Evaluation of malted barley with different degrees of fermentability using the Rapid Visco Analyser (RVA)

by

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Declaration

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Abstract

The relationship between malt fermentability and rheological variables, measured by means of the Rapid Visco Analyser (RVA) and application of multivariate data analysis, was investigated.

The RVA Kilned Malt method was optimised to achieve maximum rheological discrimination between malt samples, differing in fermentability. Five concentrations and two particle sizes were used to investigate each malt sample. Data were analysed by two different data analysis techniques, namely principal component analysis (PCA) and analysis of variance (ANOVA). Rheological variables for peak-height, -width, -area and time occurrence, were able to discriminate between high (Metcalfe, Flagship), intermediate (SSG 585, PUMA) and low malt fermentability (SSG 506, SSG 564). Variation in particle size showed insignificant ($P>0.05$) fermentability discrimination. The malt to water ratio of 1:1.5 provided the best discrimination in fermentability. PCA applied to the entire dataset was the superior data analysis technique.

Partial least squares (PLS) regression and Soft Independent Modeling of Class Analogy (SIMCA) were applied to predict malt fermentability. Recorded RVA data was regressed with both apparent attenuation limit (AAL) and free amino nitrogen (FAN), independently. Developed PLS calibration models were validated by test set and segmented cross-validation for AAL and FAN, respectively. The SIMCA classification model developed was based on different malt fermentability classes, each PCA validated independently by test set validation. A strong correlation between RVA analysis and AAL was obtained ($r=0.92$), while FAN delivered a weak correlation ($r=0.59$). Regarding the SIMCA model; the proportion of test set samples correctly classified in terms of malt fermentability was 83%. South African malt blends were predicted to have low malt fermentability. Simulated blends were predicted to have high fermentability when using a minimum of 80% Metcalfe blended with SSG 506. Blends containing higher percentages of the low malt fermentability cultivar (SSG 506) were predicted to have an overall intermediate fermentability.

Different experimental conditions were investigated during RVA analysis (i.e. instrument model; time/temperature profile, enzyme activity and heating/cooling rate). Rheological measurement using different RVA models gave similar PCA results, indicating adequate sensitivity of the older instrument for discrimination purposes. Matching the time/temperature profile used in the commercial brewery mashing process was rejected due to increased analysis time and rheological noise while reducing fermentability discrimination. Inactivating malt enzymes prior to RVA analysis provided useful sample information, such as the large starch granule's mean diameter, extract and starch content, by measuring peak height and

time to peak. The amount of starch damage inflicted on a malt sample increased after repeated centrifugal milling, but was unaffected by the sieve size used.

Multivariate data analysis is a suitable statistical technique applied to rheological data and provided more relevant information than traditional univariate techniques. The RVA can be considered an ideal instrument within a grain laboratory as it allowed the investigation of different operating conditions. It is beneficial to use an inexpensive, routine method of analysis to measure various interacting factors. RVA rheological measurement demonstrated to be a decisive monitor of malt fermentability and is highly recommended to be incorporated within the barley breeding, malting and brewing industries.

Uittreksel

Die verwantskap tussen mout fermenteerbaarheid en reologie-veranderlikes, gemeet met die “Rapid Visco Analyser” (RVA) en toepassing van meerveranderlike data analise is ondersoek.

Die RVA “Kilned Malt” metode is geoptimeer om maksimum reologiese diskriminasie, tussen gars kultivars van verskillende fermenteerbaarheid, te lewer. Vyf konsentrasies en twee partikel groottes is gebruik in die ondersoek vir elke mout monster. Data is deur beide hoof komponent analise (HKA) en variansie-analise (ANOVA) ondersoek om die verskillende data analise metodes met mekaar te vergelyk. Reologiese veranderlikes vir piek-hoogte, -wydte, -area en -vormingstyd, kon diskrimineer tussen hoë (Metcalfe, Flagship), intermediêre (SSG 585, PUMA) en lae (SSG 506, SSG 564) mout fermenteerbaarheid. Variasie in partikel grootte kon nie beduidende diskriminasie in fermenteerbaarheid aantoon nie. Die mout-tot-water konsentrasie van 1:1.5 het die beste diskriminasie in fermenteerbaarheid gelewer. Die toepassing van HKA op die hele datastel was die beter analitiese tegniek.

Parsiële kleinste kwadrate (PKK) regressie en Sagte Onafhanklike Modellering van Klas Analogie (SIMCA) is toegepas om mout fermenteerbaarheid te voorspel. Regressie tussen RVA data en skynbare attenuasie limiet (AAL), sowel as vrye amino stikstof (FAN) inhoud, is afsonderlik uitgevoer. Die geldigheid van regressie modelle is deur middel van toets stel en gesegmenteerde kruis-validasie vir AAL en FAN onderskeidelik uitgevoer. SIMCA klassifikasie modelle is gebaseer op verskillende mout fermenteerbaarheids-klasse, waarvan elke HKA klas individueel geldig is. RVA analise het ‘n sterk korrelasie met AAL (r=0.92), maar ‘n swak korrelasie met FAN (r=0.59) getoon. Die SIMCA model het 83% van toets stel monsters as korrek geklassifiseer in terme van mout fermenteerbaarheid. Suid Afrikaanse mout mengsels is voorspel as swak fermenteerbaar. Nagebootste mengsels is voorspel as hoogs fermenteerbaar wanneer minimum 80% Metcalfe met SSG 506 vermeng word. Sodra ‘n hoër persentasie van die swakker fermenteerbaarheids-kultivar (SSG 506) bygevoeg is, word intermediêre fermenteerbaarheid voorspel.

Tydens RVA analise is verskillende eksperimentele toestande ondersoek (byvoorbeeld instrument model; tyd/temperatuur profiel; ensiem aktiwiteit en verhittings/verkoelings tempo). Die gebruik van verskillende RVA modelle het soortgelyke HKA resultate gelewer. Dus bevat die ouer instrument aanvaarbare sensitiwiteit vir diskriminasie doeleindes. Nabootsing van die tyd/temperatuur profiel in die kommersiële brouproses is verwerp, aangesien analise tyd en reologiese geraas toegeneem het, terwyl fermenteerbaarheids-diskriminasie verminder het. Inaktivering van mout ensieme voor RVA analise lewer nuttige monster inligting; deur veranderlikes soos piek-hoogte en piek-tyd te meet, kan die groot stysel korrel se gemiddelde deursnit, ekstrakwaarde en stysel inhoud verkry word. Herhaalde

sentrifugale maling van ‘n mout monster lei tot beskadiging van stysel, maar dit word nie deur sif grootte beïnvloed nie.

Die toepassing van meerveranderlike data analise op reologiese data is waardevol en lewer meer relevante inligting in vergelyking met tradisionele eenveranderlike data analise. Die RVA is ‘n ideale instrument vir gebruik in ‘n graan laboratorium aangesien dit verskillende operatiewe kondisies kan ondersoek. Die gebruik van ‘n enkele, goedkoop, roetine analitiese metode is voordelig en het die potensiaal om ‘n magdom interaktiewe faktore te meet. RVA reologiese meting demonstreer die vermoë as ‘n deurslaggewende tegniek vir die bepaling van mout fermenteerbaarheid, gevolglik word toepassing sterk aanbeveel binne die gars teëlings-, vermoutings- en brouers-industrieë.

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Contents

Language and styles used in this thesis are in accordance with the requirements of the *International Journal of Food Science and Technology*. This thesis represents a compilation of manuscripts where each chapter is an individual entity and some repetition between chapters has, therefore, been unavoidable.

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Chapter 1

Introduction

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Introduction

Malted barley is most commonly used as bulk fermentable material in beer production (Hough, 1985a). It serves as a source of starch and nutrients for optimal yeast growth and subsequently aids conversion of fermentable materials [i.e. mono-, di- and trisaccharides (Muller, 2000)] to alcohol (MacGregor & Fincher, 1993; Briggs *et al.*, 2004). A variety of beer styles exist, from the typical German Reinheitsgebot beer made only from malt, hops and water to North American beers, which substitutes a portion of malt with starch adjuncts (in ratios up to 55 to 45%) (Briggs *et al.*, 2004; Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2010). When unmalted grain, such as solid maize, is used to substitute a large portion of the malt during the mashing process (i.e. adjunct brewing) a special type of malt is required (i.e. highly fermentable malt) (Hough, 1985b). Highly fermentable malt provides a large amount of enzymes for starch hydrolysis. It also supplies vital nitrogenous nutrients essential for yeast metabolism, accompanied with acceptable levels of extract (Edney, 1999). Malting barley cultivars marketed as highly fermentable are sought after for adjunct brewing, but are usually expensive mostly due to transportation costs. In 2010 a tonne of Metcalfe barley cost approximately R2 150 (ZAR) per tonne, while transportation cost of barley from Vancouver (Canada) to Cape Town (South Africa) was approximately R350 to R500 (ZAR) per tonne [exchange rate: $1(USD) = R7.14 (ZAR)] (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2010). It should be considered that prices vary from year to year depending on the malting grade production yield.

Such malting barley cultivars are not commercially available in South Africa and are therefore imported from countries such as Canada at a relatively high price. It would be of great financial benefit if local industries could develop equivalent cultivars through barley breeding programmes. Breeding for high malt fermentability as a quality trait requires evaluation during a breeding programme, usually from the F6 generation when enough homozygous seed is available for micro-malting (Potgieter & Meijering, 2009).

Malts from different malting barley cultivars are frequently blended in the malthouse (Wainwright, 1997). This allows the formulation of a specific malt blend able to comply to required brewing specifications (Briggs *et al.*, 2004). The fermentability of malt is influenced by environmental factors during barley cultivation (Kenn *et al.*, 1993), but also the malting process (Gunkel *et al.*, 2002; Briggs *et al.*, 2004), owing to quality differences between batches of the same cultivar. It is therefore essential to evaluate malt fermentability within a barley breeding programme and also in the malthouse before brewing on a larger commercial scale.

The extent of brewhouse fermentation is measured by a progressive decrease in wort density (i.e. specific gravity) (Briggs *et al.*, 2004). This allows the calculation of the attenuation limit, which indicates when the maximum amount of alcohol is produced and therefore when fermentation is

complete. Wort density decreases as soluble sugars, present within the wort, are consumed by the yeast and converted to alcohol. Fermentation is considered complete when density stabilises. It is important not only to monitor malt fermentability during actual brewing, but also to predict it, thus warning brewers of potential fermentability problems.

Fermentability of a malt sample can be measured by means of a laboratory based attenuation test, prior to its use in the brewery, but cannot measure the fermentation rate. As can be expected, laboratory conditions do not reflect actual brewing conditions and therefore the industry considers results as unrepresentative and unreliable gradually discontinuing their application (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009; Mr P van der Vyver, SAB, Cape Town, South Africa, Personal communication, 2009). Fermentation analyses [such as locally used malt fermentability indicator test (MFIT)] are often unable to indicate brewing abnormalities, causing production related problems (Calman *et al.*, 2008). There is a great need by the breeding, malting and brewing industries to obtain a faster, practical and more accurate malt fermentability prediction method.

Rotational viscometers are able to measure rheological properties of a large variety of materials, and widely used in the food industry. Applications include rheological measurement of sauces, mayonnaise, soups, dairy beverages, hydrocolloids, proteins, ready-to-eat breakfast cereals, snack foods and animal feed (i.e. pet and fish foods) (Anonymous, 2005). These instruments are considered easy to operate, while being relatively inexpensive (Rao, 1999). Rotational viscometers minimise product separation and are therefore widely used on fluids which contain suspended particles (Rao, 1999). Rotational viscometers may be used for fundamental rheological characterisation of starch pasting properties, and are considered ideal to measure the quality attributes of starch-based materials such as cereal products (Anker & Geddes, 1944; Yoshida & Yamada, 1970; Voisey *et al.*, 1977; Deffenbaugh & Walker, 1988; Evers & Stevens, 1988; Bason *et al.*, 1993; Bhattacharya *et al.*, 1999; Collado & Corke, 1999; Hermann & Sommer, 2001; Limpisut & Jindal, 2002; Zhong *et al.*, 2009).

The Rapid Visco Analyser (RVA) is a type of rotational viscometer, which possesses variable temperature and shear capabilities (Agu *et al.*, 2006). Its methodology is based on the Searle system, which means the stirrer, which is also the sensor element, rotates at a defined speed within a stationary container (Naé, 1993). The RVA can be regarded as an empirical method to measure the pasting properties of starch and consequently does not allow fundamental rheological analysis (Goode & Arendt, 2006) unless it is calibrated by means of a liquid of known viscosity. The RVA has notable advantages over similar viscometers, such as the Brabender Visco-amylograph, Bohlin rheometer and Rheoswing RSD instrument. In contrast to the aforementioned viscometers, the RVA uses a smaller sample size combined with shorter analysis times, while allowing alteration of operating conditions (such as heating rate and rotational speed) (Mijland *et al.*, 1999; Mariotti *et al.*, 2005; Zhou & Mendham, 2005). The rheological curve generated by the RVA reflects starch structural changes during various processes such as gelatinisation,

retrogradation or even enzyme hydrolysis. Each starch-based sample can be identified by its unique pasting profile (Thomas & Atwell, 1999; Mariotti *et al.*, 2005). Monitoring viscosity changes, by RVA, provides an in-depth insight into raw material quality and the condition of products being processed, resulting in a more efficient quality and process control tool within the relevant industry (Goode *et al.*, 2005a).

Of particular interest is the RVA's capability to monitor barley and malt quality, enabling quality selection within a barley breeding programme. Many studies have investigated relationships between the viscogram data from barley or malt and their malting quality analyses (Glennie Holmes, 1995b; Glennie Holmes, 1995c; Allan *et al.*, 1997; Dunn *et al.*, 1997; Stuart *et al.*, 1998; Zhou & Mendham, 2005). In a series of papers published by Glennie-Holmes, the effects of varying physical conditions, chemical conditions (Glennie Holmes, 1995a) and modification level (Glennie Holmes, 1995b) on RVA viscograms were investigated. This research established possible relationships between RVA starch characteristics and malting quality (Glennie Holmes, 1995c; Glennie Holmes, 1995d). Work was also conducted to simulate the brewery mashing process by means of an adapted RVA time/temperature profile (Goode *et al.*, 2005b; Goode & Arendt, 2006). In addition, the authors investigated interactions between grain components (purified/non-purified) and amylolytic enzymes, glucanolytic enzymes, and mash pH.

Malt starch gelatinisation temperature, the presence of smaller B-type starch granules, enzyme degradation and enzyme thermostability, greatly influence rheological measurement, but more importantly, can affect malt fermentability (Barrette *et al.*, 1973; Palmer, 1989; Eglinton *et al.*, 1998; Evans *et al.*, 2005; Calman *et al.*, 2008; Evans *et al.*, 2010). Considering the RVA's advantages over similar viscometers it can be considered an ideal instrument for routine analysis in the breeding industry, while allowing fundamental research within a laboratory context.

As mentioned above, an extensive range of RVA applications have focused on barley and malt quality. However, only a few studies related rheological variables (generated by a rotational viscometer) with attenuation limit values (Yoshida & Yamada, 1970; Calman *et al.*, 2008). Only Calman *et al.* (2008) considered brewhouse malt fermentability, by adjusting the laboratory mashing procedure to mimic attenuation results found in the brewery.

The majority of RVA research has been based on data analysis using a limited number of rheological variables (such as temperature at onset of gelatinisation, peak height, peak area, trough viscosity, final viscosity), without consideration of other variables. Multivariate techniques, such as principal component analysis (PCA), allow the variation between samples to be described by an unlimited number of variables, therefore utilising all of the rheological data generated. Multivariate techniques enable model development for prediction purposes, and subsequently, the ability to assess which variables are of importance. Only a few studies in the field of Rapid Visco analysis refer to the use of multivariate data analysis techniques (such as multiple linear regression), but most still resort to measuring a limited number of rheological variables (Cole *et al.*, 1992; Mijland *et al.*, 1999; Juhasz *et al.*, 2005; Calman *et al.*, 2008).

The aim of this study was to demonstrate the application of rheological measurement, by means of the RVA, in combination with multivariate data analysis, to evaluate malted barley in terms of brewhouse fermentability. The specific objectives of this study were therefore to:

- optimise the standard RVA Kilned Malt method, by altering malt to water concentration ratio and sample preparation (milling), to deliver maximum discrimination amongst malt samples differing in fermentability;
- develop and validate a multivariate regression model able to predict malt fermentability from all the rheological variables measured; and
- investigate the effect of RVA experimental conditions (such as instrument model, time/temperature profile, enzyme activity, heating rate, starch damage) on rheological measurements.

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CHAPTER 2

Literature Review

Contents

Chapter 2
Literature Review

1. Introduction

Malted barley has wide spread uses ranging from feed for livestock (downgraded malt i.e. out of specification), milk based non-alcoholic beverages (such as Horlicks), soft drinks, confectionaries (such as Maltesers and Bar-one), vinegar, baked products (such as bread), as well as a source of syrup extract (Bamforth & Barclay, 1993; Agu *et al.*, 2007). However, the largest production demand and usage is attributed to the brewing (96% for products such as beer) and distilling (3% for products such as whiskey and neutral spirits) industries (Kent & Evers, 1994). In general, the bulk fermentable material used for beer production is malted barley; native African beers are made instead from sorghum or millets, and Weissbier from a malted barley and wheat mixture (Hough, 1985). With regards to South African beer production, a tenfold increase in production output was experienced during the period of 1970 to 1990 (Anderson, 2006). An output of 25 million hl was recorded in 2004 (Meussdoerffer, 2009).

Starch is the major carbohydrate present in barley and accounts for up to 65% of the grain weight (MacGregor & Fincher, 1993). With regards to malting and brewing, starch plays a pivotal role in providing fermentable material as substrate for alcoholic fermentation (MacGregor & Fincher, 1993). During the malting process, barley kernels synthesise enzymes for the hydrolysis of protein, starch and other grain components into a form specially suited for yeast metabolism (Kent & Evers, 1994). Malted barley is therefore widely used as bulk material in the brewing process of beer.

Maximum conversion of starch into fermentable material occurs during the mashing process of brewing. During mashing; at a temperature of 60-64°C starch is gelatinised which renders it more susceptible to enzyme hydrolysis (Allan *et al.*, 1997). Each enzyme group has a specific temperature range for optimal efficiency. A further increase in temperature causes enzyme inactivation, and therefore, plays a crucial role in mashing. Enzyme hydrolysis, starch gelatinisation, retrogradation and stirring greatly influence viscosity when considering a malt water mixture.

In barley breeding programs it is important to assess the quality of lines by means of rapid accurate methods. An extensive range of analytical techniques [such as free amino nitrogen content, diastatic power, apparent attenuation limit, viscosity and fine grind extract (definitions provided in Table 1.2, Appendix 1)] are available to predict malt brewing quality, but they still prove to be inadequate or insufficiently informative (Bamforth & Barclay, 1993). Rheological changes, originating from the previously mentioned processes, can relay malted barley quality information. Instrumentation based on the measurement of rheology, such as the Rapid Visco Analyser (RVA), has been investigated in breeding programs and in some instances implemented in the malting and brewing industries to measure the quality of barley, malt and mash (Glennie Holmes, 1992;

Bason *et al.*, 1993; Glennie Holmes, 1995e; Goode *et al.*, 2005c; Goode & Arendt, 2006; Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009).

In this literature review the RVA's ability to measure malt quality and predict brewing performance is considered. To set the background, the malting and brewing processes, with special emphasis on the mashing phase, will be discussed. This will be followed by methods of barley, malt and beer quality determination, drawing attention to deficiencies experienced within certain methodologies. The ability of the RVA to measure malt quality will be investigated with an in-depth discussion on the origin of malt viscosity changes experienced. Additional RVA history and methodology will be given, accompanied with a short overview of the application of multivariate data analysis to rheological results. This review will be concluded by emphasising the potential of the RVA to predict malt fermentability or brewing performance, without resorting to actual brewing.

2. Malting process

Malt is produced by germinating cereal grains, usually barley (*Hordeum vulgare* L.), for a limited period of time. It is then dried to arrest the physical germination process, and accompanying biochemical processes of enzyme modification (Hough, 1985).

The aim of malting is to activate and produce enzymes able to degrade endosperm cell wall components (predominantly (1→3, 1→4)-β-glucan) and storage proteins. This action allows starch granules to be released from the endosperm protein matrix. Malting is also important to develop the desirable colour and flavour of malt (Briggs, 1998; Home *et al.*, 2001a; Home *et al.*, 2001b).

The production of malt entails three processes: **steeping**, **germination** and **kilning**. The conditions of each process are dependent on the malting barley cultivar, age of the grain, barley specifications and targeted malt specifications for a brewing style. Therefore, many variations of these processes exist. A typical South African malting process can take six days (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009).

2.1 Steeping

Typical South African steeping entails a 35 (irrigated barley cultivars malted in Alrode, Northern Cape, South Africa) to 43 (dryland barley cultivars malted in Caledon, Western Cape, South Africa) hour process of submerging cleaned barley in water at 14 to 15°C (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). Steeping allows water to enter the kernel through or near the micropyle, after which it penetrates through the husk (consisting of the lemma and palea) (Fig. 2.1). The embryo hydrates quickly; water then distributes through the aleurone layer and finally reaches the endosperm through which it slowly penetrates (Hough, 1985). This encourages the emergence of the root tip (MacGregor & Bhatty, 1993). It is the purpose of steeping to achieve a sufficient moisture content able to activate metabolism in the

embryonic and aleurone tissues, thus stimulating the production of hydrolytic enzymes (Bamforth & Barclay, 1993). Barley cultivars differ in the amount of moisture required to germinate kernels. Most require a steeping regime that allows a moisture content of at least 42-46% to be reached (Bamforth & Barclay, 1993). Carbon dioxide and ethanol are produced by the respiratory metabolism of the embryo and aleurone tissue, which can inhibit germination (Bamforth & Barclay, 1993). The steeping process is interrupted periodically by draining off steep water to allow air rests (Table 2.1), which permits the removal of these substances (Bamforth & Barclay, 1993). Additional aeration is achieved by bubbling oxygen through the steep water (French & McRuer, 1990). The moisture content of barley is increased to 32% after the first steep (normally received at 12%) and reaches a final moisture content of 46% after the second steep (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009).

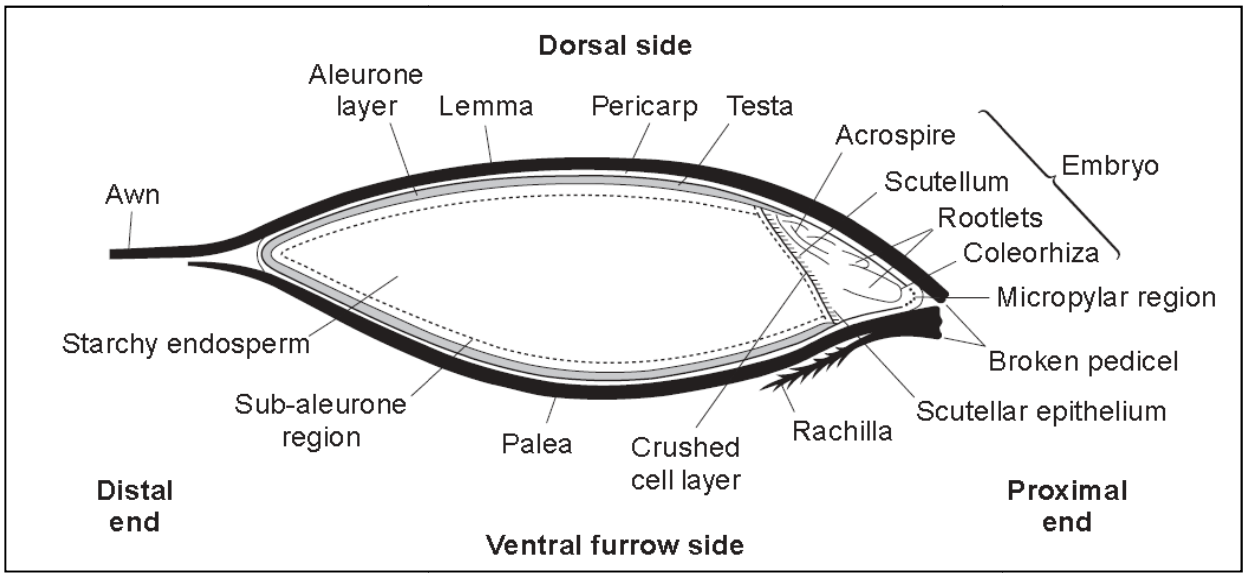

Figure 2.1 Longitudinal section through a barley kernel (Briggs *et al.*, 1981; Briggs *et al.*, 2004).

Table 2.1 A typical STEEPING cycle for the malting barley cultivar SSG 506 (Mr X Mthembu, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009)

Parameter	Time/temperature
1st wet stand (steep)	5 h
1st dry stand (aeration)	20 h
2nd wet stand	11 h
2nd dry stand	7 h
Air-on[1]	17°C
Air -off [2]	19°C

[1]the air temperature below the grain bed
[2]the air temperature above the grain bed

2.2 Germination

South African germination is a seven to eight day process which facilitates chit and rootlet development by providing cool (15-18°C) humid air to a bed of steeped kernels (i.e. pneumatic malting) (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009; Evans *et al.*, 1997). The aim of germination is to generate a maximum amount of extractable material by encouraging endosperm modification through the development, distribution, and action of enzymes (Bamforth & Barclay, 1993). The starchy endosperm of a barley kernel consists of separate cells, bound by walls constituting mainly β-glucans and pentosans (Fig. 2.2). The material inside these cells consists of starch granules embedded within a protein matrix. Embryo exposure to moisture stimulates secretion of plant hormones (i.e. gibberellins), which diffuse to the aleurone layer. Gibberellin production reaches a maximum after the first two days of germination with gibberellic acid playing a major role in controlling endosperm modification (Hough, 1985; Bamforth & Barclay, 1993; Boulton & Quain, 2001a; Briggs *et al.*, 2004). Gibberellins stimulate enzyme synthesis in the aleurone layer and perhaps also the scutellum (MacLeod *et al.*, 1964; MacLeod & Palmer, 1966; Bamforth & Barclay, 1993). Enzymes develop in the sequence of (1) cell wall degrading enzymes, (2) proteases and (3) amylases (MacLeod *et al.*, 1964). These are secreted into the starch endosperm to attack the cell walls, protein matrix and starch granules inside the cells (Wainwright, 1997). At the beginning of germination, the starch granules are covered by the protein matrix. However, within a day of the start of germination, degraded proteins leave these granules exposed (Fig. 2.2) (Hough, 1985). This process facilitates the degradation of starch granules at discrete points until larges holes, penetrating to the inside, are formed. Gradual solubilisation of the inside eventually causes the outer shell to collapse, rendering the grain friable and readily milled (Bamforth, 1999). Enzymatic breakdown of the endosperm therefore proceeds from the embryo (proximal) end of the kernel to the distal end, and from the outer to the inner regions (Hough, 1985; Bamforth, 1999). This physical weakening of the endosperm structure and the accompanied biochemical degradation are referred to as modification, a term commonly used to describe the extent of enzymatic degradation (Hough, 1985). Seventy-five percent of β-glucan and 40% of protein within the endosperm is solubilised by the end of germination. Contrary to belief, only 10% of the starch is degraded, leaving the rest to be extracted during wort production (Boulton & Quain, 2001a).

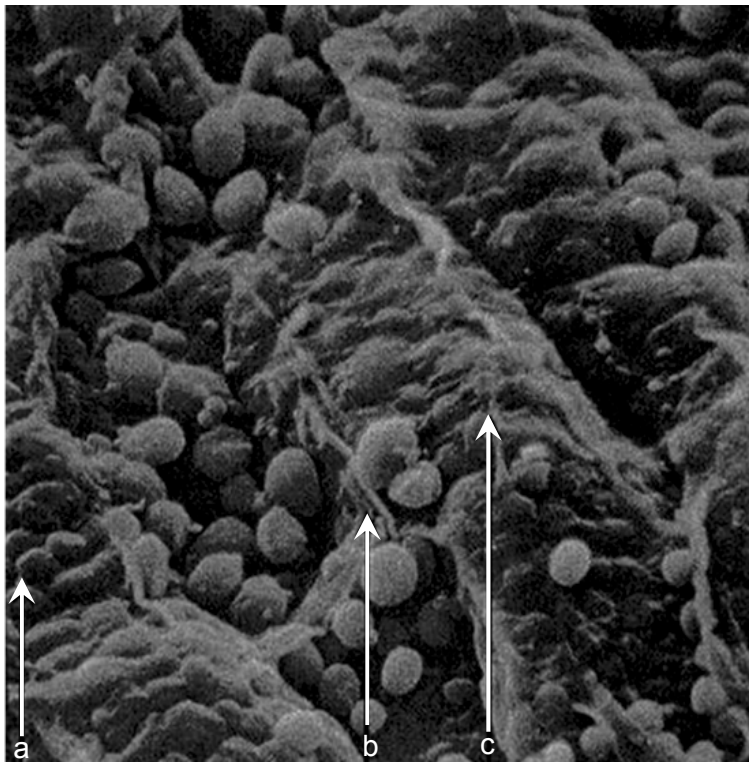

Figure 2.2 Endosperm of malted barley, consisting of: (a) open cells containing starch granules imbedded within a protein matrix; (b) open cells with hydrolysed protein matrix and (c) intact cells (Wainwright, 1997).

2.3 *Kilning*

Kilning is the controlled drying process of green malt to reach a final moisture content of 2-5%, rendering the malt stable for storage. During kilning, the temperature and humidity of the air is strictly controlled to ensure the survival of heat liable enzymes (Table 2.2). A gradual increase in temperature combined with a gradual decrease in malt moisture content is required to allow most enzymes (present in malt) to survive (Bamforth & Barclay, 1993). Exposure to excessive heat, when the grain is still wet, results in heat inactivation of enzymes (Hough, 1985; Bamforth & Barclay, 1993). Higher temperatures helps to facilitate the development of more prominent flavour and colour characteristics, mainly through non-enzymatic reactions (i.e. Maillard reaction), accompanied by other chemical reactions involving reductones (Bamforth & Barclay, 1993; Eßlinger, 2009). Different kilning cycles are used for different types of malt. Pale malt contains a large content of active enzymes due to lower kilning temperatures, while darker, more flavourful malt, contains little to no active enzymes due to higher kilning temperatures (Hough, 1985; Bamforth & Barclay, 1993; Briggs *et al.*, 2004). After kilning, malt is typically screened to remove rootlets and directly transferred to storage (Bamforth & Barclay, 1993). Malt may be stored for several months or even years after processing, depending on storage conditions (ideal conditions: moisture content under 5% and temperatures between 10-15°C) (Bamforth & Barclay, 1993).

Table 2.2 Typical South African malt kilning process (Mr X Mthembu, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009)

Kilning phases	Temperature and times
1. Free drying	63°C for 4 h (followed by temp. ramped up to 68°C for 4 h)
2. Forced drying	76°C for 12 h
3. Curing	80°C for 4 h
TOTAL TIME	24 h + 1 h additional for cooling off

3. Brewing process

During the brewing process malt is **crushed** to coarse flour, i.e. grist. Warm water is added to the grist to form a porridge-like **mash** in which enzymes further degrade the endosperm. Solid adjunct (such as maize grist) can be added during the mashing process. Maize grist must be cooked in a cereal cooker before extraction, as maize starch granules have a higher gelatinisation temperature than barley (Hough, 1985; Bamforth & Barclay, 1993). Starch in the adjuncts is converted to fermentable carbohydrates using enzymes present in malt (Bamforth & Barclay, 1993). With the addition of hot water to the mash, the liquid extract (**wort**) is separated from the solid material (spent grains) during which the husk acts as a filter bed (Hough, 1985). The wort is then **boiled** with **hops**. Boiling inactivates enzymes, sterilises the wort and coagulates some proteins, while hops lend distinctive bitterness, flavours and aromas (Hough, 1985). Liquid adjuncts (sugar syrup such as pure glucose) can be added along with the hops during the boiling process (Boulton & Quain, 2001a). The boiled wort is **clarified**, **cooled** and **aerated** to create an ideal fermentation medium (Hough, 1985). It is then inoculated with yeast, a process termed **pitching**, to convert carbohydrates to alcohol and carbon dioxide (**fermentation**), while also developing flavours and aromas. The beer is then **matured** and **clarified**. Finally, beer is **filtered**, **pasteurised** and **bottled** (Hough, 1985).

3.1 *Mashing*

The mashing procedure is considered to be the central process in beer production, due to final beer quality being strongly influenced by this procedure (Scheuren *et al.*, 2008). During mashing crucial viscosity changes occur due to enzyme activity and starch pasting properties. For this reason, the mashing phase will be considered in more detail.

The key enzymatic process during mashing is the breakdown of starch polymers (amylose and amylopectin, see section 6.1 pg. 20) into fermentable (i.e. maltose, glucose, maltotriose) and non-fermentable (i.e. maltodextrose, higher dextrins and limit dextrins) sugars (Briggs *et al.*, 1981; Briggs *et al.*, 2004). This is achieved by enzymes either present in barley or developed during malting such as α-amylase, β-amylase, debranching enzymes (R-enzyme, limit dextrinase) and α-glucosidase. The endo-enzyme α-amylase, is able to solubilise both amorphous and crystalline regions within a starch granule, splitting α-1,4 glycosidic bonds anywhere along a polymer starch

chain (Fig. 2.3). The branch points in amylopectin are formed by α-1,6 glycosidic bonds. These bonds cannot be hydrolysed by α-amylase nor α-1,4 glycosidic bonds in close proximity to a branch point. β-amylase is an exo-enzyme which only splits terminal bonds in a polymer starch chain. It removes one maltose unit at a time from the non-reducing end of a starch molecule (Fig. 2.3). Upon reaching the α-1,6 bonds of amylopectin β-amylase activity is arrested and yields β-limit dextrin. Only debranching enzymes are capable of hydrolysing α-1,6 glycosidic bonds (Thomas & Atwell, 1999a; Mousia *et al.*, 2004). α-glucosidase is also an exo-enzyme able to split α-(1-4) linkages and therefore hydrolyses small maltodextrins to produce glucose (MacGregor *et al.*, 2002b; Guerrero, 2009). The starch-degrading action of α-glucosidase is of great importance during malting, but is one of the least studied enzymes in malted barley (Sun & Henson, 1991; Bamforth, 2009).

The action of α-amylase primarily yields complex carbohydrates i.e. branched and unbranched dextrins (Fig. 2.4). β-amylase yields dextrins too, but more importantly maltose (Hough, 1985). Limit dextrinase, which splits α-1,6 glycosidic bonds in dextrins, allows more of the starch to be converted to fermentable sugars (Wainwright, 1997). Starch hydrolysis is strongly dependent on the physical state of starch. Amorphous regions within starch granules are more accessible for enzyme hydrolysis compared to crystalline regions. Starch gelatinisation improves starch susceptibility to enzyme hydrolysis and therefore plays an important role during starch conversion (Mousia *et al.*, 2004).

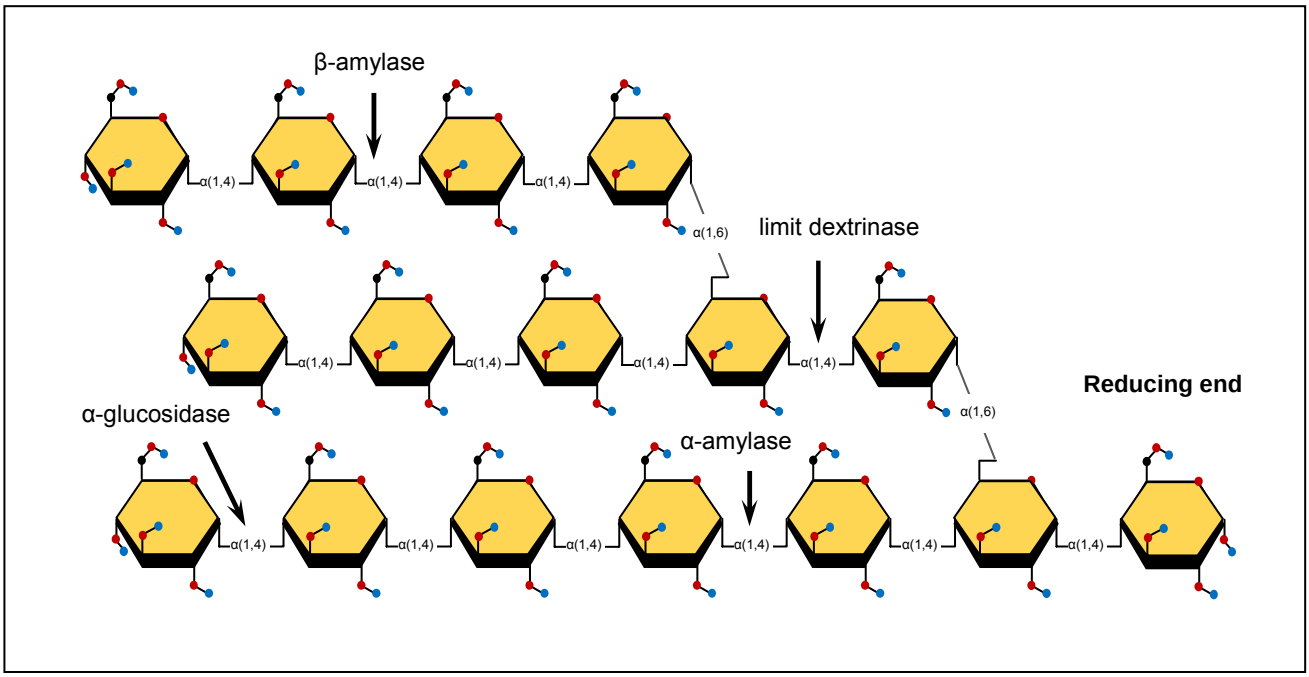

Figure 2.3 The four diastase enzymes hydrolysing specific bonds on a starch chain, consisting of branched D-glucose polymers, adapted from (Marchal, 1999).

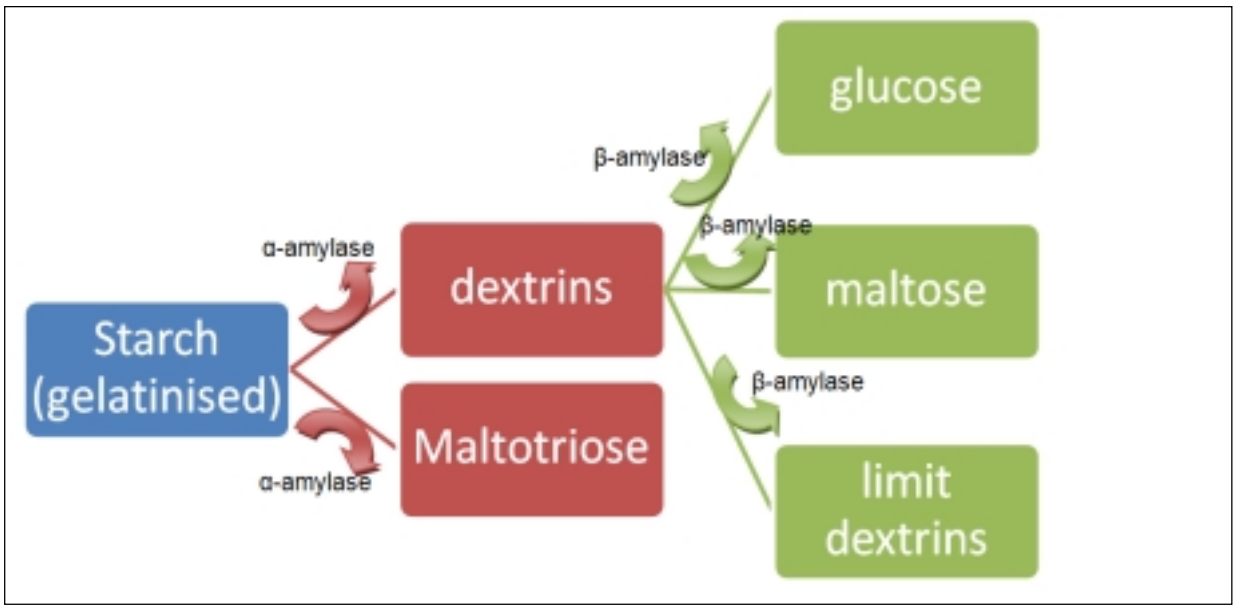

Figure 2.4 A schematic representation of the action of α- and β-amylase, breaking down the starch polymer to sugars, adapted from (Scheuren *et al.*, 2008).

Slight differences in mashing **pH**, **concentration** and **temperatures** can have a considerable effect on the carbohydrate composition of the wort thus also its fermentability (Wainwright, 1997). The effects of each parameter should be examined. Mashing **pH** greatly influences enzyme activity. Mashing pH is usually within 5.4-5.7, which is favourable for amylolysis and need not be corrected by the addition of acid or alkali (Schur, 1980). Kunze (1996) suggests a pH range of 5.5-5.6 as optimal with regards to the mash attenuation limit, protein breakdown, viscosity and lautering rate. While a pH range of 5.3-5.4 (Briggs *et al.*, 1981) and 5.4-5.8 (Bamforth & Simpson, 1995) was suggested for maximum fermentability; which is sligthly higher than the pH used to attain maximum extract (Bamforth, 2001). Certain processes or substances can influence the pH of wort or mash. These typically include: the degree of malt modification, the extent of proteolysis (which occurs during mashing), the mashing style used, the nitrogen content of malt, water alkalinity and even adjunct addition (Bamforth, 2001). Enzymes operate best at their respective pH optima. For α-amylase, the optimal pH is about 5.3, determined at room temperature (Briggs *et al.*, 2004). Most enzymes display considerable activity at either end of their pH optimum. Therefore complete hydrolysis can be attained even when the pH is not optimal, provided that enzymes survive long enough in mash (Bamforth, 2001).

Commonly used grist to liquor ratios range between 1:3.5 and 1:4.5 (Schur, 1980), whereby a more **concentrated** mash provides a protective environment against thermal inactivation of enzymes (Muller, 2000).

Mash **temperature** has a strong effect on starch degradation (Schur, 1980). There are different mashing time and temperature regimes, but all have the general aim to optimise enzyme activity and gelatinise starch (Hough *et al.*, 1982). Temperatures above 60°C are required to gelatinise starch (Kunze, 1996; Stenholm & Home, 1999). This is a compulsory process which

renders starch granules susceptible to enzyme hydrolysis (discussed in detail in section 6.1.1, pg. 24). Enzyme activity and thermal inactivation is also an important consideration. A temperature rest at 50°C optimises proteolysis (hydrolysis of proteins into peptides and free amino acids by endoproteases), β-glucan hydrolysis, and pentosanase activity (hydrolysis of arabinose and xylans). A rest at 63°C optimises β-amylase activity and at 70°C for α-amylase activity. Rapid inactivation of β-amylase and limit dextrinase occurs at temperatures of 65-70°C and above, but for α-amylase at 78°C and higher (Sjoholm *et al.*, 1995; Yang *et al.*, 2009). The substrate present affects thermal inactivation of enzymes i.e. malt endoprotease which has different temperatures of thermal inactivation dependent on hordein or glutelien content (Osman *et al.*, 2002).

3.1.1 Mashing styles

In the mash tun system, often called **infusion mashing**, only one vessel is used. The mash temperature is held virtually constant at 65°C (Hough, 1985). Before the grist falls into the tun, the vessel is heated and partly filled with hot water. Hydrated grist falls into the mash tun, somewhat like aerated porridge and has a tendency to float (Hough, 1985). The mash is held at a constant temperature for one to two hours (Mackenzie, 1927). This particular mashing style suits highly modified malts typically used for the production of ale worts. Substantial protein degradation already occurred during malting of well modified malts. Therefore a lower temperature rest which encourages proteolysis is unnecessary (Hough, 1985). The relatively high and constant temperature used during infusion mashing is sufficient to produce adequate amounts of fermentable sugars and total soluble nitrogen (Boulton & Quain, 2001a).

Traditional German brewing typically dealt with poorly modified malt and therefore implemented **decoction mashing** (Fig. 2.5). Poorly modified malts present brewing problems, therefore brewers prefer to make use of satisfactorily to well modified malts. This has led to a decline in the use of the decoction mashing style (Briggs *et al.*, 2004). During the mashing procedure grist is mashed in at a low starting temperature of 35-40°C. At certain intervals, portions of the mash are withdrawn to a kettle and boiled (kettle mash). A step-wise increase in temperature of the mash occurs by adding the boiling kettle mash to the remainder of the mash (i.e. main mash). A first decoction typically increases the main mash to a temperature of 50-54°C. This can be succeeded by a second decoction, leading to an overall temperature of ±65°C. A final decoction can give rise to a main mash temperature of 73-76°C (Hough, 1985; Briggs *et al.*, 2004). These temperature rests provide optimum activity conditions for the previously mentioned enzyme groups (Hough, 1985). Lager beer is traditionally brewed using less-modified malt. A disadvantage of this style is the malt enzymes of the boiled portion are prematurely denatured.

An alternative and now widely adopted method is programmed temperature mashing. This method gradually increases temperature in a series of steps to allow progressive enzymic degradation of proteins and carbohydrates (Boulton & Quain, 2001a).

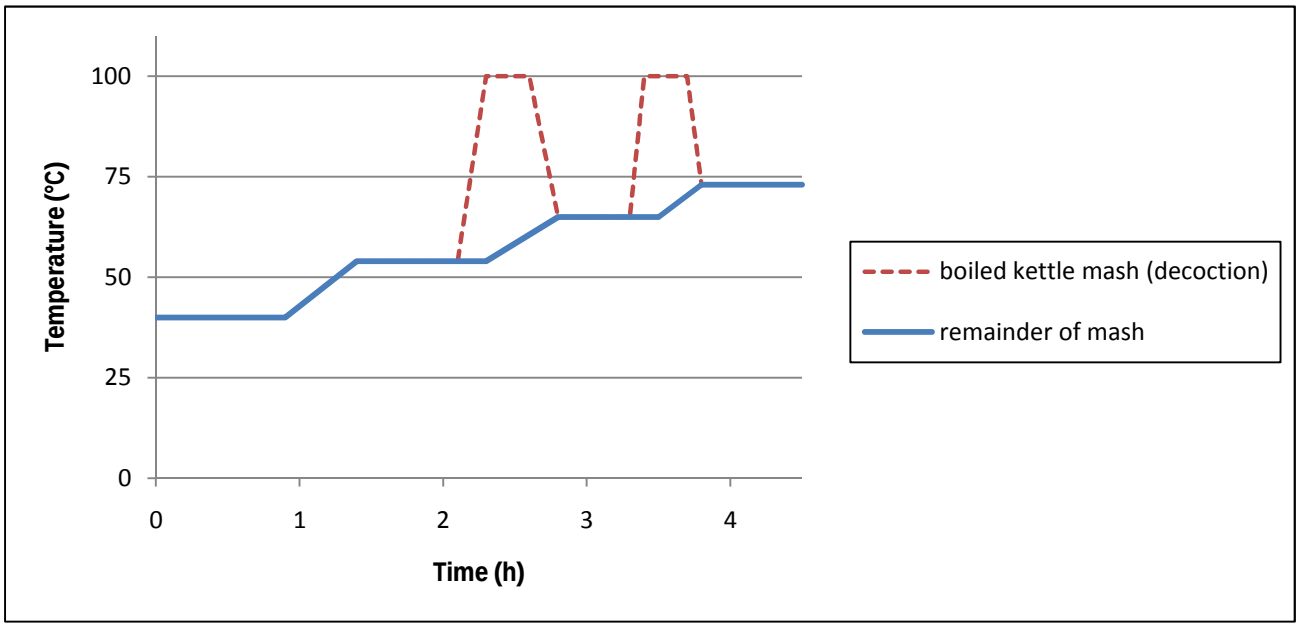

Figure 2.5 A typical decoction mashing time and temperature regime (Hough, 1985).

The demand for malting barley in the United States has always been for high protein, 6-rowed barley (Edney, 1999). Therefore most American malts tend to be well modified and contain high levels of enzymes and nitrogenous materials. Consequently, brewers add large amounts of cereal adjunct to these well modified malts. Adjuncts utilise malt enzymes and dilute the high levels of nitrogenous compounds. The American **double mash system** (Fig. 2.6) involves separate cereal cookers (operating at 65-70°C) which contain adjunct grist mixed with a small amount of malt. The addition of malt allows enzymes present to reduce the viscosity of the paste before boiling the mixture (Hough, 1985). The main mash is mashed in at 38-45°C, which encourages proteolysis and some starch hydrolysis (Hough, 1985; Briggs *et al.*, 2004). When the contents of the cereal cooker are added to the main mash, the temperature rises to ca. 67°C. This causes rapid breakdown of both malt and adjunct starch. The mash is then heated to ca. 72°C to reduce viscosity and pumped into the lauter tun or mash filter where the wort is separated from the spent grain (Hough, 1985).

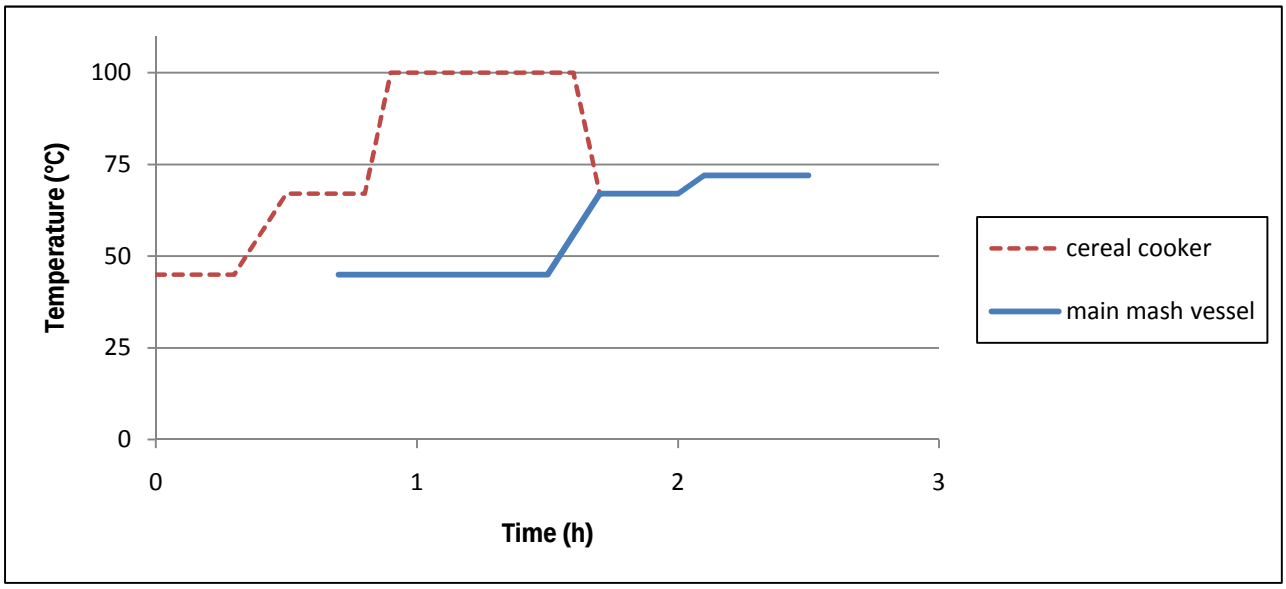

Figure 2.6 A typical double mashing system's time and temperature regime (Hough, 1985).

Most breweries use the double mash system, but some terminate it by raising the temperature from 67°C to 72°C with a single decoction (Hough, 1985). This mashing system is known as **temperature programming infusion mashing** and is gradually replacing older mashing systems. An initial mashing temperature of 35°C, for poorly modified malt, or 50°C and higher, for better modified malt, is used. Typically temperature rests at 50°C, 65°C and 75°C are incorporated. The sweet wort is subsequently collected using a lauter tun or a mash filter (Briggs *et al.*, 2004).

4. Malt fermentability

For the purpose of this review, the production steps following mashing will not be discussed in detail. However, the definition of malt fermentability, with reference to brewing, must be examined in order to define the term **highly fermentable malt**. In general, fermentability with regards to brewing describes the ability of yeast to turn sweet wort into alcohol (Yang *et al.*, 2009). It therefore describes the proportion of wort carbohydrate that may be converted to ethanol by yeast (Boulton & Quain, 2001b). Efficiency of this process governs the alcohol yield and will in turn determine the amount of beer produced (Yang *et al.*, 2009). Wort is required to provide a suitable environment for yeast growth. Necessary nutrients must be provided for fermentation to allow maximum conversion of fermentable materials (i.e. mono-, di- and trisaccharides) to alcohol. In reality however, the total extract obtainable from wort is hardly ever fully converted to alcohol (Fig. 2.9) (Mr P van der Vyver, SAB, Cape Town, South Africa, Personal communication, 2009).

