NOVEL METHODS FOR THE DETERMINATION OF COLOUR AND TRANSLUCENCY IN SELECTED ALCOHOLIC BEVERAGES

LEI CAO

A thesis submitted for the fulfillment of the requirements for the degree of

DOCTOR of PHILOSOPHY

International Centre for Brewing and Distilling

School of Life Sciences

Heriot-Watt University

Januray 2013

Abstract

At present, the various beverage industries have a significant challenge to effectively monitor the colour and translucency of their products, whether this is by visual assessments or by physical measurements. The visual assessment of translucent beverages is hampered in that the different industry sectors often have their own words/scales for translucency assessments, with specificity across a narrow range of products making them not easily transferred to other sectors of the beverage industry. Several industrial sensory evaluation methods were compared and correlated in this study. Similarly, there is a lack of accurate instrumental measurements either of the colour or the translucency of translucent beverage products in samples that are both coloured and translucent. A new multiple path-length cell together with a digital imaging system have been designed. The cell was built to deliver a specified analytical path length distribution, the overall light scattering performance of the liquid body would behave to the expected analytical path. This cell was applied at-line to determine the colour and translucency critical control points (CCPs) throughout beer production.

In this study, descriptive language and psychophysical models to assess colour and translucency were investigated using twelve so-called pseudo-beers (solutions of colorants and scatterers), six commercial beers and fifteen red wine samples. The colour appearance attributes of these samples were assessed by trained experts, who scaled lightness and hue compositions with less variation than their scaling of colourfulness. From the observers' performance, it was indicated that beers presented in glasses of different volumes and geometries had different visual colour appearance and that the perceived colour appearance was affected by different levels of scatterers. For the investigation of translucency, observers demonstrated that they could correlate different words or methodologies used for translucency evaluations. But they performed more consistently on the term "transparency", and thus, scaling "transparency" can reasonably be used as a term by which the translucency of a

beverage might be judged.

The magnitudes of colour/translucency changes between any two of the adjacent samples taken during the production of pilot-brewed beer indicated critical control points (CCPs) on colour/translucency monitoring throughout the brewing process. Five CCPs were established. Here, the novel cell and the digital imaging system were set as a off-line monitoring instrument to make determinations at each of the CCPs. The findings clearly showed that the colour/translucency results of most samples followed similar trends. Commercial final products were also tested with the new system, and the results were consistent with conventional methodology results and human observations. In comparison with conventional instruments and methods used for beer colour/translucency analyses, this novel system was demonstrated to be more sensitive, allowing for the simultaneous monitoring of colour of both in-process streams and final products. Some more improvements of the new system are still needed on translucency correlating with conventional methods, e.g. with EBC turbidity.

ACADEMIC REGISTRY

Research Thesis Submission

Name*:*	Lei Cao		
School/PGI:	School of Life Science		
Version: *(i.e. First, Resubmission, Final)*	Final	Degree Sought (Award **and** Subject area)	PhD Brewing

Declaration

In accordance with the appropriate regulations I hereby submit my thesis and I declare that:

1) the thesis embodies the results of my own work and has been composed by myself
2) where appropriate, I have made acknowledgement of the work of others and have made reference to work carried out in collaboration with other persons
3) the thesis is the correct version of the thesis for submission and is the same version as any electronic versions submitted*.
4) my thesis for the award referred to, deposited in the Heriot-Watt University Library, should be made available for loan or photocopying and be available via the Institutional Repository, subject to such conditions as the Librarian may require
5) I understand that as a student of the University I am required to abide by the Regulations of the University and to conform to its discipline.

* *Please note that it is the responsibility of the candidate to ensure that the correct version of the thesis is submitted.*

Signature of Candidate*:*	Lei Cao	Date:	22/01/2013

Submission

Submitted By *(name in capitals):*	
Signature of Individual Submitting:	

Date Submitted:	

For Completion in the Student Service Centre (SSC)

Received in the SSC by *(name in capitals):*			
Method of Submission *(Handed in to SSC; posted through internal/external mail):*			
E-thesis Submitted (**mandatory for final theses)**			
Signature:		Date:	

Acknowledgements

For the completion of this project, I would like to thank:

My supervisor, Professor Paul Hughes for supervising my project,

Dr Wei Ji for any critical comments on my work,

Mr Graham Mckernan for patient instruction,

Professor David Hopkins, Dr Peter Morris, Ms Vicky Goodfellow, Ms Dorothy Haston and Ms Anne Anstruther, for their kindness to help,

And finally my family, for their encouragement and support to finish this study.

CONTENTS

Chapter 1. INTRODUCTION

Upon pouring a beer into a glass, the drinker will make judgments on the acceptability of the product, based solely on what his or her eyes are seeing. The visual quality of beer depends on its clarity, colour and foam characteristics (Hughes and Baxter, 2001; Shellhammer and Bamforth, 2008; Spooner, 1996, 1997).

1.1 Background

In food and beverage products, the consumers often assess the initial quality of product by their colour and appearance, e.g. clarity. The colour and appearance of these products are thus the primary indicators of perceived quality (Lawless and Heymann, 2010). Some studies (DuBose et al., 1980; Shankar et al., 2009 cited in Lawless and Heymann, 2010) have shown that the colour of the products affect our perception of other attributes, e.g. aroma and flavour. Beer's colour conveys important messages to the consumer. They often conflate dark colour with stronger flavour impact, higher alcohol content and greater heaviness (O'Brien, 2006). Light colour conveys the opposite impression (Lewis and Bamforth, 2006). While interpretations of colour by consumers are by no means correct in all cases, beer colour and consistent beer colour are important quality criteria (Lewis and Bamforth, 2006). For the most part consumers expect clear beers; they might suspect poor quality and reject beer that is not clear. Producing beers that are clear and remain clear in the trade is therefore an essential requirement of almost all brewers. Nevertheless, at present there are limitations in our understanding of the theoretical and technological aspects of the measurement of both colour and translucency of liquid food products, including beers and other alcoholic beverages. In red wine industries, on the stage of filtration, two important factors must be considered, the degree of clarity and the amount of colour lost (Vine et al., 2002).

Traditionally, many industries rely on the Kubelka-Munk theory for colour measurement based on reflectance measurements. However, this theory does not work successfully for food materials for the following reasons:

1. The Kubelka-Munk theory has been successfully used for the evaluation of ceramic materials (Hogg and Nobel, 1979), soils (Barron and Torrent, 1986) and ink/papers (Yang and Kruse, 2004) for an empirical understanding of product colour and opacity formulation. This theory is the classic tool for understanding the optical properties of ink layers (Nobbs, 1997). The theory works well because these industries use ingredients that are closely controlled in terms of light scattering properties and pigment absorption properties, i.e. the average particle size of pigments is controlled between 0.4-0.7 μm (Leach and Pierce, 1993), and are uniform in thickness and appearance. But such ideal materials do not occur in foods, which contain light scattering particles of a wide range of sizes and absorption properties, and mixtures of natural pigments having ranges of absorption characteristics. Therefore, the assumptions of the Kubelka-Munk theory do not allow a complete understanding of food systems in terms of perceived colour and translucency, which requires monodisperse particles with well defined absorption properties.

2. The Kubelka-Munk theory is applied to conventional spectrophotometric analyses used for sample measurement. The spectrophotometer can only make trans-reflection measurements at a single site on a specimen. This limits application for liquid food products for which we need to investigate changes of scattering and absorption properties with depth, and ideally in real-time.

This second limitation restricts the use of current industrial instrumentation. For many years, the beer and juice industries, for example, have used the nephelometer or turbidimeter (Section 2.3.1) for gaining a rough estimate of translucency terms, i.e haze. Measurements made by such instruments can be misleading. They depend on

measurements made at specific angles and, as the optimum angle for measurement changes with particle size, instrumental assessment of haze can be substantially different from that perceived visually (Morris, 1987). Hence, instrumental readings should ideally be checked against visual observations. To understand the origins of the difficulties that arise from the application of the Kulbelka-Munk theory to food systems, we outline the specific case of beer production below.

Different standard methods existing to determine colour in beer are introduced in Section 2.2.3: Comparator method, EBC colour scale method, and *CIE* $L^*a^*b^*$ method. There are potential accurate problems and disadvantages on measurements by these methods. The comparator method and EBC colour scale method are based on EBC colour scale; thus, the definition only goes part way to completely expressing the colour of beer since no indication is given of lightness or darkness of the colour (Smedley, 1995). The comparator method provokes problems due to the variation of observer performance, variation in colour of illumination and discs due to ageing. It is difficult to determine meaningful colour by EBC scale method, because is based at a single wavelength (430 nm) when scattering light exists caused by temporal and dynamic changes. Beer colour measured by *CIE* $L^*a^*b^*$ method approach requires examining the entire visible spectrum which can be time intensive. It should be pointed out that beer or wort samples must be free from any particles which scatter light if the beer colour is measured by all these three methods. It is inconvenient and almost impossible during production.

The measurement of colour is no simple task. The sensory specialist should be very carefully report the specific conditions used in a test, and the panellists should be fully trained with experience. It is always prudent to cross-reference instrumental values to human perception on beverages' translucency measurement. But some situations arise when instrumental-sensory correlations break down, e.g. when the human responds but the instrument does not, or when the instrument responds, but the human does not (see Section 2.3.1). On another hand, the visual assessment of translucent beverages is

hampered in that the different industry sectors often have their own word/scales for translucency assessments, with specificity across a narrow range of products making them not easily transferred to other sectors of the beverage industry.

Therefore, a new DigiEye digital imaging system was developed (Image group of the Colour Science Department in Univeristy of Leeds; VeriVide Ltd) to develop robust instrumentally based scales for the independent measurement of colour and translucency of liquid food products to overcome the above problems. Part of this PhD work is to verify this system by measuring the selected liquid samples.

1.2 Aims

There are six thesis aims which are divided into three categories and each category follows by the bullet points:

Category A: To perform psychophysical evalution

- To determine visual colour appearance attributes of specific liquids, i.e. commercial beers, pseudo-beer matrices and red wines, correlated with relevant instrumental measurements, as part of database on model developments of the digital imaging system.
- To determine the relationships between 'opacity', 'transparency', 'clarity' and a categorical 5-point scale 'bright-clear-dull-hazy-cloudy' in psychophysical terms with specific liquids, i.e. commercial beers (with different colour appearance attributes), pseudo-beer matrices and red wines (with different colour appearance attributes and presumed with different haze contents caused by filtration).

Category B: To monitor brewing processing at critical control points

- To determine the critical control points governing colour and translucency development during the brewing process, using current instrumentation, quantify colour and turbidity through pilot brewing in ICBD.

Category C: To test instruments in beer production and products.

- To investigate the use of the digital imaging system as off-line instrument in pilot brewing.
- To correlate the digital imaging system method to conventional standard measurement methods used of beer products on colour and turbidity.
- To correlate the novel methods for the determination colour and translucency in beers between DigiEye digital imaging system, Telespectroradiometer (TSR) and psychophysical methods.

1.3 Thesis Structure

The whole thesis is divided into six chapters. Abrief account of each chapter is described below.

Chapter 1: Introduction.

In this chapter, the background of the limitation of conventional instrumentation and technology on colour/translucency characteristics used in beverage industries (Section 1.1) are introduced, and thus, the aim of this research (Section 1.2) is built to establish new technology/methodology to solve these problems.

Chapter 2: Literature Survey.

In this chapter, literature survey is covered: Overview of the brewing process (Section 2.1); beer colour (Section 2.2); beer translucency (Section 2.3); psychophysics and sensory evaluation (Section 2.4; on colour vision and colour/translucency evaluation)

and how DigiEye digital imaging system is developed (Section 2.5).

Chapter 3: Materials and Experimental.

Materials/samples and experimental established are introduced in this chapter. Six commercial beers, pseudo-beer matrix and commercial red wines are tested by instruments/sensory on colour/translucency characteristics (Section 3.1). As part of the whole research work, the obtained results are used for digital imaging system software development.

Three pilot brewed beers (two lagers-under different brewing process and with different sample preparations and one ale) which are assessed and analysed (Section 3.2). Based on the background theory and the analysed results, critical control points governing and affecting colour/translucency in brewing are concluded.

The DigiEye digital imaging system is verified on its repeatability (Section 3.3.1) and then introduced in pilot brewing line as off-line instrument. Pilot-brewed wort products at critical control points concluded (in Section 3.2.1), pilot-brewed beers (Section 3.3.2), and some commercial beers (Section 3.3.3) are evaluated. Comparison between this system, visual tests and conventional standard methods are made (Section 3.3.4).

Chapter 4: Results and Discussion

In this chapter, the results obtained in the research work are listed and discussed.

Chapter 5: Conclusions

According to the results and discussion, the conclusions are listed in this chapter.

Chapter 6: Future Work

Some further works are still needed to improve the novel methods before introducing this system/technology to production line. In this chapter, some future work is listed in the brewing area.

Chapter 2. LITERATURE SURVEY

2.1 Overview of the Brewing Process

In the broadest sense the word ‘brewing’ may bay be defined as ‘the combined processes of preparing beverages from the infusion of sound grains that have undergone sprouting, and the subsequent fermentation of the sugary solution produced, by yeast—whereby a proportion of the carbohydrate is converted to ethanol and carbon dioxide.’ The modern connotation of ‘brewing’ would imply ‘production of beer’ (Hornsey, 1999).

For many centuries, beer was the staple drink across Northern Europe. In some places, such as parts of Germany, it is still the drink of choice for accompanying food (Bamforth, 2003). Almost everywhere, though, “beer is the great drink of relaxation and moderation” (Bamforth, 1998). The speed and efficiency of brewing have been enhanced by improved technology, but the four stages of brewing (malting, mashing, boiling and fermentation) are still essential to the process (Laing and Hendra, 1977).

Traditionally the raw materials of brewing are water, malt, hops and yeast. While today most brewers still rely predominantly on malted barley as a source of fermentable sugars, many also use additional adjuncts that provide an alternative source of sugars and/or various processing aids such as enzymes to generate additional fermentable sugars (Lewis and Young, 1995, 2001).

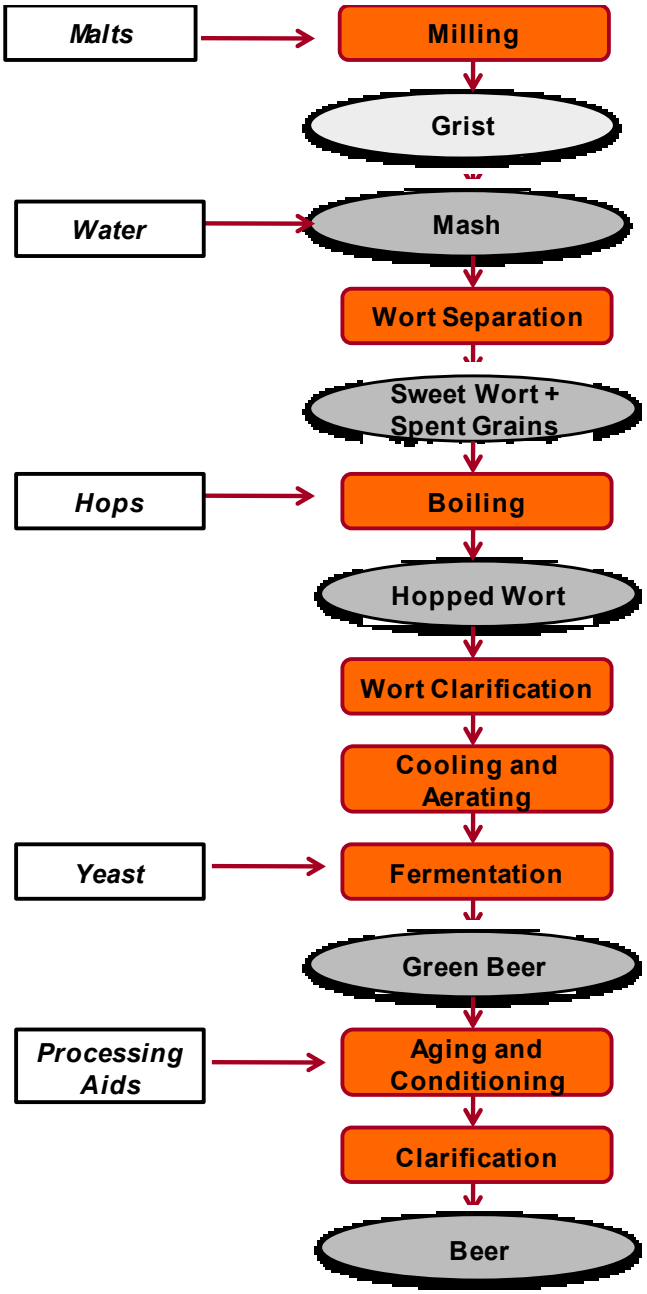

Figure 1.1.1 *Simplified flow diagram of the brewing process (edited from Hughes and Baxter, 2001).*

Malting

The malting process converts the raw barley by controlled steeping, germination and kilning into a product that is much more friable, with increased enzyme levels and with altered chemical and physical properties. The malting process involves the collection of stocks of suitable barley, the storage of the cereal until it is required, steeping the grain

in water, germinating the grain and finally drying and curing it on the kiln (Briggs et al., 1981).

- Steeping:

The purpose of steeping is to evenly hydrate the endosperm mass and to allow uniform growth during germination. Steeping begins by mixing the barley kernels with water to raise the moisture level and activate the metabolic processes of the dormant kernel by spraying or sprinkling (Kramer, 2006). Air rests are used between steeps, which provides the grain with oxygen, removes the growth-inhibiting carbon dioxide and removes some of the heat generated by the metabolizing grain. Steeping temperatures vary, with 16°C being a typical temperature (Palmer, 2006). About 70-75% soluble protein in wort is produced in malting, and the proteolysis is more effective at 15-16°C. The optimal starting moisture of the germination process is about 45-46% (Palmer, 2006).

- Germination:

The onset of germination is indicated by the appearance of the white chit. Germination was traditionally carried out on a germination floor (floor malting), but today is almost always performed in a germination compartment (pneumatic malting). A feature of the germination process is that the relative humidity of the airflow through the grain bed should be as close to 100% as possible, which removes carbon dioxide whilst avoiding water loss that reduces the rate of modification of the grain.

During germination, some important changes take place:

a) Development of the grain's enzyme systems
b) Breakdown of proteins into simpler structures
c) Breakdown of starch into simpler carbohydrates.

In this process, hydrolytic enzymes are produced, including amylolytic enzymes, which break down starch, proteolytic enzymes, which attack the protein, and cellulytic enzymes, which break down cell walls. In well-modified malt, about 90%

of β-glucan is broken down. It has been suggested that the undermodified grains of malt always contain higher levels of β-glucan and proteins than well-modified grains (Palmer, 1999). This suggests that slow β-glucan breakdown seems to be associated with the high protein grains or grains that break down their proteins slowly (Palmer, 2000). Blending of high-protein barleys with low-protein barleys is likely to produce malts whose modification is inhomogeneous, which can cause unexpected problems such as slow wort separation and beer filtration (Palmer, 1999). Haze development may also be a feature of uneven malt modification (Lalor, 2002).

- Kilning:

The green malt is dried (the moisture content decreasing from about 43% to 5%) in a kiln to prevent further enzyme activity inactive many microorganisms (Palmer, 2006). The kilned malt is stable for storage and has a friable texture suitable for the milling process which precedes brewing.

During kilning, there is a development of colour, and an increase of flavour. The development of colour results from Maillard reactions between reducing sugars and amino acids of the malt to form melanoidins (Palmer, 1989, Fayle and Gerrard, 2002). The temperature of this process should be controlled, as it affects flavour, colour and enzyme activities (Table 2.1.1). From the standpoint of adding colour to beer, malts can be categorized in three groups: colour, caramel and roasted malts (Coghe et al., 2003). Dimethyl sulfide is a flavour compound of lagers but is generally lower in ales (Hughes and Baxter, 2001). Malts kilned below 65°C can develop high levels of dimethyl sulfide, and those kilned between 80 and 82°C develop dimethyl sulfide during fermentation process (Chandra et al., 1999).

Table 2.1.1 *Typical analyses of coloured and roasted malts and barley (Palmer, 2006).*

	Moisture (%)	Colour (*EBC)	Final Kilning Temperature (°C)
Ale	4.0	5.0	100
Lager	4.5	2.0	80
Light Crystal	7.0	25-35	75
Crystal Malt	4.0	100-300	75
Amber/Brown Malt	2.0	100-400	150
Chocolate Malt	1.5	900-1100	220
Roasted Malt	1.5	1100-1400	230
Roasted Barley	1.5	1000-1550	230

* Absorbance at 430nm for single-wavelength beer colour measurement is the standard method of the European Brewery Convention (EBC, 2004).

Milling

The aim of the milling process is to prepare the grain so that it can absorb water effectively during mashing. All grains must be milled before mashing to expose the macromolecules for enzymic break down. The milled grain is particularly rich in starch and also in enzymes capable of degrading it rapidly when water is added to it (Briggs et al., 1981). The size reduction is normally achieved by using either hammer or roller mills. Hammer mills are used for providing grist suitable for use in higher-pressure mash filters, whilst for a lauter tun or mash tun, the coarse grist content is higher, and is often prepared using roller mills (Briggs et al., 1981).

Mashing

The aim of mashing is to hydrate the grist particles and to convert the insoluble materials in the grist into soluble materials, thereby the contents of the malts are brought into solution and the extract obtained. By definition, mashing is the process whereby

grist is mixed with warm brewing water or liquor such that a fermentable extract is produced that will support yeast growth and metabolism, with the subsequent production of beer (Briggs et al., 1981).

Heating from the use of warm water during mashing affects the colour, carbohydrate composition and particle content of wort (Taidi, 2002). There are several methods of mashing to obtain satisfactory wort, with the infusion mashing system is the simplest, which is common and traditional in the UK (Briggs et al., 1981). From the point of view of unit operations, infusion mashing entails mixing, grist hydration, enzyme reactions, liquid-solid separation, elution and liquid-solid separation (Hind, 1950). This process is carried out in mash conversion vessels. Liquor temperature is critical. It is known that water in the range of 65-70°C is optimal (Hornsey, 1999). At this temperature, control is exercised on the ratio of water/solids used has a thick porridge-like consistency. The temperature is held constant for a period which may be as short as 30 min or may extend for several hours (Briggs et al., 1981). The first worts are cloudy and are re-circulated. But as the runoff is continued, the wort becomes bright, because it is filtered through the bed of grist particles. Then the bright wort is either collected in a holding vessel or moved directly to a copper, where the wort is boiled with hops.

There is another common mashing system, the so-called decoction mashing system, which is common in Germany and Central Europe. This process is used for brewing with the less well, or irregularly modified malts, favoured by continental brewers, or for grists with high levels of adjunct (Hornsey, 1999). It is carried out with more finely ground grists. Decoction mashing uses three separate vessels:

- A stirred mash mixing vessel, where liquor and grist are mixed;
- A decoction vessel or mash cooker, where heating takes place;
- A wort separation device—lauter tun or a mash filter.

In the decoction mashing programme, the grist is mashed in to give an initial temperature of 35°C. One-third of the mash is then removed to the mash cooker, where

it is heated to boiling. The boiling mash is pumped back to the mash mixing vessel and is mixed with the vessels contents, raising the temperature to 50°C. One-third is then removed to the mash cooker, boiled, held for a short period and then returned to the mash. This will raise the mixed mash temperature to around 65°C, and amylolysis occurs. After a last decoction with one-third of the mash, the mash temperature increases to about 76°C. The mash is then transferred to a lauter tun or a mash filter (Briggs et al., 1981).

The most notable change in mashing is the dissolution and hydrolysis of starch to yield the greater part of the wort carbohydrates, which are fermentable. Cereal starch consists of typically 25% amylose (linear polysaccharide, made up of α-1,4 linked chains of D-glucopyranose, about 1600-1900 residues long) and 75% amylopectin (branched polysaccharide, made up of α-1,4 and α-1,6 linked D-glucopyranose). During mashing the starch is broken down into fermentable sugars by amylases and also yielding a small proportion of unfermentable carbohydrates (dextrins). The α-amylase from the malt attacks the α-1,4 links within the starch chains, producing glucose, maltose and a complex mixture of branched and unbranched oligosaccharides and dextrins. In mashing, α-amylase liberates the dextrins that are the substrate for the saccharogenic β-amylase. The β-amylase catalyses the hydrolysis of the α-1,4 links of the non-reducing chain ends of amylose and amylopectin, with the release of the reducing disaccharide maltose, the most abundant sugar in wort. However, the enzyme will not hydrolyse α-1,4 links near to α-1,6 branch points in amylopectin or dextrins. If the mash is made with β-amylase and pullulanase (a debranching enzyme), and only small amounts of α-amylase, a highly fermentable wort, rich in maltose and maltotriose, can be obtained (Enevoldsen, 1970, 1975 and Howling, 1979).

Cereals contain a range of protein types, broadly classified by their solubility properties (Briggs, 1998). When degraded by hydrolysis, proteins give rise to polypeptides, oligopeptides, and eventually free amino acids. The amounts of soluble nitrogen depend on the malt and the way that it is mashed. In mashes made at 65°C, about 50% of the

total soluble nitrogen and 30-50% of the free amino is formed by enzyme action during mashing (Briggs, 1998). The enzymes responsible for initial degradation malt protein are endo-peptidases that degrade the proteins into smaller polypetides. They are deactivated by high temperature during malting. Therefore, most proteolysis occurs during malting and not mashing, with insufficient proteinase concentrations surviving. In contrast, the carboxypeptidases (exo-peptidases) are heat stable to endure kilning in substantial concentrations to raise amino acids. They cleave individual amino acids from the carboxyl termini of peptides and polypeptides. There is normally an 'excess' of carboxypeptidase activity, and so the rate-limiting activity is due to limiting amounts of endo-peptidases. Thus, at the end of mashing, there is always a substantial amount of protein remaining in the spent grains and so a lack of substrate is not what limits the generation of soluble nitrogen (Briggs et al., 1981). The remaining proteins and polypeptides contribute to beer in terms of its mouthfeel, foaming characteristics, and haze formation. Maillard reactions between the sugars and amino-compounds during the wort boil give rise to coloured and flavoured substances in beer (Briggs et al., 2004).

Wort Separation

The resultant worts are separated from the spent grist, and the main reasons are (Hornsey, 1999):

- To obtain maximum extraction of soluble fermentable sugars
- To obtain bright worts with minimum suspended solids
- To minimise dissolved oxygen concentration in the wort.

A wide range of equipment has been used for wort separation and the majority of breweries in the worldwide use a lauter tun or mash filter rather than a mash tun for the purposes such as:

- They increase the turnaround time of the brewhouse
- They reduce the moisture content of spent grains
- They reduce effluent production.

Generally the lauter tun is cylindrical with a slotted base or profiled wedge wire and run-off pipes through which the wort is recovered. The hot liquor (at about 75-78°C) is run into the vessel so that it rises to an about an inch above the false bottom (Briggs et al., 1981). This ensures that no air is trapped under the plates, and it also serves to cushion the mash. Then the mash is transferred to the bottom of the vessel from the mash tun carefully to minimize oxygen uptake. Hot liquor is used to rinse out the mash tun and delivery pipes. The first wort is turbid, thus it is withdrawn and recycled to the top of the mash from the base of the vessel. After 5-10 minutes, the wort becomes clear and the wort is diverted to the wort collection vessel. The wort recycle may begin when about 50% of the mash has been transferred, and the wort collection begins when a filter layer of spent grains is established. Finally, sparge liquor (77°C) is sprayed onto the grains to ensure that the sugars and other dissolved materials are not left trapped in the spent grains (Bamforth, 2003).

Mash filters have polypropylene frames (consisting of a hollow chamber separated by two elastomer membranes) and plates supporting woven filter cloths (Hornsey, 1999). The whole system is enclosed in a stainless steel case and the plates are closed by a hydraulic cylinder. In process, a filter is preheated to about 80°C with steam or hot water. Then the mash is pumped from the mashing vessel and is loaded into the frames through bottom of the filter. Until filling is complete, the taps or outlets from the plates are kept open and the wort escapes through the filter cloths and is circulated to the mashing vessel until it is clear and then is collected. After the first wort is collected, sparge liquor is introduced and is distributed over the entire grain bed. When all the wort has been driven out, the sparge liquor passed through the mash. When sparging has been sufficient, the last sparge liquor is driven through the spent grains by compressed air.

Wort Boiling

Wort boiling is a process unique to beer production (Eaton, 2006). The objectives of

wort boiling include:

- Sterilization of the wort. Any micro-organisms present in wort will be destroyed at wort-boiling temperature.
- Inactivation of any remaining enzyme activity. Most enzymes present during mashing are inactivated by the sparge liquor. However, a small percentage will persist, and theses will be inactivated by coagulation during boiling (Ryder and Power, 2006).
- Extraction of the bittering materials and aroma compounds from hops and effective dispersal of oils and aroma compounds from the other additions.

The pH of wort typically ranges from 5.0 to 5.3, and wort colour is highly dependent, on the materials used and varies between pale brown to almost black (Boulton and Quain, 2001). Hops contain hundreds of components, with most of the brewing value of hops coming from their resins and hop oils (Goldammer, 2008). Based on their solubility, hop resins are subdivided into hard (contribute little to brewing) and soft (contribute to flavour and preservative properties of beer). The α- and β-acids together with the desoxy-α-acids are all present in the soft resins (Roberts and Wilson, 2006). When hops are added to the boiling wort, the alpha-acids are isomerised to form their isomeric iso-alpha-acids, the major contributors to beer bitterness.

a) α-Acids

α-Acids are responsible for about 90% of bitterness in beer, although they are not bitter in themselves (Goldammer, 2008). They are the precursors of beer bitterness since they are converted into iso-α-acids in during wort boiling. There are three major components of alpha acids are humulone, cohumlone and adhumulone (Goldammer, 2008).

b) β-Acids

β-Acids (lupulone, colupulone and adlupulone) are the second group of acids contained in hops. The bitter compounds derived from the degradation of these beta acids make a marginal contribution (Goldammer, 2008).

