,C\times T_s - 1.1\times10^{-4}\,C\times pH - 3.1\times10^{-4}\,T_s\times Mal - 2.16\times10^{-3}\,T_s\times pH \quad (7\text{-}19)$$

where Mal is the maltose concentration (% w/w) and T_s is the shearing temperature (°C). Wort Sheared at higher temperatures filtered faster than worts sheared at lower temperatures ($p<0.001$) due to the interactions of shearing temperature with other variables. The decreased resistance can be explained by the lower wort viscosity.

Wort separation in the brewhouse is normally done at a high temperature (≈76°C) which reduces the effect of wort viscosity on filtration. Wort viscosity at 76°C is approximately 1/3 of that at 20°C (Figures 3.3 and 3.5). The changes in wort viscosity caused by high concentrations of biopolymers were also smaller (Figure 3.5). Therefore, the influence of dissolved polymers such as β-glucans and proteins on wort filtration are minimized at 76°C. It was noted that β-glucans (31-443 kDa at 50-1000 mg/L) dissolved in wort retarded wort filtration mainly because of their increased viscosity. Beta-glucan molecules were found not to be retained by the filter cake (i.e., DE bed) because the concentrations of β-glucans determined by Congo red dye did not change after filtration ($p>0.05$; results not shown). In addition, the viscosity of wort measured at 20°C did not change after filtration ($p>0.05$; results not shown). This suggests that Poiseuille's law governs the process of wort filtration. Examination of the permeability of the mash bed is also considered to be another important factor affecting wort filtration. However, the study of mash bed permeability was beyond the scope of this study.

7.3.3 Effect of Shearing, MW and Concentration of β-Glucans on the Membrane Filtration Rate (20°C) of Wort

Wort samples were also filtered through 0.45 μm membranes at 20°C to evaluate their resistance to (membrane) filterability. As an alternate to the DE filtration test, the membrane filtration flux, (i.e., the flow rate of wort in m^3/m^2hr) was constant with 10 mL

volumes. In such cases, the parameters of V_{max} and Q_{init} could not be accurately estimated because the membranes were not clogged and the flow rates were constant. Also, V_{max} and Q_{init} have no practical use in wort filtration assessment. The wort flux values were of limited use without a reference in evaluation of wort filterability. Thus, the "relative flux" was defined as the percentage of flux of a wort sample filtered relative to that of water (%) under the same conditions. It normalizes the "filterability" of samples and is comparable among samples under different conditions.

The relative flux of the β-glucan-free wort reached 96% (Figure 7.6a). The addition of the 31 kDa β-glucan up to a concentration of 1000 mg/L lowered the relative flux to 72%. Higher concentrations of high MW β-glucans in the range of 31-443 kDa at 50-1000 mg/L decreased the relative flux ($p<0.001$). The relative flux of the wort containing 1000 mg/L of 443 kDa β-glucan was as low as 41% (Figure 7.6a). Shearing of wort samples at 20°C also lowered the relative flux ($p<0.01$; Figure 7.6b). The following empirical model helps to explain the effect of MW, concentration of β-glucans and shearing on the relative flux ($R^2=0.766$; $n=160$; $p<0.001$):

$$R_f = 104.83 - 0.1483\ MW - 0.0620\ C - 5.060\ S + 2.3\times10^{-3}\ MW^2 + 2.0\times10^{-5}\ C^2 \quad (7\text{-}20)$$

where R_f is the relative flux (%). The membrane filterability of wort was lowered by increased viscosity because wort viscosity was increased by high MW and high concentration of β-glucans. Shearing wort was previously found to cause both higher viscosity (Figure 3.10b) and larger apparent particle size of β-glucans in wort (Figure 4.2). Thus, shearing led to lower relative flux, which can be predicted by Poiseuille's law (Eq. 7-2). Since the 31 kDa β-glucan had less serious influence on wort flux (Figure 7.6), the species was excluded in the multiple linear regression leading to improved predictions of the flux ($R^2=0.963$; $n=128$; $p<0.001$):

$$R_f = 0.565\ MW - 2.37\times10^{-2}\ C - 8.8\times10^{-4}\ MW^2 - 1.37\times10^{-2}\ S\times C - 6.0\times10^{-5}\ MW\times C \quad (7\text{-}21)$$

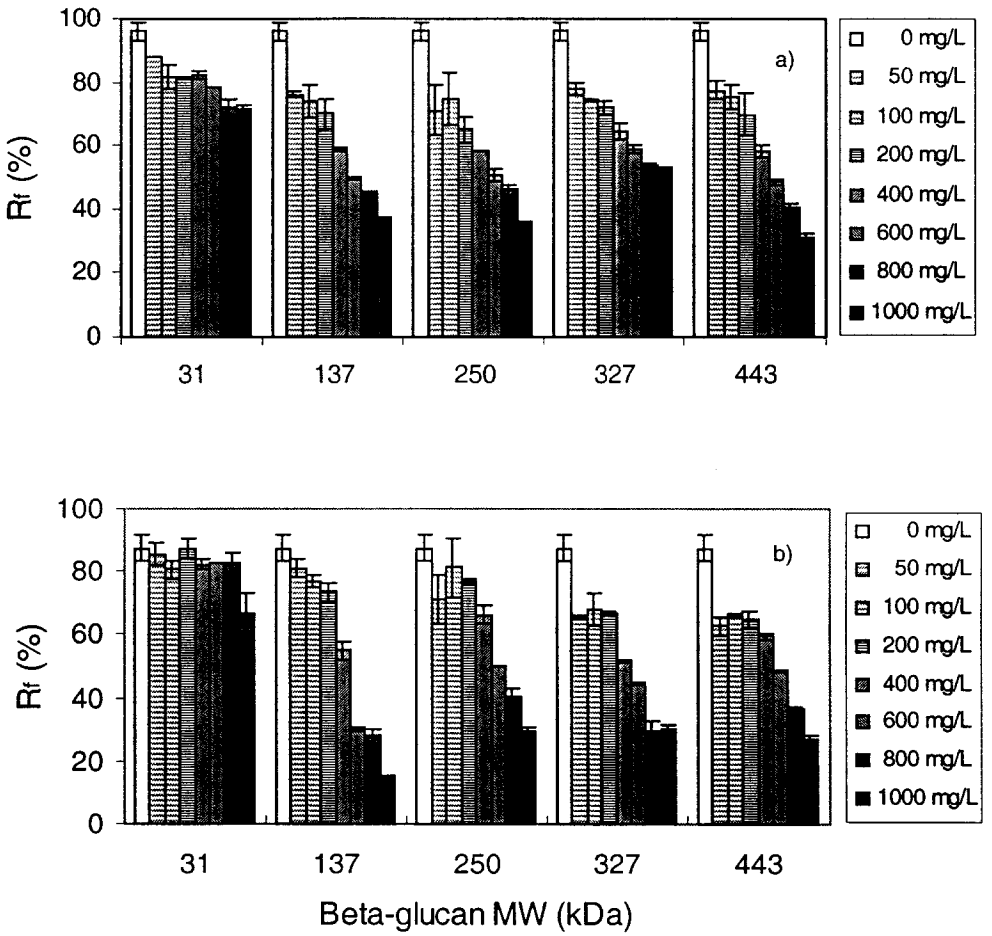

Figure 7.6 Effect of MW and concentration of β-glucans on the relative flux of 0.45 μm membrane filtration of (a) unsheared wort and (b) wort sheared at 20°C. Values are given as means ± S.D. of duplicate experiments.

The majority of β-glucan polymers (31-443 kDa) were smaller than 0.1 μm in the unsheared wort (Figure 4.1). After shearing, the apparent particle size was greater, where some were found to be >0.1 μm (Figure 4.2) but <0.45 μm as determined by the membrane categorization. It is unlikely that the 31-443 kDa β-glucans lowered flux by clogging the membranes because they had apparent diameters smaller than the nominal pore size of the membranes. It is also noteworthy that the wort used in this thesis was brilliant in clarity (20 FTU) and the effect of suspended solids (i.e., hazes) on the filtrations was minimized. However, even without the addition of β-glucans, shearing still reduced wort flux to 87% of water (Figure 7.6b); it is hypothesized that this was due to the higher turbidity and resulting particulate load from sheared proteins.

7.3.4 Effect of β-Glucan (443 kDa at 600 mg/L), pH, Maltose Level and Shearing Temperature on the Membrane Filtration Rate (20°C) of Wort

The effect of β-glucan, pH, maltose and shearing on the relative flux of membrane filtration was similar to their effects on DE filtration performance. However, the relative flux at 20°C was more sensitive to changes in wort conditions than the filtration index F_i (76°C). The difference may be due to the fact that the viscosities of worts at 20°C are approximately three times higher than those at 76°C, resulting in greater variations in the flux of membrane filtration (20°C). As illustrated in Figure 7.7, the relative flux of wort samples was lowered by an addition of the 443 kDa at 600 mg/L ($p<0.001$), higher maltose levels ($p<0.001$), and lower pH ($p<0.01$). The multiple linear regression has provided an empirical model of the relative flux ($R^2=0.856$; $n=108$; $p<0.001$):

$$R_f = 113.77 - 2.91\times10^{-2}\,C - 3.0158\,Mal - 3.91\times10^{-3}\,T_s^2 - 0.5709\,pH^2 + 8.10\times10^{-2}\,T_s\times pH + 2.0\times10^{-4}\,C\times MW\times pH \quad (7\text{-}22)$$

The membrane filterability of wort was reported to be lower when it was acidified to pH value close to that of beer (Siebert *et al.*, 1984). At higher pHs, the viscosity of wort was higher (Figure 3.11) whereas wort turbidity was much lower (Figure 6.3). The difference

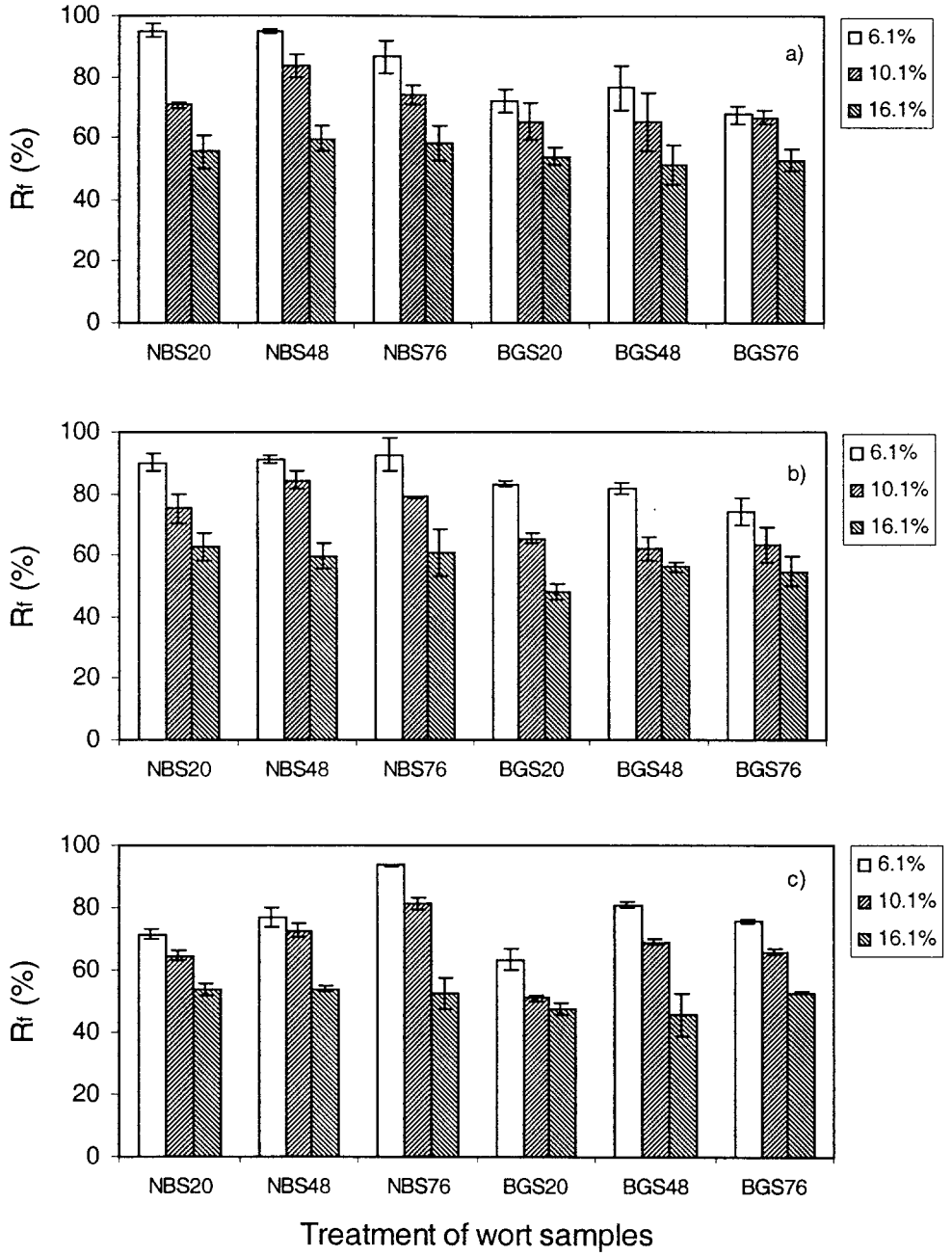

Figure 7.7 Effect of β-glucan, maltose content and shearing temperature on the relative flux of wort samples at pH values of (a) 4.0; (b) 5.4 and (c) 6.8. NB = no β-glucan; S20 = sheared at 20°C; S48 = sheared at 48°C; S76 = sheared at 76°C; BG = wort containing 600 mg/L of the 443 kDa β-glucan. Values are given as means ± S.D. of duplicate experiments.

in wort turbidity at various pH values was hypothesized to affect the relative flux (Eq. 7-22) as well as the filtration index observed in this study (Eq. 7-19). Shearing temperature also affected the relative flux ($p<0.05$). Shearing wort at higher temperatures led to easier filtration at 20°C through the 0.45 μm membranes (Figure 7.7). This is in agreement with the trend of changes in filtration index at 76°C (Eq. 7-19). Although a higher shearing temperature did not alter the particle size distribution (Figure 4.5), the β-glucan particle size may have changed within the size category (e.g., 0.01-0.1 μm).

7.3.5 Correlation between Wort DE Filtration and Membrane Filtration Performance

Both DE filtration test at 76°C and membrane filtration test at 20°C were able to detect the variations in filtration performance of wort. The results were negatively correlated with each other as shown in Figure 7.8 ($r=-0.595$; $n=152$; $p<0.001$). A higher filtration index value (F_i) at 76°C corresponded to the lower flux of membrane filtration observed at 20°C ($p<0.001$). The filtration index was subject to a large scatter. DE filtration of the wort samples was mainly affected by viscosity, while β-glucans dissolve better at high temperatures (Letters, 1995a; Esser, 1996; Annemüller and Schnick, 1999) and do not plug the flow channels in the DE bed. When filtered with 0.45 μm membranes at 20°C, however, flux was influenced by both wort viscosity and particulate matter such as protein hazes (i.e., cold trub).

The DE filtration device has an advantage over the membrane filtration test in evaluating wort filterability of malt samples. Besides testing the filterability of wort, it can also be used to assess the permeability of suspended solids in mash. A certain volume of the Congress mash can be applied onto filtration device to form a mash bed. After draining the wort, water or a buffer can be used as a reference to filter through the mash bed. The flow rate for water indicates the permeability of the mash bed tested. This approach may be more useful as a quality assurance technique to monitor the changes in wort filterability and the mash bed permeability.

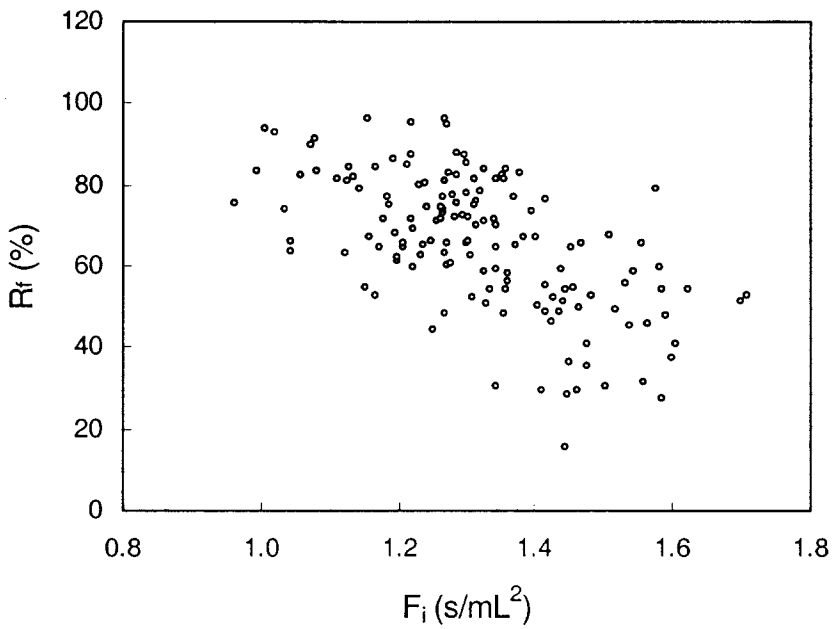

Figure 7.8 Correlation of the relative flux of 0.45 μm membrane filtration at 20°C with the filtration index at 76°C (n=152). The line represents the best fit of the linear relationship.

7.4 Conclusions

The presence of high MW β-glucans retarded wort filtration performance at 76°C ($p<0.001$). Beta-glucans with higher MWs at higher concentrations increased the filtration index ($p<0.001$). Shearing was found to increase the filtration index ($p<0.001$) whereas shearing wort at 20°C resulted in slower filtration than shearing at higher temperatures (48-76°C) ($p<0.001$). Higher concentrations of maltose also retarded wort filtration at 76°C due to increased wort viscosity ($p<0.001$). Resistance of wort to filtration was also higher when pH was adjusted to pH 4.0 than at normal wort pH, 5.4 or higher ($p<0.001$).

When wort samples were filtered through 0.45 μm membranes at 20°C, similar trends of the influence of β-glucans and environmental factors on wort filterability (expressed as relative flux) were observed. Membrane filterability at 20°C correlated negatively with the filtration index at 76°C. It is important to note that the wort used in this study was brilliant in clarity and membranes were not clogged after filtration of 10 mL of wort. In the future, it would be interesting to investigate the relationship of the membrane filterability of wort and beer that is fermented from the same wort.