Malt can be supplemented with solid- or liquid-adjunct. Adjuncts are either used for the purpose of cost saving or to impart specific flavour or colour characteristic to beer (Hough, 1985; MacGregor & Bhatty, 1993; Home *et al.*, 2001b). Solid adjunct is commonly obtained from barley, wheat, rice or maize (Hough, 1985; Boulton & Quain, 2001b). These come in various forms and usually require some means of processing before being added to the mash (Boulton & Quain,

2001a). Liquid adjunct, in the form of various sugar syrups, can be added to the wort kettle for boiling (Hough, 1985; Boulton & Quain, 2001b). Adjuncts contribute virtually no enzymes (Kent & Evers, 1994) or nitrogenous compounds to the worts (Hough, 1985). Therefore hydrolysis of adjunct starch and the supply of nitrogenous material, needed for yeast fermentation, depends almost entirely upon malt. When adding a large percentage of adjuncts during brewing, it is of great importance to use a malt type that can supply the necessary amount of enzymes and nitrogen for adequate yeast fermentation.

Higher levels of protein and enzymes, and adequate levels of soluble protein combined with acceptable levels of extract, give the sought after quality of some international malting barley cultivars. These quality traits have been especially appreciated by adjunct brewers who require the mentioned enzyme and protein levels (Edney, 1999; Home *et al.*, 2001b; Yang *et al.*, 2009). For definition's sake, **highly fermentable malt** is able to provide the necessary **enzymes** for hydrolysis of a large amount of adjunct starch, as well as **soluble nitrogen** (especially in the form of free amino nitrogen) for yeast fermentation, thus ensuring maximum conversion of fermentable materials to alcohol.

5. Quality: barley, malt and beer

It is a traditional belief that "good beer can only be made from good malt, and good malt from good barley" (Hunter, 1962). For trading, quality-control and predictive purposes (i.e. predicting end product quality before a particular production process, e.g. malt quality from barley or beer quality from barley or malt, section 5.1, pg. 24), all raw materials used in the production of beer (i.e. the barley, malt, water, hops, wort and beer) are routinely analysed.

Analyses of these materials are usually conducted according to the standard methods of one of four institutions, i.e. the Institute of Brewing (**IOB**) [IOB and the Institute and Guild of Brewing (IBG) merged to form the Institute of Brewing & Distilling (IBD) in 2009], the American Society of Brewing Chemists (**ASBC**), the European Brewery Convention (**EBC**) or the "MitteleuropaÈischen Brautechnischer Analysen Kommission" (**MEBAK**) (Bamforth & Barclay, 1993; Briggs *et al.*, 2004). These methods are similar, but based on different mashing styles (mentioned in section 3.1.1, pg. 19) originating from a given country's brewing conditions. For example, in the United Kingdom malt used for ale brewing in a traditional mash tun is relatively well-modified. A single high-temperature mash is thus used to determine the hot water extract (a common analytical test performed on malt to determine the amount of extract or degree of modification). Therefore the methodology laid down in the *Recommended Methods of Analysis* of the IOB reflects this (Bamforth & Barclay, 1993). In contrast, continental lager malt is usually less-modified. A range of increasing temperatures, typically implemented by the decoction mashing style, is optimal for such malt types. Small-scale mashes, used to establish analytical parameters on less-modified malts, reflect these conditions. Hence, the **EBC Congress mash**, commonly used to estimate malt extract, employs a system of temperature increases (Bamforth & Barclay, 1993; Guerrero, 2009). Results obtained

from these methods often differ in both value and units expressed. Conversion factors, used to interconvert analytical results, are mostly unavailable or otherwise unreliable (Briggs *et al.*, 2004).

A brewer buying malt wished to select the best quality malt at a competitive price; the malt must yield a large amount of extract, present minimal production problems and deliver a consistent good quality beer. A certain type of malt is needed to produce a certain type of beer. It is the brewers responsibility to select the set of malt analyses best suited to define the certain malt type needed to produce the sought after beer (Briggs *et al.*, 2004). Malt analysis allows the easy identification of malt compliance on arrival and throughout the production process to ensure end quality. The ongoing search for, and introduction of improved methods have led, through the decades, to the development of a long list of malt analyses (Briggs *et al.*, 2004). Brewers disagree on the specific set of analyses used to adequately define a required malt. According to Briggs *et al.* (2004) the brewer may specify the barley varieties from which the malt is made, the harvest year, whether or not abrasion or additives may be used, details of the kilning cycle, and a minimum period between production and delivery. Analysis specifications can contain upper or lower limits to which malt must comply (Briggs *et al.*, 2004). Depending on the requirements the barley, malt and beer are expected to fulfil, a vast number of analytical measurements can be conducted by breeding, malting and brewing industries to ensure a consistent end product. A set of specifications for the South African barley, malting and brewing industry is given in Table 2.3. The selected set of analyses must be “logically chosen, kept to a minimum, mutually compatible and individually informative” (Bamforth, 1999).

Table 2.3 Typical analyses conducted on barley, malt and beer by local South African barley breeding, malting and brewing industries (Mr F Potgieter, SABBI, Caledon, South Africa, Personal communication, 2009; Mr P van der Vyver, SAB, Cape Town, South Africa, Personal communication, 2009).

Agronomic characteristics of barley	**Malt quality**	**Brewing quality**
yield	Fine grind extract	fermentability
plumpness	modification (Kolbach index)	brewhouse throughput
grain protein	apparent attenuation limit	brewing yield
ripeness	viscosity	starch gelatinistion temperature
straw length	friability	filterability
harvestability	free amino nitrogen	haze potential
scald resistance	diastatic activity	taste stability
net blotch resistance	β-glucan	colour
leaf rust resistance		

5.1 Prediction of brewing performance

Establishing the quality of barley, malt and beer is important, but prediction of brewing performance or beer quality is of equal concern. Subsequently, correlations between malt and brewing analyses were investigated by numerous authors (Gromus, 1980; Maule & Crabb, 1980; Schildbach, 1980; Schur, 1980; Steiner, 1980).

The course of amylolysis during mashing cannot be predicted by amylolysis related criteria such as saccharification rate or attenuation limit of the congress wort (explained in section 5, pg. 22), α-amylase activity and the diastatic power. Much more valuable information is obtained from the iodine value of the laboratory spent grains, the fine coarse difference, the viscosity, and the activities of the endo-β-glucanases and exo-peptidases (Schur, 1980). Brewhouse yield has been shown to correlate with fine grind extract (Steiner, 1980), coarse grind extract (Maule & Crabb, 1980) and extract difference of malt (Maule & Crabb, 1980; Schildbach, 1980). In the predictive equation generated by Maule & Crabb (1980), little additional variance in brewhouse yield was explained by the inclusion of other variables such as nitrogen or α-amylase content. Lautering time correlates more strongly with extract difference than malt extract (Schildbach, 1980). Indications of beer filterability can be given by wort viscosity and the β-glucan content (Eyben & Hupe, 1980). Fermentability and beer head retention are influenced by protein content, modification and barley origin (Gromus, 1980). The quality characteristics of the beer, such as colloidal stability, foam and reducing substances, can be predicted to some extent from modification characteristics, e.g. the Kolbach (KI) and Hartong 45° index, and viscosity. A relationship can be found between the colour of the beer and the colour of the fine grind extract (Steiner, 1980). The conversion of starch into fermentable carbohydrates during brewing is affected by malt's amylolytic enzyme content and gelatinisation temperature combined with the actual temperature program of the mash (MacGregor *et al.*, 2002a). Malt fermentation performance can be predicted by measuring α-amylase, limit dextrinase, β-amylase combined with β-amylase thermostability and KI (Evans *et al.*, 2008). Mentioned correlations were obtained from malt analyses based on standardised laboratory mashing procedures (EBC or IOB) and brewing analyses based on infusion or decoction mashing procedures. Such analyses may be unrepresentative of a specific brewing condition and consequently nullify such correlations (Axcell, 1998; Home *et al.*, 2001b). Therefore, some researchers claim malt analyses are highly empirical (Goode & Arendt, 2006).

Malt analysis has been under scrutiny for many years and still remains a controversial subject. As early as 1962, Cook stated "despite the development of a large array of methods to analyse malt, it cannot be guaranteed by analysis alone that a given malt can be brewed without trouble to yield a satisfactory beer". Processing or quality problems encountered in practice cannot always be predicted from analytical results (Drost *et al.*, 1980; Axcell, 1998; Home *et al.*, 2001b). In essence, malt analyses indicate the theoretical behaviour of the malt, but in the brewery, malt can behave completely different (Mr P van der Vyver, SAB, Cape Town, South Africa, Personal communication, 2009). The extensive range of analytical techniques available to establish malt

quality and predict brewing performance is still inadequate or insufficiently informative (Bamforth & Barclay, 1993; van Nierop *et al.*, 2008). Fermentation and brewing performance can most accurately be predicted by replicating the actual brewing process on a smaler pilot scale. Such a fermentability test was developed and implemented in the 1980's at a local malting company, i.e. South African Breweries Maltings (SABM). This test was named the malt fermentability indicator test (MFIT) (Kruger *et al.*, 1982).

5.2 Malt Fermentability Indicator Test

In practice, most brewery laboratories still resort to a small-scale fermentability test or mashing procedure, which in reality is an attenuation test to predict brewing performance (Gilliland, 1951; Dixon, 1967; Bamforth, 1999). The similarity between standard laboratory fermentation methods and an actual brewery fermentation process is questionable. This led to the development of a modified laboratory fermentation system tailored to South African Breweries (SAB) brewing processes, which is in routine use at SABM. The MFIT, also referred to as micro-brewing, is a laboratory based test that reproduces the brewing process on a smaller scale to measure the ability of malt to ferment. It is used as a prediction tool to detect fermentation problems which require process adjustments in the brewery. The test evaluates the performance of the malt during fermentation through five main steps: (1) milling, (2) mashing, (3) lautering, (4) boiling and (5) fermentation (Kruger *et al.*, 1982).

The MFIT comprises of wort preparation steps (1 to 4 above), followed by a ten day fermentation. During these steps, the malt and wort is continuously evaluated for deviation from specifications. The most significant measurement is the real degree of fermentation (RDF) (Fig.2.8) calculated after the 10^{th} day of fermentation (Mr D Fisher, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009).

Wort can only be fermented by yeast to a certain limit, i.e. the limit of attenuation, after which yeast flocculates. The relative density of the extract is measured to determine the amount of sugars present, by means of Plato tables (conversion tables which allow the calculation of a sample's sugar content, expressed in g/100g, by means of specific gravity). When the relative density of the extract is measured in the presence of alcohol, the residual extract is referred to as apparent limit of attenuation (AAL) (Briggs *et al.*, 2004). However, when the relative density is measured after the alcohol is distilled off, correcting the sample mass to its original mass with purified water, the extract is referred to as the true limit of attenuation. The percentage attenuation (RDF) is used to express the percentage of the extract converted to alcohol out of the total original extract (OE). This enables the estimation of the amount of sugar consumed and thus the amount of ethanol produced (Fig. 2.9). In general, the remaining extract (LE) indicates the amount of extract which cannot be fermented by the specific strain of brewers' yeast used. To some extent, a beer's character is defined by its LE, which is brand specific (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009).

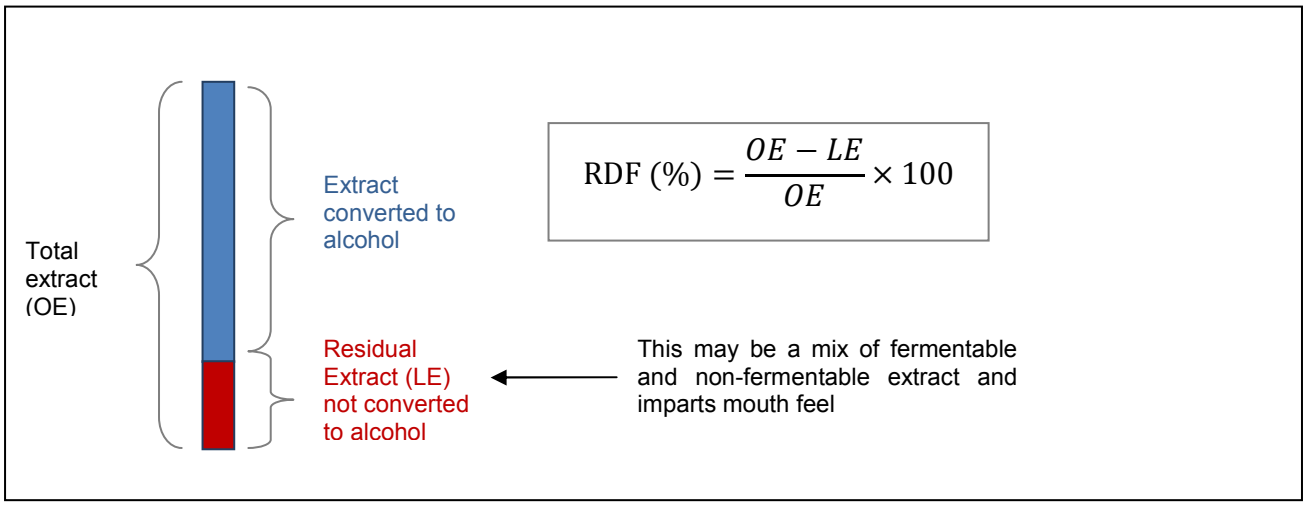

Figure 2.7 Graphical illustration of the real degree of fermentation (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009).

End-results are affected not only by malt quality and condition, but also by different yeast batches (Phaweni *et al.*, 1992), operator skill and instrumentation (which do not represent commercial equipment) (Bamforth, 1999; Mr P van der Vyver, SAB, Cape Town, South Africa, Personal communication, 2009). Thus, MFIT does not exclusively report the quality and condition of malt, and its effectiveness to predict performance is questionable, at least.

6. Rheology

Rheology is the study of the deformation and flow of materials under the influence of external forces (Naé, 1993b). Different instruments for rheological viscometric measurements have been proposed for use in the assessment of barley, malt and beer quality (Goode *et al.*, 2005c). These include the Brabender-Viscograph (amylograph) (Yoshida & Yamada, 1970), the Falling Number Apparatus (Lorenz & Kulp, 1981), Ottawa Starch Viscometer (Voisey *et al.*, 1977), Rheoswing RSD (Senge *et al.*, 1996), Bohlin CS-50 rheometer (Goode *et al.*, 2005a; Goode *et al.*, 2005b) and the Rapid Visco Analyser (Glennie Holmes, 1995a; Glennie Holmes, 1995b; Glennie Holmes, 1995c; Glennie Holmes, 1995d; Glennie Holmes, 1995e). Rotational viscometers are widely used in the food industry because most are simple to operate, allow electronic manipulation of data and are low in cost (Rao, 1999b). This type of viscometer is widely used on fluids that contain suspended particles due to settling and product separation being minimised (Rao, 1999b). A rheometer can be seen as a high quality or more sensitive viscometer. Since its mechanical friction is much less, it enables measurement of low viscosity fluids.

To understand the ability of such instruments to measure malt quality, in relation to fermentability, it is important to examine instrument methodology and the sample's rheological properties (i.e. rheological changes which occur in a malt-water mixture when applying a certain time and temperature profile/regime).

Viscosity (η) can be associated with the tendency of a fluid to resist flow (internal friction of a fluid) or in laymen's terms, as a measure for the "thickness" of a fluid. Shear stress (σ) is the stress component applied tangentially to the fluid (Rao, 1999a). Shear rate $(\dot{\gamma})$ is the velocity's spatial gradient, perpendicular to the direction of flow, established in a fluid as a result of an applied shear stress (σ), or the rate at which the velocity changes within a fluid (Dobson, 2008). To clarify these definitions: consider a homogenous fluid with a layer-thickness x, between plates of length l_0 and width w_0, proving an area A, which is large enough to avoid edge effects (Fig. 2.9). Assume the bottom plate is stationary and the top is movable along the horizontal direction. Suppose laminar flow of the fluid between the two plates and its viscosity will allow the top plate to move in the steady state at a constant velocity u, due to the force *F*. The relationship for Newtonian fluids between these variables is given by:

$$F = \mu A \frac{u}{x}$$

The pulling action is the shear stress (σ), which is defined as the force *F* over the area $(A = l_0 \cdot w_0)$ (Naé, 1993b):

$$\sigma = \frac{F}{A}$$

The units of shear stress are Newtons per square meter (N/m^2) = Pascal (Pa) (Naé, 1993b). This allows the relationship between shear stress σ, the viscosity μ, and the shear rate $\dot{\gamma}$, to be written in differential form as

$$\sigma = \mu \frac{\partial u}{\partial x} = \mu \dot{\gamma}$$

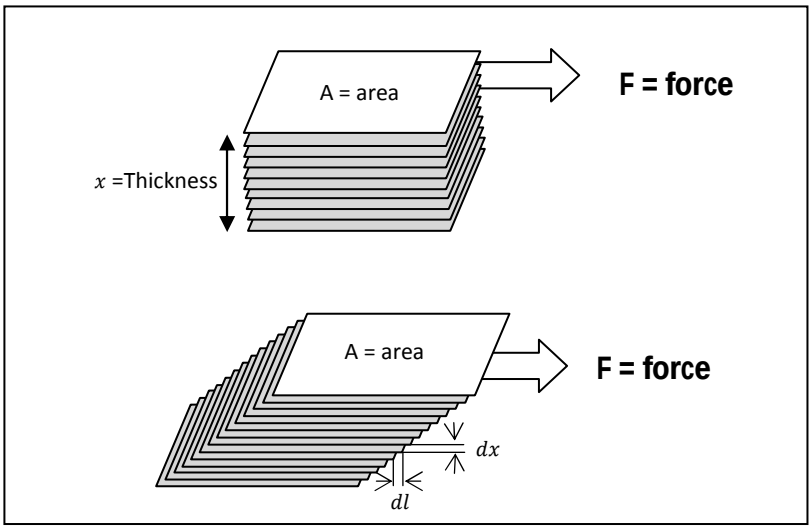

Figure 2.8 Illustration of shear stress applied to a material (Naé, 1993b).

Newtonian fluids contain compounds of low molecular weight (e.g. sugars) and lack high concentrations of dissolved polymers (e.g. pectins, proteins or starches) or insoluble solids. For

Newtonian fluids, the shear rate ($\dot{\gamma}$) is directly proportional to the shear stress (σ) (Fig. 2.9a) and therefore their viscosities are independent of shear rate (Fig. 2.9b). Viscosity measurement of non-Newtonian fluid foods (such as tomato paste, chocolate mixtures, soups) is shear rate dependent (Fig. 2.9b) (Rao, 1999b). For non-Newtonian fluids a non-linear relationship exist between shear stress and shear rate (Fig. 2.9a). Wort and beer samples display Newtonian behaviour, while mash displays non-Newtonian behaviour. This is due to the presence of colloidal matter in mash systems, consisting of soluble and insoluble particles with different sizes, shapes, and densities (Goode *et al.*, 2005a).

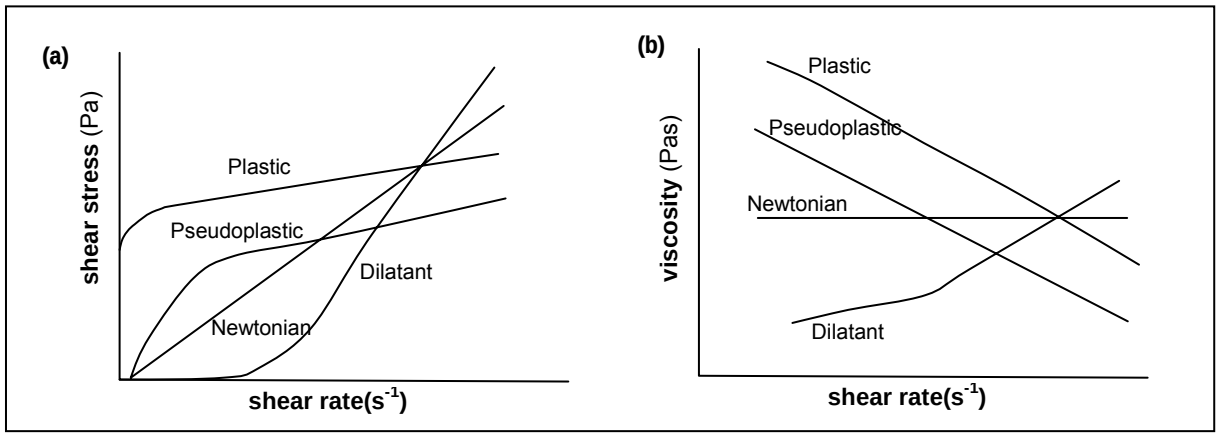

Figure 2.9 The relationship between (a) shear stress-shear rate and (b) viscosity-shear rate adapted from (Naé, 1993b).

Shear thinning or pseudoplastic behaviour of mash systems has been observed; a consequence of mash being non-Newtonian (Senge *et al.*, 1996; Goode *et al.*, 2005a). Mash particles are not perfectly spherical, but heterogeneously shaped. These particles orientate themselves in the direction of the force applied to the mash (Newport Scientific, 1995). This orientation reportedly yields a smaller internal friction of the carrier medium, resulting in a viscosity decrease (Goode *et al.*, 2005a). Shear thinning implies the resistance of a material to flow reduces, consequently decreasing the amount of energy required to sustain flow (Naé, 1993b). It therefore results from the alignment of molecules of soluble starch in the direction of stirring (Agu *et al.*, 2006). Generally, the more soluble the starch, the more it will thin upon shearing (Hoseney, 1994).

Most viscometers in use are based on rotating the sample and measuring its response to the applied stress by a variety of sensors (Naé, 1993a). The Rapid Visco Analyser, similar to other rotational viscometers, measures material viscosity by using a precision electric motor to continuously rotate at a constant speed (Newport Scientific, 1995).

6.1 Starch biology and rheology

To fully understand the role starch plays in rheological changes experienced during mashing the biological structure of starch in barley and malt must first be considered. Barley kernels consist of

approximately 55-60% starch (Pollock, 1962), while the starch portion of a mature endosperm contains about 25% amylose and 75% amylopectin (Duffus & Cochrane, 1993).

As mentioned previously (section 2.2, pg. 14), the starch endosperm of a barley kernel can be considered a cellular structure whereby each separate cell is filled with starch granules (indicated by ellipse in Fig. 2.10a). These granules are contained within thin cellulose walls, consisting of β-glucans and pentosans (Fig. 2.10b) (Wainwright, 1997). Starch granules are imbedded within a protein matrix (Barlow *et al.*, 1973; Olkku & Rha, 1978). This is thought to lend the granular appearance in scanning electron microscopy (SEM) images of barley endosperm (Fig. 2.10). Membranes existing around the starch granule are thought to be residues of the original amyloplast and endoplasmic reticulum.

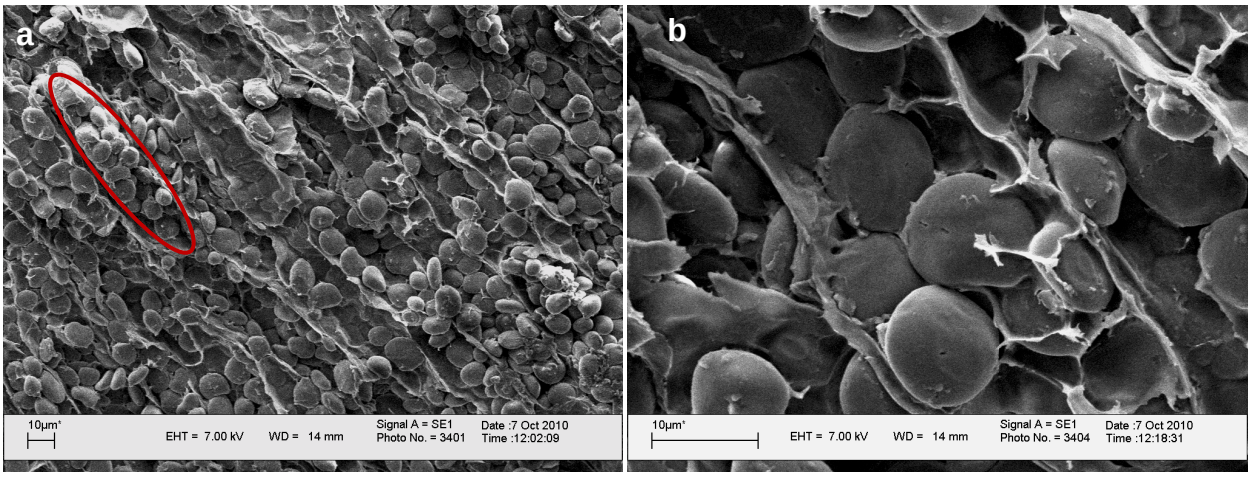

Figure 2.10 A SEM images illustrating the starch endosperm of a barley kernel consisting of (a) separate cells (b) bound by walls, containing the starch granules.

Two types of starch granules exist, namely a larger A-type (15-25 µm) and a smaller B-type (<10 µm) granule (Fig. 2.11) (MacGregor & Fincher, 1993). Within mature endosperm as much as 90% of the starch **volume** consists of A-type granules, while the total **amount** of A-type granules are found to be less than 5% (Duffus & Cochrane, 1993).

Starch granules are considered to be a mixture of hard and soft material, consisting of alternating semi-crystalline (amorphous) and crystalline shells (Yamaguchi *et al.*, 1979; French, 1984; Gallant *et al.*, 1997). A starch granule is largely composed of D-glucose polymers which organise in such a way as to form amylose (virtually linear) or amylopectin (extensively branched). These polysaccharide chains associate to form zones of differing degrees of order within each granule. Amylopectin chains are grouped to form crystalline zones (clusters), each separated by a narrow amorphous region containing α-1,6 branches (Robin *et al.*, 1974; Gallant *et al.*, 1997). Amylopectin and amylose organise to form radially orientated crystals, giving rise to the phenomenon of birefringence (Eliasson & Tatham, 2001b). The presence of a Maltese cross, a

consequence of their crystallinity, can be observed under polarised light (Fig. 2.11c) (Mariotti *et al.*, 2005).

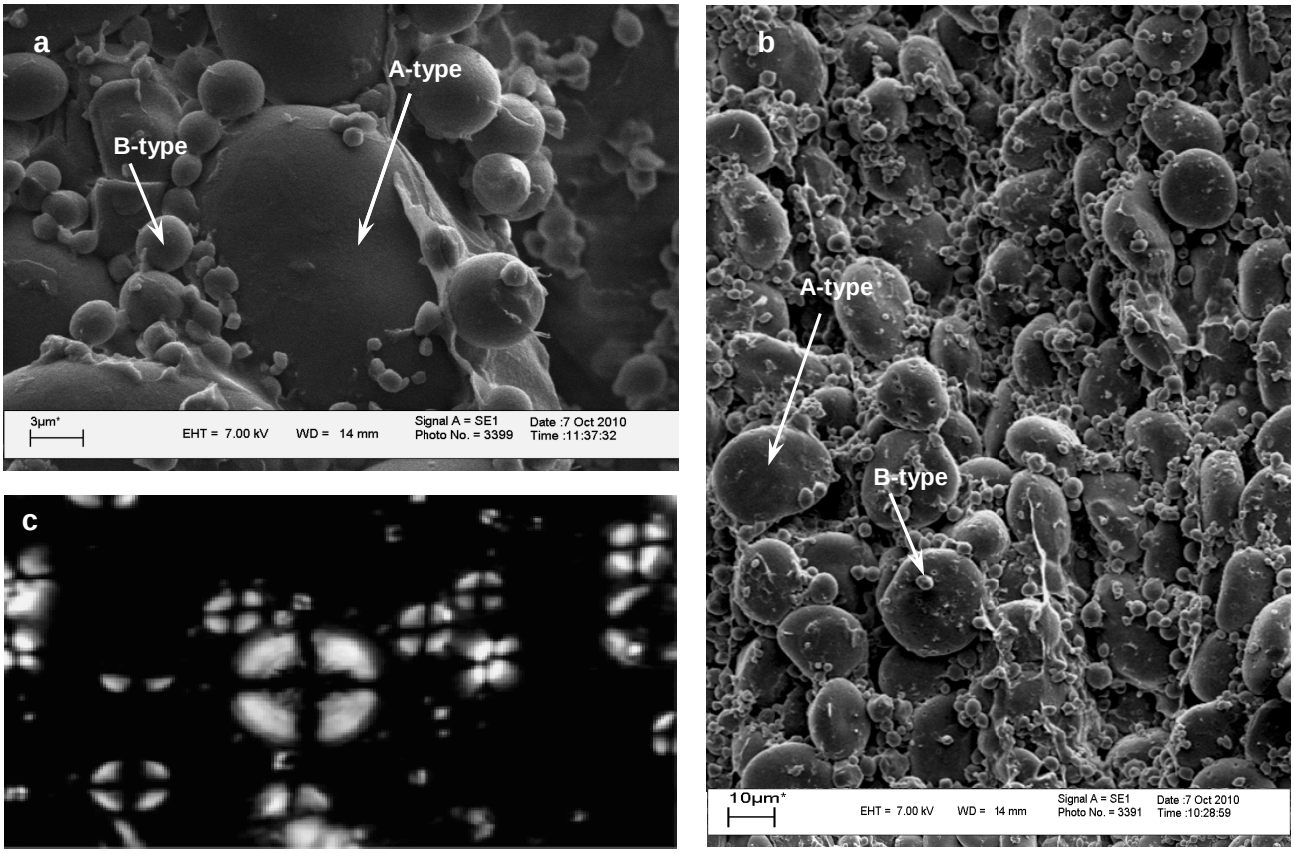

Figure 2.11 (a) SEM image illustrating the larger A-type and smaller B-type starch granules, embedded within a protein matrix, (b) within the endosperm, (c) which are birefringent when observed under polarised light (Mariotti *et al.*, 2005).

During the malting process, smaller B-type starch granules are preferentially degraded, therefore raw unmalted barley usually contains higher levels of small starch granules compared to malted barley (Bathgate & Palmer, 1973). It has been well documented; smaller barley starch granules have a higher gelatinisation temperature which can cause a decrease in enzyme digestibility (Ellis, 1976; MacGregor & Fincher, 1993). The presence of a large amount of these granules subsequently has a negative effect on mashing (Ellis, 1976). It has also been found; different size granules possess different amylose:amylopectin ratios (Takeda *et al.*, 1999) and thus varying physicochemical properties (Tang *et al.*, 2000; Mousia *et al.*, 2004).

The progression of hydrolysis during the malting process destroys nearly all the smaller starch granules, while the larger granules remain structurally intact, but appear perforated by multiple discrete endo-corrosion channels (Bathgate & Palmer, 1973; Palmer, 1989). Numerous SEM images have shown the characteristic pitting of the larger A-type starch granules in malt (Fig. 2.12) (Palmer, 1972; Pomeranz, 1972; Lorenz & Kulp, 1981; Palmer, 1987; Palmer, 1989). Enzymes

diffuse onto the surface of a starch granule, flow to the inside of a granule through these surface-to-core channels by means of capillary action, and commence amylolysis at enzyme-substrate contact points (Benmoussa *et al.*, 2006; Mahasukhonthachat *et al.*, 2010). Amylolysis has been defined as the extensive breakdown of starch and dextrin molecules by α- and β-amylases acting simultaneously, to yield soluble sugars (Glennie Holmes, 1995d). Amylolysis proceeds centripetally, creating additional pores and channels to the granule core, but also centrifugally from inside the granule core (Benmoussa *et al.*, 2006; Mahasukhonthachat *et al.*, 2010). Therefore, the formation of such channels supposedly accounts for the more rapid digestion of malt compared to barley starch (Palmer, 1989; Glennie Holmes, 1995d).

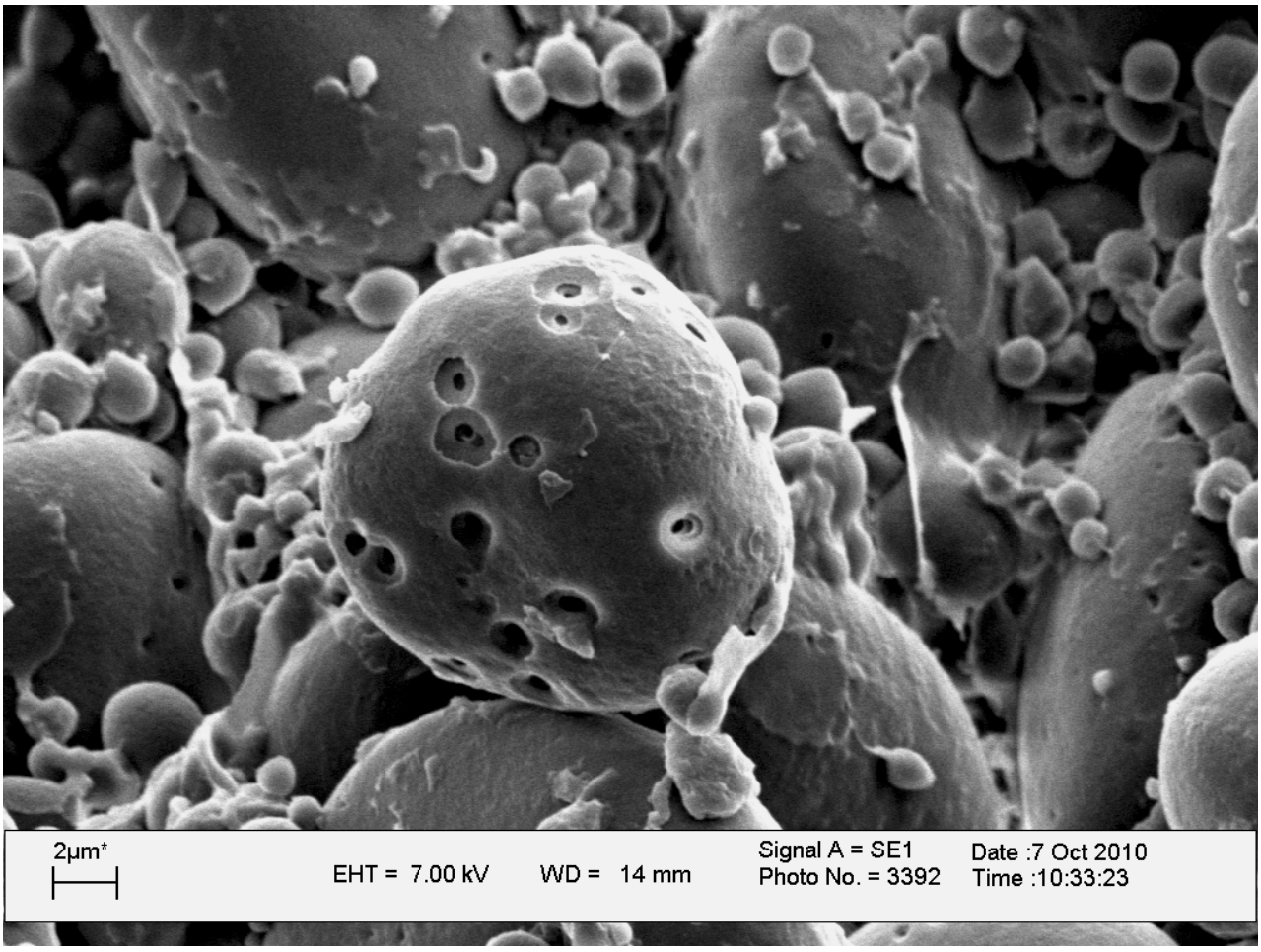

Figure 2.12 SEM image illustrating the characteristic pitting of the large (A) starch granules of malt due to amylolytic hydrolysis.

The most common way to fingerprint starch is via its pasting profile (Dengate, 1984): a starch suspension is subjected to a defined heating and cooling program and the viscosity is recorded as a function of temperature and time. The curve generated (Fig. 2.13) can be seen as a reflection of the structural changes of the malt starch granules that occur during starch **gelatinisation**, **retrogradation** (Thomas & Atwell, 1999b) and endogenous enzyme activity (particularly α-

amylase) (Mariotti *et al.*, 2005). Therefore, the rheological effect of these processes must be investigated.

6.1.1 Gelatinisation

When milled malt is mixed with water at room temperature, the starch granules absorb water and start to swell (Point 1, Fig. 2.13). This is a reversible process and can easily be undone without permanent change to the starch granule (Hoseney, 1994). Hydrogen-bonding forces hold the constituent molecules of starch granules together. When aqueous suspensions of granules are heated a temperature is reached at which these forces are weakened, allowing the absorption of water (Olkku & Rha, 1978; Dengate, 1984). Increasing the temperature of a malt-water mixture to approximately 60-64°C (Allan *et al.*, 1997) leads to irreversible swelling of starch granules and simultaneous loss of birefringence (Olkku & Rha, 1978; Hoseney, 1994). This change is an endothermic reaction and leads to a viscosity increase which continues as the temperature rises (Points 2 and 3, Fig. 2.13) (Kent & Evers, 1994).

Weaker hydrogen bonding in the amorphous areas of starch granules (mentioned in section 6.1. pg. 28-30) causes the onset of gelatinisation (Dengate, 1984). As the temperature of the aqueous suspension is increased, the crystalline areas start to melt. These areas possess different degrees of order, causing transition to take place over a temperature range (Lelievre, 1976; Olkku & Rha, 1978). The disruption of hydrogen bonding forces continues as the temperature increases above the gelatinisation range. Water molecules become attached to hydroxyl groups and the granules continue to swell (Olkku & Rha, 1978). Part of the starch is solubilised inside the granule, over a relatively narrow temperature range, before it escapes (Olkku & Rha, 1978). The swollen state of starch granules renders them more susceptible to shear disintegration (Olkku & Rha, 1978). The change in the crystalline structure of amylopectin molecules together with further weakening of the hydrogen bonding forces, causes the granules to become more susceptible to enzyme degradation (Kent & Evers, 1994; Allan *et al.*, 1997). Starch granules begin to rupture with continued swelling and heating (Point 4, Fig. 2.13), releasing the solution inside. The discharge of exudate from granules is considered to be the primary cause of viscosity increase during gelatinisation (Point 3, Fig. 2.13) (Olkku & Rha, 1978). This causes fully hydrated starch particles to separate from the micellar network and disperse into the aqueous medium (Point 5, Fig. 2.13) (Olkku & Rha, 1978). Therefore, the more soluble amylose chains leach out of the granule into the solution (Kent & Evers, 1994; Thomas & Atwell, 1999b). In some cases this process is followed at a slower rate by the amylopectin fraction (Thomas & Atwell, 1999b).

The sharp viscosity increase observed in the RVA viscogram (peak formation, Point 3, Fig. 2.13) is attributed to: the swelling behaviour of the starch granules, which reduces the mobile phase surrounding it; the accompanying leaching of starch polymers (amylose) into the mobile phase; and the resultant competition for free water between exuded amylose and the remaining granules (Dengate, 1984; Hoseney, 1994; Kent & Evers, 1994). It has also been suggested for

barley and maize, the thermal breakdown of an amylose-lipid complex could contribute to peak formation during gelatinisation (Goering *et al.*, 1975; Dengate, 1984).

Changes after starch gelatinisation (loss of birefringence) are termed pasting (Hoseney, 1994; Thomas & Atwell, 1999b; Nelles *et al.*, 2000). However, Zeng et al. (1997) refer to the term pasting as: “viscosity changes that occur just before, during and after the event of gelatinisation” and consequently includes the process of retrogradation. Batey & Curtin (2000) defined pasting as the absorption of water by starch granules to initiate swelling and, subsequently, a viscosity increase. Gelatinisation only occurs when losing the internal crystalline structure of the granule by increasing the temperature (observed as a loss of birefringence). For regular maize and wheat starch, gelatinisation occurs well before pasting (Batey & Curtin, 2000).

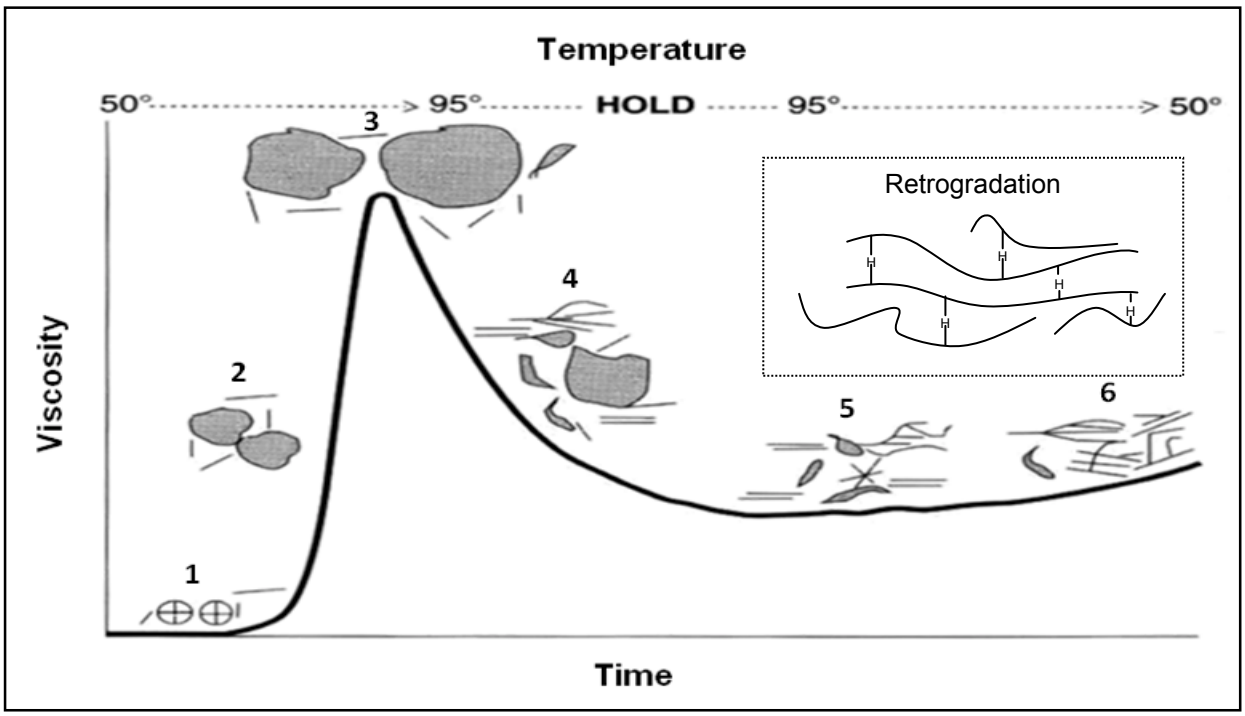

Figure 2.13 A schematic representation of starch granular changes in relationship to viscosity (Thomas & Atwell, 1999b).

6.1.2 Retrogradation

Retrogradation is the re-association of starch molecules and is typically influenced by the amount (Olkku & Rha, 1978; Leman *et al.*, 2006), the length or molecular weight (Leman *et al.*, 2006), and dispersion of amylose chains (Olkku & Rha, 1978; Leman *et al.*, 2006). Therefore retrogradation is largely due to the rapid crystallisation of amylose, forming gels at concentrations as low as 1.5% (Olkku & Rha, 1978; Dengate, 1984), but also to some extent slower amylopectin crystallisation, both actions causing a viscosity increase (Point 5 & 6, Fig. 2.13). The linear structure of amylose, allows greater mobility of the starch polymer (Taggart, 2004). Chains, leached from the swollen starch granule during gelatinisation, become entangled in the water medium at high enough concentrations (Kent & Evers, 1994; Frazier, 2009). The randomly oriented molecules of amylose

start to align themselves into a parallel network. As the starch-water mixture cools, the affinity of hydroxyl groups in one molecule for those in another increases. Therefore, starch polymer-water hydrogen bonds are replaced with polymer-polymer hydrogen bonds (i.e. hydrogen bonding between the aligned chains) (Zeng *et al.*, 1997; Taggart, 2004). Translational motion is lost by the entangled chains, causing water to be trapped within the three dimensional amylose network (Fig. 2.13) (Kent & Evers, 1994).

The viscosity increase, typically observed in a barley pasting profile (end viscosity), is thus the transformation of starch from a solvated, dispersed, amorphous state to an insoluble, aggregated or crystalline condition (Kent & Evers, 1994; Allan *et al.*, 1997). The action of α-amylase, present in malt, rapidly decreases the size of starch molecules (Mariotti *et al.*, 2005), thereby reducing the peak and final viscosity in a malt pasting profile. Degradation of 0.10% of the internal bonds within a starch molecule (especially due to α-amylase hydrolysis of α-1,4 glycosidic bonds) is claimed to cause a 50% decrease in viscosity (Glennie Holmes, 1995a). Thus a small change in starch structure results in a considerable rheological change. The activity of α-amylase modifies starch to an extent which causes a decrease in viscosity upon cooling. It is suggested the outer branches of amylopectin are hydrolysed, thus preventing the formation of large amylopectin crystals. Hydrolysed amylopectin is therefore incapable of promoting a viscosity increase upon cooling (Mariotti *et al.*, 2005). It has also been suggested the interaction between amylose and amylopectin chains affect retrogradation (Kurakake *et al.*, 2008). However, the mechanism of network formation is still not well understood.

7. The Rapid Visco Analyser

7.1 History

The RVA was first developed in 1985 by Newport Scientific (Mrs B Elliott, Newport Scientific, Australia, personal communication, 2010) to test for sprouting damage in wheat after 4.7 million tonnes (22.5% of average crop) of Australian wheat was downgraded from the milling category to general purpose grade due to weather damage in 1983 to 1984 (Ross *et al.*, 1987). This viscometric instrument has since been used in a vast range of applications in the food industry. Of particular interest is its capability to monitor quality in cereals due to the large amount of starch present in grain. This historical development of the RVA, with regards to cereals, has provided the fundamental basis for the development of malt analysing methods (Table 2.4).

Table 2.4 RVA application on different types of cereal products

Cereal Commodity:	Investigated factor or characteristic measured:	Reference
Wheat	Estimating sprouting damage	(Ross *et al.*, 1987; Watanabe & Suzuki, 1991)
Germinated wheat	Intercorrelation between NIR and RVA characteristics as influenced by germination	(Juhasz *et al.*, 2005)
Wheat: wholemeal grist, flour and starch	Noodle quality selection in breeding lines	(Panozzo & McCormick, 1993)
Wheat: waxy (low amylose) and non waxy flours	The effect of amylose content on starch pasting properties	(Zeng *et al.*, 1997; Sasaki *et al.*, 2000; Kiribuchi-Otobe *et al.*, 2004; Yanagisawa *et al.*, 2004; Mu *et al.*, 2006; Yasui, 2004),
Durum wheat	End-quality prediction (cooking loss of pasta)	(Sissons & Batey, 2003)
Wheat noodles	Relating pasting properties to noodle texture for quality selection	(Ross *et al.*, 1997; Nagamine *et al.*, 2003)
Rice noodles	End-quality prediction (texture)	(Bhattacharya *et al.*, 1999; Bason & Blakeney, 2007)
Rice starch	The effect of amylose content on starch pasting properties	(Chen *et al.*, 2003)
Maize: waxy and high amylose starch	The effect of amylose content on starch pasting properties	(Juhasz & Salgo, 2008)
Bread products	Detecting starch properties of end-products	(Yasui, 2004)
Maize	Effects of kernel hardness on pasting properties	(Almeida-Dominguez *et al.*, 1997; Landry *et al.*, 2000; Landry *et al.*, 2001; Bason & Blakeney, 2007; Narvaez-Gonzalez *et al.*, 2007)
Buckwheat	Effects of pre-harvest sprouting	(Hara *et al.*, 2007)
Sweet potatoes	Estimate α-amylase activity	(Collado & Corke, 1999)
Sweet potatoes	Genotype differentiation	(Collado *et al.*, 1999)
Barley	Detect early non-visible sprouting/sprouting damage	(Bason *et al.*, 1993; Bueckert *et al.*, 2007)
Barley	Sprouting resistance	(Hori *et al.*, 2007)
Barley	Amylose and amylopectin content of different genotypes	(Swanston *et al.*, 2001).

Table 2.4 CONTINUED

Barley and malt	Quality selection in a barley breeding programme	(Glennie Holmes, 1992; Glennie Holmes, 1995a; Glennie Holmes, 1995b; Glennie Holmes, 1995c; Glennie Holmes, 1995d; Zhou & Mendham, 2005; Zhou *et al.*, 2008)
Purified barley starch	Quality selection in a barley breeding programme	(Allan *et al.*, 1997; Dunn *et al.*, 1997; Stuart *et al.*, 1998)
Barley and malt	Influence of corn size distribution	(Agu *et al.*, 2007)
Malt	Effect of enzyme and adjunct addition	(Glennie Holmes, 1995e)
Malt	Simulating the brewery mashing process: explaining and characterising rheological changes	(Goode *et al.*, 2005c; Goode & Arendt, 2006)

7.2 Methodology and analysis

The RVA has been described as a "rotational, continuously recording viscometer, with heating, cooling and variable shear capabilities" (Agu *et al.*, 2006). It is specifically configured for starch-based materials and its methodology is based on the Searle system (Goode *et al.*, 2005c). This implies the stirrer, which is also the sensor element, rotates at a defined speed within a stationary container (Fig. 2.14a) (Naé, 1993a). The stirring paddle design intensifies the effect of non-laminar or turbulent flow which prohibits absolute viscosity measurements (Goode & Arendt, 2006) at high rotational speeds. The RVA is regarded as an empirical viscometer (Goode & Arendt, 2006) measuring relative viscosity. Viscosity for the RVA is commonly recorded in Rapid Visco Analyser units (RVU), but can be converted to centipoise (cP) [e.g. 1 RVU is approximately equal to 10 cP (1 cP = 1 mPa•s)] if a calibration is performed at a low constant rotational speed (160 rpm) (Lai *et al.*, 2000).

To run a RVA analysis, the plastic stirrer is placed in a disposable aluminium canister (Fig. 2.14b) which contains the mixed sample of water and product. The stirrer with canister is then fitted into the coupling (Fig. 2.14c). The tower is pushed down into the instrument and the aluminium canister is hydraulically clamped inside a hot copper block. Electrical heating elements mounted within the block cause a temperature increase, while cold tap water, flowing through machined channels, allows it to cool. As the block is heated or cooled, so too is the canister and its contents (Newport Scientific, 1995). The viscosity and temperature changes, which occur in the malt-water sample, are measured and displayed as a viscogram generated by the computer which is linked to the RVA instrument. The resistance to stirring, recorded as a function of time and temperature, is related to the rheological properties of the mixture.

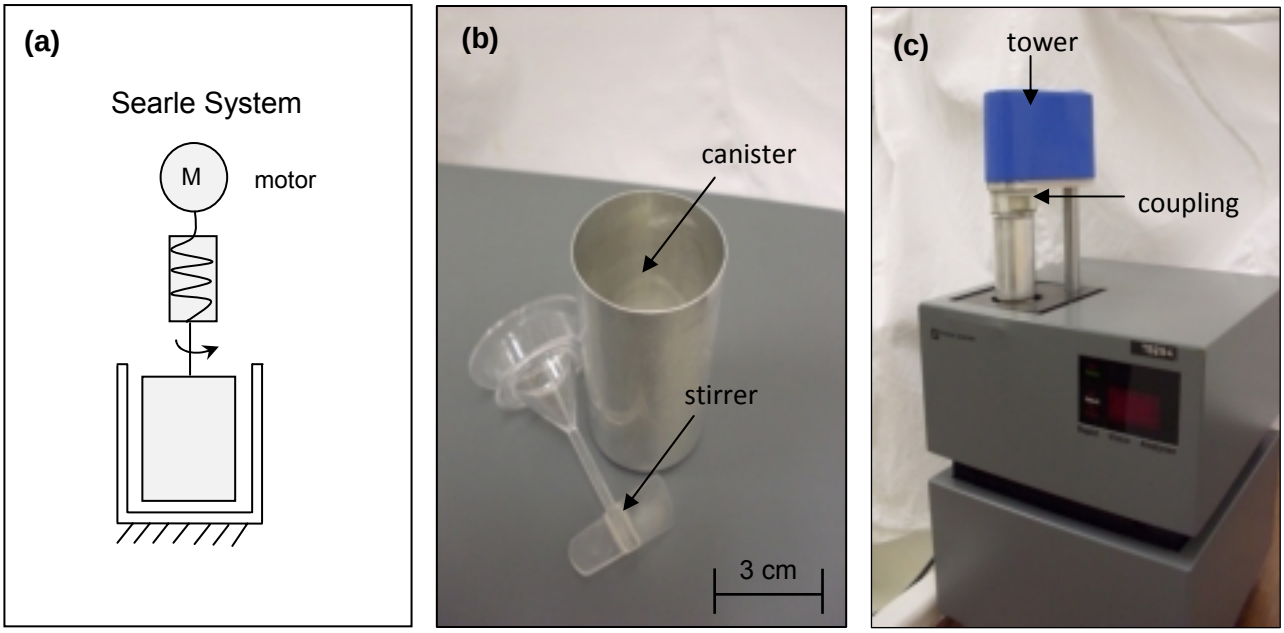

Figure 2.14 Illustration of the RVA methodology which is based upon (a) the Searle system (Naé, 1993a) and consists of (b) a stirrer placed in a canister, containing the sample, and then attached to (c) the coupling after which the tower is pressed down to initiate a test.