Processed hops have largely replaced the traditional whole cone hops (Bamforth, 2003), and the first stage in the formation of bitterness is the extraction of resins. The resins are not very water soluble, but they do dissolve in hot wort. The degree of extraction and isomerisation is expressed in terms of percentage hop utilisation, (Roberts and Wilson, 2006):

$$\%\ \ Hop\ \ Utilisation\ \ =\ \ \frac{c_1}{c_2}\times\ 00 \qquad \text{Eq.1}$$

where c_1 is weight of iso-alpha acids in wort/beer, and c_2 is weight of alpha acids added to wort, usually expressed in terms of mg/l.

The utilization of the bitter substances rarely exceeds 40% in commercial breweries and can be as low as 25% (Stewart and Russell, 1985).

Beer bitterness is commonly expressed as International Bitterness Units (IBUs), which represent a measurement of the intensity of the bitterness of the beer. 1 IBU is usually assumed to be equivalent to 1 mg of iso-alpha acid in 1 litre of water or beer (Goldammer, 2008).

1 IBU = 1 mg/l isomerised alpha acid

$$Weight\ \ of\ \ Hops\ \ =\ \ \frac{IBU\ \ \times\ \ Volume\ \ Brewed}{Alpha\ \ acid\ \ \times\ \ Utilisation} \qquad \text{Eq.2}$$

- Coagulation of excess proteins.

  Boiling causes coagulation of much of the protein. Proteins are removed which might otherwise precipitate out in the beer as haze. The proteins cross-link with tannins (polyphenols) to produce trub particles (also known as hot-break).

- Contribution to flavour formation.

  During the boil, Maillard reactions occur between free amino nitrogen comounds (e.g. amino acids) and reducing sugars (Hodge, 1953). The Maillard reaction

produces many volatile compounds, some of which have very low flavour thresholds and can influence the flavour of beer, such as the N-heterocycles (e.g. pyrazines and pyrroles), which haven described as roasted/bready (Odhay, 2004).

- Contribution to colour formation by two main routes, Maillard reactions and oxidation of polyphenols.

Wort boiled with or without hops increases in colour due to Maillard reactions or non-enzymatic browning. The pigments formed by Maillard reactions are called melanoidins and the ultimate product is caramel (Nursten, 2005; see also Section 2.2.4).

Phenols and polyphenols are extracted into boiling wort from both malt and hops. Some of them will react with proteins to form the trub, and others undergo a variety of reactions including the production of coloured compounds. Oxidation of phenols and polyphenols occurs, and quinones and their derivatives form.

a) Phenolic acids. In this class gallic acid, ferulic acid, vanillic acid, *p*-coumaric acid and chlorogenic acid are the most important, and the last three are present in both malt and hops. All of these phenolic acids are present in trace amounts but are regarded as being insignificant in terms of beer flavour (McMurrough et al., 1984).
b) Flavanols, of which kaempferol and quercetin are most widely distributed, which are hop-derived. There is little evidence that they have any effect on flavour, colour or stability of beer (McMurrough and Delcour, 1994).
c) Anthocyanogens and the related anthocyanidins. There are two groups of anthocyanogens according to the number of flavanoid units involved. Those derivatives with one unit are leucoanthocyanidins, and those with two or more are proanthocyanidins, which are most significant, being found in barley, malt and hops.

- Removal of undesirable volatiles by evaporation.

Boiling of wort drives off some undesirable volatiles including dimethyl sulfide, aldehydes and hydrocarbon components of the hop oils. Dimethyl sulfide is rapidly lost through evaporation.

- Concentration of sugars by evaporation.

The wort must be concentrated by evaporation, because the water used in mashing and sparging has produced wort lower in specific gravity than the target gravity.

Wort Clarification

Following the boil, the coagulated solids or trub, together with the insoluble spent hop material are removed. The collected trub will be in the region of 0.21-0.28 kg/hl wet weight and contain 80-85% water (Hough et al., 1982). Trub typically contains 50-60 % crude protein, 20-30% tannin, 15-20% resins and 2-3% ash (dry weight; Andrews, 1992).

The devices most commonly installed for wort clarification in breweries are whirlpool tanks, developed by the Molson Breweries in Canada (Hudston, 1969). They consist of a vertical cylindrical vessel into which the wort is pumped. Hot wort is injected tangentially, which causes the wort in the vessel to rotate. During rotation, particles in the wort are driven outwards by centrifugal force. The vertical pressure of the raised liquid at the edges tends to drive the liquid downwards and the particles strike the wall, move down with the liquid flow and move towards the base of the middle of the vessel. Deposited particles spiral downwards towards to the centre of the base to form a cone, which can be separated from the clear wort.

Cooling and Aerating

After clarification, the hot wort must be brought down to fermentation temperatures

before yeast is pitched. Traditionally this is about 6-12°C for lagers and 15-22°C for ales (Briggs et al., 2004). In modern breweries, this is achieved through a plate heat exchanger. The wort flows turbulently on one side of the plates, with a cooling medium, e.g. chilled water, flowing, often counter-currently, on the other.

When wort is chilled, it becomes cloudy due to the separation of the cold break or trub from solution. The cold break does not flocculate, but is considered to form at levels of 40-350 mg/l, containing about 50% protein, 15-25% polyphenols and 20-30% of wort carbohydrates (Briggs et al., 2004). Cold break does not normally cause problems during ale fermentation. In fact, it can be beneficial (Hornsey, 1999), as it seems that, fine-flavoured beers benefit from the removal of some of the cold break. Cold breaks promote a vigorous fermentation by acting as a nucelation site where carbon dioxide bubbles form. The bubbles keep yeast in suspension and therefore in contact with wort for sustained and efficient fermentation (Bamforth, 2003). Cold breaks can be removed by Kieselguhr or perlite filtration, centrifugation, sedimentation or flotation, but many breweries no longer make additional efforts for cold break removal (Briggs et al., 2004). In the initial stage of fermentation, wort is inevitably exposed to relatively high oxygen concentrations (ranging from 4 - 14 mg/l; Depraetere et al., 2007), which is required for the early stages of yeast growth. The concentration of oxygen required depends on the wort and yeast strain (Hough et al., 1982). Many brewers believe that gas injected into the hot wort prior to cooling improves cold break formation. However, when injected into wort at the hot end of a heat exchanger, there is a risk of wort resulting in darker-coloured worts (Hough et al., 1982; Wilson, 1978). In addition, oxygenation of hot wort can contribute to off-flavours in the beer (Palmer, 2002).

Fermentation

There are two main types of traditional fermentation systems: top fermentation and bottom fermentation. Top-fermenting yeasts used to be a characteristic of British-style

ale breweries with fruity and estery aroma (Briggs, 1998), while bottom fermentation is traditionally associated with the production of lager-style beers. Lager fermentation tends to provide beers typically with a lighter and partly sulfurous aroma (Yoshida et al., 2008).

The main objective of this stage is to ferment wort to the desired gravity. The proportion of the wort dissolved solids (extract) which can be fermented is called the percentage fermentability of the wort:

$$Fermentability\ (\%) = \frac{OG - FG}{OG} \times 100 \qquad \text{Eq.3}$$

where *OG* is original gravity and *FG* is final gravity.

The original gravity can be expressed in Plato (^{o}P), which measures the concentration in weight/weight terms as grammes of solids per 100 grammes of wort, or in ^{o}Sacch, which relates the specific gravity of the wort to that of water taken as 1000. Final gravity means the gravity of the wort when it is fully fermented such that adding more yeast or leaving it longer will lead to no further fall in gravity.

During fermentation, yeast cells use the sugars from the malt starch and adjuncts to produce ethanol and carbon dioxide as major products of metabolism. Sucrose is hydrolysed to fructose and glucose, which are assimilated simultaneously. The main fermentation sugar, maltose, is then taken up. When the maltose concentration falls to undetectable levels, maltotriose is assimilated (Eßlinger, 2009). The yeast also produces a series of minor metabolites such as esters, higher alcohols and organic acids that contribute positively to flavour (Ugliano and Henschke, 2009). The optimal level of pitching rate is $10\text{-}12*10^{6}$.

Yeast converts simple nitrogen compounds (i.e. amino acids and ammonium ions) from wort to its own cellular substances. High molecular weight polypeptides tend to become insoluble, and those will be filtered out (Eßlinger, 2009). Yeast contributes to beer

colour indirectly by absorbing colour materials to their cell wall, and affects colour measurement and perception if they confer turbidity on the final product (Shellhammer, 2008).

It has been established that aspects of beer colour relate to wort production rather than fermentation (Smedley, 1992). The colour of beer is generally dominated by malt kilning/roasting and wort boiling, as well as the oxidation of polyphenolics in mashing and boiling. Beer colloidal susceptibility is largely established upstream of the fermenter in terms of polypeptides, tannoids, oxalate, etc (Bamforth, 1999). During fermentation, pH decreases (Coote and Kirsop, 1976), as organic acids are produced and buffering compounds e.g. amino acids are consumed, and then remains essentially constant. In the first days of fermentation, the colour of beer becomes lighter. Some substances change their colour as the pH drop. Some are adsorbed on the surface of the yeast and are removed with the settling yeast. The decreasing pH can also cause colloidally dissolved bitter substances and polyphenols precipitate (Eßlinger, 2009).

Maturation

At the completion of primary fermentation, beer is said to be ‘green’, which is physically and microbiologically unstable (Goldammer, 2008), and can have the aroma of green apples (Stewart, 2004). It is hazy due to protein-tannin complexes and yeast cell (Briggs, 1998), it can lack sufficient carbonation, and its taste and aroma need to be improved (Briggs, 1998). In order to refine green beer it must be matured or conditioned.

Traditionally, maturation involves secondary fermentation of the remaining fermentable carbohydrates at a reduced rate, controlled by low temperatures and a low yeast count in the green beer. Small quantities of fermentable carbohydrate may be added in the form of ‘priming sugar’. In some systems freshly fermenting wort is added to provide the fermentable material in a process known as krausening. The carbon dioxide that is

produced dissolves in the beer because the vessel is closed and the beer is 'conditioned'. Some flavour changes occur during the maturation of beer with consequent positive effects, such as reduction of vicinal diketones such as diacetyl, and losses of volatile sulfur compounds, aldehydes, and volatile fatty acids (Briggs et al., 2004).

During maturation, some clarification takes place. This is by natural sedimentation of polypeptides and polyphenol complexes, but this process can be enhanced by physical and chemical means. To remove polypeptides or polyphenols and to improve its physical stability, a number of methods are employed for reducing chill haze. This stabilization procedure is often referred to as "chillproofing", such as chillproofing agents have been used to enhance beer haze stability (i.e. reduction in the concentration of beer proteins and/or polyphenols; see also Section 2.3).

Clarification

Although conditioning - maturation, clarification, and stabilization - plays an important role in reducing yeast and haze loading materials, a final beer filtration is needed in order to achieve colloidal and microbiological stability (Boulton and Quain, 2001; Kołtuniewicz and Drioli, 2008). Clarification of beer involves the removal of yeast and the sedimented protein and polyphenol haze material derived from beer stabilization techniques and cold break.

Summary

"Malting and brewing are not simple processes. They are marked by a complex blend of vegetative and technical stages, at any of which there is plenty of opportunity for things to go wrong" (Bamforth, 2003). Colour and clarity of beer final products can be influenced by many factors, i.e. raw materials, process plant selection and processing parameters. Brewers commonly use sensors to make measurements together with associated control systems that respond to the values measured wherever possible. If

values are out of specification, adjust a relevant parameter in order to push the process back on track (Bamforth, 2003).

2.2 Beer Colour

2.2.1 Colour Perception and Measuring Colour

"We perceive the world in which we live by our five senses, vision, hearing, touch, taste and smell, of which the sense of vision is usually the first used in detecting events and objects around us in the visual world" (MacDougall, 2002). The initial attraction or rejection of food depends on its looks, because the first impression of food is formed by its appearance, including colour, shape, size, etc. Most of our traditional colour concepts affect our reaction to food, which is one way to make a safe judgment the food quality. For example, a green colour is associated with unripe fruit such as orange, while a brown banana is thought to be spoilt (Mudambi et al., 2006).

There are three key attributes of colour: a source of illumination/light, an object to interact with the light which comes from this source and a human eye to observe the effect which results. The human eye transmits information that the brain will interpret as colour (Hari et al., 1994).

Light itself has no colour and colour does not exist by itself, it only exists in the mind of viewer (Delgado-Vargas and Paredes-López, 2003). Visible light is characterised by its wavelength, which ranges from around 360 to 780 nm (Christie, 2001). Wavelengths from about 400 to 450 nm appear violet; 450 to 490 nm, blue; 500 to 575 nm, green; 575 to 590 nm, yellow; 590 to 620 nm, orange; and 620 to 700 nm, red. In order to evaluate the colour of an object, it must be illuminated and upon interacting with the object the incident light can be transmitted, reflected, refracted, absorbed and/or

scattered (Shellhammer, 2008; Gilbert and Haeberli, 2008). When light strikes an object, part of the light is reflected from the sample surface. The mirror-like reflection is called specular reflection which is directed back toward the energy source, whereas the diffuse reflection reflected light emerges from the surface at random angles through 180° (Penner, 2010). Absorption and scattering are the most important influences on colour by the interactions between light and objects. Absorption is the process by which radiant energy is utilised to raise molecules in the object to higher energy states. Scattering is the interaction by which light is re-directed as a result of multiple refractions and reflections (Christie, 2001). The interactions involved (Figure 2.2.1.1) are:

- From the surface — specular and diffuse reflections, and refraction into the body of the object
- Within the object — internal diffusion (scattering) and absorption
- Through the object — regular and diffuse transmission

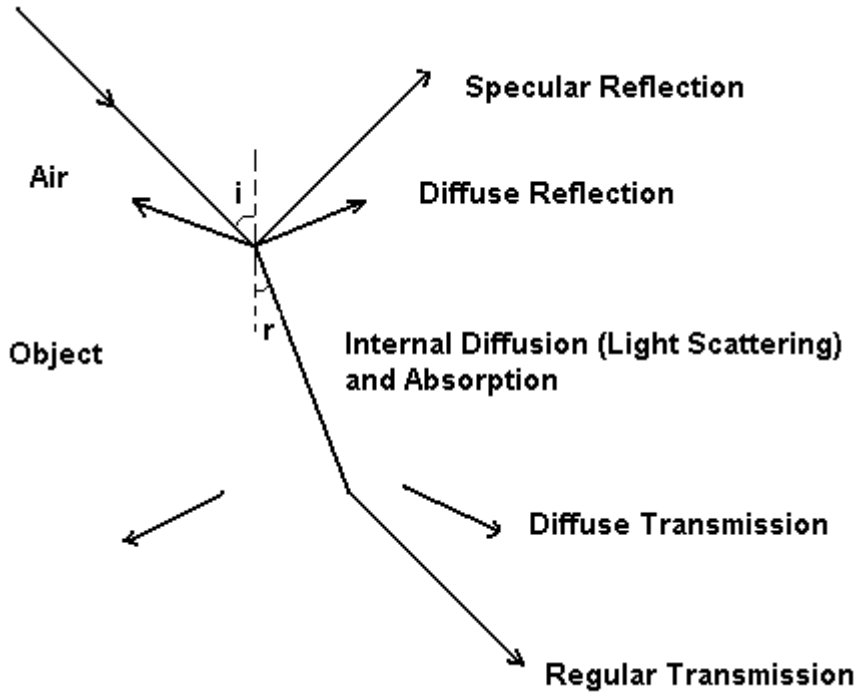

Figure 2.2.1.1 *The interaction of light with an object containing light absorbing and scattering elements (Hutchings, 1999).*

For a liquid, if all wavelengths of light are absorbed, the liquid will appear black and if none of the light is absorbed, it will appear transparent (no scattering existing), i.e. filtered water, or translucent or opaque in white (depending on the degree of existed

scattering). Beer owes its colour to the selective absorption of certain wavelengths of visible light, whilst the remaining wavelengths of light are transmitted or scattered, thus giving to the observed colour.

Our perception of colour arises from the composition of light that enters the eyes. The retina owes rods and cones, which are light sensitive cells. It is the rods and cones that translate the optical image into a pattern of nerve activity that is transmitted to the brain by the fibers in the optic nerve (Christie, 2001). At low levels of illumination, only the rods are active, which perception only darkness and lightness. At medium or high levels of illumination, only cones are sensitive. Three types of cones are classified in eyes, as short (sensitive to blue light), medium (sensitive to green light) and long (sensitive to red light). The cones, which absorb the visible light, allow us to distinguish between different colours, by responding individually to green, red or blue light, and these three responses are interpreted by the brain as colour (see also Section 2.4.2).

Light used for general illumination is white, but there are many types of white light. To standardize the variations among the illuminations, the International Commission on Illuminants, known as CIE (Commission Internationale de l'Eclairage) has established standard illuminants, which has been defined by a spectral power distribution (SPD) and correlates with a colour temperature. The colour temperature is determined from the temperature in Kelvin to which a black body that absorbs all energy that falls onto it needs to be heated to emit light of a spectral distribution characteristic of the specific light source. The light emitted by the black body changes as the colour temperature changes. For example, CIE C has a spectral power distribution, $S_{(\lambda)}$, (Figure 2.2.1.2) that correlates with a temperature of approximately 6740^{o}K (Delgado-Vargas and Lopez, 2003). The three most commonly reported for appraisal of foods are defined as illuminants A, C, and D_{65}. Illuminant A is provided by a gas-filled tungsten lamp, and has a yellow aspect. Illuminant C, designed to represent daylight from an overcast sky, is tungsten light filtered, and is bluer than illuminant A. Source D65 is based on measurements of total daylight of sun plus sky and is also bluer than illuminant A.

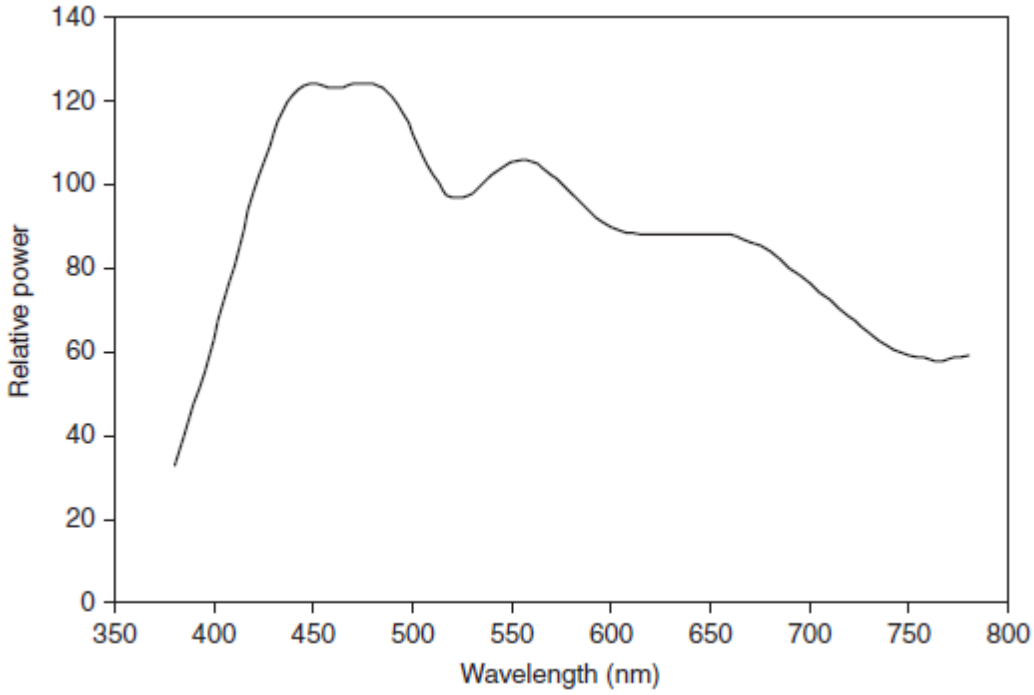

Figure 2.2.1.2 *Spectral power distribution, $S_{(\lambda)}$, of CIE illuminant C (ASTM, 2001).*

The CIE also provides the recommendations of the standard colorimetric observers based on experiments with a number of people possessing normal colour vision in order to assess the spectral sensensitivity of the human eye (Figure 2.2.1.3). In 1931 the CIE defined the 2^{o} Standard Colour Observer and 1964 defined a second colour known as the 10^{o} Supplementary

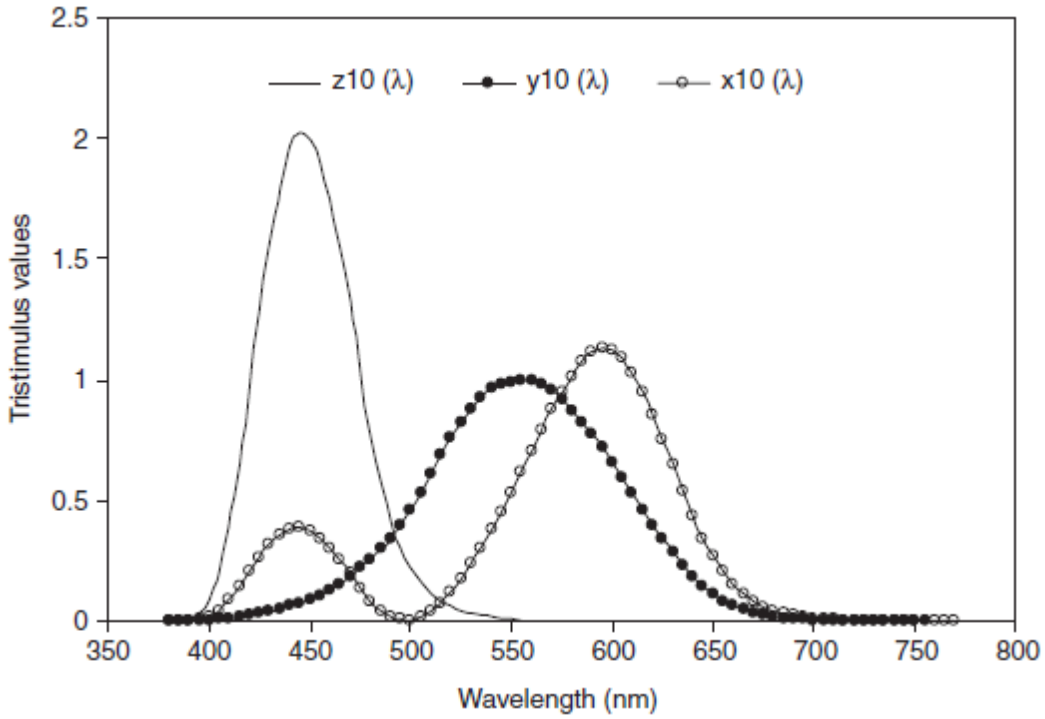

Figure 2.2.1.3 *CIE colour matching functions $x_{10(\lambda)}$, $y_{10(\lambda)}$, $z_{10(\lambda)}$ for the 10° Standard Observer (ASTM, 2001).*

With the adoption of standard observer functions and standard illuminants, it became possible to convert the spectral transmission curve of any object to three numerical values. These numbers are known as CIE tristimulus values, X, Y and Z, the amounts of red, green, and blue primaries required to give a colour match, using the following generic form:

Eq.4

In the case of beer colour measurements, a spectrophotometer measures the spectral transmission, $T_{(\lambda)}$, of the sample, at distinct wavelengths. These data can be combined with the spectral power distribution, $S_{(\lambda)}$, and the standard observer spectral sensitivities, $x_{10(\lambda)}$, $y_{10(\lambda)}$, $z_{10(\lambda)}$ at the same vavelengths using Equation 4 and integrated over the entire visible spectrum to yield the CIE tristimulus values, X, Y and Z, as Equation 5.

Eq.5

where k is a normalizing factor calculated as:

———————— Eq.6

In 1976, the CIE defined the CIELAB ($L^*a^*b^*$) colour space as a reference colour space, and the L^*, a^* and b^* were defined by the equations:

— Eq.7

— —

— —

where

— — if — — Eq.8

— — — — if — —

and

— — if — —

— — — — if — —

and

— — if — —

— — — — if — —

where X, Y and Z are tristimulus values of the test object colour stimulus considered and X_n, Y_n and Z_n are the tristimulus values of specified white object colour stimulus.

The three coordinates of CIELAB (Figure 2.2.1.4) represent the lightness as L^*, which has the range from 100 (lightest colour, white) to 0 (darkest colour, black). The a^* reading refers to the red-green axis, shifting from red (positive) to green (negative), and the b^* reading refers to the yellow-blue axis, shifting from yellow (positive) to blue

(negative), and zero values are neutral.

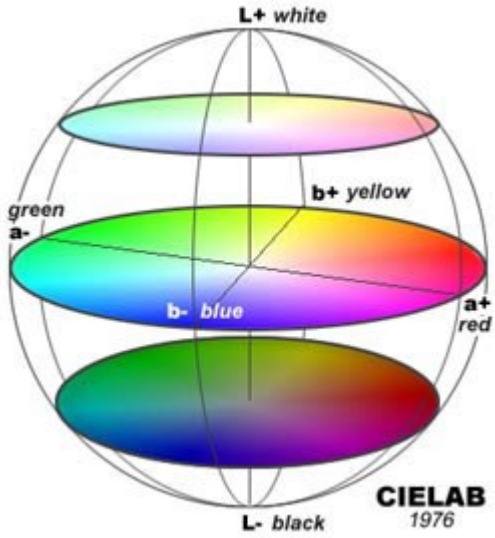

Figure 2.2.1.4 *CIELAB colour space.*

Three parameters define the position of a specified colour within a roughly spherical colour space which contains all perceivable colours, as hue, lightness and chroma.

Hence, any colour can be specified within the colour space by the three parameters, which can be expressed as lightness (L^*), chroma (C^*_{ab}), and hue angle (h_{ab}). These three parameters represent the colour in three dimensions: L^* and C^*_{ab} are scalar measurements of distance within the colour space, while h_{ab} is a measurement of angular rotation on the horizontal plane of the colour space. Correlating of perceived attributes lightness, chroma and hue are calculated as:

as defined in Equation 7.

Eq.9

The magnitude of differences in colour between two samples of, for instance, beer 1 and beer 2, can be estimated by deriving the Euclidean distance ($\Delta E^*_{a,b}$) between their locations in CIELAB space, i.e.:

Eq.10

where L^*, a^* and b^* are the CIELAB colour parameters of the two test samples (Figure 2.2.1.5). The smaller is the distance, the closer in colour are the two samples.

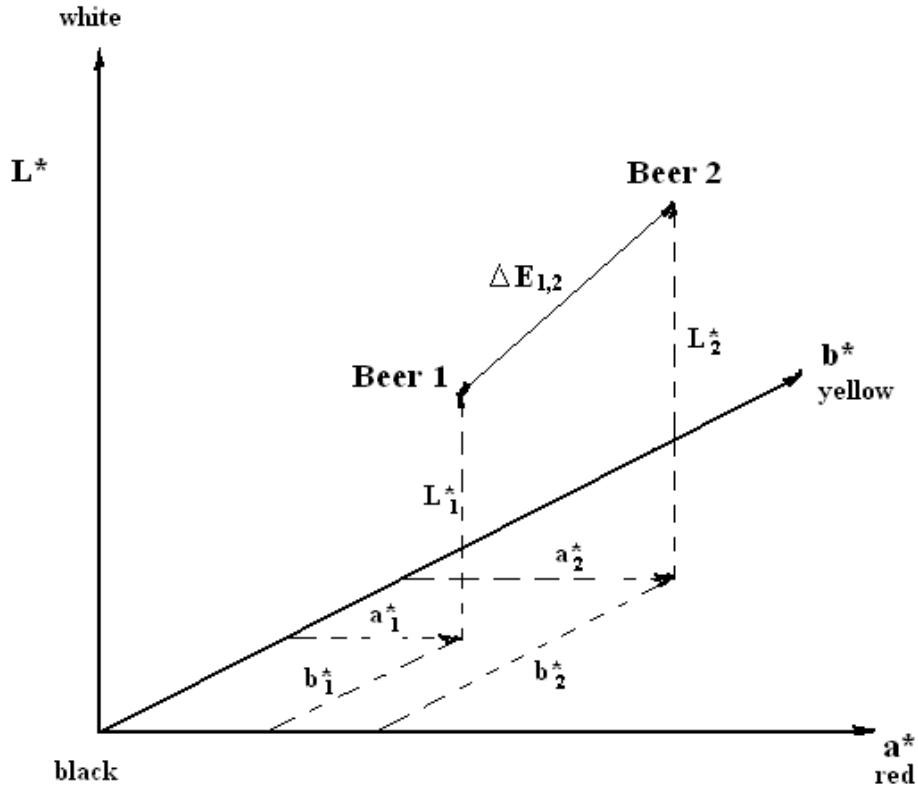

Figure 2.2.1.5 *Difference in colour between two samples in the CIELAB colour space, represented by the $\Delta E_{1,2}$ parameter (Smedley, 1995b) .*

The CIE recommendation (1995) was suggested that moderate colour differences were between 0 – 5 CIELAB units, under which human could distinguish colour differences effectively. In theory, for beers, the colour threshold range from 0.7 to 4.0 (Smedly, 1995).