8. EFFECT OF β-GLUCANS AND PROCESS CONDITIONS ON MEMBRANE FILTRATION PERFORMANCE OF BEER

Membrane filtration is a recently established technology for the production of sterile, un-pasteurized beer, which is believed to provide better flavour and flavour stability versus traditional heat pasteurization. Compared to the traditional deep bed filtration, membrane filtration is more susceptible to clogging by colloidal particles in beer due to their smaller cutoff pore sizes. Laboratory membrane filtration tests can be used to evaluate beer filterability. An extended filtration test with 0.45 μm size membranes was employed in this study to investigate the influence of β-glucans, shearing, pH, ethanol content and lagering on beer filterability.

8.1 Introduction

Biological stability of beer has been mainly achieved by pasteurization (Back, 1997). It is accepted that pasteurization may cause impaired flavour (e.g., cooked flavour) and reduced flavour stability (i.e., accelerated staling). Membrane filtration and cold sterile packaging avoids the negative effect of heat pasteurization on the beer flavour. It also lowers the pseudo- or invisible hazes of beer (Feische and Polzer, 1999; Fillaudeau, 1999; Leeder and Houldsworth, 1988; Parkes, 1999). However, the membrane filtration systems possess low productivity and short filter operation life (Back, 1997) as compared to the traditional separation methods. Membrane filtration of beer can be operated as crossflow membrane filtration (CFMF) under an applied static pressure. This tangential flow pattern minimizes fouling of the membranes and improves productivity and the filter operation cycle time (Belfort *et al.*, 1994).

Choosing an appropriate nominal pore size for beer sterile filtration is usually difficult. The nominal pore rating (i.e., equivalent diameter) must be as small as possible to guarantee safe retention of microorganisms present in beer. On the other hand, the pores

should be large enough so that the longest possible filtration cycle life can be achieved (Ilberg *et al.*, 1995). Microbiological tests have shown that it is necessary to use a nominal pore diameter of 0.45 μm so that beer spoilage microorganisms are assuredly retained, although membranes with this pore size can be readily blocked depending on level of particulates present in the beer (Ilberg *et al.*, 1995; Westbrook, 1992).

With the same filter area, a 0.2 μm membrane filter can filter in one batch approximately 1% of the beer volume compared to industrial DE filters (Esser, 1996). These limitations are a direct result of membrane fouling, which is the blockage of the pores by certain material particularly macromolecular "gels" (McKechnie, 1995). However, the filtration flux and the clogging of membranes are also dependent upon the physical and chemical properties of the beer being filtered (McKechnie, 1995; Siebert *et al.*, 1984). The filtrate flux of CFMF has a major impact on its economics (Lenoël *et al.*, 1993; 1994; McKechnie, 1995). With a filtration cycle time of 10-15 hours, its permeate flux varies between 20-100 L/m^2hr. Another important cost-effective parameter is membrane life. The membrane can last over 3 years (Fillaudeau, 1999; McKechnie, 1995).

Both beer flux and membrane life are related to clogging or fouling of the membranes. The main components responsible for membrane fouling are yeast cells, colloidal hazes, carbohydrates such as pentosans and β-glucans, high MW proteins and calcium salts of oxalate (Fillaudeau, 1999; Meier *et al.*, 1995). Beta-glucans have been reported to affect membrane filtration (Jin *et al.*, 2001; Krüger *et al.*, 1989; Meier *et al.*, 1995; Oonsivilai, 2000; Oonsivilai *et al.*, 1999; 2000; Patelakis, 1999; Patelakis *et al.*, 1999; Siebert *et al.*, 1984; Stewart *et al.*, 1998; Sudarmana *et al.*, 1996). High MW fractions and those with high contents of β-1,4-linkages (mainly in the 65°C water-soluble fraction) have higher potential to cause problems in filtration and clarity of beer due to their interchain aggregation (Izydorczyk *et al.*, 1998a).

Since an early study by Esser (1972), membrane filtration tests have been adopted by many researchers (Annemüller and Schnick, 1999; Back, 1997; Egi, 2002; Egi *et al.*, 2001; Eyben and Duthoy, 1979; Jin *et al.*, 2001; Meier, 1992; 1995; Oonsivilai, 2000; Oonsivilai *et al.*, 1999; Patelakis, 1999; Patelakis *et al.*, 1999; Reid *et al.*, 1990; Siebert, 1984; Stewart *et al.*, 1998; Sudarmana *et al.*, 1996). The objectives of this study were to determine how the MW and concentration of β-glucans affect beer membrane filtration performance; to investigate if shearing beer influences the efficiency of membrane filtration; and to study the effects of pH, ethanol content and lagering on beer filterability.

8.2 Materials and Methods

Materials and methods employed in this study have been partly described in section 3.2. Details of the materials used in this study are described in section 3.2.1. A commercial lager beer (Labatt Blue, product code E10H11C, UBC 062067351013, Oland Breweries Ltd., Halifax, NS) was used to prepare a beer base free of β-glucans (section 3.2.4). This β-glucan-free beer base was then used to prepare beer samples varying in β-glucan concentration (0-1000 mg/L), pH (3.8-4.6), and ethanol content (0-10% v/v) as described in section 3.2.5. Barley β-glucans purchased from Megazyme International Ireland Ltd. (Bray, IRL) having molecular weights of 31, 137, 250, 327 and 443 kDa were used to prepare beer samples with β-glucan concentrations in the range of 0-1000 mg/L. Shearing of beer samples was described in section 3.2.6. Beta-glucan concentration was determined with the Congo red binding assay described in section 3.2.7.

A membrane filtration test under a constant pressure was modified from the method used by Patelakis (1999): (1) "AcetatePlus" (supported, plain) membranes with a nominal pore size of 0.45 μm (Cat. No. A04SP02500, Material No. 1215635, Batch No. 088439, 087551 and 093062; Osmonics Inc., Minneapolis, MN) was used with a 25 mm Swinnex filter holder (Millipore Inc., Bedford, MA); (2) filtration was carried out under a pressure of 7 kPa (i.e., 1 psi) instead of 5 psi; (3) 40 mL of beer was applied and (4) filtration

temperature was controlled at 5.0°C unless otherwise specified. The mass of beer samples (g) filtered was recorded over the filtration time and converted into volume (mL). According to Hermans and Bredée (1936), the effect of clogging on filtration can be described as follows:

$$t/v = (k/2)\, t + 1/S_0 \qquad (8\text{-}1)$$

where t is the filtration time; v is the volume of filtrate collected; k is a constant and S_0 is the initial flow rate. The constant k/2 was defined as $1/V_{max}$ and S_0 as Q_{init} (Egi, 2002; Egi *et al.*, 2001; Jin *et al.*, 2001; Meier *et al.*, 1992; Oonsivilai, 2000; Patelakis, 1999; Sudarmana *et al.*, 1996). Thus, the model was rewritten as:

$$t/v = t/V_{max} + 1/Q_{init} \qquad (8\text{-}2)$$

where V_{max} is the maximum volume of sample which can be filtered through a membrane filter and Q_{init} is the initial flow rate. However, the membrane filtration results have revealed a nonlinear relationship between t/v and t at the beginning of filtration (Patelakis, 1999; Sudarmana *et al.*, 1996). The first several data points were discarded to force the data with linear regressions (Patelakis, 1999; Sudarmana *et al.*, 1996). This altered slope and intercept of the plots, leading to under-estimation of V_{max} and over-estimation of Q_{init}. To avoid such an error, a double reciprocal plot was derived from the above model (Eq. 8-2):

$$1/v = (1/Q_{init})\, 1/t + 1/V_{max} \qquad (8\text{-}3)$$

This transform showed a linear relationship over all data points and was believed to be more accurate in estimating the filtration parameters. A double reciprocal plot for the β-glucan-free beer is exemplified in Figure 8.1. From the slope and intercept of the regression, V_{max} and Q_{init} were found to be 277 mL and 0.16 mL/s, respectively. Since these parameters are dependent on the filter area, it is necessary to standardize them into volume of maximum filtrate per unit filter area and initial flow rate per unit area of filter membrane. The effective filtration area of the membranes (25 mm in diameter) used was 3.80 cm^2 (effective diameter was 22 mm in the membrane holder). For convenience of comparisons with industrial filters, V_{max} was expressed as m^3/m^2 and Q_{init} was expressed as m^3/m^2hr in this thesis. "Relative V_{max} (%)" and "relative Q_{init} (%)" were defined as the

percentage of V_{max} or Q_{init} of beer containing β-glucans relative to that of the β-glucan-free filtered beer under the same conditions:

$$\text{Relative } V_{max}\ (\%) = (V_{max\ BG} / V_{max\ NB}) \times 100 \quad (8\text{-}4)$$

$$\text{Relative } Q_{init}\ (\%) = (Q_{init\ BG} / Q_{init\ NB}) \times 100 \quad (8\text{-}5)$$

where $V_{max\ BG}$ is the V_{max} of beer containing β-glucans; $V_{max\ NB}$ is the V_{max} of the β-glucan-free beer; $Q_{init\ BG}$ is the Q_{init} of beer containing β-glucans and $Q_{init\ NB}$ is the Q_{init} of the β-glucan-free beer.

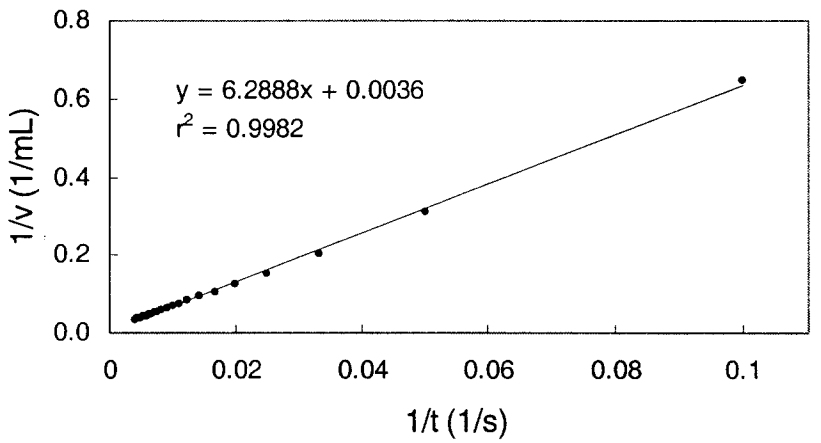

Figure 8.1 Modeling the membrane filtration data with double reciprocal plot for the β-glucan-free beer.

The experimental design of this study is displayed in Table 8.1. Duplicate experiments were carried out and mean values and standard deviations were reported. Linear regressions and ANOVA of the experimental results were conducted with SYSTAT version 5.05 (SPSS Inc., Chicago, IL).

Table 8.1 Experimental design for the studies on beer membrane filterability

Sample	Factor	Level
Beer [a)]	Shearing	Sheared / unsheared
	MW of β-glucan	31, 137, 250, 327 and 443 kDa
	Concentration of β-glucan	50-1000 mg/L
Beer	β-Glucan (443 kDa)	0, 600 mg/L
	Shearing temperature	0, 5, 10°C
	pH	3.8, 4.2, 4.6
	Ethanol	0, 5, 10% v/v
Sheared beer	Cold storage	4°C for 2 weeks

[a)]: Beer at pH 4.2 containing 3.3% w/w of real extract and 5.0% v/v of ethanol.

8.3 Results and Discussion

The membrane filterability of beer includes the maximum volume of beer that can be filtered through a given area of membrane (V_{max}) and the initial flow rate (Q_{init}) (Esser, 1972; Sudarmana *et al.*, 1996). By using the transformed filtration model (Eq. 8-3), it becomes possible to predict the volume of beer filtered at any time when the two parameters are known. As long as the membrane filtration test is carried out under the same conditions as the production situation, the filterability can be scaled up. Otherwise, a correlation must be established between the laboratory test and the production results. Another purpose of the membrane filtration test is to detect batches which may prove difficult to filter, providing a warning to brewing managers. The latter purpose is the main reason for most membrane filtration studies. Results of this study have shown that β-glucans, low pH and low ethanol content reduced the V_{max} of beer samples. Addition of β-glucans and high ethanol content lowered the Q_{init} of beers but pH did not affect the initial flux. Shearing of beer samples resulted in contradictory changes in beer filterability. Details of the experimental results are discussed in the following sub-sections.

8.3.1 Effect of Shearing, MW and Concentration of β-Glucans on Membrane Filtration Performance of Beer

Beta-glucans (31, 137, 250, 327 and 443 kDa) at 50-1000 mg/L lowered both V_{max} and Q_{init} of beer during the 0.45 μm membrane filtration at 5°C (Figures 8.2 and 8.3). Both MW and concentration of β-glucans significantly decreased beer filterability ($p<0.001$). Higher concentrations of β-glucans of higher MWs resulted in lower V_{max} and Q_{init} of beer samples. The control (β-glucan-free) beer had a V_{max} at 0.75 m^3 of beer per m^2 filter area (Figure 8.2a). The addition of the 31 kDa β-glucan at 1000 mg/L decreased the V_{max} to 0.31 m^3/m^2 whereas 1000 mg/L of 137, 250, 327 and 443 kDa β-glucans led to V_{max} values around 0.1 m^3/m^2 (Figure 8.2a). This finding agrees with the literature reports (Nischwitz *et al.*, 1999; Oonsivilai, 2000; Stewart *et al.*, 1998). Shearing beer at 5°C resulted in lower V_{max} values for all samples ($p<0.001$; Figure 8.2b). In the literature, shearing due to centrifugation of beer caused a decline in membrane filterability by 75% (Siebert *et al.*, 1984) and shearing of a model beer resulted in lower V_{max} (Patelakis, 1999). In this study, the stepwise multiple regression indicated that shearing, MW and concentration of β-glucans were significant ($p<0.001$) in determining the V_{max} ($R^2=0.799$; $n=160$; $p<0.001$):

$$V_{max} = 0.808 - 1.59\times10^{-3}\,MW - 1.10\times10^{-3}\,C - 0.326\,S + 2.52\times10^{-6}\,MW^2 + 6.58\times10^{-7}\,C^2 + 2.9\times10^{-4}\,S\times MW + 1.70\times10^{-4}\,S\times C \qquad (8\text{-}6)$$

where MW is the molecular weight of β-glucans (kDa); C is the concentration of β-glucans (mg/L); and S is shearing treatment (S=0 and 1 for the unsheared and sheared beers, respectively). The control beer had a lower V_{max} at 0.41 m^3/m^2 (i.e., a 45% reduction) after shearing. The decreased filterability of the control was caused by an increased haze level in sheared beer (Figure 6.4b) because haze particles retard membrane filtration by clogging (Siebert *et al.*, 1984). Beta-glucans (31-443 kDa) at a "normal" concentration of 200 mg/L in sheared beer decreased the V_{max} to 0.1-0.3 m^3/m^2 (Figure 8.2b). High MW β-glucans (137-443 kDa) at ≥600 mg/L led to V_{max} values of 0.02-0.1 m^3/m^2. This implies that an industrial scale filter having cartridges of 320 m^2

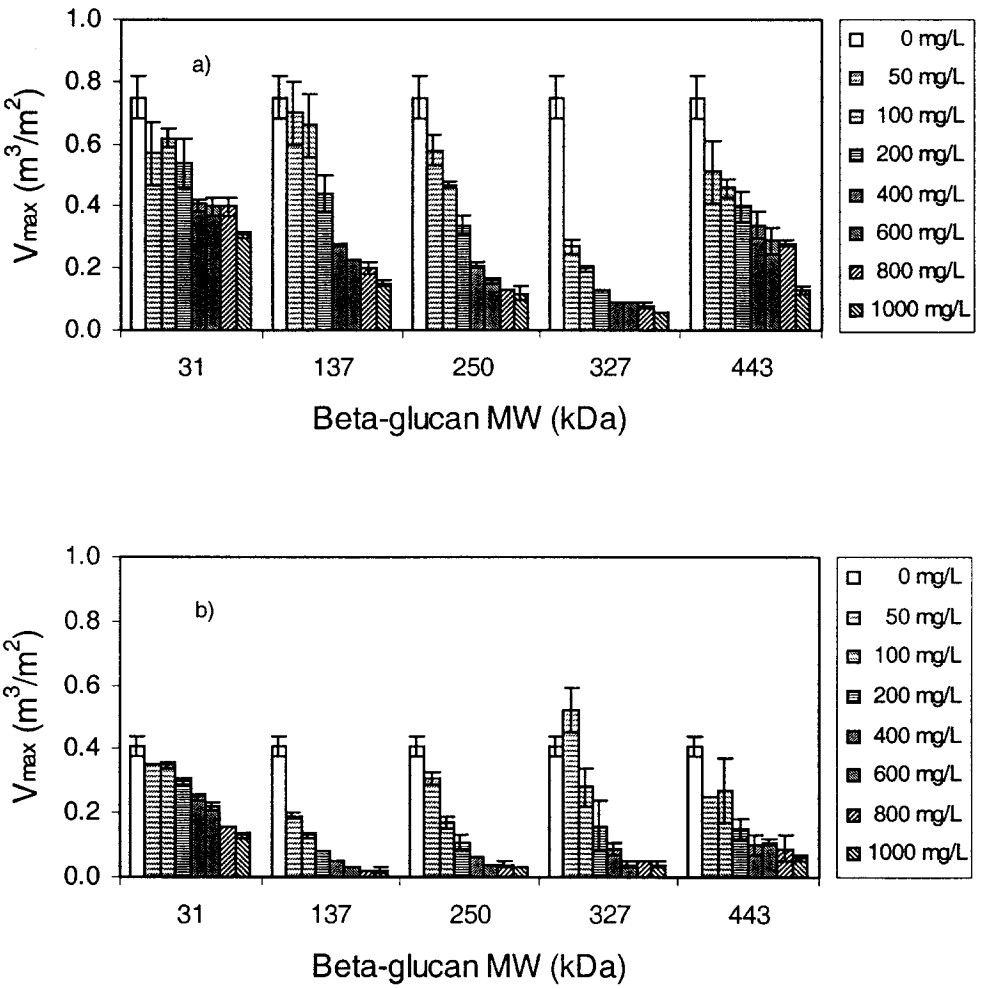

Figure 8.2 Effect of MW and concentration of β-glucans on the V_{max} of (a) unsheared and (b) sheared beer. Values are given as means ± one standard deviation (S.D.) of duplicate experiments.