A viscometer must be capable of providing readings that can be converted to shear rate ($\dot{\gamma}$) and shear stress (σ) in the proper units of s^{-1} and Pa, respectively, otherwise results are highly empirical (Naé, 1993a; Lai *et al.*, 2000). Viscosity measurement is performed under laminar flow conditions, because turbulent flow leads to higher measurements (Naé, 1993a).

In rotational viscometer geometries (concentric cylinder, cone-plate, parallel disk) **shear stress** (σ) can be calculated from the measured **torque** and the dimensions of the test geometry being used (height and radius of the rotor) (Naé, 1993a). The measured torque is directly proportional to the sensor measuring a **signal** (such as current drawn by the electric motor). It is assumed that average **shear rate** ($\dot{\gamma}$) around the paddle is directly proportional to the **rotational speed** (Rao, 1999b). This assumption has been supported for the RVA by Lai *et al.* (2000). The value of k_s (a constant that must be determined for each paddle in order to obtain shear rate) was determined for the impeller-cup combination of the RVA, enabling the measurement of shear rate (Rao, 1999b; Lai *et al.*, 2000). To summarise, the relative viscosity of fluids is determined via the RVA by continuously rotating an electric motor at constant speed (constant shear rate). As the motor load changes due to changes in viscosity of the fluid under investigation, the power, necessary to maintain the constant speed, is measured electronically. This generates a torque signal (shear stress) which is converted to a relative viscosity value by the linked computer (Newport Scientific, 1995).

7.3 Advantages and disadvantages

The main advantages of the RVA compared to similar viscometers, such as the Brabender Visco-amylograph, is that it requires a small sample size (RVA: 5 to15 g, amylograph: 500 g (Mijland *et al.*, 1999)), it has shorter test runs (15 min compared to 2 hours (Mariotti *et al.*, 2005)), the ability to set time and temperature profiles (Mariotti *et al.*, 2005; Zhou & Mendham, 2005), variable shear capabilities and electronic data output, therefore allowing electronic manipulation of data (Zhou & Mendham, 2005). The Falling Number Apparatus is unable to measure changes in viscosity with respect to time and produces high errors (>5.0%) between duplicates. The disadvantage of the RVA is that long calibration and preparation time is still required in order to run a test (Thiewes & Steeneken, 1997). A total analysis time of 15 minutes, though shorter than two hours, is not considered a rapid method.

7.4 RVA viscograms

A typical RVA viscogram of malt consists of an initial high-speed mixing phase, a gelatinisation peak, and a viscosity decrease as malt enzymes and stirring degrade starch to less viscous products (Fig. 2.15) (Glennie Holmes, 1995a).

The curve generated (Fig. 2.15) is therefore a reflection of the granular changes during starch gelatinisation, retrogradation and enzyme hydrolysis. It is well known: different starches generate different viscosity profiles, but the same flour or the same starch can also give rise to different curves if it is subjected to different operating conditions (Mariotti *et al.*, 2005).

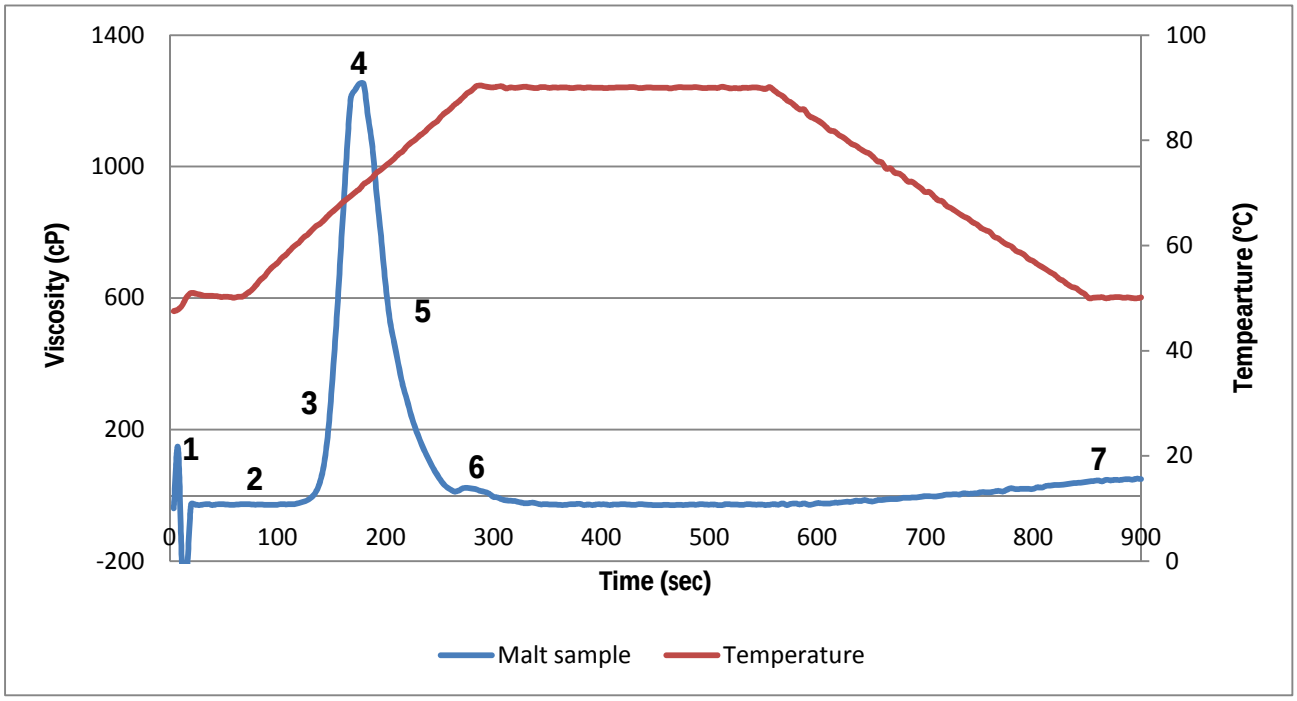

Figure 2.15 A typical RVA viscogram of malt.

A large degree of “viscosity noise” can be observed at the beginning of a test (**point 1**, Fig 2.16). The initial variation in viscosity represents the high speed stirring of the paddle (960 rpm). This helps to thoroughly disperse malt particles, facilitates water absorption by starch granules and prevents clump formation (which obstructs accurate viscosity reading). After mixing at 960 rpm for 10 seconds, the rest of the test is performed under a constant speed of 160 rpm. Laminar flow is encountered at 160 rpm, while turbulent flow occurs at 960 rpm, hence the large viscosity deviation initially observed (Lai *et al.*, 2000; Mrs B Elliott, Newport Scientific, Australia, personal communication, 2010).

Glucanolytic, proteolytic, and pentosanolytic enzymes with reported optimum temperature activities at approximately 50°C are most active at **point 2** (Fig. 2.15) (Narziss, 1992; Goode *et al.*, 2005a).

The sharp increase in viscosity (**point 3**, Fig. 2.15) is due to the onset of gelatinisation of starch (Kent & Evers, 1994). A peak is formed (**point 4**, Fig. 2.15) by reaching an equilibrium between viscosity increasing (swelling) and decreasing (rupture) processes and is considered to be related to the swelling potential of starch granules, when inhibiting enzyme activity (Allan *et al.*, 1997).

Decreasing viscosity (**point 5**, Fig. 2.15) is due to granule rupture after gelatinisation, subsequent polymer alignment as a result of mechanical shear (shear thinning), and enzyme degradation which is mostly attributed to α-amylase (Newport Scientific, 1995; Goode *et al.*, 2005a).

A small increase in viscosity can be observed at **point 6** (Fig. 2.15), which is hypothesised to be due to secondary starch gelatinisation and pasting of smaller B-type starch granules (Goode & Arendt, 2006). Smaller granules have been found to gelatinise and therefore paste at higher temperatures in comparison to large granules (MacGregor & Bhatty, 1993). Increasing the level of amylase increases the rate of starch degradation and consequently decreases viscosity, which diminishes the appearance of a secondary rheological peak (Goode & Arendt, 2006). A secondary peak has also been observed in RVA analyses of maize starch suggesting the presence of phospholipids were partly responsible for the observed viscosity increase (Nelles *et al.*, 2000). Levels of lipid associated with amylose can vary significantly in normal malting barley due to environmental conditions (Allan *et al.*, 1997). Lipid complexed with amylose has been reported to significantly influence the swelling potential of starch granules, and in turn influence RVA pasting properties (Allan *et al.*, 1997). It was suggested complexes of amylose with free fatty acids, monoacyl lipids and, perhaps, even diglycerides, may also contribute to the second pasting peak phenomenon (Morrison, 1995; Nelles *et al.*, 2000).

A slight increase in viscosity can be observed near the end of the test (**point 7**, Fig. 2.15). This is due to a small amount of re-association of starch molecules (mainly amylose) during retrogradation (Olkku & Rha, 1978). A malt viscogram differs from a barley viscogram, as barley displays extensive retrogradation and therefore a drastic increase upon cooling, compared to malt

which has a low final viscosity due to the action of enzymes (Glennie Holmes, 1995a). It has been suggested, the outer branches of the amylopectin are hydrolysed and thus made unavailable for the formation of large amylopectin crystals (Krag & Poulsen, 1998; Mariotti *et al.*, 2005). These small crystallites are unable to form a three-dimensional network and therefore incapable of a viscosity increase during cooling (retrogradation) which owes to the distinct difference between malt and barley end viscosities (Eliasson & Tatham, 2001a; Mariotti *et al.*, 2005).

7.5 RVA quality measurement of barley, malt and mash

Micro-malting is a rate limiting step in the quality assessment of barley lines in a breeding program. Using methods based on barley flour or starch would reduce the need to micro-malt. This caused many authors to investigate useful relationships between the viscogram data from barley and the malting quality of their respective malts (Glennie Holmes, 1995b; Glennie Holmes, 1995c; Allan *et al.*, 1997; Dunn *et al.*, 1997; Stuart *et al.*, 1998; Zhou & Mendham, 2005).

The peak time, peak viscosity and trough viscosity (terminology explained in Fig. 2.16) of **starch** (isolated from five Australian malting barley cultivars) negatively related to the mean large starch granule diameter. Peak time showed the highest correlation (r=-0.89) (Dunn *et al.*, 1997; Stuart *et al.*, 1998). The proportion of small starch granules was positively related to peak and final viscosity, and negatively related to initial pasting time (Allan *et al.*, 1997). Malt extract (EBC, fine grind) was negatively related to peak time as well as trough and peak viscosity of barley starch (Allan *et al.*, 1997; Dunn *et al.*, 1997). The most significant correlation existed between malt extract and peak time (r=-0.95) (Allan *et al.*, 1997; Dunn *et al.*, 1997; Stuart *et al.*, 1998). This relationship was however highly dependent on cultivar (Allan *et al.*, 1997). Rapid quality assessment methods are of particular importance to breeding programs. Evaluating barley flour rather than barley starch eliminates time consuming starch purification processes. **Barley flour**, however, did not show similar trends when analysed by the RVA. No relationship was observed between malt extract and either peak time or peak viscosity of barley flour (Dunn *et al.*, 1997; Stuart *et al.*, 1998).

Zhou & Mendham (2005) indicated RVA viscosity measurements, conducted on barley flour from 60 breeding lines, closely related to malt extract (EBC method: double mash system in which 67-72°C is terminated by using a single decoction). Most correlations with fine extract, however, were insignificant and could not aid in the prediction of malting potential. A better correlation with malting quality was obtained when redefining the pasting temperature and using silver nitrate to inactivate enzymes (Zhou & Mendham, 2005).

In a series of papers presented by Glennie Holmes, studies were made on: the effect of varying physical conditions, chemical conditions (Glennie Holmes, 1995a) and modification level on RVA viscograms (Glennie Holmes, 1995b); predicting malting potential (Glennie Holmes, 1995c); the relationship between RVA starch characteristics; and malting quality (Glennie Holmes, 1995d). Peak viscosity, peak area and final viscosity, obtained from viscograms (under autolytic and enzyme-inhibited conditions), were all related to extract values (IOB, coarse concentrated hot

water extract and cold water extract). Glennie Holmes (1995c) indicated the possibility of using peak time as a cultivar-independent measure of quality potential. No correlation, suitable for testing progeny from the intermediate generations of a breeding program, could be found (Glennie Holmes, 1995c).

The brewery mashing process can be replicated during RVA analysis by using a similar time and temperature profile. This enables the RVA to be applied as a laboratory-scale rheological tool for mash viscosity assessment. The RVA was found to be sensitive enough to detect not only the major viscosity changes that occur during starch gelatinisation and liquefaction processes, but also the minor viscosity changes, found during the proteolytic and saccharification steps (Goode *et al.*, 2005c; Goode & Arendt, 2006). Both studies simulated an industrial mashing process of which the time and temperature profile resembled that of an upward infusion mashing programme. Goode *et al.* (2005b) indicated correlations between the level of grain modification and certain rheological data points. In order to simulate the different degrees of malt modification, increasing amounts of malted barley was adding to "green" unmalted barley. The peak viscosity (R^2=0.9988), the area recorded under the gelatinisation curve (R^2=0.9928) and the peak viscosity breakdown rate (R^2=0.9783) showed significant correlations with the level of barley adjunct (Goode *et al.*, 2005a). Goode & Arendt (2006) investigated interactions between grain components (purified/non-purified), and amylolytic enzymes, glucanolytic enzymes, and mash pH. Studies indicated rheological data points were affected by the level of amylase (an α-amylase preparation containing trace amounts of additional enzymatic side activities). The trends observed were a decrease in the peak viscosity, the area under the peak, and the viscosity breakdown rate as the level of amylase increased. In addition, secondary starch gelatinisation, due to the presence of smaller starch granules in purified barley starch, closely correlated with the level of mash amylase (Goode & Arendt, 2006).

Measuring the intrinsic viscosity of barley starch relays useful quality information of starch properties, such as the proportion of small starch granules (shown to positively correlate with peak viscosity and final viscosity but negatively with initial pasting time), amylose:amylopectin ratio, the relative diameter of large starch granules [shown to significantly ($P<0.001$) negatively correlate with peak time] and other pasting characteristics (Allan *et al.*, 1997; Dunn *et al.*, 1997; Stuart *et al.*, 1998)]. These properties can significantly influence malting quality parameters, such as malt extract which has been shown to significantly ($P<0.001$) negatively correlate with peak time, trough and peak viscosity. Starch isolation is a complex and time consuming procedure. The malting process only degrades approximately 10% of starch. **Inhibiting malt enzymes** during rheological analysis produces similar rheological results compared to starch extracted from the same malt sample. In this regard, enzyme inhibition is a practical alternative to rheologically investigate 'isolated' starch from malt (Glennie Holmes, 1995a). Silver nitrate is considered to be the most effective α-amylase inhibitor and is commonly utilised during RVA analysis to inactivate malt enzymes, giving greater inhibition than calcium complexing agents, acids, alkalis or barium hydroxide (Glennie Holmes, 1995a). Silver nitrate has a constant effect above 0.1 $mM.g^{-1}$

(Meredith, 1970; Glennie Holmes, 1995a), while the addition of 0.1 M silver nitrate solution (25 g) to ground barley or malt (4 g) is the generally accepted concentration used during RVA analysis (Glennie Holmes, 1995a; Zhou & Mendham, 2005; Zhou *et al.*, 2008).

When inhibiting enzyme hydrolysis during rheological analysis of a malt sample, a drastic increase in peak height accompanied with a greater degree of setback can be observed (retrogradation), therefore resembling the rheological profile of barley (Glennie Holmes, 1995a).

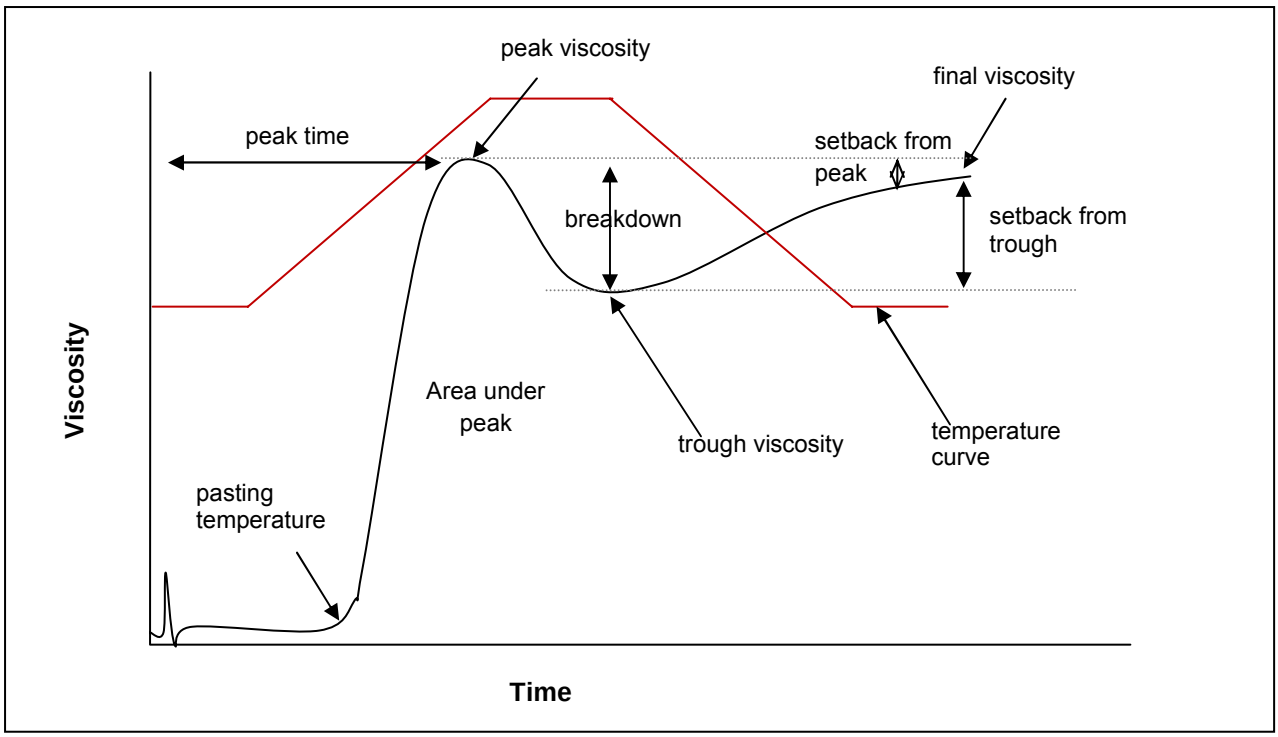

Figure 2.16 RVA terminology explained (adapted from (Newport Scientific, 1995).

7.6 Multivariate data analysis

Multivariate data analysis has been extensively applied within the field of food science (e.g. sensory analysis, near infrared spectroscopy, high-performance liquid chromatography and matrix-assisted laser desorption/ionisation-time of flight mass spectrometry) (Bro *et al.*, 2002; Drake *et al.*,2003; Westad *et al.*, 2003; Rasch *et al.*, 2010; Vaclavik *et al.*, 2011). Multivariate data analysis, such as principal component analysis (PCA), is rarely applied to the field of rapid visco analysis. Conventionally, only a few selected variables are considered (such as peak height, peak area, onset of gelatinisation) by using univariate data analysis techniques, such as analysis of variance (ANOVA). However, PCA is able to assess all rheological variables generated, as it allows the variation experienced among samples to be described by an unlimited amount of variables, and is therefore a more suitable data analysis technique.

The essential function of PCA within a data set is a reduction in dimensionality, while retaining as much variation present as possible (Cowe & McNicol, 1985). It is also the objective of PCA to transform the original data set into a more relevant co-ordinate system or new set of variables, [i.e.

the principal components (PC's)]. These components are uncorrelated and ordered, therefore retaining most of the variation present in the original variables by the first few PC's (Jolliffe, 1986; Esbensen, 2002). The first principal component (PC1) has a direction that spans the maximum variance experienced in a data set (i.e minimising the sum of all the squared transverse distances) (Esbensen, 2002). The second principal component (PC2), is orthogonal to PC1 and consequently lies in the direction of the second largest variance. Therefore, all PCs generated are orthogonal to each other and represent a successive decrease in variance. The great advantage of multivariate data analysis techniques, such as PCA, is the original data matrix X can be decomposed into a structure part (the first PCs that span the largest variance directions), and a noise part (directions in the data swarm where the variance/elongation is small enough to be neglected) in order to detect and model the "latent phenomena" in a data matrix. Data is therefore decomposed into resultant scores and loadings, which can be represented visually by means of scatter plots and line plots allowing efficient interpretation.

Multivariate data analysis, in the form of partial least squares regression (PLS), allows the development of a statistical model (through model calibration and validation when regressing a X-data matrix with Y-data), enabling future prediction.

Soft Independent Modeling of Class Analogy (SIMCA) is a classification methodology of supervised pattern recognition (Esbensen, 2002). SIMCA classification allows the assignment of new objects to a class which they show the largest similarity to, therefore modelling only the common properties between samples within the same class. SIMCA classification utilises separate bilinear modelling (most often PCA models) for each valid data class. The rationale behind calculating multivariate regression or classification models for prediction is to eliminate or replace an expensive, time consuming or impractical measurement with an easier, inexpensive one. The application of multivariate data analysis is a well-matched statistical technique applied to rheological data and with proper programming can be collaborated with the RVA software for practical implementation in the industry, as a means of predicting malt fermentability.

8. Conclusion

Viscosity changes occur during the mashing process of brewing. This can be attributed to the intricate interaction of **starch** (having its origin from the barley grain), **enzymes** (developed during the malting process) and **stirring**. An increase in viscosity is caused by starch **gelatinisation** and **retrogradation** (during subsequent cooling), while a decrease in viscosity is attributed to **enzyme hydrolysis** and **shear thinning** of soluble starch. Such processes influence fermentation efficiency, as they govern the creation of fermentable material needed for yeast fermentation and subsequently alcohol production. Therefore, measuring the rheological changes of a malt-water mixture, by means of a viscometer, reflects useful physical and chemical information. This helps to improve the understanding of more complex biochemistry underlying the transition of malt to beer and its potential influence on final beer quality. By careful selection of appropriate test conditions

the RVA, combined with univariate data analysis techniques, could have real potential for malt fermentability prediction purposes.

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Chapter 3

Optimisation of the Rapid Visco Analyser (RVA) Kilned Malt method for quality discrimination of malted barley with regards to fermentability

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Optimisation of the Rapid Visco Analyser (RVA) Kilned Malt method for quality discrimination of malted barley with regards to fermentability

Abstract

There is a lack in South African breeding, malting and brewing industries to accurately measure the brewhouse fermentability and therefore brewing performance of malt. To date, no RVA method has succeeded to discriminate sufficiently between different degrees of malt fermentability. The Rapid Visco Analyser (RVA) was investigated as an instrument potentially able to discriminate between different levels of malt fermentability. A standard RVA method of malt rheological analysis (i.e. the Kilned Malt method) was optimised in order to achieve maximum rheological discrimination between malt samples, differing in fermentability. RVA test conditions, such as concentration ratio (five different ratios) and particle size distribution (two different sieve sizes used during milling), were varied in order to identify optimal conditions. Data analysis techniques; i.e. univariate (analysis of variance: ANOVA) and multivariate data analysis (principal component analysis: PCA), were compared to identify the most suitable application to RVA data. ANOVA identified the RVA viscogram variables “peak height”, “area under the peak”, “peak width (time/temp): peak height” and “time at peak 2”, while PCA identified the variables “peak height”, “area”, “peak width (time/temp): peak height”, “change in time (Δt)” and “change in temperature (ΔT)” as potential variables being able to discriminate best between different degrees of malt fermentability. Results obtained from ANOVA and PCA were in agreement. It was indicated that particle size distribution affected all samples to the same extent, therefore not to the benefit of malt fermentability discrimination. Results indicated the malt to water ratio of 1:1.5 (w/w) allowed optimal discrimination between highly fermentable- and problematic malting barley cultivars. PCA applied to the entire data set (225 data points) is undoubtedly the most advantageous data analysis technique. In this study rheological measurement, by means of the RVA, has shown promising ability to measure malt fermentability, when altering the malt to water ratio. Correct identification of malt fermentability degree and consequently actual brewing performance could prevent production losses in the South African brewing industry. With proper programming multivariate data analysis can be incorporated in RVA software for practical implementation in the industry as a means of predicting malt fermentability.

Introduction

It is crucial for maltsters and brewers to predict the brewing performance of malted barley-breeding lines or existing commercial cultivars, without resorting to the actual brewing process. Current methods used by the breeding, malting and/or brewing industries, to predict a malt’s brewing performance either lack accuracy or is non-existent. Measuring the rheology of a malt mixture has been investigated, and in some instances, approved as an official method able to determine malt

quality (ICC, 2008). The Rapid Visco Analyser (RVA) is a rheometric instrument that has been extensively investigated as a method for monitoring barley grain and malt quality (Glennie Holmes, 1995a; Glennie Holmes, 1995b; Glennie Holmes, 1995c; Glennie Holmes, 1995d; Glennie Holmes, 1995e; Stuart *et al.*, 1998; Zhou & Mendham, 2005). The RVA Kilned Malt Method (Newport Scientific Method 16, Version 3, June 1997) is currently used by the South African malting industry for rapid estimation of the malting potential of a finished malt (Newport Scientific, 1995). It is a test which only considers a limited amount of data points from the RVA viscogram (usually six or less). It fails to discriminate adequately between malt quality of different cultivars, and therefore its application is ineffective in a barley breeding programme and has gradually become meaningless in the breeding, malting and brewing industries. To date, no RVA method has been satisfactorily optimised to discriminate between highly and poorly fermentable malted barley cultivars and/or breeding lines. Test conditions can be adjusted when running a RVA test and have an evident effect on the RVA viscogram. These conditions could potentially aid in optimal quality discrimination with regards to fermentability.

Milling and particle size have been shown to greatly affect the mashing process and therefore brewing performance of a malt (Meddings & Potter, 1971; Mousia *et al.*, 2004). Variations in particle size greatly affects rheological properties and subsequently the viscogram profile derived from the RVA (Becker *et al.*, 2001). The size of malt flour particles determine the rate of hydration (Glennie Holmes, 1995a) and consequently the transfer of material and heat between the interior of the malt particles and the mash liquid (Schur, 1980). Enzyme hydrolysis, being dependent on hydration, can therefore also be affected by particle size distribution (Mahasukhonthachat *et al.*, 2010). The type of mill together with its sieve size used is therefore another important variable affecting the RVA viscogram and should be considered (Glennie Holmes, 1995a; Becker *et al.*, 2001). Becker *et al.* (2001) illustrated larger particle sizes (>250µm) of extruded maize and wheat pellets produced higher peaks in the earlier part of the pasting profile compared to smaller particle sizes. Particle shape can also influence a RVA viscogram, because of its influence on water uptake and swelling behaviour (Becker *et al.*, 2001). Mechanical damage, typically encountered during milling, leads to an increased capacity to absorb water, increased susceptibility to amylolysis, and the loss of organised structure (Evers *et al.*, 1984a; Evers *et al.*, 1984b; Evers & Stevens, 1988). Particle size with regards to the RVA gap size between the rotating paddle ends and the canister wall (between stationary and rotating phase) is also important for accurate viscosity measurement (Goode *et al.*, 2005a). To prevent inaccurate measurement, it is vital to keep particles in suspension during rheological measurement (Rao, 1999). A gap size of ten times less the diameter of the biggest particle size has been suggested for optimal rheological measurement (Hermann & Sommer, 2001). Milling can affect the brewing process (such as sugars production during mashing and wort separation speed) and, subsequently, the final product quality (Bamforth & Quain, 1989; Mousia *et al.*, 2004).

Development of a peak; during RVA rheological analysis is considered to be barely measurable unless the malt to water ratio (malt:water ratio w/w) is 0.33 $g.mL^{-1}$ (1:3) or more concentrated (Glennie Holmes, 1995a). This is due to enzyme hydrolysis which prevents sufficient viscosity increase during gelatinisation (Glennie Holmes, 1995a). It can also be attributed to the small amount of starch unable to reduce the large amount mobile phase surrounding it (Kent & Evers, 1994). According to Glennie Holmes (1995a), malt:water ratios (w/w) more concentrated than 0.55 $g.mL^{-1}$ (1:1.8) produce a viscogram with an irregular trace, forming a peak viscosity lower than predicted. Typical malt:water ratios (w/w) utilised by breweries include 1:3 up to 1:3.5 (Hough, 1985), while the RVA Kilned Malt Method uses a malt:water ratio (w/w) between 1:2.5 to 1:3 (on a 14% moisture basis) (Newport Scientific Method 16, Version 3, June 1997). The concentration of malt in an aqueous medium can have a considerable effect on rheology. Considering malt-water systems with a limited amount of water, the gelatinisation temperature range (initial, midpoint and end point) broadens as the starch concentration increases (Ghiasi *et al.*, 1982; Dengate, 1984). This was contradictory to findings which concluded increased slurry concentrations decreased the temperature of transition (Anker & Geddes, 1944; Sandstedt & Abbott, 1964), but also decreasing the time to peak and temperatures of peak viscosity with increasing sample weights (Glennie Holmes, 1995a). The malt concentration during mashing can influence enzyme stability which directly affects rheological properties and thus the viscogram profile. Concentrated mashes provide a protective environment for malt enzymes, which show enhanced thermostability (Muller, 2000). As malting barley cultivars differ in gelatinisation temperature range, enzyme composition, content and activity; a more concentrated mash could enable better discrimination between cultivars based on their rheological properties.

The effects of malt modification on rheological properties have been reported (Yoshida & Yamada, 1970; Goode *et al.*, 2005b; Goode & Arendt, 2006). A similar rheological trend with the RVA, namely; an increase in malt modification (representing a better quality), caused a clear reduction in peak height, while a higher peak viscosity usually indicated a less modified, poorer malt quality (Glennie Holmes, 1995b; Goode *et al.*, 2005b). It was suggested the malting process selectively removes material (e.g. hydrolysis of β-glucans) with high water-binding capacity from barley (Woodward & Fincher, 1983). Differences in water-binding capacity due to varying degrees of modification can be expected to deliver different RVA viscograms. RVA thus has real potential of discriminating between malt of different barley cultivars based on the extent of modification.

Considering cultivar selection; Metcalfe is a two row Canadian malting barley cultivar released by Agriculture Canada in 1994. It is a cross between the well-known Canadian cultivars Oxbow and Manley. It modifies quickly during the malting process (Jackson, 2002), contains superior malt extract levels, moderate protein levels, high yield, good husk adherence, low levels of β-glucan, a high rate of proteolysis and displays excellent brewhouse performance (Ladish, 2005). It is regarded as having elite malting quality similar to the previously dominant Canadian cultivar Harrington

(Jackson, 2002). Flagship has beneficial malting quality drawn from European and Canadian genetics, crossed to a robust Australian feed cultivar (see pedigree in Appendix 1, Table 1.1) (Pattemore *et al.*, 2010). Its outstanding malting quality profile displays a significant quality increase over many other Australian barley cultivars, and comparable with leading international malting cultivars. The high diastatic power and fermentability of Flagship is ideally suited for high starch adjunct brewing (Anonymous, 2006). SSG 564, SSG 506, PUMA and SSG 585 are South African cultivars released from local barley breeding programmes (see pedigree in Appendix, Table 1.1). To some extent, all of the latter cultivars fail to match Metcalfe and Flagship's fermentability and present brewing problems in some form or another. Such highly fermentable and problematic cultivars can be compared rheologically to investigate the sensitivity of the RVA to detect these quality differences.

Of equal importance to an experimental procedure, is the statistical technique applied when analysing data, therefore the reliability of results obtained and conclusions drawn. RVA results are conventionally analysed using univariate methods, therefore considering one variable at a time, viewed in isolation. Multivariate data analysis is a novel application for RVA. This technique allows the variation between samples to be described by an unlimited amount of variables. It is therefore a more suitable application to RVA data, due to the number of variables generated.

The aim of this study was to optimise the RVA Kilned Malt method in order to maximise differentiation between highly fermentable malt (represented by cultivars Metcalfe and Flagship) and poorly fermentable malt (represented by SSG 564, SSG 506, PUMA and SSG 585 cultivars) by:

- evaluating the rheological effect when varying malt-water concentration and particle size distribution of the ground flour;
- identifying which test conditions allows optimal discrimination between highly fermentable and poorly fermentable malted barley cultivars; and
- comparing different data analysis techniques (univariate vs. multivariate) in order to identify the most suitable for future application.

Materials and Methods

Malted barley samples

The malt of six different malting barley cultivars, together with detailed cultivar information and malt analysis data (Tables 1.1 & 1.2 in Appendix 1), were obtained from South African Breweries Maltings (SABM) in Caledon, South Africa. The imported cultivars, Metcalfe and Flagship represented highly fermentable good quality malt. SSG 564, SSG 506, PUMA and SSG 585 represented local malting barley cultivars, presenting fermentation problems during brewing (Dr I

Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009).

Sample preparation

Samples were ground in a centrifugal mill (Retsch model ZM1, Haan, Germany) fitted with a 1 mm or 0.5 mm ring sieve size. Milling was conducted on the same day as moisture content determination and/or RVA measurement. The malt:water ratio (w/w) was varied between five different concentrations namely; 1:1.5, 1:2, 1:2.5, 1:3 and 1:3.5. These ratios were all set at 14% malt moisture content. The total sample weight (water and malt) used in RVA analysis was kept constant at 27 g (Eq. 1.1) allowing the calculation of the amount of malt and water needed (adjusted according to moisture content) (Eq. 1.2 to 1.3).

total sample weight: $M + W = 27\ g$ [1.1]

concentration ratio (example malt:water of 1:1.5): $1.5M_{14\%} = 1W_{14\%}$ [1.2]
substituting (example malt:water of 1:1.5): $M_{14\%} = 10.8\ g$ and $W_{14\%} = 16.2\ g$ at 14% MC

moisture content correction: $M_{14\%}(100 - 14) = M_x(100 - MC)$ [1.3]
substituting (example malt:water of 1:1.5): $M_x = 10.8(100 - 14)/(100 - MC)$; $W_x = 27\ g - M_x$

In which: M = malt mass; W = water mass (at any moisture content)
$M_{14\%}$ or $W_{14\%}$ = Malt or water mass at 14% moisture content (depending on concentration)
M_x or W_x = Malt or water mass to be weighed at measured moisture content
MC = measured moisture content

Moisture content determination

The moisture content of the malted barley samples were determined prior to RVA analysis according to the European Brewery Convention (EBC) method 4.2 (European Brewery Convention, 1998). Moisture dishes and their lids were dried for 30 min at 105°C in a vacuum oven (Heraeus model RVT 360, Henau, Germany). Dishes were removed from the oven and placed in a desiccator to cool. The mass of each moisture dish, with a lid, was weighed to the nearest 0.001 g and recorded (W_1). After milling, a malt flour sample was weighed (5 ± 0.001 g) in a moisture dish, the lid was placed on top and the total weight was recorded (W_2). Each moisture dish and lid (uncovered) were placed in the oven at 105-106°C. Samples were dried for three hours ± 5 min, starting from the time when the standardised temperature was regained. The lids were placed back on the dishes, removed from the oven and allowed to cool to room temperature in a desiccator for ± 30 min. The

covered moisture dishes were weighed to the nearest 0.001 g and recorded (W_3). This allowed the calculation of the moisture content (Eq. 1.4).

$$\%\ Moisture\ content = \frac{W_2 - W_3}{W_2 - W_1} \times 100 \quad [1.4]$$

In which: W_1 = Mass of moisture dish + lid
W_2 = Mass of moisture dish + lid + malt sample before drying
W_3 = Mass of moisture dish + lid + malt sample after drying

Rheological measurement

Rapid Visco Analyser (RVA)

The Rapid Visco Analyser (RVA model 3D+, Newport Scientific, Warriewood, Australia), was used to measure the pasting properties of the different malt samples. Before initiating a test, the plastic stirrer was attached to the stirring motor of the RVA and zeroed at 160 rpm against air. The RVA was calibrated on a daily basis using test starch according to the Malt Analysis Method conducted by SABM (Southern Associated Maltsters, 2004). A test starch sample consisted of unmodified dent maize flour (approximately 74% amylopectin and 26% amylose). Test starch along with aluminium sample canisters and plastic stirrers were supplied by Newport Scientific, Modderfontein, South Africa. Peak viscosity, final viscosity and trough viscosity obtained from the viscogram of the test starch sample indicated instrument calibration. The RVA was only considered to be calibrated once two of the three measurements, obtained a viscosity value within the stated range.

The required amount of water (dH_2O) and malt sample (flour) were pre-weighed (see sample preparation, p.60) into separate aluminium sample canisters, at ambient temperature (± 22°C). When commencing a test, malt was carefully added to the water and thoroughly stirred to prevent any sample clumping. The plastic stirrer and canister were reattached to the instrument, and the pre-programmed rheological profile, as used by the Kilned Malt method (Table 3.1), was initiated by pressing down on the tower.

Table 3.1 RVA temperature and time profile

Time (h:min:s)	**RVA Parameters**	**Value**
00:00:00	Temperature (°C)	50
00:00:00	Rotational speed (rpm)	960
00:00:10	Rotational speed (rpm)	160
00:01:00	Temperature (°C)	50
00:04:42	Temperature (°C)	90
00:09:12	Temperature (°C)	90

00:14:12	Temperature (°C)	50
00:15:00	End of test	-

RVA Viscograms

In each of the 180 tests, an Excel (Microsoft Office Excel 2007) table was generated containing viscosity, temperature, and rotational speed information recorded every four seconds of a 15 min test, therefore generating 225 data points per test. A viscogram of each test (containing the 225 data points) was created, from which 22 variables were selected and/or calculated (Fig. 3.1 & Table 3.2). Selection of variables was based on those identified from literature, and those the researchers considered to be of importance. Reducing the amount of variables allows the application of univariate techniques, such as Analysis of Variance (ANOVA), and is commonly used to analyse RVA data.

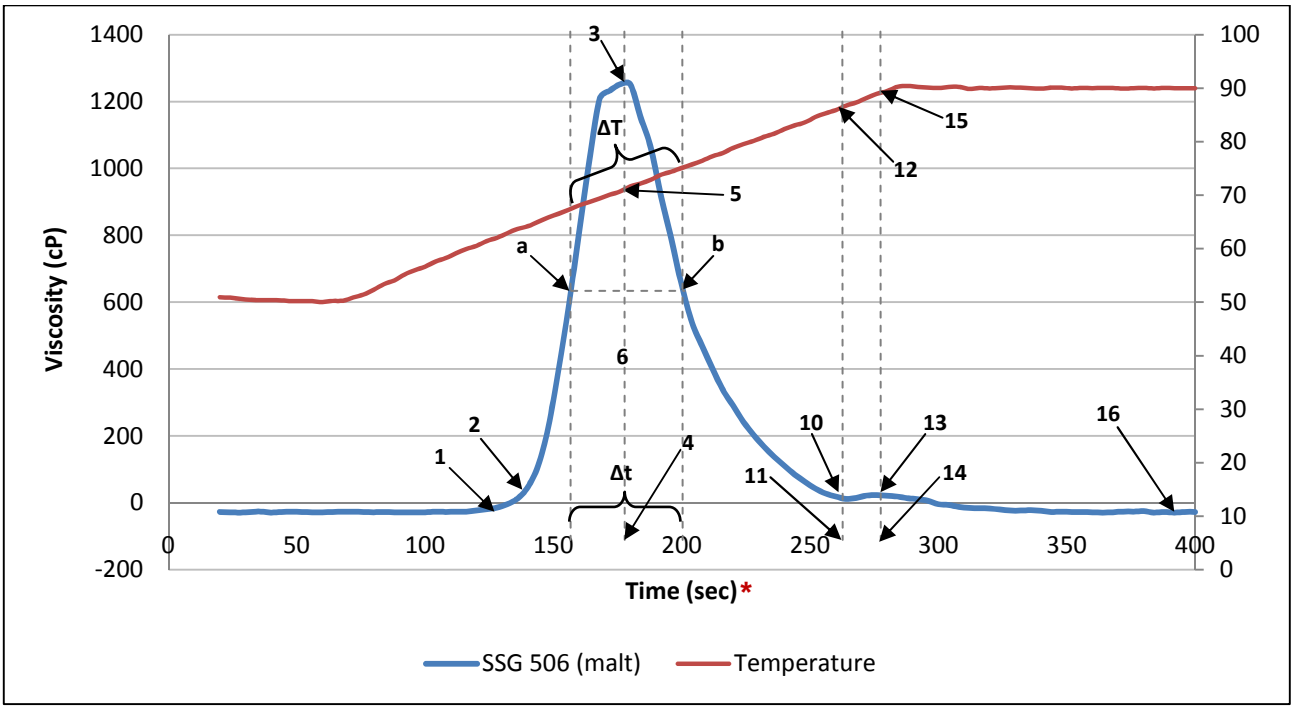

Figure 3.1 Typical RVA malt viscogram illustrating selected variables.

* For optimal display purposes, only the first 400 s are illustrated in the viscogram (with corresponding viscosity and temperature values)

Table 3.2 Description of the selected RVA viscogram variables used

Symbol	Parameter	Term description
1	Pasting temp (Newport)	The temperature recorded when the viscosity increase at a rate of 3.33 $cP.s^{-1}$
2	Pasting temp (Zhou)	The temperature recorded when the viscosity increase at a rate of 9.58 $cP.s^{-1}$
3	Peak height 1	The maximum viscosity measured when the peak height is reached
4	Time at peak 1	The time measured when the peak height is reached
5	Temp at peak 1	The temperature measured when the peak height is reached
6	Area under peak	The area measured under the peak according to the established baseline
6/3	Area : peak height [a]	This ratio allows comparison of the viscogram peak form
	Peak width (s): Peak height [a]	This ratio enables the description of broad and narrow peaks with regards to time
	Peak width (Temp) : Peak height[a]	This ratio enable the description of broad and narrow peaks with regards to temperature
10	Visc at trough 1	The trough viscosity was measured between the primary and secondary peak
11	Time at trough 1	The time measured at the trough
12	Temp at trough 1	The temperature measured at the trough
13	Visc at peak 2 [a]	The viscosity measured at the secondary peak height
14	Time at peak 2 [a]	The time measured at the secondary peak height
15	Temp at peak 2 [a]	The temperature measured at the secondary peak
16	End viscosity	The end viscosity measured in respect to the baseline
a	Time at a [a]	Point “a” was established at 50% of the peak height on the rising slope of peak 1. The time at this point was measured.
a	Temp at a [a]	The temperature at point “a” was measured.
b	Time at b [a]	Point “b” was established at 50% of the peak height on the declining slope of peak 1. The time at this point was measured.
b	Temp at b [a]	The temperature at point “b” was measured.
Δt	Δt [a]	The change in time between points “a” and “b”.
ΔT	ΔT [a]	The change in temperature between points “a” and “b”

[a] newly created viscogram variables

Experimental design and procedure

The experimental design thus consisted of six different cultivars, three replicates, two different particle size distributions and five different malt:water ratio's. Samples were analysed in a randomised order.

Univariate data analysis: Analysis of Variance

Pre-processing was applied to the data in the form of baseline offset correction in Excel (Microsoft Office Excel 2007). Statistical analyses on the 22 selected RVA viscogram variables (Table 3.2) for all the 180 tests were performed and graphs compiled using STATISTICA version 9.0. (StatSoft, Inc., Tulsa, OK, USA). Data were analysed using Analysis of Variance (ANOVA) to determine the significance of differences between samples. A three way factorial ANOVA and Fisher's least significant difference (LSD) *post-hoc* testing were used. All references to significant difference indicate a statistically significant difference at a 5% significance level ($P<0.05$).

Multivariate data analysis: Principal Component Analysis

Multivariate data analysis was performed on the 22 selected RVA viscogram variables (mentioned above), and also on the 225 data points (entire viscogram) from each of the 180 tests. Variables were imported into MATLAB version 7.8 (The MathWorks, Natick, MA). Pre-processing was applied to the data in the form of baseline offset correction and pareto scaling (van den Berg *et al.*, 2006). Principal component analysis (PCA) was performed using the PLS toolbox version 5.2 (Eigenvector, Manson, WA, USA) (Wise *et al.*, 2006).

Repeatability

It is also important to consider the similarity between replicates. It would be of little use to develop a method which showed good separation between cultivars and consequently quality, but to the detriment of accurate replication. The similarity between replicates was evaluated by calculating the Euclidean distance between replicates. This distance is zero if the three samples are identical and high if they differ by a large amount.

Results

RVA Viscograms

A clear variation in peak height was observed when comparing the different quality malted barley **cultivars** (Fig. 3.2). Metcalfe and Flagship, representing the highly fermentable malt, formed lower broader peaks. The poor quality malts formed higher narrower peaks, while SSG 506 had the highest peak. Considering Flagship and SSG 506, a slight shift of the peak in analysis time from the other samples was seen, as both form a peak earlier with regards to time (decreased time to peak). The declining slope of SSG 564 however appeared the latest with regards to analysis time. In view

of the more concentrated samples, a smaller secondary peak was observed (indicated by the elipse in Fig. 3.2).

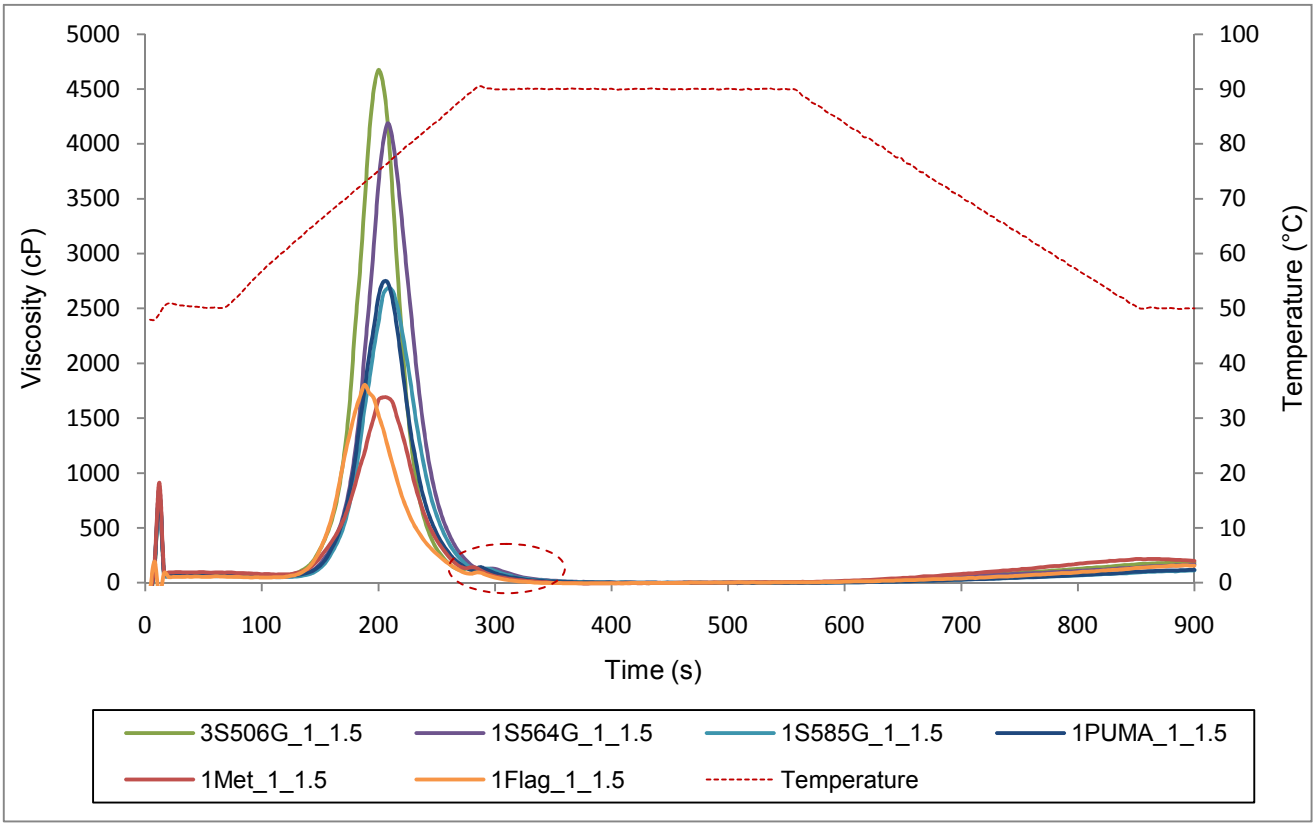

Figure 3.2 RVA viscogram of the six different cultivars compared at the 1:1.5 malt:water ratio (w/w) and 0.5 mm sieve size used during milling (average viscosity of three replicates).

When samples were more diluted (water addition), a reduction in peak height was observed (Fig. 3.3a). This illustrated the inverse-power relationship of dilution to peak height and area (Fig. 3.3b). A shift of the peak in analysis time for SSG 506 was seen between the malt:water ratio (w/w) of 1:1.5 and the other **concentrations** (Fig. 3.3a). Thus as the malt concentration increased, peak formation occurred slightly later. The same effect was observed for other cultivars (figures not included).

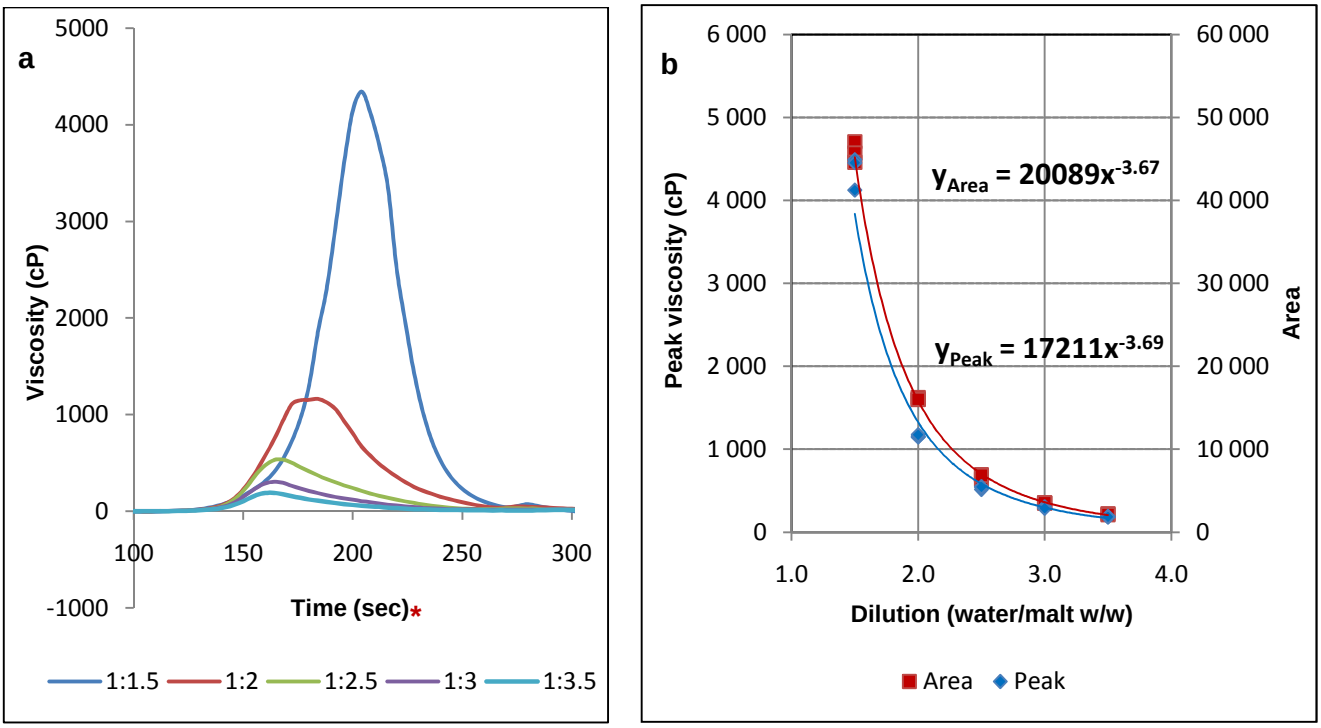

Figure 3.3 RVA viscogram of SSG 506 illustrating: (a) the five different malt:water ratios and (b) the inverse power relationship between peak viscosity/peak area and dilution (using 0.5 mm sieve size used during milling).