CIEDE2000 ΔE_{00} was developed based on CIELAB, which includes not only lightness, colourfulness and hue weighting functions, but also an interactive term between colourfulness and hue differences for improving the performance for blue colours and a scaling factor for CIELAB a^* scale for improving the performance for gray colours. It gives more accurate predictions than the previous formulae when tested using many experimental data sets (Luo et al., 2001; Xu and Yaguchi, 2004, Shen and Berns, 2011).

It also outperformed CMC and CIE94 by a large margin, and predicted better than BFD and LCD. The computational process is given below.

Step 1. Calculate the CIE L^*, a^*, b^*, and C^*_{ab} as Equation 9

Step 2. Calculate a', C' and h' Eq.11

$$L' = L^*$$
$$a' = (1+G)a^*$$
$$b' = b^*$$
$$C'_{ab} = \sqrt{a'^2 + b'^2}$$
$$h'_{ab} = tan^{-1}(b'/a')$$

where

$$G = 0.5\left(1 - \sqrt{\frac{\overline{C^*_{ab}}^7}{\overline{C^*_{ab}}^7 + 25^7}}\right)$$

where $\overline{C^*_{ab}}$ is the arithmetic mean of the C^*_{ab} values for a pair of samples.

Step 3. Calculate $\Delta L'$, $\Delta C'$ *and* $\Delta H'$ Eq.12

$$\Delta L' = L'_b - L'_s$$
$$\Delta C'_{ab} = C'_{ab,b} - C'_{ab,s}$$
$$\Delta H'_{ab} = 2\sqrt{C'_{ab,b}C'_{ab,s}}\ sin(\frac{\Delta h_b}{2})$$

where $\Delta h_b = h'_{ab,b} - h'_{ab,s}$

Step 4. Calculate ΔE_{00} Eq.13

$$\Delta E_{00} = \sqrt{\left(\frac{\Delta L}{k_L S_L}\right)^2 + \left(\frac{\Delta C'_{ab}}{k_C S_C}\right)^2 + \left(\frac{\Delta H'_{ab}}{k_H S_H}\right)^2 + R_T\left(\frac{\Delta C'_{ab}}{k_C S_C}\right)\left(\frac{\Delta H'_{ab}}{k_H S_H}\right)}$$

where

$$S_L = 1 + \frac{0.015(\bar{L}' - 50)^2}{\sqrt{20 + (\bar{L}' - 50)^2}}$$

and

$$S_C = 1 + 0.045\bar{C}'_{ab}$$

and

$$S_H = 1 + 0.015\bar{C}'_{ab}T$$

where

$$T = 1 - 0.17\cos(\bar{h}'_{ab} - 30°) + 0.24\cos(2\bar{h}'_{ab}) + 0.32\cos(3\bar{h}'_{ab} + 6°) - 0.20\cos(4\bar{h}'_{ab} - 63°)$$

and

$$R_T = -\sin(2\Delta\theta)R_C$$

where

$$\Delta\theta = 30\exp\left\{-\left[\frac{\bar{h}'_{ab} - 275°}{25}\right]^2\right\}$$

and $$R_C = 2\sqrt{\frac{\bar{C}'^{7}_{ab}}{\bar{C}'^{7}_{ab} + 25^7}}$$

Note that $\bar{L}'$, $\bar{C}'_{ab}$, and $\bar{h}'_{ab}$ are the arithmetic means of the L', C'_{ab} and h'_{ab} values for a pair of samples. For calculating the $\bar{h}'_{ab}$ value, caution needs to be taken for neutral colours having hue angles in different quadrants, for example, a standard and a sample with hue angles of 90° and 300° would have a mean value of 195°, which differs from the correct answer, 15°. This correct value can be obtained by checking the absolute difference between two hue angles. If the difference is less than 180°, the arithmetic mean should be used. Otherwise, 360° should be subtracted from the larger angle, followed before calculating of the arithmetic mean. This gives 300° – 360° = –60° for the sample, and a mean value of (90° – 60°)/2=15°. This is explained in detail by Luo et al. (2001).

2.2.2 Colour Appearance Model

Colour appearance is a array of visual phenomena, which extends basic colorimetry to the level of defining a specific colour perception of a stimuli in a wide variety of viewing conditions such as Bezold-Brücke hue shrift (hue changes with luminance), Abney effect (hue changes with colorimetric purity), Helmholtz-Kohlrausch effect (brightness depends on luminance and chromaticity), Hunt effect (colourfulness increases with luminance), Stevens effect (contrast increases with luminance), Helson-Judd effect (hue of nonselective samples), Bartleson-Breneman equations (image contrast changes with surround), discounting the illuminant, other context and structural effects, simultaneous contrast, crispening and spreading (Fairchild, 2005).

The human visual system has an ability to maintain the colour appearance of an object despite large changes in quality and intensity of the illumination (Westland et al., 2012). A red apple tends to look red whether it is viewed by daylight, tungsten light or candlelight. It is considered that the human visual system achieves colour constancy by some process that allows it to discount the effect of the illumination, and the process is described as “chromatic adaptation”. Therefore most CAMs (colour appearance model) include a chromatic adaptation transform (CAT), which is a method for computing the corresponding colour under a reference illuminant for a stimulus defined under a test illuminant. Corresponding colours are colours that have the same appearance under different illumination (Bartleson, 1978).

CIELAB can be considered to be a CAM, which makes relatively poor predictions of colour appearance. It has no luminance level dependency, thus, it is incapable of predicting luminance-dependent effects. CIELAB also has no background or surround dependency, so it cannot be used to predict simultaneous contrast or Bartleson-Breneman results showing a change in image contrast with surround relative luminance. CIE also has no mechanism for modeling cognitive effects, and does not provide correlates for the brightness and colourfulness attributes (Fairchild, 2005).

A CAM as any model is defined that includes predictors of at least the relative colour appearance attributes of lightness, chroma and hue. The colour appearance attributes are defined as (Fairchild, 2005):

- Brightness: attribute of a visual sensation according to which an area appears to exhibit more or less light.
- Lightness: the brightness of an area judged relative to the brightness of a similarly illuminated reference white.
- Colourfulness: attribute of a visual sensation according to which an area appears to exhibit more or less chromatic content.
- Chroma: colourfulness of an area judged as a proportion of the brightness of a similarly illuminated area that appears white or highly transmitting.
- Saturation: colourfulness of an area judged in proportion to its brightness.
- Hue: attribute of a visual sensation according to which an area appears to be similar to one of the perceived colours: red, yellow, green and blue, or to a combination of two of them.

The CIECAM02 is currently the most used colour appearance model for digital imaging and successor of CIECAM97s which has been revised and proposed by the CIE Technical Committee 8-01 (Li et al., 2000). This model provides a viewing condition specific means for transforming tristimulus values to or from perceptual attribute correlates. The main components of this model are its chromatic adaptation transform, CIECAT02, and its equations for calculating mathematical correlates for the six defined dimensions of colour appearance: brightness, lightness, colourfulness, chroma, saturation and hue (Moroney et al., 2002). Input data for CIECAM02 include the tristimulus values of the test stimulus (XYZ) and adapting white point ($X_wY_wZ_w$), adapting luminance, and surround luminance, and whether or not observers are discounting the illuminant (Figure 2.2.2.1). The model can be used to predict these appearance attributes or, with forward and reverse implementations for distinct viewing conditions, to compute corresponding colours.

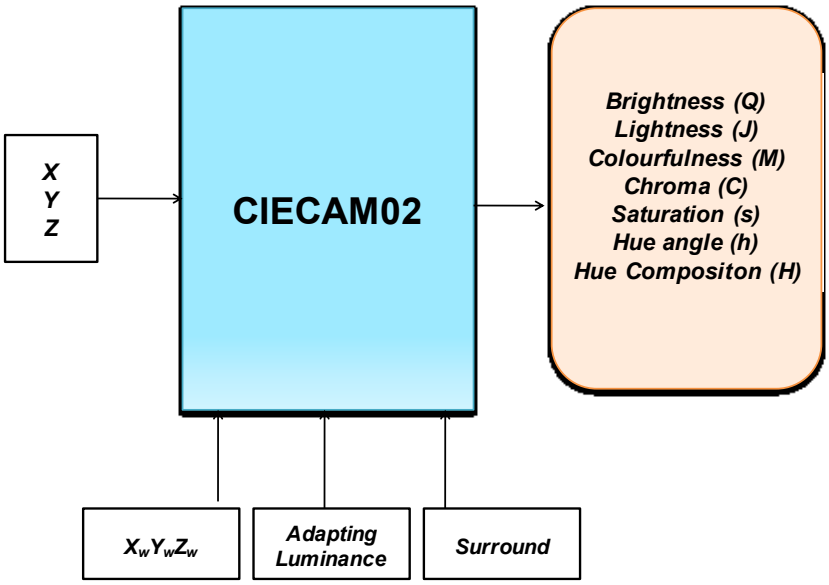

Figure 2.2.2.1 *CIECAM02 input and output parameters.*

2.2.3 Standard Methods for Measuring Beer Colour

There are three main methods that are used for beer colour measurement:

- Comparator method:

  Beer colour was simply compared by human eye to a set of colour standards. A tintometer was developed with a set of reference standards made from potassium chromate solutions. These solutions have colour characteristics reminiscent of commercial beers (Shellhammer, 2008). Coloured glass discs were developed by Lovibond in 1893, and after a series of modifications, such comparator discs were accepted by the European Brewery convention in 1951 (Hughes and Baxter, 2001) and the unit is defined as EBC (EBC, 2002). The interval between two discs is 0.5 EBC units when the colour is less than 10 EBC units, and increase to 1.0 EBC units when the EBC colour is greater than 10 (Fengxia et al., 2004). The observer makes the nearest match, thus there are potential problems leading to errors within and between laboratories (Sharpe et al., 1992; Smedley, 1992), such as ageing of the

discs, variation of observer performance, variation of light source and the dilution of highly coloured beers.

- EBC colour scale:

The American Society of Brewing Chemists (ASBC) implemented a spectrophotometric method of measuring beer colour in 1950. This is a method for assessing the colours of bright beers and worts relating absorbance measurements to the EBC scale. According to the European Brewery Convention Analysis Committee (EBC, 1998), the colour of wort and beer is measured at a wavelength of 430 nm in a 10 mm cuvette (EBC methods 9.6), and this measurement is the standard method of the ASBC (ASBC, 2004). Another wavelength, 530 nm, was also recommended as the wavelength for spectrophotometric analysis of ale colour in the UK (IOB, 1986). However, this was replaced and subsequently 430 nm was adopted by UK industry later. The EBC standard method for beer colour uses absorbance at 430 nm in a 10 mm quartz cuvette against water as reference. This standard method for beer colour measurement was defined as:

Eq.14

where A_{430} is absorbance reading of sample in a 10 mm quartz cuvette.

Samples should be free from any particles, because at a single wavelength, it is difficult to determine the meaningful colours when scattering light exists caused by temporal and dynamic changes (Fengxia et al., 2004). Some brewing industries retain this method as the conventional standard for online/offline colour measurement because this technique is rapid and easily transferable. But this technique is less satisfactory for darker beers; furthermore, it is possible for two beers with identical EBC colours to be visually different because of different transmission spectra outside of A_{430} (Smedley, 1995a). Table 2.2.3.1 present the colour ranges of beer styles in terms of EBC colour units.

Table 2.2.3.1 *Beer colour across a selection of beer styles.*

Beer Style	EBC Colour Units	Colour Description
American/Light Lager (Papazian, 2006)	3-8	Very pale to pale
Bavarian Helles (Dornbusch, 2000)	6-10	Pale to straw
American/European Malt Pilsner (Jurado, 2002a)	6-18	Gold
English Pale Ale (Papazian, 2006)	10-28	Straw to copper
Red Beers (Jurado, 2002b)	18-108	Brown/amber/reddish
Porter (Shellhammer, 2008)	40-60	Dark brown
Irish Stout (Papazian, 2006)	> 80	Black

- *CIE* $L^*a^*b^*$ method:

In many industries, tristimulus measurements (as a means of colour interpretation) are used for colour quality control. Thristimulus values were calculated from transmittance data over the range 380 nm to 780 nm. Chromaticity and CIELAB values for L^*, a^* and b^* were obtained from the X, Y and Z tristimulus values by the relationships described above.

This approach requires examining the entire visible spectrum which can be time intensive if rapid, online reading is required. It should be pointed out that beers samples must be free from any particles which scatter light.

2.2.4 Light-absorbing Species in Beer

Beer colour arises in beer primarily from the selection of raw materials which comprise the grist (e.g. malts and adjuncts). Components affecting colour can increase during wort production, and can decrease somewhat during fermentation, and can finally be

adjusted to an exact specification by addition of, for instance, caramels or coloured malt extracts.

There are four main routes for the creation of beer colour during processing, which are mainly during malting and wort production. For example, the concentration of pigmented substances in barley is low, but increases during malt kilning process. This process results in colour formation by Maillard reactions. In addition, caramelization and pyrolysis reactions can occur under conditions of high sugar concentration and temperatures. Caramelization reactions proceed as thermal decomposition of sugars at temperatures greater than 120°C, the rate of which depends on pH and type of sugar (Del Pilar Buera et al., 1987; Park et al., 1998). Pyrolysis reactions predominate when the temperature is above 200°C (Shellhammer, 2008), producing intensely black pigments.

Melanoidins

The Maillard reaction, also called nonenzymic or nonoxidative browning, is due to the reaction between reducing sugars and certain amino acids or proteins, via a series of complex reactions under heating conditions (Hodge, 1953; Palmer, 1989). Highly coloured and flavoured compounds are formed. Melanoidins are the polymeric and coloured final products of the Maillard reaction, which are formed during processing of foods, including beer. They are water soluble pigments, with the colour appearing yellow, orange and red initially and turning to brown as the Maillard reactions allowed proceeding (Nursten, 2005).

For beer production, Maillard reactions can occur at various stages of higher temperature processes, such as malt kilning, the roasting of barley, mashing, wort clarification, wort boiling, trub separation, and wort cooling (Hughes and Baxter, 2001).

All malts possess some degree of colour, from very pale yellow in lightly kilned lager

malts to extremely dark in roasted malts or barleys, which can be categorized in three groups: colour, caramel and roasted malts (Coghe et al., 2003). Colour malts (high-dried malts) are produced in a kiln. The extent of colour formation is influenced by time and temperature, but is also influenced by the degree of malt modification. In the production of caramel malt, the kilning operation is replaced with stewing and roasting. Here, green malt, having completed germination is heated to a temperature 65-75°C for enzymatic saccharification (Gretenhart, 1997). During this stewing process, starch is converted into maltose, maltrotriose and glucose, along with nonfermentable dextrins. The temperature is then increased to 80-145°C, where caramelization and Maillard reactions take place because of the high concentrations of precursors and elevated temperatures. These malts tend to be amber with red hues, which contribute significant colour when used in beer production. Roasted malts are heated to temperatures of more than 200°C, and it results in substantial colour formation from pyrolysis reactions. These malts offer limited fermentable extract, but they are used as colouring agents which offer colour ranging from brown to black (Shellhammer, 2008).

Melanoidins are water soluble, so the grist colour is substantially extracted during mashing. Mash pH affects extraction, and therefore the wort colour. Generally, low mash and wort pH helps conserve low wort colour (Lewis and Bamforth, 2006). Therefore, the colour of intensely coloured malts or roasted materials might be incompletely extracted in such a normal mash; this could lead to variability of wort colour (Lewis and Bamforth, 2006). Thus, a separate process with optimal temperature and pH (both high) is needed to extract such grist (Lewis and Bamforth, 2006). The boil is also a source of wort and beer colour pick-up due to the Maillard reaction and caramelization. However, the effect is rather small in modern short-time boils. Colour pick-up in boiling might also increase if significant oxygen entrains as the wort enters the kettle (Lewis and Bamforth, 2006).

Polyphenols

Malt also contributes polyphenols to wort along with peroxidase enzymes (Antrobus and Large, 1997) which can modify the colour of phenolic material during mashing and runoff (Clarkson et al., 1992). The quantities of polyphenols extracted from the grist increase with increasing wort pH, sparging temperatures and long run-offs (Leiper and Miedl, 2008). Further extraction of hop-derived polyphenols can occur during wort boiling. Oxidised polyphenols are another significant source of colour in beer, which generally appear red-brown, forming from polyphenols throughout the brewhouse (De Schutter et al., 2008), and these reactions may be nonenzymic or enzyme catalyzed (Lewis and Bamforth, 2006). In this case, oxygen is indispensable. Hence, aeration or oxygenation of wort whilst still hot will tend to result in colour pick-up. Colour changes due to polyphenol oxidation are most apparent in pale lager beers during storage post-packaging. Fining with polyphenol adsorbents such as polyvinylpolypyrolidone (PVPP), prior to packaging helps mitigate oxidative browning by reducing levels of potential browning polyphenolic precursors (Shellhammer, 2008).

Trace Metals

The colour of beer can also be influenced by the presence of trace metals, such as copper and iron. These metals can stimulate oxidation reactions, e.g. with polyphenols (Oszmianski et al., 1996; Makris and Rossiter, 2000). Metals could be used to modify colour in processing, e.g. aluminium (III) cations promote caramelisation by acting as catalysts, and they are used mainly for colour and flavour adjustment and now frequently in dark roasted malts (Boulton and Quain, 2001).

Other Sources

There are some other sources may contribute to beer colour changes. For example, riboflavin, which present at low level in beer, may contribute significantly to the colour of pale lager-type beers (Pozdrik et al., 2006; Briggs et al., 2004). Riboflavin appears yellow or orange yellow with peak absorbance at 445 nm.

For ale and stout production, some coloring agents such as malt extracts and caramel colouring are used, which have intense red-brown colours (Smedley, 1995a). Using caramel colouring or high molecular weight malt extracts offers brewers a convenient means of ensuring consistent analytical beer colour without modifying beer flavour.

2.3 Beer Translucency

A translucent material is one that both transmits and reflects light, and as a phenomenon, translucency occurs between the extremes of transparency and opaqueness (Hutchings, 1999, 2003).

Transparency characteristics are often used to judge the quality of a food/beverage. The haze in beers normally leads to rejection (Lewis and Bamforth, 2006), although some other preferences exist, e.g. there is a preference for the whitish-yellow hazy wheat beers of in Germany and Belgium (Jackson, 1996). Therefore, it is important to brewer to put efforts on monitoring clarity in production process.

2.3.1 Beer Haze Measurement

Turbidity is the cloudiness or haziness of a fluid caused by particles that are generally invisible to the naked eye. The unit for turbidity commonly used in the breweries is the European Brewery Convention (EBC) unit (100 EBC haze units is obtained by mixing 1 volume 1‰ hydrazine (w/v) and 1 volume 1% hexamethyene tetramine solutions). The haze content for beers should not be over 1.0 EBC, and preferably, below 0.8 EBC (Gan et al., 2001). Normally, it is commercially unacceptable above 2 EBC (Lewis and Bamforth, 2006). The measurement of turbidity is commonly based on an optical principle and is an expression of the optical property that causes light to be scattered

and absorbed rather than transmitted in straight lines through the sample. Both options are based on the same measuring method (Eßlinger, 2009). Absorption is usually measured to detect high particle concentrations, while scatter measurements tend to be applied at lower concentrations (Bretthauer et al., 2009).

Modern turbidimters use the technique of nephelometry, which work from the amount of light scattered, in which a tube containing the sample is illuminated from a set angle. Light scattered by any suspension presented is measured at another set angle, commonly 90° (Figure 2.3.1.1, although some other angles are used) to the angle of illumination. The beer looks bright, but the haze meter can give disparate results. The wavelength of the incident light also varies between instruments (350-860 nm) and some instruments are more sensitive to colour than others (Buckee et al., 1986).

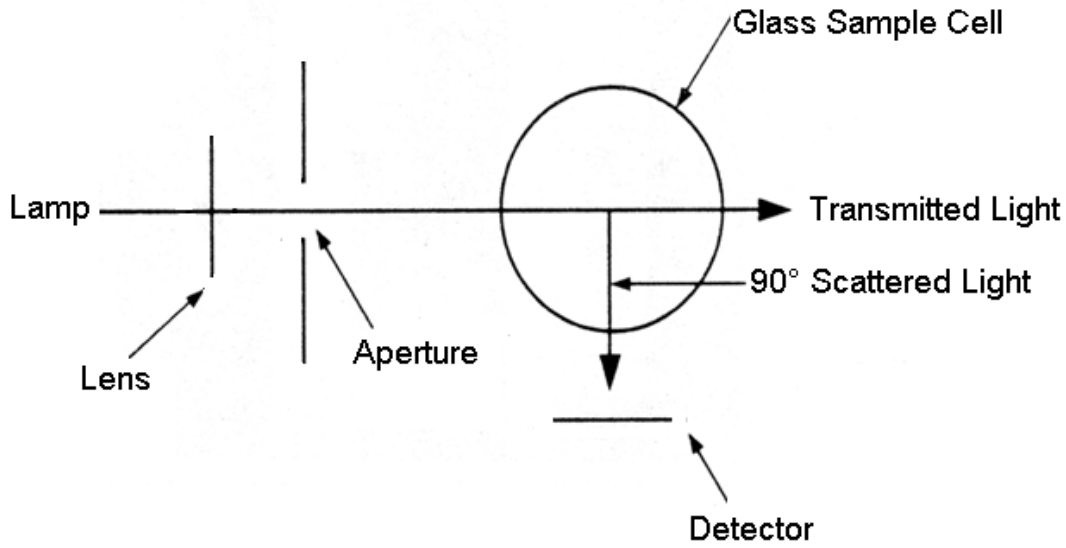

Figure 2.3.1.1 *Sketch of a nephelometer (single-beam design) with 90° detector (Sadar, 1996 cited in EPA, 1999).*

Most instruments measure light deflected at 90°, but 13° forward scatter is also used (Bamforth, 2003). In most cases visual assessment of beer haze correlates well with instrument readings for light scattered at 90°, but some beers which appear bright to the eye give substantial meter readings. Sometimes a beer may contain extremely small particles that are not readily visible to the human eye but that scatter light strongly at

90°. Morris (1987) investigated the relationship between haze and particle size. He found that the 90° haze meter gave results that can be closely related to turbidity for particles > 0.5 μm in diameter but for particles < 0.5 μm it was somewhat oversensitive. A high reading will therefore be obtained for a suspension containing particles < 0.5 μm diameter if the calibration was originally carried out with particles > 0.5 μm.

The accuracy of these instruments in quantifying haze or turbidity should not be overestimated. Turbidity will be quantified only if the light scattered at a set angle is a fixed proportion of the total light scattered from the path of the beam. It is evident (Francis and Clydesdale, 1975) that measurements made at angles of 45° and 90° will place the two particle-sized suspensions in reverse order. The usefulness of the instrument is probably based on its illumination and collection angles being wide enough to average out the scattering caused by the particle size range normally encountered in the practical production situation. The particle size of the standards used to calibrate instruments, and the particle sizes in the beers being measured, also influences the data.

ASTM D 1003 defines haze as "that percentage of light which in passing through the specimen deviates from the incident beam by forward scattering. For the purposes of this method only light flux deviating more than 2.5 degrees on the average is considered to be haze." Of possible application to the food industry are the findings of Billmeyer and Chen (1985), who have investigated the haze and optical clarity of plastic films and sheets (Hutchings, 1999). They used an integrating-sphere spectrophotometer for the accurate determination of haze and showed that valuable information regarding the origin of the haze can be gained by the measurement of transmittance. Four measurements are taken (Figure 2.3.1.2 and Table 2.3.1.1), and the haze can be calculated as:

Step 1. Calculate total transmittance, T_t Eq.15

Step 2. Calculate diffuse transmittance, T_d Eq.16

Step 3. Calculate percentage haze Eq.17

Step 4. Simplify Equation 16 Eq.18

The term in brackets in Equation 18 is particularly important when speciments with low transmittance are being measured (Billmeyer and Chen, 1985). In measurements T1 and T2, light flux from the source falls on the white standard and is reduced by reflection. Subsequently, the reflected flux falls on the internal walls of the sphere, from which it is further diffusely reflected and ultimately detected, the detector viewing the sphere wall but not either port at A or B. In measurements T3 and T4, scattered flux falls directly on the sphere wall without the intermediate step of reflection from a white standard.

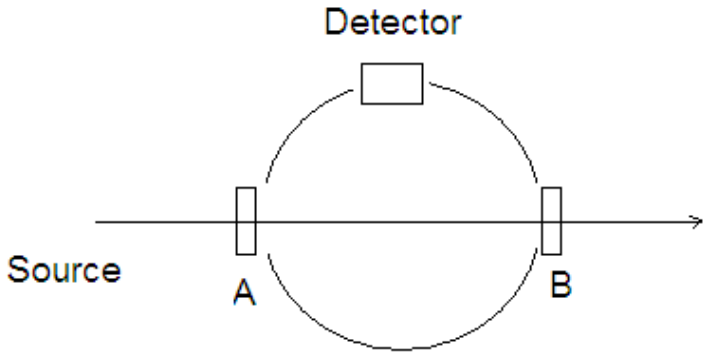

Figure 2.3.1.2 *Sketch of the geometry of the integrating sphere used in haze measurement (Billmeyer and Chen, 1985).*

Table 2.3.1.1 *Instrument arrangements used in the measurement of haze (Billmeyer and Chen, 1985).*

Measurement	Position A	Position B	Quantity Represented
T1	No specimen	White standard	Incident light
T2	Specimen	White standard	Total light transmitted by specimen
T3	No Specimen	Light trap	Light scattered by instrument
T4	Specimen	Light trap	Light scattered by instrument and specimen

Mundy and Boley (1999) concluded that there are significant differences between the haze values obtained on the same sample with different instruments, confirming that the results obtained on different instruments cannot be directly compared.

2.3.2 Composition of Beer Haze

Beer hazes can be divided into two broad types: biological and non-biological. Infection of bright beer with either bacteria, wild yeasts or other fungi will produce a biological haze due to the growth of the invading organisms when the beer will usually become sour and develop other unacceptable off-flavours (Briggs et al., 2004; Lewis and Bamforth, 2006). This biological infection and haze can be reduced by pasteurization and sterile filtration. But sterile beers may develop a non-biological haze. Before beer shows any haze at room temperature, it may form a chill haze.

The Haze Group of the European Brewery Convention defined the non-biological hazes of beer as:

‘Chill haze’: should be used to describe the haze which is formed when beer is chilled to 0°C and which redissolves when the beer is warmed up to 20°C or more.

‘Permanent haze’: haze which remains in beer at 20°C or more.

The unit for beer translucency (turbidity) commonly used in breweries is the European Brewery Convention (EBC) unit. A range of substances can cause non-biological haze in beer:

Metal Cations

Iron and copper have been considered as the metal cations most involved in the formation of metal-containing hazes (Moll, 1987). This was because of the direct contact of the raw material, the mash, the wort and the beer with containers. A concentration of copper greater than 1 mg/l exerts a catalytic action in the oxidation of beer, leading to irreversible haze (Mayer et al., 2003). However, these types of problems with iron and copper in the brewery have almost disappeared since stainless steel was introduced (Moll, 1987). But soluble iron and copper can still be introduced by substances added during the making of beer, such as hop acids, additives, stabilisers, dilution water, filter aids, etc, (Moll, 1987). Zinc also can stimulate the formation of non-biological haze, which was studied by Kühbeck et al. (2006a, c). Their work showed that the zinc concentration had a substantial effect on wort turbidity and hot trub formation during lautering, boiling, cooling and later in the process (Kreder, 1999, Kühbeck et al., 2006b).

Oxalate

Burger and Becker (1949) carried out a bibliographic study which indicated that oxalic acid is produced by simple oxidation of carbohydrates:

$$2C_6H_{12}O_6 + 9O_2 \longrightarrow 6(COOH)_2 + 6H_2O$$

Most beers are known to contain some oxalate, either as an ionic species in solution or as its insoluble calcium salt. The main source of oxalate in beer is barley malt (600 mg/kg), hops (4000 mg/kg) and brewers' yeast (500-800 mg/kg) (Burger and Becker, 1949). The presence of calcium and oxalate ions in beer may lead to the formation of an insoluble white calcium oxalate precipitate or haze (Burger et al., 1956).

A method commonly used by the brewer to decrease the oxalate content in beer is to add gypsum (calcium sulfate dihydrate) during brewing, either to the brewing water, at the mashing stage, or during wort boiling (Briggs et al., 1981). The dissolved calcium ions can combine with anionic oxalate to produce an almost totally insoluble calcium oxalate precipitate, which may be removed by settling, centrifugation or filtration before product packaging. The oxalate is not effectively precipitated if there is insufficient calcium upstream and can head to haze problems in the final beer (Hughes and Baxter, 2001).

Polyphenol-Protein Hazes

Colloidal instability in beer is most often caused by interactions between polypeptides and polyphenols (Siebert and Lynn, 1998).

Beer typically contains around 500 mg/l protein, and most of this is in the form of polypeptides that are in the 5-100 kD size range (Leiper et al., 2003; Steiner et al., 2010). A concentration of only 2 mg/l protein in beer is enough to form haze (Kaersgaard and Hejgaard, 1979). Polypeptides that are responsible for haze formation originate mainly from barley and are rich in the amino acid proline (Mikyška et al., 2002; Apperson et al., 2002; Asano et al., 1982; Siebert et al., 1996; Siebert and Lynn, 1998).