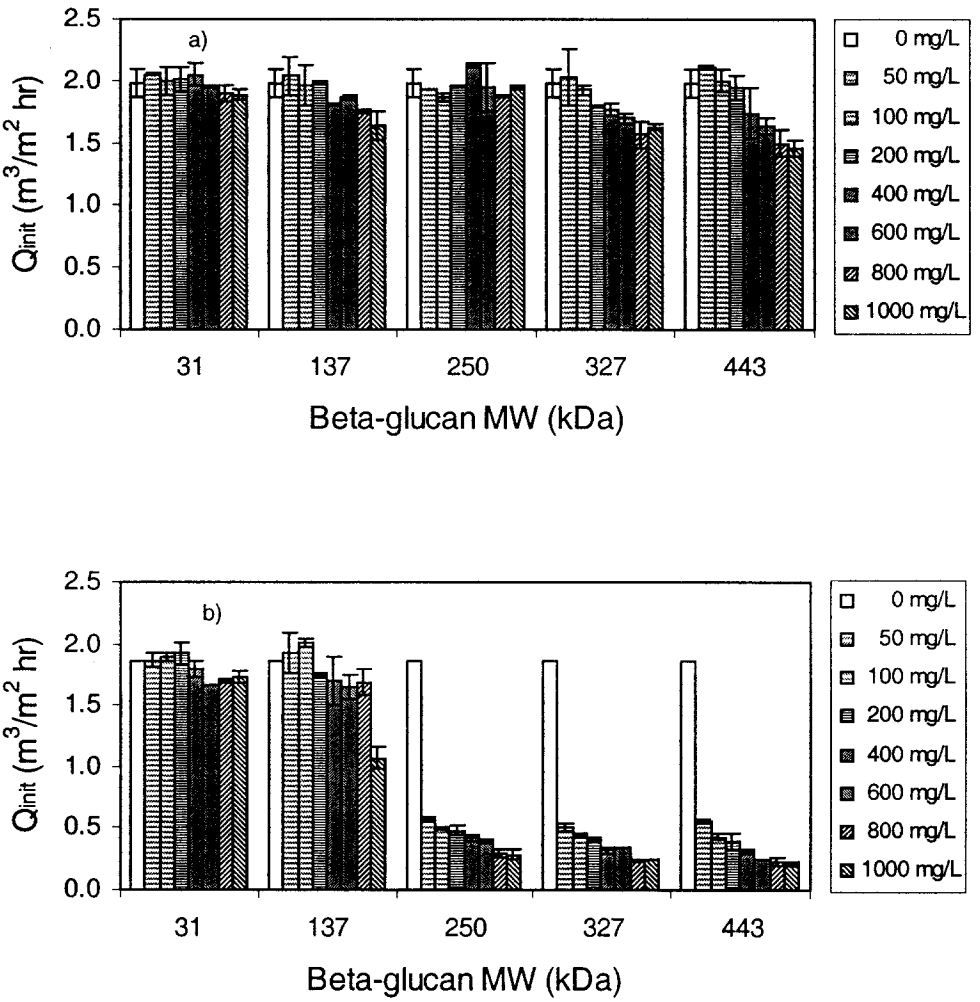

Figure 8.3 Effect of MW and concentration of β-glucans on the Q_{init} of (a) unsheared and (b) sheared beer. Values are given as means ± S.D. of duplicate experiments.

membrane area (which is designed for productivity at 35 m^3/hr according to Hoffmann, 1998) would only have a maximum filtration capacity of 6.4-32 m^3 before the filter was completely clogged. This would pose a serious problem for membrane filtration in the plant.

It is believed that only the high MW β-glucans cause "gel formation" and process difficulties in beer while the low MW β-glucans do not (Bamforth, 1994; Forrest and Wainwright, 1977; Izawa *et al.*, 1993; Letters, 1977; Stewart *et al.*, 1998). This was found to be true when viscosity, turbidity and Q_{init} were examined for unsheared beer containing low MW (i.e., 31 kDa) β-glucan (Figures 3.13a, 6.4a and 8.3). However, even the 31 kDa β-glucan in beer increased turbidity after shearing of samples (Figure 6.4b). Increasing the concentration of the 31 kDa β-glucan lowered V_{max} of both sheared and unsheared beers (Figure 8.2). At a concentration of 1000 mg/L, the 31 kDa β-glucan reduced the V_{max} by 59% and 68% (compared to their beer base controls) for the unsheared and sheared beers, respectively. In the literature, beer β-glucan level is rarely higher than 100-300 mg/L (Table 2.1). It was observed that the reactivity of 1000 mg/L of 31 kDa β-glucan with Congo red was approximately equivalent to that of the high MW (137, 250, 327 and 443 kDa) β-glucans at 100 mg/L (Figure A.3 in Appendix 4). Thus, a low reading in the β-glucan analysis can result from a low content of the high MW β-glucans or a high level of the low MW β-glucans. When the β-glucan content of beer is measured by calibration against a high MW standard, the measurement of a low level of β-glucans could be misleading (since there is no information about the MW distribution of beer β-glucans). Apparently, high levels of low MW β-glucans have an impact on V_{max} more than very low levels of the high MW fractions. This may help explain why membrane filtration may become difficult when the β-glucan level seems low. Thus, it is desirable to degrade the β-glucans to very low molecular weights, instead of only breaking down high MW β-glucan polymers into the medium or low molecular weight range.

Brewers are perhaps more interested in the flow rate of beer filtration than the maximum capacity of the filter cartridges. In the early 1990s, production lines had fluxes in the range of 0.03-0.05 m^3/m^2hr for 0.4-0.6 μm crossflow membrane filters (Kiefer, 1992). Recently 0.45 μm membrane filters have become available with 0.1-0.8 m^3/m^2hr (Fillaudeau, 1999; Hoffmann, 1998). When V_{max} and Q_{init} are determined by the membrane filtration test, the flux at any time can be predicted from Eq. 8-3, assuming the laboratory and production filtrations are operated under the same conditions (or if a scale up relationship has been established). The unsheared, β-glucan-free beer (containing 3.3% w/w of real extract and 5% v/v of ethanol, pH 4.2) showed an initial flux of 1.98 m^3/m^2hr (Figure 8.3a). The addition of 31 kDa β-glucan up to 1000 mg/L had no significant effect on the Q_{init} ($p>0.05$). However, Q_{init} was decreased by increased concentrations of 137 kDa, 327 kDa and 443 kDa β-glucans ($p<0.001$). For an unknown reason, the addition of 250 kDa β-glucan did not affect the Q_{init} ($p>0.05$). The 443 kDa at a concentration of 1000 mg/L reduced the Q_{init} to 1.46 m^3/m^2hr (i.e., a 26% reduction). The decrease in Q_{init} was caused (at least in part) by higher beer viscosity (Figure 3.13a) according to D'Arcy's and Poiseuille's laws (Eq. 7-1 and 7-2). Shearing beer lowered the Q_{init} for all samples ($p<0.001$; Figure 8.3b). Even the control beer exhibited a lower Q_{init} after shearing. Q_{init} was decreased by high β-glucan MWs and high concentrations, as well as shearing treatment ($R^2=0.650$; $n=160$; $p<0.001$):

$$Q_{init} = 2.7567 - 3.78\times10^{-3}\,MW - 1.10\times10^{-3}\,C - 0.8443\,S + 3.69\times10^{-6}\,MW^2 + 6.32\times10^{-7}\,C^2 \qquad (8\text{-}7)$$

The 250, 327 and 443 kDa β-glucans resulted in very low Q_{init} after shearing (Figure 8.3b). The different responses of various β-glucans to shearing may help explain the sporadic difficulties in beer filtration. Patelakis (1999) has reported that shearing lowered V_{max} of beer containing 0.5% w/w of 327 kDa β-glucan ($p<0.05$) but did not affect the Q_{init} ($p>0.05$). Caution must be taken with his results since the data during the first 150 s were omitted which may have led to a less accurate analysis.

8.3.2 Membrane Filtration Performance of Sheared Beer after Cold Storage

The sheared beers containing 31-443 kDa β-glucans were stored at 4°C for 2 weeks to simulate the effect of lagering on beer turbidity (Figure 6.7) and filterability (Figure 8.4). V_{max} of the sheared control beer increased after "lagering" (from 0.41 to 0.72 m^3/m^2). Sheared beers containing 31 kDa and 137 kDa β-glucans also had improved V_{max} after lagering ($p<0.001$; Figure 8.4a). However, V_{max} of the beer samples containing 250, 327 and 443 kDa β-glucans decreased after the cold storage ($p<0.001$). Such reduced filterability can be partly explained by the settling and the resulting turbidity level of the samples (Figure 6.7). Higher turbidity in the storage beer resulted in lower V_{max} values (Siebert *et al.*, 1984).

Opposite trends were observed for Q_{init} of sheared beer after cold storage (Figure 8.4b). The Q_{init} decreased after "lagering" for sheared beers containing 31 kDa and 137 kDa β-glucans ($p<0.001$) compared to sheared beer prior to cold storage. Q_{init} increased for beer samples contained 250, 327 and 443 kDa β-glucans ($p<0.001$). However, the Q_{init} values were still lower than that of unsheared beers ($p<0.001$). Thus, the lagering process can not completely reverse poor filterability attributable to β-glucans. These β-glucan polymers in beer had particle size distributions primarily in the range of 0.01-0.1 μm diameter (Figures 4.3, 4.4 and 4.6). Such colloidal particles (i.e., $d<0.1$ μm) are stable in suspension and have an impact on membrane filtration. Figure 6.8 illustrates that the majority of β-glucan polymers remained in suspension after the two-week cold storage.

8.3.3 Influence of β-Glucan (443 kDa at 600 mg/L), Shearing Temperature, pH and Ethanol Content on the Membrane Filtration Performance of Beer

The influence of β-glucan, shearing, pH and ethanol on beer filterability are shown in Figures 8.5 and 8.6. The addition of the 443 kDa β-glucan at 600 mg/L lowered V_{max} for beer containing 0-10.0% v/v of ethanol at pH 3.8-4.6 ($p<0.001$; Figure 8.5). Higher pHs

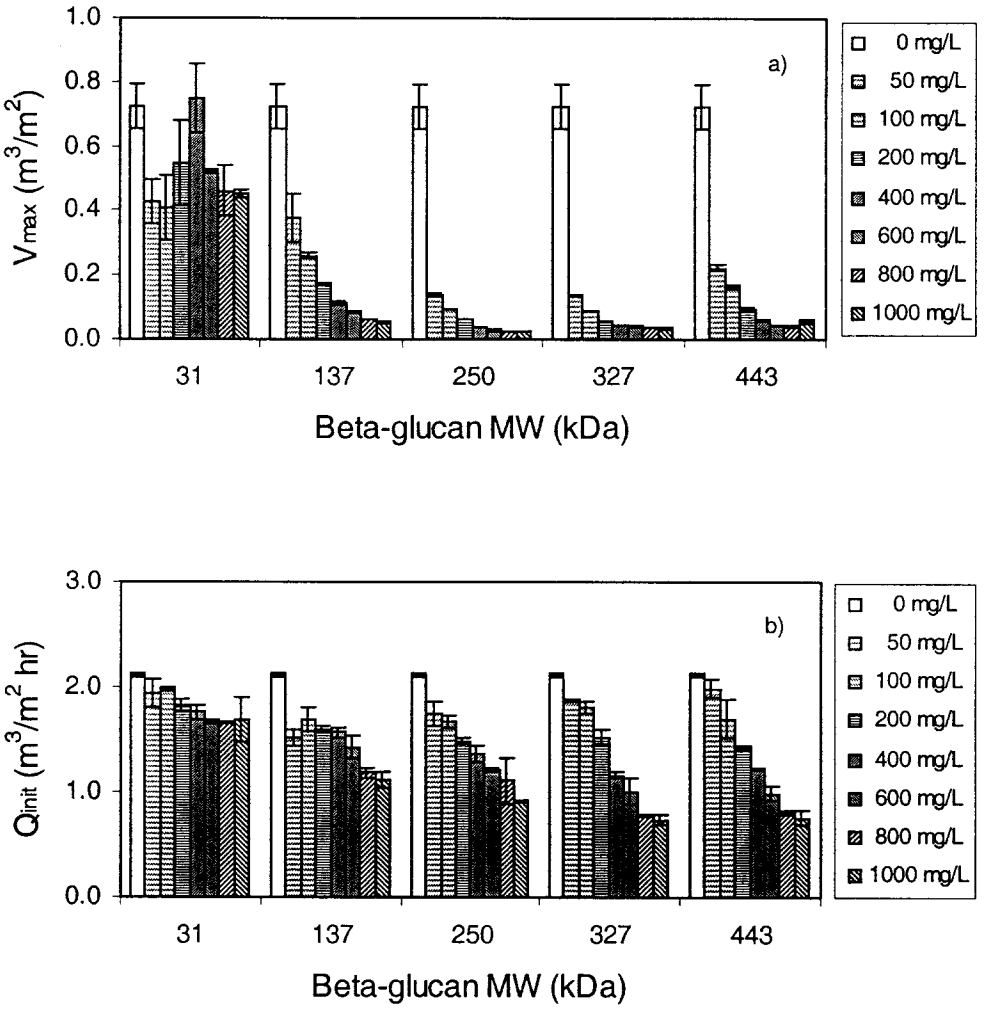

Figure 8.4 Effect of "lagering" at 4°C for 2 weeks on (a) V_{max} and (b) Q_{init} of sheared beer samples containing 0-1000 mg/L of β-glucans (31-443 kDa). Values are given as means ± S.D. of duplicate experiments.

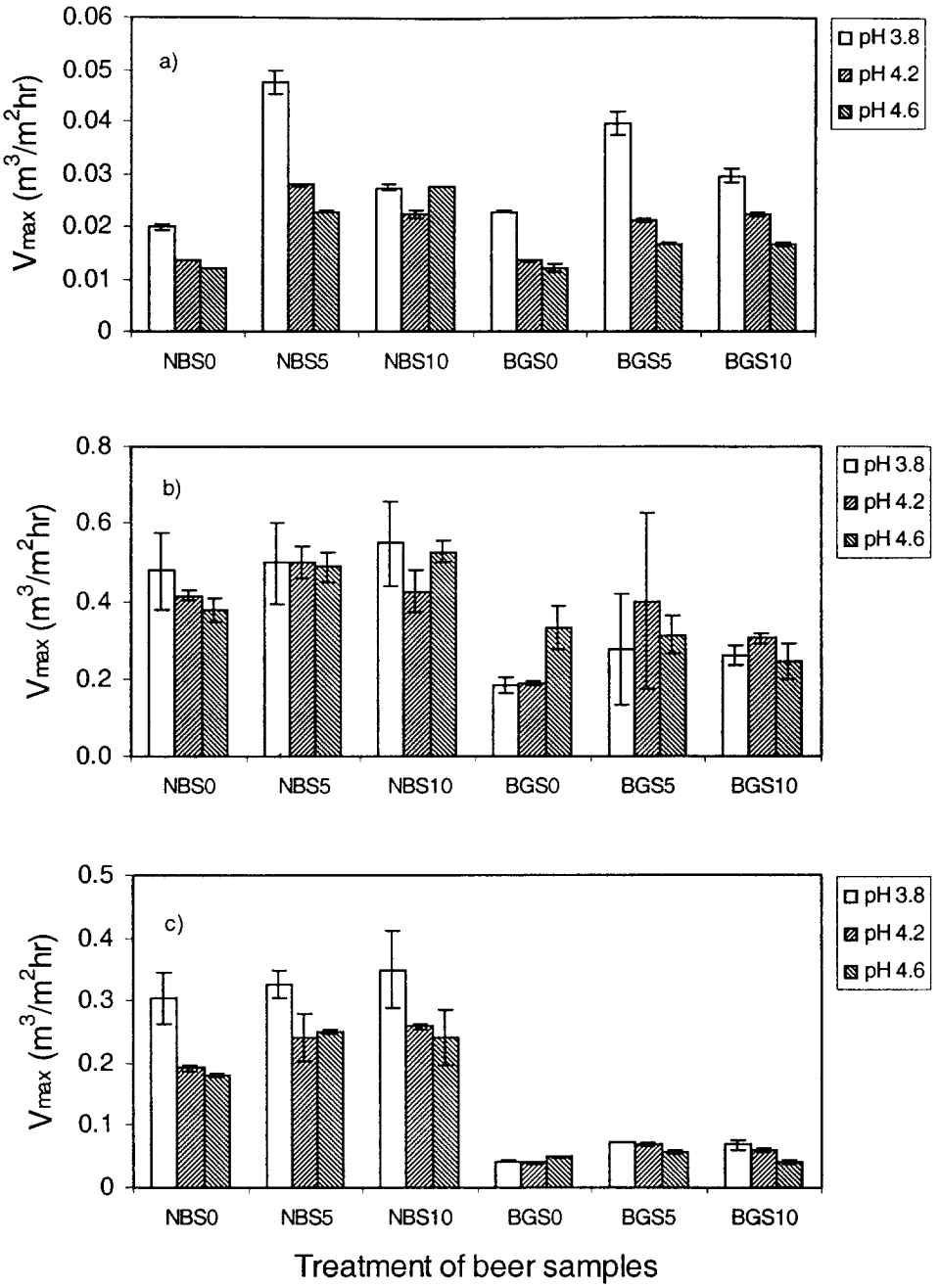

Figure 8.5 Effect of β-glucan, pH and shearing temperature on the V_{max} of beer samples containing ethanol at (a) 0%; (b) 5.0% and (c) 10.0% v/v. NB = no β-glucan; S0 = sheared at 0°C; S5 = sheared at 5°C; S10 = sheared at 10°C; BG = 600 mg/L of 443 kDa β-glucan. Values are given as means ± S.D. of duplicate experiments.

between 3.8-4.6 increased the V_{max} and Q_{init} ($p<0.001$). In the literature, Annemüller and Schnick (1999) suggested that optimum filterability could be obtained between pH 4.2-4.4. A recent study has found that the V_{max} of beer containing 5.0% v/v of ethanol and 327 kDa β-glucan at 250-5000 mg/L was higher at pH 3.6 than at pH 4.5 ($p<0.05$; Oonsivilai, 2000). The disagreement of the results may be due to the buffer system used by Oonsivilai, which contained no beer proteins.