* For optimal display purposes, only the 100 - 300 s timeframe is illustrated in the viscogram

A smaller size ring sieve used during milling produced malt flour with smaller particle size distribution. A deviation in the peak inclining (viscosity increasing rate) was observed when **particle size distribution** was varied (Fig. 3.4). As the particle size distribution decreased (1.0 mm to 0.5 mm sieve size), peak formation occurred slightly later in analysis time. To some extent peak height was also affected.

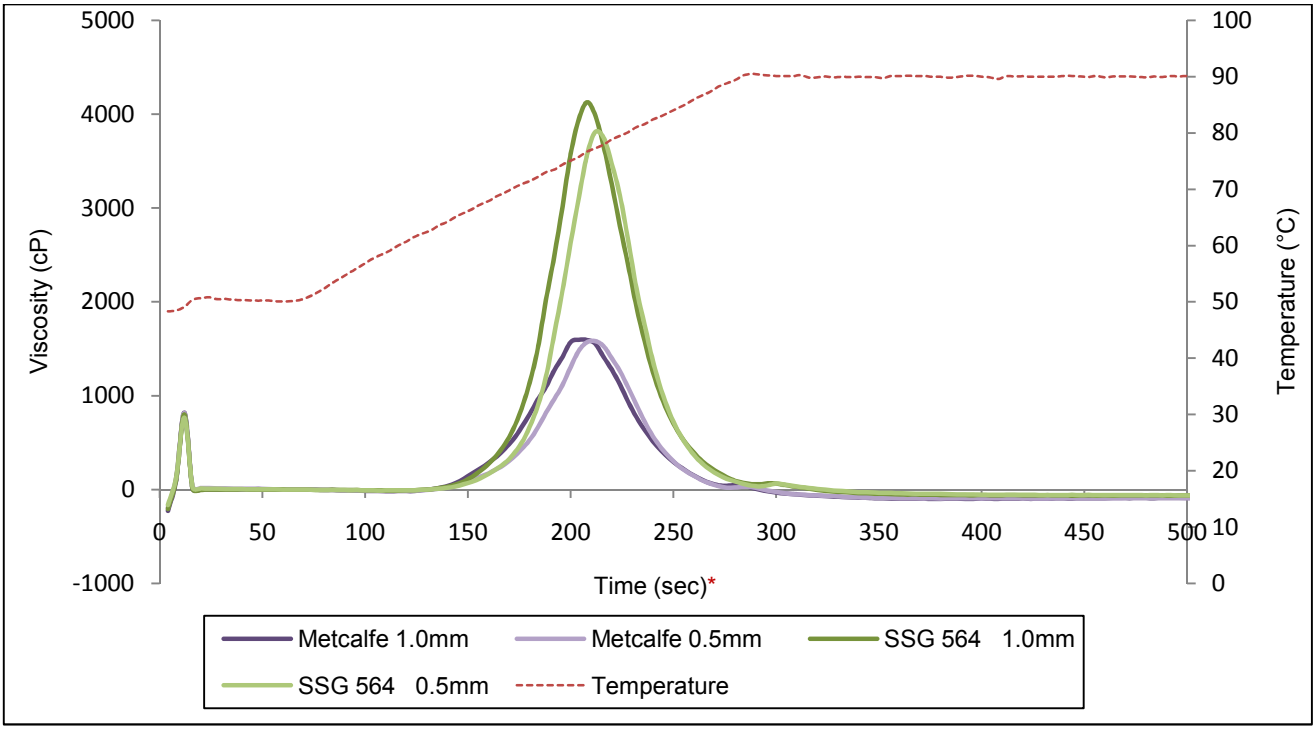

Figure 3.4 RVA viscogram illustrating the effect of different particle size distributions when considering Metcalfe and SSG 564 (at 1:1.5 malt:water ratio).

* For optimal display purposes, only the first 500 s are illustrated in the viscograms

The effect of varying particle size distribution was largely influenced by the concentration ratio [examining two malt fermentability extremes (Metcalfe vs. SSG 564)] (Fig. 3.5). Results indicated, the viscosity incline (leading to peak formation) was largely affected when varying particle size distribution at a high malt:water ratio (w/w) (**1:1.5**). Peak height seemed to be affected to a smaller extent, at such a high malt concentration (Fig. 3.5a). This effect was also observed for the other malt samples (data not shown).

At a malt:water ratio (w/w) of **1:2**, a clear difference between peak height was observed when varying particle size distribution (Fig. 3.5b). The onset of pasting seemed to be less affected compared to the more concentrated malt:water ratio (w/w) (1:1.5), but the greatest effect was observed in the incline (just before peak formation). Peaks also appeared to have a faster incline rate accompanied by a slower decline rate, compared to the 1:1.5 malt:water ratio.

For the **1:2.5** ratio, the incline (just before peak formation), peak height, but also the decline was affected by particle size distribution (Fig. 3.5c). A decrease in particle size distribution seemed to 'retard' the decline, delaying peak formation and breakdown with regards to analysis time, especially for Metcalfe. At the least concentrated malt to water ratios (**1:3** and **1:3.5**), variation in particle size distribution had a minimal affect on rheological analysis (Fig. 3.5d & Fig. 3.5e). Only the decline, after peak formation, was seemingly affected, during which samples milled with the smaller sieve size (0.5 mm) seemed to produce a delayed breakdown.

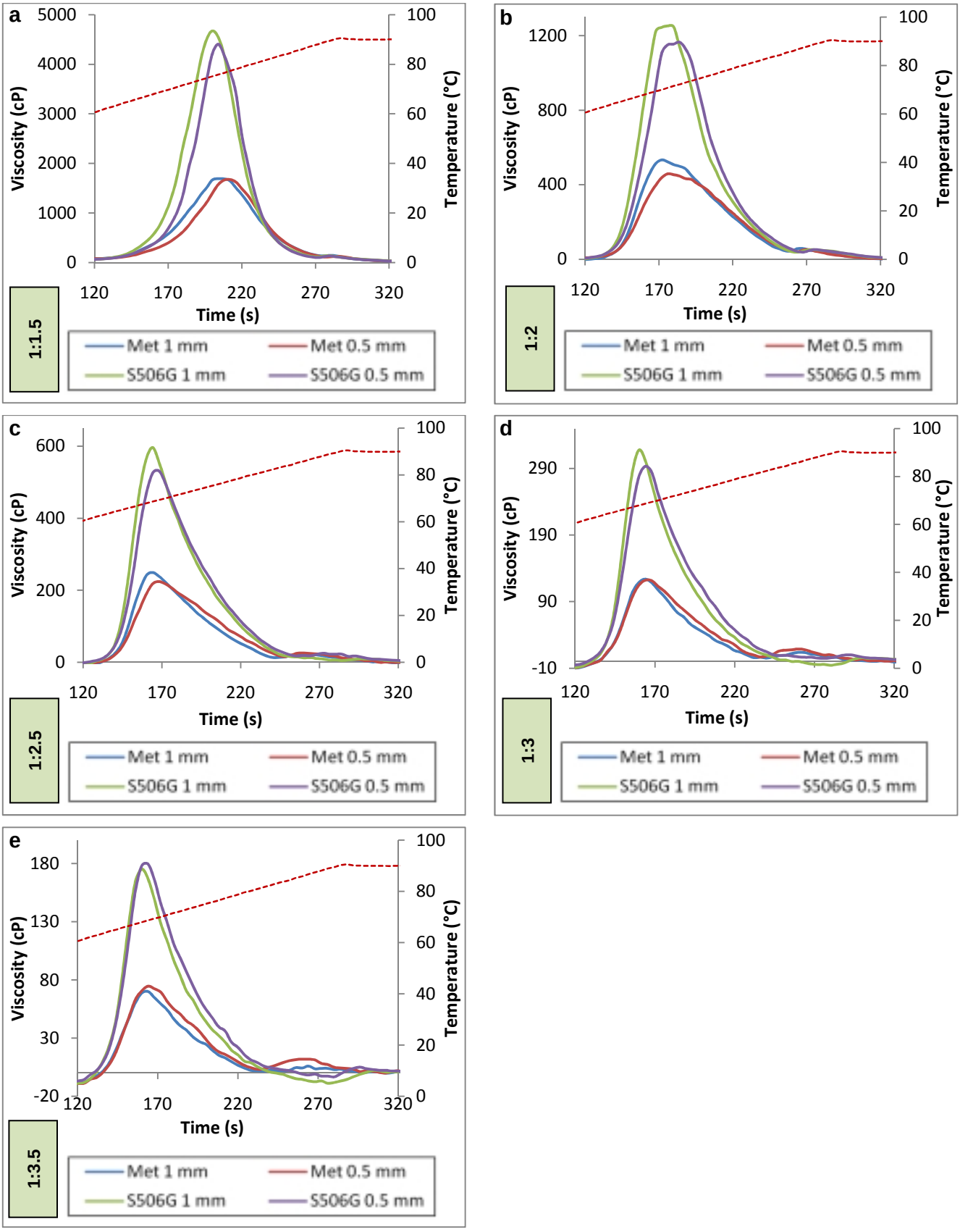

Figure 3.5 RVA viscograms of Metcalfe and SSG 564 illustrating the interaction between particle size distribution and the five different malt:water ratios of; (a) 1:1.5, (b) 1:2, (c) 1:2.5, (d) 1:3 and (e) 1:3.5.

Univariate data analysis: ANOVA (22 selected viscogram variables)

More than half of the selected viscogram variables indicated a non-significant interaction (P>0.05) between cultivar and **particle size distribution** (such as “time/temp at a”, “time/temp at peak 1”, “pasting temp (Newport)”, “temp at trough 1”, “time/temp at peak 2” and “visc at peak 2”). Considering the variable “time at a”, the same cultivar trend was observed for both particle size distributions (red line compared to blue line), indicating the same amount of variation between cultivars for both sizes were obtained (Fig. 3.6). This variable gave a probability value (P-value) larger than 0.05, for the cultivar by particle size interaction, indicating that different particle size distributions did not affect fermentability discrimination. Thus either sieve sizes (1 mm or 0.5 mm) could be used during milling for future testing.

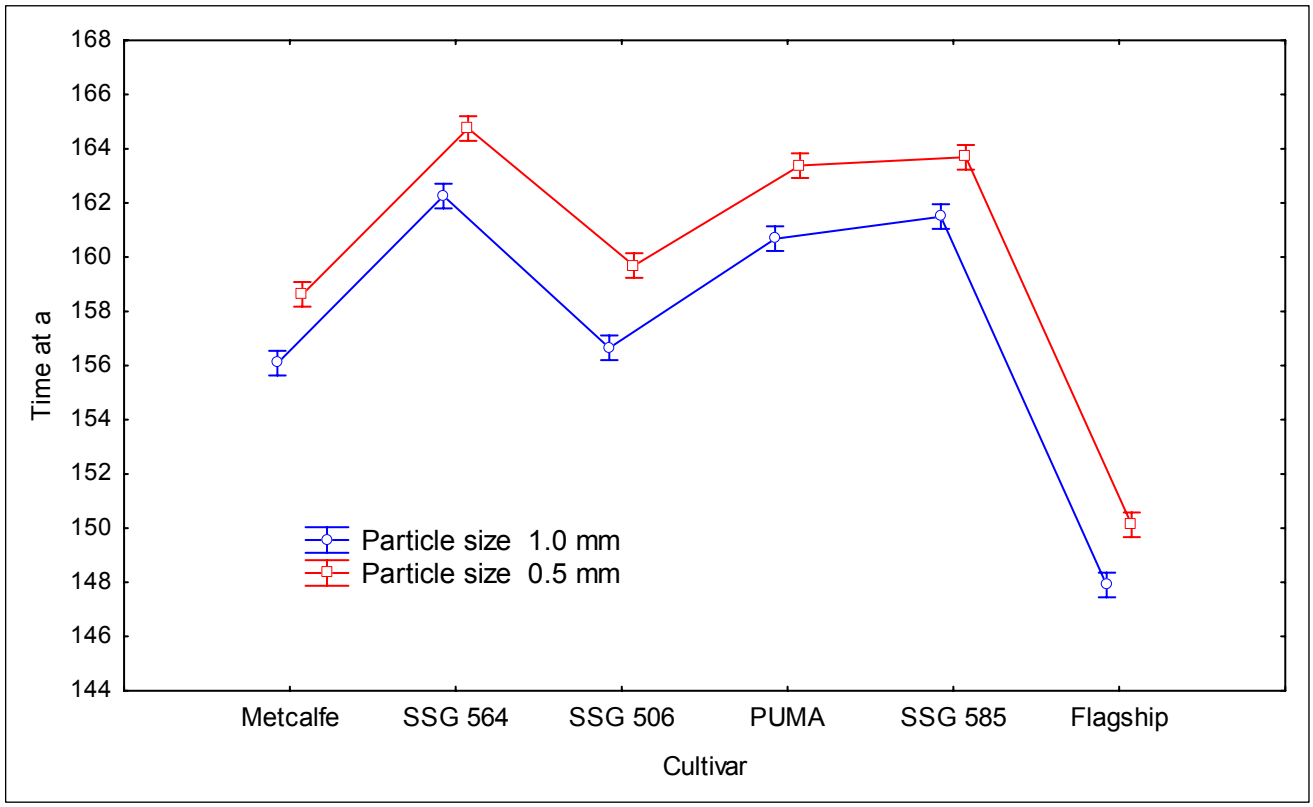

Figure 3.6 Results for cultivar by particle size interaction for the variable “time at a” as obtained with ANOVA. Vertical bars denote 0.95 confidence intervals (P = 0.46458).

The cultivar by malt:water **concentration** ratio interaction was significant (P<0.05). The mean values indicated; as the malt samples became more diluted, the peak viscosity decreased (Appendix 1, Table 1.3 to 1.24). Considering the cultivar by malt:water ratio (w/w) interaction of the variable “peak height”, a significant difference (P<0.05) was observed between five of the six different cultivars tested (Fig. 3.7). No significant difference (P>0.05) was found between Puma and SSG 585, suggesting in some way, they are alike . More importantly, a trend in fermentability was seen, with Metcalfe and Flagship grouping together (due to lower peak heights), but dissimilar to the other

four poor quality cultivars (having higher peaks). The malt:water ratio (w/w) of 1:1.5 gave the best separation of cultivars and therefore fermentability discrimination (malt fermentability indicated by Tables 1.1 & 1.2, Appendix 1).

The malt:water ratio (w/w) of 1:2 (Fig. 3.8) showed similar discrimination, but upon further dilution, the ability to discriminate between cultivars was lost, due to their smaller viscosity intensities. Other variables such as “area under the peak” (Fig. 3.9a), “time at peak 2” (Fig. 3.9b) and “peak width (time/temp):peak height” (Appendix, Table 1.10 & 1.11) showed a similar trend. These variables indicated that using the 1:1.5 malt to water ratio, optimal discrimination between different degrees of malt fermentability could be obtained (malt fermentability indicated by Tables 1.1 & 1.2, Appendix 1).

All of the variables, except those related to the secondary peak formation, displayed a highly significant (P<0.05) interaction between particle size and concentration ratio (Appendix, Table 1.3 to1.24).

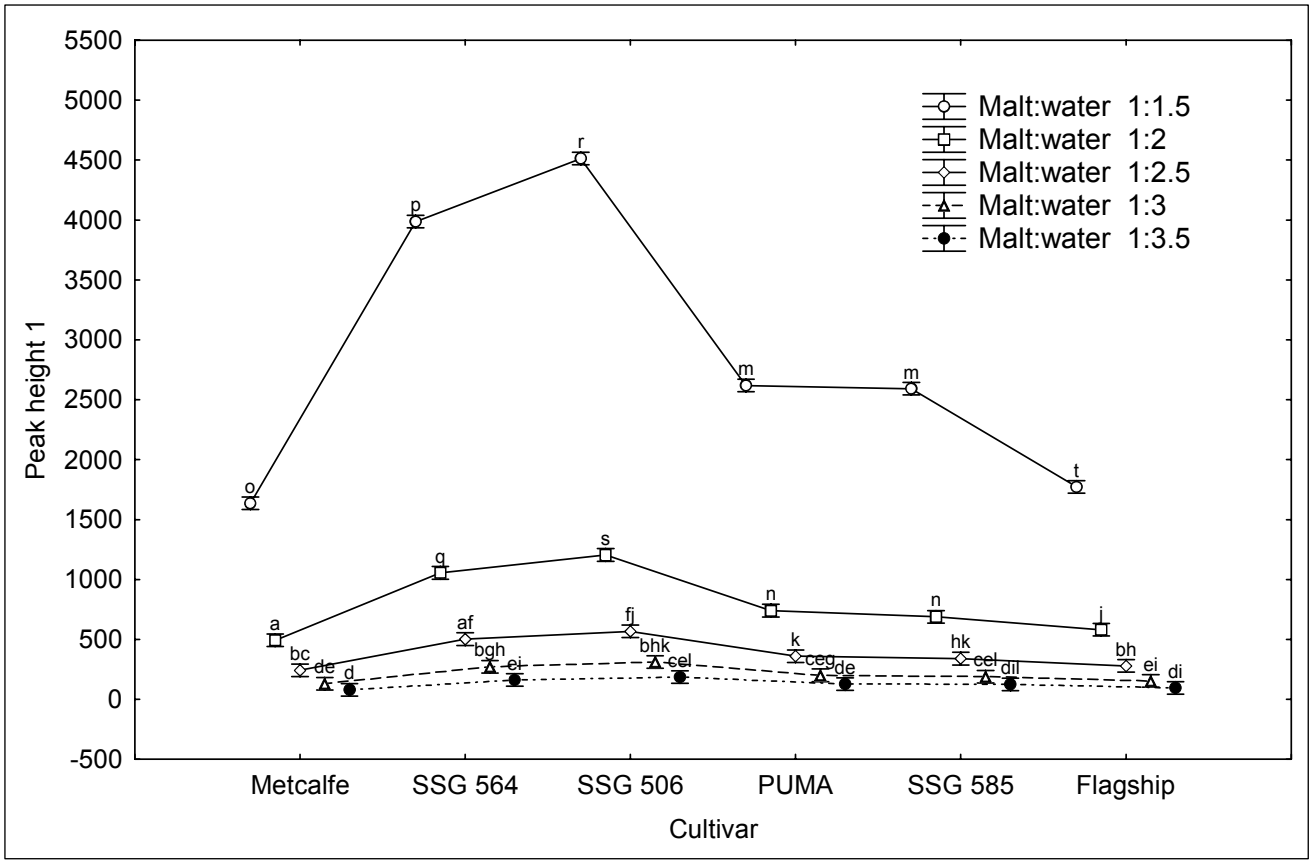

Figure 3.7 Results for cultivar by malt:water ratio (w/w) interaction of the variable “peak height” as obtained with ANOVA. Vertical bars denote 0.95 confidence intervals.

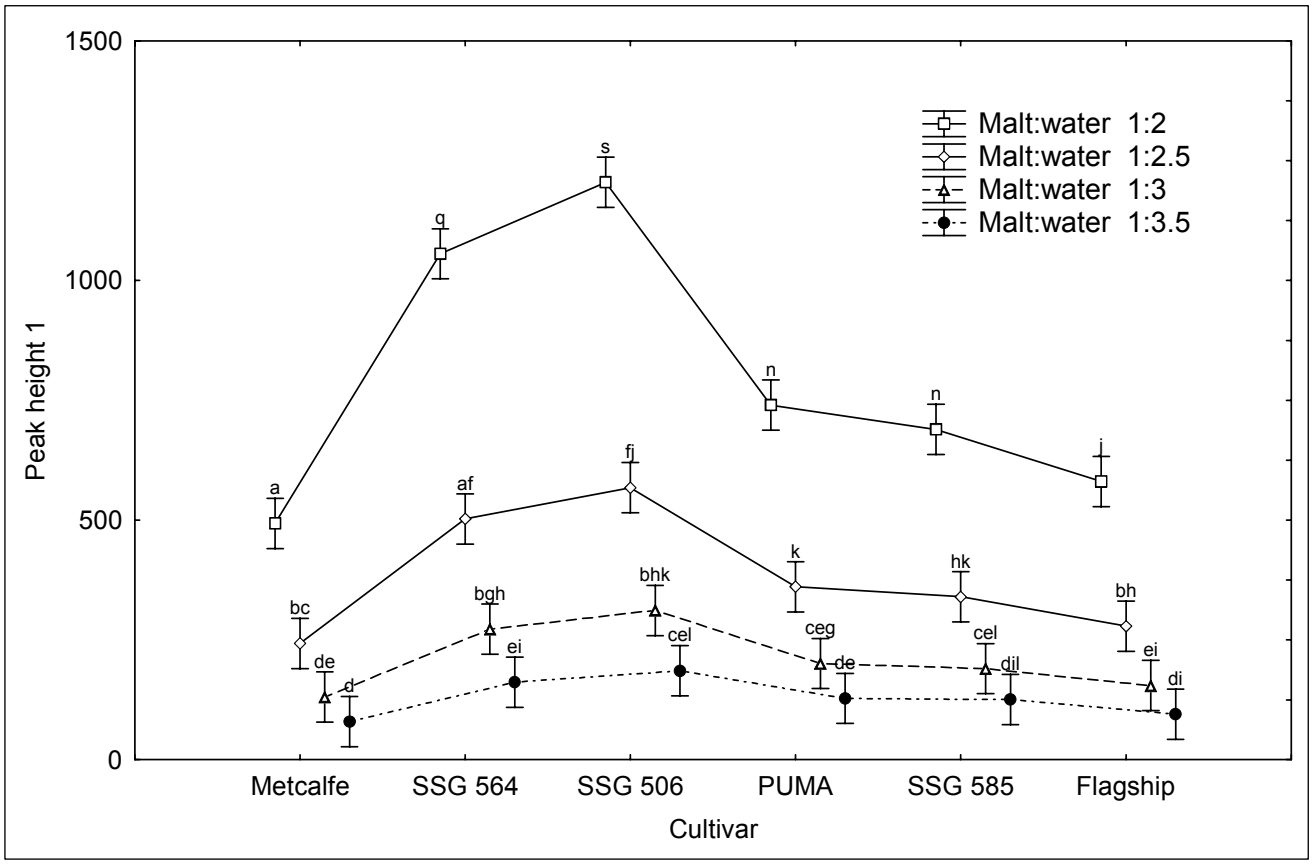

Figure 3.8 Results for cultivar by malt:water ratio (w/w) interaction of the variable "peak height" illustrating the more diluted malt:water ratios. Vertical bars denote 0.95 confidence intervals.

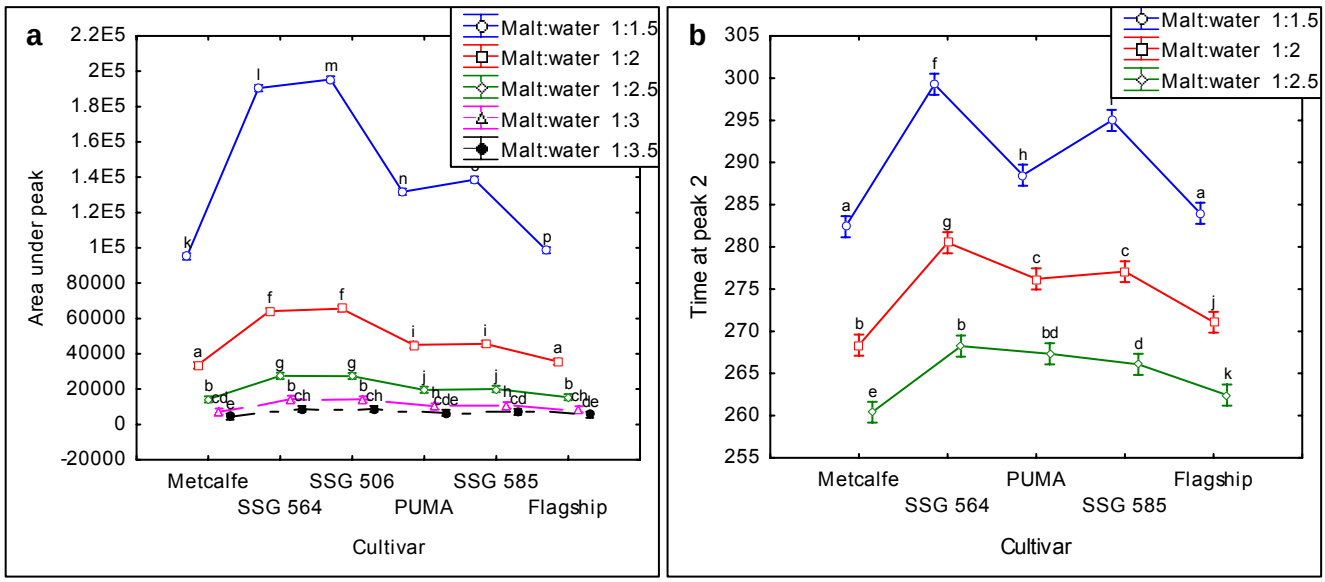

Figure 3.9 Results for cultivar by malt:water ratio (w/w) interaction of the variables (a) "are under peak" and (b) "time at peak 2" as obtained with ANOVA. Vertical bars denote 0.95 confidence intervals.

Multivariate data analysis: PCA (22 selected viscogram variables)

The 22 selected viscogram variables were examined using PCA; in order to identify variables correlating with samples and therefore able to discriminate with regards to malt fermentability. The first two principal components accounted for approximately 74% of the variation in the data set (Fig. 3.10). The PCA scores plot, containing the five different concentrations, indicated the malt:water ratio (w/w) of 1:1.5 delivered the best cultivar and quality separation due to better clustering of the cultivars (indicated by the elipses in Fig. 3.10).

Difference in malt:water ratios were observed in the direction described by PC1, while difference in particle size distribution and malting quality was observed in the direction described by PC2.

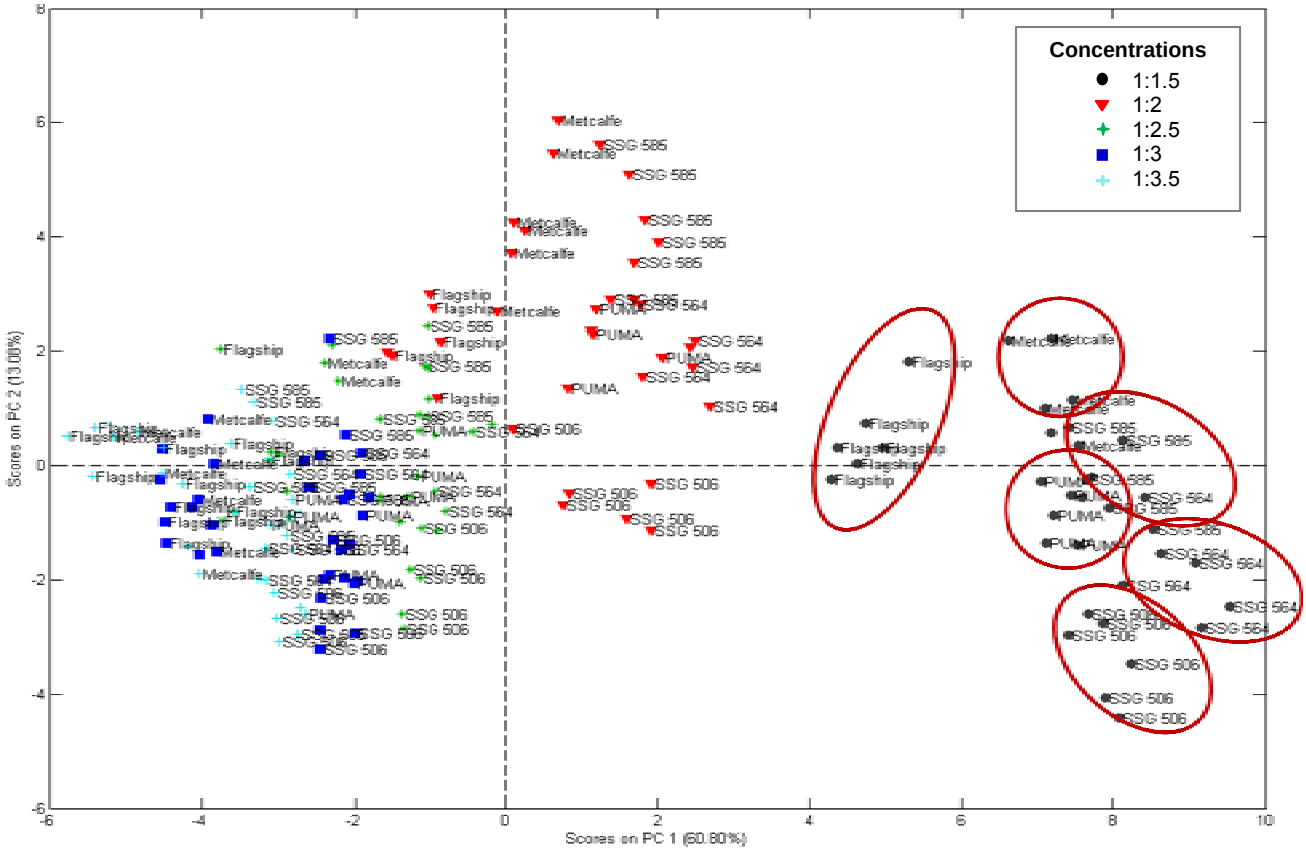

Figure 3.10 Scores plot (PC1 vs. PC2) illustrating the five different malt:water ratios of the six different cultivars when considering selected RVA viscogram variables.

A decrease in fermentability, dependent on both principal components (PC's) was seen in the direction indicated on the scores plot of the more concentrated malt:water ratio (w/w) of 1:1.5 (Fig. 3.11a). SSG 506 (indicated by the elipse in Fig. 3.11a), correlated with the variables "area under peak" and "peak viscosity" from the loadings plot (Fig. 3.11b). Metcalfe was dissimilar to SSG 506, due to the locations being on the opposite ends of the PC space in the scores plot. Metcalfe correlated with the variables "peak width (time/temp): peak height", "Δt" and "ΔT". RVA viscogram

variables describing the height, width and area of the peak, explained why SSG 506 differed from Metcalfe. SSG 564 and SSG 585 correlated with variables such as “time/temp at b”, “pasting temp (Zhou)”, “time/temp at trough 1”, “time/temp at peak 2”, “time/temp at peak 1”, “time/temp at a”. These variables describe the position of the viscogram profile with regards to analysis time. Most of these variables are located after peak formation and therefore describe the shift of the declining slope in analysis time. RVA viscogram variables linked to analysis time (especially of the declining slope), therefore explained why PUMA and SSG 585 differed from Flagship. Difference in particle size distribution was more dependent on PC 1, but gave a similar degree of variation between cultivars. It can therefore be concluded that particle size distribution did not aid in further discrimination between different degrees of malt fermentability.

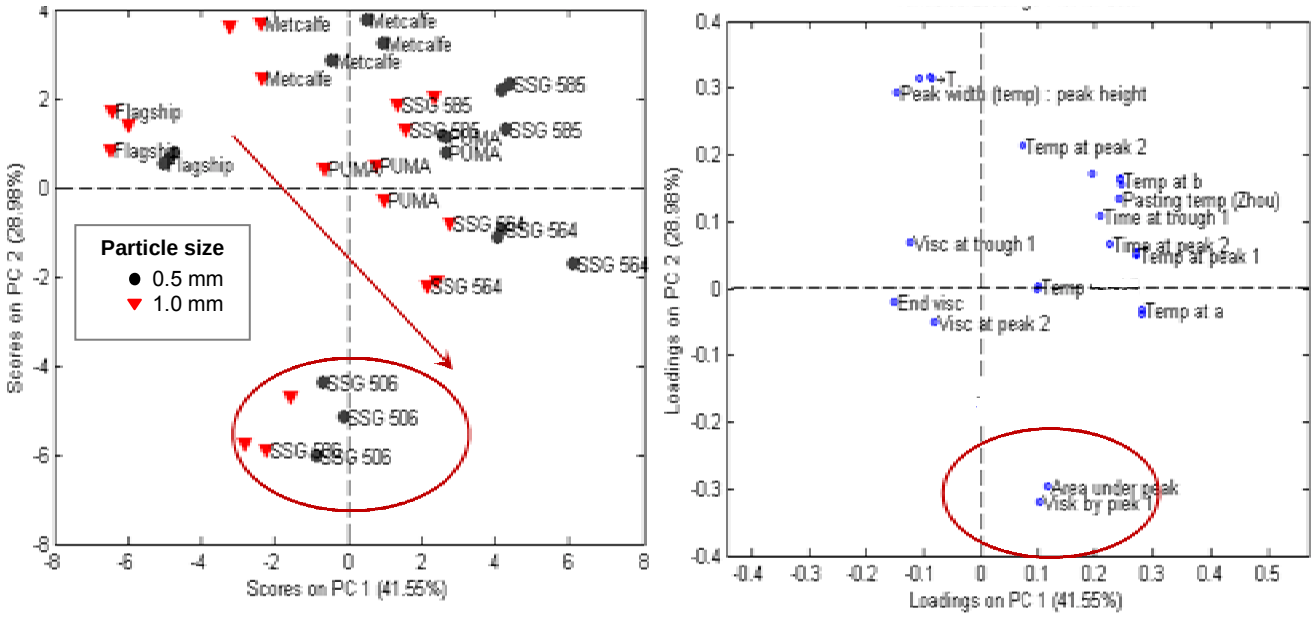

Figure 3.11 The (a) scores plot (PC1 vs. PC2) illustrating the samples, and (b) corresponding loadings plot, illustrating the variables, of the more concentrated 1:1.5 malt:water ratio.

Multivariate data analysis: PCA (225 data points)

All of the 225 data points used to construct a RVA viscogram were examined using PCA to compare with previous results obtained from less variables.

The first two principal components (PCs) accounted for approximately 91% of the variation in the data set (Fig. 3.12). More variation was explained compared to 74% of the previous scores plot (Fig. 3.10). In the scores plot of PC1 vs. PC2 (Fig. 3.12), clustering of three samples (as indicated by the elipses) represented the three replicates. Due to the close proximity of Metcalfe’s three replicates, it appeared to be more repeatable when compared to the other cultivars. Differences in malt:water ratios (especially 1:1.5 compared to the rest) were observed in the direction described by PC1. The loading line plot of PC1 represented a typical RVA viscogram (Fig. 3.13). Variation

captured in PC1, therefore concentration differences, was mainly due to differences in peak viscosity (peak height). Difference in particle size distribution was observed in the direction described by both PC's, but with PC2 being more dominant. The loading line plot of PC2 resembled the first derivative of a typical RVA viscogram. Variation captured in PC2, therefore particle size distribution, seemed to account for differences in peak occurrence in analysis time. Differences in malt fermentability were observed in the direction described by both PCs, indicating both peak occurrence (analysis time) and peak viscosity contributed to quality discrimination.

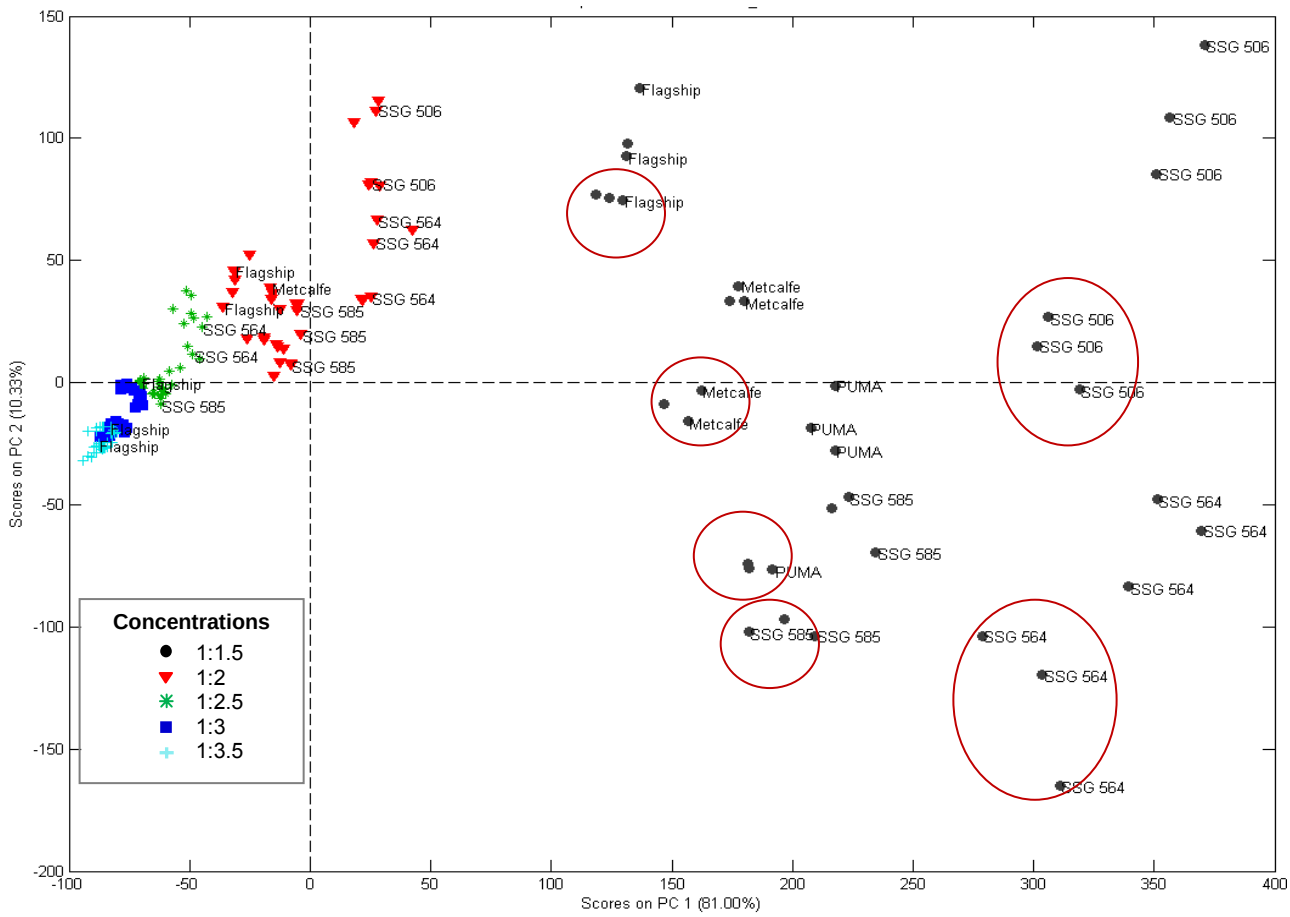

Figure 3.12 Scores plot (PC1 vs. PC2) illustrating the five different malt:water ratios of the six different cultivars when considering the 225 data points.

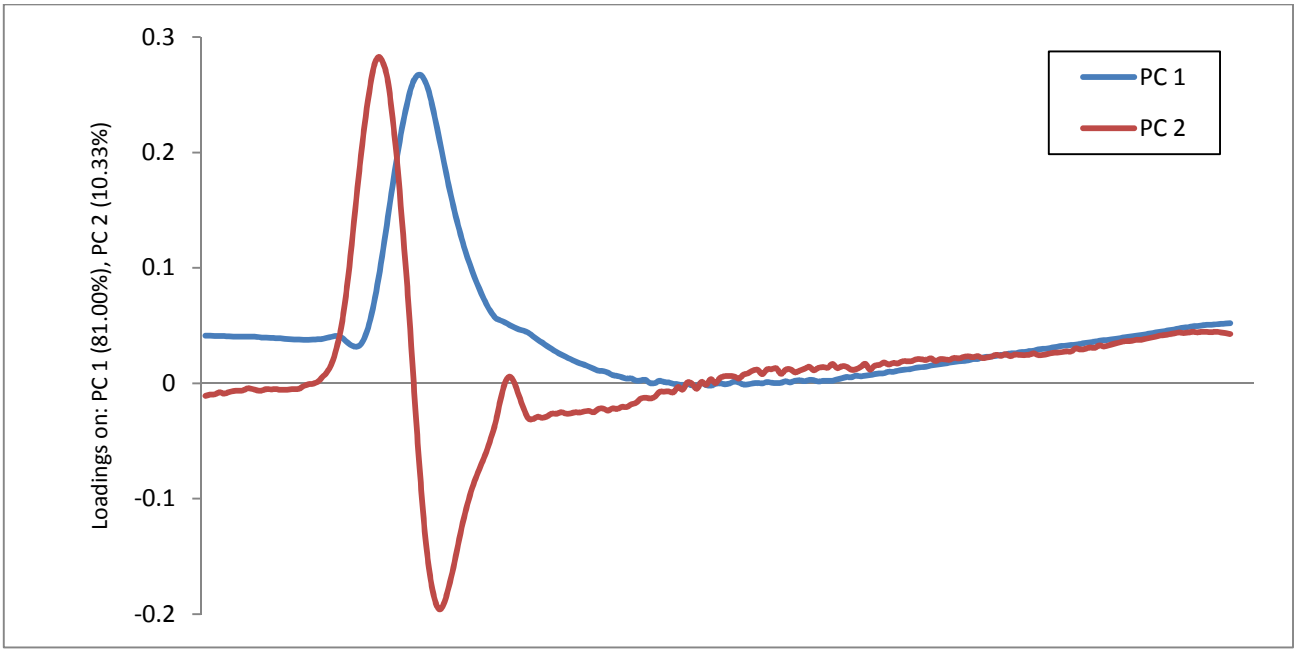

Figure 3.13 Corresponding loading line plots (PC1 vs. PC2) when considering the 225 data points.

Distinction between the malt of different malting barley cultivars was more apparent for the 1:1.5 malt:water ratio (w/w) (Fig. 3.14a) compared to the other ratios (Fig. 3.14b). A clear distinction was seen between the highly fermentable malt, i.e. Metcalfe and Flagship, and the other inadequate or poor quality malts (Fig. 3.14a).

Although a slight distinction in particle size distribution in the PC space of the scores plot was observed (Fig. 3.14a), it did not allow further discrimination between malting qualities. Either of the sieve sizes could thus be used during milling for future sample preparation.

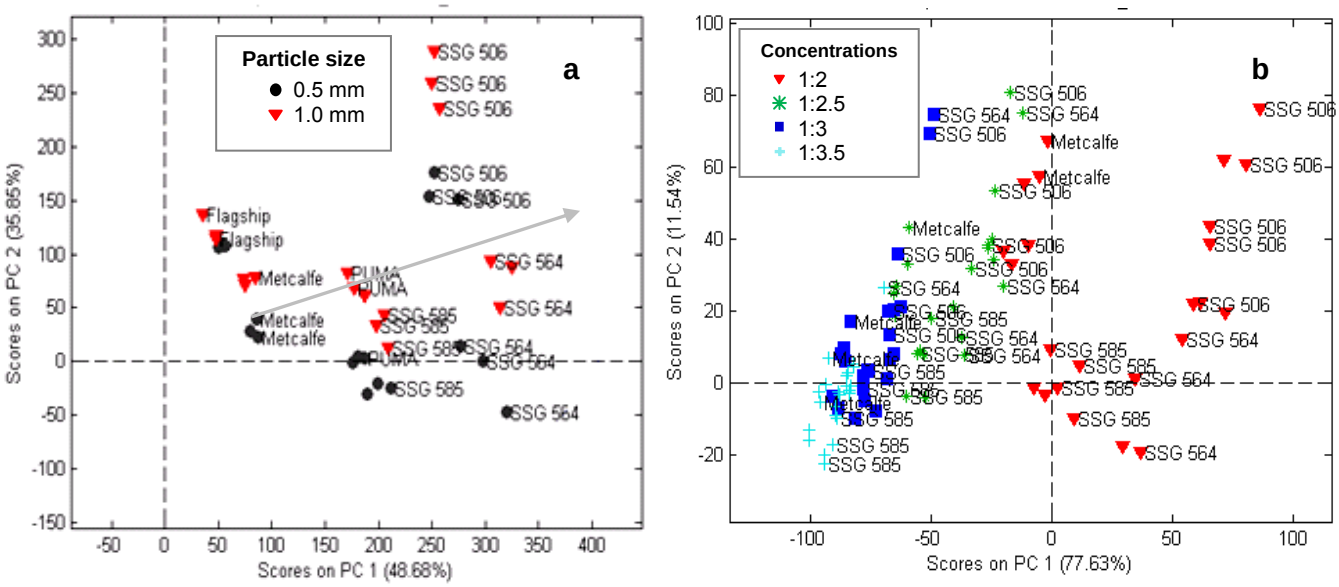

Figure 3.14 Scores plot (PC1 vs. PC2) illustrating the distinction between malting barleys for the (a) 1:1.5 malt:water ratio (w/w) and (b) the other more diluted malt:water ratios.

Repeatability

Samples from all the concentration ratios displayed the same similarity of replicates (Table 3.3 & 3.4). This indicated the malt:water ratio (w/w) of 1:1.5 was suitable for optimal quality discrimination without detriment to repeatability.

Table 3.3 Replicate similarity of the **225 data points** (entire viscogram) for the different malt:water ratios showing average of Euclidean distances between replicates

Cultivars	Malt:water ratio				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	0.21	0.25	0.34	0.32	0.38
SSG 564	0.36	0.31	0.32	0.47	0.44
SSG 506	0.28	0.27	0.37	0.50	0.23
PUMA	0.25	0.31	0.30	0.47	0.32
SSG 585	0.35	0.32	0.32	0.06	0.45

Table 3.4 Replicate similarity of the **22 selected viscogram variables** for the different malt:water ratios showing average of Euclidean distances between replicates

Cultivars	Malt:water ratio				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	0.64	0.66	0.54	0.55	0.25
SSG 564	0.55	0.71	0.56	0.51	0.57
SSG 506	0.56	0.60	0.59	0.33	0.48
PUMA	0.50	0.61	0.60	0.43	0.55
SSG 585	0.43	0.61	0.45	0.48	0.43

Discussion

Viscograms

Considering the different **cultivars**, peak height results seem to coincide with trends found in literature; namely that better modified malt's form a lower broader peak, while unmodified or poor quality malts produce long narrow peaks (Yoshida & Yamada, 1970; Glennie Holmes, 1995b; Glennie Holmes, 1995c; Goode *et al*., 2005b; Goode & Arendt, 2006). According to the results, peak

height and width relay quality information and variables within it should be examined for possible quality discrimination. SSG 506 and 564 displayed higher peak viscosities, compared to the other cultivars. The effect of adding additional sources of Diastatic Power should be investigated, and if the situation does not improve, it is further suggested that β-glucanases and/or proteases may be needed (Glennie Holmes, 1995c). According to malt analyses conducted (Kolbach index and B-glucan content) addition of β-glucanases and/or proteases is not expected to deliver better modified malts as these analyses were within malt-house specifications (Table 1.2, Appendix 1). Some literature refers to the end viscosity to relay quality information (Goode *et al.*, 2005b; Goode & Arendt, 2006). Due to the enzyme activity of malt, a barely measurable end viscosity is formed which is heavily dependent on baseline determination. To accurately link values to the end viscosity and then compare different samples to one another would be highly speculative. The shift of viscosity peak formation, with regards to analysis time, between cultivars can possibly be explained by differences in the amylose-lipid complex formation. It has been suggested that peak viscosity, for barley and maize, may occur as a result of an amylose-lipid association (Goering *et al.*, 1975; Dengate, 1984). Starch granules containing a higher content of surface lipids exhibited delayed gelatinisation and viscosity increase (Eliasson *et al.*, 1981; Dengate, 1984). Formation of a secondary peak possibly indicated secondary starch gelatinisation of smaller B-type starch granules (Goode & Arendt, 2006) or phospholipids associated with amylose (Morrison, 1995; Nelles *et al.*, 2000) (discussed in literature review, section 7.4, p. 39).

Varying **concentration ratio** greatly affected the declining slope of the RVA viscogram. Contrary to the findings of Glennie Holmes (1995a), a measurable peak was still formed at a malt:water ratio (w/w) of 1:3.5, which was more diluted than that stated of being unable to form a peak (1:3). His results also indicated that malt:water ratios more concentrated than 1:1.8 produced a viscogram with an irregular trace. The reported effect was not observed in this study, even when considering a higher malt concentration (1:1.5). This could perhaps be due to different time and temperature profiles used during RVA analysis. The concentrated malt-water samples produced a delayed peak (i.e. peak formation later in analysis time) when compared to the diluted ratios (1:2, 1:2.5, 1:3 and 1:3.5). This can perhaps be explained by the competitive effect of malt for water at the concentrated 1:1.5 ratio. At such a high malt concentration, the hydration of starch particles are hindered, subsequently also starch gelatinisation and hydrolysis (processes dependent on hydration). Another possible explanation could be, the limited amount of water caused an increased gelatinisation range, therefore viscosity increased at a slower rate, producing a delayed peak (Ghiasi *et al.*, 1982; Dengate, 1984).

Varying **particle size distribution** greatly affected the incline of the RVA viscogram. Although Becker *et al*., (2001) focused on extruded maize- and wheat-pellets, in both cases, the larger particle sizes displayed a ‘peak shift’ (in analysis time) which occurred earlier when compared to the smaller particle sizes. Almeida-Dominguez *et al.* (1997) examined the effect of varying particle size

of milled maize kernels. Smaller particles formed a higher peak which occurred earlier. The researchers' explanation to their findings were: larger, more compact particles require additional time and energy to hydrate, subsequent starch granule swelling, gelatinisation and pasting (therefore a viscosity increase) would also be delayed. However the smaller particles, which contain a larger surface area and facilitates more rapid hydration, allows for greater gelatinisation at reduced temperatures (Almeida-Dominguez *et al.*, 1997). It should however be kept in mind, the mentioned study was conducted on maize kernels, with limited to no enzyme activity present. Therefore, it can be hypothesised that smaller particles hydrate and gelatinise at a faster rate. Enzyme hydrolysis, being dependent on hydration and gelatinisation, initiates earlier and therefore the incline of the peak can be expected to occur at decreased viscosity rate in comparison to the larger particle size sample. This could cause the 'delayed appearance' in peak formation, but a similar peak decline in analysis time (due to thermal inactivation of enzymes).

It was evident from results that **concentration ratio** seemed to **interact** with the rheological effect of varying **particle size**. Similar peak heights at the 1:1.5 malt:water ratio (w/w) indicated a similar extent of enzyme hydrolysis for both particles, however the rate of viscosity increase differed between particle size distributions (at the high malt:water ratio (w/w) of 1:1.5), indicating the rate of enzyme hydrolysis was affected. The following explanation is given as to the reason for the observed effect.

A finer sieve (0.5 mm) used during centrifugal milling reduced particle size distribution, compared to using a coarser sieve (1 mm) which results in increased particle size distribution. The rate of water absorption was affected by varying the particle size distribution and smaller particles displayed faster initial absorption (Meddings & Potter, 1971). The rate of water migration subsequently affects the gelatinisation process (Mousia *et al.*, 2004). The gelatinisation process of whole rice grains was found to be delayed when compared to finer ground rice (Riva *et al.*, 1994). It has been reported malt amylases is unable to hydrolyse raw ungelatinised starch (Meddings & Potter, 1971; Olkku & Rha, 1978; Allan *et al.*, 1997; Mousia *et al.*, 2004). The biochemical process of hydrolysis takes place immediately after the formation of the gelatinised substrate (Meddings & Potter, 1971). Starch hydrolysis is controlled by starch gelatinisation, which is affected by various factors such as moisture content, additives, particle size distribution and starch damage (Mousia *et al.*, 2004). This could indicate gelatinisation as the principal cause of viscosity differences. Under enzyme inhibited conditions, peak viscosity is proportional to the cube of the starch concentration, while under autolytic conditions degrading 0.1% of the internal bonds within starch molecules causes a 50% reduction in viscosity (Glennie Holmes, 1995a). Considering the resultant effect, enzyme hydrolysis seems to have a larger overall effect, namely reducing viscosity notably more than gelatinisation increases it. Inhibiting enzyme hydrolysis, a higher viscosity is obtained compared to uninhibited conditions (starch gelatinisation and enzyme hydrolysis). Peak formation was highly retarded when using a high malt concentration ratio (**1:1.5**) (peak leaning to the left)

compared to the others (1:2, 1:2.5, 1:3 and 1:3.5) (peak leaning to the right). Highly concentrated sugar and starch conditions have been shown to inhibit gelatinisation due to increased osmotic pressure and consequently increased competition between starch and sugar components for available water (Lelievre, 1976; Eliasson, 1980; Ghiasi *et al.*, 1982; Muller, 1989). This is the mostly likely cause of different viscosity increase rates observed when varying particle size, using highly concentrated malt:water ratio (w/w) (i.e. 1:1.5).