Polyphenols are lost throughout the brewing process, particularly during mashing, boiling, wort cooling and cold conditioning. Beer contains approximately 100-300 mg/l polyphenol (McMurrough and O'Rourke, 1997), which originate from barley and hops. The flavanols group account for 10% of total beer polyphenols and contain the species related to colloidal instability (Leiper et al., 2005). Flavanols found in beer include catechin, epicatechin, gallocatechin and epigallocatechin (Siebert and Lynn, 1998; Figure 2.3.2.1). They can exist as monomers but are more commonly joined to form flavanoids as dimers, trimers or lager polymers. Two dimers have been particularly associated with haze formation: procyanidin B3 (catechin-catechin) and Prodelphinidin B3 (gallocatechin-catechin; Figure 2.3.2.1). The flavanoids can further polymerize and

oxidize to produce condensed polyphenols called tannoids (Chapon, 1994). This oxidation can occur throughout the brewing process, enzymatically during mashing or non-enzymatically during boiling.

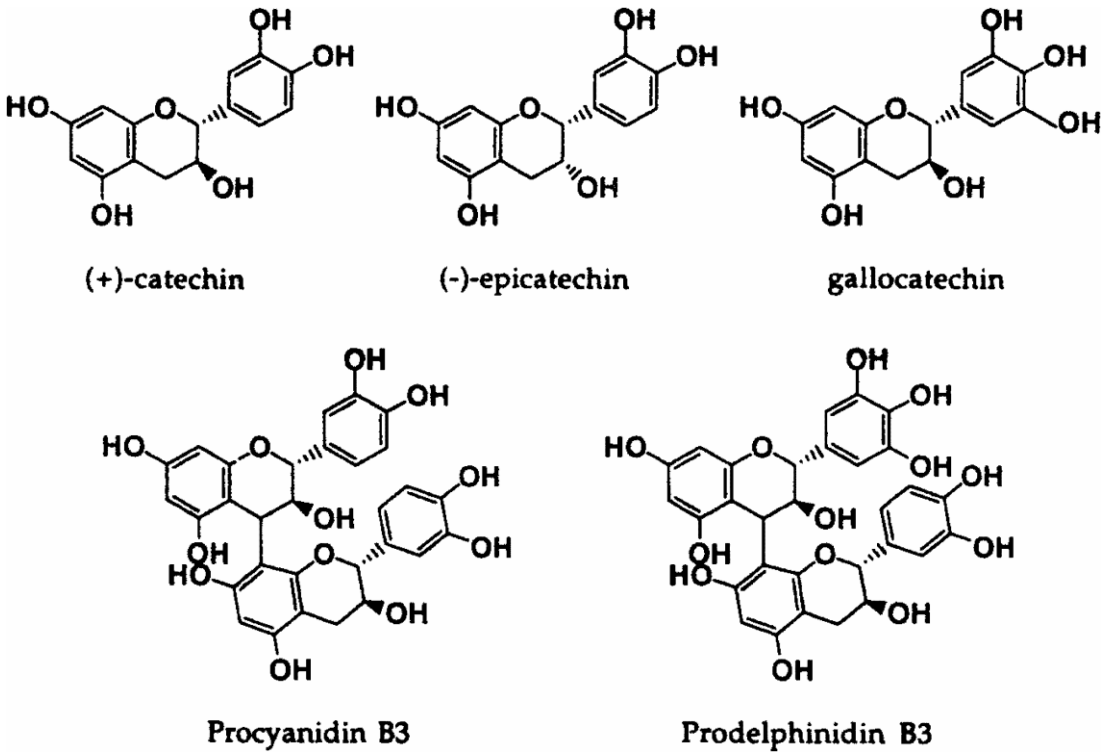

Figure 2.3.2.1 *Structures of the main beer flavanol monomers and dimers (Siebert and Lynn, 1998).*

These tannoids and proteins can form reversible chill haze by hydrogen bonding, which will redissolve if the beer is warmed (Siebert et al., 1996). Covalent bonds soon form between the tannoids and proteins, and insoluble permanent haze are then created which will not dissolve when heated. There is also scope for secondary hydrophobic interactions between polyphenols and proteins. Siebert et al. (1996, 1998) proposed a model for interactions between polyphenol and proline-rich protein in beer. In this model, the polyphenols must have at least two protein binding sites for forming large aggregations (Figure 2.3.2.2).

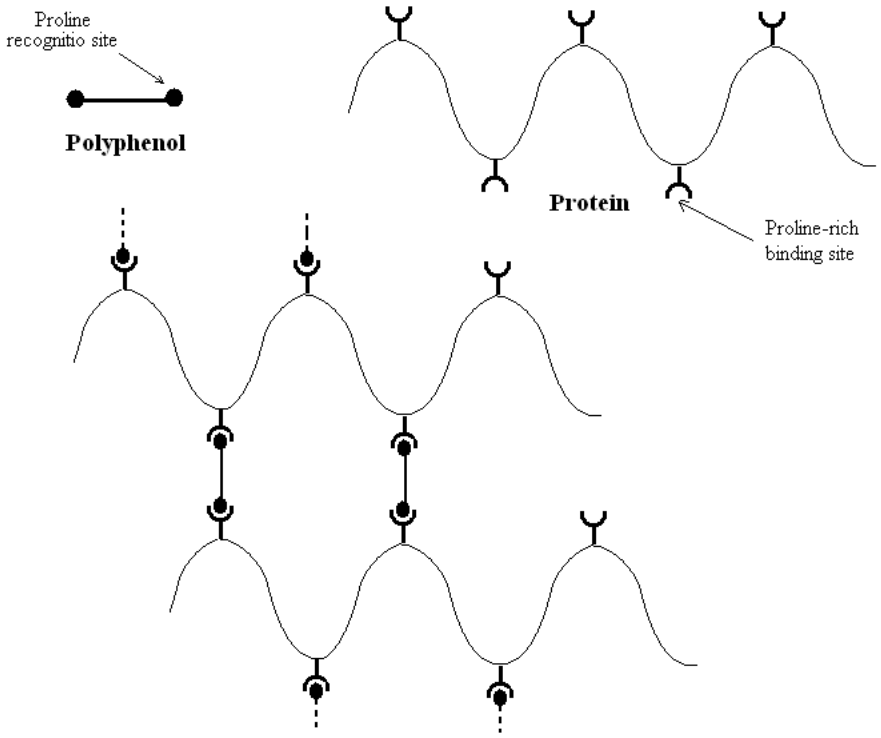

Figure 2.3.2.2 *Model proposed for polyphenol-protein interactions in beer. This model implies a more or less definite stoichiometry of cross-linking (Siebert et al., 1996 and Siebert and Lynn, 1998).*

The amount of haze formed depends on the concentrations of both the protein and the polyphenol and their ratio. When the number of polyphenol haze-active sites equals the number of protein binding sites, the largest network with the largest particles will be formed. With an excess of protein, the polyphenol will form a bridge between two peptide chains but there will be insufficient for further bridges. With an excess of polyphenol relative to protein, all the protein binding sites will be occupied and it is unlikely that the free end of the polyphenol will find a vacant protein site for further cross-linking.

Some operations have been devised to minimise or eliminate these interactions from occurring:

1. By removing the reactive species: reducing the concentration of haze active proteins, and/or reducing the concentration of haze active polyphenols.

In contrast with other beverages, beer has a significant excess of haze-active proteins and a low proportion of haze-active polyphenols (Siebert et al., 1996). Therefore, reducing the polyphenol content is an efficient way to stabilize beer (Mikyška et al., 2002). Removal of haze active polyphenols can be accomplished by adsorption using PVPP (polyvinylpolypyrrolidone) or a PVP (polyvinylpyrrolidone)-modified silica gel (Chapon, 1994). Mikyška's work (2002) showed that PVPP treatment of beer had a positive impact on the flavour stability of heat-aged beers.

The haze forming reaction can also be prevented by removing the haze active protein components with silica gel. Silica gel (SiO_2) is a highly porous structure with a large surface area. Its surface is covered with silanol (SiOH) groups that bind to proline residues in polypeptides. Silica is highly selective for haze protein adsorption due to high levels of proline present in these polypeptides. The proteins involved in foam stability, which contains little proline is thus relatively unaffected by silica (Leiper et al., 2003).

Brewers' Clarex^{TM} is a protease preparation, which is specific to haze-causing polypeptides rich in proline (van Roon and Craig, 2010). The enzyme was found to be effective in model systems and was tested in pilot-scale trials, being added to the fermentation vessel with the wort (Lopez and Edens, 2005). The beers produced showed good haze stability, and had very little effect on foam stability.

2. Chill filtration is often effective for improving the physical shelf-life of beer. The lower the temperature the more cold trub and chill haze will form. Subsequent filtration at low temperatures will remove these materials.

Carbohydrates

The carbohydrate haze in beer may be clearly visible, or scatter light in conventional

haze measurements. These types of haze are due to the presence of retrograded starch.

During mashing, an aim is that the starch should be degraded by amylolytic activity. Unconverted starch will undergo retrogradation when cooled. The retrograded starch is more resistant to enzymic breakdown than the degraded starch and then gives a haze (van der Maarel et al., 2002; Quaglia and Gennaro, 2003; Bamforth, 1985).

Malt-derived hazes usually contain high levels of β-glucans. Even though β-glucans may not always form gelatinous precipitates in beer, they can still cause haze problems (Durand et al., 2009; Speers et al., 2003). β-Glucan is known to cause reduced recovery of extract by impeding enzyme access, reduced rates of lautering, reduced filter run off rates and formation of hazes in the final beer (Bamforth, 1994).

2.4 Psychophysics and Sensory Evaluation

The consumer may use his/her eye to "taste" food. For example, colour is one of the major attributes which affect the perception of quality (Francis, 1995). The consumer assesses beer quality on colour and clarity attributes by eyes, although the results may be different as those obtained from instrumental measurements. Industries always compare these data obtained from instruments with sensory evaluation (e.g. Corby Bottlers, Coors) for accuracy.

Psychophysics, a term that was introduced by Gustav Fechner in 1860, is the study of the relationship between the psychological perception of a sensory stimulus and the intensity of the physical stimulus that causes that perception. Sensory evaluation is the utilization of psychophysical techniques in the food industry to answer three types of questions (Fisher and Thomas, 1997), and which are the outcomes of the three main types of testing:

1. Description: what are the sensory attributes of the product? How is one product different from another in quality? How do changes in the process, formulation, packaging, or storage conditions affect its perceived sensory characteristics?

The first step in studying perception is only describing what we perceive (Goldstein, 2002). Descriptive tests are used to specify appearance characteristics of products in quality or quantity. They are flexible enough to be used to describe appearance properties from the raw material, through processing, to the finished-product stages.

2. Discrimination: are there discriminable differences between two or more samples? How many people would detect it? If there is a difference, how great is it?

Fechner described a number of quantitative methods to measure the relationship between stimuli and perception, and these methods are called the classical psychophysical methods because they were the original methods that were used to measure the stimulus-perception relationship (Goldstein, 2002). Fechner's contribution was to work out the details of three important sensory test methods: the method of limits, the method of constant stimuli and the method of adjustment or average error (Boring, 1942, cited in Lawless and Heymann, 1998). Each of the three methods was closely associated with a measured response of sensory systems. The method of limits could be used to determine absolute thresholds; the method of constant stimuli used to determine difference thresholds; and the method of adjustment used to establish sensory equivalence.

Discrimination or difference tests indicate whether a difference can be distinguished between two samples. The samples can be presented in a pair or in threes, two of which are the same. These tests can be determined to a pre-defined statistical level of significance not only whether a difference can be seen but also the individual's level of sensitivity to the difference of the stimulus being offered. The absolute threshold occurring in a paired-comparison test of a graduated series of samples is normally taken as the point in the series at which 75% of the judges responses correctly (Lawless and Heymann, 1998).

3. Affective or hedonics: how much is a product enjoyed? Is it an improvement over another product? Which attributes that are liked or disliked? Which product would the consumer select?

Affective or hedonic testing involves judgments made on scales of liking, pleasure, desirability, satisfaction, etc. (Hutchings, 1999).

Magnitude estimation is used to estimate the relationship between physical intensity and sensory magnitude and to obtain comparative ratings of specific attributes (Hutchings, 1999). Magnitude estimation is a priori a ratio scaling procedure, in which subjects are instructed to assign numbers in relative proportions that reflect the strength of their sensations (Stevens, 1956). It is adapted from psychophysical research (Lawless and Heymann, 2010). The ratio between the numbers are supposed to reflect the ratios of sensation magnitudes that have been experienced. Panels are presented with a number of samples and are asked to assign a number to each based on their perception of the intensity of some quality. For example, suppose panels were asked to assign numbers corresponding to the perceived lightness of several beverages. Because the observers assign umbers based on ratios or proportions, magnitude estimation data are considered to be on a ratio scale. This method allows each subject to use a wide range of positive numbers without restriction. Two primary variations of magnitude estimation have been used (Lawless and Heymann, 2010). In one method, a reference sample is given and assigned a fixed value. All subsequent samples are rated relative to this reference. This reference must be presented first, but it may also be reintroduced later. In the other variation of magnitude estimation, no reference sample is given and a panel is free to choose any number he/she wishes for the first sample. All subsequent samples are then rated relative to the first sample. People may use different ranges of numbers, so the data have to be normalized into same range (ASTM, 2008).

Magnitude estimation data are often transformed to logs before data analyse. The general form of the relationship between physical stimulus (I) and the sensory intensity (R) is

Eq. 19

Or after log transformation:

Eq.20

where R is the sensory response intensity,

I is the physical stimulus intensity,

k is a constant of proportionality that depends upon the units of measurement,

n is the exponent of the power function or slope of the straight line in a log-log plot (Stevens, 1957 cited in Lawless and Heymann, 2010).

The data do tend to be a bit more variable than other bounded scaling methods. The unbounded nature of the scale may make it especially well suited to sensory attributes where an upper boundary might impose restriction on the panelists' ability to differentiate very intense sensory experiences in their rating (Lawless and Heymann, 2010).

Category scaling is the most popular method of sensorially assessing foods (Hutchings, 1999). The coded samples are presented simultaneously or sequentially in a balanced order which differs among the individual panels. Category scales consisting of a series of word phrases structured in ascending or descending order of intensity are used to measure the specific attribute (e.g. bright, cloudy, etc). The scales all involve a horizontal or vertical line with deliberately spaced labels and the panels' task is to make a mark somewhere along the line to indicate the strength of their perception or strength of their likes or dislikes. For analysis purposes, successive digits are later assigned to each point represented on the scale (Green et al., 1993). This follows the convention of having higher numbers represent a greater magnitude or more of a given quality.

2.4.1 Colour Vision

Trichromatic theories of colour vision

The trichromatic theory of colour vision states that colour vision depends on the activity of three different receptor mechanisms. The theory was originally proposed by Thomas Young and Hermann von Helmhotz (Goldstein, 2001) based on the results of a psychophysical procedure called colour matching. The colour matching experiments indicate that people with normal colour vision can match any spectral colour at different wavelength by mixing a certain amount of three primary lights, i.e. red, green and blue. In this theory, our eyes have three types of colour receptors, which are called cones. These three types of cones have different sensitivities to different wavelengths of light. The short cones are most sensitive to blue light; the medium cones, to green light; and long cones, to red light. The response pattern of these three types of cones allows us to see different colours, e.g. when medium cones are most strongly activated, we see green; and when a combination of different types of cones is activated, we see colours just as mixing paint of different colours produces yet other colours, e.g. when long and medium cones are stimulated at the same time, we see yellow.

Opponent-process theories of colour vision

Ewald Hering (1834-1918) used the results of phenomenological observations, in which stimuli were presented and observers described what they perceived, to propose the opponent-process theory of colour vision, which based on his work with afterimages. An afterimage is what you see if you gaze at a visual stimulus for a while and then look at a neutral surface. Like trichromatic theory, this theory suggests that our eyes have three types of colour receptors, and each type of receptor consists of a pair of opposing receptors. In this theory, some receptors are sensitive to red or green; others, to blue or yellow; and others, to black or white. According to this theory, red-green receptors do not simultaneously transmit messages for red and green. Rather, they transmit messages

for either one or the other, such as the fact that it is difficult to imagine a bluish-yellow or a reddish-green. When the red one is activated, the green one is blocked or inhibited, and so we see red.

Which model of colour vision has it right? Both theories are right to a certain extent. The trichromatic theory is correct at the receptor level, since the photochemistry of cones responds in the way described by trichromatic theory—some are sensitive to red light; others to green light and others to blue light. Hering's opponent-process theory is correct in terms of the behavior of cells that lie between the cones and the occipital lobe of the cerebral cortex—including bipolar and ganglion cells. Most authorities believe that colour vision includes elements of both trichromatic and opponent-process theories (Nevid, 2009).

Trichromats are people with normal colour vision who can discern all the colours of the visible spectrum. About one out of every 40000 people is completely colour blind, who can see only in black and white, and we classify them as monochromats. These individuals have only one type of cone, so their brains cannot discern differences in wavelengths of light give rise to perception of colour. Dichromats are more common—people who lack one of the three types of colour receptors. It is difficult for them to distinguish between certain colours. About eight percent of men and about 0.44% of women have some form of colour blindness (Lawless and Heymann, 2009). The most common form is red-green colour blindness.

2.4.2 Visual Colour and Translucency Evaluation

Sensory evaluations of colour and translucencyr are frequently performed, and sensory scientists have used the whole range of sensory testing tools to undertake visual translucency assessments and colour measurements (Lawless and Heymann, 1998).

The sensory scientist performing colour assessment should carefully standardize and control some experimental conditions (Lawless and Heymann, 1998). The CIE provides some recommendations on the concerned basic colorimetry, which should be controlled in the evaluations: the reference standard for reflectance; the use of the standard illuminants; the illuminating and viewing conditions; the standard colorimetric observers, etc.

- Background colour in the viewing area.

  Ideally the background colour should be non-reflective and neutral (ASTM, 1982).

- Illuminant and sources.

  Standard lights used in food colour evaluation tend to be CIE defined illuminants A, C and D65 (see also Section 2.2.1). The illuminant A is high in red-yellow wavelengths and low in blue-violet wavelengths. Illuminants C and D65 are both high in blues. Lights C and D65 are designed to mimic variations of daylights. Under different illuminants, the differences in perceived colour occur, because the colour depends on the absorption of light by the object and the incident spectrum's wavelengths.

- Panellists' viewing angle and the angle of light incidence on the sample. Colorimetric specifications are derived from spectral or tristimulus measurements. The measured values depend on the geometric relationships between the measuring instrument and the sample. These relationships are called "geometric conditions" or "geometry". Similarly visual appraisals of samples are affected by geometry (CIE, 2004). The geometry is described by first indicating the angle of illumination by the light, and then the angle of viewing by the detector in a format such as 45º/0º. However, when the geometry is the inverse, it is considered to be equivalent, as long as the illumination and viewing angles are exactly reversed. Usually the booth area is set up with the illuminant vertically above the samples and the panelists viewing angle when panellists are seated is about 45° to the sample, this minimizes specular reflection effects.

- Distance from the light and the product. This will be affected the amount of light incident on the sample.
- Whether the sample is lit with reflected or transmitted light.
- Panellists should be tested for colour blindness, e.g. by Ishihara colour vision test (Ishihara, 2004).

Sensory evaluation on colour is not a simple task, it is a complex process. For example, Melgosa et al. (2000) demonstrated that human were more sensitive to small ΔL*. In their research, they proved the number of correct responses tended to decrease when the lightness difference ΔL* increased, i.e. observers gave more correct responses when the lightness difference (ΔL*) was 10 units (70-90% correct) than that was 27 units (30-60% correct). From their and other research, lightness and hue were distinguished more accurately than colourfulness attribute (Melgosa et al., 2000; Ji et al., 2005).

Instrumental methods are available for turbidity measurement (Section 2.3.1), but it is always prudent to cross-reference instrumental values with human perception. If the relationship between perceived turbidity and instrumental turbidity is not well known for a product, it is recommended human assessors are tested to evaluate their sensory reactions to the product. In other words, light scattering as a physically measured phenomenon may not tell us what we need to know about perceived turbidity (Lawless and Heymann, 1998). Particle size affects light scattering, thus, it should be possible to correlate sensory clarity and distribution of suspended matter in a sample. A liquid which transmit more light will appear more transparent. However, this relationship may be complicated by other factors, such as the colour of the medium (Siebert, 2009).

2.5 DigiEye Digital Imaging System Development

Foods and beverages typically contain light scattering particles of a range of sizes and

absorption properties, and mixtures of natural pigments having ranges of light—absorption characteristics. It is not easy or accurate to measure their colour and translucency by conventional methods. The DigiEye system together with a new multiple path-length cell is designed. It is a non-contact digital-grading system for assessing colour appearance based on the CIECAM02 colour appearance model (see also Section 2.2.2; Cui et al., 2004).

The DigiEye system basically includes a computer, a coated cabinet (VeriVide Ltd; Figure 2.5.1) with an illumination source (D65 was used in this research), a digital camera and a colour sensor for calibrating displays (Luo et al., 2002; Luo et al., 2003). A novel multiple path-length cell, consisting of six different pathlengths, was introduced and discussed (see also Section 4.3) which can cover a wide range of particles sizes typically found in foods and beverages, was used together with DigiEye system.

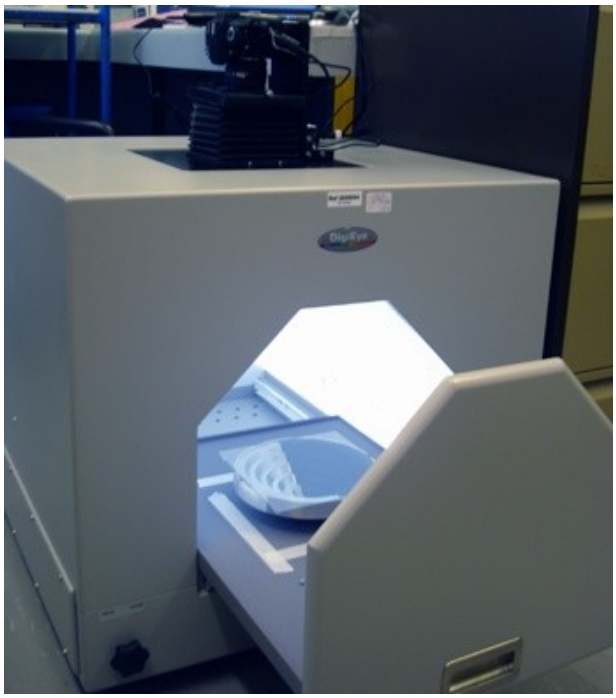

Figure 2.5.1 *Digital viewing cabinet with digital camera and multiple path-length cell.*

2.5.1 Theory for the Independent Specifications of Colour and Translucency

Particles with different sizes in a liquid will reflect and/or scatter incoming light to different extents. In a clear liquid, the absorption of light by dissolved particles dominates when light travels through the liquid body. This liquid is transparent with different intensity of colour, where Beer-Lambert law relates optical properties of light with the physical properties of liquid, i.e. optical path length and concentration. When a liquid is too dark and all the light is absorbed, the situation of opaque absorption prevails. According to the Beer-Lambert Law, the absorption of light is proportional to the concentration of absorbing particles in the liquid (Christie, 2001):

Eq.21

where: I_{in} is the intensity of the incident light;

I_{out} is the intensity after passing through the material;

T is transmittance;

A is absorbance;

ε is the molar extinction coefficient at that wavelength;

l is the distance that the light travels through the material (the path length);

c is the analyte concentration.

Many industries rely on the Kubelka-Munk theory for colour measurement based on reflectance measurements (Figure 2.5.1.1, Kubelka and Munk, 1931; Loof, 1967 cited in Broch, 2002), which allows the interconversion of light reflectance and transmittance. It was suggested that the scattering phenomenon, like the absorption phenomenon is considered (Kubelka and Munk, 1931).

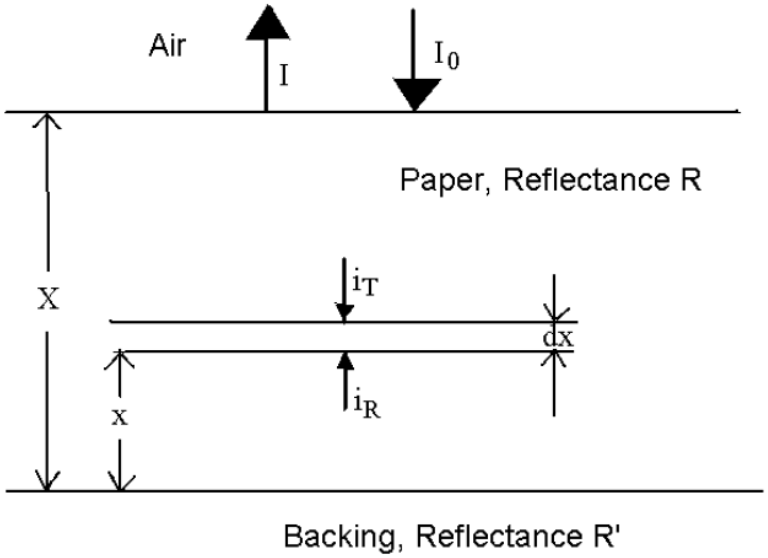

Figure 2.5.1.1 *Consider light of intensity I_0 incident on a piece of paper of thickness X and reflectance R. Behind this paper is a surface of reflectance R'. The light which re-emerges from the top surface of the paper after scattering, absorption or transmission has intensity I. At a distance x from the bottom surface of the paper there is a thin lamina of thickness dx scattered light is incident on it which is travelling both upwards and downwards through it with intensities i_R and i_T, respectively.*

where K is the absorption coefficient: the limiting fraction of absorption of light energy per unit thickness, as the thickness becomes very small,

S is the scattering coefficient: the limiting fraction of light energy scattered backwards per unit thickness at thickness tends to zero.

The effect of the material in a thin element dx on i_T and i_R is to:

decrease i_T by $i_T(S + K)dx$ (absorption and scattering)

decrease i_R by $i_R(S + K)dx$ (absorption and scattering)

increase i_T by i_RSdx (scattered light from i_R reinforces i_T)

increase i_R by i_TSdx (scattered light from i_T reinforces i_R).

So:

$$-di_T = -(S + K)i_Tdx + i_RSdx \qquad \text{Eq.22}$$

$$di_R = -(S + K)i_Rdx + i_TSdx$$

The Beer-Lambert law is valid at lower concentrations of solutes, in the absence of

significant scattering, and only if individual wavelengths are used. The Kubelka-Munk theory works well because these industries use ingredients that are closely controlled in terms of light scattering properties and pigment absorption properties (Leach and Pierce, 1993), and are uniform in thickness and appearance. Most liquid foods are translucent and both absorption and scattering processes exist when a light travels through the liquid bodies (Figure 2.5.1.2). In this situation, derivations from the Beer-Lambert law become significant due to the single wavelength dependency, and path length distribution analysis is needed.

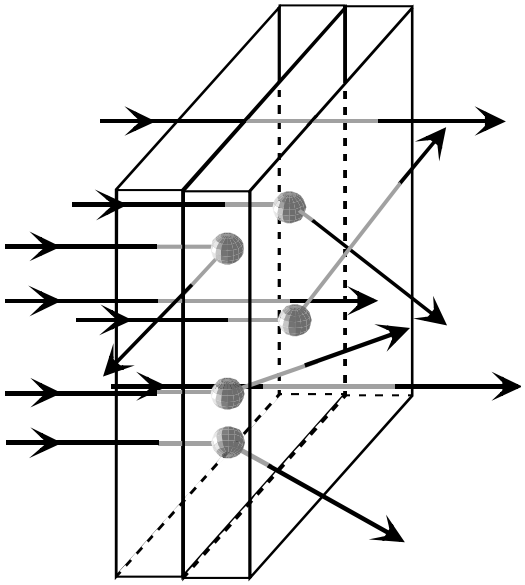

Figure 2.5.1.2 *Light passes though a liquid body with particles of different size, resulting different transmission, scattering and reflection.*

Absorbance A in the Beer-Lambert law can be measured spectroscopically, and by either changing "c" and fixing "l", or changing "l" and fixing "c", the value of ε can be estimated for this type of liquid sample under investigation, either turbid or clear. When ε is known, a set of path lengths can be estimated for that particular type of sample (Gilchrist and Nobbs, 1997). It is preferable to reduce path length rather than diluting to reduce concentration since the dilution may affect the chemistry of the system and alter its colour (Smythe and Bamforth, 2000). Thus, we focus only on changing "l" and fixing "c" to identify ε values for different samples. Therefore, the Beer-Lambert law

can be re-arranged as:

Eq. 23

With

Thus,

Eq.24

Where the *A, T, ε, c, and l* are defined as Equaiton 19.

If an optical cell is built according to the analytical path length distribution, the overall light scattering performance of the liquid body would behave to the expected analytical path, i.e. the overall colour appearance and translucency of the liquid body would close to the human observation. In other words, the colour measurement of the liquid will relate to observer judgments on colour and translucency characteristics of the samples. Statistics are applied to analyse the amount of light travels at a certain length (Gilchrist and Nobbs, 1997). In principle, the tasks are based on: determination of ε for different materials under current investigation with 10 mm as reference path length; identifying four critical wavelengths for each material; identifying optical cell depth data set which gives the required variation in transmittance at each wavelength and determining specific material ε values and adjust this back to 10 mm path length.

The design of multiple path-length cell was based on the CIE XYZ data obtained from TSR (tele-spectroradiometer). Spectroradiometry is a method of measuring the spectrum of radiation emitted by a source or object (Bentham, 1997), with a telescope lens on the front. It separates by diffraction grating the radiation of an object into single component wavelength ranges of the visible spectrum (from 380 to 780 nm with a 0.9 nm/pixel wavelength resolution; Figure 2.5.1.3—Minolta CS1000, 2005) and sequentially captures and measure their intensities. The obtained single wavelengths are converted into tristimulus XYZ values and subsequently into colour appearance attributes,

lightness, colourfulness and hue.