The V_{max} increased with higher ethanol concentrations in the range of 0-10% v/v ($p<0.001$; Figure 8.5). However, the highest V_{max} was observed at 5% v/v of ethanol content due to its quadratic response to the ethanol levels ($R^2=0.653$; n=108; $p<0.001$):

$$V_{max} = 0.1766\ E + 2.78\times10^{-2}\ pH - 3.0\times10^{-4}\ C - 1.64\times10^{-2}\ E^2 \qquad (8\text{-}8)$$

where E is the ethanol content (% v/v) and C is the β-glucan concentration (mg/L). This finding is in agreement with the results by Oonsivilai (2000) that model "beer" containing 5.0% v/v of ethanol had higher V_{max} than 4% v/v or 6% v/v of ethanol ($p<0.05$). However, the V_{max} of beer containing 0.5% w/w of 327 kDa β-glucan with 4% v/v of ethanol was found to be approximately 8 times higher than that with 5% and 6% v/v of ethanol (Patelakis, 1999). The contradictions in the findings were believed to be a result of different β-glucan concentrations used. It was also observed that shearing temperature did not show any effect on V_{max} ($p>0.05$) since the effect of shearing on filterability was dependent on beer ethanol content and temperature.

The initial filtration rate was lowered by addition of the 443 kDa β-glucan at 600 mg/L ($p<0.001$; Figure 8.6). The multiple linear regression could describe the changes in Q_{init} ($R^2= 0.974$; n=108; $p<0.001$):

$$Q_{init} = 0.486\ pH - 1.19\times10^{-3}\ C - 0.166\ E + 9.88\times10^{-3}\ E^2. \qquad (8\text{-}9)$$

An increased Q_{init} with higher beer pHs in the range of 3.8-4.6 was also observed ($p<0.001$). In simulated buffer systems, Q_{init} was higher at pH 4.5 than pH 3.6 ($p<0.05$; Oonsivilai, 2000) although another study found that pH in the range of 3.6-4.5 did not affect Q_{init} of degassed beer containing 0.5% w/w of 327 kDa β-glucan ($p>0.05$;

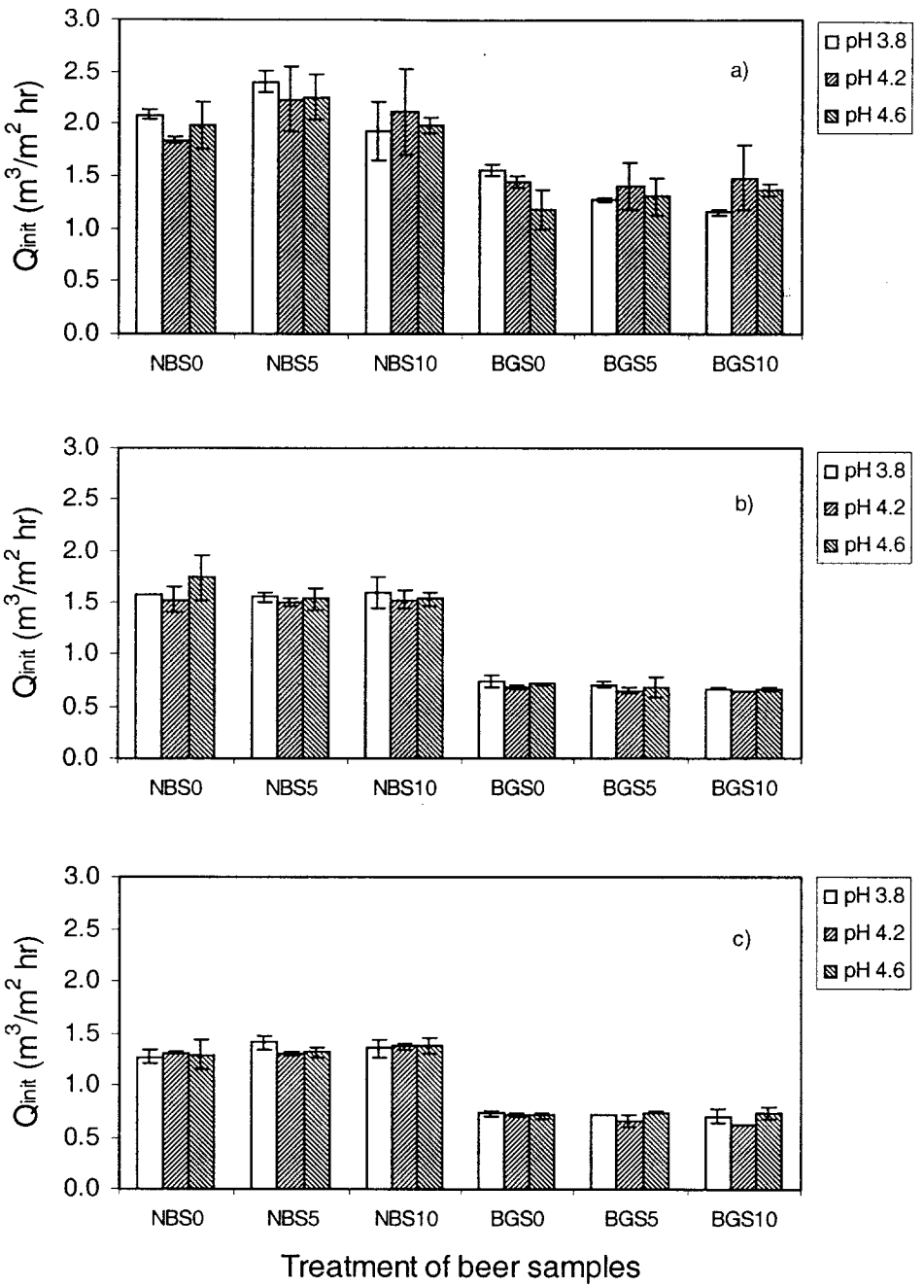

Figure 8.6 Effect of β-glucan, pH and shearing temperature on the Q_{init} of beer samples containing ethanol at (a) 0%; (b) 5.0% and (c) 10.0% v/v. Sample codes are given in Figure 8.5. Values are given as means ± S.D. of duplicate experiments.

Patelakis, 1999). Also, shearing temperature did not affect Q_{init} ($p>0.05$; Figure 8.6). Higher ethanol concentrations in the range of 0-10% v/v lowered Q_{init} for all samples ($p<0.001$). A recent study has found no effect on Q_{init} of ethanol concentrations between 4-6% v/v ($p>0.05$; Oonsivilai, 2000). In contrast, 4% v/v of ethanol resulted in a lower Q_{init} than 5% and 6% v/v of ethanol ($p<0.05$) although it showed a much higher V_{max} (Patelakis, 1999). These minor contradictions reflect the differences among beer/buffer systems used in the studies.

8.3.4 Influence of Shearing Temperature, pH and Ethanol Content on the Relative Filterability of Beer containing β-Glucans

The above results have shown that shearing and the presence of β-glucans lowered beer filterability. Filterability of the β-glucan-free beers was affected by shearing and ethanol concentration (Figures 8.2 to 8.6). In order to elucidate the behaviour of β-glucan polymers during membrane filtration, the relative filterability of beer was expressed as "relative V_{max}" and "relative Q_{init}". The relative filterability of the β-glucan-free beer was thus 100% by definition. The relative filterability indicates changes in filtration performance due to the addition of β-glucans under the same conditions. This technique cancels the effect of non-β-glucan components and avoids interference by variations among membranes from different batches.

Both relative V_{max} and relative Q_{init} were reduced by higher MW of β-glucans at higher concentrations and by shearing ($p<0.001$; Figures 8.7 and 8.8). Beta-glucans at higher MWs and higher concentrations are more viscous (Figure 3.3) and greater in their apparent particle size (Figure 4.1). Thus, the filtration rate became slower and the membranes were more readily clogged by the suspended β-glucan particles. Shearing temperature (0-10°C) did not affect the relative V_{max} or relative Q_{init} ($p>0.05$; Figures 8.9 and 8.10). Higher pH values in the range of 3.8-4.6 increased both the relative V_{max} and relative Q_{init} ($p<0.001$). Although β-glucans are theoretically neutral in charge, beer pH

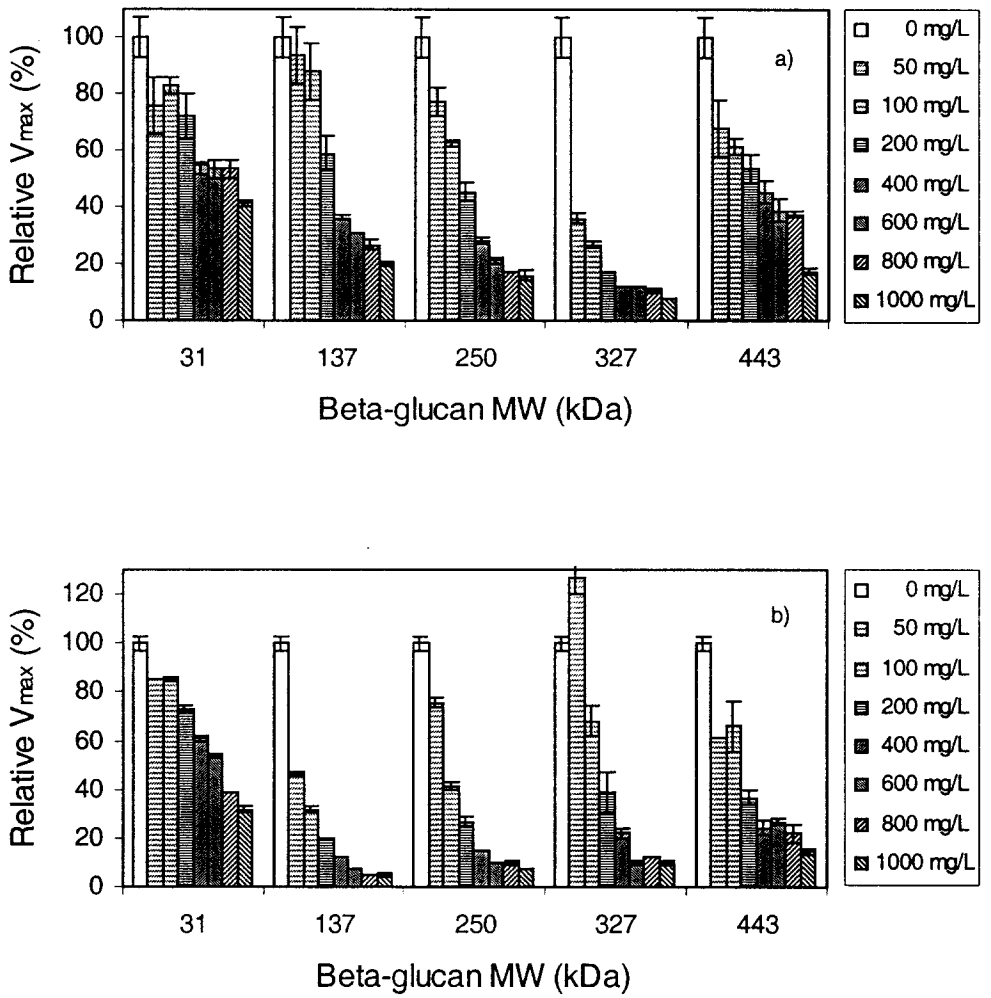

Figure 8.7 Effect of MW and concentration of β-glucans on the relative V_{max} of (a) unsheared and (b) sheared beer at 5°C. Values are given as means ± S.D. of duplicate experiments. .

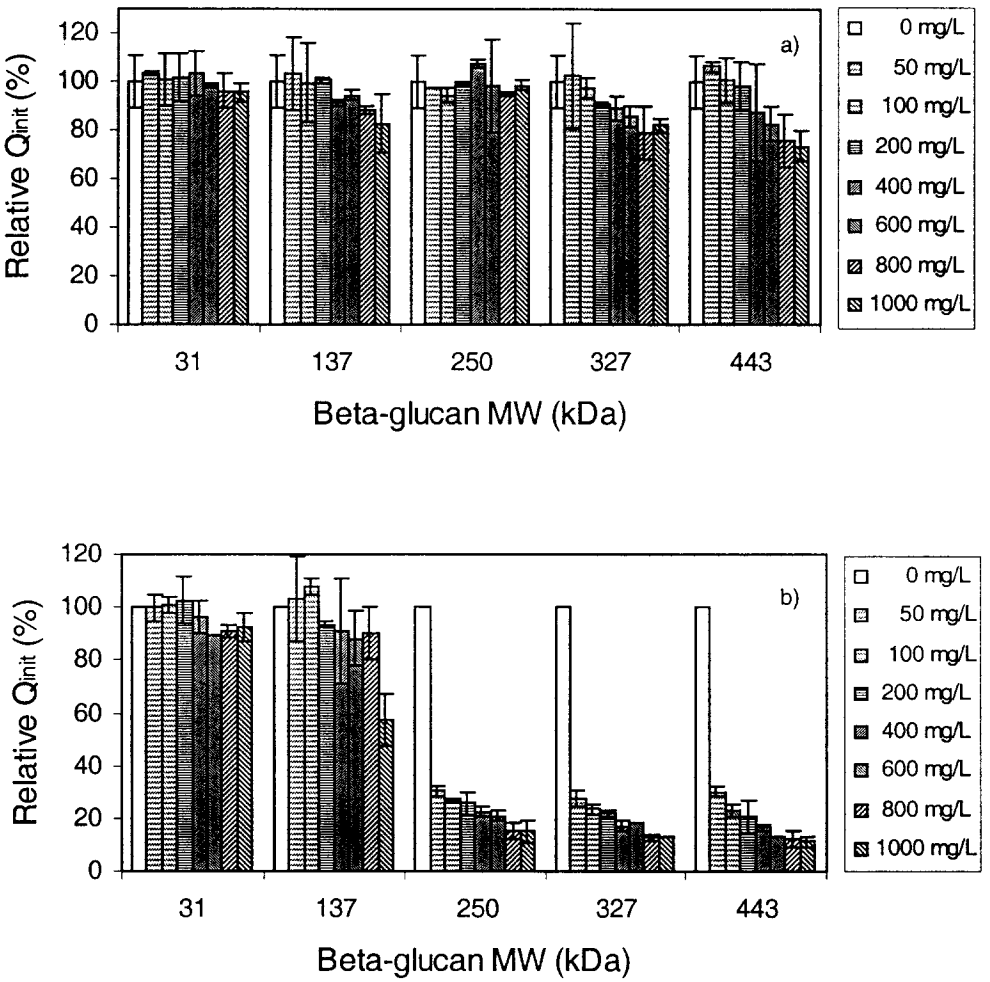

Figure 8.8 Effect of MW and concentration of β-glucans on the relative Q_{init} of (a) unsheared and (b) sheared beer at 5°C. Values are given as means ± S.D. of duplicate experiments.

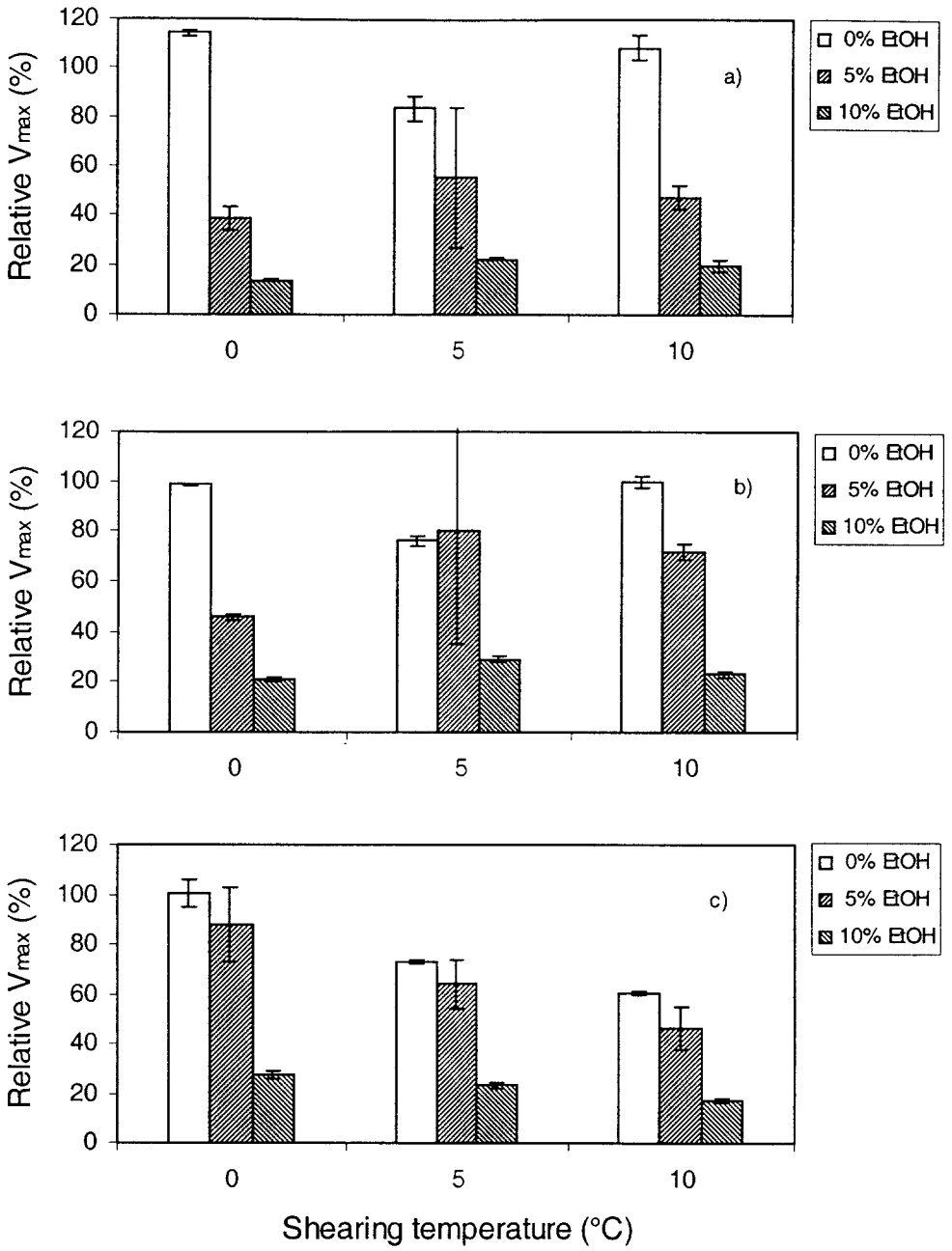

Figure 8.9 Effect of ethanol content and shearing temperature on the relative V_{max} of beer samples at (a) pH 3.8; (b) pH 4.0 and (c) pH 4.6. Values are given as means ± S.D. of duplicate experiments.