Considering the less concentrated malt ratios (especially **1:3** and **1:3.5**), initial rheological differences are virtually eliminated. This could be attributed to the presence of a large amount of water, during which consistency (mainly affected by the amount of starch exudate released) was considered to become a function of temperature (Longley & Miller, 1971; Olkku & Rha, 1978). Therefore, it can be reasoned water absorption, gelatinisation and, subsequent enzyme hydrolysis, seemed to proceed at a very similar rate. The exact reason for the delayed breakdown and earlier secondary peak formation, of the smaller particles, is unknown. Perhaps this could be due to the increased physical separation of enzyme and substrate as a result of the diluting effect of water. It can be reasoned breakdown occurred at the exact same rate for both particles. However for the smaller particles, gelatinisation (viscosity increase) proceeded to a greater extent after enzymes were already inactivated, thus reaching equilibrium height only later. This could also explain why, at the highly concentrated malt ratio (1:1.5) the breakdown appeared similar for both particles, as enzymes were allowed a greater time period before thermal inactivation due to enhanced thermostability, provided by the highly concentrated malt-water mixture (Muller, 2000).

It can be concluded the initial increase in viscosity, under concentrated conditions (1:1.5 and 1:2), was largely influenced by particle size distribution, due to the combination of effects (high concentration ratio while varying particle size distribution) on gelatinisation and enzyme hydrolysis. However, in an access amount of water, viscosity breakdown, (therefore retrogradation and shear thinning) were largely influenced by particle size distribution. Additional starch damage could have been inflicted on samples when milling with a finer sieve size (Mahasukhonthachat *et al.*, 2010). This could also have affected rheological results. Better insight with regards to the effect of particle size variation could have been obtained by the addition of one more particle size. This was not practically possible due to milling limitations. Further testing is needed to draw more concise conclusions (see Chapter 5).

Univariate data analysis: ANOVA (22 selected viscogram variables)

Analysis of variance indicated **particle size** played no role in optimising the RVA method. This suggested when working with either particle size, variation between the cultivars was affected to the same degree. However, the 0.5 mm sieve size produced a finer flour and therefore created viscograms containing a smaller amount of deviation (Appendix 1, Table 1.3 to 1.24), suggesting

larger particles can obstruct viscosity measurements. This justified using the 0.5 mm sieve size during centrifugal milling for further RVA testing.

The high malt **concentration ratio** (1:1.5), which merely served as an upper concentration limit, unexpectedly delivered the greatest separation of peaks between the different cultivars. This could indicate how competition between malt particles for water at such a high concentration influences starch pasting properties and enzyme hydrolysis of malts of different qualities. This could perhaps be explained by selective removal of material with a high water-binding capacity during modification (hydrolysis of β-glucans) (Woodward & Fincher, 1983). Less optimally-malted (poor-quality) cultivars contain more high water-binding capacity material and as a result a limited amount of water would affect these cultivars more than optimally malted (good quality) cultivars. It has been suggested deviation in enzyme content could be responsible for fermentability variation between different malts (Evans *et al.*, 2005). However based on malt analyses conducted; B-glucan content were within specification limits even though SSG 585, 506, 564 and PUMA are known to deliver brewhouse fermentability problems (Table 1.2, Appendix 1). A decrease in the thermostability of enzymes results in a less fermentable extract, suggesting diastatic enzyme thermostability was possibly linked to fermentability (Eglinton *et al.*, 1998). A concentrated mash provided enhanced thermostability for enzymes (Muller, 2000). A more concentrated mash could indicate viscosity differences between cultivars which differ in enzyme composition, content and activity. In a concentrated starch paste, the individual starch granules gelatinise and swell freely until all the available water has been absorbed. When starch granules gelatinise in the presence of a large amount of water, exudate (such as amylose) is released in amounts that cause consistency to become a function of temperature alone (Longley & Miller, 1971; Olkku & Rha, 1978). With a similar time and temperature regime applied throughout the entire experiment, rheological differences between cultivars at diluted ratios (1:3 and 1:3.5) can be expected to become a minimum. Results further indicated RVA viscogram variables such as “peak height”, “area under the peak” and “time at peak 2” relay quality information and are good measurements for establishing malt fermentability.

PUMA and SSG 585 was suggested to be similar based on their peak heights. This could indicate the same extent of modification for both samples. Both cultivars are also known to produce fairly similar fine extract values and brew in a similar fashion (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). However malt modification analysis (partially unmodified grain and whole unmodified grains) contradicts these findings (Table 1.2, Appendix 1).

The significant interaction found between particle size and the malt:water ratio (w/w) substantiates previous deductions made (pg. 78) on the rheological effect of varying particle size, which seemed to be affected by the malt concentration.

Multivariate data analysis: PCA (22 selected viscogram variables)

Results from this study once again confirm previously published data, i.e. good quality malt forms a low but broad peak, while the poor quality malts form a high narrow peak (Yoshida & Yamada, 1970; Glennie Holmes, 1995b; Glennie Holmes, 1995c; Goode *et al*., 2005b; Goode & Arendt, 2006). Therefore allowing peak height and area, or any relationship between them, to discriminate amongst malt qualities.

Samples (in the PCA scores plot) correlating with variables (in the PCA loadings plot) are expected to have a large amount of those variables in comparison to dissimilar samples. As previously stated, the sample Metcalfe is dissimilar to SSG 506. The sample SSG 506 correlated with the variables “area under peak” and “peak viscosity” from the loadings plot (Fig. 3.11b, p.73). Metcalfe correlated with the variables “peak width (time/temp): peak height”, “Δt” and “ΔT”. From the PCA results, SSG 506 was therefore expected to have a larger area and higher peak in comparison to Metcalfe, which was expected to have a broader, shorter peak. This deduction was confirmed with previously mentioned viscogram results (Fig. 3.2, p.65). As previously stated, SSG 564 and SSG 585 correlated with variables such as “time/temp at b”, “pasting temp (Zhou)”, “time/temp at trough 1”, “time/temp at peak 2”, “time/temp at peak 1”, “time/temp at a”. These variables describe the position of the viscogram profile with regards to analysis time. From the PCA results, SSG 564 and SSG 585 are therefore expected to occur later in time (having a larger amount of the stated variables) compared to Flagship, which is expected to occur the earliest. Again these deductions were confirmed by previous viscogram results (Fig. 3.2, p.65).

The malt:water ratio (w/w) of 1:1.5 provided the best separation of malt quality, allowing malts to be rated in terms of degree of fermentability efficiency. Particle size did not aid in further quality discrimination due to the same amount of variation between samples.

It is interesting to note that the variables mentioned in ANOVA, able to discriminate between the different degrees of fermentability, were the same variables able to distinguish SSG 506 from Metcalfe (variables: “area under the peak”, “peak height” and “peak width (time/temp): peak height”) and SSG 585, SSG 564 and PUMA from Flagship (variable: “time at peak 2”), identified from PCA. Similar conclusions can thus be drawn from both data analysis techniques.

Multivariate data analysis: PCA (225 data points)

Principal component analysis on the entire viscogram explained more variation compared to analysis on fewer variable, due to more variables available to explain more sample variation experienced. By conducting multivariate data analysis, the time consuming selection of variables from RVA results (conventional method) was eliminated. PC2 represented the first derivative of a typical RVA viscogram. A loading line plot shows the largest loadings which correspond to the most important diagnostic variables in a range. This implicated rheological variables from the inflection points of both the peak incline and decline were considered of importance (Fig. 3.13, p.75). It has

been stated “the first derivative of RVA curves represent the rate of viscosity change as a function of time” (Juhasz & Salgo, 2008). Therefore, more variables describing peak formation, with regards to time, are considered important. Variation captured in PC2 is thus able to account for differences in peak occurrence in analysis time. Results obtained from PCA on the entire viscogram indicated separation between different malt:water ratios could largely be explained by variables forming the peak such as peak height. Separation of particle size can be explained by a shift of the peak with respect to analysis time. Discrimination based upon cultivar (thus degree of fermentability) was not only dependent on variables associated with peak formation, but also linked to peak occurrence in analysis time. Metcalfe appeared to be more repeatable compared to the other cultivars. This could possibly be due to its smaller peak viscosity and therefore smaller standard deviation. Considering the more concentrated ratio (Fig. 3.14a, p.75), the same degree of malt fermentability separation can be seen when compared to the previous scores plot of the 22 selected variables (Fig. 3.11a, p.73). This indicated the small amount of variables were accurately chosen to represent or summarise the 225 data points, as similar fermentability discrimination was found.

Once again, PCA on the 225 data points indicated the 1:1.5 malt:water ratio (w/w) at either 1 or 0.5 mm sieve size optimised the RVA Kilned Malt method for maximum fermentability discrimination. Multivariate data analysis indicated the potential of PCA to not only identify good and poor malt fermentability, but also to rate these malts in terms of degree of fermentability efficiency (indicated by the arrow, Fig. 3.14a, p.75). It is thus possible that SSG 585 and PUMA have a fermentability of intermediate efficiency.

Repeatability

Results indicated replications were fairly similar when compared across the different malt:water ratios, for the entire curve (Table 3.3, p.76) and the selected variables (Table 3.4, p.76). The three replicates, as seen on previous scores plots (Fig. 3.11, 3.12 and 3.14, p.73-75), seemed to be spread out far from each other, especially when considering the more concentrated ratio (1:1.5). This ratio yielded the highest peaks and thus the largest absolute difference between replicates (high standard deviation). This distance is therefore arbitrary and when standardised to eliminate the effect of high intensities, and large deviation, samples at this concentrated ratio are just as repeatable as the other ratios.

Conclusion

The rheological effect of varying malt concentration and particle size was investigated. The objective was to identify optimal RVA sample conditions, accompanied with a compatible data analysis technique, to deliver optimal fermentability discrimination. Both particle size distribution and malt concentration ratio was found to affect rheological analysis, especially with regards to peak formation. Due to the interaction of concentration ratio and particle size distribution, milling proved to

be more than a sample preparation step affecting rheological measurement. However, variation in particle size distribution affected all the cultivars (which differed in terms of malt fermentability) to the same degree, thus not contributing to better fermentability discrimination. For future studies, it might prove useful to intensify the viscosity signal of the secondary peak to possibly extract useful information from it.

Results obtained from ANOVA (selected variables), PCA (selected variables) and PCA (225 data points), were all in agreement. These methods indicated that by using the 1:1.5 malt to water ratio at any of the stated sieve sizes (1 mm or 0.5 mm using a centrifugal mill), the RVA Kilned Malt Method was optimised to discriminate between malts of high, intermediate and low malt fermentability. Multivariate data analysis in the form of PCA on the entire data set eliminated the selection of variables from the viscograms. This greatly reduced pre-treatment of RVA data, as calculation of data points took a great deal of time and effort to determine. PCA conducted on the entire data set explained more variation within it.

PCA (on the entire data set) has the potential to be a rapid, more versatile data analysis technique and with the right programming, can be linked with RVA software to create a powerful novel tool for practical implementation in the industry. Results proved the ability of viscosity measurement by means of the RVA to distinguish between malting barley cultivars of different fermentability degrees. If such a method is implemented in malting and brewing industries, the brewing performance of unknown malting barley cultivars could be predicted. Such a rapid test, which exclusively reports on the condition of malt and requires less operator skill, could avoid severe losses as a result of wrongful identification of highly fermentable malts.

It should be noted that this research had a limited sample size and contained only six different cultivars. Before such a method can prove to successfully predict samples of unknown malt fermentability, a model should be developed and properly validated using an extended sample set.

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Chapter 4

Model development, validation and prediction of malts with different degrees of fermentability using the optimised Rapid Visco Analyser (RVA) Kilned Malt method

Chapter 4

Model development, validation and prediction of malts with different degrees of fermentability using the optimised Rapid Visco Analyser (RVA) Kilned Malt method

Abstract

Laboratory malt fermentability tests, i.e. AAL are not representative of commercial brewing conditions and consequently should not be used to predict fermentability on an industrial brewing scale. This instigated the development of a multivariate classification model based on different malt fermentability classes. Rheological analysis conducted by means of the Rapid Visco Analyser (RVA) was regressed with malt fermentability in order to develop multivariate regression models able to predict this characteristic. Using the Kilned Malt method combined with previously identified sample conditions (Chapter 3), rheological data were regressed with apparent attenuation limit (AAL) and free amino nitrogen (FAN), on an individual basis. Calibration models developed were validated by a random test set and segmented cross-validation for AAL and FAN, respectively. A strong correlation between RVA analysis (the entire viscogram) and AAL was obtained (r=0.92), while FAN delivered a weak correlation (r=0.59) and should not be used for predictive purposes. These classes were identified by means of principal component analysis (PCA) and malt fermentability information provided by local maltsters. Classification models, i.e. Soft Independent Modeling of Class Analogy (SIMCA), were developed by creating individual PCA models of each distinct data class. Individual PCA models were validated separately by means of test set validation in order to identify the optimal model dimensionality. In terms of malt fermentability, the proportion of test set samples correctly classified was 83%. Local malt blends, were all predicted to have low malt fermentability. Simulated blends were predicted to have high fermentability when using a minimum of 80% Metcalfe and maximum of 20% SSG 506. Blends with higher proportions of the low malt fermentability cultivar SSG 506 were predicted to have an overall low fermentability. RVA analysis thus showed great potential to give further insight on malt fermentability.

Introduction

At present a diverse range of beers and flavoured alcoholic beverages are produced and sold by South African Breweries (SAB). It is hard to comprehend that as recently as twenty years ago only two major brands existed. Fifteen years ago a specific South African beer brand claimed the majority market share only to be replaced with a new brand, with different characteristics (Potgieter & Meijering, 2009). This shift in South African consumers' beer preference and the need to substitute expensive imported malting barley cultivars with similar local cultivars, have driven local barley breeding programmes to develop new cultivars with a

high malt fermentability (Potgieter & Meijering, 2009). Local breeding programmes use marker selection to develop these new cultivars. However, malt fermentability in early generation lines is not currently evaluated due to testing constraints (Mr. F Potgieter, Senior Barley Breeder, SABBI, Caledon, South Africa, Personal communication, 2010). Quality evaluation methods in barley breeding programmes must be suitable for the analysis of a large number of samples in a short time period, while only using a small amount of sample (Ellis, 1986; Molina-cano, 1991; Han *et al.*, 1997). To avoid production losses, fermentability of new commercial cultivars must be evaluated before being used in commercial brewing. During brewing, it is a general practice to mix the malt of different malting barley cultivars, which differ in quality and fermentability. Therefore, of equal concern is the evaluation of a malt blend's fermentability.

Wort fermentability refers to the efficiency of yeast to convert sugars (present in the wort) to alcohol. Fermentability, governs the alcohol yield during brewing and, therefore, the amount of beer produced per tonne of malt (Fox *et al.*, 2001; Evans *et al.*, 2010). Fermentability is commonly analysed by conducting a mini fermentation test and subsequently measuring the apparent attenuation limit (AAL) (see detailed discussion in Chapter 2, section 5.2, p.26). This procedure entails the fermentation of wort under controlled conditions in an excess amount of yeast. Specific gravity (density) is measured before and after fermentation which allows the calculation of AAL (Fox *et al.*, 2001; Briggs *et al.*, 2004).

These attenuation tests have several shortcomings, most importantly laboratory mashing conditions are unrepresentative of large scale brewhouse mashing conditions. This results in poor correlation between attenuation tests and actual brewhouse performance (Calman *et al.*, 2008). Breeding programmes require rapid quality assessment of a large number of samples. Conducting attenuation tests is considered time consuming (Fox *et al.*, 2001; Evans *et al.*, 2010), e.g. the malt fermentability indicator test (MFIT), which requires a 10 day fermentation period (Mr. D Fisher, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). Attenuation tests are usually expensive to conduct. The yeast used and accelerated fermentation conditions differ from that practiced in the brewhouse, giving potentially biased results (Evans *et al.*, 2010). Fermentation also requires large volumes of wort, therefore its application in breeding programmes is impractical, particularly in early generation testing (Fox *et al.*, 2001; Briggs *et al.*, 2004). This implies only lines in the most advanced stages of the breeding programme can be tested for fermentability (Fox *et al.*, 2001).

Due to testing constraints, replacing fermentability analysis (AAL) by more practically suitable analyses have been investigated. Therefore relationships have been established by developing multilinear regression models able to predict AAL (Evans *et al.*, 2005; Evans *et*

al., 2010). Such malt analyses typically include: diastatic power enzymes (i.e. α-amylase content, total limit dextrinase content, total β-amylase content and β-amylase thermostability), the Kolbach index (i.e. the ratio of total soluble nitrogen to total nitrogen) and free amino nitrogen content (FAN) (Evans *et al.*, 2010). Starch gelatinisation temperature has also been identified as a useful measurement for the prediction of malt fermentability (Evans *et al.*, 2010).

The FAN value indicates the availability of nitrogen compounds (mainly amino acids and small peptides). FAN must be sufficiently high to support yeast growth and metabolism and therefore ensure fermentation is not restricted by a lack of nitrogenous nutrients (Briggs et al., 2004). Adjuncts contribute relatively little soluble nitrogen to the wort. When brewing with a large amount of adjuncts, wort nitrogen content is diluted, thus requiring higher FAN values from malt (Briggs *et al.*, 2004).

Enzyme content, activity and thermostability, together with starch gelatinisation temperature, are important factors determining malt fermentability (Evans *et al.*, 2010). Variation in these factors will drastically influence viscosity measurements recorded when gradually heating a malt water mixture above its gelatinisation point. If viscosity measurements are sensitive enough, this would enable discrimination between samples which differ in malt fermentability. The Rapid Visco Analyser (RVA) has the sensitivity needed to measure malt fermentability (Chapter 3, pp. 76-82). Principal component analysis (PCA) applied to the entire viscogram allowed the fermentability to be rated and therefore enabled discrimination between samples with different degrees of malt fermentability.

Partial least squares (PLS) regression, a form of multivariate data analysis, allows the development of a statistical model to enable future prediction. Firstly, a multivariate calibration model is developed by acquiring a large number of samples, representative of the future population. In the calibration set, each sample in the X-matrix (viscosity measurements) must contain corresponding Y-values (fermentability degree). In this study, regression was performed using rheological measurement (RVA) and malt analyses (AAL and FAN individually). Regression allows the prediction of one or several quantitative variables, but when the response is a category variable, classification enables the useful allocation of samples to a class (Esbensen, 2002).

Soft Independent Modeling of Class Analogy (SIMCA) is a classification method of supervised pattern recognition. SIMCA classification allows the assignment of new objects to a class which they show the largest similarity to, therefore modelling only the common properties between samples within the same class. SIMCA classification utilises separate bilinear modelling, most often PCA models, for each valid data class. During the training stage, individual PCA models of the recognised data classes are created. The subsequent classification stage utilises the established class models to assess which classes new

objects belong to. Results from the classification stage allow the inspection of the modelling and discrimination power of the individual variables (Esbensen, 2002).

All calibrations must be validated, either with cross-validation or test set validation. Correct validation is essential to ensure the development of a successful model, able to work in future for similar new data sets. Validation is vital to indicate the optimal dimensionality of a multivariate model (X,Y), and therefore to avoid either over-fitting or under-fitting. Test set validation uses a completely new data set, with corresponding Y-values, for validation. The calibration model predicts the Y-values of the new data set and then compares these independently predicted values with the known real Y-values. Test set is the best form of validation, but often its application is impractical due to the requirement of a large number of samples (i.e. samples are required for both the training and test sets). Cross-validation involves dividing the sample set (objects with known Y-values) into different segments; the number of samples within these segments, and the way in which the samples are selected, varies, e.g. in full cross-validation each segment consists of one sample. A series of sub-models are calculated, where each segment is used as the validation set once (i.e. the calibration is based on all samples not in the segment and the validation is based on samples in the segment). The final calibration model is developed using all objects in the sample set, the prediction error statistics of this calibration are the averaged error statistics of the series of sub-models (Esbensen, 2002).

The rationale behind calculating multivariate regression models for prediction is to eliminate or replace an expensive, time consuming or impractical measurement with an easier, inexpensive measurement. If malt fermentability or its brewhouse performance can be predicted by measuring viscosity, then malt fermentability can be evaluated with ease; whether it be within in a breeding programme or in the malt house. Consequently, the aim of this study was to develop and validate a multivariate calibration model and subsequently predict the malt fermentability of new malt samples (i.e. samples not used during model development) having unknown fermentability.

Materials and Methods

Malted barley samples

The malt of 53 different samples (in total) were used for rheological analysis. The samples consisting of malting barley cultivars, blends or pieces (n=49) were obtained from South African Breweries Maltings (SABM, Caledon, South Africa), while four malt samples were barley breeding lines obtained from collaborators in Australia (Perth and Adelaide) (Table 4.1). Different malt samples were used during calibration and validation of the different multivariate models developed.

Table 4.1 Malt sample information and layout used during model development

Sample information			
Sample name	Sample type[2]	Fermentability[3]	Country of origin
(1)[1]Metcalfe	C	HF	Canada
(2)Metcalfe	C	HF	Canada
(1)Flagship	C	HF	Australia
(2)Flagship	C	HF	Australia
(1)Gairdner	C	HF	Australia
(2)Gairdner	C	HF	Australia
Baudin	C	HF	Australia
SSG 506	C	PF	South Africa
(1)SSG 564	C	PF	South Africa
(2)SSG 564	C	PF	South Africa
(1)SSG 585	C	PF	South Africa
(2)SSG 585	C	PF	South Africa
(1)PUMA	C	PF	South Africa
(2)PUMA	C	PF	South Africa
(1)Canadian malt	C	HF	Canada
(2)Canadian malt	C	HF	Canada
Cocktail: Hartswater	C	unknown[4]	South Africa
Cocktail: Douglas	C	unknown	South Africa
Line A	L	HF	Australia
Line B	L	HF	Australia
Line C	L	HF	Australia
Line D	L	HF	Australia
Voyage	C	HF	Australia
Sebastian	C	unknown	Denmark
Hamelin	C	HF	Australia
Henricke	C	unknown	South Africa
PUMA/Clipper	B	unknown	Clipper: Australia
B29	B	unknown	South Africa
B30	B	unknown	South Africa
B31	B	unknown	South Africa
B32	B	unknown	South Africa
B33	B	unknown	South Africa
B35	B	unknown	South Africa

Table 4.1 CONTINUED

B38	B	unknown	South Africa
B107	P	unknown	South Africa
B115	P	unknown	South Africa
B117	P	unknown	South Africa
B119	P	unknown	South Africa
B121	P	unknown	South Africa
B123	P	unknown	South Africa
B124	P	unknown	South Africa
B125	P	unknown	South Africa
B126	P	unknown	South Africa
B127	P	unknown	South Africa
B132	P	unknown	South Africa
B134	P	unknown	South Africa
B137	P	unknown	South Africa
B139	P	unknown	South Africa
B90:10[5]	B	unknown	-
B80:20[5]	B	unknown	-
B70:30[5]	B	unknown	-
B60:40[5]	B	unknown	-
B50:50[5]	B	unknown	-

[1](1) or (2) = Malt from same cultivar but of different batches,

[2]sample type: C = Cultivar (pure), L = Breeding line, B = Blend (mixture of cultivars), P = Piece (the amount within one malting silo, usually a pure cultivar),

[3]fermentability: HF = High malt fermentability, PF = Poor malt fermentability (brewing problems experienced at local breweries),

[4]samples with unknown malt fermentability used for prediction,

[5]Blends made from different percentages of Metcalfe:SSG 506

Sample preparation

Samples were ground using a mill (Retsch model ZM1, Haan, Germany) fitted with a 0.5 mm sieve size. The malt to water ratio was kept constant at 1:1.5 (14% moisture content). Malt and water weights were determined according to Eq. 1.1-1.3 (Chapter 3; p.60). The blending of malt was simulated by mixing different percentages of Metcalfe with SSG 506 (i.e. 100% Metcalfe: 0% SSG 506, 90:10, 80:20, 70:30, 60:40 and 50:50]

Moisture content determination

The moisture content of the malt samples was determined prior to RVA analysis according to the European Brewery Convention method 4.2 (European Brewery Convention, 1998a) (see detailed methodology in Chapter 3, pg. 60).

Malt analyses

Apparent attenuation limit (AAL)

The fermentability of malt extract in a congress mash (European Brewery Convention, 1998b) was determined by measuring the AAL, when using a consistent yeast source (i.e. dried lager yeast, from quality assured suppliers). Analysis was conducted on 19 different malt samples by Agri-Science Toowoomba, Queensland, Australia using the standard EBC method (European Brewery Convention, 1998c).

Free amino nitrogen (FAN)

The FAN content of wort (European Brewery Convention, 1998d) was analysed by spectrophotometry for 15 different malt samples. FAN analysis were not conducted on the Australian barley breeding lines due to samples size limitations, therefore the number of samples were less in comparison to AAL analysis. These measurements were conducted by Agri-Science Toowoomba, Queensland, Australia.

RVA rheological measurements

A Rapid Visco Analyser (RVA model 3D+, Newport Scientific, Warriewood, Australia) controlled with RVA Thermocline for Windows (version 3.11, Newport Scientific Pty Ltd., Warriewood, Australia) was used to measure the pasting properties of all the malt samples (Table 4.1) (see detailed description of methodology in Chapter 3, p. 61).

Multivariate data analysis

Rheological variables (entire viscogram: 225 data points) were imported into Excel (Microsoft Office Excel 2007). Pre-processing was applied to the data in the form of baseline offset correction. Viscosity data (i.e. X-variables) were imported into The Unscrambler (version 9.2, CAMO, Oslo, Norway). For regression purposes, malt analyses data (AAL and FAN) (i.e. Y-variables) were also imported.

Regression of rheological data and malt analysis (AAL/FAN)

Partial least squares (PLS) regression models were developed using PLS1. The application of test set validation to FAN measurement was seen as impractical due to the small sample set (less samples having known FAN values; n=15). Test set validation is the strictest form of

validation and therefore applied to AAL measurement, which contained slightly more samples with known values (n=19). Therefore different validation methods were used to measure the models' prediction ability. The calibration model for AAL comprised of 13 randomly selected malt samples and were subsequently validated by means of a random test set consisting of 6 malt samples. Calibrated models for FAN were validated by using random cross-validation, containing 5 segments with 3 samples per segment and including uncertainty testing. Inspection of the residual Y variance of validation (see statistical term in Appendix 2, Table 2.1) allowed the selection of the appropriate number of components, indicated by means of the typical "V" (elbow) shape, to obtain optimal model complexity. Adequate models were identified by examining the correlation coefficient (r) (which ideally should be 1) root mean square error of calibration (RMSEC), root mean square error of prediction (RMSEP) (which should be as low as possible), and the bias (which should be close to 0) (see statistical term in Appendix 2, Table 2.1).

Cross-validation allowed the application of uncertainty testing in the form of modified jack-knifing (Martens & Martens, 2000). This enabled the identification of significant variables and score plot stability.

Table 4.2 Samples used for calibration and validation for AAL and FAN

AAL		FAN	
Test set validation	**Sample name**	**Segmented cross-validation**	**Sample name**
Training set (Calibration)	(1)SSG 564[1] (2)SSG 564 (1)SSG 585 (1)PUMA (2)PUMA (1)Flagship (2)Flagship (1)Gairdner (1)Canadian malt (2)Canadian malt Line A Line C Line D	**Segment 1**[2]	SSG 506 (1)Canadian malt Voyage
		Segment 2	(1)SSG 585 (2)Canadian malt (1)Metcalfe
		Segment 3	(1)PUMA (2)PUMA (1)Flagship
Test set (validation)	(1)Metcalfe (2)Metcalfe SSG 506 (2)SSG 585 Line B Voyage	**Segment 4**	(2)SSG 585 (1)SSG 564 (1)Gairdner
		Segment 5	(2)Metcalfe (2)Flagship (2)SSG 564

[1](1) or (2) = Malt from same cultivar but of different batches,

[2]Cross-validation involves dividing the sample set (known Y-values) into different segments where each segment is used as the validation set once. The final calibration model is developed using all objects in the sample set.

Classification

As AAL is considered to be unrepresentative of actual brewing performance, multivariate classification models were based on malt brewing performance as experienced by local brewers. Principal component analysis (PCA) was applied to the viscosity data of all the samples (Table 4.1). The generated scores plot, together with additional fermentability information supplied by SABM (Table 1.1, Appendix 1), allowed the supervised classification of samples according to fermentability degree. SIMCA classification models were developed by creating individual PCA models of each distinct data class. Individual PCA models of each class were validated by dividing the samples into a test and training set, both with known X (viscosity data) and Y-values (fermentability classes) (Table 4.3). A SIMCA model was generated by combining these validated PCA models to predict the fermentability of unknown malt samples based on a 5% significance level.

Table 4.3 Allocated malt fermentability classes, divided into a test and training set

	Malt fermentability classes		
	1: "High malt fermentability"	2: "Intermediate malt fermentability"	3: "Low malt fermentability"
Training set	(1)[1]Metcalfe	Cocktail Douglas	(2)SSG 564
	(1)Flagship	(1)Gairdner	SSG 506
	(2)Flagship	(2)Gairdner	(2)SSG 585
	Line A	(1)PUMA	
	Line C	(1)SSG 585	
	Line D		
	(1)Canadian malt		
	(2)Canadian malt		
Test set	(2)Metcalfe	Cocktail Hartswater	(1)SSG 564
	Baudin	(2)PUMA	
	Line B		

[1](1) or (2) = Malt from same cultivar but of different batches

Results

Malt analyses

Most AAL and FAN values obtained (Table 4.4) were within a reported range of barley specifications for malting purposes: 78-86% AAL and 140-180 mg.L^{-1} for FAN (EBC analyses) (Fox *et al.*, 2003). The samples (2)PUMA, (1)SSG 585 and (1)SSG 564 had low fermentability values compared to the average measurement (81.6%). Gairdner, Voyage and (1)SSG 564 had low FAN values, compared to the average measurement (147.8 mg.L^{-1}) and could possibly present brewing problems. Malt from the four different barley breeding lines (A,B,C and D) along with Metcalfe, Flagship, Gairdner and Voyage delivered high AAL values. Unexpectedly, PUMA (batch 1), SSG 506 (batch 1) and SSG 564 (batch 2) delivered high AAL values accompanied with adequate FAN levels. SSG 564(1) displayed a low FAN value, while Voyage had an extremely low value.

Table 4.4 Measurement of apparent attenuation limit and free amino nitrogen

Cultivar: batch	**Average**	
	AAL (%)	**FAN (mg.L^{-1})**
(1)[1]Metcalfe	82.3	149.2
(2)Metcalfe	81.2	151.4
Flagship	82.3	142.1
(a)Canadian Malt	82.2	145.7
(b)Canadian Malt	82.1	155.4
(2)Gairdner	81.7	137.3
Voyage	81.5	110.4
(1)Puma	80.4	168.7
(2)Puma	77.6	162.6
(1)SSG 585	76.4	166.0
(2)SSG 585	75.1	159.6
(1)SSG 506	82.5	143.6
(1)SSG 564	77.9	126.1
(2)SSG 564	81.7	151.4
Line A	87.3	-
Line B	86.1	-
Line C	85.2	-
Line D	85.2	-
Minimum	**75.1**	**110.4**
Average	**81.6**	**147.8**
Maximum	**87.3**	**168.7**

[1](1) or (2) = Malt from same cultivar but of different batches

Multivariate data analysis: model development

Regression of rheological data and malt analysis (AAL)

The loading line plot of component 1 resembled the basic structure of a RVA viscogram, while the loading line plot of component 2 resembled the first derivative of a typical RVA viscogram (Fig. 4.1). Differences in malt fermentability were observed in the direction described by both components, indicating both variance in peak height and peak formation time allowed discrimination. The PLS loading line plot (Fig. 4.1) were similar to previous results (Chapter 3, Fig. 3.13 pg. 75) which considered PCA loading line.

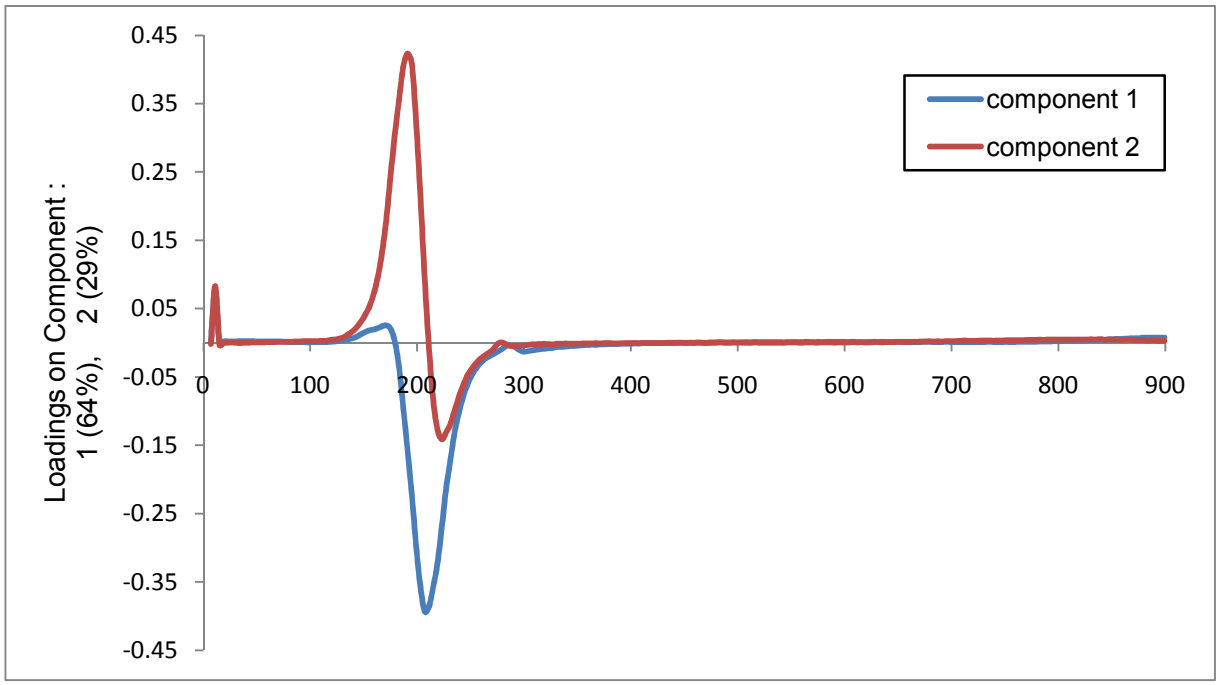

Figure 4.1 Loading line plots of the first two PLS components.

The first PLS component described 64% of the variation experienced within the X-data, which explained 56% of the Y-data. The second component described 29% of X-variation, which explained only 5% of Y-variation. In the scores plot, it was interesting to notice similar cultivars but of different batches differed according to their placement in the component space (Fig. 4.2). This was also clearly seen from the malt viscograms (Fig. 4.3) especially considering peak formation with regards to height (SSG 585) and analysis time (SSG 564).

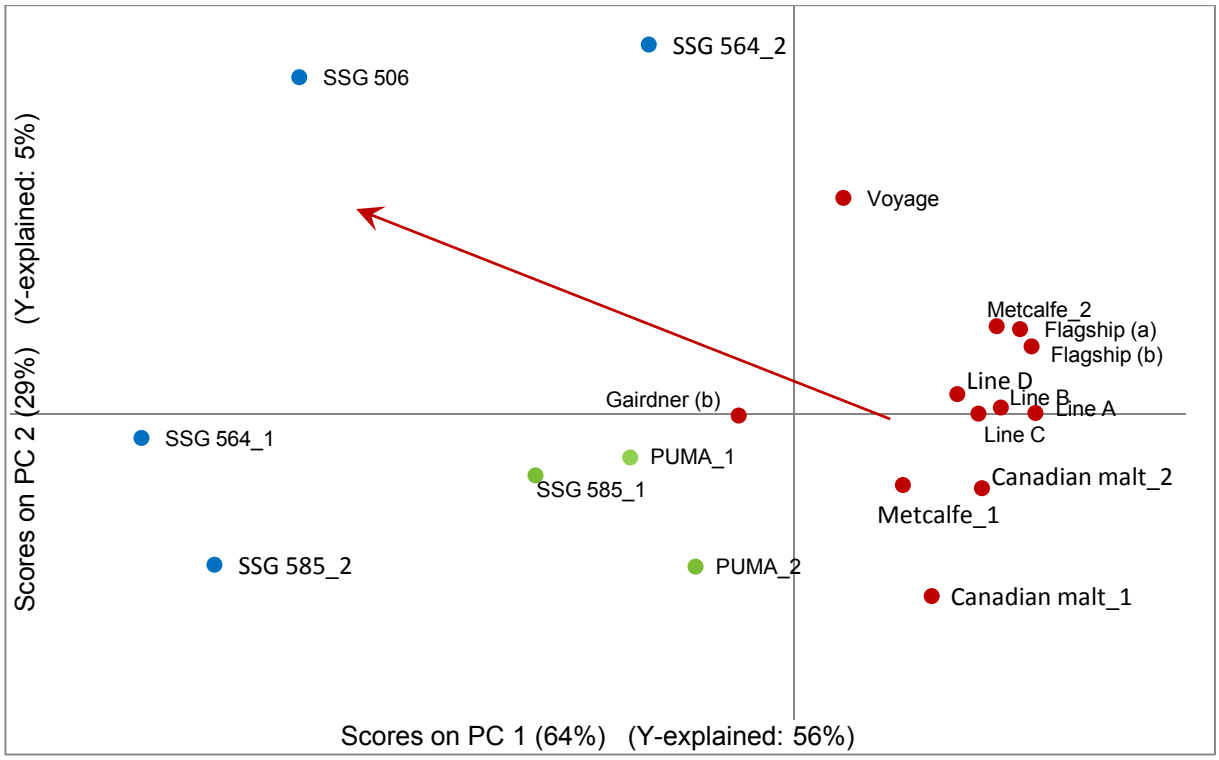

Figure 4.2 Scores plot of the first two PLS components, illustrating the samples.

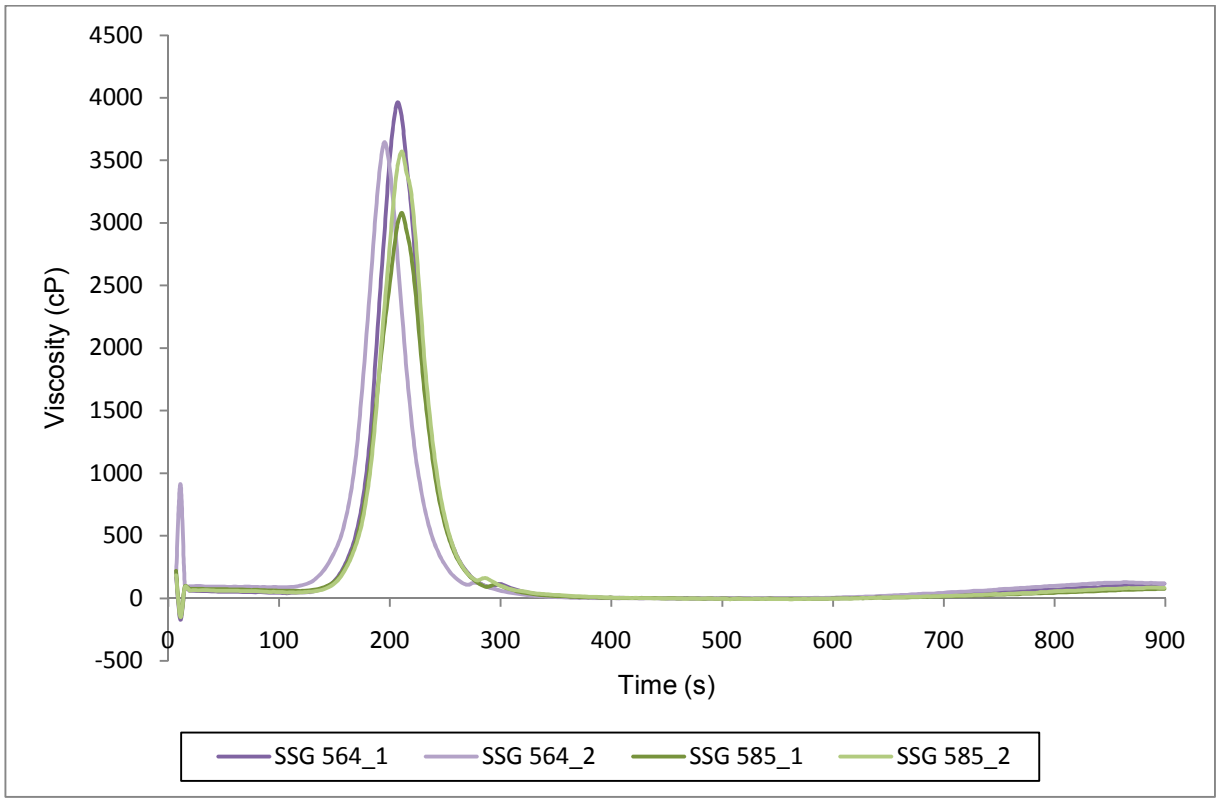

Figure 4.3 Viscograms illustrating different batches of the same malting barley cultivar.

As more components were added, calibration residual Y-variance (see statistical term in Appendix 2, Table 2.1), thus modelling error, typically decreased (Fig. 4.4a).

From the validation residual Y-variance six components were identified as the optimal dimensionality (Fig. 4.4b). This could be identified as the lowest residual Y-variance before an increase was experienced.

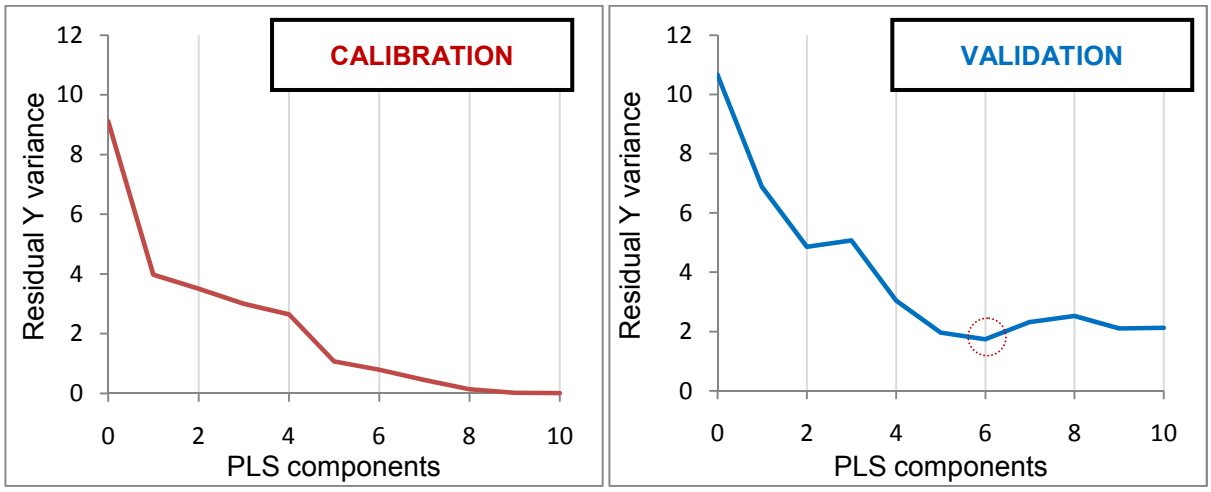

Figure 4.4 The residual Y variance plots of (a) calibration and (b) validation indicated the modelling and prediction error in Y, respectively (circle indicating optimal components).

The correlation coefficient (r) associated with both calibration and validation was high, 0.96 and 0.92, respectively (Fig. 4.5 & Fig. 4.6). For the calibration, the bias was close to zero, but increased for validation (Bias = -0.2570). The RMSEC (see statistical term in Appendix 2, Table 2.1) was measured just below 1% which was not unreasonably high, as the AAL analysis had a repeatability of 1% and a reproducibility of 3%. As expected the SEP was higher than the SEC (see statistical terms in Appendix 2, Table 2.1). The RPD (see statistical term in Appendix 2, Table 2.1) was equivalent to 2.523 (see calculation of standard deviation in Appendix 2, Table 2.2).

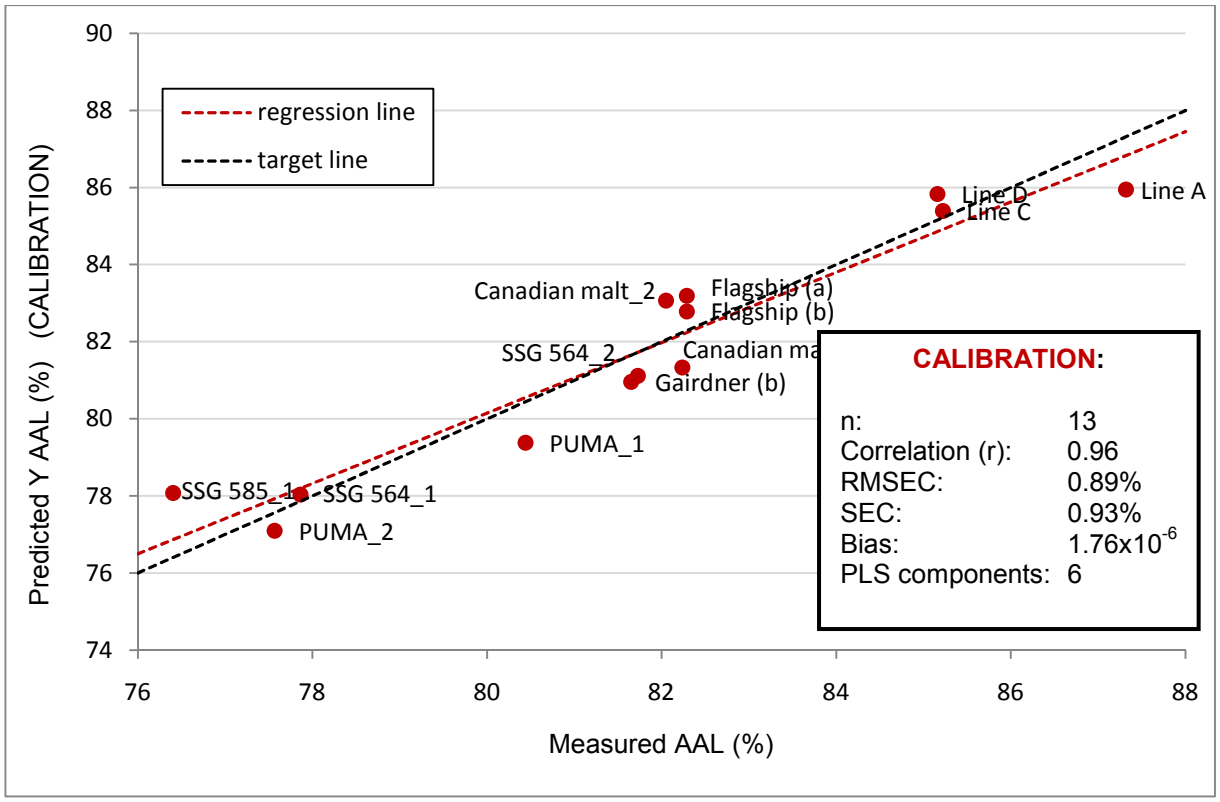

Figure 4.5 Predicted versus measured percentage of apparent attenuation limit (calibration).

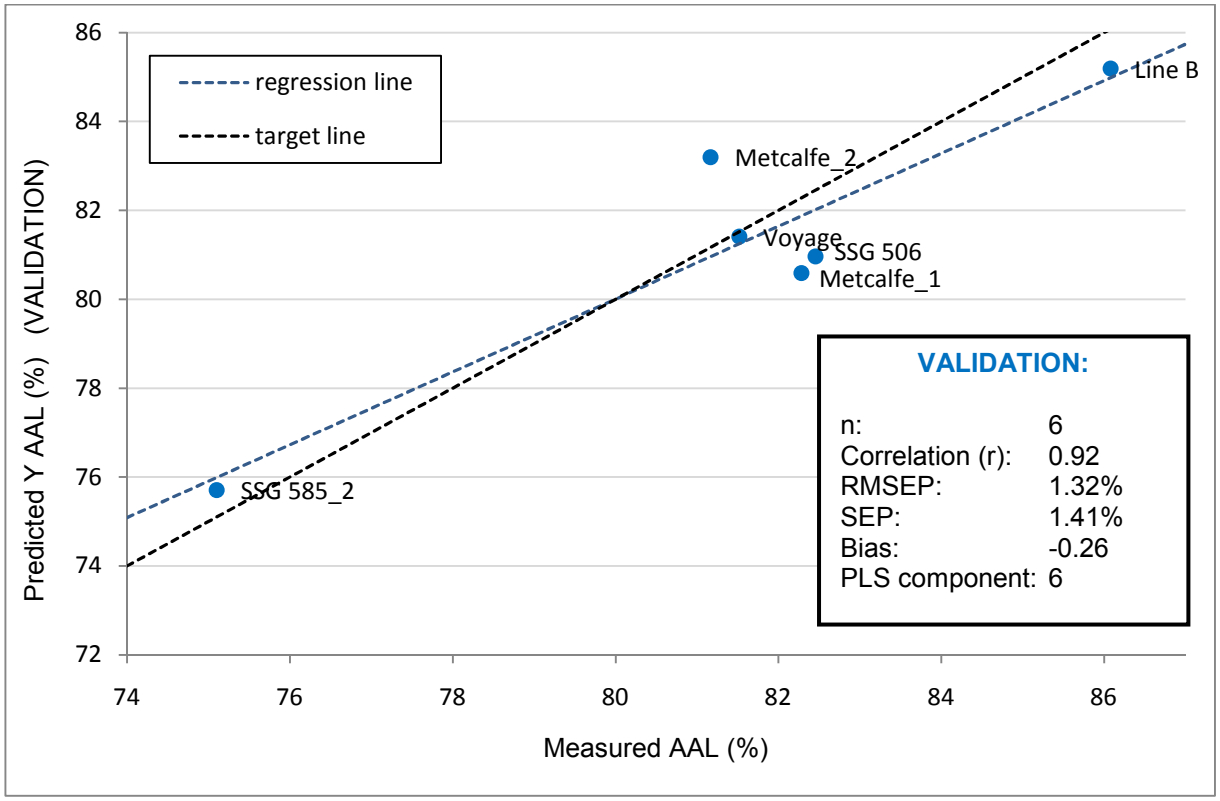

Figure 4.6 Predicted versus measured percentage of apparent attenuation limit (validation).

Regression of rheological data and malt analysis (FAN)

For the FAN models, a less rigorous validation method was used, leading to more optimistic results in comparison to previous test set validation. Sample instability was observed in the scores plot, whereby a given sample (such as SSG 506) was far away from the rest of the data swarm (Fig. 4.7).