Figure 2.5.1.3 *Minolta CS-1000 Tele-spectroradiometer (Minolta CS-1000 Tele-spectroradiometer, 2003).*

2.5.2 The Digital Imaging System

A stainless steel cell was designed and constructed (by Department of Colour Science of University of Leeds) together with relavant models (e.g. optical model, multiflux model and translucency model). This cell consists of six different path lengths, which was designed to cover a wide range of particles sizes typically found in foods and beverages.

With all analysis, the path length distribution by the optical cell (Figure 2.5.2.1) was decided as 2 mm cell, 5 mm cell, 10 mm cell, 20 mm cell, 30 mm cell and 50 mm cell. The 2 mm depth was specially designed for dark colour samples, such as red wine samples, to produce a thin layer liquid body. A 50° slope surface is made at each depth to trap the light inside the liquid body. This cell can be further modified incorporation a pump for continuous or stepped in line sampling.

Figure 2.5.2.1 The *robust optical cell made from stainless steel with six internal steps.*

The Nikon D80 digital camera is used in this system. Different digital cameras produce different RGB responses for the same scene, and the output RGB signals do not directly correspond to the device-independent tristimulus values based on the CIE standard colorimetric observer (Hong et al., 2000). A software was developed (by Department of Colour Science of University of Leeds) to establish a relationship between digital camera's RGB signials and CIE XYZ values. The Nikon D80 digital camera used in the system was calibrated against a GretagMacbeth® DC chart as shown in Figure 2.5.2.2. The performance of the camera is assessed through colour difference analysis by CIEDE2000 ΔE_{00} colour difference formulae. The desired median value is less than one.

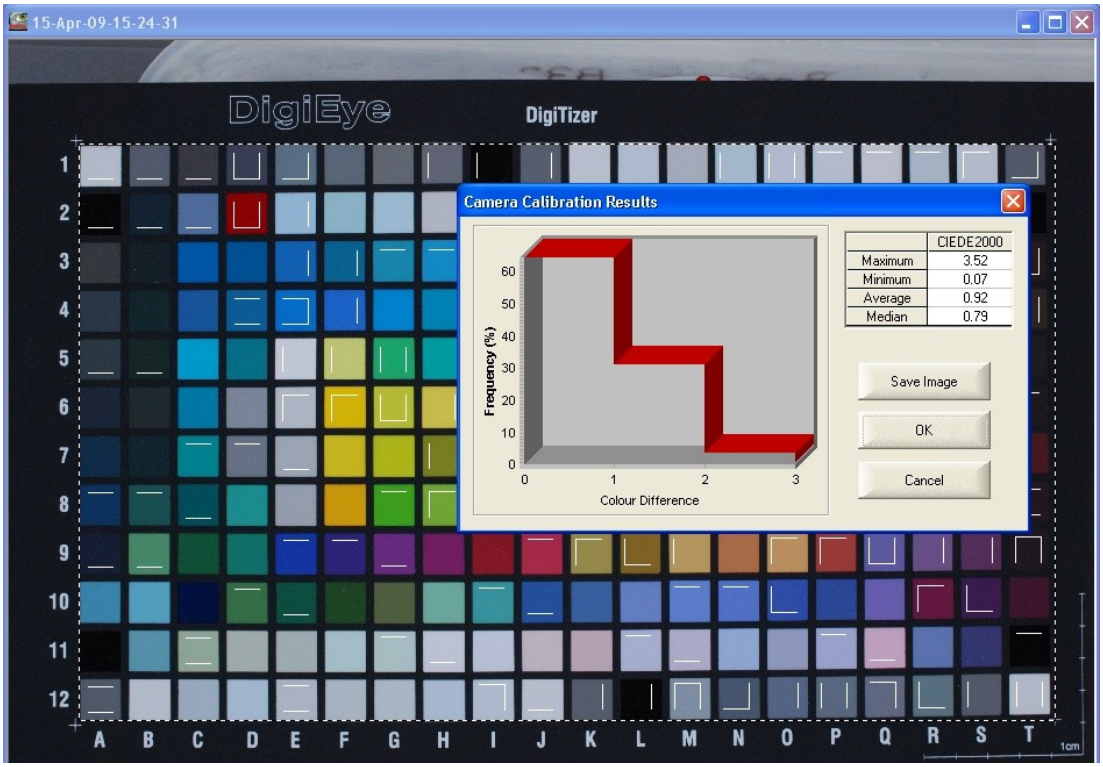

Figure 2.5.2.2 *DigiEye characterisation performance against a GretagMacbeth DC chart.*

According to the Beer–Lambert law, for a given sample in a particular cell, the ε and c terms are constants. In the six-depth cell, averaged S (εc) is converted back to CIE XYZ at standard depth. By comparing the CIE XYZ of a sample in highball glass measured by tele-spectroradiometer (TSR), this standard depth can be optimised. This optical modelling refining process optimise the minimum colour difference between a predicted CIE XYZ on a standard depth cell using digital with measured CIE XYZ on a highball glass using TSR.

The accompanying software was also developed (by Department of Colour Science of University of Leeds) to automatically evaluate transparency (T), lightness (J), colourfulness (M), and hue composition (red (R), yellow (Y), green (G) and blue (B)) data for each sample in the six depth (as RING 1 to RING 6; the output is as Figure 2.5.2.3), which are converted from CIE XYZ values. These data were used as input to the mathematic model to correlate with observer data to opacity, clarity, etc. The

software developed is called “six-stage transformation”. The measurement of process could be summarised as (Figure 2.5.2.4):

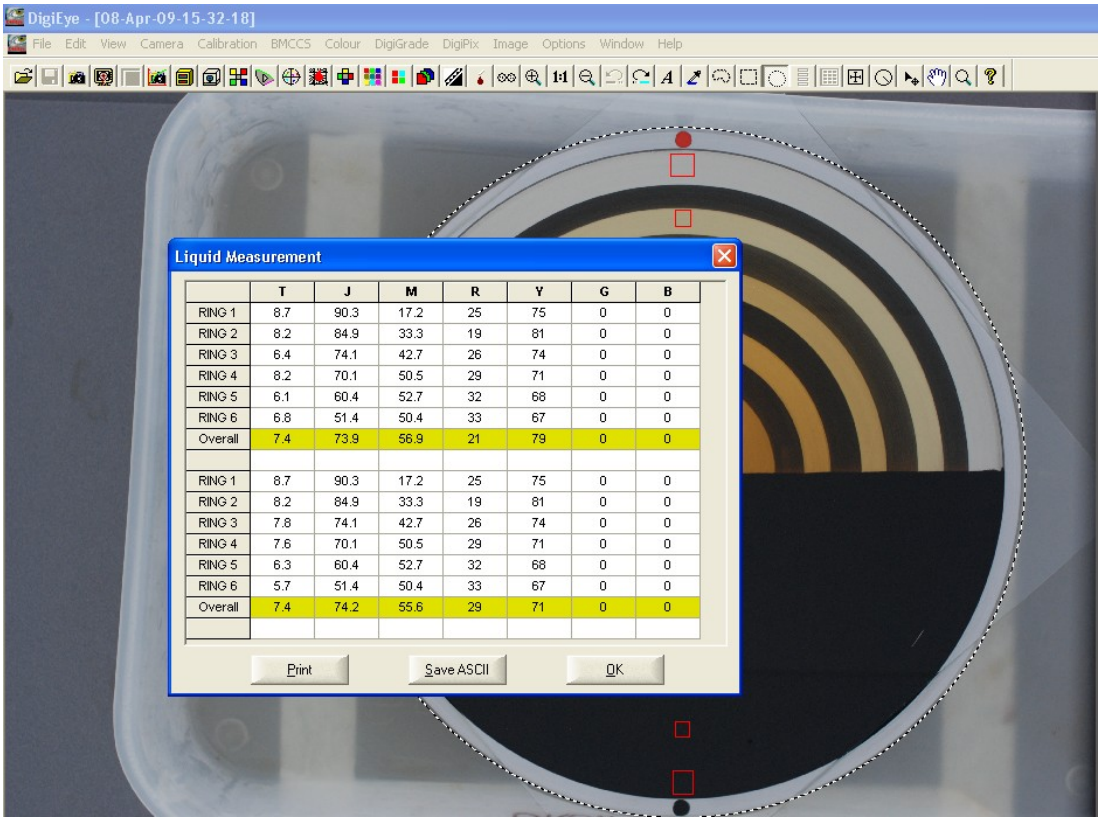

Figure 2.5.2.3 *DigiEye sub-sampling image at six different depths over white/black background.*

- Calibration of digital camera to transfer device dependent camera RGB signal to standard CIE XYZ values.
- Captures an image of a sample to obtain one set of six XYZ data over white background and another set of six XYZ values over black background. Each of the six values is corresponding with six different depths in the desined metal cell.
- An optical model (by Department of Colour Science of University of Leeds) is introduced to transfer six different depth of XYZ values to a standard depth of XYZ under a desire condition.

- From the XYZ values of sample calculate CIECAM02 colour appearance descriptors, lightness (J), colourfulness (M) and hue (H) composition.
- Applying multiflux optical model (by Department of Colour Science of University of Leeds) to quantify liquid translucency properties through liquid reflectance (R_o) and internal transmittance (T_o).
- R_o and T_o are further computed to translucency scale through a translucency model (by Department of Colour Science of University of Leeds).

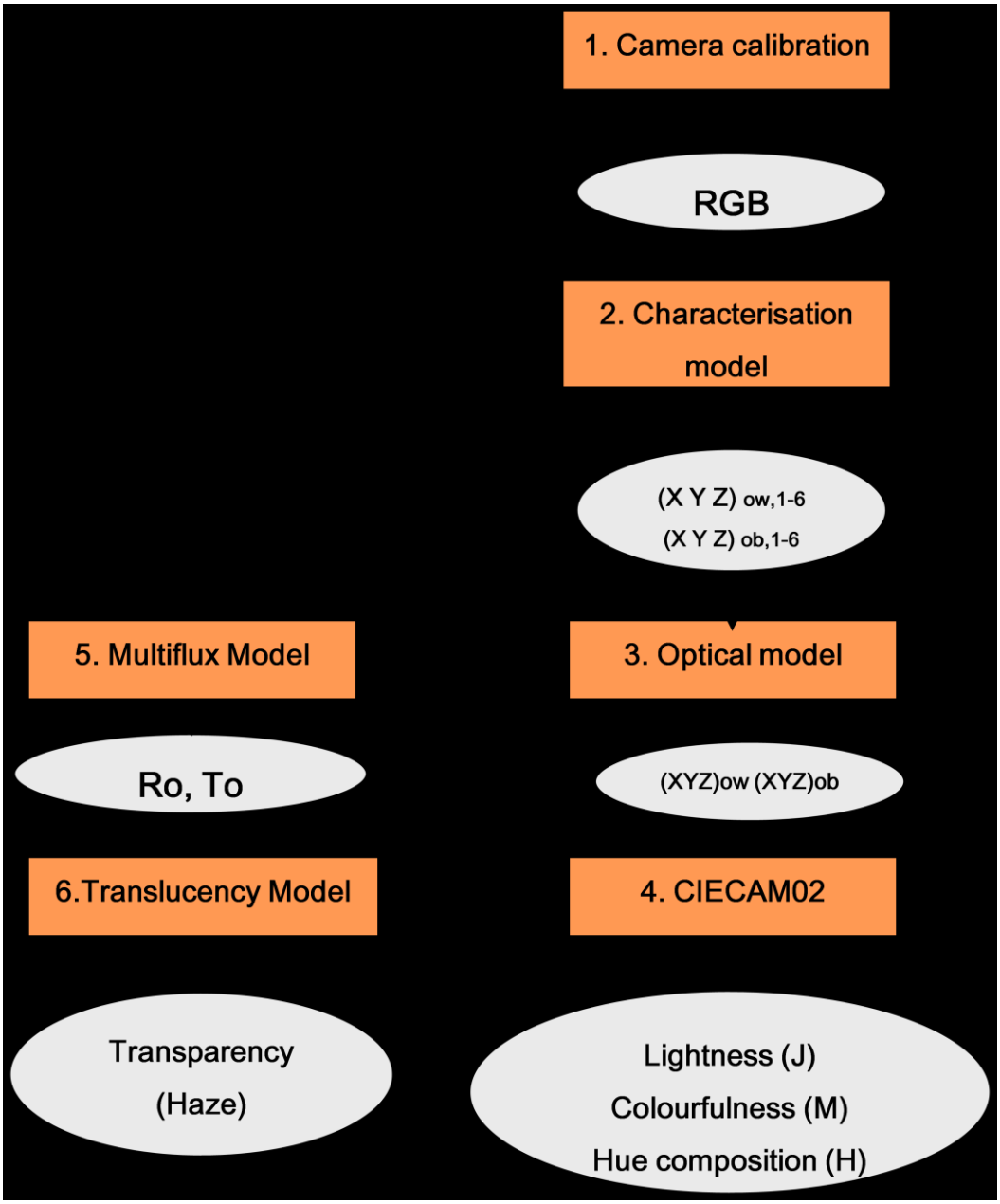

Figure 2.5.2.4 *Digital system measurements by "six-stage transformation".*

The measurement of the liquid samples in the cell proceeded as follows:

- The D65 illuminants in the DigiEye viewing cabinet were turned on and warmed up for twenty minutes.
- Camera was set up and calibrated against a GretagMacbeth® DC chart.
- The cell is then put into the DigiEye viewing cabinet. The cell position is pre-defined so that the mirror image of the cabinet viewing window is eliminated.
- The samples were carefully poured into the cell until surface tension raises the liquid surface above the cell edge.
- A sheet of optically flat glass was used to cover the liquid. This was to ensure a parallel top and bottom surface liquid layer. Care was also taken to ensure that no air bubbles were caught underneath the glass, as they will introduce random light scattering.
- An image of the sample in the cell was taken and automatically imported into a named software package. After defining the measuring area, the software automatically allocates 12 measuring area in squares, as 6 squares over white and other 6 over black, with the depths of 2 mm, 10 mm, 20 mm, 30 mm, 40 mm and 50 mm.
- The software then reports relevant appearance attributes of transparency, lightness, colourfulness, hue composition as redness, yellowness, greenness and blueness.

Chapter 3. MATERIALS AND EXPERIMENTAL

The performances of the instruments used in this research were tested in repeatability. In each test, distilled water was used as sample and tested by thirty times, and the average value was compared to each individual.

3.1 Colour and Translucency Evaluations of Beverages

3.1.1 Beer Experiment

Three lagers (Coors, Carling and Grolsch-all malt) and three ales (Worthington, Caffrey's, and Stones) sourced from Coors Brewing Ltd. (Burton-Upon-Trent, UK) were assessed. The colours of these six beers ranged from "light" to "dark" and all the commercial samples were supplied in cans:

Coors: Coors Light, alcohol content 5.0%ABV

Carling: Carling Black Label, alcohol content 4.1%ABV

Grolsch: Grolsch Premium Lager, alcohol content 5.0%ABV.

Worthington's: Worthington's Creamflow Draught Bitter, alcohol content 3.6%ABV

Caffrey's: Caffrey's Irish Ale, alcohol content 4%ABV.

Stones: Stones Bitter, alcohol content 3.7%ABV.

All the beer samples were stored at room temperature and in dark conditions. Before the experiment, the beer samples were degassed, so that gas bubbles did not affect the visual sensory and transmittance measurements. A Whatman® No. 1 filter paper was used, with a particle retention diameter of 11 μm, to degas the beers. The samples were assessed within two hours of filtration.

The colour stability of beer samples was evaluated by using GretagMacbeth CE7000A spectrophotometer. The transmittance data then were transformed into CIE XYZ values

applying a CIE D65 10 degree observer weighting table (CIE, 2004). By comparing measurement from each day results with that obtained on the first day, the stability of the industrial samples was indicated using colour difference CIEDE2000 ΔE_{00} and CIE LAB ΔE^*_{ab}.

All analyses (including instrumental and psychophysical experiments) were performed within three days of the cans being opened.

Commercial beer samples were measured using Dr. Lange LTP6B Haze Meter (Figure 3.1.1.1; Robin Instruments Limited) for haze determination (in EBC units).

Figure 3.1.1.1 *Dr. Lange LTP6B haze meter.*

In the previous research taken in University of Leeds, a large matrix was made by different combination of colorants (yellow, blue and red) and scatters. In this research, a pseudo-beer matrix was made with a range of yellow colorants and scatterers based on the previous research in University of Leeds (Figure 3.1.1.2). Viewing models were developed using a range of standard solubilised colorants and scatterers (Sensient® Colours, Kings Lynn, UK; Unilever®, Sharnbrook, UK) suspended in aqueous media. Colorant (Quinoline Yellow 311744) and scatterers (Neutral scatterer 1000940) were

used in different concentration combinations to create a matrix of simulated beers. Solutions and suspensions were buffered with citrate/hydrogen phosphate (pH 4.0).

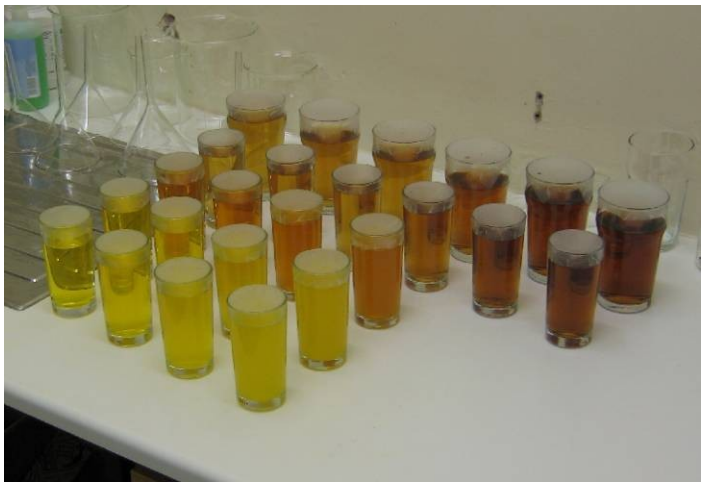

Figure 3.1.1.2 *Pseudo-beer matrix and commercial beer samples.*

Buffer solution: pH 4.0 (Scorpio, 2000):

The buffer solution contained disodium hydrogen phosphate (10.51 g/l) and citric acid (12.10 g/l).

The colorant solution:

The concentration of saturated yellow colorant solution was 4.33% (w/w), i.e. 100 g of this solution contains 4.33 g Quinoline Yellow solids. Here the saturated colorant solution is referred to 100% solution. The saturated solution (100%) was then diluted by adding buffer, and expressed in concentrations of 60%, 90% and 100%.

Table 3.1.1.1 *The colorant solutions in different concentrations:*

Concentration	Add Colorant Solution	Add buffer solution (g)
60%	90 g of 100% solution	805.9
90%	140 g of 100% solution	690.8
100%	4.33%	0.0

The scatterer solution:

The concentration of saturated scatterer solution was 0.267% (w/w; i.e. 100 g of this solution contains 0.267 g Neutral scatterer solids).

Table 3.1.1.2 *The scatterer solutions in different concentrations:*

Concentration	Added Scatterer Solution	Added buffer solution (g)
20%	50 g of 100% solution	797.7
60%	200 g of 100% solution	670.3
100%	0.267%	0.0

Each pseudo-beer sample (200 ml) contained 100 ml colorant solution and 100 ml scatterer solution. For pure colour solutions, there was 100 ml colorant solution and add extra 100 ml buffer solution, and for pure scatterer solutions, there was 100 ml scatterer solution and extra 100 ml buffer solution.

The pseudo-beer matrix (12 samples) was made as indicated in Table 3.1.1.3:

Table 3.1.1.3 *Pseudo-beer matrix codes:*

100ml +100ml	**Colour 60%**	**Colour 90%**	**Colour 100%**
Scatterer 0%	Y6S0	Y9S0	Y10S0
Scatterer 20%	Y6S2	Y9S2	Y10S2
Scatterer 60%	Y6S6	Y9S6	Y10S6
Scatterer 100%	Y6S10	Y9S10	Y10S10

In order to detect the effect caused by different glasses, normal pint glasses with the vertically curved glass body were used for the beer samples following the highball glasses during the experiments.

Psychophysical Colour Appearance Attributes Assessment:

The commercial beer samples and pseudo-beer matrix were presented in highball glasses (Figuer 3.1.1.2) and assessed by a panel of ten observers in terms of colour appearance attributes: lightness, colourfulness and hue composition. This was carried out in a controlled viewing cabinet with black walls and using a half black/half white chart as background for contrast (described in the following paragraphs). These observers were fully trained and experienced for the determination of visual attributes, all of whom were based in the image group of the Colour Science department at Leeds University. They all passed the initial colour blindness test by the Ishihara colour vision test (Section 2.4.2), and they were trained to establish the concept of colour appearance in their mind by different colour models, such as the Munsell colour space, in the preceding two to three years. The observers were seated in a chair 800 mm from the sample placed in the viewing cabinet and facing the central area at the back of the cabinet.

A magnitude estimation method (Section 2.4; Luo et al., 1991) and categorical judgment method (Section 2.4; Gescheider, 1997) were used here. Each observer assessed each of the six beers and 12 pseudo-beers in the highball glasses (twice) and pint glasses. Here observer colour appearance data were designated as visual lightness (L_v), visual colourfulness (C_v) and visual hue (H_v).

Lightness: "An area exhibited more or less light relative to a reference white."

Lightness is the amount of light reflected from a sample against the reference background white. For scaling lightness, observers were told that the white background reference card had a lightness of 100. The observers scored 0 for complete darkness as they could imagine. Therefore, the scale from a hundred to zero was set, and the observers were able to score the samples, including real and pseudo-beers.

Colourfulness: "An area exhibited as more or less chromatic."

A standard colour was set with colourfulness as 40. Observers were asked to score all samples according to reference. The observers scored the samples against the white background only.

*Hue***:** *"An area appears to be similar to one of the four unitary hues: red, green, yellow and blue, or a combination of them."*

The observers scored the samples against the white background. They scored hue according to pure hues: red yellow, green and blue. The colour produced by the mixture of the standard reference hues, it was reported as percentage composition, e.g. 40% red and 60% yellow.

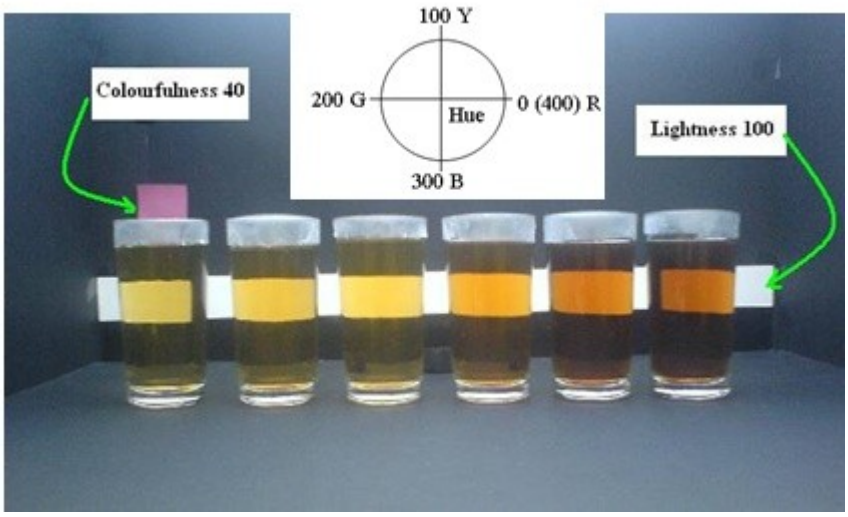

Figure 3.1.1.3 *Beer samples in viewing cabinet.*

The L_v, C_v and H_v ranged from 0 (black) to 100 (white), from 0 (neutral colours) to unlimited, and from 0 (red), 100 (yellow), 200 (green), 300 (blue) to 400 (red), respectively. The C_v and H_v data were used to calculate observer a_v and b_v, showing as the following equations:

$a_v = C_v \cos(h_v)$

$b_v = C_v \sin(h_v)$

$h_v = 0.9 \times H_v$, where h_v is to convert H_v from the scale of 0-400 to 0-360 (hue angle).

Figure 3.1.1.4 shows the format of the experimental instructions presented to the observers for the psychophysical assessment of colour appearance of real and pseudo-beer samples.

Experimental Instructions

Thank you for taking part in this visual assessment.

The aim of this session is to investigate the colour appearance of semi-transparent liquid. Magnitude Estimation method and categorical judgment method will be used in this psychophysical experiment.

You will see different glasses containing coloured liquid individually in the viewing cabinet. A reference colourfulness solid sample will be shown in the viewing cabinet before and throughout the experiment.

Glasses containing liquid will be put in the viewing cabinet of dark surround. A White/Black card is placed behind the glass. You need to focus on the part of the liquid where white card is behind to evaluate the **Lightness**, **Colourfulness**, and **Hue**.

All the samples will be scaled based on the overall appearance of the glass with the liquid inside.

Please Note:

For **Lightness**, the white part of the card has the lightness value of 100 while the zero value of lightness is your imaginary perfect black.

For **Colourfulness**, the colourfulness value of the reference is 40, zero value is the neutral colour while there is no top limit for colourfulness.

For **Hue**, the estimated value should be given as the hue composite, which is one, or to proportions of two of the perceived primaries: Red, yellow, Green and Blue. (For example, 60R40Y means combination of 60% red and 40% yellow).

Figure 3.1.1.4 *Format of the experimental instructions for the psychophysical assessment of total colour appearance.*

Table 3.1.1.4 *Visual colour appearance attributes estimation of beer samples.*

	Colour Appearance (L_v, C_v, H_v)	Beer Samples*	Observers	Total Estimation
Number	3	30	10	**900**

* Sample number 30 = 12 (pseudo sample) + 6 * 2 (commercial sample in highball glasses tested twice) + 6 (commercial samples in pint glasses)

A Minolta CS-1000 TSR was used to measure these samples placed in a viewing cabinet, employing the same geometry as the visual assessments, and the results were compared with those visual assessments.

Psychophysical Translucency Characteristics Assessment

The terms of opacity, transparency, and clarity were assessed. In addition, an ordinal scale with five steps, as bright-clear-dull-hazy-cloudy, was used to describe the turbidity of the liquid products. Opacity and transparency judgments were made at different times to ensure that observers separated the two attributes (Ji et al., 2005). Two reference samples were shown in the viewing cabinet before and throughout the experiment: a highball glass of distilled water and a black card.

Opacity: the ability of a specimen to prevent the transmission of light.

One highball glass containing clear liquid (distilled water) was used as the reference for opacity 0. Another glass containing a black card was used as the reference for opacity 10. The observers were asked to score each sample according to these two references.

Categorical 5-point scale: Five categorical terms were used to describe whether the liquid food clear or not (Kotschevar and Luciani, 2006; Grainger, 2009):

Bright- clear-dull-hazy-cloudy

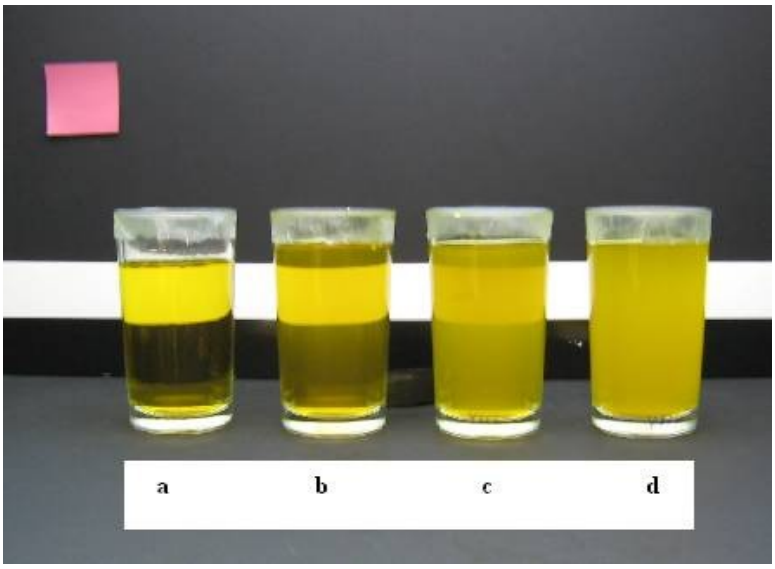

Figure 3.1.1.5 *Example to illustrate the clarity of liquids.*

Here observers were to score the samples using one of these five descriptions against the contrast line. A glass containing liquid (distilled water) was used as reference, and we set as "bright" (score 1). The observers were asked to imagine the "cloudy" liquid themselves (scored 5). The terms "clear", "dull" and "hazy" were scored as 2, 3 and 4, respectively. Before the experiment, some samples were given to the observers to familiarise them with the experiment. From the example in Figure 3.1.1.5, samples become increasingly cloudy from left to right.

Transparency: is used to describe the passage of light through the liquid.

Analogously to the opacity experiment, distilled water was set as a reference, which was transparency 10, and a black card was set as transparency 0.

During training, the observers were not trained to relate transparency to opacity. Rather, they were trained on each attribute independently. The opacity and transparency experiments were performed at different times to minimize their any interaction between these measurements.

Clarity: is used to describe the turbidity.

The clarity scales were set from 10 to 0. A glass of distilled water was set as 10, which was considered as the liquid with the highest clarity. The observers were asked to imagine the liquid with the lowest clarity and score it 0.

In the psychophysical experiment, we presented the twelve observers with the samples in random order. This was to minimize the possibility that observers may remember the result for previous sample, giving rise to carry over effects. In total, 1440 observations on translucency characteristics were accumulated (12 observers judged the four translucency characteristics of each of the 30 samples).