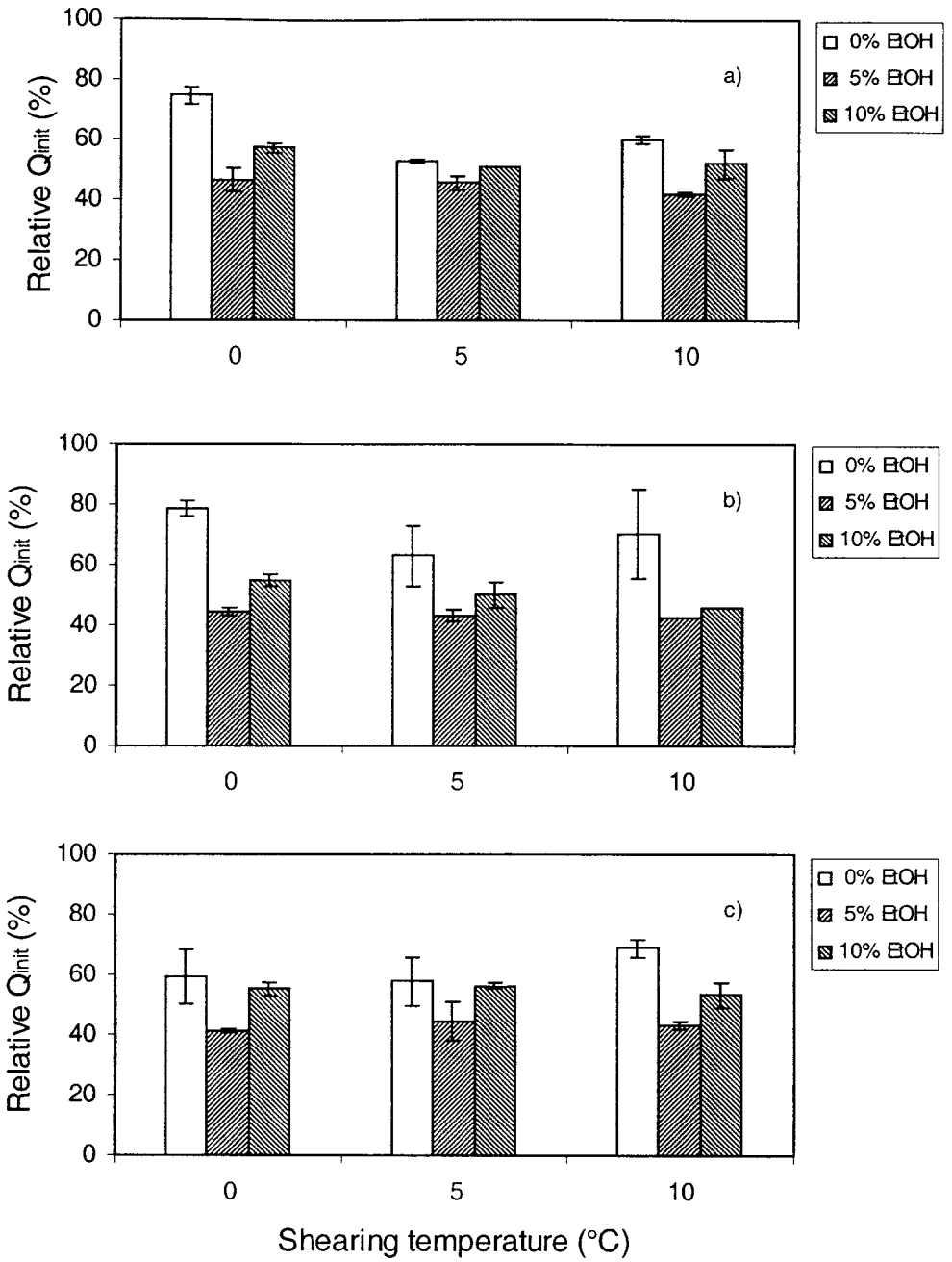

Figure 8.10 Effect of ethanol content and shearing temperature on the relative Q_{init} of beer samples at (a) pH 3.8; (b) pH 4.0 and (c) pH 4.6. Values are given as means ± S.D. of duplicate experiments.

may have affected their behaviour in membrane filtration by influencing the electrostatic interactions of the protein associated with β-glucan molecules. The 443 kDa β-glucan was found to contain 3.34% of protein (Table A.1 in Appendix 3) although it was not clear if the protein was bound to β-glucan molecules.

Ethanol content of beer affected both relative V_{max} and relative Q_{init} ($p<0.001$; Figures 8.9 and 8.10). Although the addition of ethanol improved V_{max} (Figure 8.5), it reduced the relative V_{max} and relative Q_{init} of beer ($p<0.001$; Figure 8.9). Thus, β-glucans have a larger impact on filterability at high ethanol concentrations. The filtration difficulties (i.e., reduced V_{max} and Q_{init}) due to β-glucans was worse at high ethanol concentrations (Figure 8.9 and 8.10). The mechanism of decreased filterability of the alcohol-free beer warrants further investigations.

8.4 Conclusions

Studies on membrane filtration have shown that the presence of β-glucans resulted in lower beer filterability. The trends of beer filterability corresponding to changes in β-glucan MW and concentration as well as processing conditions are summarized in Table 8.2. Both V_{max} and Q_{init} were lowered by higher β-glucan MWs and concentrations ($p<0.001$). Shearing beer decreased both V_{max} and Q_{init} ($p<0.001$) while shearing temperature (0-10°C) was not important ($p>0.05$). Between pH 3.8-4.6, higher pH values improved beer filterability ($p<0.001$). The addition of ethanol at 5-10% v/v decreased the initial filtration rate ($p<0.001$), but improved the V_{max} ($p<0.001$). Apparently, alcohol-free beer had much lower V_{max}. It was noted that lower filterability was associated with higher haze levels. However, the addition of ethanol at 5-10% v/v decreased the relative V_{max}, indicating that β-glucan caused filtration problems were worse at higher ethanol concentrations. Results also revealed that cold storage at 4°C for 2 weeks did not improve beer filterability ($p>0.05$).

Table 8.2 Effect of β-glucan and processing conditions on beer filterability

Treatment	Level	Response of V_{max}	Response of Q_{init}
β-Glucan MW	31-443 kDa	↓*** [a)]	↓***
β-Glucan conc.	0-1000 mg/L	↓***	↓***
Shearing	Unsheared / sheared	↓***	↓***
Shearing T	0, 5 and 10°C	NS	NS
pH	3.8, 4.2 and 4.6	↑***	↑***
Ethanol	0, 5, and 10% v/v	↑*** [b)]	↓*** [b)]
Cold storage	4°C for 2 weeks	(31 & 137 kDa ↑***; 250-443 kDa ↓***)	(31 & 137 kDa ↓***; 250-443 kDa ↑***)

a): The symbol ↑ represents an increased response upon a higher level of the treatment; ↓ indicates a lower response to the higher treatment levels; *** represents a significance level of $p<0.001$; NS denotes not significant ($p>0.05$); b): The addition of ethanol at higher concentration also resulted in lower values of both relative V_{max} and relative Q_{init} ($p<0.001$).

9. RAPID TECHNIQUES FOR THE IDENTIFICATION OF TECHNICAL PROBLEMS CAUSED BY β-GLUCANS

Barley β-glucan (443 kDa) was added to wort (12°P, pH 5.4) and beer (3.3% w/w of real extract and 5.0% v/v of ethanol, pH 4.2) in the range of 200-1000 mg/L at an interval of 200 mg/L. Samples were treated with shearing, hydrolysis by lichenase, addition of urea (3% w/v), and heating at 70°C for 1 hour. The physical and chemical properties of the samples including β-glucan content, viscosity, turbidity and filtration performance were then examined.

9.1 Introduction

Predictive measurements of wort and/or beer properties have become a major goal during brewing research on barley β-glucans. Determining the total β-glucan content alone cannot always predict the performance of beer filtration (Narziβ *et al.*, 1989a; Stewart *et al.*, 1998; Sudarmana *et al.*, 1996). Only the level of high MW β-glucans in wort and beer is believed to be correlated with beer filterability (Narziβ *et al.*, 1989a; Stewart *et al.*, 1998; Sudarmana *et al.*, 1996). Based on the hypothesis that hydrogen bonding is involved in association of β-glucan molecules, urea at 3% has proved to break up the non-dialyzable, high MW aggregates of β-glucans (Hinchliffe and Box, 1985; Letters, 1977). The difference in dialyzed β-glucans with and without 3% urea reflected the amount of aggregated β-glucans which, in turn, affected beer filterability (Hinchliffe and Box, 1985).

Shearing beer provides another technique to predict the potential problems of beer (Letters *et al.*, 1985). Turbulent shear (i.e., blending) of a 0.5% w/w β-glucan solution enhanced haze formation (Patelakis, 1999; Patelakis *et al.*, 1999). The tendency of β-glucan precipitation can also be indicated by viscosity reduction after lichenase treatment

(Grimm *et al.*, 1995b). Large viscosity reductions due to degradable β-glucans indicate a high risk of β-glucan precipitation (Grimm *et al.*, 1995b).

Upstream prediction methods such as wort membrane filtration prior to fermentation would be preferred by brewers. However, high deviations of membrane filterability of production wort made the detection of processing problems difficult (Siebert *et al.*, 1984). Lowering wort pH to that of beer and adding ethanol to wort at a level of beer ethanol content followed by equilibrating for about 18 hours improved the reliability of the membrane filtration prediction (Siebert *et al.*, 1984). An earlier detection method for problematic β-glucans has been proposed by analysis of the fine/coarse β-glucan difference (Narziβ, 1993; Wagner, 1999). Similar to measuring the fine/coarse extract difference, the β-glucan difference between the worts prepared from fine and coarse ground malt may indicate an extractable level of high MW β-glucans (which are expected to be greater in under-modified or unevenly modified malt; Narziβ, 1993; Wagner, 1999). However, it is difficult to accurately predict beer filterability since this depends on the malt used, the mashing and brewing conditions applied and the yeast employed (Wainwright, 1997). Also, when barley adjuncts are used, with or without exogeneous β-glucanases, the effect of malt high MW β-glucans on beer filterability becomes less apparent.

When a brewer is alerted to filtration difficulties related to β-glucans, appropriate actions may be taken to eliminate the potential problem. Beta-glucanases can be used to degrade β-glucan polymers (Forage and Letters, 1986; Sudarmana *et al.*, 1996). However, most commercial β-glucanase preparations are contaminated with protease activities, which may degrade beer proteins and affect beer foaming properties. Heat dissociation of the aggregated β-glucans has been proposed as a short-term means to eliminate filtration problems caused by β-glucans in the packaging plant. Heating beer to 70°C or flash heating beer to 76-80°C solubilizes β-glucan aggregates and filtration becomes easier

upon cooling down the beer (Esser, 1996; Annemüller and Schnick, 1999). These observations agree with the finding that β-glucan precipitates dissociate during heating (Letters, 1995a). However, heat treatment is only a temporary solution and may affect the beer flavor stability. In practice, if beer is heat treated above the pasteurization temperature, there is no reason to filter it through membranes. Ideally, the membrane filtration difficulties caused by β-glucans (and/or other polymers) can be identified before beer arrives at the packaging plant, allowing the personnel to decide what actions they can take.

The purpose of this study was to develop a quick diagnostic method to predict process difficulties due to β-glucans. An objective was to evaluate the effectiveness of the predictive treatments in identification of the technical problems caused by β-glucans.

9.2 Materials and Methods

Wort free of β-glucans was prepared from pale malt as described in section 3.2.2. A barley β-glucan at 443 kDa purchased from Megazyme International Ireland Ltd. (Bray, IRL) was used to prepare wort and beer samples with β-glucan concentrations in the range of 0-1000 mg/L at intervals of 200 mg/L. A commercial lager beer (Labatt Blue, product code E10H11C, UBC 062067351013, Oland Breweries Ltd., Halifax, NS) was used to prepare a beer base free of β-glucans (section 3.2.4). This β-glucan-free beer base was used in preparation of beer samples varying in β-glucan concentration.

Wort and beer samples were treated with shearing, 0.1 U/mL of lichenase, (i.e., (1→3)(1→4)-β-D-glucan 4-glucanohydrolase from Megazyme International Ireland Ltd., Bray, IRL), 3% w/v of urea (equivalent to 0.5*M*), and heating in a 70°C water bath for 1 hour followed by cooling in water baths to 20°C for wort and 5°C for beer, respectively. Shearing of wort and beer samples was carried out with the procedure described in section 3.2.6. The preliminary experiments indicated that 30 minutes is long enough to

hydrolyze β-glucans up to 1000 mg/L in wort and beer samples with 0.1 units of lichenase activity per mL (U/mL) at 50°C and natural pH values (i.e., pH 5.4 for wort and pH 4.0 for beer). One unit of lichenase activity is defined as the amount of enzyme required to release 1 μmol glucose per minute from 10 mg/mL of barley β-glucan substrate at pH 6.5 and 40°C (McCleary and Mugford, 1997). An example of the hydrolysis of beer β-glucans (443 kDa) is shown in Figure A.4 in Appendix 5. Beta-glucan concentration of samples was determined with the Congo red binding assay (section 3.2.7). Wort and beer viscosities were determined with a VOR rheometer (sections 3.2.8). Turbidities of wort and beer were measured by 90° light scattering at 580 nm (section 6.2). Methods of wort DE filtration and beer membrane filtration were described in sections 7.2 and 8.2, respectively. Colour of the membranes after filtration of the β-glucan-free beer was measured after the membranes were naturally dried in the open air at ambient temperature for 2 hours. Colour was determined with a Chroma Meter CR-300 (Minolta Camera Co. Ltd., Osaka, JPN) and recorded as the Hunter (L, a, b) co-ordinates. The instrument was calibrated with a white calibration plate CR-A43 number 20233133 giving (L, a, b) co-ordinates at (97.75, -0.17, 2.29).

The experimental design of this study is illustrated in Table 9.1. Duplicate experiments were carried out and mean values and standard deviations were reported. Regressions and ANOVA of experimental results were conducted with statistical software SYSTAT version 5.05 (SPSS Inc., Chicago, IL).

9.3 Results and Discussion

To identify processing difficulties due to β-glucans, wort (12°P, pH 5.4) and beer (pH 4.2 containing 3.3% w/w of real extract and 5% v/v of ethanol) were treated with lichenase hydrolysis, shearing, urea (3% w/v) and heating at 70°C for 1 hour. Results of viscosity, turbidity and filterability are reported and discussed in this section.

Table 9.1 Treatments of wort and beer to predict problems caused by β-glucans

Sample	Treatment	Level
Wort [a)]	Control	–
	Shearing	Blender setting "60" for 35 s, sheared at 20°C
	Lichenase	0.1 U/mL, treated at 50°C for 30 minutes
	Urea	3% w/v at 20°C
	Heating	Heated in a 70°C water bath for 1 hour
Beer [b)]	Control	–
	Shearing	Blender setting "60" for 35 s, sheared at 5°C
	Lichenase	0.1 U/mL, treated at 50°C for 30 minutes
	Urea	3% w/v at 5°C
	Heating	Heated in a 70°C water bath for 1 hour

[a)]: Wort (12°P) at pH 5.4, containing the 443 kDa β-glucan at 0-1000 mg/L;
[b)]: Beer having 3.3% w/w of real extract and 5.0% v/v of ethanol at pH 4.2, containing 31-443 kDa β-glucans at 0-1000 mg/L.

9.3.1 Effect of Diagnostic Treatments on Viscosities of Wort and Beer

When correlated with β-glucan MW and concentration, the viscosity of wort and beer can indicate potential problems due to β-glucans. Following treatments with shearing, lichenase, urea and heating, wort and beer viscosities were measured at 20°C and 5°C, respectively. Beta-glucan (443 kDa) was more viscous in beer than in wort (Figure 9.1; results of specific viscosity not shown), which is in agreement with earlier findings (Chapter 3). Shearing wort at 20°C and shearing beer at 5°C did not affect viscosity ($p>0.05$). Incubation with lichenase at 50°C for 30 minutes hydrolyzed β-glucans to undetectable levels by Congo red dye (Figure A.4) and reduced wort and beer viscosities close to the control samples (Figure 9.1). Wort viscosity increased after the addition of urea at 3% w/v ($p<0.001$) and heating at 70°C for 1 hour ($p<0.01$). However, beer viscosity was not affected by either urea or heating ($p>0.05$). Shearing resulted in higher wort viscosity previously (Figures 3.3 and 3.10a) but did not show any effect in this experiment. It is difficult to explain the inconsistent effects of shearing on wort viscosity. While using viscosity as an indicator, only lichenase hydrolysis was able to distinguish

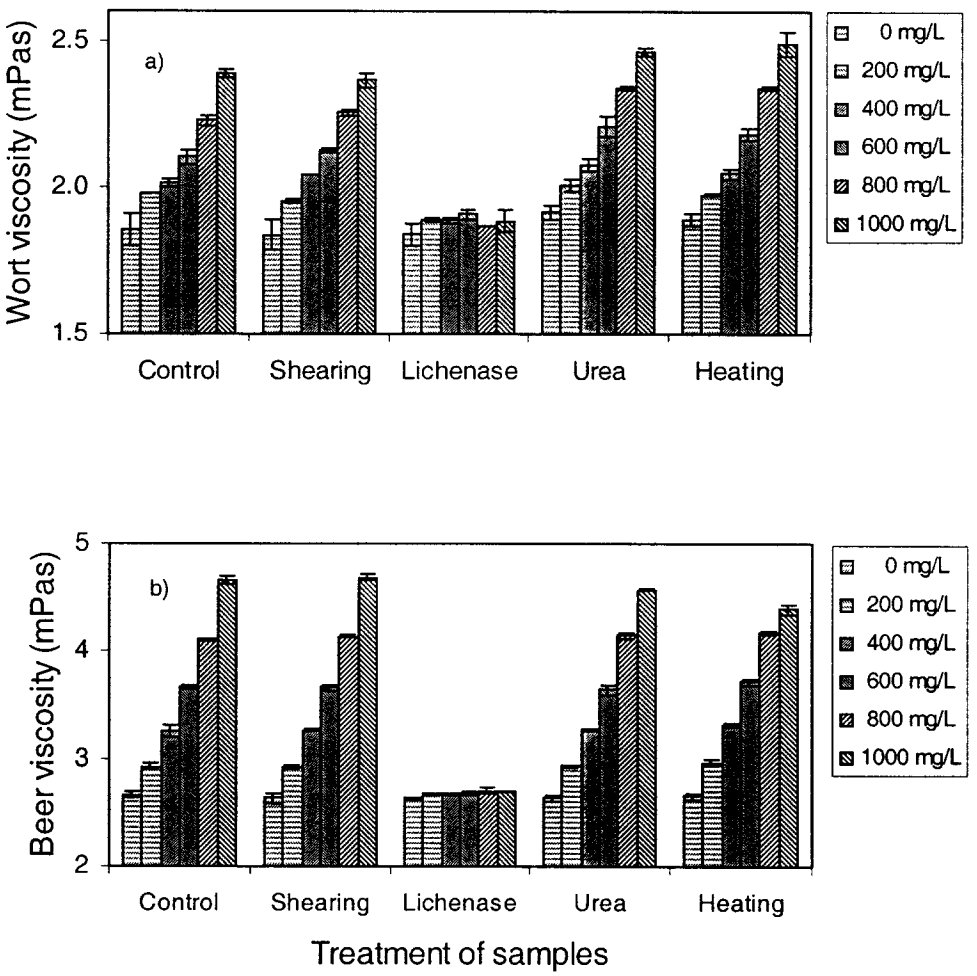

Figure 9.1 Effect of diagnostic treatments on viscosities of (a) wort at 20°C and (b) beer at 5°C. Values are given as means ± standard deviation (S.D.) of duplicate experiments.

the samples containing β-glucan from β-glucan-free samples. Such a viscosity differential represents the viscosity due to β-glucan in wort and beer and is a function of solvent conditions and the MW and concentration of β-glucans. Thus, the β-glucan viscosity difference is a better parameter than the concentration of total β-glucan or the high MW fraction of β-glucans. Although addition of urea and heat treatment increased wort viscosity, they did not affect the viscosity of beer. Only hydrolysis by lichenase was effective for distinguishing samples with and without β-glucan.