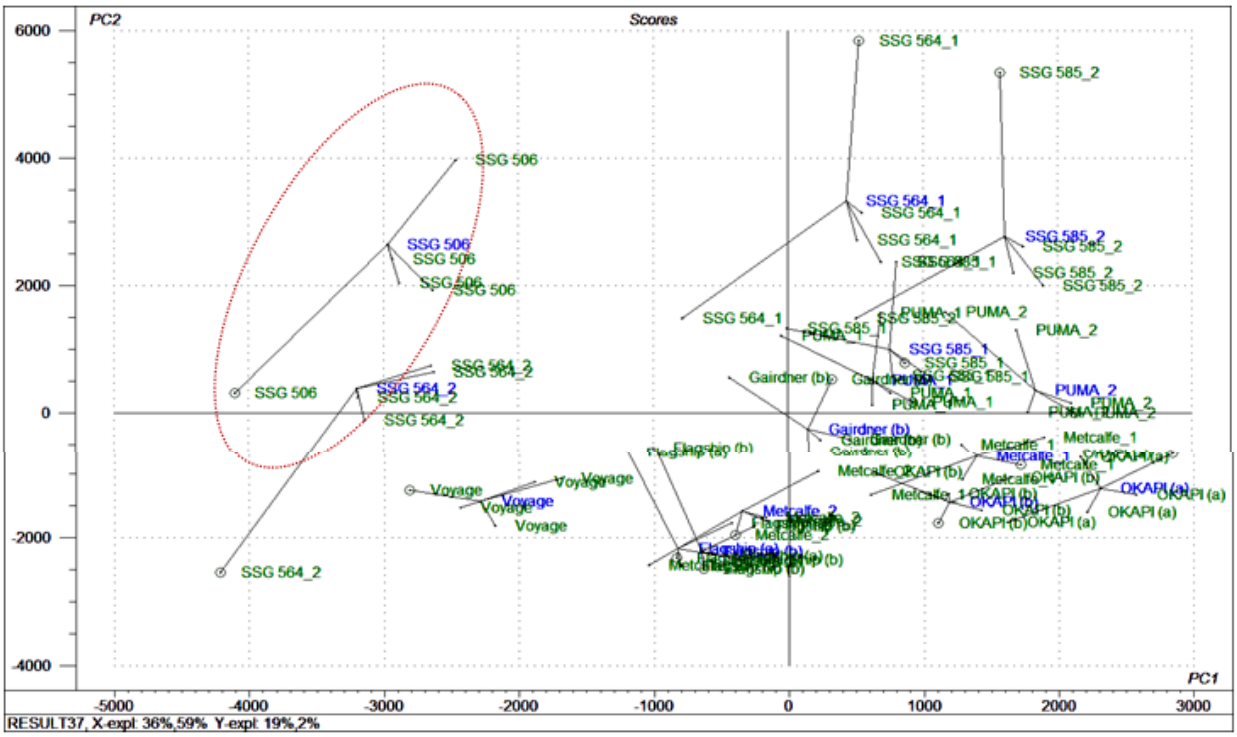

Figure 4.7 Stability plot illustrating instability of a given sample (such as SSG 506 indicated by circle) by being far away from the rest of the data swarm.

When comparing the residual Y variance of calibration to validation, a large difference was observed (Fig. 4.8). Residual Y variance of validation drastically increased after the first component and was still fairly high after using 5 components.

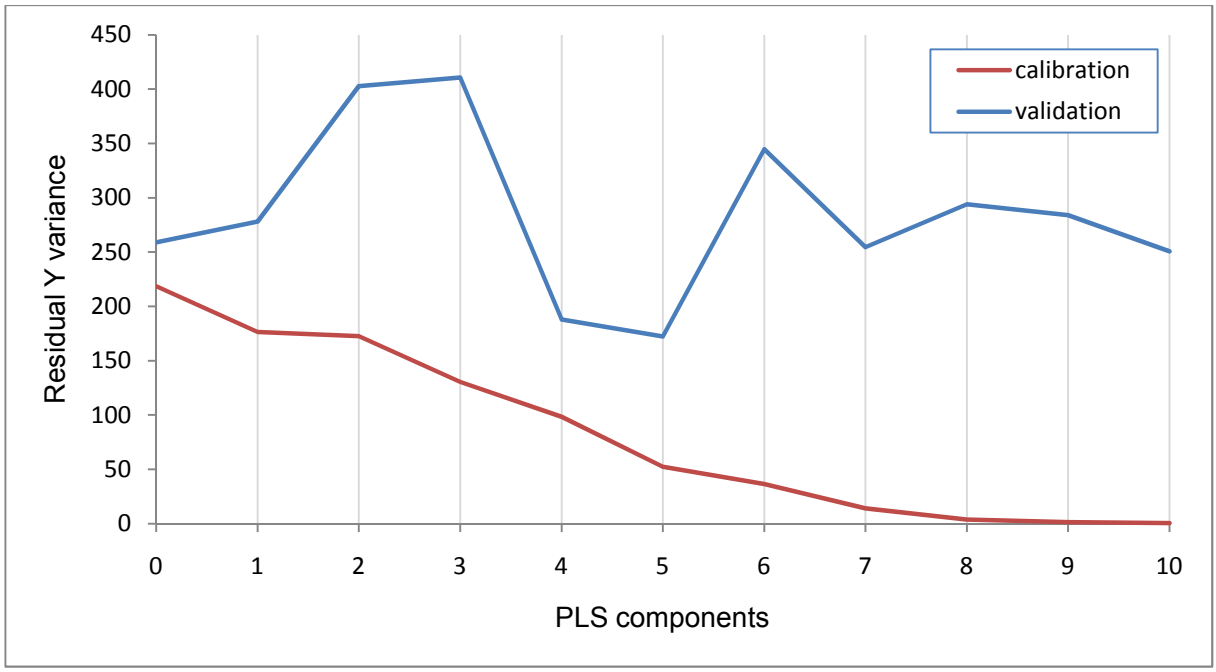

Figure 4.8 The residual Y variance plots of calibration and validation indicated the modelling and prediction error in Y, respectively.

The calibrated model displayed a high correlation coefficient (r=0.87), a RMSEC of 7.24, accompanied with a low bias (-1.27×10^{-5}) (Table 4.5). Therefore, the model seemed to be well fitted (Fig. 4.9). In contrast to calibration, validation indicated the model's poor performance to predict new Y-values (Table 4.5). The validation coefficient of determination was very low (i.e. R^2=0.346) indicating a poor correlation. The RMSEP was extremely high (13.12 $mg.L^{-1}$), in contrast to the very low RPD (1.170) and fairly low bias (1.43) (see calculation of standard deviation in Appendix 2, Table 2.2).

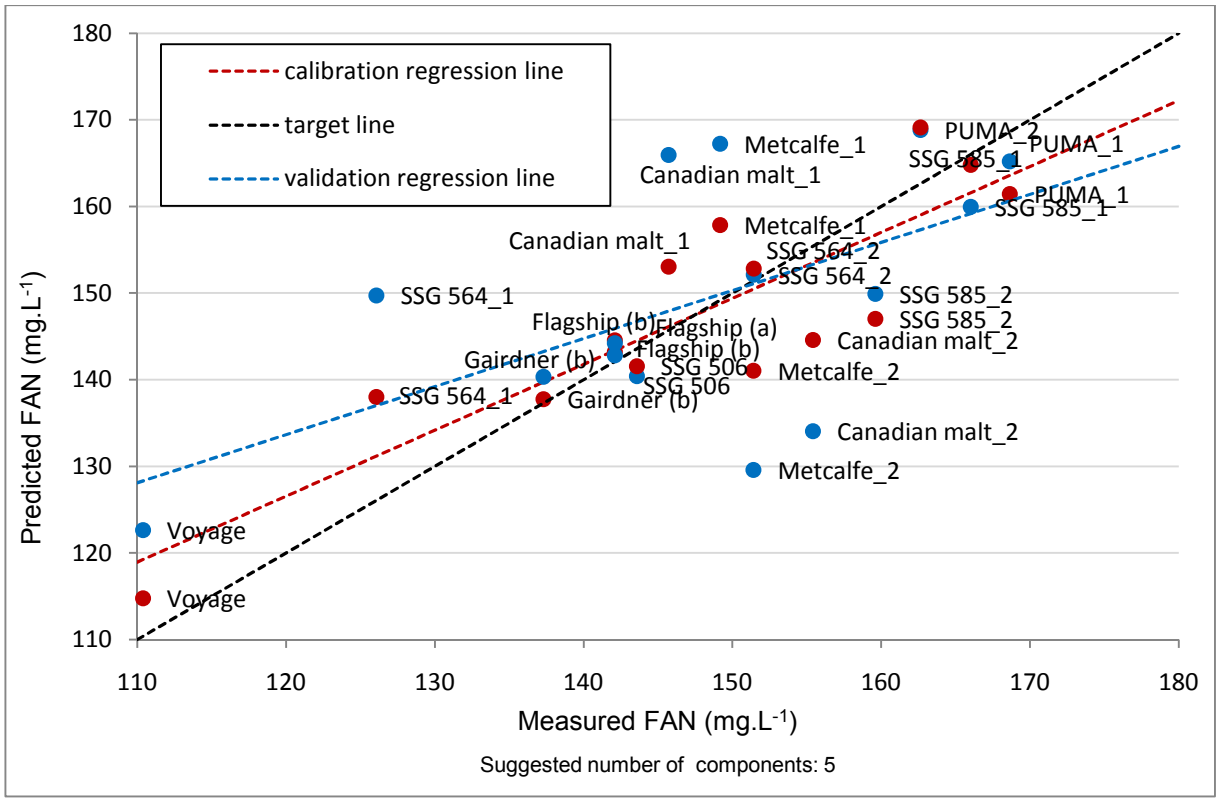

Figure 4.9 Predicted versus measured free amino nitrogen content (calibration indicated by red, validation indicated by blue).

Table 4.5 Predicted versus measured plot statistics

	Calibration	**Validation**
N	15	15
Correlation (r)	0.87	0.59
RMSEC/P[a]	7.24 mg.L^{-1}	13.12 mg.L^{-1}
SEC/P[b]	7.48 mg.L^{-1}	13.50 mg.L^{-1}
Bias	-1.27x10^{-5}	1.43

a: RMSEC for calibration; RMSEP for validation
b: SEC for calibration; SEP for validation

Classification: PCA plots

Principal component analysis (PCA) was performed first in order to identify outliers, non linearities or any other abnormal behaviour. Henricke was consequently removed from the PCA model as this sample displayed a high residual variance accompanied with a high leverage in the influence plot (data not included).

From the score plot and fermentability information supplied, three different clusters could be identified based on a fermentability degree (Fig. 4.10). This allowed the allocation of classes, of which class 1 represented the “high malt fermentability”; class 2 the “intermediate malt fermentability”; and class 3 the “low malt fermentability” (Table 4.3, p.96).

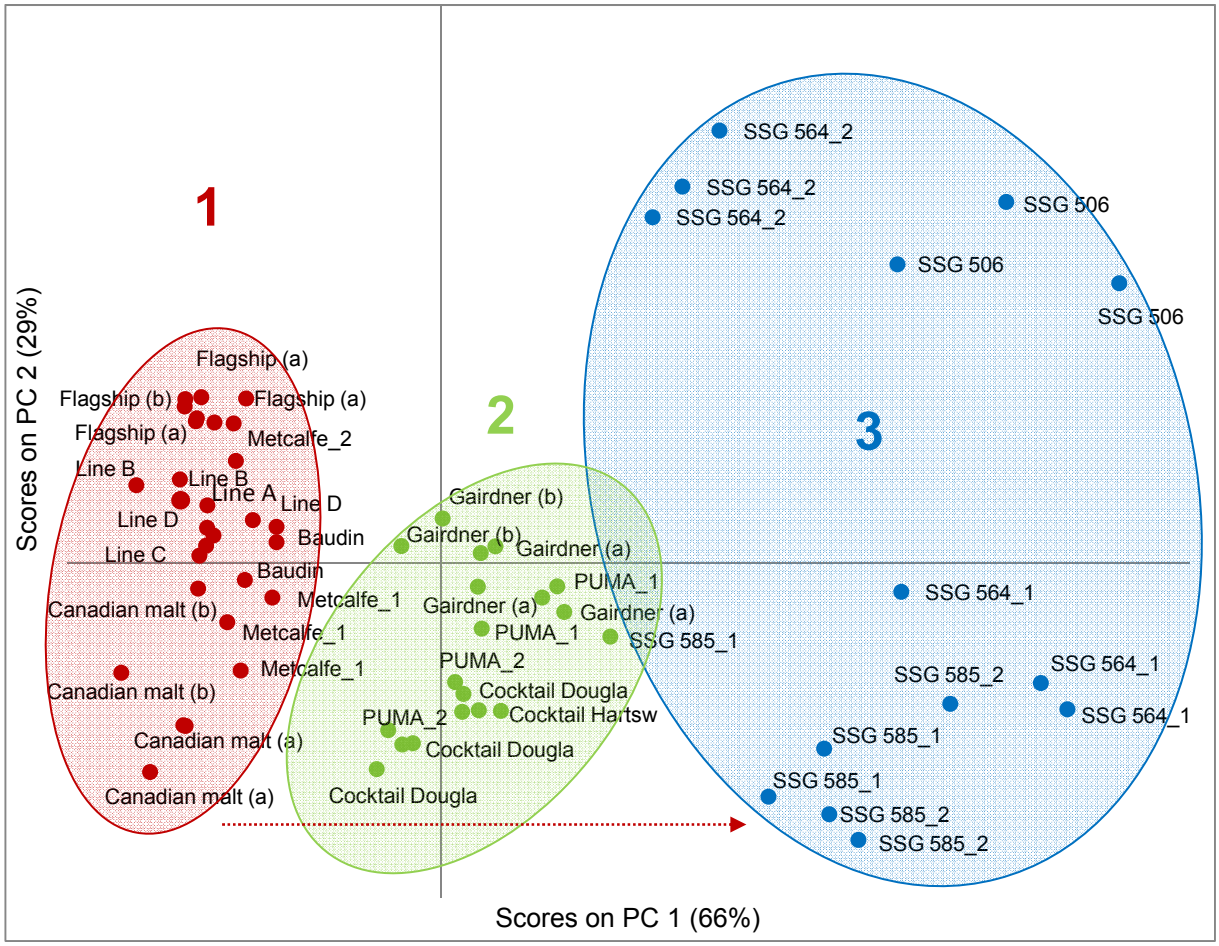

Figure 4.10 PCA scores plot (PC1 vs. PC2) illustrating different malt fermentability groupings (decrease in fermentability indicated by arrow).

Classification: SIMCA

Three different PCA models were developed based on the three different malt fermentability classes, while incorporating all the sample replications. Faulty replicates were identified as outliers by means of the score and influence plot. Viscograms of replicate outliers were examined and removed if measurements proved to be faulty (Fig. 4.11). The optimum number of components for each class were identified. Considering the first malt fermentability class, i.e. “High malt fermentability”, 95.82% of the X variance was described by 6 components (data not shown). For the second malt fermentability class, i.e. “Intermediate malt fermentability”, 93.75% of the X variance was described by 4 components, while 96.75% of the X variance was described in total by 2 components for the third malt fermentability class, i.e. “low malt fermentability”. The PCA models were used to classify new unknown samples by means of SIMCA classification. Test set samples correctly classified were 83%. All three models were proved to be significantly different ($P<0.05$) from one another as indicated by the model distance plot.

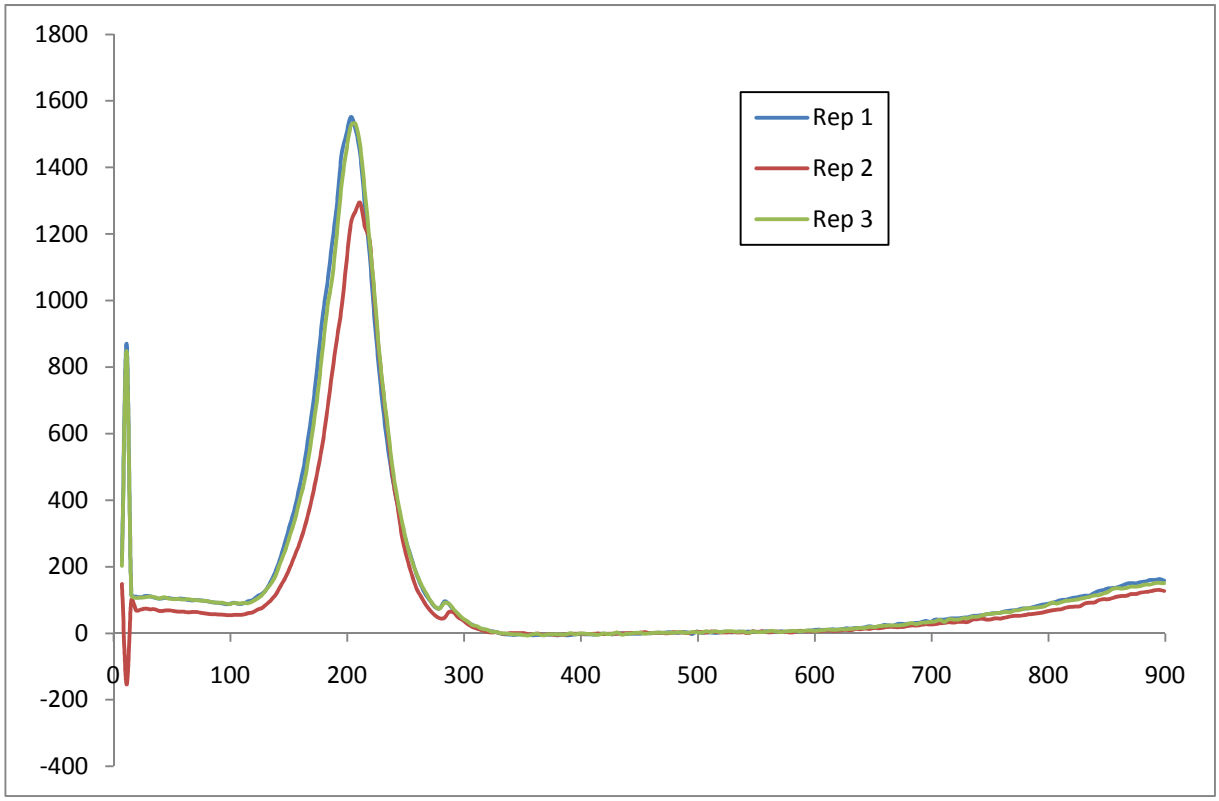

Figure 4.11 Viscograms of the three replications of the sample '(2)Canadian malt' showing the incorrect measurement.

Prediction of unknown cultivars

Of all the cultivars having unknown malt fermentability, only Hamelin and one replicate of Voyage were assigned to a fermentability class. These samples were allocated to group 3, therefore predicted to have a low malt fermentability. The other unknown cultivar samples (i.e. Sebastian, Henrick and PUMA/Clipper) did not fit into any class within the given limits.

Prediction of malt industry blends

The malt fermentability of 21 local malt blends, were predicted (Table 4.6). All three replicates of blend 107 were classified as fermentability group 2 and therefore predicted to have an intermediate malt fermentability. One or two of the replicates of blends 29, 123, 125 and 127 were classified as group 2, while the other replicates of the same samples were predicted to belong to group 3. All of the other blended samples were allocated to group 3 and therefore predicted to have a low malt fermentability.

Table 4.6 Classification of malt industry blends (B) to different malt fermentability classes

Group1	Group2	Group3	Not classified
-	B29 (2)[a]	B29 (1,3)	B30 (3)
	B107 (1,2,3)	B30 (1,2)	B35 (2)
	B123 (3)	B31 (1,2,3)	B38 (2,3)
	B125 (3)	B32 (1,2,3)	B115 (1)
	B127 (1,2)	B33 (1,2,3)	B124 (1)
		B35 (1,3)	B125 (1)
		B38 (1)	B127 (3)
		B115 (2,3)	B132 (1)
		B117 (1,2,3)	B134 (1,2,3)
		B119 (1,2,3)	B139 (1,2,3)
		B121 (1,2,3)	
		B123 (1,2)	
		B124 (2,3)	
		B125 (2)	
		B126 (1,2,3)	
		B132 (2,3)	
		B137 (1,2,3)	

[a] Indicating in brackets the different replications

Prediction of simulated blends

Considering the predicted low fermentability degree of SABM blends and the lack of constituents information (thus what could be expected from those samples in terms of fermentability) it was decided to simulate malt blends by mixing different proportions of a high malt fermentability (Metcalfe) with an low fermentability malt (SSG 506). The Metcalfe:SSG 506 blends of 90:10 and 80:20 were predicted to have a high malt fermentability, while 70:30, 60:40 and 50:50 were more similar to group 2 (intermediate malt fermentability) (Table 4.7). One replicate of the blend 80:20 (Metcalfe:SSG 506) was predicted to belong to group 2.

Table 4.7 Classification of simulated blends to different malt fermentability classes

Group1	Group2	Group3	Not classified
Blend 90:10 (1,2,3)[a]	Blend 80:20 (1)	-	Blend 70:30 (3)
Blend 80:20 (2,3)	Blend 70:30 (1,2)		Blend 60:40 (2,3)
	Blend 60:40 (1)		
	Blend 50:30 (1,2,3)		

[a] Indicating in brackets the different Breplications

Discussion

Malt analysis

Different malting barley cultivars are expected to deliver malt which differs in fermentability (AAL values), due to the environmental effect on barley during cultivation, but also the malting process itself. Metcalfe, Flagship, Gairdner and Voyage were expected to deliver high AAL values, as these cultivars are known and marketed as having high malt fermentability. The unexpectedly high AAL values obtained from PUMA (batch 1), SSG 506 (batch 1) and SSG 564 (batch 2) suggested these cultivars could convert a large amount of starch extract to alcohol. Malt samples containing high AAL percentages, but accompanied with a very low FAN content (i.e. Voyage) could still present brewing problems compared to a cultivar having similar AAL, but higher FAN [i.e. (2)Gairdner]. As the low level of nitrogen nutrient can inhibit yeast metabolism and therefore restrict fermentation (Briggs *et al*., 2004). The previous cultivars [i.e (1)PUMA, (1)SSG 506, (2)SSG 564] displayed adequate levels of FAN and therefore indicated yeast fermentation would not be restricted due to a nitrogen nutrition deficiency. These results contradicted brewhouse findings, as PUMA, SSG 506 and SSG 564 are known to deliver fermentation problems during adjunct brewing (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). This indicated the inadequacy of malt analysis (such as AAL and FAN) to accurately predict the brewhouse fermentability or brewing performance of malt and confirms literature findings (Phaweni *et al.*, 1992; Bamforth, 1999; Calman *et al.*, 2008). Low FAN values obtained from SSG 564 and Voyage indicated these samples could possibly produce brewing problems, such as reduced alcohol yield when used in conjunction with a large amount of adjunct (Briggs *et al.*, 2004). Typical high-gravity lager wort with a specific gravity of 1.060 contains approximately 150 g/l fermentable sugar and 150 $mg.L^{-1}$ free amino nitrogen (Briggs *et al.*, 2004).

Multivariate data analysis: model development

Regression of rheological data and malt analysis (AAL)

Different batches of a specific malting barley cultivar implied a difference in barley cultivation. Environmental conditions have been shown to influenced the RVA pasting curves of malting barley cultivars (Dunn *et al.*, 1997). Significant environmental trends have been found for peak viscosity and trough viscosity of isolated barley starch, while genotypic trends were observed for peak time (Dunn *et al.*, 1997).

Test set validation is usually used to assist with the selection of the number of components to avoid model over-fit or under-fit. Over-fitting implies the addition of too many components and therefore modelling noise, while under-fitting implicates the use of too few components, therefore excluding vital information (Esbensen, 2002). A large number of PLS components were needed to minimise the prediction error. This was expected since AAL is a complex characteristic. Thus specific gravity is not necessarily portrayed in a RVA viscogram (given by component 1 & 2, Fig. 4.1, p.98), and subsequently needs a larger number of components to be explained.

The high correlation coefficient obtained in both calibration and validation indicated a large percentage of the variance in X could be accounted for by the variance in Y (Williams, 2001). The low calibration bias indicated there was little to no systematic difference between the average predicted and measured values of the training set and the validation set (Esbensen, 2002). The SEP was smaller than twice the SEC, the contrary could have indicated a high degree of over-fitting or an erroneous validation sample. Due to the low bias value, SEC/P was almost equal to RMSEC/P, respectively, indicating a reasonable precision.

The use of test set validation, the strictest form of validation, indicated the calculated RPD is not over optimistic. The RPD value obtained (2.523) was fairly low, but according to Williams (2001), this is good enough for rough screening purposes. This suggests further model development with a greater number of samples is warranted.

Due to constraints on sample size and diversity, the calibration model developed consisted only of 13 samples, while it was validated by merely six samples. The accuracy can therefore be questioned, as the model is expected to change when expanding it with more known samples. Laboratory measured AAL do not represent experience brewhouse fermentability during actual high adjunct brewing and therefore should not be used as a valid Y-reference method to predicting future malt fermentability. Actual brewhouse AAL values or any other additional information able to explain fermentability differences between SSG 506, 564, 585, PUMA and Metcalfe should be used as actual reference method. However, the current multivariate regression model developed indicated the potential of rheological analysis to contain fermentability information.

Regression of rheological data and malt analysis (FAN)

Uncertainty testing indicated the FAN model was unstable. Sample instability can be observed as a given sample, such as SSG 506, being far away from the rest of the data swarm. This indicated the sub-model without the sample was very different from the other sub-models, meaning the particular sample influenced all other sub-models due to its uniqueness (Esbensen, 2002). The scores plot indicated that samples removed within a particular cross-validation segment, significantly affected the common model. Martens' uncertainty testing allowed the identification of significant rheological (X) variables which describes Y (data not included). Variables representing the initial viscosity, along with a variable close to the inflection point upon viscosity increase, were identified as significant in the model describing FAN (Y). These variables seemed suspicious as the initial viscosity measurement was highly dependent on baseline determination and therefore could not be the only significant X variables describing Y.

Ideally, a simple model should be obtained where the residual variance decreases to zero within the least number of components possible (Esbensen, 2002). An initial increase in the residual Y variance is unacceptable in PLS and usually indicates the presence of an outlier. Residual Y variance of validation was still fairly high after 5 components indicating that variation in Y was poorly described by the X variables.

This indicated that although the calibration data were well fitted (displaying a high correlation coefficient, small residual calibration variance, RMSEC and bias), the model was unable to accurately describe new data (displaying a large residual validation variance, RMSEP and small coefficient of determination). The RPD was 1.17 which is classified as being very poor and not recommended for application, especially in a breeding programme (Williams, 2001).

Results of future predictions can be presented as "predicted values ± 2•RMSEP" if new samples were similar to those used in calibration (Esbensen, 2002). This gave a confidence interval of 26 $mg.L^{-1}$ in FAN values of newly predicted samples which were unacceptable. As mentioned previously, the model was validated by means of cross validation which is a "less strict" method of validation giving more optimistic results. This indicted rheological measurement, as used in this study, cannot explain the variation experienced in the free amino nitrogen content of malt samples as these small nitrogenous compounds are not expected to cause rheological changes.

PCA plots evaluation

Henricke was identified as an outlier from the influence plot and scores plot (data not shown). Investigation of this sample revealed it was heavily infested with weevils. These insects thrive on malt by consuming the modified starchy endosperm, leaving a larger percentage of

husk. The sample Henricke was therefore expected to deliver anomalous rheological results. A number of factors could have attributed to faulty viscosity measurement of a replication. It was mostly likely thought to be due to incorrect measurement of malt and water samples weights, or poor sample mixing (malt with water) before RVA analysis.

Classification: SIMCA

Faulty measurements of replicates were thought to be due to inaccurate mixing of malt with water, or a small amount of malt spilled before adding water, essentially giving a lower malt concentration. Samples used during test set validation of the individual PCA models were kept out of the final model. Predicting the fermentability of test set samples allowed the calculation of the percentage of correct class allocations and indicated model performance. Of the test set samples 83% were correctly classified.

Prediction of unknown cultivars

Samples belonging to a particular group, such as Hamelin which were predicted to belong to group 3, indicated the distance to the next closest class was larger than the accepted distance with respect to group 3 (Esbensen, 2002). Samples predicted to belong to none of the groups most probably belonged to a “new” unknown class which was not used during model development, or alternatively they may have been outliers (Esbensen, 2002). A single replication of Voyage was allocated to Group 3. This replicate displayed an unusually high peak height compared to the other Voyage replicates. This could have resulted in the false positive allocation of the particular replication to Group 3 (i.e. falsely predicted to belong to Group 3 when in reality it did not belong to any grouping), while the other replications were not classified.

Prediction of malt industry blends

Replicates of the same malt samples, which were allocated to different fermentability classes were either predicted to belong to different classes (i.e. replicates differed rheologically) or a single sample was predicted to belong to either of two closely related classes (i.e. replicates did not necessarily differ to a great extent from one another, but rather classes were closely related). Prediction results could not be verified in terms of malt fermentability degree as the precise constituents of the blends, along with their respective fermentabilities (laboratory measured AAL or actual brewhouse performance), were unknown. If predictions are correct this could implicate problems for local breweries if these blends are used during adjunct brewing, as they were predicted to deliver fermentation problems similar to PUMA and SSG 506.

Prediction of simulated blends

Results indicated the possibility that a highly fermentable malt such as Metcalfe could be substituted up to 20% with a low malt fermentability such as SSG 506. Differences between replicates in the 80:20 blend indicated a small error made during sample blending caused a detectable rheological deviation. This could also have indicated the 80:20 blend was near the specification limits of group 1.

Conclusion

Malt fermentability is a complex characteristic and proved throughout literature to be a difficult characteristic to describe by means of malt analysis such as AAL, FAN, malt extract and diastatic power. AAL showed a strong correlation with RVA rheological analysis. AAL is strongly linked to sugar concentration which is a result of starch degradation, the principal factor being monitored during RVA analysis. Regression models built were based on only a small sample set. For improved calibrations, the current sample set must be extended and contain a wider range of fermentability (AAL) values.

Rheological analysis contained little to no relevant information with regards to FAN values. This was expected as FAN is dependent on the amino acid and peptide content of malt. These substances vary between samples in miniscule amounts and cannot be expected to cause great viscosity changes. It is also thought fermentability is only affected once the FAN levels are below a certain concentration. Thus increasing the FAN content does not increase fermentability. Rheological and FAN measurements therefore cannot be expected to correlate above such a concentration.

Environmental changes had an evident effect on the rheological properties of malting barley cultivars. Further, stressing the need for routine malt fermentability evaluation, regardless of the particular cultivar.

SABM blends were all predicted by SIMCA classification models to have intermediate to low malt fermentability. This stresses the alarming possibility that locally developed malting barley cultivars, believed to be of high malt fermentability, might present fermentation problems during adjunct brewing. Of great economic importance, highly fermentable malts, such as Metcalfe, could possibly be blended with malt of low fermentability by up to 20% (SSG 506). In practice, highly fermentable malt used during the brewing process is always blended to a certain specification (dependent on a beer profile) with a malt being less fermentable (i.e. standard larger malt). Perhaps for future studies it would prove to be more useful to incorporate such blends in the sample set, if the overall brewing performance of the blend is known, as well as the fermentability/performance of individual cultivar constituents.

Once again, it must be stressed that developed models display great potential for future application, but conclusions drawn and practical implementation require further work. It is suggested to expand the current sample set, accompanied with detailed sample information with regards to malt fermentability or actual brewing performance within the brewhouse (malt samples subjected to the particular beer profile in question). Since rheological analysis is influenced by various factors and gives a general overview of interactions, it is perhaps ideal for the measurement of a complex characteristic, such as malt fermentability. Rheological analysis, by means of the RVA should be considered a noteworthy measurement to predict malt fermentability.

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Chapter 5

Effect of different analysis conditions on Rapid Visco Analyser malt viscograms

Chapter 5
Effect of different analysis conditions on Rapid Visco Analyser malt viscograms

Abstract

Viscosity measurements of malt samples were made using different RVA analysis conditions (i.e. instrument model; brewhouse time/temperature profile; heating/cooling rate and enzyme activity) of the Rapid Visco Analyser. This study was conducted to investigate the rheological effect when varying these operational conditions. Results can provide additional sample information (physical and biochemical condition) or possibly indicated better sample discrimination with regards to malt fermentability. Conditions were varied on individual basis while the rheological response was measured for malt samples differing in fermentability degree and/or particle size distribution. Rheological measurement using the RVA 3D+ gave similar results compared to the RVA-4, indicating adequate sensitivity of the former instrument for discriminatory purposes. Using the time/temperature profile of a commercial brewery mashing process for rheological analysis was rejected. This adjustment caused a substantial increase in analysis time, increased rheological noise and caused a reduced peak height, which consequently diminished the degree of fermentability discrimination. Inactivating malt enzymes during RVA analysis allowed the assessment of barley viscograms and therefore the comparison of starch, which had the largest influence on rheology. However, this eliminated the ability to discriminate on the basis of malt fermentability. Increasing or decreasing the heating rate influenced the time available for enzyme action before thermal inactivation. This affected the degree of malt fermentability discrimination and gave further insight to physical and biochemical processes (hydration, gelatinisation and enzyme hydrolysis) affected by differences in sample particle size distribution. The amount of starch damage caused by milling a malt sample was unaffected by the sieve size used. However, additional damage occurred after repeated sample milling with a centrifugal mill. A pH increase of 0.1 was observed during RVA analysis. As the measured pH was within the range normally observed during mashing, adjustment was unnecessary. It is of great benefit to use a single, inexpensive, routine analysis to measure the effect of various factors. In this study, calculated variation of conditions delivered insightful information which is of real use for future RVA research exploration.

Introduction

The Rapid Visco Analyser (RVA) offers great versatility compared to other rotational viscometers. It requires a small sample size, to which additives can easily be added; it has the ability to operate at different time and temperature profiles, allowing shorter test runs; it

contains variable speed control; and allows electronic output and manipulation of data. The RVA can be considered an ideal instrument within a grain laboratory as it allows the investigation of different operating conditions. Evaluating the obtained results (viscograms); the RVA can be optimised for any given rheological characteristic of interest. Viscosity measurement is influenced by numerous interacting variables, and therefore can be considered a macroscopic view of material properties. Minor adjustment to analysis conditions cause large changes to recorded rheological measurements. With careful alteration of experimental conditions a useful latent phenomenon can be identified through additional testing.

In RVA analysis, it has been suggested to simulate the commercial processing conditions, which a sample undergoes (with regards to brewhouse: pH, malt:water ratio and time/temperature profile) (Mrs B Elliott, Newport Scientific, Australia, personal communication, 2010). **Simulating the brewery mashing process** during RVA analysis (in a time and temperature manner) allows the ability to follow enzyme degradation processes (Goode *et al.*, 2005a). Studies have been conducted to simulate a commercial mashing process of which the time and temperature profile resembled that of an upward infusion mashing programme (Goode *et al.*, 2005c; Goode & Arendt, 2006). Goode *et al.* (2005b) indicated correlations between the level of grain modification and certain rheological data points. The RVA has been used to detect major viscosity changes, due to starch gelatinisation and liquefaction processes, and minor viscosity changes, such as proteolytic and saccharification activity (Goode *et al.*, 2005b; Goode *et al.*, 2005c; Goode & Arendt, 2006).

Enzyme inhibition causes malt viscograms to resemble the rheological profile of barley (Glennie Holmes, 1995a). It can be reasoned that starch has the largest rheological influence on a barley viscogram, however the extent of physical damage, the presence of nonstarch polysaccharides, phytochemicals (i.e. tannins) and the gelatinisation temperature can also play a role (Woodward & Fincher, 1983; Morrison & Tester, 1994; Zhu et al., 2008). In this regard, enzyme inhibition is therefore a practical alternative to rheological investigation of malt starch (Glennie Holmes, 1995a). Silver nitrate is considered the most effective α-amylase inhibitor and is commonly used during RVA analysis to inactivate malt enzymes. Silver nitrate has a constant effect above 0.1 $mM.g^{-1}$ (Meredith, 1970; Glennie Holmes, 1995a), while the addition of 0.1 M silver nitrate solution (25 g) to 4 g of barley or malt, is generally used during RVA analysis (Glennie Holmes, 1995a; Zhou & Mendham, 2005; Zhou *et al.*, 2008). Rheological differences based on starch content could give further insight to the effects of milling and the influence, if any, on malt fermentability.

Viscosity is recorded as a function of **time and temperature**. The curve generated has been described as: “a reflection of the structural changes of the granules that occur during

starch gelatinisation and retrogradation, but also of endogenous enzyme activity, more particularly α-amylase" (Mariotti *et al.*, 2005). **Increasing the heating** rate consequently leads to faster thermal inactivation of enzymes and thus an increased peak viscosity (Mariotti *et al.*, 2005). Decreasing the time available for both starch gelatinisation and enzymic hydrolysis relative to commercial practice, may accentuate differences between malt samples (Glennie Holmes, 1995b). The action of α-amylase rapidly decreases the size of starch molecules to an extent proportional to the amount of time allowed before thermal inactivation. **Decreasing the heating rate** leaves samples more exposed to the prolonged effect of **shear** and **enzyme hydrolysis**, causing a lowering of peak viscosity (Mariotti *et al.*, 2005). Adjusting the time/temperature regime during RVA analysis drastically affects rheological measurement (Yun & Quail, 1999; Batey & Curtin, 2000). The rheological effect of heating/cooling rate alterations can provide further insight to the consequences of milling and variation in malt fermentability.

The main effect of **milling** is a reduction in **particle size** and consequently, an increase in surface area to volume ratio. This affects reaction rates such as water binding, solubilisation, heat transfer, swelling, gelatinisation and amylolysis. Particle size and the type of grinding action used (cryomilled vs. hammer-milled) do not affect the gelatinisation temperatures of sorghum samples, therefore initial viscosity increase is independent of particle size (Mahasukhonthachat *et al.*, 2010). However, particle size and the grinding action used affect starch digestion in sorghum, therefore differences in peak formation (incline, peak height and decline) could be expected to occur during rheological assessment. The molecular and structural properties of starch, protein and cell wall components are affected by the frictional heat and mechanical energy of milling (Morrison & Tester, 1994; Tester & Morrison, 1994; Tester, 1997; Kerr *et al.*, 2000).

Milling leads to the formation of **damaged starch** which influences its rheological and functional properties (Morrison & Tester, 1994; Tester & Morrison, 1994; Tester, 1997; Leman *et al.*, 2006; Mahasukhonthachat *et al.*, 2010). The RETSCH ZM series mills are centrifugal mills, in which size reduction takes place through a two step grinding process involving impact (sample falling onto the rotor) and shearing (between the rotor and the fixed ring sieve) (Anonymous, 2009). The feed material (e.g. whole grain malt) falls onto the rotor, after which centrifugal acceleration throws it outwards to the wedge-shaped rotor blades to be pre-crushed on impact. It is then finely ground between the rotor and the ring sieve and collected in the surrounding pan. The feed material remains in the grinding chamber for only a short time, providing gentle but fast processing.

Optimising the **pH** during the **mashing** process produces several benefits to brewers, such as increased extract, fermentability and lautering run-off speed (Bamforth, 2001; Goode *et al.*, 2005b). The pH of mash is usually in a range of 5.4 to 5.7 which is favourable for

amylolysis and needs no further correction (Schur, 1980). Most enzymes display considerable activity above and below their pH optimum, with α-amylase having an optimum at approximately pH 5.3 (room temperature) (Briggs *et al.*, 2004). Complete hydrolysis can be achieved even when the pH is not optimal, provided enzymes survive long enough in the mash. Thus, heat tolerance of the enzymes is a more significant experimental factor than pH (Bamforth, 2001).

The aim of this study was to determine the effect of **various RVA analysis/operating conditions** on the **rheological response** of malt samples differing in **fermentability degree** and **particle size**. Additionally, **mash pH** was measured to investigate if future adjustment was indeed necessary. Thus, the rheological effect on malt samples was investigated when varying:

- instrument models (RVA 3D+ vs. RVA-4);
- time/temperature profile (to simulating the brewery mashing process);
- enzyme activity;
- heating rates; and
- degree of starch damage

Materials and Methods

Malted barley samples

Malt samples were obtained from South African Breweries Maltings (SABM) in Caledon (Table 5.1) (see detailed cultivar description in Appendix 1, Table 1.1). For pH measurement three different local barley malt pieces (i.e. 360-3000 tonnes of malt contained within a silo, usually from a pure malting barley cultivar) were used (Table 5.1).

Sample preparation

Samples were ground with a centrifugal mill (Retsch model ZM1, Haan, Germany) fitted with a 0.5 mm or 1 mm ring sieve (Table 5.1). Malt enzymes were inactivated using 0.1 M silver nitrate (KIMIX, Analytical Reagent Grade, Cape Town, South Africa). The aqueous solution was prepared by weighing the amount of silver nitrate dependent on the total volumetric amount needed. The malt:water ratio was set at 1:1.5 (14% malt moisture content) for autolytic testing, allowing the calculation of the amount of malt and water needed according to equation 1.1 to 1.3 (Chapter 3 p. 60). However, under enzyme inactivated conditions (i.e. silver nitrate addition), a ratio of 1:6 (14% malt moisture content) was used allowing the calculation of the amount of malt and silver nitrate solution (0.1 M) needed according to equation 5.2 to 5.4. The total sample weight (water and malt) used in RVA analysis was kept constant at 27 g ± 0.002 g.

total sample weight: $M + AgNO_3 = 27\ g$ (at any malt moisture content) [5.2]

concentration malt:water ratio at 1:6: $6M = 1W_{AgNO_3}$ (at 14% malt MC) [5.3]

substituting: $M_{14\%} = 3.857\ g$ and $AgNO_{3_{14\%}} = 23.142\ g$ (at 14% malt MC)

moisture content correction: $M_{14\%}(100 - 14) = M_x(100 - MC)$ [5.4]

substituting: $M_x = 3.857(100 - 14)/(100 - MC)$; $M_x + AgNO_{3_x} = 27\ g$

In which: M = mass of malt; $AgNO_3$ = mass of silver nitrate solution (0.1 M) at any malt moisture content

$M_{14\%}$ or $AgNO_{3\ 14\%}$ = mass of malt or silver nitrate solution (0.1 M) at 14% malt moisture content

M_x or $AgNO_{3\ x}$ = Malt or silver nitrate solution (0.1 M) mass to be weighed at measured malt moisture content

MC = measured malt moisture content

Table 5.1 Experimental layout of RVA conditions, malt samples and sieve size used

	Condition varied:	Difference between malt samples:	Malt samples used:	Sieve size used:
1	instrument model: (RVA 3D+ vs. RVA-4)	degree of fermentability	Metcalfe, Flagship, PUMA, SSG 585, SSG 564, SSG 506	0.5 mm
2	time/temperature profile: simulated brewery mashing process	degree of fermentability	Metcalfe, PUMA, SSG 506	0.5 mm
3	enzyme activity	degree of fermentability	Metcalfe, Flagship, PUMA, SSG 585, SSG 564, SSG 506	0.5 mm
4	enzyme activity	particle size distribution	Metcalfe, PUMA, SSG 506	1 mm
5	heating rate: increased[1]	particle size distribution & degree of fermentability	Metcalfe, SSG 506	1 mm & 0.5 mm
6	heating rate: decreased[1]	particle size distribution & degree of fermentability	Metcalfe, SSG 506	1 mm & 0.5 mm
7	starch damage	Amount of times milled	Metcalfe	0.5 mm
8	Malt-water pH measurement	Measurement before and after RVA analysis	Alrode piece[2] 138, 141, 142	0.5 mm

[1]Increased or decreased heating rate is relative to the heating rate used in the time/temperature profile of the Kilned Malt method (0.18°C.s^{-1})

[2]piece refers to a unit of malt contained within a silo, usually of a pure malting barley cultivar

Moisture content determination

The moisture content of all malted barley samples was determined prior to RVA analysis according to the European Brewery Convention method 4.2 (European Brewery Convention, 1998) as described in Chapter 3, p. 60.

RVA Measurements

The RVA (model 3D+, Newport Scientific, Warriewood, Australia), was used to measure the pasting properties of different malt samples. The RVA 4 (model 4, Newport Scientific, Warriewood, Australia) was also included in this study to allow comparison between older and newer RVA models. The amount of water (dH_2O) or aqueous silver nitrate solution (0.1 M) was pre-weighed in separate aluminium canisters to which a pre-weighed amount of malt was added, for autolytic or enzyme inhibited conditions, respectively (weights determined according to equation 5.2-5.3 p.120 and in Chapter 3, equation 1.1 to 1.3, p.60). The Kilned Malt method's (Newport Scientific Method 16, Version 3, June 1997) time and temperature profile was used during rheological analysis, except when the brewery mashing process was simulated (Table 5.2), the heating rate was increased (Table 5.3) or decreased (Table 5.4) compared to Kilned Malt method's. Methods for rheological measurement were as described in Chapter 3, p.61.

Table 5.2 RVA time/temperature profile: simulation of commercial brewery mashing process

Time (hrs:min:s)	**RVA Parameters**	**Value**
00:00:00	Temperature (°C)	63
00:00:00	Rotational speed (rpm)	160
00:43:00	Temperature (°C)	63
00:44:00	Temperature (°C)	72
01:09:00	Temperature (°C)	72
01:10:00	Temperature (°C)	76
01:11:00	End of test	-

Table 5.3 RVA time/temperature profile: when heating rate was increased

Time (hrs:min:s)	**RVA Parameters**	**Value**
00:00:00	Temperature (°C)	50
00:00:00	Rotational speed (rpm)	960
00:00:10	Rotational speed (rpm)	160
00:01:00	Temperature (°C)	50
00:03:20	Temperature (°C)	90
00:09:12	Temperature (°C)	90
00:14:12	Temperature (°C)	50
00:15:00	End of test	-

Table 5.4 RVA time/temperature profile: when heating rate was decreased

Time (hrs:min:s)	**RVA Parameters**	**Value**
00:00:00	Temperature (°C)	50
00:00:00	Rotational speed (rpm)	960
00:00:10	Rotational speed (rpm)	160
00:01:00	Temperature (°C)	50
00:04:42	Temperature (°C)	60
00:10:00	Temperature (°C)	60
00:13:00	Temperature (°C)	90
00:15:00	End of test	-

Repeated milling (starch damage)

A malt sample (Metcalfe) was milled in a Retsch mill with a 0.5 mm sieve size (milling time was instantaneous to the feed rate). A subsample was taken from the milled sample and milled twice more to obtain a sample with a higher level of starch damage.

Mash pH

Before pH measurements were recorded the pH electrode and its temperature probe (pH 211R Microprocessor pH meter, Hanna Instruments, Italy), were calibrated (immersion into pH 7 and pH 4 buffered solutions). The pH and temperature of the malt-water mixtures were simultaneously recorded before and after RVA analysis.

Multivariate data analysis: Principal component analysis

Rheological variables were imported into The Unscrambler (version 9.2, CAMO, Oslo, Norway) and principal component analysis (PCA) was performed. Methods for multivariate data analysis were as described in Chapter 4, p.94.

Results and discussion

Comparison of RVA models

It is inevitable that new models of measuring equipment would become available. The RVA 3D+ was used during the majority of the research conducted and described in the earlier chapters of this thesis. The RVA 4, a newer model, has since been developed. It was essential to evaluate the two instruments in terms of adequate sensitivity to discriminate between malt samples with different degrees of fermentability, enabling transferability of the prediction models developed on the RVA 3D+ to the newer RVA 4. Previous rheological

analyses were conducted under highly concentrated malt conditions (Chapters 3 & 4), therefore the obtained results were validated with a more sensitive RVA instrument to ensure optimal sample discrimination was still obtained without detriment to viscosity signal. The **RVA 4** is able to measure viscosity at varying shear rates with greater sensitivity at both viscosity ends. Thus, it allows viscosity measurement of materials with nearly ten times higher or approximately ten times lower viscosity (below 500 cP), compared to the RVA Series 3 (Anonymous, 1995). The validity and reproducibility of data, between different instrument models, were therefore examined.

Similar viscograms were recorded using both RVA instruments. However, one of the replicates of Flagship was identified as an outlier in the PCA influence plot (Fig. 5.1a), displaying a high residual X variance and high leverage. This was confirmed by the PC scores plot (Fig. 5.1b) and viscogram (Fig. 5.2). This sample was removed from the PCA model as the difference was due to a faulty measurement, unrelated to the instrument.

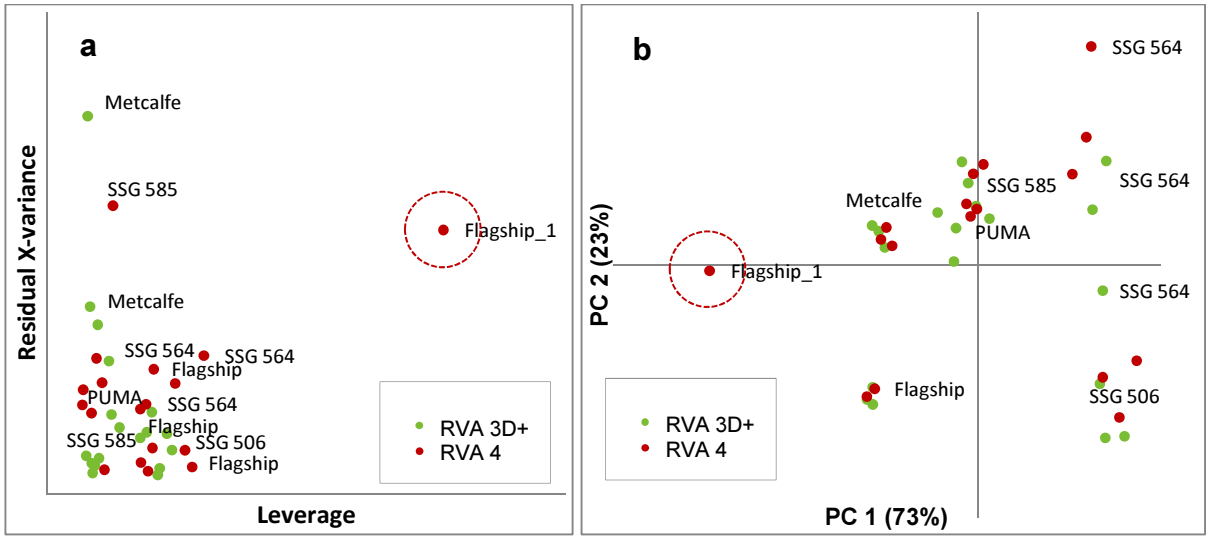

Figure 5.1 Flagship (replicate 1; indicated by red circle) identified as an outlier from (a) the influence plot (PC 4) and (b) the scores plot (PC1 vs PC2).

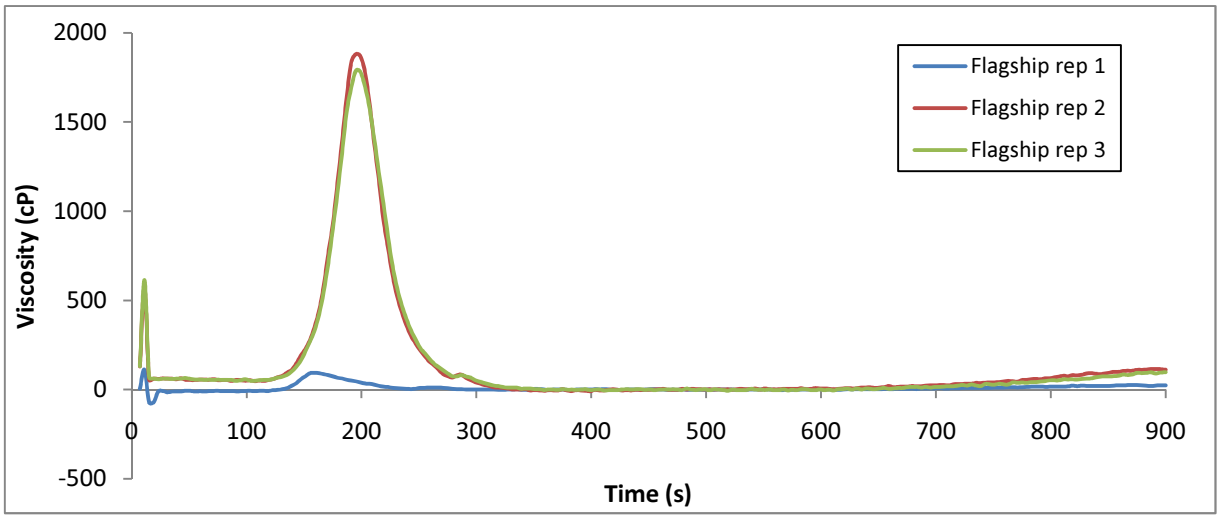

Figure 5.2 Viscograms of the three replicates of Flagship, illustrating the erroneous viscogram of replicate one.

The RVA 3D+ instrument is part of the first model series developed by Newport Scientific (Mr F Meyer, Ronin, Modderfontein, South Africa, personal communication, 2010; Mrs B Elliott, Newport Scientific, Australia, personal communication, 2010). It is considered to be less sensitive to rheological changes in comparison to later versions. Increasing the malt concentration, during RVA analysis, can intensify viscosity noise and subsequently viscosity deviation amongst replicate samples.

Clusters observed in the PC scores plot showed sample grouping was based on cultivar type and not due to the different instrument models used (Fig. 5.3). Therefore, both instruments delivered similar discrimination between malt samples differing in degree of fermentability. This indicated, rheological measurement obtained by means of the RVA 3D+ was sufficient to discriminate between different malt samples. This was in spite of its lower sensitivity compared to the newer RVA-4 instrument. This also indicated malt fermentability results can easily be reproduced when using different instrument models.