Table 3.1.1.5 *Visual translucency characteristics estimation of beer samples.*

	Translucency Description	Beer Samples*	Observers	Total Estimation
Number	4	30	12	**1440**

* Sample number 30 = 12 (pseudo sample) + 6 * 2 (commercial sample in highball glasses tested twice) + 6 (commercial samples in pint glasses)

Figure 3.1.1.6 shows the format of the experimental instructions presented to the observers for the psychophysical assessment of translucency characteristics of real and pseudo-beer samples.

Experimental Instructions

Thank you for taking part in this visual assessment.

The aim of this session is to investigate the translucency character of semi-transparent liquid. Magnitude Estimation method and categorical judgment method will be used in this psychophysical experiment.

All the samples will be scaled based on the overall appearance of the glass with the liquid inside.

One glass containing clear liquid is used as the reference for Opacity 0. Another glass containing a black card is used as the reference for Opacity 10.

Opacity	10	9	8	7	6	5	4	3	2	1	0

One glass containing distilled water was used as reference “bright”, and please imagine “cloudy” yourself. Some samples will be presented to help you to familiar these terms.

Ordinal 5-point scale	bright	clear	dull	hazy	cloudy

One glass containing clear liquid is used as the reference for Transparency 10. Another glass containing a black card is used as the reference for Transparency 0.

Transparency	0	1	2	3	4	5	7	3	8	9	10

One glass containing clear liquid is used as the reference for Clarity 10. Please imagine the liquid with the lowest clarity and score it zero.

Clarity	10	9	8	7	6	5	4	3	2	1	0

Figure 3.1.1.6 *Format of the experimental instructions for the psychophysical assessment of translucency characteristics.*

3.1.2 Red Wine Experiment

Five types of red wine (Corby bottler, UK) from different processing stages, were evaluated (Table 3.1.2.1).

Table 3.1.2.1 *Red wine samples list.* (See below for an explanation of process stage codes).

Reference	Red Wine	Corby Rotation	Process stage	Packaging	Quantity in Volume (L)
A1	Italian Red	06/5873	1*	Bottle	2 x 0.75
A2	Italian Red	06/5873	2*	Bottle	2 x 0.75
A3	Italian Red	06/5873	3*	Box	3
B1	Bordeaux Rouge	06/5957	1*	Bottle	2 x 0.75
B2	Bordeaux Rouge	06/5957	2*	Bottle	2 x 0.75
B3	Bordeaux Rouge	06/5957	3*	Bottle	1 x 0.75
C1	Cote Du Rhone	06/5849	1*	Bottle	2 x 0.75
C2	Cote Du Rhone	06/5849	2*	Bottle	2 x 0.75
C3	Cote Du Rhone	06/5849	3*	Box	3
D1	Cote Du Rhone	06/5850	1*	Bottle	1 x 0.75
D2	Cote Du Rhone	06/5850	2*	Bottle	1 x 0.75
D3	Cote Du Rhone	06/5850	3*	Box	3
E1	Italian Red	06/6031	1*	Bottle	2 x 0.75
E2	Italian Red	06/6031	2*	Bottle	2 x 0.75
E3	Italian Red	06/6031	3*	Box	3

The processing stages are illustrated in Figure 3.1.2.1:

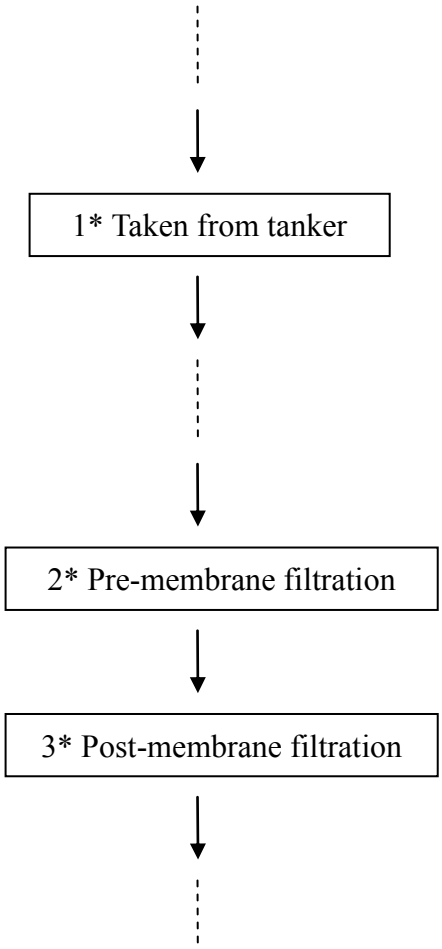

Figure 3.1.2.1 *The red wine samples at which stages were collected in the processing.*

1*: Taken from tanker:

The red wine samples were taken from tanker directly without any further processing.

2*: Pre-membrane filtration:

After being taken from the tanker, the red wine samples were processed through several stages to reach prefiltration.

3*: Post-membrane filtration:

These samples were collected after they had been membrane filtered.

All the samples were kept at around 10°C, and all experiments including physical and psychophysical measurements, were carried out within two days of opening.

The red wine samples were measured using Dr. Lange Haze meter for the haze content (in EBC units). Ten measurements were taken for each of sample.

A Petri dish was used in the red wine sensory experiments, and at a depth of 5 mm was for each sample (Gonzalez-Miret Martin et al., 2007). Again, this experiment was performed in a controlled viewing cabinet (Figure 3.1.2.2), and using a half black/half white chart as background.

Figure 3.1.2.2 *The cabinet used in the red wine experiment.*

Psychophysical Colour Appearance Attributes Assessment

The red wine samples were put into the Petri dishes and assessed by a panel of 10 observers in terms of colour appearance attributes: lightness, colourfulness and hue composition. This was performed in a controlled viewing cabinet with black walls and using a half black/half white chart as background (Figure 3.1.2.3).

Lightness: A reference was set by a Petri dish of distilled water against the white background, which should be scored as 100. And the observers scored 0 for complete darkness. Therefore, the scale from a hundred to zero was set, and the observers were able to score the samples.

Colourfulness: A standard colour was set with colourfulness at 40 as a reference.

Hue: The observers scored the samples against the white background.

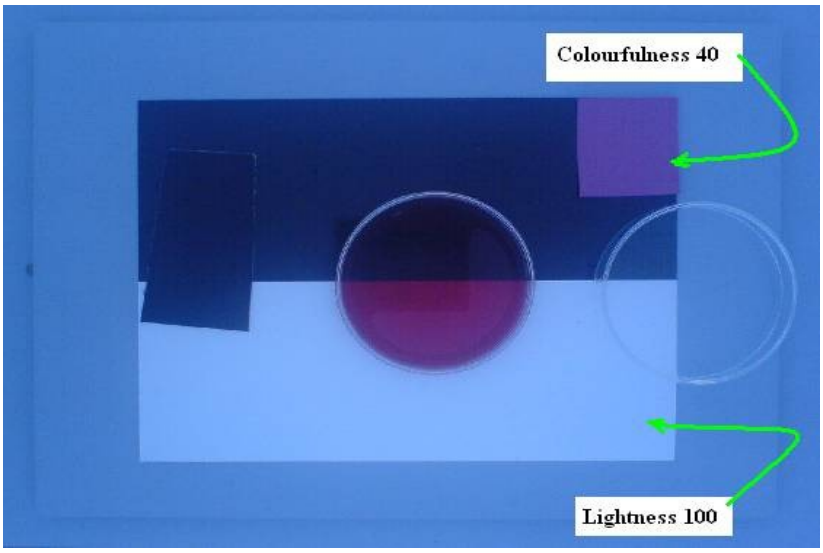

Figure 3.1.2.3 *Wine sample in the cabinet with black walls and using a half black/half white chart as background.*

Analogous to the beer experiment, each observer assessed each of the fifteen red wines in Petri dishes. Here observer colour appearance data were designated as visual lightness (L_v), visual colourfulness (C_v) and visual hue (H_v). The L_v, C_v and H_v ranged from 0 (black) to 100 (white), from 0 (neutral colours) to unlimited, and from 0 (red), 100 (yellow), 200 (green), 300 (blue) to 400 (red), respectively. The C_v and H_v data were calculated (see Section 3.1.1).

In this part, 450 measurements were taken (15 red wine samples in Petri dishes were judged by 10 observers on three colour appearance attributes—L_v, C_v and H_v).

Table 3.1.2.2 *Visual colour appearance attributes estimation of red wines.*

	Colour Appearance (L_v, C_v, H_v)	Red Wine	Observers	Total Estimation
Number	3	15	10	**450**

Experimental Instructions

Thank you for taking part in this visual assessment.

The aim of this session is to investigate the colour appearance of semi-transparent liquid. Magnitude Estimation method and categorical judgment method will be used in this psychophysical experiment.

You will see different Petri dishes containing red wine individually in the viewing cabinet. A reference colourfulness solid sample will be shown in the viewing cabinet before and throughout the experiment.

Petri dishes containing red wines will be put in the viewing cabinet of dark surround. A White/Black card is placed under the Petri dish. You need to focus on the part of the liquid where white card is behind to evaluate the **Lightness**, **Colourfulness**, and **Hue**.

All the samples will be scaled based on the overall appearance of the dish with the liquid inside.

Please Note:

For **Lightness**, the white part of the card has the lightness value of 100 while the zero value of lightness is your imaginary perfect black.

For **Colourfulness**, the colourfulness value of the reference is 40, zero value is the neutral colour while there is no top limit for colourfulness.

For **Hue**, the estimated value should be given as the hue composite, which is one, or to proportions of two of the perceived primaries: Red, yellow, Green and Blue. (For example, 60R40Y means combination of 60% red and 40% yellow).

Figure 3.1.2.4 *Format of the experimental instructions for the psychophysical assessment of total colour appearance of red wines.*

A Minolta CS-1000 TSR was used to measure these samples placed on a viewing cabinet, employing the same geometry as the visual assessments, and the results were compared with those visual assessments.

Psychophysical Translucency Characteristics Assessment

These samples were assessed in a viewing cabinet with dark surround. A white/black card was placed under the Petri dishes. All the samples were scaled based on the overall appearance of the dish with the liquid inside.

Similar to the beer experiment, the terms of opacity, transparency, and clarity were scaled. The ordinal scale with five steps, as bright-clear-dull-hazy-cloudy, was used to describe the appearance of the liquid products.

Two reference samples were shown in the viewing cabinet before and throughout the experiment: a Petri dish of distilled water and a black card (Figure 3.1.2.5).

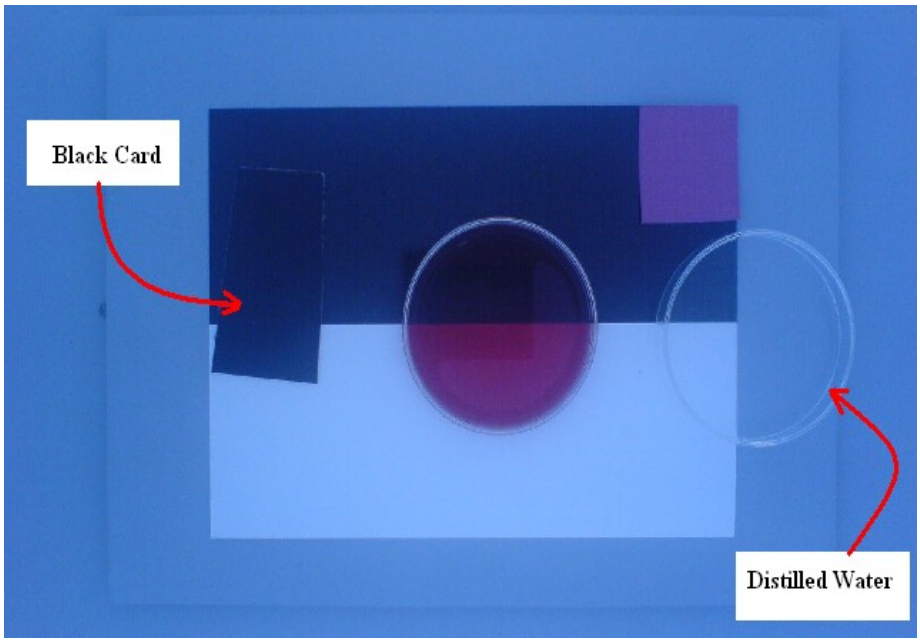

Figure 3.1.2.5 *Wine sample in the cabinet.*

Opacity: One Petri dish containing clear liquid (distilled water) was used as the reference for opacity 0. Another dish containing a black card was used as the reference for opacity 10. The observers were asked to score each sample according to these two references.

Categorical 5-point scale: There are five categorical terms (see Section 3.1.1) that have been used to describe whether the liquid food being clear or not (information is supplied by Corby Bottlers):

Bright- clear-dull-hazy-cloudy

Transparency: Analogous to opacity experiment, we set clear liquid as reference, which was transparency 10, and the black card was set as transparency 0.

Clarity: Clarity scales were set from 10 to 0. Distilled water in a Petri dish was set as 10, which was considered as the liquid with the highest clarity. The observers were asked to imagine the liquid with the lowest clarity and score it 0.

Table 3.1.2.3 *Visual translucency charateristics estimation of red wines.*

	Translucency Description	Red Wine	Observers	Total Estimation
Number	4	15	12	**720**

Figure 3.1.2.6 shows the format of the experimental instructions presented to the observers for the psychophysical assessment of translucency characteristics of red wine samples.

Experimental Instructions

Thank you for taking part in this visual assessment.

The aim of this session is to investigate the translucency character of red wines. Magnitude Estimation method and categorical judgment method will be used in this psychophysical experiment.

All the samples will be scaled based on the overall appearance of the Petri dish with the liquid inside.

One dish containing clear liquid is used as the reference for Opacity 0. Another dish containing a black card is used as the reference for Opacity 10.

Opacity	10	9	8	7	6	5	4	3	2	1	0

One dish containing distilled water was used as reference “bright”, and please imagine “cloudy” yourself.

Ordinal 5-point scale	bright	clear	dull	hazy	cloudy

One dish containing clear liquid is used as the reference for Transparency 10. Another glass containing a black card is used as the reference for Transparency 0.

Transparency	0	1	2	3	4	5	7	3	8	9	10

One dish containing clear liquid is used as the reference for Clarity 10. Please imagine the liquid with the lowest clarity and score it zero.

Clarity	10	9	8	7	6	5	4	3	2	1	0

Figure 3.1.2.6 *Format of the experimental instructions for the psychophysical assessment of translucency characteristics.*

3.2 Identification of Brewing Process Control Points

All the samples used for monitoring the control points for brewing were from the pilot brewery of ICBD.

3.2.1 Brewing

Lager Brewing A

Materials and Processing Parameters

The standard lager was brewed, and the recipe was as follows:

Lager Malt: 34.2 kg

Additions: Hallertau Magnum 99 g

Yeast type (creamy): Tennents lager 2.0 kg (12 x 10^6 cells/ml)

Water: water was used for brewing to produce 200 litre lager.

Milling

The required malt was hammer milled (77.5% particles size ≤ 0.15 mm).

Mashing

The malt grist was cooked at 65°C for 60 minutes, then the temperature was increased to 75°C.

Mash Filtration

The wort produced was clarified by mash filtration. Samples were taken after collection of every 20 litres from the filter. After the third wort is collected, sparge liquor, which

ensured that the sugars and other dissolved materials are adequately recovered from the spent grains, was introduced and was distributed over the entire grain bed. When all the wort has been driven out, the sparge liquor was passed through the mash.

Boiling and Whirlpool

The wort was boiled in the kettle for 60 minutes, and hops required were added for the bitterness of beer. Hallertau Magnum hops (99 g) was added at the start of boil. After boiling, the wort was left in the kettle for whirlpool separation of the trub, taking about 25 minutes. Samples were collected before and after boiling, and after whirlpool.

Wort Cooling

After whirlpool, the wort was pumped through heat-exchanger for cooling. Chilling fluid media was applied to cool down the hot wort to 11°C, whilst oxygen was supplied to dissolve in the liquid (target of 12 mg/l). The wort was then transferred into the fermentation vessel.

Fermentation and Conditioning

The fermentation was started at 9.5°C and was allowed to rise up to 13°C. The product was held at 13°C until the gravity was about 1009° (after approximately 7 days) then cooled to 5°C for 7 days for conditioning. Samples for analysis were collected every day.

Filtration

The product was filtered through Carlson XE400 filter sheets. Prefiltered and postfiltered samples were collected.

Gravity

Original: 1048.0°

Final: 1009.7°

Sampling, Sample Preparation and Analysis

Following the brewing processing stages, the samples were taken from mashing stage to the final chill filtration, as:

- After mashing
- Mash filtration: the samples were taken after every collection of 20 litres of wort were clarified during the mash filtration stage (total of 200 litres)
- Before and after wort boiling
- Before and after wort cooling
- Fermentation: the samples were taken every day during the fermentation period (7 days)
- Conditioning: the samples were taken every day during the conditioning period (7 days)
- After final chill filtration.

All samples collected from each brewing steps (except that collected from postfiltration, which was without particles and clear) were divided into two groups.

First group: the samples were measured untreated for colour and turbidity. Some samples were collected at higher temperatures (e.g. from mashing, mash filtration, boiling, whirlpool), whereas some samples were collected at lower temperatures (e.g. after cooling, during fermentation, conditioning and after final filtration). After being collected, these samples were left to cool to about 20°C, and then further physical

measurements (colour appearance and turbidity) were taken.

Second group: the samples were filtered through Whatman® No. 1 filter paper (with a particle retention diameter of 11 μm), to separate most large particles (e.g. yeast: their largest and smallest axes vary between 5 and 13 μm (Posada, 1987)), then they were centrifuged to remove the other particles left. After reaching about 20°C, these clear samples were assessed for colour appearance measurement.

The two groups of samples were measured with a Shimadzu (UV-1601) spectrophotometer (Figure 3.2.1.1) in terms of transmittance (path length 10 mm). Transmission data were collected at 10 nm intervals in the region 400 nm to 700 nm. Based on *CIE* $L^*a^*b^*$ method (Section 1.2.1), CIE L^*, a^* and b^* values were calculated. Turbidimeter (Figure 3.2.1.2, HACH2100N Laboratory Turbidimeter, EPA, 115 Vac) was used to analyse the turbidity of the untreated samples' turbidity in EBC units.

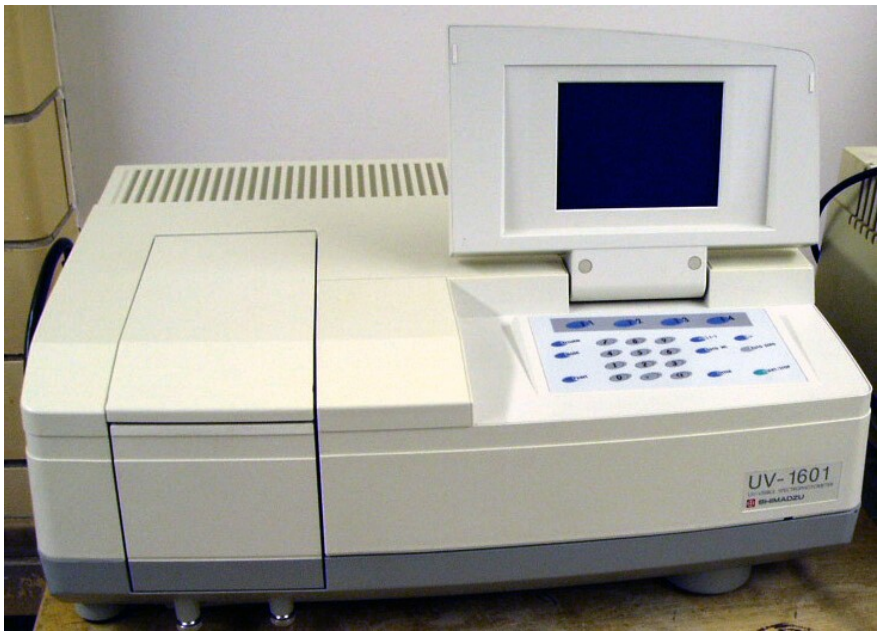

Figure 3.2.1.1 *Shimadzu (UV-1601) spectrophotometer.*

Figure 3.2.1.2 *HACH2100N Laboratory Turbidimeter, EPA, 115 Vac* .

Lager Brewing B
Materials and Processing Parameters

The standard lager was brewed, and the recipe was as:

Malt: 34.2 kg

Additions: Hallertau Magnum 99 g

Yeast type (creamy): Tennents lager 2.0 kg (12 x 10^6 cells/ml)

Water: water was used for brewing to produce 200 litre lager.

Milling

The malt was roller milled (81.0% particles size ≥ 0.60 mm).

Lautering

All the wort was re-circulated in the lauter tun, until it appeared bright, then the bright liquid was diverted into the kettle. Samples were taken when every 20 litres wort were diverted, and after the third wort is collected, sparge liquor was introduced. A total 200 litres of wort were collected, and samples were taken for the further measurement on colour and translucency.

Fermentation and Conditioning

The processing of these two steps for lager A and B are the same, except the length of the time (see details in the Sections 'Sampling, Sample Preparation and Analysis').

Gravity

Original: 1048.8°

Final: 1011.9°

Sampling, Sample Preparation and Analysis

The samples were taken from mashing stage to the final chill filtration, at the following points:

- Before and after mashing,
- Lautering: the samples were taken every 20 litre wort were clarified during the filtration stage (total of 200 litres)
- Before and after wort boiling
- Before and after wort cooling
- Fermentation: the samples were taken every day during the fermentation period (6 days)

- Conditioning: the samples were taken every day during the conditioning period (6 days)
- After final chill filtration.

The samples were prepared as same as lager brewing A. Shimadzu (UV-1601) spectrophotometer was used to measure the transmittance of the clarified samples (treated as the second group of samples of lager brewing A), and CIE L^*, a^* and b^* values were calculated. Turbidity of the lager brewing B samples were measured with the method the same as that of lager brewing A.

Ale Brewing

Materials and Processing Parameters

The standard ale was brewed, and the recipe was as:

Malt: 34.2 kg

Roast Barley: 0.39 kg

Additions: Hallertau Magnum 99 g

Yeast type (creamy): Caledonian brewery ale strain 1.7 kg (12 x 10^6 cells/ml)

Water: water was used for brewing to produce 200 litre ale.

Most of the brewing steps for the ale and lager A were similar, except the time taking for fermentation and conditioning.

Gravity

Original: 1048.7°

Final: 1010.6°

The samples were taken from mashing stage to the final chill filtration, at the following points:

- After mashing
- Mash filtration: the samples were taken every 20 litre wort were clarified during the filtration stage (total 200 litres)
- Before and after wort boiling
- Before and after wort cooling
- Fermentation: the samples were taken every day during the fermentation period (7 days)
- Conditioning: the samples were taken every day during the conditioning period (6 days)
- After final chill filtration.

The ale producing samples were prepared and assessed similarly as lager B.

3.3 Instrument Testing in Beer Production and Products

3.3.1 System Repeatibility Testing

3.3.1.1 Fixed Aperture Lens *vs* Unfixed Aperture Lens Testing

Two types of camera aperture lens (one fixed aperture lens and one unfixed aperture lens) were introduced into the digital imaging system, and the system repeatability working under the two types lens were tested and compared.

One pilot brewed ale final product was used as the sample for the system repeatibility

testing. Each test took four hours, and five images were taken every fifteen minutes. Thus, 17 groups of images were taken, and totally, there were 105 images for each test in the four hours (17 5 = 85). The samples were degassed through filter paper (Whatman No. 1) before each test. All the samples were tested at room temperature (approximately 23°C).

3.3.1.2 Pilot Brewed Samples Testing

Final products of one pilot brewed lager and one pilot brewed ale were set as samples to evaluate the system repeatibility. The samples were degassed by filtration through filter paper (Whatman No. 1). Each test evaluation took four hours, and each image was taken every fifteen minutes. Three repeated evaluations were carried for reliability and accuracy by both lager and ale samples, i.e. lager was tested three times, and thus, 255 images were taken (17 5 3 = 255) in the twelve hours; the same evaluations were repeated for ale. All the samples were tested at room temperature (approximately 23°C).

3.3.2 Pilot Brewing Testing

All the pilot brewed samples used for system testing were brewed in the ICBD brewery. The materials and brewing procedures were determined by avoiding any critical process factors which may interfere with the parameters. The most issue deciding the colour of the beer product is the malt/barley selected in brewing (see Section 2.1). In this study, in order to cover a wider range of colour appearance, paler and darker lagers were brewed, and thus, lager malt, rice and caramel malt, etc. were used as ingredients to produce paler to darker products. Based on the facilities and operation capacities of the pilot brewery in the ICBD, and colour of lagers intended to produce, the amounts of

malt/barley and relevant hops and yeast type/amount were decided. The materials of the pilot brewed lagers (200 litres) and ales (200 litres) are listed in Tables 3.3.2.1 and 3.3.2.2

Table 3.2.2.1 *Materials of pilot brewed lagers.*

	Malt/Barley	(kg)	Additions Type	(g)	Yeast Type 11.5 to12 x 10^6 (cells/ml)	(kg)
Lager 1	Lager Malt Crystal Malt Rice	34.4 0.6 8.0	Hallertau Magnum Saaz	72 100	Tennents Lager	1.6
Lager 2	Lager Malt Crystal Malt Rice	34.4 0.6 8.0	Hallertau Magnum Saaz	72 100	Tennents Lager	2.0
Lager 3	Lager Malt 3 Crystal Malt Rice	34.4 0.6 8.0	Hallertau Magnum Saaz	72 100	Tennents Lager	2.5
Lager 4	Lager Malt	30.0	Hallertau Magnum	89	Tennents Lager	1.5
Lager 5	Lager Malt	30.0	Hallertau Magnum	89	Tennents Lager	1.5
Lager 6	Lager Malt Crystal	29.7 0.3	Hallertau Magnum	89	Tennents Lager	1.5
Lager 7	Lager Malt Crystal Malt	29.7 0.3	Hallertau Magnum	89	Tennents Lager	2.5
Lager 8	Lager Malt Crystal Malt	29.4 0.6	Hallertau Magnum	89	Tennents Lager	2.5
Lager 9	Lager Malt Crystal Malt	29.4 0.6	Hallertau Magnum	89	Tennents Lager	2.5
Lager 10	Lager Malt Caramel Malt	24.0 2.4	Hallertau Magnum	77	Tennents Lager	2.5
Lager 11	Lager Malt Caramel Malt	24.0 2.4	Hallertau Magnum	77	Tennents Lager	2.5
Lager 12	Lager Malt Caramel Malt	24.0 2.4	Hallertau Magnum	77	Tennents Lager	2.7
Lager 13	Lager Malt Caramel Malt	24.0 2.4	Hallertau Magnum	77	Tennents Lager	2.7
Lager 14	Lager Malt Caramel Malt	22.8 7.2	Hallertau Magnum	77	Tennents Lager	2.7
Lager 15	Lager Malt Caramel Malt	22.8 3.6	Hallertau Magnum	77	Tennents Lager	2.7

Table 3.3.2.2 *Materials of pilot brewed ales.*

	Malt/Barley	(kg)	Additions Type	(g)	Yeast Type 10 to 22 x 10^6 (cells/ml)	(kg)
Ale 1	Malt	44.80	Challenger	200	Belhaven	2.3
	Crystal Malt	0.24	Golding	300		
	Gypsum	0.24				
Ale 2	Malt	44.80	Challenger	100	Caledonian	2.4
	Roast Barley	0.24	Golding	100		
	Gypsum	0.24				
Ale 3	Malt	24.30	Hallertau Magnum	157	Caledonian	2.5
	Amber	1.55				
	Crystal	1.55				
	Black Malt	1.10				
	Wheat Malt	1.10				
Ale 4	Malt	29.30	Hallertau Magnum	205	S&N	3.5
	Caramel malt	0.7	Challenger	308		
	Crystal Malt	1.10	Cascade	100		
	Wheat Malt	1.50	Golding	50		
Ale 5	Malt	29.30	Hallertau Magnum	119	S&N	2.6
	Caramel malt	0.70	Challenger	173		
	Crystal Malt	1.10	Cascade	57		
	Wheat Malt	1.50	Golding	30		
Ale 6	Malt	30.94	Hallertau Magnum	167	S&N	3.2
	Wheat Malt	2.52	Challenger	175		
	Crystal Malt	5.20				
Ale 7	Malt	30.0	Hallertau Magnum	118	S&N	2.6
	Crystal Malt	1.10	Challenger	174		
	Wheat Malt	1.50				
Ale 8	Malt	28.80	Challenger	320	S&N	3.0
	Wheat Malt	7.68	Fuggles	468		
	Caramel Malt	2.10				
Ale 9	Malt	23.69	Challenger	195	S&N	2.3
	Wheat Malt	6.43	Fuggles	400		
	Caramel Malt	1.76				
Ale 10	Malt	30.94	Hallertau Magnum	167	S&N	3.2
	Wheat Malt	2.52	Challenger	175		
	Crystal Malt	5.20				
Ale 11	Malt	24.28	Hallertau Magnum	127	S&N	2.0
	Wheat Malt	1.72	Challenger	140		
	Crystal Malt	4.08				

Table 3.3.2.2 *(continued)*

	Malt/Barley	(kg)	Additions Type	(g)	Yeast Type	(kg)
Ale 12	Pale Malt Lager Malt Amber Crystal Malt	16.40 16.10 3.98 1.30	Hallertau Magnum Challenger	187 256	S&N	2.8
Ale 13	Pale Malt Lager Malt Amber Crystal Malt	12.60 12.30 3.05 1.00	Hallertau Magnum Challenger	106 200	S&N 1.8	1.8
Ale 14	Malt Barley Roast Barley	32.31 4.26 4.50	Hallertau Magnum	357	S&N 3.6	3.6
Ale 15	Malt Barley Roast Barley	23.70 3.12 2.96	Hallertau Magnum	203	S&N 2.7	2.7

Fifteen lagers and fifteen ales (including two dark ales) were pilot brewed, and the general brewing processes were showing in Tables 3.3.2.3 and 3.3.2.4. The digital imaging system was introduced into fifteen lager pilot brewing and fifteen ale pilot brewing as the off line colour/transparency test instrument (Figures 3.3.2.1, 3.3.2.2 and 3.3.2.3).