9.3.2 Effect of Diagnostic Treatments on Turbidities of Wort and Beer

The presence of β-glucans in wort and beer resulted in higher turbidity (Chapter 6). As a quick diagnostic test, 12°P wort (pH 5.4) and degassed beer (pH 4.2, containing 3.3% w/w of real extract and 5.0% v/v of ethanol) were examined for their changes in turbidity (20°C) after the various treatments (Figure 9.2). Shearing wort (20°C) and beer (5°C) caused higher turbidity levels ($p<0.001$). This is in agreement with the results in Figures 6.2 and 6.4 and in the literature (Patelakis, 1999). However, the increased turbidity of beer was independent of the β-glucan level. This disagrees with an early finding that the increased turbidity caused by shearing was proportional to β-glucan concentration (Figure 6.4b). Thus, it was found that shearing does not always cause an increase of beer turbidity by β-glucans.

Heating samples at 70°C for 1 hour decreased their turbidity ($p<0.001$). It is believed that heating at 70°C of wort and beer (followed by cooling to 20°C) breaks up the associated macromolecules of β-glucans and proteins. The turbidity of β-glucan-free wort decreased by 1.7 FTU after heat treatment. Wort samples containing 200-1000 mg/L of β-glucan had an "average" decrease of turbidity by 2.9 FTU. Heat treatment of the β-glucan-free beer caused a decrease in turbidity of 23.5 FTU, while the average decrease in turbidity

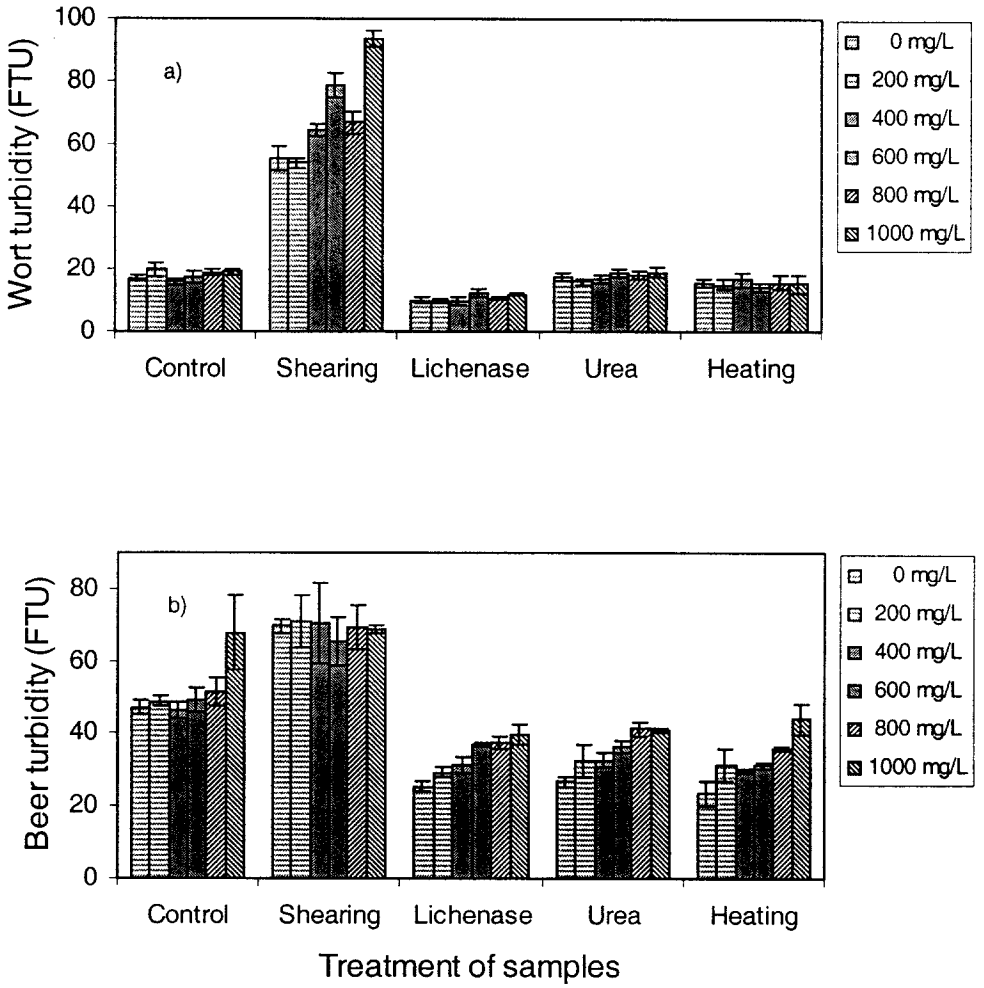

Figure 9.2 Effect of diagnostic treatments on the turbidities of (a) wort and (b) beer samples. Values are given as means ± S.D. of duplicate experiments.

for beers containing 200-1000 mg/L β-glucan (443 kDa) was 18.3 FTU. This suggests that the decrease of turbidity caused by heat treatment be mainly due to the effect on other components in beer rather than on β-glucan. Similarly, the addition of 3% w/v urea decreased turbidity by 20.5 FTU for the β-glucan-free beer and 16.0 FTU for beers containing β-glucan. This indicates that the urea-induced decrease in turbidity was a result of protein dissociation since urea is a destabilizer of hydrogen bonding.

The hydrolysis of β-glucans with lichenase lowered the turbidities of wort and beer ($p<0.001$). Interestingly, the decrease of turbidity caused by lichenase was 7.1 FTU for the β-glucan-free wort and 7.5 FTU for wort containing β-glucan, respectively. With beer samples, the decrease in turbidity after lichenase treatment could not distinguish β-glucan-free beer (21.7 FTU) and samples containing β-glucan (a mean value of 17.8 FTU). Incubation with lichenase at 50°C for 30 minutes lowered turbidity due to the heat treatment. Also, the lichenase preparation (1000 U/mL) was supplied in a suspension of 3.2 *M* $(NH_4)_2SO_4$. The addition of lichenase at 0.1 U/mL introduced an amount of 0.32 m*M* of ammonium sulfate, which may have a salting-in effect on proteins involved in wort turbidity and beer haze particles. It is unlikely that the enzyme preparation contained trace proteolytic activity which lowered the protein haze level, since lichenase was purified by affinity chromatography and all bands of the enzyme from isoelectric focusing had endo-(1-3)(1-4)-β-glucanase activity (McCleary, personal communication, 2002; Megazyme, 1999). From the above results, it can be concluded that turbidity is not a satisfactory parameter to employ as a diagnostic for the detection of β-glucan problems.

9.3.3 Effect of Diagnostic Treatments on the Filtration Performance of Wort and Beer

Worts containing 200-1000 mg/L of 443 kDa β-glucan were examined for their DE filtration at 76°C and 0.45 μm membrane filtration at 20°C (using methods discussed in sections 7.2.2 and 7.2.3) after the diagnostic treatments (Figure 9.3). Shearing wort at 20°C lowered the membrane filtration rate ($p<0.001$) but did not affect DE filtration

index at 76°C ($p>0.05$) because the filtration index decreased for worts containing 200-600 mg/L of β-glucan but increased at 800-1000 mg/L of β-glucan after shearing ($p<0.001$). Lichenase treatment lowered the DE filtration index ($p<0.05$) and improved the relative flux of membrane filtration ($p<0.01$). The addition of urea and heat treatment did not affect the rate of membrane filtration ($p>0.05$), but increased the DE filtration index ($p<0.001$). The increased wort "lautering" difficulty was believed to be due to the higher viscosity caused by urea and heating (Figure 9.1). Combined with shearing or lichenase treatment, the relative flux of wort membrane filtration is one potential diagnostic parameter for identification of the problems caused by β-glucans. The filtration index (i.e., slope of the DE filtration plot), however, is not a suitable indicator.

The membrane filtration performance of beer is one of the parameters that is readily affected by β-glucans. In beer "sterile filtration", membranes with 0.45 μm or smaller pore ratings are used. The membrane filtration test is an easy and direct way to predict the filterability of a particular batch of beer. Combined with one of the above treatments, it can also determine if a filtration problem is caused by β-glucan. In this study, membrane filterability was examined at 5°C for control beer (pH 4.2, containing 3.3% w/w of real extract and 5.0% v/v of ethanol) and beer containing 200-1000 mg/L β-glucan (443 kDa) to study the feasibility of the proposed diagnostic treatments. Shearing beer at 5°C decreased the initial filtration rate but increased the V_{max} ($p<0.001$; Figure 9.4). The decrease in Q_{init} was caused by higher turbidity after shearing (discussed in Figure 9.2). However, the increase of V_{max} after shearing is a contradictory finding compared to the results in Figure 8.2 where shearing lowered the V_{max}. Even the β-glucan-free beer had an increased V_{max} (Figure 9.4a). V_{max} of beers containing β-glucan relative to that of β-glucan-free beer, however, was lower after shearing ($p<0.001$). After treatment with lichenase, beer samples, with or without β-glucan, showed improved V_{max} and Q_{init} values ($p<0.001$). The increased filterability of the β-glucan-free beer was due in part to lower turbidity after treatment (Figure 9.2). The addition of lichenase did not shift filtration rate (Q_{init}) of β-glucan-free beer from those containing very high concentrations

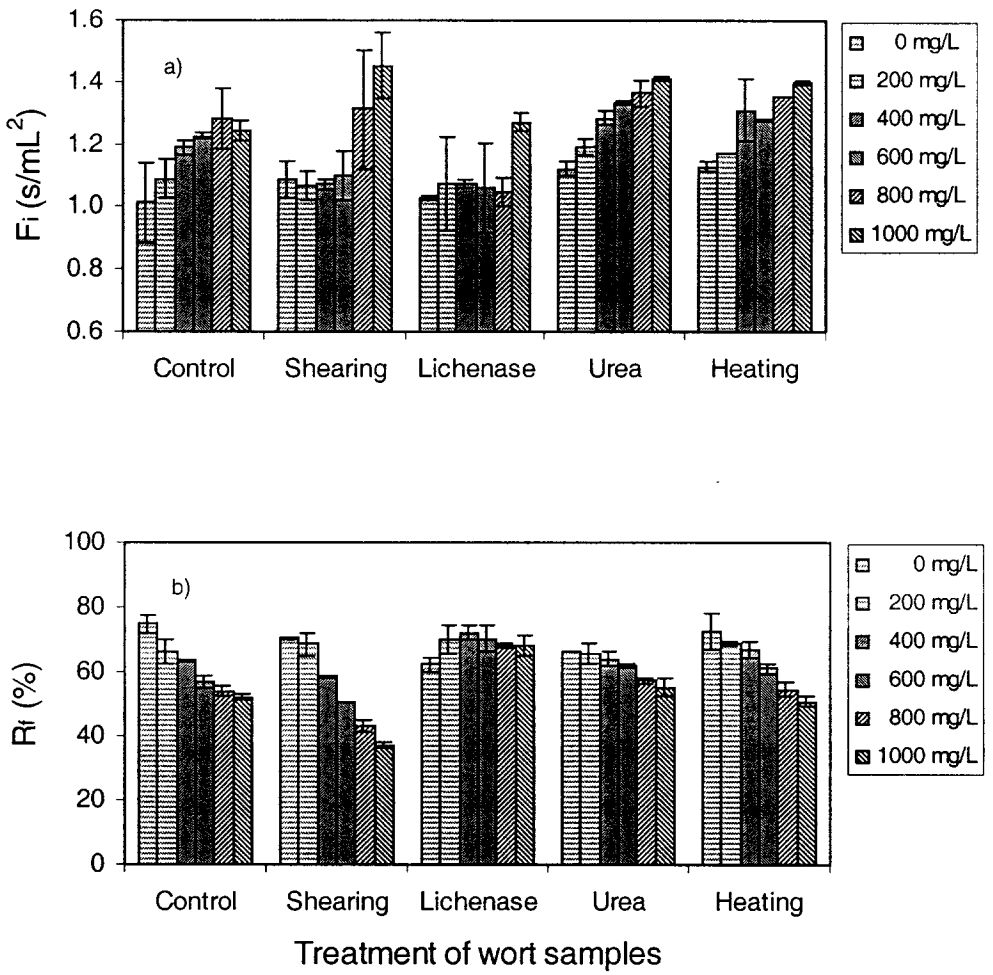

Figure 9.3 Effect of shearing, lichenase, urea and heating on (a) DE filtration index at 76°C and (b) relative flux at 20°C of wort samples. Values are given as means ± S.D. of duplicate experiments.

of high MW β-glucans. Early studies have shown that β-glucanase can improve beer membrane filterability (Forage and Letters, 1986; Sudarmana *et al.*, 1996). However, the effect of β-glucanase on beer filterability at low β-glucan levels has not been examined in the literature. The addition of urea at 3% w/v led to much better filterability of all beers ($p<0.001$). This is in agreement with the lowered beer turbidity (Figure 9.2). However, adding urea is not an effective method to differentiate between potential problematic samples. Heating beer at 70°C for 1 hour increased Q_{init} ($p<0.001$). Heating also increased V_{max} of β-glucan-free beer (p0.001) but decreased V_{max} of beer containing 200-1000 mg/L of β-glucan ($p<0.01$). It is hypothesized that heating at 70°C followed by cooling to 5°C was favourable to the initial filtration rate but lowered the total volume of beer which can be processed through a filter. Thus, heat treatment and membrane filtration can not be used to predict problematic beers due to β-glucan because the filterability of the β-glucan-free control was also affected.

It was noted that some of the treatments affected the appearance of the retentates on membranes. The colour and darkness of the retentates after filtration of 40 mL of beer varied among the treatments but these were not affected by the levels of the 443 kDa β-glucan. The membranes shown in Figure 9.5 occurred after filtration of the β-glucan-free beer. Their appearance was evaluated by the Hunter (L, a, b) tristimulus colourimetry (Table 9.2). The L value represents lightness ranging from black (L=0) to white (L=100). Values of the a axis indicate red (positive readings) and green (negative readings). Values of the b axis reflect yellow (positive readings) or blue (negative readings). The darkness of the membrane surface was increased by shearing, lichenase and heating ($p<0.001$) as well as urea ($p<0.05$). All treatments increased the redness of the membrane colour, (i.e., A values; $p<0.001$; Table 9.2). The yellowness of the membranes was increased by shearing, lichenase and heating ($p<0.001$) but reduced by urea ($p<0.001$). Lichenase treatment caused the least change in the appearance of the retentates. It is implied that lichenase causes less changes in the non-β-glucan components and is preferred as a diagnostic analysis of beer filterability.

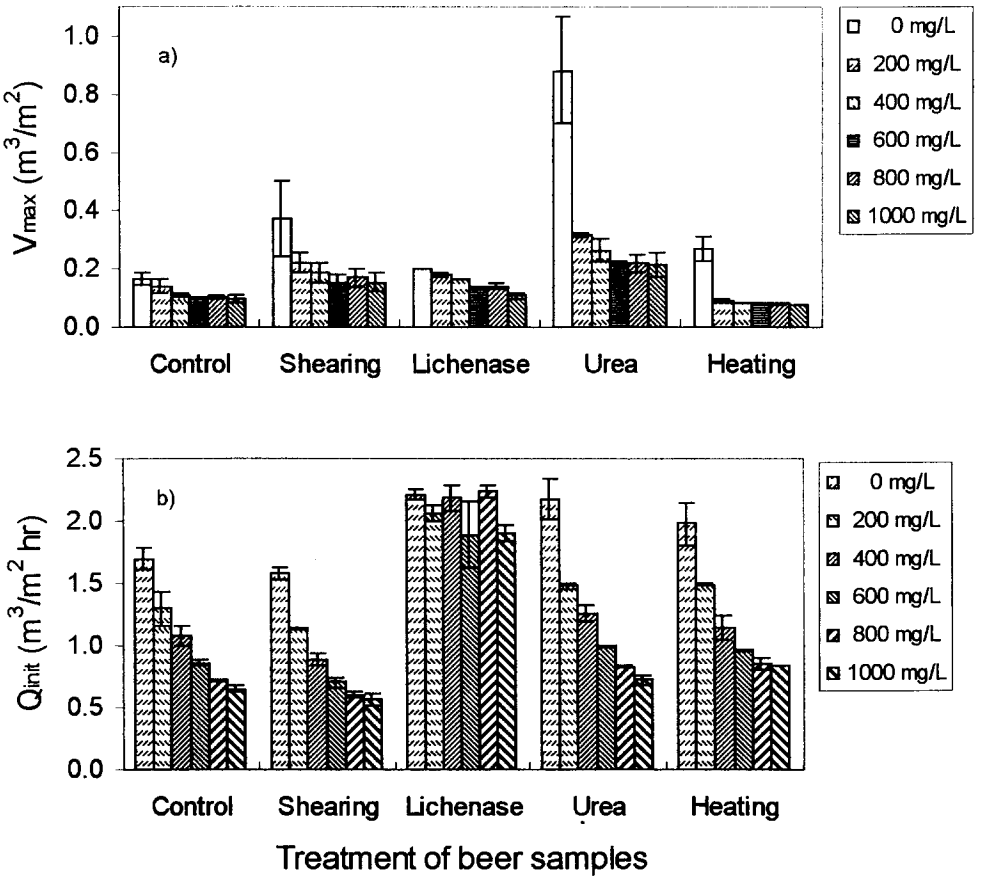

Figure 9.4 Effect of shearing, lichenase, urea and heating on beer filterability (a) V_{max} and (b) Q_{init} at 5°C. Values are given as means ± S.D. of duplicate experiments.