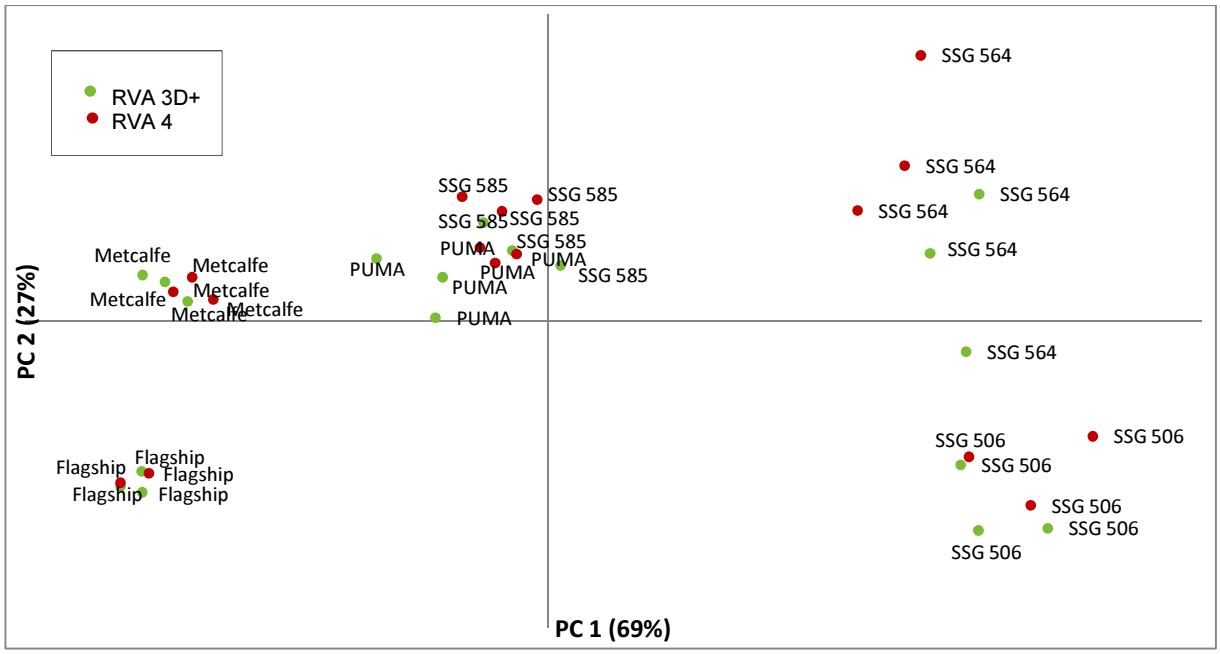

Figure 5.3 Principal component scores plot illustrating the similarity between results obtained when two different RVA models (RVA 3D+ and RVA 4) were compared.

Simulation of the commercial brewery mashing process in terms of time/temperature profile

Using the time/temperature profile of a commercial brewery mashing process substantially lowered peak heights when compared to peaks normally attained by the time/temperature profile of the Kilned Malt method (Fig. 5.4). This was due to the longer available time for enzymes to hydrolyse gelatinised starch before being inactivated at higher temperatures (commercial brewery mashing time: 1h 11 min.; while Kilned Malt method's time: 15 min.). According to Mariotti *et al.* (2005), increasing the temperature of a starch and water suspension at a slower rate, increases the time during which α-amylase can act on starch granules, resulting in lower peak viscosities. In addition, starch samples were exposed for a prolonged period to shear thinning, which led to further viscosity reduction (Batey & Curtin, 2000). This diminished the rheological differences between malt samples differing in fermentability.

In the commercial brewery mashing process, there is no final decrease in temperature (cold pasting) as typically encountered during RVA analysis. This decrease in temperature gives information on the retrogradation behaviour of a malt-water mixture and can possibly identify problematic viscosity increases. Previous results (Chapter 3, p.78), however, indicated little information was given by the final viscosity of a malt-water mixture as it was heavily dependent on base line determination. The most useful information was obtained from variables associated with peak formation (i.e. peak incline, height, area and decline) (Chapter 3, pp. 83-84). Therefore difference in peak height can be used to discriminate between malts based on degree of fermentability.

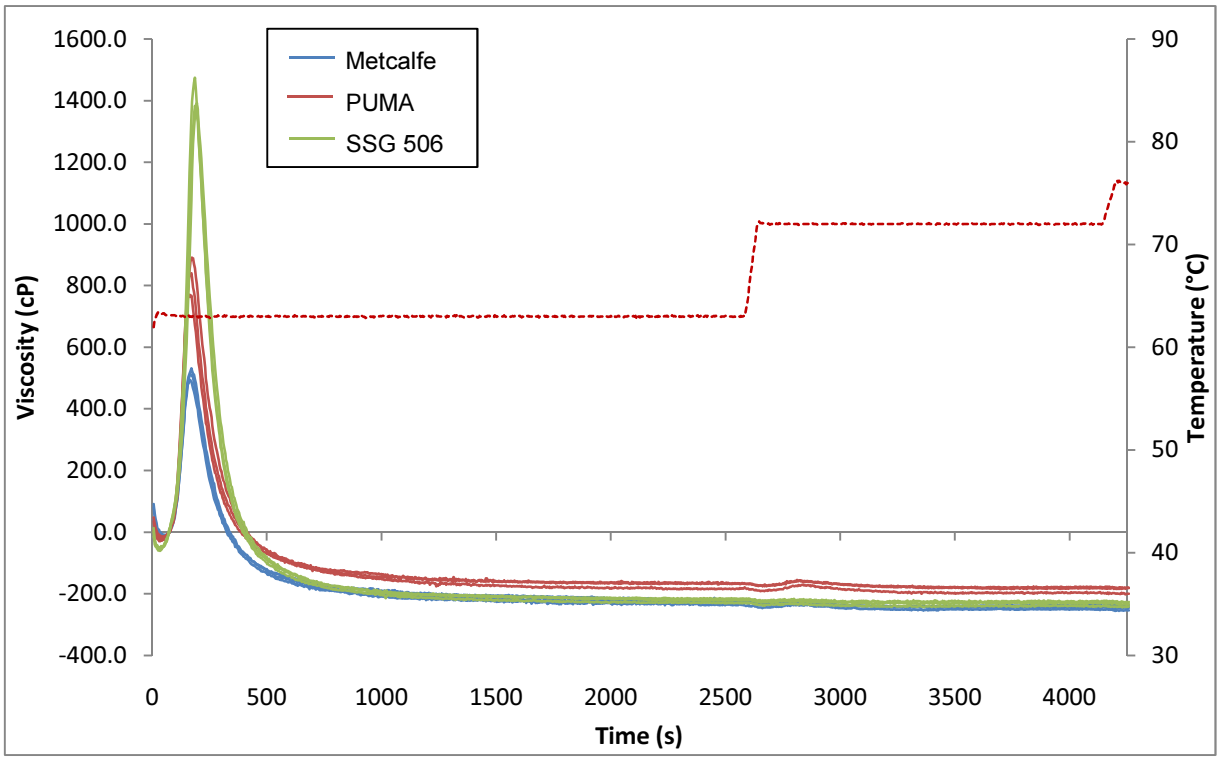

Figure 5.4 Viscograms of 3 different malt samples (3 replicates), differing in fermentability degree, measured using the the time/temperature profile of the commercial brewery mashing process.

Considering the same viscogram when the commercial brewery mashing process was simulated (Fig. 5.4), a slight increase in viscosity around 2750 s (at 72°C) was observed for all of the malt samples (Fig. 5.5). This peak may represent the gelatinisation of smaller B-type starch granules; which has been observed to occur around ca. 68-70°C (Goode *et al.*, 2005b). This conclusion should be confirmed by additional analytical testing, such as microscopic observations with normal and polarised light (Mariotti *et al.*, 2005). The increase in viscosity could also have been the result of interaction between amylose and lipids, as high temperatures cause the melting of the amylose-lipid complex and can be measured by means of differential scanning calorimetry (Eliasson, 1994; Sasaki *et al.*, 2000). This amylose-lipid complex has also been reported to inhibit starch swelling (Tester & Morrison, 1990; Tester, 1997; Han *et al.*, 2002; Matsuguma *et al.*, 2009).

The long analysis time did not justify the quality of information obtained, especially from the low viscosity end of the viscogram. This long time/temperature profile (1 h 11 min) is not recommended for the discrimination of malt sample fermentability. However, this time/temperature profile may prove more useful for less-modified malt samples. Utilising a time/temperature profile which optimises enzyme activities, as used during decoction

mashing (Chapter 2, pp. 20-21), might deliver more rheological changes and therefore offer more information.

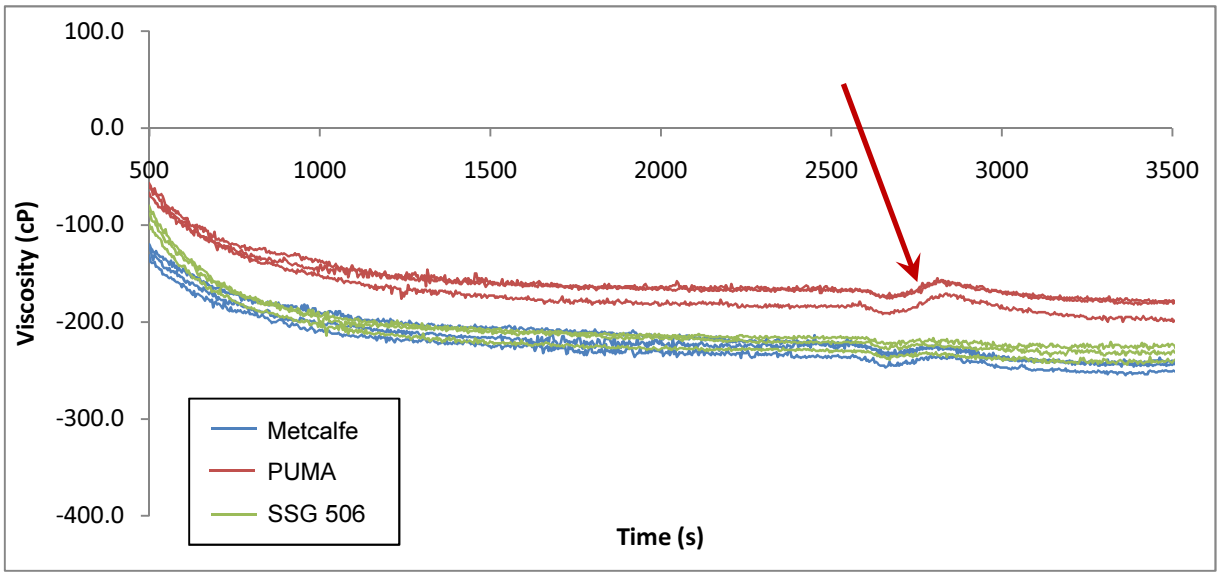

Figure 5.5 Viscogram 'low viscosity end' recorded from three malt samples differing in fermentability degree and measured using the time/temperature profile of the commercial brewery mashing process (note the small viscosity increased at ca. 2750 seconds).

Effect of enzyme inhibition on malt samples differing in fermentability degree

When inactivating malt enzymes through silver nitrate addition, Flagship and SSG 506 produced the highest peaks, Metcalfe delivered an intermediate peak height, while SSG 564, PUMA and SSG 585 delivered the lowest peaks (Fig. 5.6). The intrinsic viscosity of starch, contained within malt can be measured by inactivating malt enzymes during rheological analysis (Glennie Holmes, 1995a). Viscosity is stated to increase by the cube of the starch concentration within the malt, and gives an indication of the amount of extract possibly attained (Glennie Holmes, 1995b). A larger peak viscosity is also thought to indicate starch is less restricted within a sample (Mahasukhonthachat *et al.*, 2010). This suggested Flagship and SSG 506 contained the highest starch content, with a more 'open' starch structure and expected to deliver a large extract. Inactivation of malt enzymes produced viscograms similar to barley. This can be explained by the malting process which converts starch to an intermediate form that is still capable of gelatinising (Glennie Holmes, 1995b). Approximately 10% of starch is degraded during the malting process, therefore delivering such great peak heights accompanied with a large degree of retrogradation (Glennie Holmes, 1995b). Under enzyme inhibited conditions malt samples could not be separated into groups representing degree of fermentability. This was expected, since pasting properties are greatly affected by

α-amylase activity (Dengate, 1984), or even enzyme thermostability; these factors have a large influence on malt fermentability.

The onset of gelatinisation is not necessarily indicated by the temperature recorded when viscosity begins to increase. Contradiction exists as to what the initial rise in viscosity represents, where some term it the gelatinisation starting point (Tester *et al.*, 1991; Goode *et al.*, 2005c; Goode & Arendt, 2006; Mariotti *et al.*, 2009) and others believed it to be due to pasting, as gelatinisation of malt and wheat samples occurs before pasting (Batey & Curtin, 2000). Perhaps the increase does not represent the actual point where birefringence is initially lost, but rather closely related to the onset of gelatinisation (i.e. an earlier rise in viscosity, due to pasting, might indicate earlier onset of gelatinisation which occurred at a lower temperature, therefore rheological measurement can indicated on a relative basis the onset of gelatinisation). When enzymes were inhibited the onset of pasting (initial rise in viscosity) was independent of cultivar, and therefore more a general characteristic of malted barley (Fig. 5.6).

Flagship displayed the highest rate of viscosity increase during the onset of pasting. SSG 506, Metcalfe, PUMA and SSG 585 ranked second (with regards to rate), while SSG 564 displayed a delayed increase (Fig. 5.6). This initial increase in viscosity is related to the swelling potential of the starch granules (Allan *et al.*, 1997). As a consequence of the diversity in starch granules, swelling occurs over a range of temperatures. Complete enzyme hydrolysis during mashing is dependent on this inherent swelling potential of a sample. The different rates of viscosity increase could also indicate differences in the gelatinisation temperature ranges of the different cultivars. This corresponds to some extent with previous findings (Chapter 3, p. 65) when the same cultivars were examined under autolytic conditions. This could suggest a lower gelatinisation temperature range for Flagship compared to the other cultivars.

Flagship and Metcalfe appeared to have a peak maximum slightly later than the other cultivars (Fig. 5.6). Peak time has been suggested to relate to the relative swelling potential of starch granules (Allan *et al.*, 1997). A significantly negative relationship between peak time and mean large starch granule diameter has been found. Large starch granules with larger mean diameters swell more easily, and thus decrease peak time. Peak time also has a significantly negative relationship with malt extract (Allan *et al.*, 1997). This can be verified in practice by the cultivar SSG 506, which displayed a decreased peak time and is known by the South African malting industry to have a high number of large round starch granules, delivering a high fine-extract value (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). PUMA delivers a good fine-extract value, followed by a slight decrease for SSG 585, while Metcalfe delivers an intermediate

fine-extract value, which agrees with the peak time observations (Fig. 5.6) and malt analysis (FGE results in Table 1.2, Appendix 1).

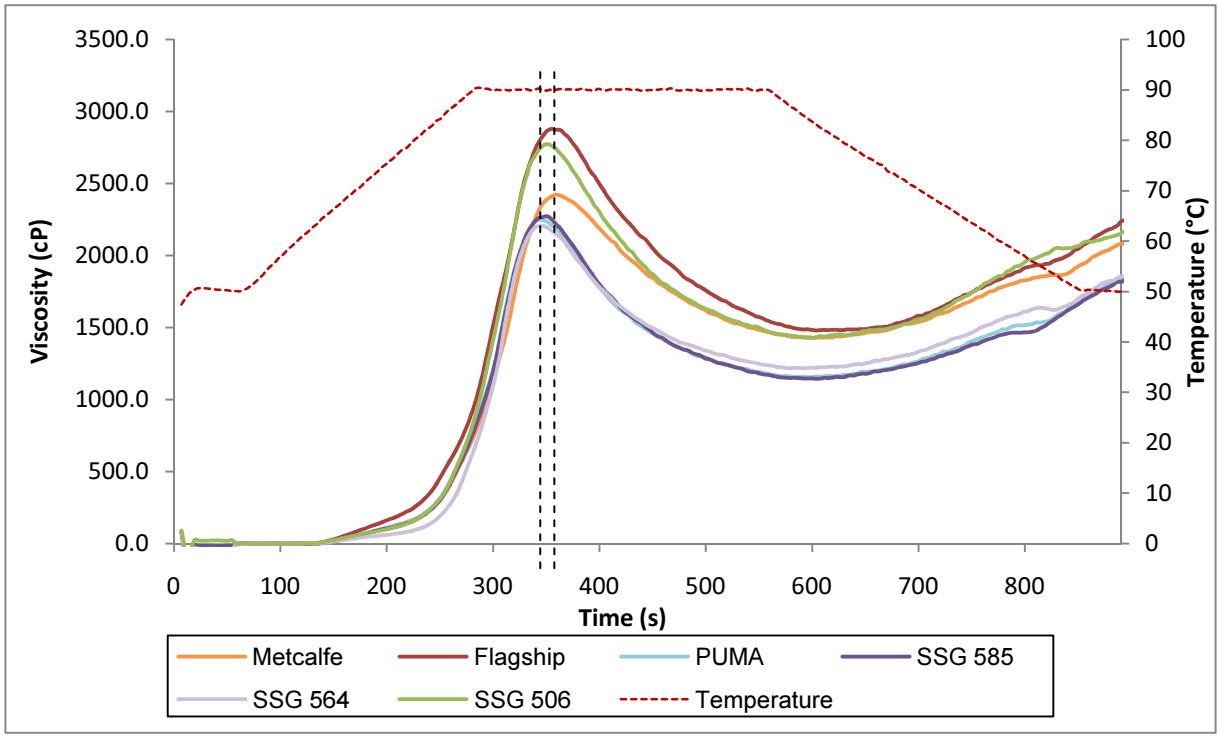

Figure 5.6 Viscograms of 6 different malt samples (3 replicates averaged), differing in degree of fermentability, recorded under enzyme inhibited conditions (dashed lines indicate 'peak time').

Malt samples displayed extensive retrogradation when enzymes were inhibited compared to autolytic conditions (Fig. 5.7). This was due to α-amylase activity which hydrolysis starch, causing a decrease in viscosity upon cooling (Mariotti *et al.*, 2005). The outer branches of amylopectin are hydrolysed, thereby preventing the formation of large amylopectin crystals, capable of promoting a viscosity increase upon cooling. The interaction between amylose and amylopectin chains has also been suggested to play a role during retrogradation (Kurakake *et al.*, 2008). However, the precise mechanism of network formation is still unexplained. Under inhibited conditions, starch polymers re-associate upon cooling, resulting in a substantial viscosity increase (Olkku & Rha, 1978; Leman *et al.*, 2006). This phenomenon is governed by the concentration, length and state of dispersion of amylose chains (Olkku & Rha, 1978; Leman *et al.*, 2006). Metcalfe displayed the smallest amount of breakdown and setback (from peak), while Flagship and SSG 506 delivered the greatest amount of breakdown. Based on limited data the amylose content was found to be negatively related to peak time, peak viscosity and trough viscosity (Allan *et al.* 1997). Amylose content of normal malting barley remains fairly consistent, but environmental

conditions can vary the levels of lipid associated with amylose (Allan *et al.*, 1997). Lipophospholipid associated with amylose can reduce the swelling potential of starch granules, therefore affecting rheological measurement by increasing gelatinisation temperature (Tester *et al.*, 1991; Stuart *et al.*, 1998). This could have a negative effect on malt extract.

The viscograms of six different malting barley cultivars recorded under enzyme inhibited conditions were compared to previous results collected under autolytic conditions (Fig. 5.7). Under enzyme inhibited conditions, the peak viscosity is proportional to the cube of the starch concentration, while under autolytic conditions degrading 0.1% of the internal bonds within a starch molecules causes a 50% reduction in viscosity (Glennie Holmes, 1995a). Thus, a small change in starch structure resulted in a considerable rheological change (Glennie Holmes, 1995a). Therefore peak height, or any viscosity scaling, should not be compared between the two conditions as different starch concentrations were utilised to compensate for α-amylase activity.

Under enzyme inhibited conditions, the onset of gelatinisation/pasting occurred later and over a wider temperature range, while under autolytic conditions gelatinisation/pasting commenced slightly earlier and over a narrower temperature range. This effect has been reported several times, but is not well understood (Glennie Holmes, 1995a; Allan *et al.*, 1997; Goode *et al.*, 2005a; Leman *et al.*, 2006).

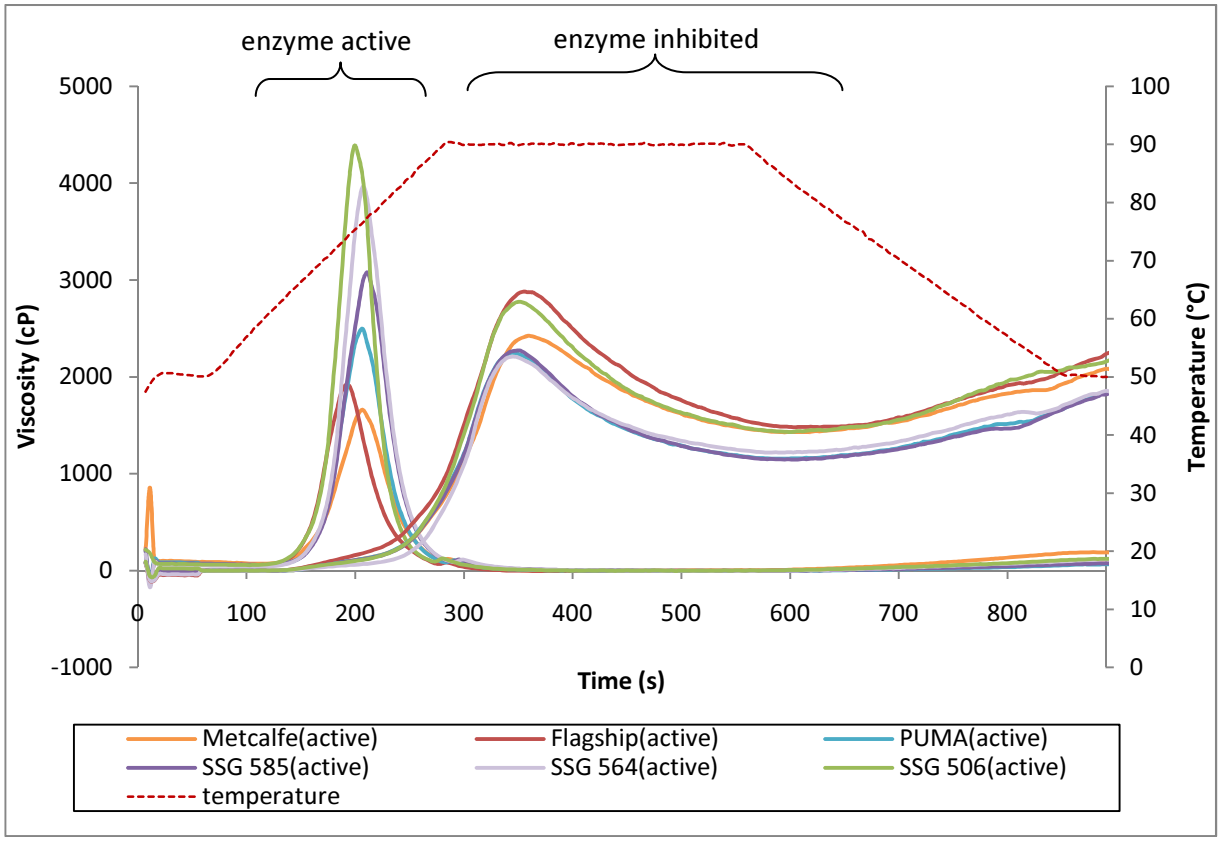

Figure 5.7 Viscograms of 6 different malt samples (3 replicates averaged), differing in degree of fermentability, when comparing malt enzyme activity (autolysis vs. enzyme inhibition).

Effect of enzyme inhibition on malt samples, differing in particle size distribution

The main effect of milling is essentially a reduction in particle size. This effect increases the surface area available for many processes (i.e. water binding, solubilisation, heat transfer, swelling and amylolysis) (Mahasukhonthachat *et al.*, 2010).

Under enzyme inhibited conditions, peak height of SSG 506 and PUMA was fairly similar irrespective of particle size distribution (Fig. 5.8). However, Metcalfe exhibited an increase in peak height when the sieve size was reduced from 1 to 0.5 mm. Variations in particle size have been shown to affect peak viscosity, with the greatest effect being observed when particle size was below 300 µm (Mahasukhonthachat *et al.*, 2010). The change in viscosity observed in this study could be non-significant as the deviation amongst the three replicates is large (Fig 5.9).

The onset of gelatinisation/pasting was similar for all the samples (Fig. 5.8) and is in accordance with a recent study (Mahasukhonthachat *et al.*, 2010). Starch gelatinisation/pasting was unaffected by particle size and the type of milling action used. Further, the viscosity incline differed according to cultivar, thus viscograms of the same

cultivar but with different particle size distributions superimposed onto one another. The subsequent swelling of starch granules (following gelatinisation) were largely unaffected by particle size. This indicated the malt concentration was low enough not to exercise further constraint on the gelatinisation process (refer to Fig. 3.4 and 3.5, Chapter 3 pp.78-79). Enough water was present causing the consistency (mainly affected by the amount of starch exudate released) to became a function of temperature alone (Longley & Miller, 1971; Olkku & Rha, 1978). Under highly concentrated autolytic conditions starch gelatinisation seemed to be the primary process inhibited. This verified previous results in which five different malt concentrations were used (Fig 3.5, Chapter 3, p. 68). Malt amylases cannot hydrolyze raw ungelatinised starch. The reaction of enzyme hydrolysis occurs rapidly once substrate becomes available (Meddings & Potter, 1971). Under highly concentrated conditions starch gelatinisation is hindered due to the competitive effect of starch and hydrolysed product (such as glucose and maltose) for water. Therefore, under such concentrated conditions, the rate limiting step was the amount of gelatinised substrate available and not the rate of enzyme hydrolysis. Only under these extreme conditions, does particle size seem to have an effect.

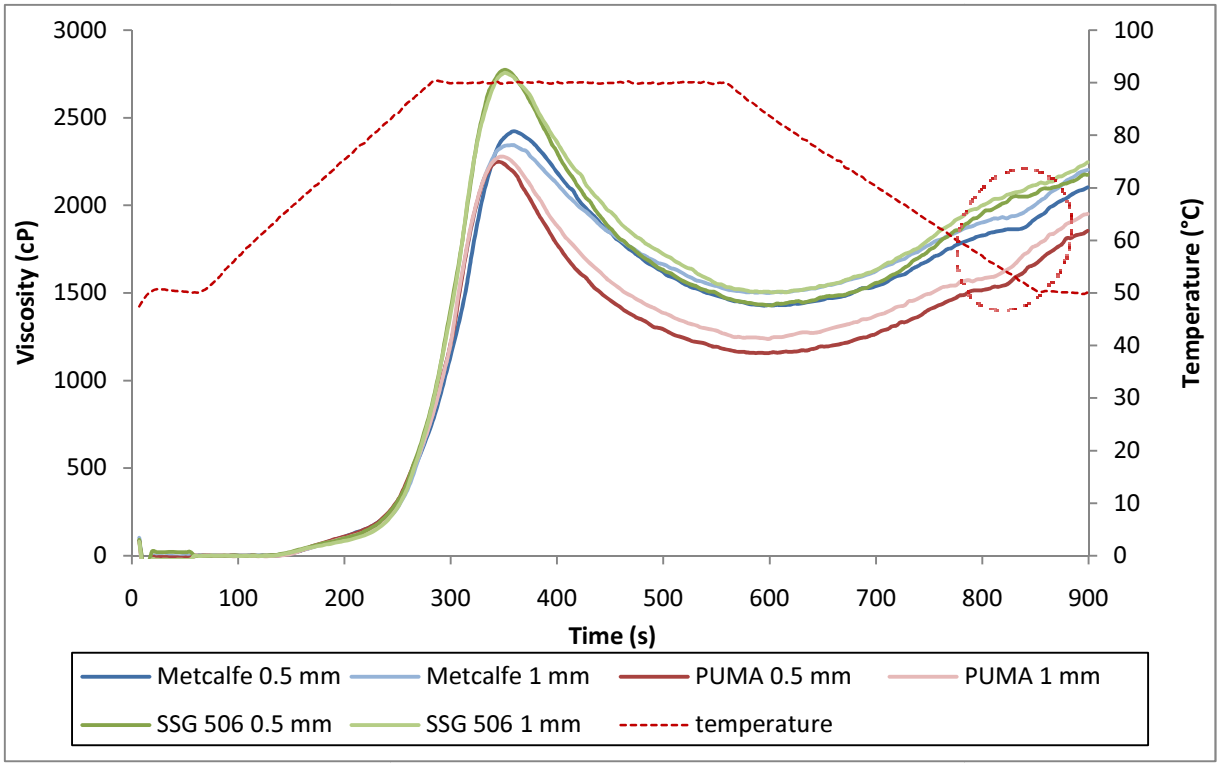

Figure 5.8 Viscograms of 3 different malt samples of 2 different particle size distributions (3 replicates averaged), recorded under enzyme inhibited conditions.

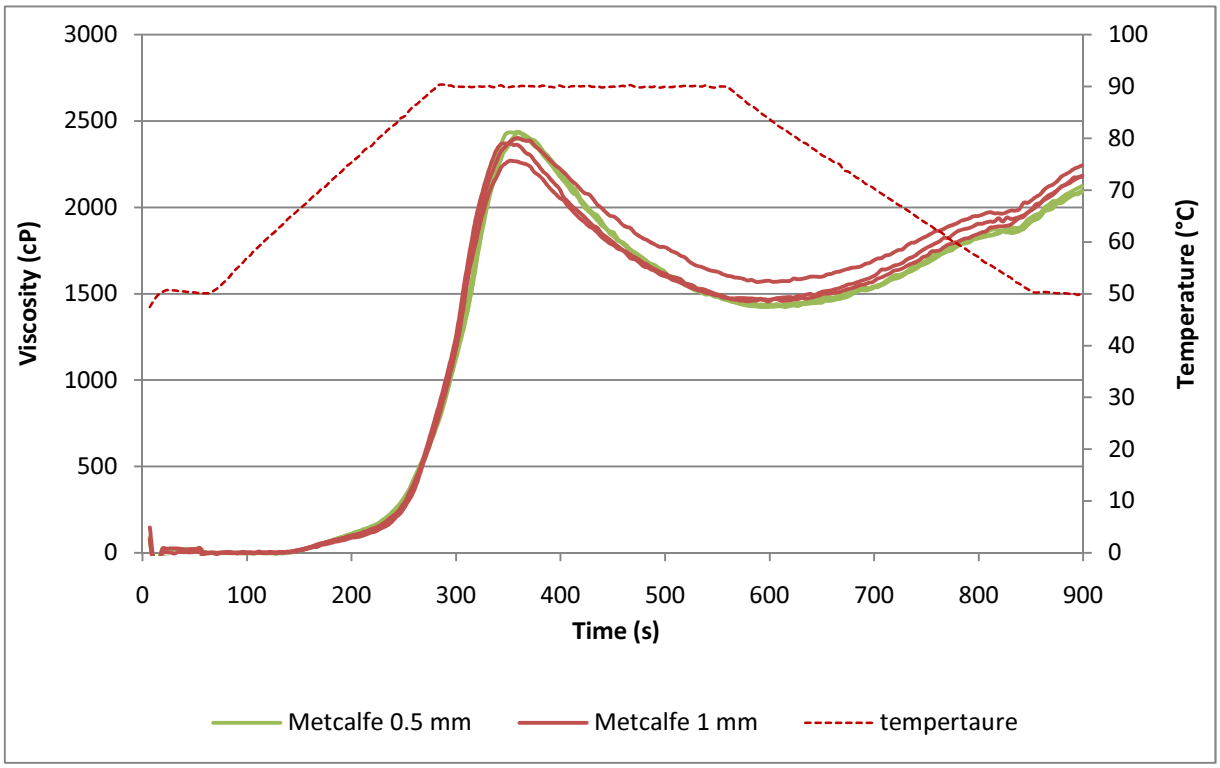

Figure 5.9 Viscograms illustrate deviation amongst replicates of 3 different Metcalfe replicates of 2 different particle size distributions, recorded under enzyme inhibited conditions.

Setback and final viscosity were slightly affected by particle size variation; the samples milled with the smaller sieve size (i.e. 0.5 mm) had a lower final viscosity. This suggests a small amount of starch-damage occurred during milling with the smaller sieve. A small amount of mechanical damage can cause starch to lose its crystallinity and yield low molecular weight materials (Mahasukhonthachat *et al.*, 2010).

The viscograms of two wheat samples with differing degrees of starch damage (Fig. 5.10) (Leman et al. 2006) displayed a similar pattern to that observed for the different particle size distribution samples (Fig. 5.8). This suggested that starch damage played a role as a result of different sieve sizes. The final viscosity of sorghum samples was found to be particle size dependent (Mahasukhonthachat *et al.*, 2010). The reduction of final viscosity observed in this study may be a function of starch damage or particle size dependence.

A viscosity deviation was once again observed during setback (cold paste viscosity increase) for all three cultivars tested.

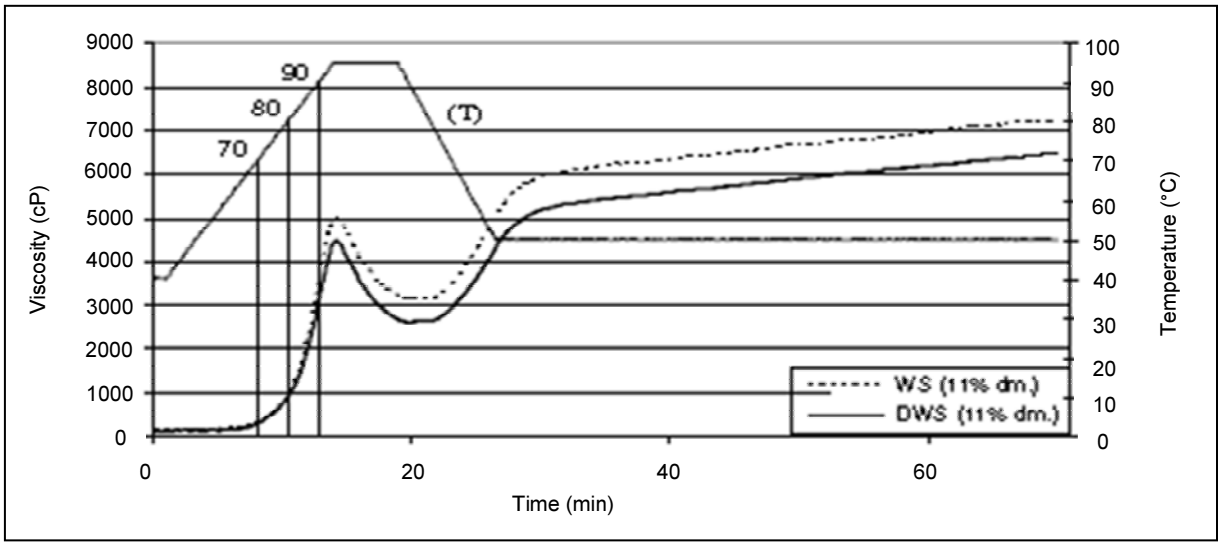

Figure 5.10 RVA viscograms illustrating the effect of wheat starch damage (WS = wheat starch containing a low level of damaged starch, DWS = wheat starch containing a high level of damaged starch) (Leman *et al.*, 2006).

Effect of increased heating rate on malt samples

Samples: one malting barley cultivar with two different particle size distributions

Previous work indicated particle size distribution influenced all malt samples to a similar degree, therefore the rheological effect of increased heating rate was only considered on one sample, differing in particle size distribution. A substantial increase in peak height (from approximately 1500 to 2300 cP) was observed when the heating rate was increased (Fig. 5.11) compared to results obtained using the Kilned Malt method's time/temperature profile (Fig. 5.7; enzyme active). This results in a decreased time period, during which α-amylase can act on starch granules, thus, hydrolysing less starch and delivering a higher peak viscosity (Mariotti *et al.*, 2005).

Samples milled with the smaller sieve size (i.e. 0.5 mm) displayed a slight delay in viscosity incline (Fig. 5.11). Enhanced enzyme activity of the 0.5 mm compared to the 1 mm sieve size samples is thought to occur. These results substantiated previously drawn conclusions (Chapter 5, p. 15 & Chapter 3, Fig. 3.5, pp.78-79) which explained the interaction between particle size distribution and malt to water concentration ratio.

Reaction processes (water binding, solubilisation, heat transfer, swelling) were constrained at such a high malt:water concentration (1:1.5) (Glennie Holmes, 1995a; Mousia *et al.*, 2004). Additionally, faster thermal inactivation, due to the increased heating rate, posed further time constraints. As particle size decreases, surface area and therefore rate of water absorption increased (Mahasukhonthachat *et al.*, 2010). The rate of gelatinisation and subsequently enzyme hydrolysis are dependent on the rate of hydration. The increased

hydrolysis rate of finer particles causes an increased rate of degradation, thus producing an overall effect of a slightly reduced incline and peak viscosity.

This assumption of enhanced hydrolysis rate, due to a smaller particle size distribution, must be confirmed by further analytical testing. It would be of interest to measure the amount of product formed (such as glucose) to assess the rate of hydrolysis (Meddings & Potter, 1971). Prolonged enzyme activity, by decreasing the heating rate, can alleviate some of the time constraints posed on the processes of gelatinisation and enzyme hydrolysis. Starch would fully hydrate, thus allowing the maximum rate for the gelatinisation of starch and subsequent enzyme hydrolysis to be reached for both particles size distributions. Thus diminishing viscosity differences between different particle size distributions during the onset of gelatinisation.

The final viscosity of the 0.5 mm sieve size sample was lower than the 1 mm. This is in agreement with previous findings (Fig. 5.8, p.132). Again, this may have been an indication of a small amount of starch damage inflicted during milling with the finer sieve size. More frictional heat and mechanical energy was shown to be inflicted on sorghum samples when grinding with a 1 mm retention sieve compared to a 2 mm retention sieve (using a Hammer mill) (Mahasukhonthachat *et al.*, 2010). Samples with a larger proportion of damaged starch, (due to the thermal and mechanical action of milling) are expected to deliver higher initial viscosities due to increased water absorption and swelling (Mahasukhonthachat *et al.*, 2010). Starch damage seemed to be an unlikely cause of the observed final viscosity decrease, due to similar initial viscosities observed (Fig. 5.11). A reduction in particle size could also have caused a lowering of the final viscosity. Due to the RETCSH mill's gentle but fast grinding action, it is believed little to no further starch damage could be expected when grinding with a finer ring sieve (Anonymous, 2009).

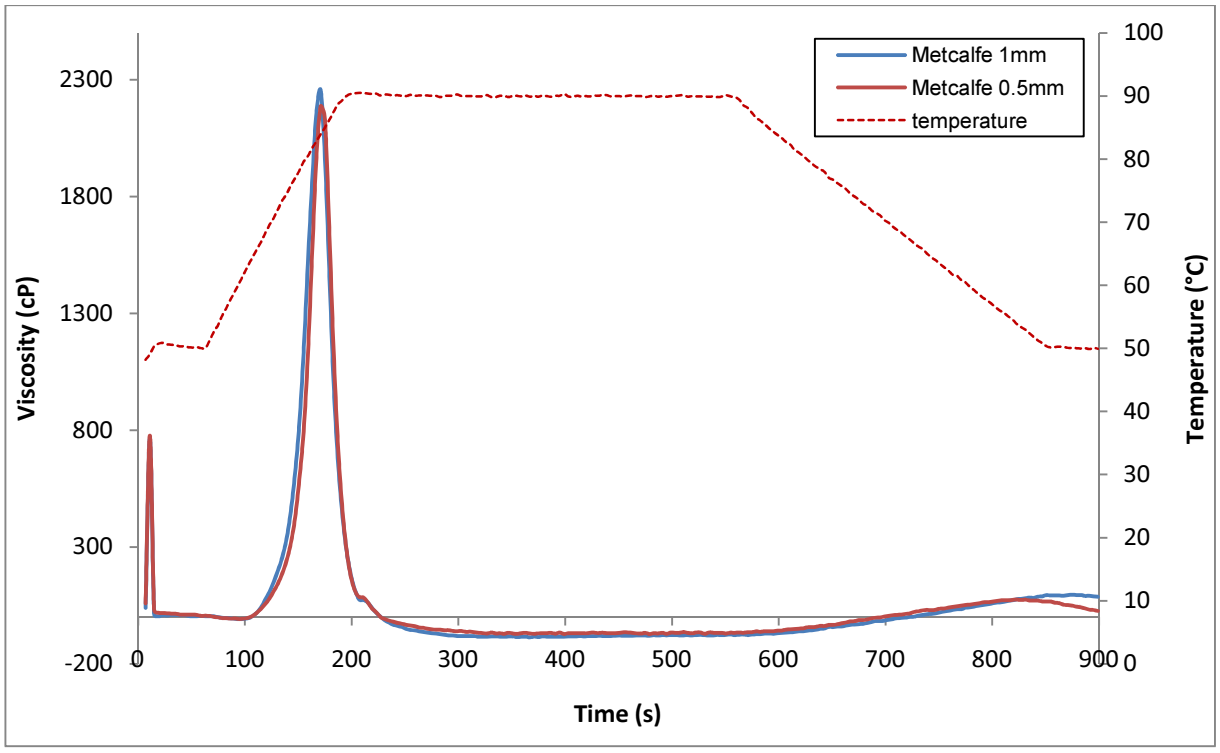

Figure 5.11 Viscograms of two different particle size distributions of the cultivar Metcalfe (replicates averaged), when increasing the heating rate.

Samples: two different malting barley cultivars

Only two malt samples of differing malt fermentability were investigated (Metcalfe and SSG 506). These malt samples represented fermentability extremes, while the other 4 (Flagship, SSG 585, SSG 564 and PUMA) are presumed to fall within these 'rheological limits'. The increased heating rate (Fig. 5.12), and Kilned malt method time/temperature profiles produced fairly similar viscogram patterns. For the time/temperature profile in which the heating rate was increased, the degree of discrimination seemed large in quantity or magnitude, but not necessarily better in comparison to the time/temperature profile of the Kilned Malt Method. The increased heating rate reduced peak width. This was due to earlier onset of thermal inactivation, causing a narrow peak to form. As shown from previous work (Chapter 3 and Chapter 4) information vital to discrimination between different degrees of malt fermentability lies within the viscogram rheological variables forming the peak. When the peak narrows, less rheological variables represents peak formation. Thus, when increasing the heating rate some fermentability information is lost (in comparison to a time/temperature profile which delivers high broad peak). This 'lost information' is related to enzyme hydrolysis, a crucial discriminatory factor based on fermentability.

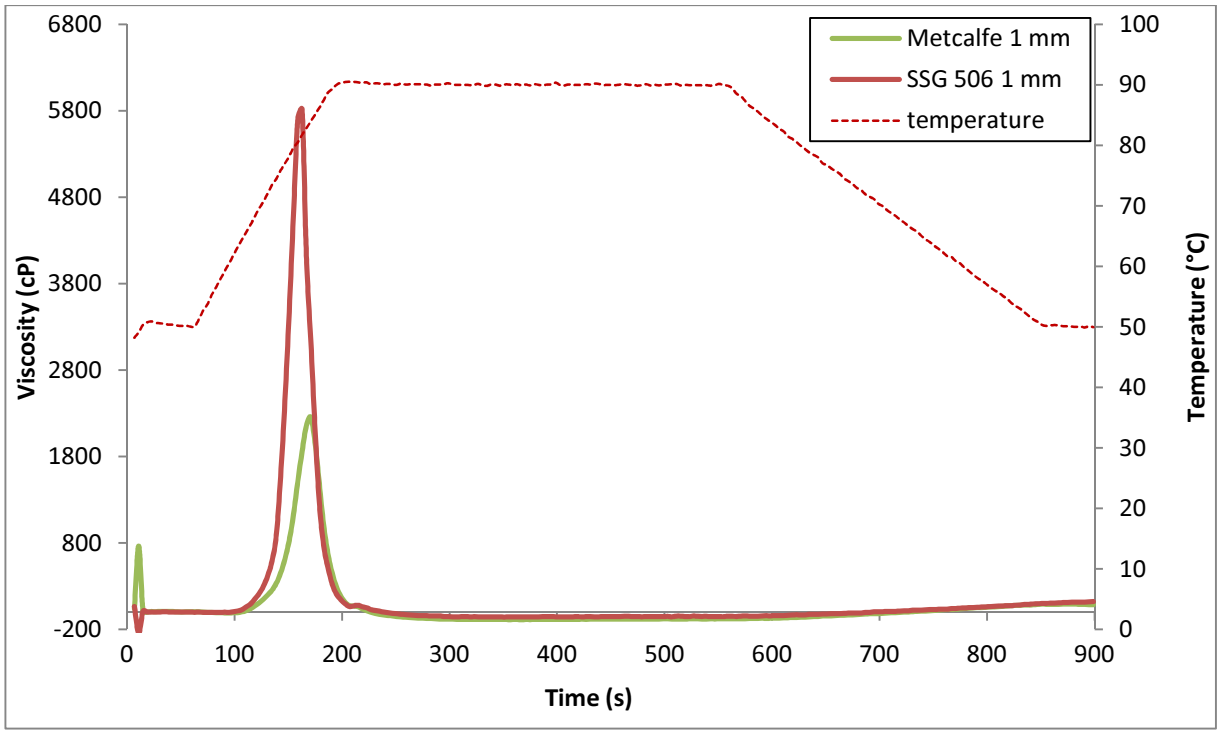

Figure 5.12 Viscograms of 2 different malt cultivars (replicates averaged), when using a RVA time/temperature profile in which the heating rate was increased.

Effect of decreased heating rate on malt samples

Samples: one malting barley cultivar with two different particle size distributions

A decreased heating rate substantially reduced peak height due to prolonged enzyme activity before thermal inactivation (Fig. 5.13). This promoted a greater degree of starch granule rupture due to prolonged shear and accompanied α-amylase action on swollen granules, leading to a lower final viscosity (Mariotti *et al.*, 2005).

The formation of three peaks was observed, which illustrated the transition of gelatinisation over a range of increasing temperatures. This is due to the crystalline areas within starch granules possessing different degrees of order (Lelievre, 1976; Olkku & Rha, 1978). The third peak, which was smallest (Fig. 5.13), was thought to represent the smaller B-type starch granules which gelatinise at a higher temperature and over a wider range (Meddings & Potter, 1971). Larger, highly swelled starch granules are more prone to starch damage (due to shear and enzyme hydrolysis) than physically smaller B-type starch granules. Hence, B-type starch granules may remain intact while larger granules are disrupted, especially when using low temperatures and longer analysis times (Mariotti *et al.*, 2005). Formation of the secondary starch gelatinisation peak (due to the smaller B-type starch granules) was expected to be largely concealed or considerably reduced, due to the prolonged enzyme action allowed by the slower heating rate. Hence, the third peak may not necessarily have been due to secondary starch gelatinisation.

Examining the cultivar Metcalfe, variation in particle size had little rheological effect (Fig. 5.13). This was expected as adequate time was given for samples to properly hydrate and gelatinise, accompanied with prolonged enzymes action, especially of α-amylase. This allowed enough time for samples of both particle size distributions to fully hydrolyse the maximum amount of substrate, eliminating any rheological differences between the two particle sizes. Malt milled with the smaller sieve size (i.e. 0.5 mm) displayed a slightly lower height for peak 1, but fairly similar heights for peak 2 and 3. The smaller particle size distribution produced slightly delayed peaks 1 and 2, but peak 3 seemed to occur earlier. The reasons for the differences observed for these three peaks are not yet understood.

The initial increase in malt viscosity occurred around the same temperature for both an increased and decreased heating rate (ca. 58-60°C). This verified that pasting temperature, during gelatinisation, is largely unaffected by the heating rate (Mariotti *et al.*, 2005).

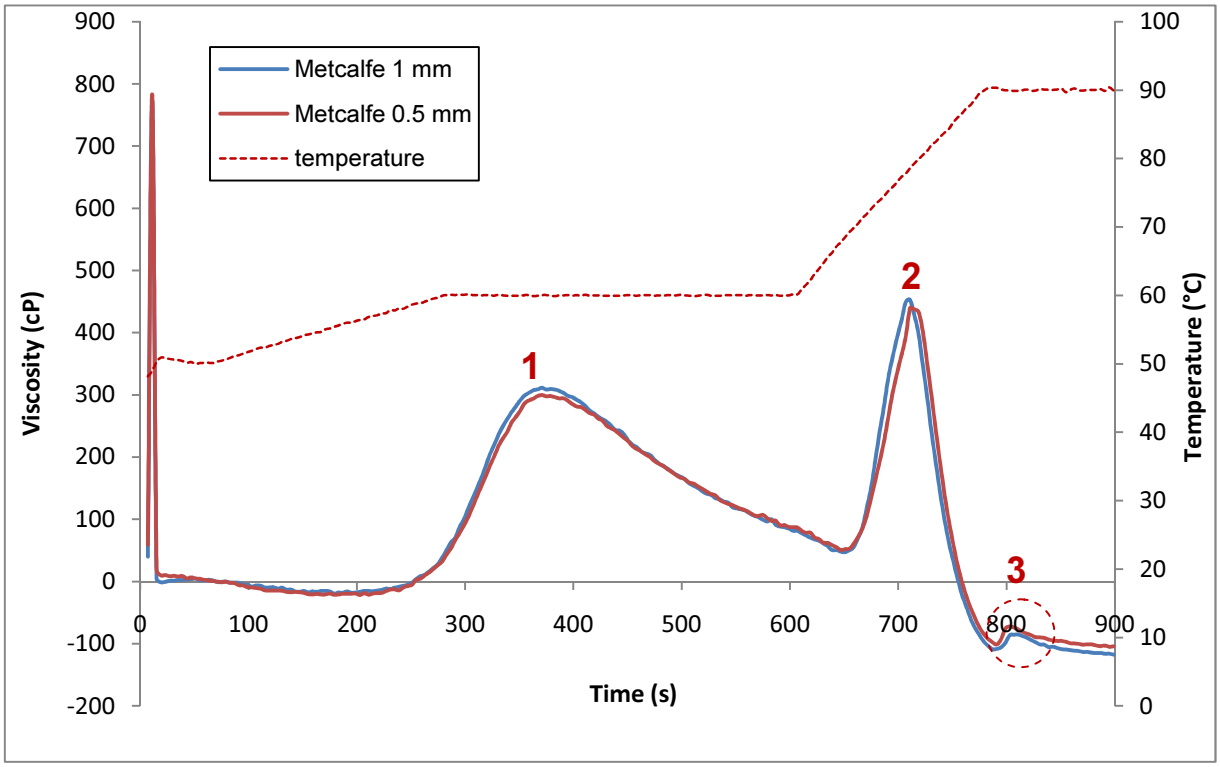

Figure 5.13 Viscograms of two different particle size distributions of the cultivar Metcalfe (replicates averaged), when decreasing the heating rate, (3rd peak indicated by circle).

<u>Samples: two different malting barley cultivars</u>

Only two malt samples were investigated which differed in malt fermentability degree to the greatest extent (Metcalfe and SSG 506). The first peak formed seemed to discriminate between different degrees of fermentability (Fig. 5.14). However, due to prolonged enzyme activity lower peaks were produced, thus the degree of discrimination was notably less

compared to results obtained from the time/temperature profile according to the Kilned Malt Method. The second and third peaks appeared fairly similar for both cultivars, indicating rheological differences (due to enzyme activity, starch gelatinisation or even the physical structure of the granules, i.e. modification) between these peaks were cultivar independent.