Table 3.3.2.3 *Pilot brewed lagers processing data. (CC: Cereal Cook, MM: Malt Mash).*

Lagers	Mashing		Mash Clarification	Boiling	Fermentation/ Conditioning	Gravity (°)	
	°C	min		min	days	Original	Final
1	(CC) 56.4 (CC) 85.9 (CC) Boil (MM) 47.3 Transfer CC to MM (MM) 67.0 (MM) 71.4 (MM) 75.6	5 5 10 15 40 1 1	Lautering	60	7/7	1046.1	1007.6

Table 3.3.2.3 ***(continued)***

2	(CC) 57.2 (CC) 85.0 (CC) Boil (MM) 47.7 Transfer CC to MM (MM) 67.0 (MM) 71.0 (MM) 75.9	2 5 10 15 40 1 1	Lautering	60	7/7	1048.0	1007.1
3	(CC) 59.9 (CC) 85.0 (CC) Boil (MM) 47.6 Transfer CC to MM (MM) 67.0 (MM) 71.0 (MM) 75.5	2 5 10 15 40 2 1	Lautering	60	8/7	1046.2	1005.6
4	66.8 74.1	45 1	Filtration	60	7/7	1048.9	1008.4
5	67.0 75.0	45 1	Filtration	60	6/7	1047.8	1008.1
6	67.2 75.5	45 1	Filtration	60	7/7	1049.1	1011.9
7	67.3 75.0	45 1	Filtration	60	7/7	1048.1	1009.6
8	67.2 74.5	45 1	Filtration	60	8/7	1046.8	1009.8
9	67.3 75.3	45 1	Filtration	60	7/7	1048.2	1008.2
10	66.9 74.7	45 1	Filtration	60	7/7	1049.3	1007.8
11	67.1 75.2	45 1	Filtration	60	6/7	1048.8	1008.5
12	62.9 75.2	60 1	Filtration	60	7/7	1049.5	1008.6
13	63.5 75.4	60 1	Filtration	60	7/7	1047.8	1009.0
14	66.8 75.2	45 1	Filtration	60	8/7	1050.5	1008.9
15	66.9 75.4	45 1	Filtration	60	7/7	1046.5	1009.5

Table 3.3.2.4 *Pilot brewed ales processing data.*

Ales	Mashing		Mash Clarification	Boiling	Fermentation/ Conditioning	Gravity (°)	
	°C	min		min	days	Original	Final
1	66.2 75.8	55 1	Lautering	60	6/7	1061.2	1017.0
2	64.9 74.7	60 1	Lautering	60	5/6	1063.3	1012.4
3	63.9 75.1	60 1	Filtration	60	6/6	1042.7	1008.3
4	63.9 74.9	60 1	Filtration	60	8/7	1069.9	1016.9
5	64.2 75.2	60 1	Filtration	60	9/6	1048.8	1011.4
6	64.0 75.6	60 1	Filtration	60	8/7	1069.8	1016.2
7	65.0 74.8	60 1	Filtration	60	8/7	1051.3	1013.6
8	63.6 75.2	60 1	Filtration	60	7/7	1071.0	1017.8
9	63.6 75.0	60 1	Filtration	60	7/7	1047.4.2	1010.8
10	63.6 75.6	60 1	Filtration	60	8/7	1074.0.3	1017.9
11	63.8 74.9	60 1	Filtration	60	7/7	1042.6	1010.1
12	64.8 75.3	60 1	Filtration	60	7/7	1074.2.5	1016.6
13	64.0 74.8	60 1	Filtration	60	6/7	1040.8	1008.4
14	64.1 76.0	60 1	Filtration	60	7/7	1074.8	1021.1
15	64.0 76.0	60 1	Filtration	60	7/7	1040.8	1009.7

Based on the results obtained of research about CCPs in brewing (see Section 3.2 and 4.2), the five CCPs were decided. The samples were collected at the CCPs governing colour and translucency development in process for beer:

- Mash clarification (end)

- Boil start point
- Boil end point
- Pre-final filtration
- Post-final filtration.

All of the final beer products were degassed before testing by filtration through filter paper (Whatman No. 1). EBC colours were measured according to the EBC Recommended Method, where by light absorbance is measured at 430 nm at path length 10 mm against a reference of distilled water, and were also assessed visually using a Lovibond Comparator. All the samples were collected at the CCPs apart from final products which were centrifuged at 27504×g (RCF) twice to separate the grist or yeast contents. All of the samples were tested at room temperature (approximately 23°C).

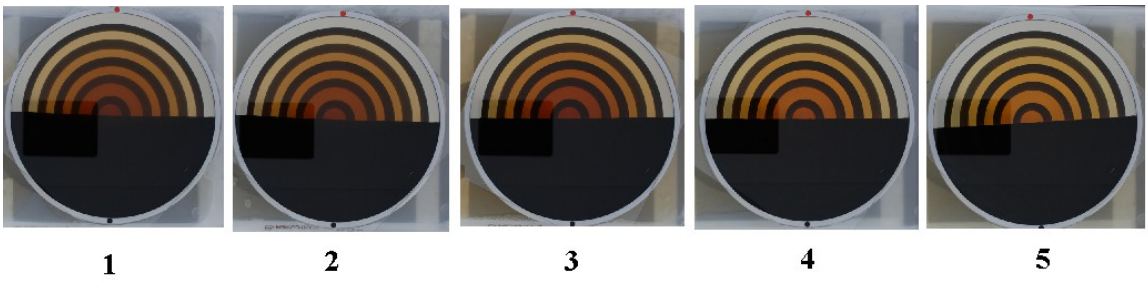

Figure 3.3.2.1 *Pilot brewed lager samples collected at CCPs during processing monitored using the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

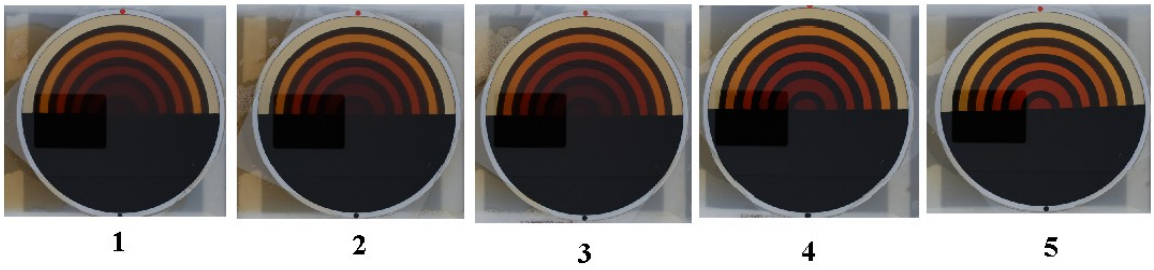

Figure 3.3.2.2 *Pilot brewed ale sample collected at CCPs during processing monitored using the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

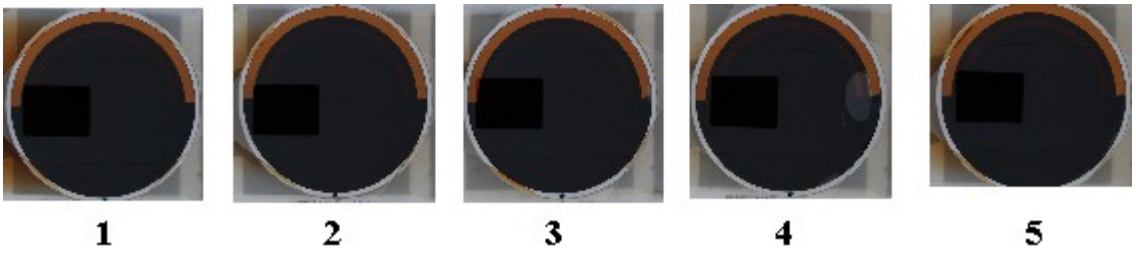

Figure 3.3.2.3 *Pilot brewed dark ale sample collected at CCPs during processing monitored using the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

3.3.3 Commercial Beer Samples

Fifty commercial beers were involved for the digital imaging system test, including twenty-five lagers (Table 3.3.3.1, Figure 3.3.3.1) and twenty-five ales (Table 3.3.3.2, Figure 3.3.3.2). These beers were selected to cover the colour of beer from pale to dark. Some of these beers were largest of beer markets by value, e.g. Carling Black Label was the top lager brand in UK, with 6.1% of the market, Skol was number two, with 5.1% of the market and Heineken was the third, with 4.9%. A newer launch in the UK is ice beer. Foster's Ice was the first brand in the UK (Datamonitor London, 1995).

Table 3.3.3.1 *Commercial lager samples*

Lagers	Alc Vol (%)	ml
Budvar Czech Premium Lager	5.0	500
Foster's In-can Scuba	4.0	440
Foster's The Amber Nectar	4.0	440
Carlsberg Export Premium Lager	5.0	275
Carlsberg	3.8	440
Heineken Lager Beer	5.0	500
Carling Black Label	4.1	500
Beck's Vier	4.0	440
Budweiser Genuine Lager	5.0	473
Cobra Lager	5.0	330
Asahi	5.0	330
Red Stripe	4.7	400
Skol	3.0	440
Tiger	4.8	330
Cusquena	5.0	330
Staropramen	5.0	330
Coors Light	4.5	300
Coors Light Active Can	4.5	440
Coors Light Silver Premium	4.5	440
Tennent's Super	9.0	440
Tuborg	4.6	275
Sanmigurel Premium Lager	5.0	330
Peroni Nastro Azzurro	5.1	330
Corona Extra	4.6	330
Beck's Beer	5.0	440

Figure 3.3.3.1 *Commercial lager samples.*

Table 3.3.3.2 *Commercial ale samples*

Ales	Alc Vol (%)	ml
Greene King IPA	5.0	500
ASDA Gentleman Jack Ale	5.0	500
Hobgoblin Ale	5.2	500
Old Speckled Hen Strong Fine Ale	5.2	500
Newcastle Brown Ale	4.7	550
Shepherd Neame 1698 Bottle Conditioned Strong Ale	6.5	500
Tangle Foot Premium Ale	5.0	500
Deuchars IPA	4.4	500
Belhaven Twisted Thistle IPA	5.3	500
Harviestoun Bitter & Twisted Blond Beer	4.2	500
Black Sheep	5.7	500
Belhaven Best	3.5	440
John Smith's Extra Smooth	4.0	440
McEwan's Export Ale	4.5	500
Tennent's Special Ale	3.5	500
Tennent's Draught Velvet	3.5	440
Ridley's Old Bob	5.1	500
Badger Golden Glory	4.5	500
Badger Fursty Ferret	4.4	500
Wells Banana-Bread Beer	5.2	500
Brewdog Punk IPA	6.0	330
Brewdog Trashy Blonde	4.1	330
Belhaven St Andrews Ale	4.6	500
Broughton Old Jock Ale	6.7	500
Badger Golden Champion	5.0	500

Figure 3.3.3.2 *Commercial ale samples.*

The commercial products were degassed before being tested. All the samples were

tested at room temperature (approximately 23°C). Each product was assessed by digital imaging system three times for repeatability and accuracy. These products were also assessed by EBC Recommended Method (Shimadzu (UV-1601) Spectrophotometer) on colour attributes, and turbidity (HACH2100N Laboratory Turbidimeter, EPA, 115 Vac) on translucency characteristics, which were correlated to those data obtained from digital imaging system.

3.3.4 Correlation between the Colour Appearance Measuring Methods

Colour appearance attributes measured by DigiEye imaging system, Minolta CS-1000 tele-spectroradiometer (TSR) and visual tests (see Section 3.1.1) were compared. Three lagers (Coors, Carling and Grolsch-all malt) and three ales (Worthington, Caffrey's, and Stones) sourced from Coors Brewing Ltd. (Burton-Upon-Trent, UK) were assessed, and these samples were prepared in the same way as Section 3.1.1. All the beer samples were stored at room temperature and in dark conditions. Before the experiment, the beer samples were degassed by Whatman® No. 1 filter paper. The TSR was used to measure these samples placed in a viewing cabinet, employing the same geometry as the visual assessments (see Section 3.1.1). The assessing procedure by DigiEye imaging system was the same as Section 3.3.3.

Chapter 4. RESULTS AND DISCUSSION

This study began with attempts to develop relationships between psychophysical evaluations of visual perception and various instrumental measurements of colour and translucency before using the methodology developed to assess beer production at identified critical control points. Finally the developed methodology was used to appraise a wide range of commercial final products.

The performances by repeatability of the instruments used in this research were tested, and recorded as Table 4.1. Distilled water was used as sample and measured thirty times. Turbidity was tested by Dr. Lang LTP6B Haze meter and HACH 2100N Laboratory Turbidimeter, and lightness was tested by the two spectrophotometers and tele-spectroradiometer. The details of calculation of repeatability see Section 4.1.1.2 (Equation 25).

Table 4.1 *The repeatability of the instruments used in this research.*

Instruments	Tests	Repeatability
CE7000A Sepctrophotometer	Colour difference	1.21
Dr. Lang LTP6B Haze Meter	Turbidity (EBC)	4.01
Minolta CS-1000 Tele-spectroradiometer	Colour difference	2.47
Shimadzu (UV-1601) Spectrophotometer	Colour difference	1.69
HACH 2100N Laboratory Turbidimeter	Turbidity (EBC)	3.50
Digital Imaging System	See Section 4.3.1	See Section 4.3.1

4.1 Colour and Translucency Evaluations of Beverages

As discussed earlier, existing colour theories, specifically the Kubelka-Munk theory, are not particularly effective for the appraisal of scattering, polydisperse samples, such as alcoholic beverages. The relationship between visual perception and instrumental analyses was explored using as test samples six different commercial canned beers and five red wines. The latter had been collected in three processing stages (see also Section 3.1).

4.1.1 Psychophysical Evaluations and Instrumental Measurements on Beers

4.1.1.1 Instrumental Measurements

In order to maintain consistency, beer samples were taken from the same cans to reduce variation, and the stability of the degassed samples was established prior to the main experiment.

A. Colour Stability of Commercial Beer Samples

The colour stability of beer samples was assessed by using GretagMacbeth CE7000A spectrophotometer in terms of transmittance.

Each of the six beers was measured for the transmittance twice a day for three days, and the data converted to the tristimulus values and L^*, a^* and b^*. Figures 4.1.1.1 and 4.1.1.2 generally show the colour appearance results on a^*b^* and $L^*C^*_{ab}$ colour plates and Figures 4.1.1.1, 4.1.1.2 and 4.1.1.3 show the details of the colour appearance of six commercial beers on L^*, a^* and b^* in the three days. Mean values and 95% confidence

intervals of these attributes were showing in Tables 4.1.1.3, 4.1.1.4 and 4.1.1.5.

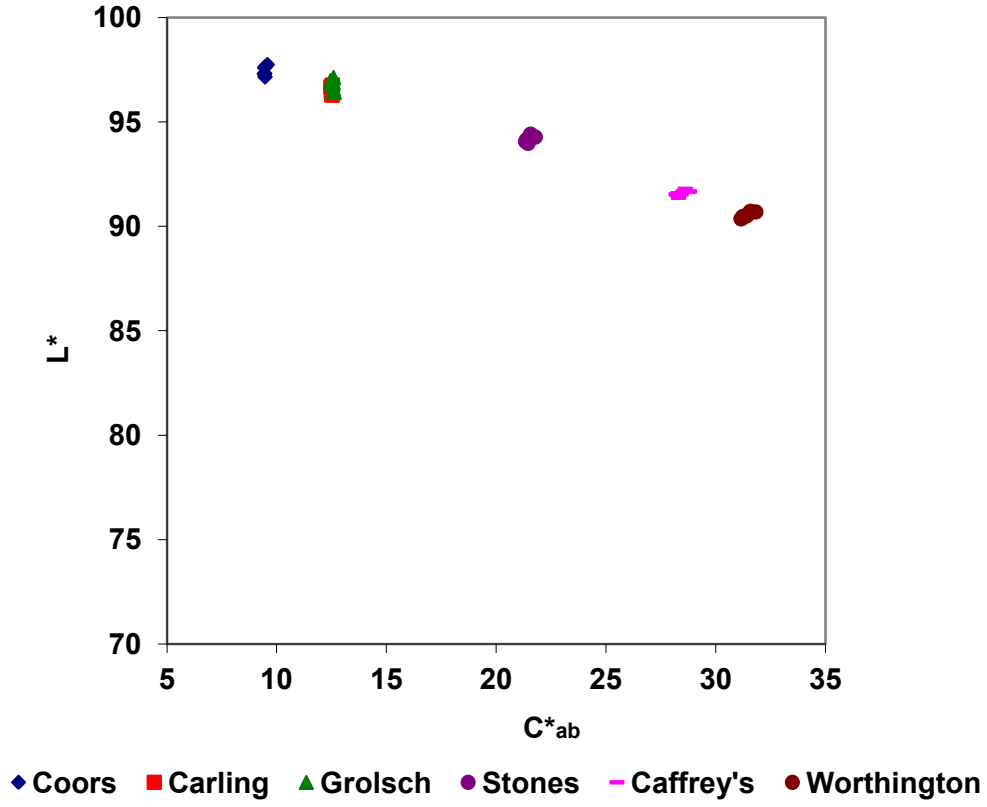

Figure 4.1.1.1 *Six commercial beers plotted in CIELAB $L^*C^*_{ab}$ colour space.*

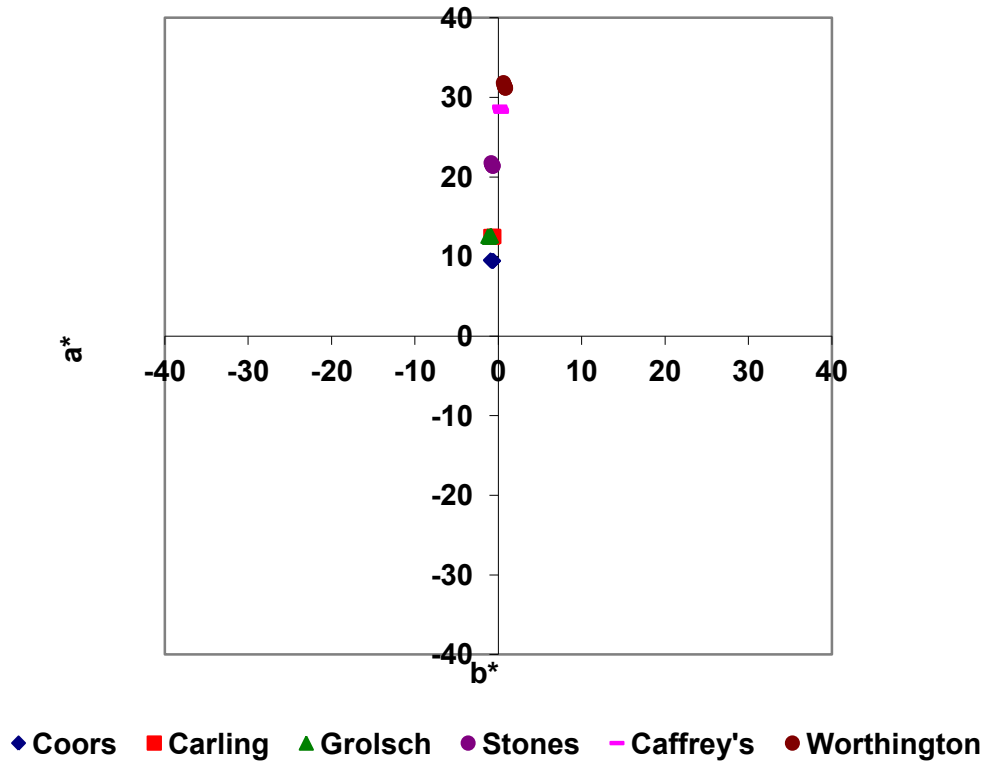

Figure 4.1.1.2 *Six commercial beers plotted in CIELAB a*b* colour space.*

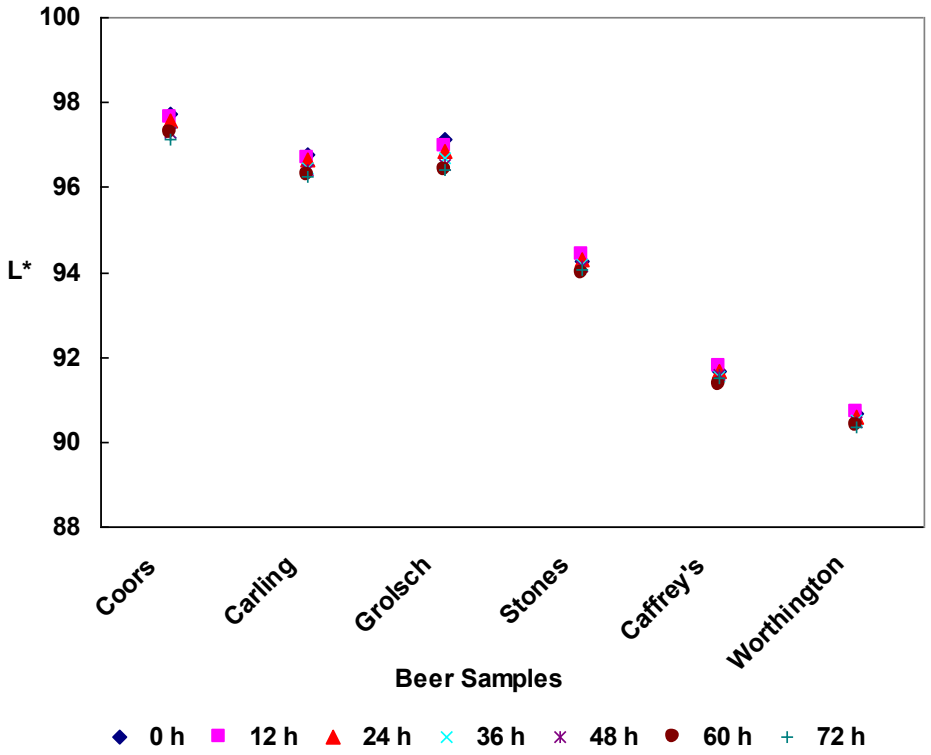

Figure 4.1.1.3 *CIELAB L* parameter of the six commercial beers tested over three days.*

Table 4.1.1.1 Mean and *95% confidence limits of CIELAB L* parameter of the six commercial beers tested over three days.*

Beers	Coors	Carling	Grolsch	Stones	Caffrey's	Worthington
Mean (seven days)	97.43	96.50	96.71	94.19	91.59	90.53
95% Confidence intervals	0.10	0.08	0.13	0.07	0.06	0.06

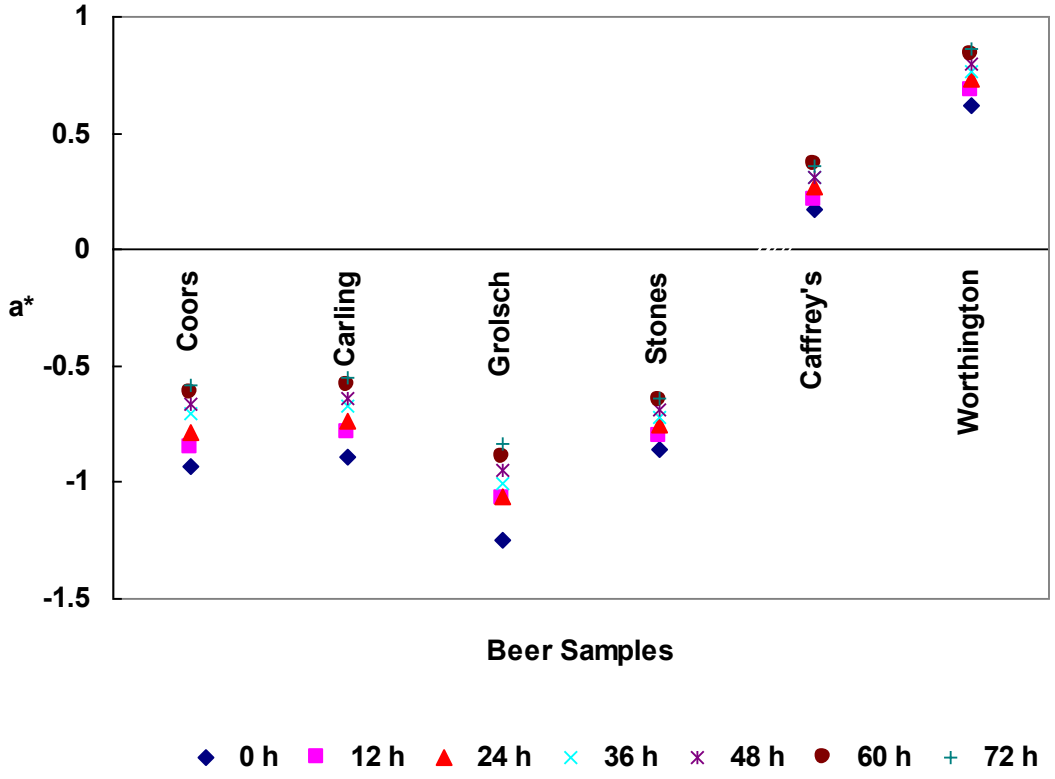

Figure 4.1.1.4 *CIELAB a* parameter of the six commercial beers tested over three days.*

Table 4.1.1.2 *95% confidence limits of CIELAB a* parameter of the six commercial beers tested over three days.*

Beers	Coors	Carling	Grolsch	Stones	Caffrey's	Worthington
Mean (seven days)	-0.73	-0.70	-1.01	-0.73	0.29	0.76
95% Confidence intervals	0.06	0.05	0.09	0.03	0.03	0.04

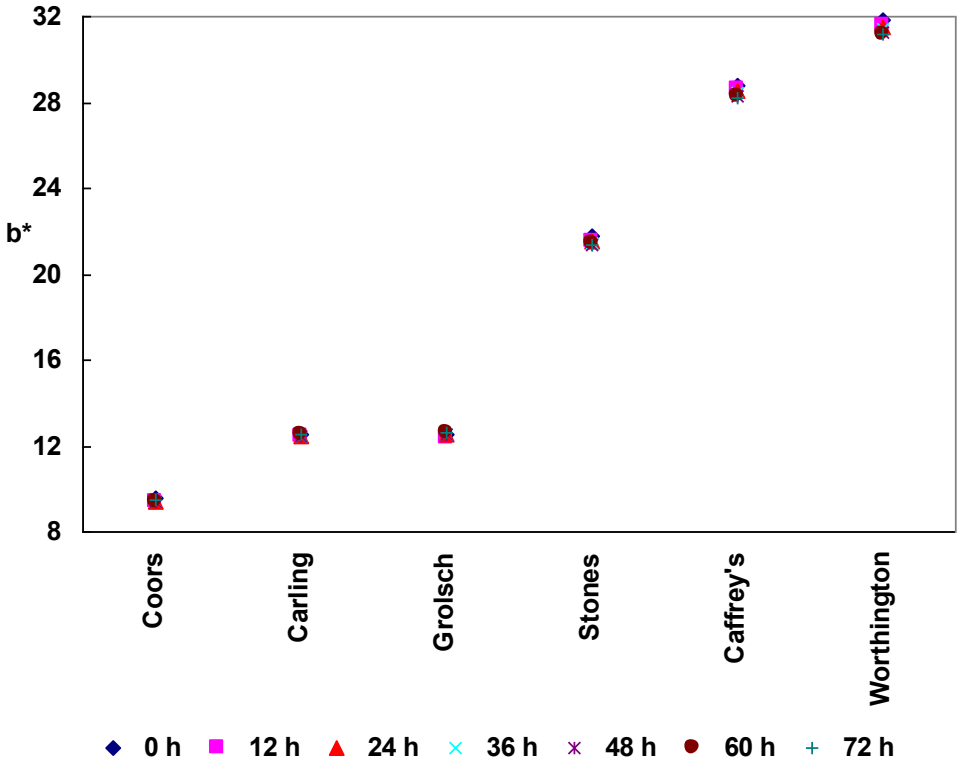

Figure 4.1.1.5 *CIELAB b^* parameter of the six commercial beers tested over three days. The maximum range of measurements was 0.47 b^* units (for Worthington).*

Table 4.1.1.3 *95% confidence limits of CIELAB b^* parameter of the six commercial beers tested over three days.*

Beers	Coors	Carling	Grolsch	Stones	Caffrey's	Worthington
Mean (seven days)	9.44	12.48	12.53	21.49	28.45	31.41
95% Confidence intervals	0.02	0.09	0.03	0.06	0.09	0.10

Each of the six samples was tested seven times in terms of colour measurement over three days. From Figures 4.1.1.3 - 4.1.1.5, there were not obvious differences for any attribute of any sample. To confirm these observations, the CIEDE2000 ΔE_{00} values were calculated, as described in Section 2.2.1. The samples tested at the second time to the seventh time points were compared with those tested the first time (the "freshest" samples) on ΔE_{00}. The samples were tested at seven time points resulting in six comparisons (Figure 4.1.1.6).