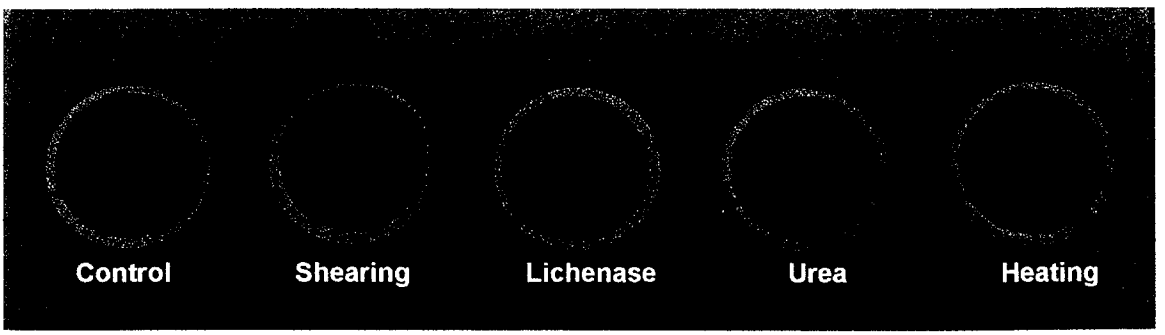

Figure 9.5 Appearance of the membranes after filtration of β-glucan-free beer.

Table 9. 2 Hunter (L, a, b) colourimetric measurement of membranes [1)]

Treatment	L [2)]	a [3)]	b [4)]
Unsheared	77.39 ± 0.20	4.34±0.04	20.97 ± 0.07
Sheared	68.97 ± 0.12	7.01±0.06	23.99 ± 0.04
Lichenase	72.30 ± 0.10	6.03±0.06	23.83 ± 0.11
Urea	77.00 ± 0.13	4.61±0.05	17.05 ± 0.14
Heating	67.54 ± 0.31	7.52±0.14	24.91 ± 0.20

[1)]: Values are given as means ± S.D. of replications (n=5); new membranes had (96.99, -0.35, -0.61); [2)]: Urea treated sample differed from the control (unsheared) ($p<0.05$) and all other sample pairs differed from one another at $p<0.001$; [3)]: All differed from one another ($p<0.001$); [4)]: The sheared and lichenase treated beer did not differ ($p>0.05$) while other sample pairs differed ($p<0.001$) to one another.

9.4 Conclusions

Results of the diagnostic study of β-glucans in wort and beer are summarized in Table 9.3. Shearing, urea and heat treatments are not satisfactory in predicting process problems due to β-glucan. Although freezing and thawing of beer enhances precipitation of β-glucans (Haas and Fleischman, 1964; Letters, 1977; Casey and Ingledew, 1985; Skinner *et al.*, 1993; Takayanagi *et al.*, 1969; Tanaka and Sakuma, 1999), the test process is time consuming. Also, the method is non-specific (i.e., the precipitate contains proteins and α-glucans as well; Tanaka and Sakuma, 1999).

Examination of wort and beer viscosities before and after lichenase treatment (0.1 U/mL, natural pH of sample, 50°C for 30 minutes) is recommended as a diagnostic test to predict potential haze and filtration problems due to β-glucans. Since the viscosity of β-glucans in wort and beer is related to their MW and concentration in a given solvent system, it is not required to separate the low and high MW fractions. In practice, dialysis of the lichenase preparation can avoid any possible interference by the ammonium sulfate stabilizer present in the enzyme preparation.

Table 9.3 Effect of the diagnostic treatments on the processing properties of beer

Sample	Treatment	Viscosity	Turbidity	Filterability	
Wort				*Lautering slope*	*Relative flux*
	Shearing	NS	↑***	NS	↓***
	Lichenase	↓***	↓***	↓*	↑**
	Urea	↑***	NS	↑***	NS
	Heating	↑**	↓***	↑***	NS
Beer				V_{max}	Q_{init}
	Shearing	NS	↑***	↑***	↓***
	Lichenase	↓***	↓***	↑***	↑***
	Urea	NS	↓***	↑***	↑***
	Heating	NS	↓***	↓**	↑***

Note: The symbol "↑" represents an increased response to the treatments; "↓" indicates a decreased response to a treatment; ***: $p<0.001$; **: $p<0.01$; *: $p<0.05$; NS = not significant ($p>0.05$).

Caution must be taken since the above diagnostic test was based on the results from pure barley β-glucan. The method warrants further study with regard to behaviour of the "native" β-glucans in wort and beer. There is a report that haze particles <0.22 μm in beer contained half β-glucan, 25% α-glucan and 25% protein, and the β-glucan was not susceptible to β-glucanase due to the protein coating (Jackson and Bamforth, 1983). Some processing problems may be caused by a combination of various polymers in beer.

10. SUMMARY

The findings of this thesis are generally discussed and summarized in this chapter. Further research needs are also briefly discussed.

10.1 Overall Discussion and Concluding Remarks

The behaviour of barley β-(1→3)(1→4)-D-glucans in wort and beer was examined to investigate their influence on the brewing process and beer quality. The objectives were to determine the effect of shearing on the particle size distribution of β-glucan polymers in wort and beer and their contribution to viscosities as well as haze of wort and beer; to test the hypothesis that wort and beer filterability is affected by β-glucan MW and concentration; to develop a rapid and simple upstream method for the detection of problematic β-glucan polymers; and lastly to examine the effect of β-glucans on the interfacial and foaming properties of wort and beer.

Beta-glucans of MWs of 31, 137, 250, 327 and 443 kDa at concentrations of 50, 100, 200, 400, 600, 800 and 1000 mg/L were added to β-glucan-free wort and beer and subjected to various levels of shearing (sheared and unsheared, shearing at 20, 48, and 76°C for wort, at 0, 5 and 10°C for beer), pH (4.0, 5.4 and 6.8 for wort, 3.8, 4.2 and 4.6 for beer), maltose concentration (6.1, 10.1 and 16.1% w/w for 8, 12 and 18°P worts, respectively), and ethanol concentration (0, 5 and 10% v/v in beer at pH 4.2 and containing 3.3% w/w of real extract). The parameters examined include viscometric properties, apparent particle size of β-glucans, wort and beer turbidity, wort DE filtration index, beer membrane filtration V_{max} and Q_{init}, surface tension of wort and beer, beer foamability and foam stability. A better understanding of β-glucan behaviour in brewing is of help to develop predictive and diagnostic tests for the problems due to β-glucans. Diagnostic treatments including heating, addition of urea, endo-(1→3)(1→4)-β-glucanase from *Bacillus subtilis* (i.e., lichenase) and shearing were employed to

investigate their feasibility to predict the processing difficulties due to β-glucans.

The apparent particle size of β-glucans in wort and beer increased with their MW and concentration, leading to higher turbidity and lower filtration efficiency ($p<0.001$). Solvent conditions affected the distribution of β-glucan particles, turbidity, and filtration ($p<0.001$). As an overall trend, β-glucans had greater size, and higher viscosity and turbidity in beer than in wort ($p<0.001$). Beta-glucans had greater particle size at higher pH values in wort and beer ($p<0.001$). Maltose showed no significant influence ($p>0.05$) on β-glucan particle size distributions in wort. Ethanol at higher levels increased the proportion of the 0.01-0.1 μm ("equivalent diameter") fraction and decreased the amount of >0.1 μm fraction of the 443 kDa β-glucan in beer. Shearing of wort and beer resulted in a decrease in the <0.01 μm fraction of β-glucans and an increase in the 0.01-0.1 μm β-glucan fraction ($p<0.001$). However, shearing temperature did not show any significant effect on β-glucan particle size distribution ($p>0.05$). The fraction of >0.1 μm β-glucans accounted for only 12-14% in all the samples studied. This implies that purified β-glucans (31-443 kDa) in beer are not likely to initiate plugging of the 0.45 μm membrane pores because only particles >0.45 μm in beer can retard the filterability by clogging (Ilberg *et al.*, 1995). However, if other beer components such as proteins and protein-polyphenol complexes plug the membrane pores in the early stage of filtration, the actual pore size will decrease and thus retain β-glucan polymers on the membrane surface or capillary path of flow. Consequently, clogging of the 0.45 μm membranes by β-glucan polymers could be observed later.

Experiments were carried out with clear wort and beer. Addition of β-glucans increased turbidity of wort and beer at higher MWs and concentrations ($p<0.001$) although the samples were brilliant as evaluated by the brewing practice (EBC, 1987). Shearing increased the turbidity of both wort and beer ($p<0.001$). Turbidity of wort decreased at higher maltose levels and higher pH values ($p<0.001$). Shearing increased the turbidity of

wort at pH 5.4 and 6.8 but decreased the turbidity at pH 4.0 ($p<0.001$). Shearing at higher temperatures decreased wort turbidity ($p<0.001$). The hazes formed in the β-glucan-free worts were believed to be caused by proteins, whereas the increased hazes in worts containing 443 kDa β-glucan derived from both proteins and β-glucans. Beer turbidity was not affected by shearing temperature ($p>0.05$). In the sheared beer, addition of ethanol (5-10% v/v) decreased turbidity ($p<0.001$). Filtration through 0.45 μm membranes removed non-β-glucan haze particles but did not lower hazes caused by β-glucans (Figure 6.6) because they are smaller than 0.45 μm in the apparent diameter. After cold storage, turbidity of sheared beers containing lower MWs (31 and 137 kDa) and low concentrations (50-200 mg/L) of β-glucans was reduced ($p<0.001$). In contrast, the turbidity of beers containing higher β-glucan MWs (250, 327 and 443 kDa) at concentrations higher than 400 mg/L increased ($p<0.001$). Thus, hazes caused by high MW β-glucans at high concentrations can not be removed by 0.45 μm membrane filtration or lagering at 4°C for 2 weeks. These high MW β-glucans in beer must be eliminated or minimized in malting and mashing to avoid haze problems downstream.

The presence of high MW β-glucans retarded DE filtration of wort at 76°C ($p<0.001$) by increasing filtration resistance. Shearing wort resulted in higher filtration index, an indicator of the resistance to filtration, particularly when the wort was sheared at a low temperature (20°C). As could be expected, higher concentrations of maltose increased the filtration index due to increased wort viscosity. The filtration index was also higher at pH 4.0 than at a normal wort pH of 5.4.

When wort samples were filtered through 0.45 μm membranes at 20°C, influences of β-glucans and environmental factors on membrane filterability expressed as relative flux were opposite to that of the filtration index. The relative flux at 20°C correlated negatively to the filtration index at 76°C. Wort samples used in this study were essentially brilliant in clarity. If wort turbidity is too high, however, membranes can become clogged instantly and the filtration test became difficult (Egi, 2002). For

example, adjusting wort pH to 4.0 increased turbidity dramatically and the membrane filtration efficiency was much lower. However, this acidification partly improved the usefulness of the wort membrane filtration test in predicting beer membrane filtration performance (Siebert *et al.*, 1984).

Results have shown that the presence of β-glucans impaired the membrane filtration performance of beer. Both V_{max} and Q_{init} were lowered by higher MWs and concentrations of β-glucans ($p<0.001$). Shearing beer decreased both V_{max} and Q_{init} ($p<0.001$) while shearing temperature (0-10°C) did not have an effect ($p>0.05$). In the range of 3.8-4.6, higher pHs resulted in improved filterability of beer ($p<0.001$). The addition of ethanol at 5-10% v/v decreased the Q_{init} ($p<0.001$), but improved the V_{max} ($p<0.001$). The alcohol-free beers had much lower V_{max} even when no β-glucan was present. It is hypothesized that the lower filterability was due to higher haze levels of the beer containing no ethanol. When the relative V_{max} was used to evaluate the effect of β-glucans, the addition of ethanol at 5-10% v/v was found to lower the relative V_{max} ($p<0.001$). Thus, filtration problems caused by β-glucans are worse in the presence of higher ethanol concentrations. It was also found that cold storage at 4°C for 2 weeks did not improve beer filterability ($p>0.05$).

The effects of β-glucans on interfacial and foaming properties were also investigated in this thesis (Chapter 5). Beta-glucans of MWs of 31-443 kDa (1000 mg/L) lowered the surface tension of their aqueous solutions. The effect of β-glucan MW and concentration, as well as shearing on the surface tension of wort differed from that in beer. Beta-glucans at high concentrations lowered wort surface tension despite their molecular weights ($p<0.05$). Surface tension of wort decreased at higher maltose concentrations and lower pH values ($p<0.001$). Beer surface tension, however, decreased at higher ethanol concentrations ($p<0.001$) while the presence of high MW β-glucans increased beer surface tension ($p<0.01$). Beer pH value and β-glucan concentration showed no significant influence on surface tension ($p>0.05$). Shearing of samples lowered wort

surface tension but increased beer surface tension ($p<0.001$). However, the surface tension of both wort and beer were not affected by shearing temperature ($p>0.05$).

Although β-glucans are able to lower surface tension, the presence of 50-1000 mg/L of β-glucans (31-443 kDa) were found to be detrimental to beer foamability and foam stability ($p<0.01$; Table 5.2). Foamability of beer was enhanced ($p<0.001$) by higher temperatures (0-10°C) and pHs (3.8-4.6). The addition of ethanol at 5-10% v/v lowered beer foamability ($p<0.01$) and foam stability ($p<0.001$). Beer foam stability decreased at lower pHs and higher ethanol contents ($p<0.001$) but was not affected by foaming temperature ($p>0.05$). A Pearson correlation indicated that lower surface tension favoured beer foamability while better foam stability can be expected with greater foaming capacity. It is noteworthy that the foaming test used in this thesis was to agitate degassed samples instead of pouring beer from bottles because the sample volume was limited when commercially purified β-glucans were studied.

Other important physical characteristics affecting the brewing process are the rheological properties of wort and beer. The addition of β-glucans (31-443 kDa) increased the viscosities of wort and beer although β-glucans contributed less to wort and beer viscosities than the non-β-glucan components. In the range of 50-1000 mg/L, viscosity of β-glucans increased linearly with MW and concentration. The effect of temperature on wort viscosity followed the Arrhenius relationship. Shearing increased wort viscosity but decreased beer viscosity ($p<0.001$). However, wort viscosity caused by β-glucans was lowered by shearing ($p<0.001$) and beer viscosity due to β-glucans was not affected by shearing ($p>0.05$). Wort viscosity increased at high pHs in the range of pH 4.0-6.8 ($p<0.001$) but pH in the range of 3.8-4.6 did not affect beer viscosity ($p>0.05$). Maltose in wort and ethanol in beer increased the viscosity of β-glucans ($p<0.001$). Beta-glucans had higher intrinsic viscosities (5°C) in beer than in wort ($p<0.001$). The Mark-Houwink equation has proved to govern the influence of the β-glucan MW on intrinsic viscosity. The estimated critical overlap concentrations of β-glucans studied were 2.5-8.3 g/L in

wort and 1.3-4.7 g/L in beer, respectively. These values are higher than literature reported C* values for β-glucans in water and acetate buffer systems (Linemann and Krüger, 1997; Oonsivilai, 2000).

Beta-glucan specific viscosity (i.e., the viscosity due to β-glucans relative to the solvent system or water) was a useful indicator for the potential processing difficulties caused by these polymers. It has been shown that viscosity caused by β-glucan (and resulting process parameters such as turbidity level, V_{max} and Q_{init}) can be detected by lichenase hydrolysis. Examination of wort and beer viscosities before and after lichenase treatment (0.1 U/mL, natural pH of sample, 50°C for 30 minutes) is recommended as a diagnostic test to detect potential haze and filtration problems due to β-glucans. This supports the hypothesis that large viscosity reductions after enzymatic hydrolysis correspond to a high risk of β-glucan precipitation (Grimm *et al.*, 1995b). Shearing, urea and heat treatments were found to be unsatisfactory in predicting the process difficulties (Chapter 9) because these treatments did not specifically effect β-glucan polymers. Shearing, urea and heating also affect the physiochemical properties of beer proteins and pentosans. Shearing is a difficult process to standardize as regards control of shear rate. When 31-443 kDa β-glucans were added, even using the same blender to shear samples under identical conditions increased wort viscosity (Figures 3.10) but decreased beer viscosity (Figure 3.15). A contradictory result was also found where shearing did not affect the viscosity of wort containing 443 kDa β-glucan (Figure 9.1). Thus, shearing causes inconsistent changes of viscosity for wort and beer samples and can not be used as a predictive technique when only viscosity is examined.

In the literature, urea at a concentration of 3% w/v has been suggested to identify the non-dialyzable β-glucans in beer (Hinchliffe and Box, 1985; Letters, 1977). The difference between the non-dialyzable β-glucan levels with and without 3% w/v of urea was reported to indicate the amount of aggregated high MW β-glucans that can correlate to beer filterability (Hinchliffe and Box, 1985). In this thesis, addition of urea at 3% w/v

increased wort viscosity but did not affect beer viscosity (Figure 9.1; Table 9.2). Urea also decreased beer turbidity ($p<0.001$) but not wort turbidity ($p>0.05$) (Figure 9.2; Table 9.2). It is believed that 3% w/v of urea led to dissociation of beer biopolymers including β-glucans, proteins and protein-polyphenol complexes because it is a structure destabilizer of hydrogen bonding and can induce unfolding of these polymers. Interestingly, wort DE filtration at 76°C was slower after the addition of urea ($p<0.001$) while both V_{max} and Q_{init} of beer membrane filtration at 5°C were enhanced ($p<0.001$; Figures 9.3, 9.4 and 9.5). Also, treatment with urea was not specific to the presence of β-glucans (i.e., the β-glucan-free beer had improved filtration performance; Figure 9.4).