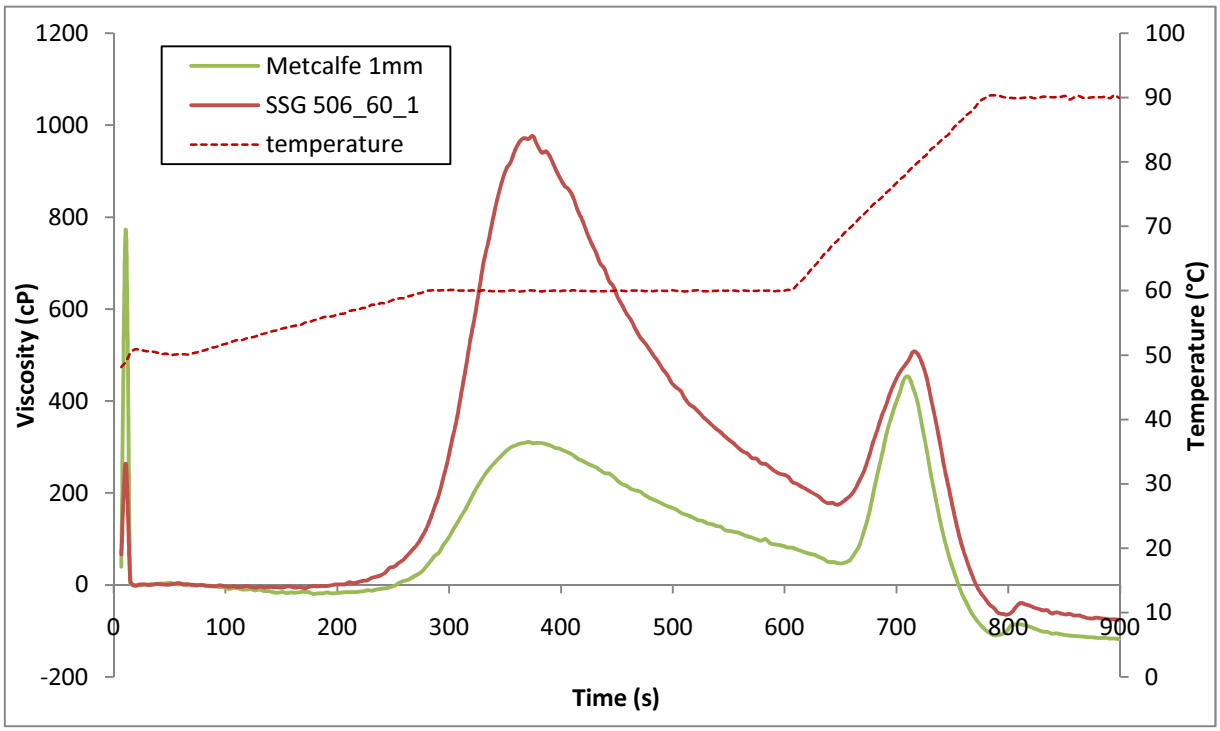

Figure 5.14 Viscograms of 2 different malt cultivars (replicates averaged), when using a RVA time/temperature profile in which the heating rate was decreased.

Effect of repeated milling on a malt sample

The milling action of the RETSCH mill probably inflicts very little starch damage on a sample and consequently was expected to be undetected by means of rheological measurement.

A clear viscosity difference was observed for all rheological variables recorded after repeated milling (Fig. 5.15). Peak height was reduced, while initial and trough viscosity increased. This was in agreement with the results published by Leman *et al.* (2006), in which, the effect of amylase addition (α-, β- and maltogenic amylase were obtained from *Bacillus subtilis*, sweet potatoes and *Bacillus stearothermophilus*, respectively) to damaged wheat starch and native wheat starch was compared. Samples with a larger degree of starch damage are known to be more susceptible to enzymic hydrolysis. However, the onset of pasting/gelatinisation remains unaltered (Morrison & Tester, 1994; Leman *et al.*, 2006).

Mechanically-damaged starch swells considerably more than native starches, delivering a noticeably higher initial viscosity during rheological analysis (Mahasukhonthachat *et al.*, 2010). Thus, the higher initial viscosity (between 0 to 2 min) for the repeatedly milled sample

was a clear indication of starch damage (Fig. 5.15). The peak incline was largely unaffected between the two samples. This indicated the degree of starch damage was not responsible for the different incline rates observed under the high heating rates and malt concentration conditions, but rather that particle size reduction was the cause of viscosity deviation. Consequently, milling with a finer sieve size during centrifugal milling did not produce a greater level of starch damage as was the case for repeated milling.

The trough viscosity (illustrated by the 350-700 s time frame in the viscogram) was slightly lower for the malt sample containing a lower degree of starch damage, but similar final viscosities were obtained (Fig. 5.15). This supports the assertion; the decrease during breakdown and final viscosity was due to a decrease in particle size rather than degree of starch damage.

Additional analytical testing is required to confirm this conclusion. The degree of starch damaged can be measured using extraction procedures (“Blue Value”), an amperometric method (i.e. an iodine absorption method by means of the Chopin Rapid FT instrument) (Dubat, 2004), dye-staining procedures, NIR procedures (Osborne & Douglas, 1981), and enzyme digestion procedures (i.e Megazyme assay procedure). Of these, the enzyme digestion procedures is preferred. Only starch granules which were enzymatically or mechanically damaged during malting or milling, respectively, are hydrolysed below the temperature of gelatinisation (Eßlinger, 2009). Therefore enzyme procedures should not be used to compare starch damage between different malt batches, as a slight difference in the extent of modification (during malting) would produce different degrees of starch damage, irrespective of mechanical damage (during milling). The degree of starch damage of the same malt sample, in which particle size distribution differs, can be compared by means of a Megazyme assay procedure.

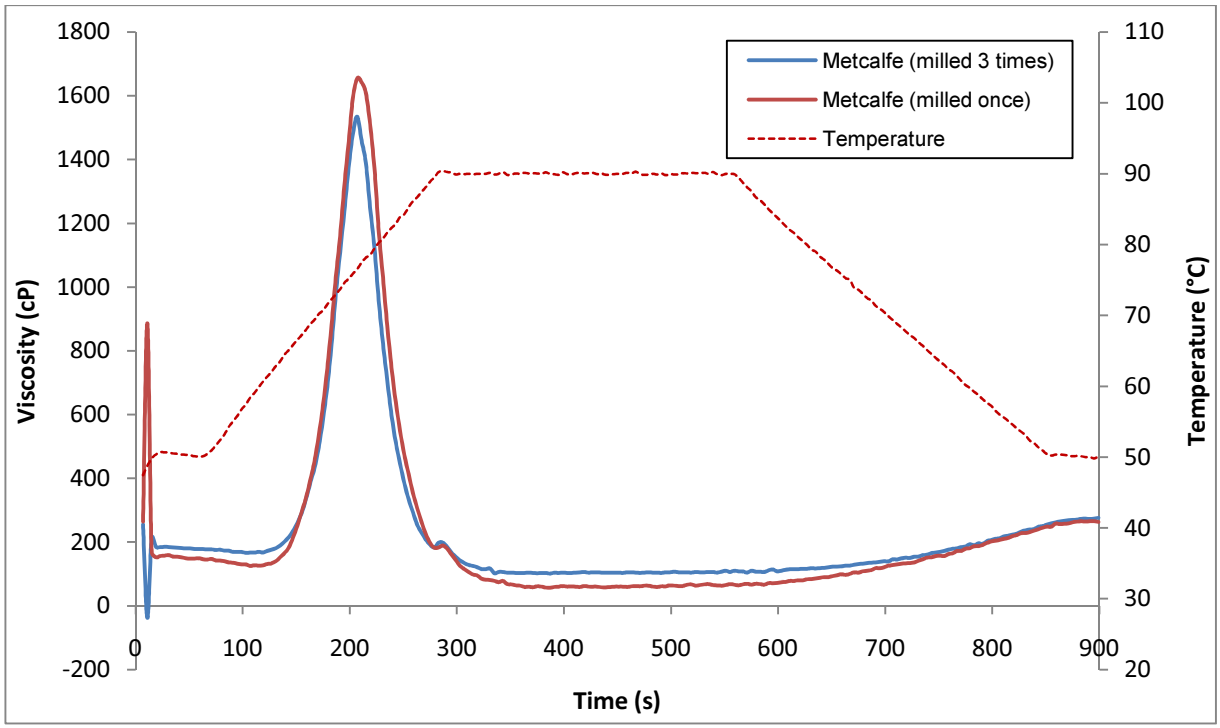

Figure 5.15 Viscograms of two Metcalfe samples which differed in the amount of times milled, illustrating the effect of starch damage.

Malt-water pH measurement

The malt concentration did not have a large effect on pH, but as expected, the corresponding temperature did (Table 5.5). After conducting RVA analysis, pH seemed to increase, probably due to a short period of proteolysis (Bamforth, 2001). The pH fluctuated slightly between different malt samples. This was expected as pH is influenced by several factors, such as protein and free amino nitrogen content, as well as the malting regime (steeping, germination, degree of malt modification and kilning). Results substantiated the statement of Schur (1980); that pH need not be corrected as it usually occurs in a range of 5.4 to 5.7.

Table 5.5 pH of malt-water mixtures before and after RVA analysis

Malt sample	Before RVA run		After RVA run (allowing time to cool)	
	pH	Temp (°C)	pH	Temp (°C)
Alrode Piece 138	5.79	22.9	5.88	22.1
Alrode Piece 138 (at higher malt concentration)	5.79	23.5	-	-
Alrode Piece 141	5.57	25.1	5.63	25.7
Alrode Piece 142	5.59	27.0	5.70	29.2
Alrode Piece 142 (at higher malt concentration)	5.63	26.7	-	-

Conclusion

Rheological measurements are well configured to test overall quality of samples containing a large amount of starch such as grains. Therefore, it is ideal to analyse the condition of a malt-water sample and to give greater insight into various chemical, physical and biochemical reactions taking place. The RVA is a rotational viscometer which allows the alteration of operational conditions. It is especially well suited for exploratory research into rheological response to varying conditions.

Time/temperature profile, heating rate, enzyme activity and sample preparation all had a rheological effect. As a result of centrifugal milling; particle size reduction was the primary cause of viscosity differences between samples. Starch damage only played a role after repeated milling, illustrating the centrifugal mill's gentle action and compatibility with RVA analysis by minimising starch damage.

In all the RVA measurements conducted, a viscosity increase (ca. 17-24 cP) after the main peak was observed. This could have been due to secondary starch gelatinisation of smaller B-type starch granules. However, due to its height being unaffected by the extent of enzyme action (eg. when decreasing the heating rate), cultivar, enzyme activity, degree of starch damage, it was suspected to be due to some other unknown occurrence, such as amylose-lipid complex dissociation.

Further rheological testing can be conducted to see if the viscosity increase after the main peak was due to amylose-lipid dissociation. The amylose-lipid dissociation is a reversible process, however gelatinisation is not. A malt-water sample can merely be

analysed by the RVA twice using the current time/temperature profile of the Kilned Malt method (allowing time to cool before the second rheological analysis). If a small peak is once again observed at a high temperature (ca. 90°C) it is most likely due to the amylose-lipid complex formation.

It is of great benefit to use a single, inexpensive, routine analysis method to measure the effect of various factors. Small adjustments of analysis conditions can bring about great rheological changes. This proved to affect sample discrimination, but also deliver additional information on a sample's biochemical and physical properties.

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Chapter 6

General discussion and conclusion

Chapter 6
General discussion and conclusion

The brewing process of South African beers can range from a German lager beer type to a North American type, which requires a substantial amount of solid maize adjunct, i.e. 40% of total mash (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). For efficient fermentation during high adjunct brewing, highly fermentable malt is needed (Hough, 1985; Edney, 1999). Such malting barley cultivars are not commercially available in South Africa and are imported from countries such as Canada and Australia (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). These cultivars are expensive compared to local cultivars, mainly due to transportation costs, availability and global demand [e.g. imported barley: R2 150(ZAR) per tonne with additional cost of R350 to R500 (ZAR) per tonne for transportation from Vancouver to Cape Town; local barley: R2 300 to R2 400 (ZAR) per tonne, exchange rate: $1(USD) = R7.14 (ZAR)]. Therefore, it would be beneficial for local barley breeding programmes to breed malting cultivars having a range of malt fermentability to expand the local beer industry, while eliminating high transportation costs.

The fermentability of barley lines, evaluated in a breeding programme, must be accurately determined to ensure released commercial cultivars deliver the expected fermentability performance in the brewhouse. Different malts are generally blended, prior to brewing, to comply to required brewing specifications (Wainwright, 1997; Briggs *et al.*, 2004). The fermentability of different malt batches of the same cultivar can deliver a range of values due to both environmental differences (Kenn *et al.*, 1993) and the malting process (Gunkel *et al.*, 2002; Briggs *et al.*, 2004). Therefore, of equal concern is routine malt fermentability evaluation of different batches and/or blends in the malting industry. Fermentability is currently assessed by the local malting industry with an adapted fermentability test. This test is considered too long and labour intensive to be used during rapid screening or quality evaluation and therefore its application was removed from local breeding programmes (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). The standard EBC malt analyses (wort viscosity, free amino nitrogen (FAN), diastatic power and fine grind extract, β-glucans, Kolbach Index and more) is also carried out, but does not give the brewer or the breeder information on brewing performance (Dr I Meijering, Chief Maltster, SAB Maltings Pty Ltd, Caledon, South Africa, Personal communication, 2009). Current analyses often indicate cultivars and blends, or in some instances breeding lines to have a high malt fermentability. However, only after being brewed commercially are fermentability or other brewing inadequacies revealed (Cook, 1962;

MacGregor & Bhatty, 1993; Wainwright, 1997); Mr P van der Vyver, SAB, Cape Town, South Africa, Personal communication, 2009). Inadequate prediction of malt fermentability or a lack of warning to potential fermentability problems causes downstream production losses within the local brewing industry (such as alcohol yield and vessel occupancy). It is therefore important to accurately predict malt fermentability of barley breeding lines, existing cultivars and blends in relation to actual fermentability experienced during commercial brewing. At present, several malting barley cultivars have been developed in South Africa containing a high malt fermentability, but still not equivalent to highly fermentable malt imported from North America.

The Rapid Visco Analyser (RVA) is a type of rotational viscometer, which possess heating, cooling and variable shear capabilities (Agu *et al.*, 2006). Its methodology is based on the Searle system which implies the stirrer, which is also the sensor element, rotates at a defined speed within a stationary container (Naé, 1993). It is advantageous over similar viscometers as it uses smaller sample sizes and shorter analysis times, while varying operating conditions (i.e. time and temperature profile, rotational speed and sample condition) (Mijland *et al.*, 1999; Mariotti *et al.*, 2005; Zhou & Mendham, 2005). The RVA has been shown to possess the required sensitivity to measure important rheological changes experienced during gelatinisation, retrogradation and enzyme hydrolysis, allowing quality discrimination amongst starch-based samples (Batey & Curtin, 2000; Goode *et al.*, 2005; Goode & Arendt, 2006; Leman *et al.*, 2006; Agu *et al.*, 2007). Of particular interest is its capability to monitor barley and malt quality, enabling quality selection within a barley breeding programme (Glennie Holmes, 1995b; Glennie Holmes, 1995c; Allan *et al.*, 1997; Dunn *et al.*, 1997; Stuart *et al.*, 1998; Zhou & Mendham, 2005).

A promising correlation was observed between rheological measurements of malt and laboratory measured malt fermentability, i.e. apparent attenuation limit (AAL). This served to highlight the hopes of replacing unreliable malt fermentability testing with rheological measurement of malt, using the RVA, which could potentially prevent substantial production losses. In this research rheological measurement by means of the RVA was investigated as a method potentially able to discriminate among different degrees of malt fermentability as experienced in a laboratory fermenter or the local brewery. Firstly, a standard RVA procedure was optimised to deliver maximum malt fermentability discrimination. Secondly, using the previously identified sample conditions a multivariate regression model was developed; allowing future prediction of malt fermentability (laboratory and brewhouse) solely by analysing the malts' rheological properties. Finally, a preliminary investigation was conducted to establish the effect of experimental conditions on rheological measurements.

Sample concentration and particle size were varied using five different malt to water ratios and two different sieve sizes (delivering two different flour particle size distributions), respectively. The highly concentrated malt:water ratio of 1:1.5 merely served as an experimental upper limit as previous studies indicated such a high malt concentration would produce viscograms with an irregular trace (Glennie Holmes, 1995a). Unexpectedly this ratio delivered the best malt fermentability discrimination. Increasing the malt concentration above 1:1.5 should be investigated further, perhaps a higher malt concentration could deliver even beter fermentability discrimination. This would also help to establish the upper concentration limit after which further malt addition delivers a negative effect on rheological results. Data were analysed by both principal component analysis (PCA) and analysis of variance (ANOVA), allowing the comparison of multivariate to univariate data analysis techniques, respectively. For ANOVA conventional viscogram variables along with newly created variables were used. Conventional regressions between RVA data and malt analyses (such as AAL) were conducted using conventional viscogram variables. Results indicated new variables, never before considered in literature, such as “peak width:peak height” and “time at peak 2” to possess the ability of malt fermentability discriminate. This proves the advantage of multivariate techniques, as all variables generated are considered and the model finds those variables of particular importance able to explain the subject of interest. Results indicated varying particle size distribution affected all the cultivars to the same degree and therefore did not deliver greater malt fermentability discrimination. Multivariate data analysis enables future prediction which is a fundamental advantage above univariate techniques.

Even though it was possible to distinguish between different degrees of malt fermentability, in order to use this information in the breeding and/or malting industry, the results had to be properly validated. For this reason, regression was applied (in the form of partial least squares regression) to determine whether good discrimination between malt fermentability could be estimated. Rheological data obtained by the RVA were regressed with both apparent AAL and FAN, independently. Calibration models developed were validated by random test set and segmented cross-validation for AAL and FAN, respectively. The strictest form of validation still delivered a strong correlation between RVA analysis and AAL (r^2=0.84). This was higher than the correlation obtained between AAL (determined as described by (De Clerck, 1957) and final viscosity and peak area as determined by the Brabender-Viscograph (Yoshida & Yamada, 1970). However, FAN delivered a weak correlation (r^2=0.35) and should not be used for prediction purposes. In addition, a multivariate classification model, i.e. Soft Independent Modeling of Class Analogy (SIMCA), was developed based on different malt fermentability classes. These classes were identified

by means of exploratory PCA in conjunction with malt fermentability information provided by local maltsters and brewers. Simulated blends were developed after prediction could not be verified due to the lack in available sample information. Results suggested a malt sample with high fermentability should not be blended with more than 20% of a sample having low fermentability, as further addition will cause an overall intermediate malt fermentability. The models generated displayed great potential for future application, but for practical implementation in the breeding, malting and brewing industry further work is required. Regression and classification models built were based on a small sample set; thus for future work the sample set must be extended, in order to generate more representative classification models and regression statistics (r^2, RMSEC/P, SEC/P and bias). These malt samples must cover a wider range of AAL values (e.g. 60 to 90%), while having detailed sample information about factors which greatly influence pasting properties; such as the presence/extent of physical damage, starch content, nonstarch polysaccharides, phytochemicals (i.e. tannins) and starch gelatinisation temperature. Laboratory measurement of malt fermentability must generate results which are comparative (fermentability between different malt samples), but also representative (performance in brewhouse) if it is to be used as the Y-reference method. This implies having the correct order of samples with regard to fermentability degree during actual brewing conditions, thereby allowing ranking but not necessarily the exact numerical value or range of fermentability. Alternatively, current laboratory mashing regimes used before attenuation testing can be changed to yield similar AAL values to that experienced in the brewhouse (Calman *et al.*, 2008). Current research (Chapter 3) identified the ability of rheological peak variables to discriminate amongst malt samples differing in fermentability. For future work, only these rheological peak variables should be correlated with malt fermentability and compared to current correlations found in Chapter 4 (which used all the rheological variables recorded). If similar relationships are found, the current RVA method can be shortened substantially (with regards to analysis time) to exclude trough and final viscosity measurement.

Different experimental conditions were used during RVA analysis, i.e. instrument model; time and temperature profile, heating/cooling rate and enzyme activity. Current work (Chapter 3) considered different sample conditions (malt concentration and particle size distribution) which differed from preliminary investigation conducted (Chapter 5). The cause of interesting rheological occurrences identified in Chapter 3 was further investigated by varying experimental conditions. Additionally the effect of these conditions on malt fermentability discrimination were also examined. Conditions were varied on an individual basis and the subsequent effect on the rheological responses of different malt samples assessed. Rheological measurement using the RVA 3D+ gave similar results to the RVA-4,

indicating adequate sensitivity of the former instrument for discrimination purposes. This also implicates inter-instrument agreement/transferability. Thus similar instrument types but of different versions are able to measure the same values, allowing the use of prediction models between different instrument versions. Inactivating malt enzymes, prior to RVA analysis, delivered useful biochemical information of malt samples with regards to their amount of extract. Varying the heating rate delivered useful physical information (i.e. interaction between particle size and concentration ratio) and its effect on biochemical reactions (i.e. gelatinisation and enzyme hydrolysis). The heating rate also influenced the degree of malt fermentability discrimination. The amount of starch damage inflicted on a malt sample during milling was not affected by the sieve size used. However, additional damage was caused after the sample was milled repeatedly with a centrifugal mill. As the measured pH was always within the range normally observed during mashing, adjustment was unnecessary during RVA analysis and therefore its rheological effect was not investigated. It is of great benefit to use a single, inexpensive, routine method of analysis to measure the effect of various factors. Minor adjustment to analysis conditions caused large changes to the recorded rheological measurements. Experimental conditions should only be altered after careful consideration, as results become difficult to interpret when varying more than one factor at a time. In this study, calculated variation of conditions delivered insightful information.

Near-infrared spectroscopy (NIRS) have been used to predict rheological variables recorded by the RVA (Meadows & Barton, 2002; Juhasz *et al.*, 2005). For future work it is suggested to correlate NIRS with RVA rheological variables recorded which could allow substituting RVA rheological measurement with NIR spectroscopic techniques. If strong correlations are found after proper model validation, this could allow rapid non-destructive analysis of malt fermentability which is ideal for barley breeding programmes.

Rheological measurement by means of the RVA has shown the ability to measure malt fermentability. Fermentability of malt is a complex characteristic and cannot be sufficiently assessed by means of a single measurement. For example important factors impacting on fermentability, such as FAN content, cannot be measured by means of rheology. Therefore, additional complementary analyses must be used in conjunction with the RVA in order to fully describe malt fermentability. The application of multivariate data analysis is a well-matched statistical technique applied to rheological data and delivers more relevant information than traditional univariate techniques. Rheological measurement has proved to be a vital technique in fermentability assessment and it is highly recommended to be incorporated within the breeding, malting and brewing industries. The simplistic approach of measuring viscosity changes of a malt-water mixture unravelled intricate interactions of various physical

and biochemical factors, enabling the RVA an holistic overview able to describe complex quality attributes such as malt fermentability.

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APPENDICES

APPENDIX 1

Table 1.1 Malt characteristics explaining problems experienced (Potgieter, 2009; Potgieter & Meijering, 2009; Van der Vyver, 2009) Smit, 2010 personal communication

Cultivars	Characteristics	Pedigree
Metcalfe	North-American (Canada) barley cultivar, excellent quality, highly fermentable malt (HFM), expensive.	Oxbow/Manley
Flagship	Flagship (Australia) has good malting quality drawn from European and Canadian genetics, crossed to a robust Australian feed cultivar. A highly fermentable malt (HFM).	Chieftan/Barque//Manley/VB9104
SSG 564	Local barley cultivar currently in production in the Southern Cape (dry-land) region. It is seen as a type of "HFM", but has a lower extract than the imported equivalent and is thus not an equal replacement. Problems are experienced with dormancy and there are fermentability issues especially in the case of Carling Black Label. Good agronomical characteristics.	Triumph/Schooner
SSG 506	Local barley cultivar phased out in 2008 and produced in the Southern Cape (dry-land) region. It is described as having poor quality, being extremely dormant, and needs an extra 12 hours of processing time during malting. Good agronomic characteristics: good plumpness and straw strength.	Psaknon/2*Dampier//Schooner/3/CI 3097/Triumph
PUMA	Local barley cultivar produced in the Northern Cape (irrigation), contains a high % of small starch granules thus needs longer mashing times to ensure gelatinisation and conversion. High quality cultivar with very good fermentability and high enzyme levels which produces low viscosity worts (low β-glucan).	A cultivar of Agricultural Research Council (ARC) with German origin
SSG 585	Local barley cultivar withdrawn before final release. Production is mainly Northern Cape (irrigation) region. It requires long brewhouse cycle (mashing) times. May give high FANs. Similar to PUMA.	Psaknon/2*Dampier//Clipper/3/Triumph/4/Clipper

Table 1.2 Malt analysis sheet

Variety	TN[1] (dry)	TSN[2]	KI[3]	FAN[4]	VIS[5]	FGE[6] (as is)	FGE[6] (dry)	FRIAB[7]	WUGs[8]	PUGs[9]	BET1[10]	MOIST[11]	COL[12]	PUGS/ WUGS	FAN:TSN
SSG 564	1.49	0.63	42	151	1.56	76.0	79.6	93.3	0.06	0.22	190	4.5	4.5	0.28	24.0
SSG 506	1.71	0.77	45	184	1.51	77.2	80.8	88.3	0.00	0.64	224	4.5	4.2	0.64	23.9
Metcalfe	2.00	0.83	42	180	1.52	75.7	79.6	65.1	2.50	4.64	207	4.8	4.7	7.14	21.7
SSG 585	1.83	0.91	50	232	1.53	78.0	81.7	-[a]	2.26	2.90	306	4.5	9.7	-	25.5
PUMA-H	1.70	0.77	45	191	1.47	78.7	82.2	-	0.06	0.28	99	4.3	4.2	-	24.8
SABM MEAN	**1.71**	**0.77**	**42.9**	**191**	**1.51**	**-**	**80.0**	**91.8**	**0.21**	**0.34**	**186.3**	**4.94**	**3.8**	**-**	**-**

[a]Some values missing

[1]The total nitrogen content on dry basis (0% malt moisture content)

[2] The total soluble nitrogen content, related to total protein; which represents all the nitrogenous matter in the malt, including insoluble forms

[3]The Kolbach Index is a ratio of soluble/total nitrogen and indicates the degree of proteolysis. The higher the number, the more highly modified the malt.

[4]Free amino nitrogen content indicates the availability of nitrogen compounds (mainly amino acids and small peptides).

[5]Viscosity of wort is a measure of the breakdown of ß-glucans (endosperm cell walls) during malting, indicating the degree of run off during sparging.

[6]Fine grind extract indicates the maximum soluble yield possible for a malt; relatively indicating the solubility of the malt and the husk and protein content

[7] Friability which indicates the degree of malt modification and therefore the ability to crumble when subjected to crushing. It is related to mealiness of malt.

[8]Whole unmodified grains: glassy kernels

[9]Partly unmodified grains

[10]Beta-glucan content indicates the degree of malt modification, as they are present within the cell walls and must be hydrolysed by means of β-glucanases

[11]Moisture content of malt

[12]Colour of malt measured according to a visual method developed by the European Brewing Convention.

Table 1.3 Mean values (± standard deviation) for the variable: **TIME AT PEAK 1**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	209 ± 3 **a**	174 ± 2 **b**	166 ± 2 **c**	165 ± 2 **cd**	163 ± 2 **de**
Flagship	190 ± 2 **s**	163 ± 2 **em**	157 ± 2 **n**	157 ± 2 **no**	155 ± 2 **o**
SSG 585	213 ± 4 **r**	179 ± 3 **l**	171 ± 2 **gi**	171 ± 2 **hi**	169 ± 2 **jk**
PUMA	209 ± 3 **af**	179 ± 3 **l**	170 ± 2 **hij**	169 ± 2 **hjk**	166 ± 2 **c**
SSG 564	211 ± 3 **f**	181 ± 4 **p**	173 ± 2 **bg**	170 ± 2 **hij**	168 ± 0 **k**
SSG 506	203 ± 2 **q**	179 ± 7 **l**	165 ± 2 **c**	162 ± 2 **em**	161 ± 2 **m**

Table 1.4 Mean values (± standard deviation) for the variable: **PEAK HEIGHT 1**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	1636 ± 61 **o**	493 ± 45 **a**	243 ± 18 **bc**	131 ± 5 **de**	80 ± 3 **d**
Flagship	1772 ± 38 **t**	580 ± 40 **j**	279 ± 9 **bh**	155 ± 3 **ei**	95 ± 3 **di**
SSG 585	2592 ± 119 **m**	689 ± 62 **n**	340 ± 22 **hk**	190 ± 10 **cel**	126 ± 4 **dil**
PUMA	2619 ± 128 **m**	740 ± 49 **n**	361 ± 24 **k**	201 ± 3 **ceg**	128 ± 4 **de**
SSG 564	3987 ± 256 **p**	1056 ± 92 **q**	502 ± 58 **af**	273 ± 20 **bgh**	162 ± 8 **ei**
SSG 506	4513 ± 249 **r**	1205 ± 62 **s**	568 ± 41 **fj**	312 ± 18 **bhk**	186 ± 4 **cel**

Table 1.5 Mean values (± standard deviation) for the variable: **TEMPERATURE AT PEAK 1**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	77 ± 0.49 **a**	70 ± 0.47 **b**	69 ± 0.42 **c**	69 ± 0.30 **cd**	69 ± 0.33 **de**
Flagship	73 ± 0.40 **p**	68 ± 0.45 **e**	67 ± 0.30 **n**	67 ± 0.25 **n**	67 ± 0.29 **n**
SSG 585	77 ± 0.67 **g**	71 ± 0.55 **hm**	70 ± 0.31 **ik**	70 ± 0.32 **ik**	70 ± 0.26 **j**
PUMA	77 ± 0.55 **af**	71 ± 0.56 **m**	70 ± 0.31 **jk**	70 ± 0.28 **jk**	69 ± 0.33 **cl**
SSG 564	77 ± 0.56 **fg**	72 ± 0.69 **h**	70 ± 0.36 **bi**	70 ± 0.35 **jk**	69 ± 0.06 **jl**
SSG 506	76 ± 0.42 **o**	71 ± 1.16 **m**	69 ± 0.38 **c**	68 ± 0.35 **e**	68 ± 0.41 **e**

Table 1.6 Mean values (± standard deviation) for the variable: **PASTING TEMPERATURE (NEWPORT)**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	63 ± 0.42 **a**	64 ± 0.21 **bc**	64 ± 0.23 **d**	65 ± 0.15 **e**	65 ± 0.31 **f**
Flagship	62 ± 0.24 **il**	63 ± 0.29 **l**	63 ± 0.20 **j**	63 ± 0.32 **k**	64 ± 0.21 **ab**
SSG 585	65 ± 0.37 **e**	64 ± 0.14 **e**	65 ± 0.21 **e**	65 ± 0.23 **h**	65 ± 0.23 **f**
PUMA	64 ± 0.37 **e**	65 ± 0.30 **e**	64 ± 0.13 **e**	65 ± 0.19 **h**	65 ± 0.09 **f**
SSG 564	64 ± 0.50 **dg**	64 ± 0.26 **dg**	64 ± 0.22 **g**	64 ± 0.09 **e**	65 ± 0.15 **h**
SSG 506	62 ± 0.34 **i**	63 ± 0.18 **j**	63 ± 0.10 **k**	64 ± 0.13 **ab**	64 ± 0.15 **cd**

Table 1.7 Mean values (± standard deviation) for the variable: **PASTING TEMPERATURE (ZHOU)**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	66 ± 1.40 **ab**	65 ± 0.29 **ac**	66 ± 0.55 **abd**	– [a]	–
Flagship	64 ± 0.32 **cdgh**	64 ± 0.20 **g**	59 ± 6.95 **n**	–	–
SSG 585	67 ± 0.70 **b**	66 ± 0.31 **abd**	66 ± 0.19 **abf**	–	–
PUMA	67 ± 0.67 **be**	66 ± 0.41 **abd**	66 ± 0.23 **abfh**	67 ± 0.23 **ab**	–
SSG 564	66 ± 0.84 **ab**	65 ± 0.35 **ace**	65 ± 0.27 **ace**	66 ± 0.27 **abf**	–
SSG 506	65 ± 0.64 **cdfg**	64 ± 0.30 **cg**	64 ± 0.24 **cdg**	65 ± 0.20 **cdfg**	66 ± 0.33 **abfh**

[a] Some means not estimable

Table 1.8 Mean values (± standard deviation) for the variable: **AREA UNDER PEAK**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	95129 ± 5284 **k**	33511 ± 2099 **a**	14225 ± 156 **b**	7292 ± 403 **cd**	4432 ± 438 **e**
Flagship	98609 ± 2956 **p**	35597 ± 2067 **a**	15436 ± 228 **b**	8585 ± 191**ch**	5510 ± 378 **de**
SSG 585	138604 ± 8474 **o**	45712 ± 2724 **i**	20112 ± 1094 **j**	10833 ± 402 **h**	7143 ± 314 **cd**
PUMA	131726 ± 7057 **n**	45075 ± 2150 **i**	19594 ± 665 **j**	10284 ± 604 **h**	6594 ± 315 **cde**
SSG 564	190486 ± 14882 **l**	64059 ± 4973 **f**	27513 ± 2505 **g**	14436 ± 336 **b**	8743 ± 364 **ch**
SSG 506	195382 ± 14307 **m**	65549 ± 2071 **f**	27417 ± 1261 **g**	14252 ± 431 **b**	8790 ± 254 **ch**

Table 1.9 Mean values (± standard deviation) for the variable: **PEAK HEIGHT**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	58 ± 3 **ab**	68 ± 3 **o**	59 ± 4 **a**	56 ± 3 **cd**	56 ± 4 **cde**
Flagship	56 ± 2 **cde**	61 ± 1 **g**	55 ± 2 **cen**	56 ± 2 **cen**	58 ± 2 **ab**
SSG 585	53 ± 2 **ij**	67 ± 2 **q**	59 ± 2 **ah**	57 ± 2 **bd**	57 ± 3 **bdn**
PUMA	50 ± 1 **m**	61 ± 2 **g**	54 ± 3 **ceij**	51 ± 3 **m**	52 ± 2 **km**
SSG 564	48 ± 2 **f**	61 ± 1 **gh**	55 ± 2 **cei**	53 ± 3 **jk**	54 ± 3 **eij**
SSG 506	43 ± 2 **p**	54 ± 1 **ceij**	48 ± 2 **f**	46 ± 2 **l**	47 ± 1 **fl**

Table 1.10 Mean values (± standard deviation) for the variable: **PEAK WIDTH (time) : PEAK HEIGHT**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	0.0306 ± 0.0021 **ab**	0.1288 ± 0.0178 **c**	0.2113 ± 0.0350 **d**	0.3400 ± 0.0282 **m**	0.5715 ± 0.0334 **n**
Flagship	0.0270 ± 0.0017 **abl**	0.0942 ± 0.0089 **g**	0.1722 ± 0.0157 **i**	0.2991 ± 0.0138 **q**	0.5175 ± 0.0176 **r**
SSG 585	0.0183 ± 0.0008 **be**	0.0920 ± 0.0122 **g**	0.1577 ± 0.0151 **h**	0.2583 ± 0.0299 **j**	0.3640 ± 0.0284 **p**
PUMA	0.0166 ± 0.0009 **el**	0.0750 ± 0.0074 **k**	0.1324 ± 0.0164 **c**	0.2146 ± 0.0185 **d**	0.3268 ± 0.0236 **o**
SSG 564	0.0104 ± 0.0009 **e**	0.0511 ± 0.0055 **f**	0.0977 ± 0.0144 **g**	0.1634 ± 0.0229 **hi**	0.2689 ± 0.0298 **j**
SSG 506	0.0083 ± 0.0006 **e**	0.0385 ± 0.0032 **af**	0.0730 ± 0.0083 **k**	0.1215 ± 0.0155 **c**	0.2028 ± 0.0088 **d**

Table 1.11 Mean values (± standard deviation) for the variable: **PEAK WIDTH (temperature) : PEAK HEIGHT**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	0.0055 ± 0.00040 **ab**	0.0229 ± 0.0033 **c**	0.0374 ± 0.0062 **d**	0.0605 ± 0.0051 **m**	0.1020 ± 0.0055 **n**
Flagship	0.0048 ± 0.0003 **abl**	0.0168 ± 0.0016 **g**	0.0307 ± 0.0029 **i**	0.0531 ± 0.0021 **q**	0.0931 ± 0.0040 **r**
SSG 585	0.0033 ± 0.000 **be**	0.0165 ± 0.0022 **g**	0.0280 ± 0.0025 **h**	0.0457 ± 0.0048 **j**	0.0643 ± 0.0056 **p**
PUMA	0.0030 ± 0.0002 **el**	0.0134 ± 0.0013 **k**	0.0236 ± 0.0029 **c**	0.0382 ± 0.0032 **d**	0.0580 ± 0.0038 **o**
SSG 564	0.0019 ± 0.0002 **e**	0.0090 ± 0.0009 **f**	0.0175 ± 0.0026 **g**	0.0290 ± 0.0042 **hi**	0.0476 ± 0.0056 **j**
SSG 506	0.0015 ± 0.0001 **e**	0.0069 ± 0.0006 **af**	0.0130 ± 0.0016 **k**	0.0214 ± 0.0028 **c**	0.0360 ± 0.0019 **d**

Table 1.12 Mean values (± standard deviation) for the variable: **TIME AT TROUGH 1**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	277 ± 2 **ab**	260 ± 3 **cd**	245 ± 3 **ef**	238 ± 2 **eg**	235 ± 3 **g**
Flagship	277 ± 2 **ab**	262 ± 2 **cdl**	248 ± 3 **fj**	240 ± 1 **ef**	248 ± 31 **fj**
SSG 585	287 ± 2 **hm**	270 ± 2 **ail**	255 ± 1 **dj**	247 ± 1 **fn**	241 ± 1 **efg**
PUMA	281 ± 1 **bm**	268 ± 3 **ci**	255 ± 1 **djn**	257 ± 14 **d**	258 ± 18 **dk**
SSG 564	291 ± 2 **h**	273 ± 2 **abi**	260 ± 2 **cd**	249 ± 3 **fj**	243 ± 2 **efg**
SSG 506	271 ± 1 **ai**	265 ± 2 **cik**	271 ± 13 **ai**	272 ± 20 **ai**	270 ± 7 **ail**

Table 1.13 Mean values (± standard deviation) for the variable: **VISCOSITY AT TROUGH 1**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	86 ± 14 **l**	40 ± 5 **a**	18 ± 2 **b**	10 ± 1 **cd**	– [a]
Flagship	55 ± 6 **k**	25 ± 3 **h**	13 ± 1 **cdi**	9 ± 1 **cde**	5 ± 3 **e**
SSG 585	55 ± 13 **k**	42 ± 2 **af**	23 ± 3 **gh**	17 ± 1 **bi**	13 ± 1 **cdi**
PUMA	65 ± 11 **j**	38 ± 3 **a**	20 ± 2 **bg**	– [a]	–
SSG 564	45 ± 17 **f**	42 ± 8 **af**	23 ± 3 **gh**	16 ± 4 **bi**	12 ± 1 **cd**
SSG 506	65 ± 7 **j**	31 ± 2 **m**	–	–	–

Table 1.14 Mean values (± standard deviation) for the variable: **TEMPERATURE AT TROUGH 1**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	89 ± 0.28 **a**	86 ± 0.48 **b**	83 ± 0.45 **cd**	82 ± 0.40 **ef**	– [a]
Flagship	89 ± 0.28 **ai**	86 ± 0.30 **bo**	84 ± 0.50 **ck**	82 ± 0.21 **df**	83 ± 3.42 **dl**
SSG 585	90 ± 0.03 **g**	88 ± 0.32 **hm**	85 ± 0.34 **jq**	84 ± 0.17 **ckl**	83 ± 0.23 **dfr**
PUMA	90 ± 0.23 **ag**	87 ± 0.48 **mn**	85 ± 0.16 **pq**	–	–
SSG 564	90 ± 0.06 **g**	88 ± 0.41 **hi**	86 ± 0.44 **bj**	84 ± 0.39 **ck**	83 ± 0.28 **dl**
SSG 506	88 ± 0.30 **hm**	87 ± 0.44 **no**	–	–	–

[a] Some means not estimable

Table 1.15 Mean values (± standard deviation) for the variable: **TIME AT PEAK 2**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	282 ± 3 **ab**	268 ± 2 **cde**	260 ± 1 **fgh**	259 ± 4 **fh**	– [a]
Flagship	284 ± 1 **b**	271 ± 2 **e**	262 ± 3 **fgm**	259 ± 2 **h**	265 ± 14 **djm**
SSG 585	295 ± 2 **s**	277 ± 1 **ikl**	266 ± 1 **cdj**	261 ± 2 **fgh**	259 ± 2 **h**
PUMA	289 ± 2 **q**	276 ± 3 **kl**	267 ± 2 **cd**	–	–
SSG 564	299 ± 3 **n**	280 ± 2 **abi**	268 ± 2 **cde**	263 ± 1 **gj**	260 ± 1 **fh**
SSG 506	279 ± 1 **aik**	275 ± 1 **l**	–	–	–

Table 1.16 Mean values (± standard deviation) for the variable: **VISCOSITY AT PEAK 2**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	95 ± 12 **a**	52 ± 6 **bc**	28 ± 1 **de**	19 ± 2 **fg**	– [a]
Flagship	69 ± 5 **i**	38 ± 3 **s**	26 ± 2 **ekl**	19 ± 2 **fg**	12 ± 5 **h**
SSG 585	76 ± 11 **r**	53 ± 4 **bc**	33 ± 1 **j**	26 ± 1 **ekl**	20 ± 3 **fg**
PUMA	89 ± 10 **p**	49 ± 3 **c**	29 ± 3 **dej**	–	–
SSG 564	66 ± 13 **i**	54 ± 8 **b**	32 ± 2 **dj**	27 ± 1 **ek**	22 ± 1 **gl**
SSG 506	96 ± 5 **a**	43 ± 1 **m**	–	–	–

[a] Some means not estimable

Table 1.17 Mean values (± standard deviation) for the variable: **TEMPERATURE AT PEAK 2**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	90 ± 0.46 **ab**	87 ± 0.40 **cd**	86 ± 0.20 **ef**	86 ± 0.77 **e**	– [a]
Flagship	90 ± 0.12 **ab**	88 ± 0.29 **d**	87 ± 0.65 **f**	86 ± 0.43 **e**	87 ± 1.96 **f**
SSG 585	90 ± 0.11 **b**	89 ± 0.19 **hj**	87 ± 0.23 **ck**	86 ± 0.48 **ef**	86 ± 0.35 **e**
PUMA	90 ± 0.10 **b**	89 ± 0.44 **hi**	87 ± 0.24 **ck**	–	–
SSG 564	90 ± 0.08 **ab**	90 ± 0.45 **ag**	88 ± 0.35 **cd**	87 ± 0.18 **f**	86 ± 0.15 **e**
SSG 506	89 ± 0.23 **gh**	89 ± 0.23 **ij**	–	–	–

[a] Some means not estimable

Table 1.18 Mean values (± standard deviation) for the variable: **END VISCOSITY**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	201 ± 14 **n**	90 ± 4 **ab**	38 ± 15 **c**	13 ± 6 **de**	3 ± 3 **f**
Flagship	139 ± 12 **g**	61 ± 7 **i**	38 ± 1 **cl**	29 ± 1 **jm**	22 ± 2 **k**
SSG 585	89 ± 15 **b**	41 ± 5 **cl**	28 ± 1 **jm**	23 ± 1 **km**	18 ± 2 **dk**
PUMA	95 ± 15 **a**	45 ± 5 **l**	29 ± 1 **jm**	21 ± 2 **k**	11 ± 3 **e**
SSG 564	133 ± 23 **g**	66 ± 9 **hi**	36 ± 5 **c**	30 ± 2 **j**	24 ± 2 **jk**
SSG 506	161 ± 20 **o**	72 ± 3 **h**	36 ± 2 **c**	18 ± 4 **dk**	4 ± 3 **f**

Table 1.19 Mean values (± standard deviation) for the variable: **TIME AT 'a'**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	183 ± 4 **a**	156 ± 1 **b**	151 ± 1 **c**	150 ± 1 **d**	148 ± 1 **e**
Flagship	169 ± 3 **q**	148 ± 1 **e**	144 ± 1 **r**	143 ± 1 **s**	141 ± 1 **t**
SSG 585	188 ± 3 **p**	161 ± 2 **j**	156 ± 1 **b**	155 ± 1 **fk**	153 ± 1 **ghi**
PUMA	187 ± 4 **o**	161 ± 2 **j**	156 ± 1 **bk**	154 ± 1 **fgh**	153 ± 1 **i**
SSG 564	191 ± 4 **l**	163 ± 2 **m**	156 ± 1 **b**	154 ± 0 **fg**	153 ± 1 **hi**
SSG 506	184 ± 4 **a**	158 ± 2 **n**	151 ± 1 **c**	150 ± 1 **d**	148 ± 1 **e**

Table 1.20 Mean values (± standard deviation) for the variable: **TEMPERATURE AT 'a'**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	72 ± 0.81 **a**	67 ± 0.25 **bc**	66 ± 0.24 **de**	66 ± 0.11 **f**	66 ± 0.16 **g**
Flagship	70 ± 0.49 **s**	66 ± 0.17 **g**	65 ± 0.24 **t**	65 ± 0.28 **u**	65 ± 0.18 **v**
SSG 585	73 ± 0.63 **r**	68 ± 0.31 **l**	67 ± 0.16 **b**	67 ± 0.23 **chm**	67 ± 0.09 **ijk**
PUMA	73 ± 0.71 **q**	68 ± 0.43 **l**	67 ± 0.18 **bc**	67 ± 0.14 **ijm**	67 ± 0.14 **k**
SSG 564	74 ± 0.63 **n**	69 ± 0.40 **o**	67 ± 0.23 **bh**	67 ± 0.08 **ci**	67 ± 0.14 **jk**
SSG 506	72 ± 0.62 **a**	68 ± 0.40 **p**	66 ± 0.24 **d**	66 ± 0.15 **ef**	66 ± 0.17 **g**

Table 1.21 Mean values (± standard deviation) for the variable: **TIME AT 'b'**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	233 ± 2 **a**	218 ± 4 **b**	201 ± 6 **c**	194 ± 3 **de**	193 ± 4 **def**
Flagship	217 ± 1 **bg**	202 ± 3 **cm**	192 ± 4 **f**	189 ± 2 **n**	190 ± 2 **n**
SSG 585	236 ± 3 **q**	223 ± 4 **r**	209 ± 3 **s**	203 ± 4 **hm**	199 ± 3 **j**
PUMA	230 ± 3 **p**	216 ± 3 **g**	203 ± 4 **ch**	197 ± 4 **ik**	194 ± 2 **e**
SSG 564	233 ± 2 **a**	216 ± 3 **g**	204 ± 3 **h**	199 ± 3 **ij**	197 ± 3 **k**
SSG 506	221 ± 2 **o**	204 ± 4 **h**	192 ± 3 **df**	187 ± 4 **l**	186 ± 2 **l**

Table 1.22 Mean values (± standard deviation) for the variable: **TEMPERATURE AT 'b'**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	81 ± 0.28 **a**	78 ± 0.87 **b**	75 ± 1.03 **c**	74 ± 0.61 **de**	74 ± 0.59 **de**
Flagship	78 ± 0.25 **bf**	76 ± 0.47 **ck**	74 ± 0.66 **e**	73 ± 0.39 **l**	73 ± 0.43 **l**
SSG 585	82 ± 0.48 **o**	79 ± 0.63 **p**	77 ± 0.51 **q**	76 ± 0.69 **gk**	75 ± 0.55 **h**
PUMA	81 ± 0.59 **n**	78 ± 0.62 **bf**	76 ± 0.64 **gk**	75 ± 0.67 **hi**	74 ± 0.37 **d**
SSG 564	81 ± 0.44 **a**	78 ± 0.51 **f**	76 ± 0.57 **g**	75 ± 0.63 **h**	74 ± 0.65 **i**
SSG 506	79 ± 0.43 **m**	76 ± 0.70 **g**	74 ± 0.64 **de**	73 ± 0.66 **j**	73 ± 0.43 **j**

Table 1.23 Mean values (± standard deviation) for the variable: **Δ TIME**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	50 ± 3 **ab**	63 ± 3 **c**	51 ± 5 **a**	44 ± 3 **de**	46 ± 3 **df**
Flagship	48 ± 2 **knp**	54 ± 2 **ij**	48 ± 3 **knp**	46 ± 2 **fn**	49 ± 2 **abp**
SSG 585	47 ± 1 **kno**	63 ± 3 **c**	53 ± 2 **j**	49 ± 4 **bk**	46 ± 3 **dfo**
PUMA	43 ± 1 **e**	55 ± 2 **i**	47 ± 3 **kn**	43 ± 3 **eh**	42 ± 2 **ghl**
SSG 564	41 ± 2 **gh**	54 ± 1 **ij**	48 ± 2 **bk**	44 ± 3 **de**	43 ± 3 **el**
SSG 506	37 ± 2 **m**	46 ± 2 **fn**	41 ± 2 **g**	38 ± 3 **m**	38 ± 1 **m**

Table 1.24 Mean values (± standard deviation) for the variable: **Δ TEMPERATURE**

Cultivars	Mean ± standard deviation				
	Malt to water concentrations (particle size averaged)				
	1:1.5	**1:2**	**1:2.5**	**1:3**	**1:3.5**
Metcalfe	8.94 ± 0.53 **ab**	11.16 ± 0.64 **c**	9.00 ± 0.87 **a**	7.91 ± 0.56 **def**	8.13 ± 0.53 **dfg**
Flagship	8.55 ± 0.38 **jlo**	9.71 ± 0.31 **im**	8.54 ± 0.54 **jlo**	8.20 ± 0.24 **fgn**	8.84 ± 0.35 **abo**
SSG 585	8.51 ± 0.26 **jln**	11.25 ± 0.58 **c**	9.46 ± 0.38 **i**	8.65 ± 0.59 **bj**	8.07 ± 0.52 **dfg**
PUMA	7.86 ± 0.22 **de**	9.86 ± 0.34 **m**	8.46 ± 0.53 **jln**	7.66 ± 0.57 **eh**	7.41 ± 0.36 **h**
SSG 564	7.45 ± 0.35 **h**	9.48 ± 0.16 **i**	8.67 ± 0.43 **bj**	7.83 ± 0.59 **de**	7.67 ± 0.62 **eh**
SSG 506	6.74 ± 0.35 **k**	8.28 ± 0.39 **gl**	7.36 ± 0.44 **h**	6.64 ± 0.52 **k**	6.68 ± 0.31 **k**

APPENDIX 2

Table 2.1 An explanation of multivariate statistical terms and related equations (Williams, 2001; Esbensen, 2002)

Multivariate statistical term	Explanation and equations
Residual Y variance	Calculated for calibration & validation. Plot based on validation: specify optimal number of components to avoid model over- or under-fitting (indicated by "elbow" formation). $$Residual\ variance_{val} = \frac{\sum(\hat{Y}_{val} - Y_{val})^2}{n}$$
Root mean square error of calibration (RMSEC)	Direct estimate of modelling error in Y. Calculated from calibration objects only. Expressed in original measurement units. $$RMSEC = \sqrt{\frac{\sum_{i=1}^{n}(\hat{Y}_{i,cal} - Y_{i,cal})^2}{n}}$$
Root mean square error of prediction (RMSEP)	Direct estimate of the prediction error in Y. Express average error expected in future predictions. Unusually high RMSEP accompanied with reasonable data structure: indicate bad model specification. If bias is also large: non-representative validation set. $$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(\hat{Y}_{i,val} - Y_{i,val})^2}{n}}$$

Table 2.1 CONTINUE

Standard error of calibration (SEC)	Computed as standard deviation (SD) of differences between rheological and reference samples in calibration sample set, corrected for bias.
Standard error of prediction or performance (SEP)	Precision of results. Computed as the standard deviation of residuals, corrected for bias. Increases when Y (reference) values are inaccurate. SEP > SEC (theoretically). $$SEP = \sqrt{\frac{\sum(X-Y)^2}{n} - \frac{\sum^2(X-Y)/n}{n-1}}$$
Bias	Average difference between predicted & measured Y-values for all samples. Validation bias: accuracy measurement of a prediction model. Bias ≈ 0 (ideally) $$RMSEP^2 \approx SEP^2 + Bias^2$$
Ratio of (standard error of) **P**rediction (validation) to (standard) **D**eviation (RPD)	Enables relative evaluation of SEP in terms of SD (reference data). Ideally the ratio of the SD : SEP should be 5 or higher (SEP << SD) $$RPD = \frac{SD(validation\ samples)}{SEP}$$

$\hat{Y}$ = predicted malt analysis value

Y = measured malt analysis value

n = number of samples

X = measured rheological value

SD = standard deviation

Table 2.2 Summary of the AAL and FAN reference data for the different parameters (indicating standard deviation)

Parameter	Total Sample set				Calibration set				Validation set			
	n	Range	Avg.[a]	SD[b]	n	Range	Avg.	SD	n	Range	Avg.	SD
AAL	19	75.10-87.32	81.59	3.27	13	76.4-87.32	81.67	3.27	6	75.1-86.08	81.45	3.57
FAN	15	110.4-168.7	146.51	15.8	-[c]	-	-	-	-	-	-	-

[a] Average

[b] Standard deviation

[c] Cross validation was used for FAN, therefore calibration and validation values were omitted