Heating wort and beer at 70°C for 1 hour decreased turbidity ($p<0.001$) and improved the filtration rate of beer ($p<0.001$) but lowered beer V_{max} ($p<0.01$) and wort filtration performance ($p<0.001$; Table 9.2). Since the turbidity of β-glucan-free beer was also lowered by heat treatment, it is hypothesized that heating dissolved the chill-haze particles of non-β-glucan polymers in beer. Thus, heating can not be used to differentiate the potential problems due to β-glucans from that caused by other biopolymers.

To summarize, the presence of high MW β-glucans increased viscosity, turbidity, filtration index and difficulties in membrane filtration of wort and beer. Beta-glucans exhibited greater particle size in beer than in wort. Processing conditions such as shearing, temperature, pH, maltose concentration of wort and ethanol content of beer also affected the behaviour of β-glucans. Results have shown no enhancing or stabilizing effects of β-glucans on beer foam. To predict the potential difficulties caused by β-glucans, only the changes in β-glucan specific viscosity after lichenase hydrolysis can be considered as a diagnostic treatment. One may set a 5% difference as the significance level to differentiate viscosity between the treated and un-treated samples. This test can be applied to both wort and beer for the purpose of predictive analysis.

Variables were controlled throughout this thesis to investigate their effects on some

parameters of the brewing process and quality of wort and beer. However, several factors must be considered when interpreting the results of these studies. First of all, it was assumed that the amount of pentosans in the wort and beer did not interfere with the experiments. The concentration of total arabinoxylans in the 12°P wort was determined to be 39.5 mg/L (Egi, 2002). The pentosans present in wort and beer were also assumed to have no interactions with the added commercial β-glucans. Possible contributions of the wort and beer pentosans to viscosity, turbidity, and filtration difficulty are included in the background values of the β-glucan-free samples. Secondly, the wort and beer samples used in this thesis were very low in haze levels. Thus good sensitivity to the changes of turbidity and filterability due to β-glucans was achieved. If hazy wort and beer were used, the "invisible" β-glucan haze may not be detected efficiently. Thirdly, the measured β-glucan particle size was a fast categorization of the polymers and only represented the range of size distribution "sieved" by the nominal pore size of the membranes used. However, this technique is better than light scattering which does not differentiate β-glucan particles from other polymers in wort and beer. It is also better than using liquid chromatography, which only provides information about the retention time of a very broad peak but there are no separated individual peaks (Flores, 1997; Megazyme, 1999). Fourthly, the β-glucans used in this thesis were commercially purified and were polydisperse in their MW distributions. Chromatograms of some selected β-glucan standards are shown in Figure A.5 (Megazyme, 1999) although data are not available for the rest of β-glucans used. One must be cautious when interpreting the effect of β-glucan MW on wort and beer properties because these polymers are not sharply separated peaks. For example, the polydispersity of β-glucans may partly explain why MW was not found important in wort surface tension. Lastly, the membrane filtration of beer was carried out under a pressure lower than that applied in breweries. Thus, the initial filtration rate would be lower than what can be observed in brewing practice although V_{max} values are expected to be similar.

10.2 Recommendations for Future Research

To further elucidate the behaviour of β-glucan polymers in brewing, it is worthy to examine the properties of "native" β-glucans in wort and beer. Isolation of the native β-glucans from commercial malt and adding them into wort and beer would provide further information about the behaviour of these polymers which are reported to be crosslinked with proteins and pentosans (Forrest and Wainwright, 1977; Izydorczyk and MacGregor, 2000). It is expected that these native β-glucan complexes would have larger particle sizes and higher sensitivity to changes in brewing process conditions because of the attached protein moieties (and probably pentosans as well). Membrane pores used in filtration tests are expected to be more readily clogged by these crosslinked polymers due to their greater size.

The interactions between β-glucans and other individual components such as proteins and pentosans in wort and beer also warrant further investigations. Megazyme β-glucan preparations can be used in model beers (i.e., buffers) together with various types and amounts of proteins and pentosans followed by examination of their response to the processing conditions explored in this thesis. Knowledge about such interactions will provide brewers with better understanding of the behaviour of biopolymers in brewing.

It is also worthy to study the relationship between membrane filterabilities of wort and beer as well as the changes of turbidity, viscosity, foaming properties and filterability during fermentation. Investigating the carryover of β-glucans during fermentation will help brewers to develop technologies of eliminating β-glucan problems during fermentation and lagering. It must be recognized that other wort parameters such as total nitrogen content should be controlled. Although the β-glucan problems occur sporadically, it can be foreseen that brewers will be able to minimize the risk of these polymers as additional information on the behaviour of β-glucans in brewing becomes available.

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12. APPENDICES

Appendix 1. Effect of Shearing Speed and Time on the Turbidity of β-Glucan Solutions

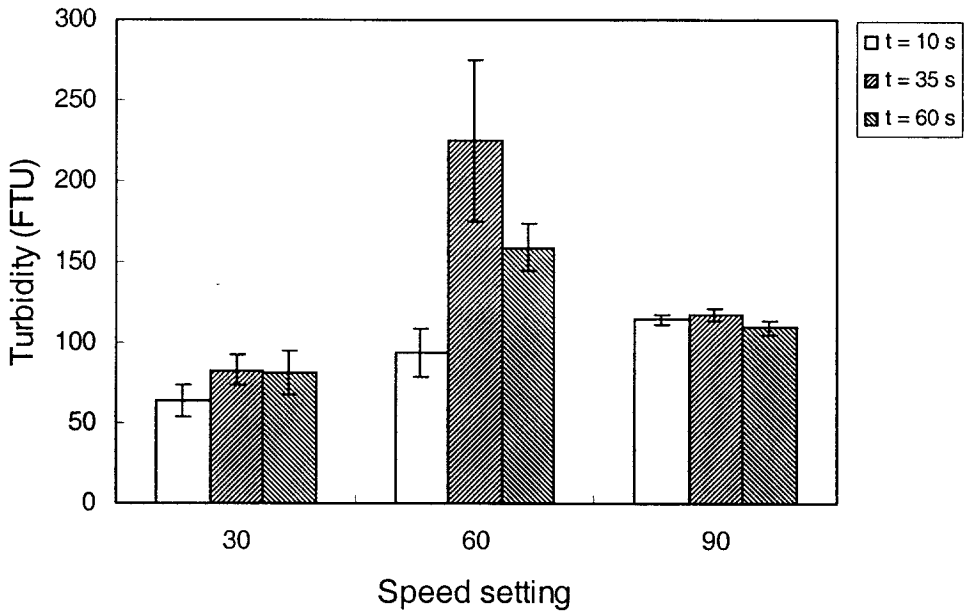

Figure A.1 Effect of shearing speed and time on the turbidity of β-glucan solutions. Values are given as means ± one standard deviation (S.D.) of duplicate experiments.

Sample tested was a 443 kDa β-glucan solution (1000 mg/L) dissolved in 100 m*M* acetate buffer (pH 4.0, containing 5.0 % v/v of ethanol). The unsheared sample had a haze level of 44.7±6.2 FTU. An amount of 10 mL solution was sheared at 20°C and the turbidity measured with the intensity of 580 nm light scattered at 90° to the incident. Turbidity was raised by shearing ($p<0.001$) and affected by speed setting ($p<0.01$) and shearing time ($p<0.05$).

Appendix 2. Viscosity of 2-24°P Worts at 20°C

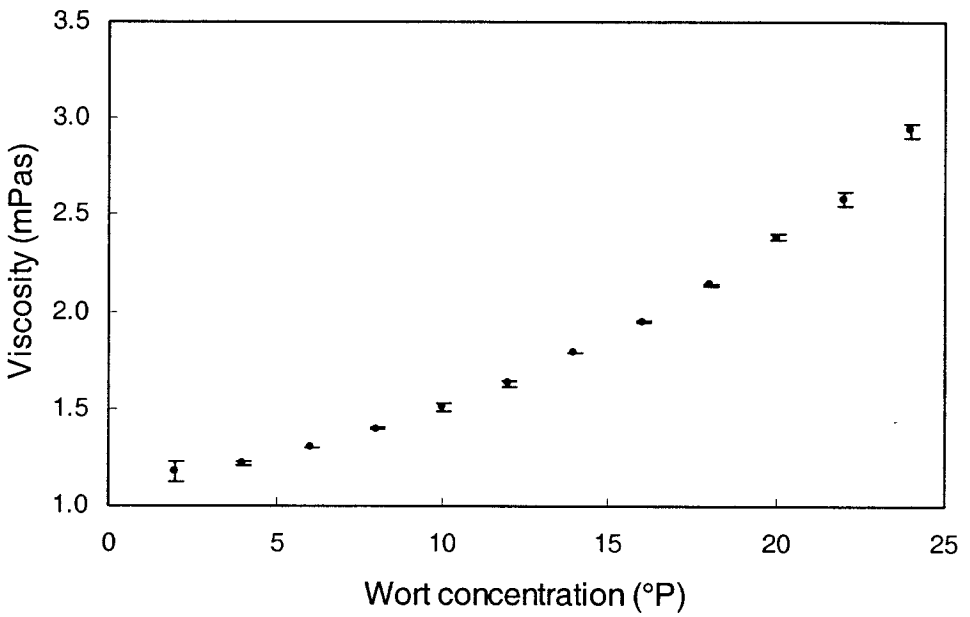

Figure A.2 Effect of wort concentration on viscosity at 20°C.

The wort samples were prepared from the β-glucan-free base wort by concentration (500 mL wort was boiled for 15 minutes) to 30% w/w and serial dilutions were prepared. Values are given as means ± S.D. of duplicate experiments.

Appendix 3. Protein Content of Commercial β-Glucans

Table A.1 Protein content of commercial β-glucan preparations determined by the Lowry assay [a)]

β-Glucan MW (kDa)	Lot number	Protein content (% d.b.) [b)]	Standard deviation
31	41101	9.35	0.07
137	90401	2.89	0.45
250	60501	1.39	0.21
327	40301	0.72	0.15
443	90501	3.34	0.28

[a)]: Lowry reagents were calibrated with BSA standards; [b)]: means of duplicate samples.

Appendix 4. Reactivity of β-Glucans (31-443 kDa) with Congo Red

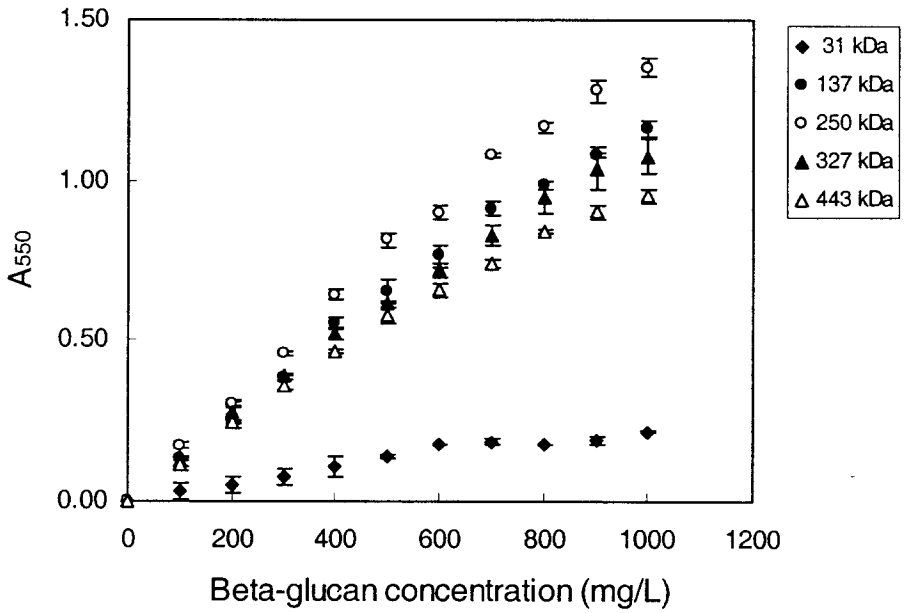

Figure A.3 Responses of Congo red dye to β-glucans.

Beta-glucans were dissolved in water (0.5% w/v) and further diluted to 1000 mg/L in 100 mM NaAc buffer (pH 4.0, containing 5% v/v of ethanol). Serial dilutions were obtained with the same acetate buffer. Duplicate experiments were carried out and results are reported as means ± S.D.

Appendix 5. Degradation of β-Glucans (31-443 kDa) by Lichenase

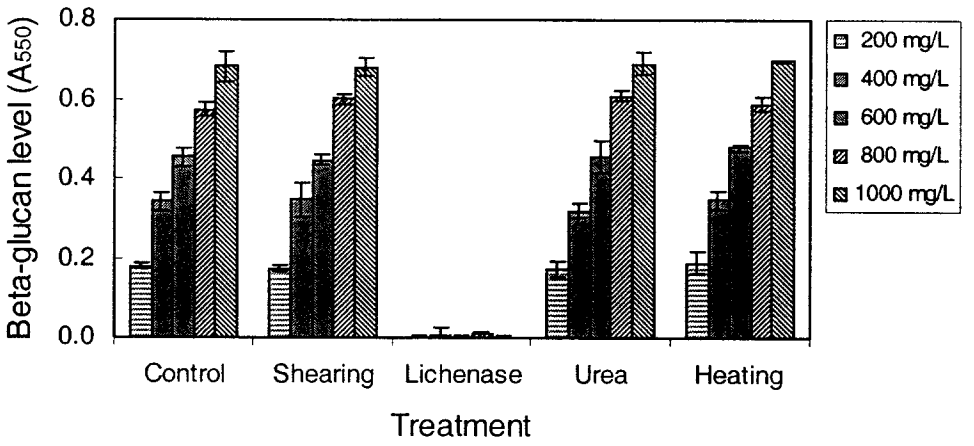

Figure A.4 Lichenase hydrolysis of 443 kDa β-glucan in beer

Lichenase at 0.1 U/mL was added to beer containing 200-1000 mg/L of the 443 kDa β-glucan. Hydrolysis was carried out at 50°C for 30 minutes. The levels of β-glucan were expressed as the absorbance at 550 nm with Congo red binding. Results are given as means ± S.D.

Appendix 6. Size Exclusion Chromatograms of β-Glucan Standards

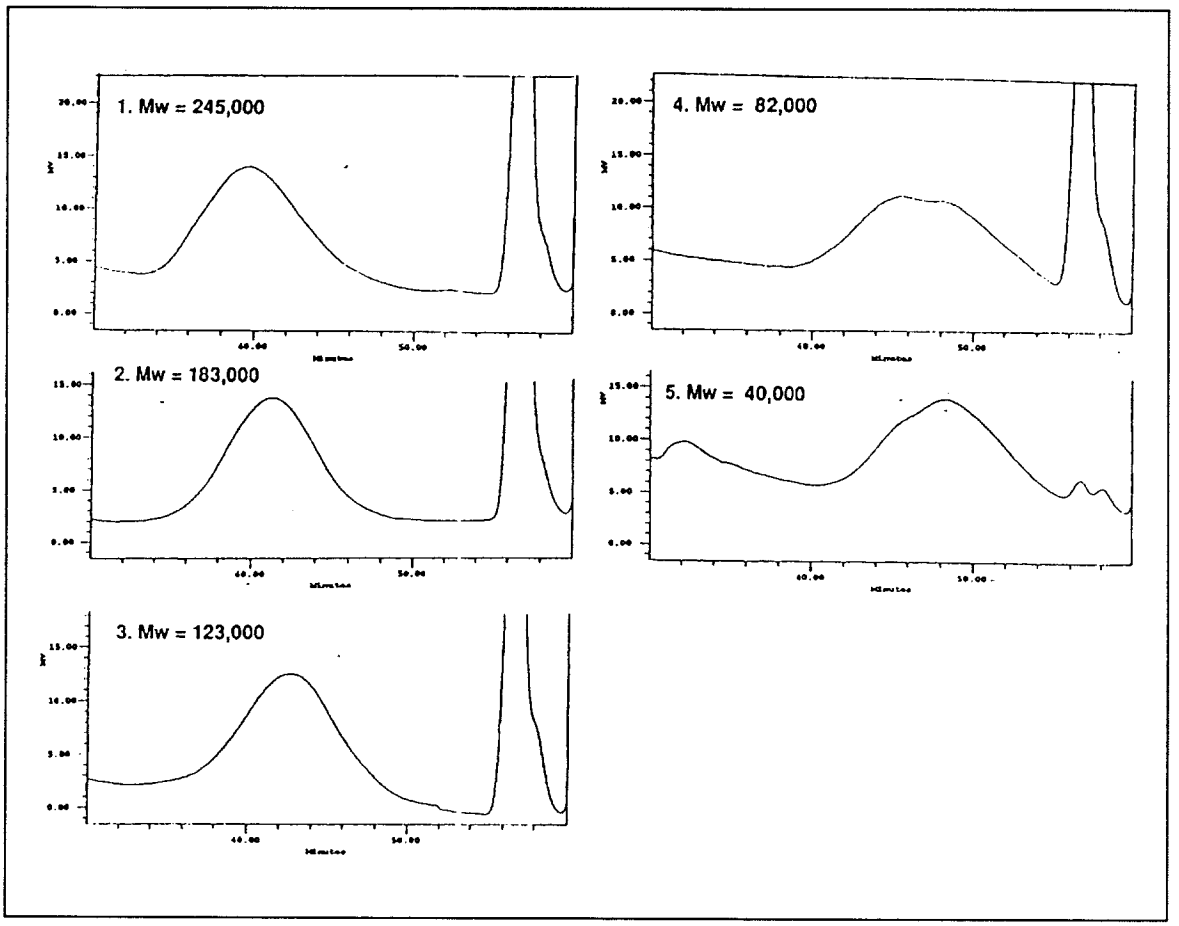

Figure A.5 Comparison of the chromatograms of β-glucan standards (Megazyme, 1999).

The chromatography system consisted of Hydrogel 200, 500 and 2000 columns at 70°C, and the eluent was 50 m*M* sodium hydroxide. Measurements were performed with a PRECISION DETECTORS 2000 dual angle light scattering detector fit inside a WATERS M422 refractive index detector (Megazyme, 1999).