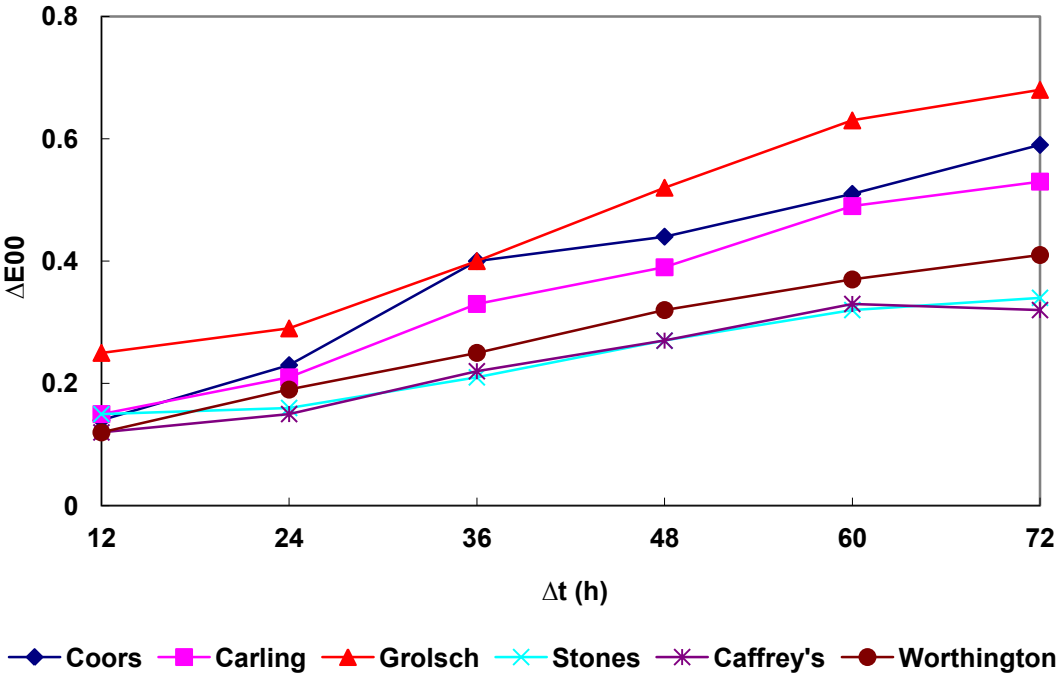

Figure 4.1.1.6 (a) *CIEDE2000 ΔE_{00} of the six commercial beers tested over three days.*

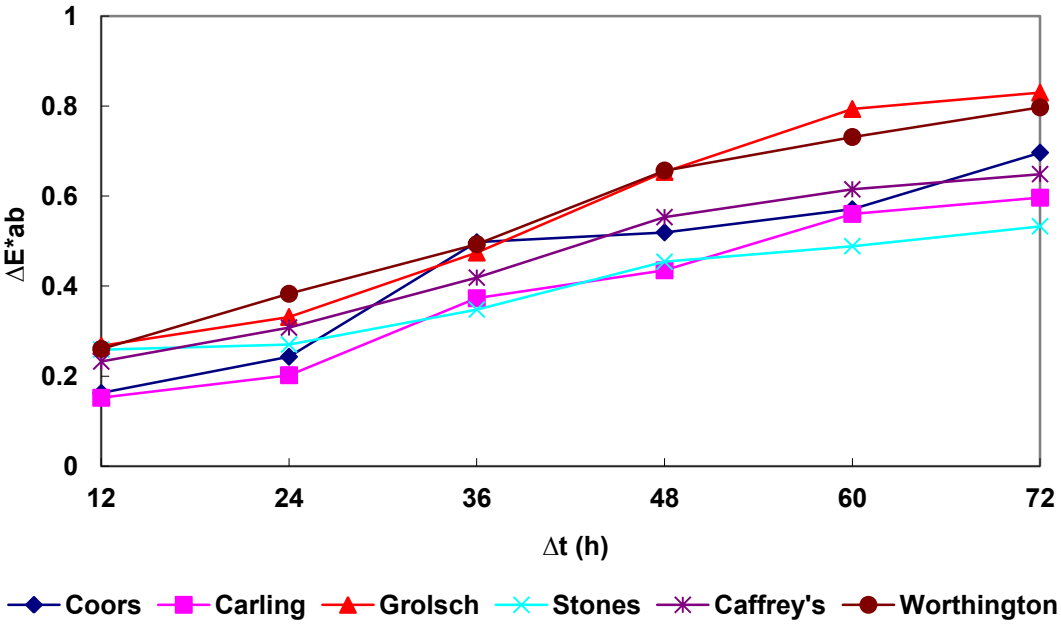

Figure 4.1.1.6 (b) *CIELAB ΔE^*_{ab} of the 6 commercial beers tested over three days.*

12 hours: The comparison between the samples measured in the first afternoon and measured in first morning; 24 hours: The comparison between the samples measured in the second morning and measured in first morning; 36 hours, 48 hours and 60 were similar comparison, until the 72 hours comparison: the comparison between the samples measured in the fourth morning and measured in first morning. The colour changes are systematic with all CIELAB coordinates increasing with exposure to air (oxygen).

From previous research, calculation of colour differences of the same samples by ΔE_{00} and ΔE^*_{ab} can yield different results and, normally. Although the samples were the same, the colour difference values obtained by different calculations were different: CIEDE2000 ΔE_{00} outperforms than CIELAB ΔE^*_{ab} as many evidences shown that the former fits better to all visual experimental results than the latter (Section 2.2.1). Many evidences showed that (see Section 2.2.1) rather than its lower value. From Figures 4.1.1.6 (a) and 4.1.1.6 (b), we can see the colour difference CIEDE2000 (ΔE_{00}) results for colour difference between fresh and "ageing" beer, of which the maximum value is 0.68 and, by CIELAB ΔE^*_{ab}, the maximum value is 0.83. For food materials, it is considered that there is no detectable colour difference if CIELAB ΔE^*_{ab} is less than 1, and for most beers, the detection threshold values for CIELAB ΔE^*_{ab} range from 0.7 to 4.0 (Section 2.2.1). Therefore, for the six beer samples, their colour was considered to be stable over three days for the purposes of this study. This was therefore established the maximum time from sample preparation to experimental sensory assessment.

B. Haze Stability of Commercial Beer Samples

Commercial beer samples were measured using Dr. Lange Haze meter for the haze content (in EBC units) after they had been opened for three days (Figure 4.1.1.7).

The haze content for beers should not be over 1.0 EBC, and preferably, below 0.8 EBC (Section 2.3.1), the latter representing "brilliant" (absolutely clear) beer. Beers are considered "slightly hazy" above 0.8 EBC and are normally commercially unacceptable above 2 EBC (Section 2.3.1). Most of the beer samples supplied satisfied this stipulated brilliant standard. The haze values of the six beers tested were below 0.9 after they had been opened for three days, and this period was set for sample preparation to experimental sensory assessment.

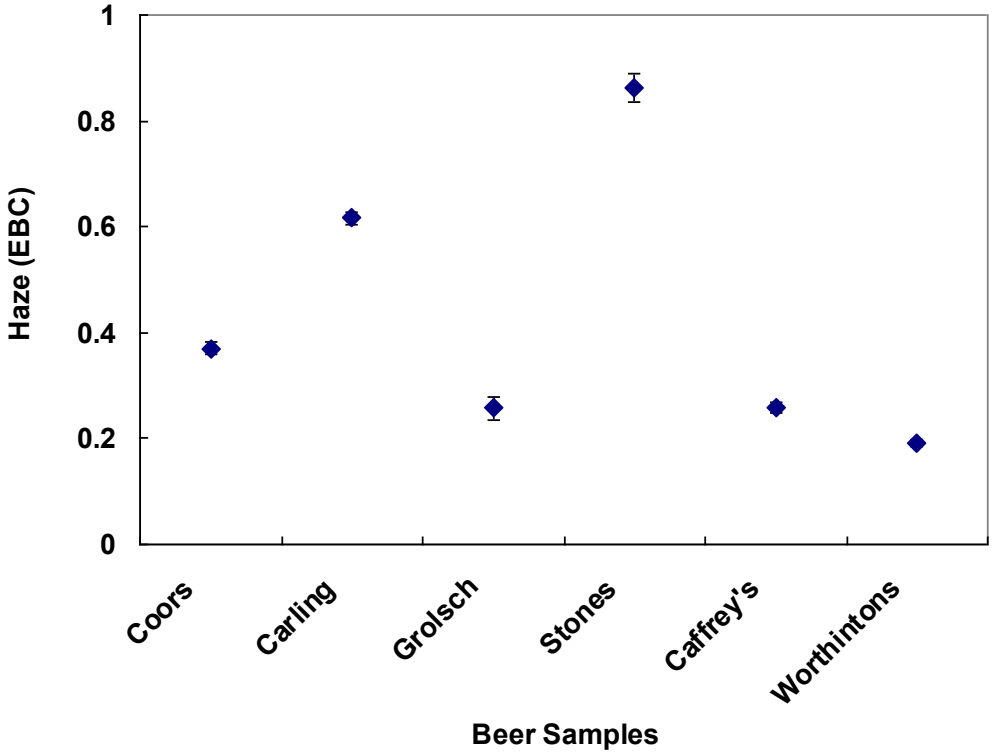

Figure 4.1.1.7 *Haze content of the six beer samples after they had been opened for three days.*

4.1.1.2 Psychophysical Determinations

The pseudo-beer matrix was made and assessed by observers in the psychophysical evaluations on colour appearance attributes and translucency characteristics. This pseudo-beer matrix was made with a range of colorants and scatterers (Figure 3.1.1.2) in aqueous media. From the increasing levels of colorants, it might be defined the relationship between the colorants content and observers' visual results both on colour appearance attributes and translucency characteristics.

A. Colour Appearance Attributes Evaluation

The following results (Figures 4.1.1.8 – 4.1.1.11) were obtained from a panel of ten observers (Section 3.1.1) by 30 samples (12 pseudo-beer samples, six commercial beer samples in pint glasses and six commercial beer samples which were tested twice in highball glasses). Thus 900 observations on colour appearance attributes were made.

From Figure 4.1.1.8, it can be seen that an increase of colorant concentration from 60% to 90%, caused no obvious change in visual lightness (L_v) and colourfulness (C_v). A further increase of colorant concentration to 100% caused decreasing visual lightness (L_v). With each colorant concentration, an increase of scatterer concentration generally resulted in increasing colourfulness (C_v). This increasing scatterer concentration also caused initially decreasing lightness (L_v) and finally a little increasing. In general words, observers thought the sample Y6 and Y9 were similar on lightness (L_v) values which were higher than those of Y10, and they also thought the samples were more colourful as colorant concentration increasing (moving along the arrows).

From Figure 4.1.1.1, lightness (L^*) values of the six commercial beers measured by instrument were between 90 to 98 units, which covered eight units, and thus ΔL^* was eight units. Human have the ability to distinguish these lightness change (Section 2.4.2). From Figures 4.1.1.8 and 4.1.1.9, our observers detected the lightness of the six commercial beers changed, between 40 to 80 units (thus ΔL_v was 80).

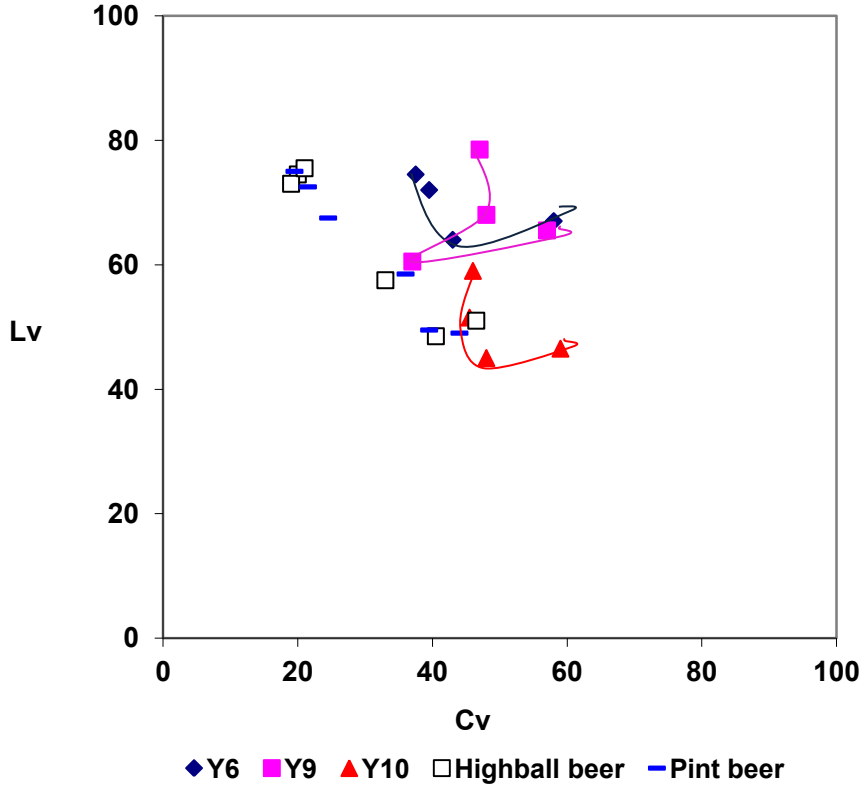

Figure 4.1.1.8 *Visual results on lightness (L_v) and colourfulness (C_v) of the beer samples.*

Y6: the pseudo-beer samples composing 60% yellow colorant, and the levels of scatterers were 0%, 20%, 60% and 100%; Y9: the pseudo-beer samples composing 90% yellow colorant, and levels of scatterers were 0%, 20%, 60% and 100%; Y10: the pseudo-beer samples composing 100% yellow colorant, and levels of scatterers were 0%, 20%, 60% and 100%, along the arrows. Highball beer: six commercial beers in highball glass. Pint beer: six commercial beers in pint glass.

From Figure 4.1.1.9, hue values of Y10 were the highest in the pseudo-beer samples, and those of Y6 were the lowest. It means that as the colorant concentration increases the hue values decrease. It also can be seen that, moving along the arrows, for Y6, Y9 and Y10 samples, lightness decreased when scatterer concentrations were increased from 0% to 60%, and then increased when the concentration reached 100%.

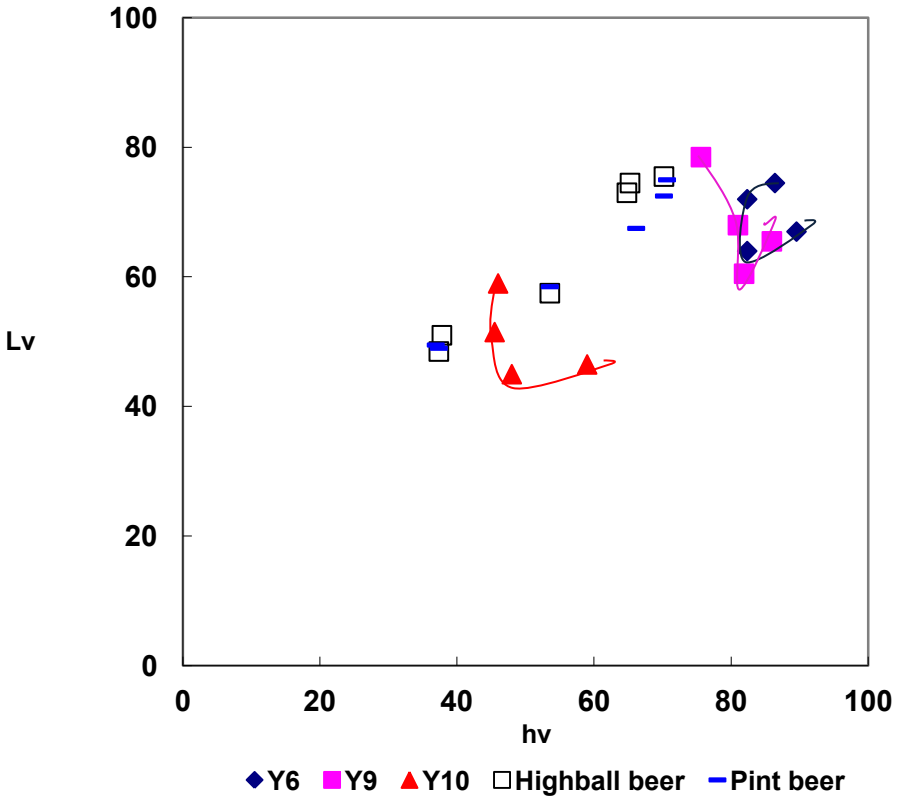

Figure 4.1.1.9 *Visual results on lightness (L_v) and hue (h_v) of the beer samples.*

Y6: the pseudo-beer samples composing 60% yellow colorant, and the levels of scatterers were 0%, 20%, 60% and 100%; Y9: the pseudo-beer samples composing 90% yellow colorant, and levels of scatterers were 0%, 20%, 60% and 100%; Y10: the pseudo-beer samples composing 100% yellow colorant, and levels of scatterers were 0%, 20%, 60% and 100%, along the arrows. Highball beer: six commercial beers in highball glass. Pint beer: six commercial beers in pint glass.

It was observed in Figures 4.1.1.8 and 4.1.1.9 that, moving along the arrows (under each colorant concentration), "U shape" effects were caused on the relationship between L_v and C_v, and L_v and H_v by increasing concentration of scatterer. In these cases, L_v deceased when increasing the scatterer concentration from 0 to 60%, and finally increased slightly when the scatterer concentration reached 100%. These L_vs' routes may be caused by the different ratio of absorbed and scattered light. In other words, there was more light being absorbed as the scatterer concentration increasing from 0 to 60%, while some more light was scattered by the particles when their concentration increasing from 60% to 100%.

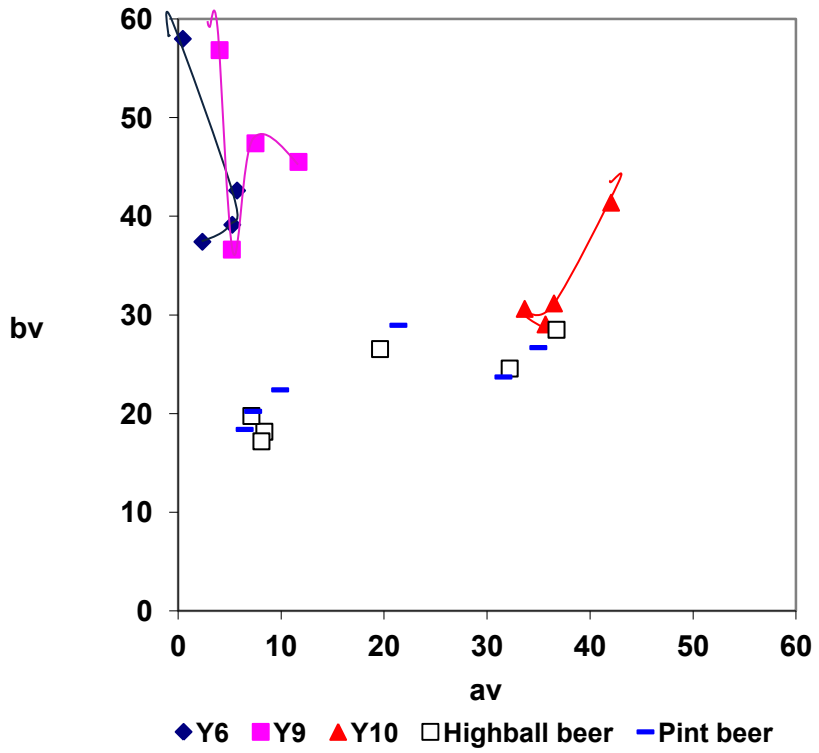

Figure 4.1.1.10 *Visual hue composition a_v and b_v of the beer samples.*

Y6: the pseudo-beer samples composing 60% yellow colorant, and the levels of scatterers were 0%, 20%, 60% and 100%; Y9: the pseudo-beer samples composing 90% yellow colorant, and levels of scatterers were 0%, 20%, 60% and 100%; Y10: the pseudo-beer samples composing 100% yellow colorant, and levels of scatterers were 0%, 20%, 60% and 100%, along the arrows. Highball beer: six commercial beers in highball glass. Pint beer: six commercial beers in pint glass.

The corresponding a_v value visually indicates red ($a_v > 0$) or green ($a_v < 0$), and the b_v value visually indicates yellow ($b_v > 0$) or blue ($b_v < 0$) hues. From Figures 4.1.1.9 and 4.1.1.10, all of the samples appeared reddish yellow (hue values were between red and yellow but closer to yellow) or yellowish red (hue values were between yellow and red but closed to red) visually according to their hue values (30 to 95 units) hue composition a_v values (0 to 42 units) and b_v values (17 to 60 units).

From Figure 4.1.1.10, Y10 samples had higher a_v values, which means they were redder than Y6 and Y9 samples. In other words, increasing the colorant concentration resulted a_v increasing, and the samples were redder. When individual Y6, Y9 or Y10 samples

were considered, moving along the arrows, high scatterer concentrations resulted in higher values of b_v and, therefore, the samples were yellower.

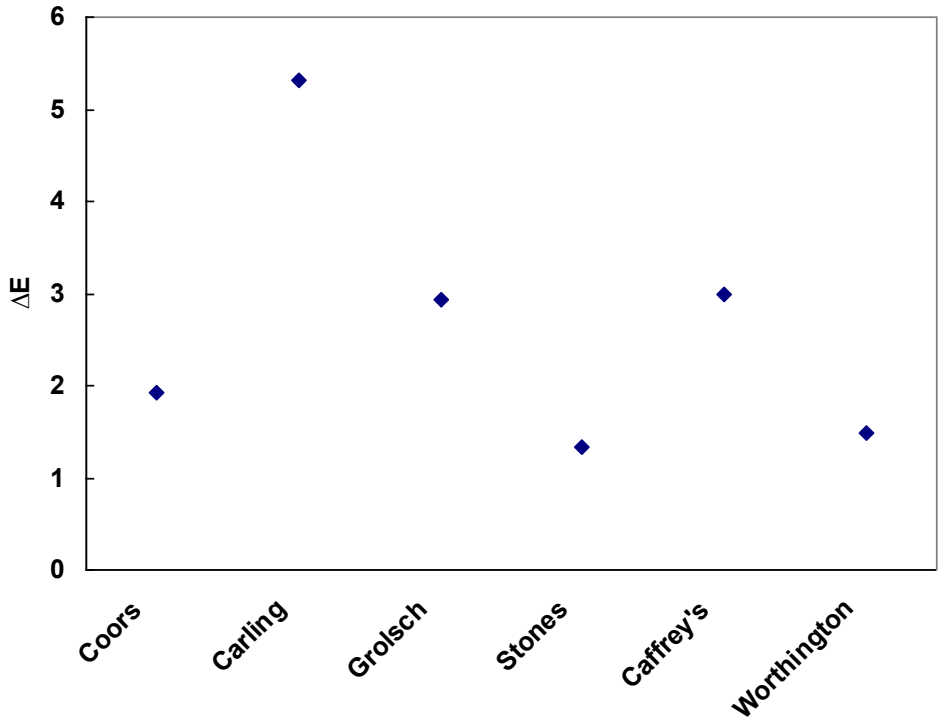

Figure 4.1.1.11 *ΔE_v (visually tested) between the beers in highball glasses and pint glasses.*

From Figures 4.1.1.8 to 4.1.1.11, for the same commercial beer samples, observers perceived differences between the same samples presented in highball glasses or pint glasses on the colour appearance attributes of visual lightness, colourfulness and hue compositions. When ΔE^*_{ab} varies, the response of the human visual system is not the same. Thus, the CIE recommendation (Section 2.2.1) was suggested that moderate colour differences were between 0 – 5 CIELAB units, under which human could distinguish colour differences effectively. The CIELAB ΔE^*_{ab} (between 1 – 5.2 CIELAB units) of the six commercial beers on colour appearance attributes on visual tested in highball glasses and pint glasses are shown in Figure 4.1.1.11, which varied significantly in their visual performance when they were presented in highball glasses and pint glasses.

The observer performances on beer samples, denoted here as observer accuracy, were quantified by the determination of coefficients of variation (*CV*; Table 4.1.1.4). The *CV* values were used as a statistical measure to investigate the agreement between any two sets of data, say *x* and *y*. The coefficient of variation is a measure of the distance along the *y* axis of the points from the 45° line in the (*x*, *y*) plot. It expresses the root-mean-square deviation of the mean value of the *y* set. It can be thought of as relative percentage error, and was calculated using following equations. For a perfect agreement between two sets of data, the *CV* should equal zero.

$$CV = 100\times \frac{\sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - x_i)^2}}{\overline{Y}}$$

$$\text{and } \overline{Y} = \frac{1}{N}\sum_{i=1}^{N} y_i \qquad \text{Eq.25}$$

In our case, we compared each score given by observers to the relevant mean value. No comparison for colour appearance attributes in pint glasses was made as the glasses were not suitable for assessing colour appearance attributes with the vertically curved glass body, in contrast to the highball glasses which have vertically straight walls.

Table 4.1.1.4 *Observer accuracy on visual colour appearance attributes by beers: lightness, colourfulness and hue.*

	L_v	C_v	H_v
CV (%)	14.7	23.4	14.7

Discernment of the psychological attributes of colour appearance has proven difficult, although the definitions are relatively easily understood, and lightness and hue were distinguished more accurately than colourfulness attribute in agreement with previous research (Section 2.4.2). From Table 4.1.1.4, the overall mean *CV* values were 14.7, 23.4 and 14.7 for lightness, colourfulness and hue, respectively, which means that there

were 14.7%, 23.4% and 14.7% variation on these three attributes of observers' answer, which were lower compared with those found in previous studies of Gonzalez-Miret Martin et al. and Ji et al. It indicated that the lightness and hue results were more consistent, and the colourfulness results indicated the colourfulness was the most difficult attribute to scale in this case. Similar results have been found in many studies (Section 2.4.2). Compared with lightness and hue, colourfulness is a term which is less used in our daily life, so presumably the data given by observers is subject to greater variation.

To further develop the technique, the visual results were compared with the TSR measurements (CS-1000) in a highball glass.

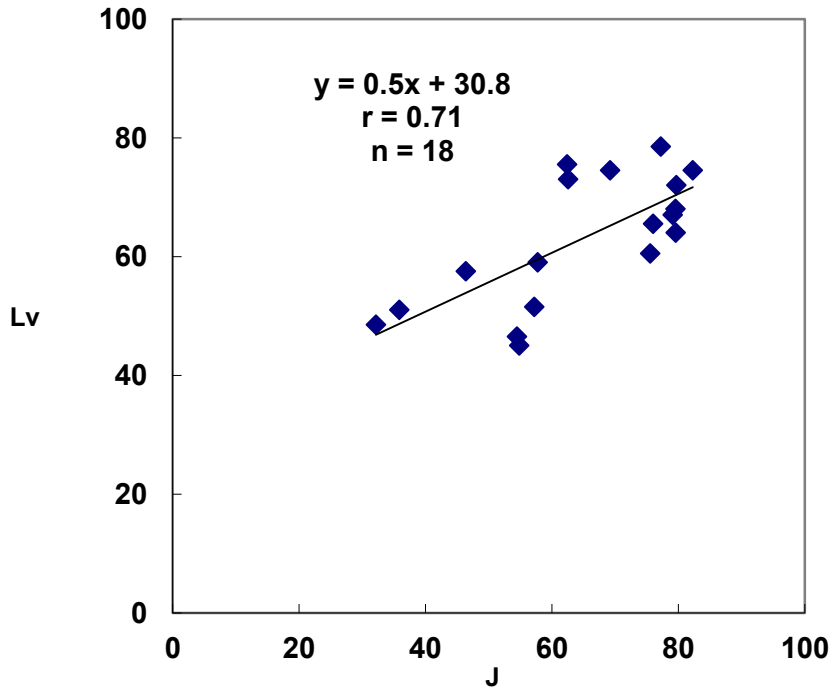

Figure 4.1.1.12 *CIECAM02 descriptor of J measured by TSR plotted as function of visual* L_v.

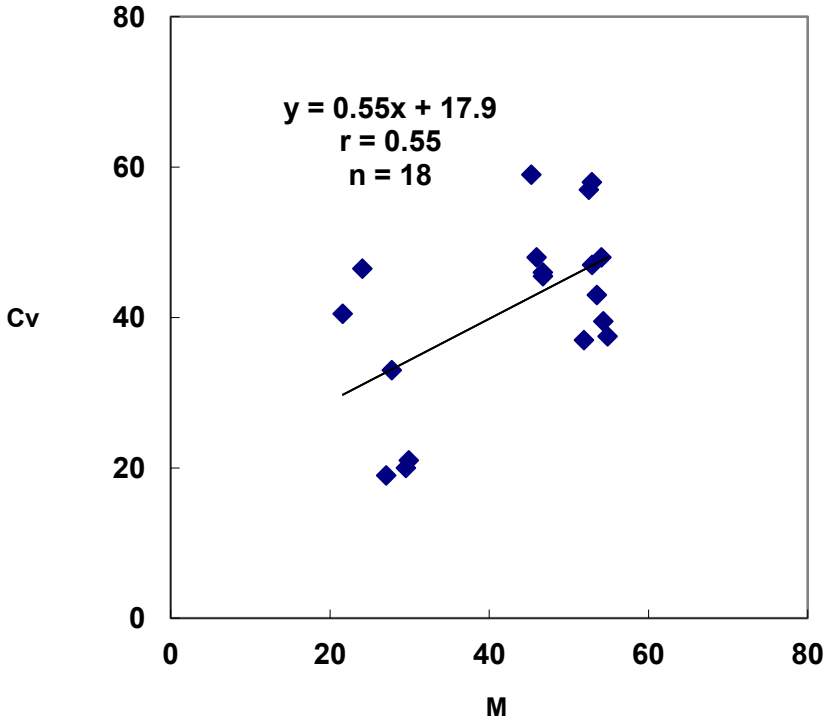

Figure 4.1.1.13 *CIECAM02 descriptor of M measured by TSR plotted as function of visual* C_v.

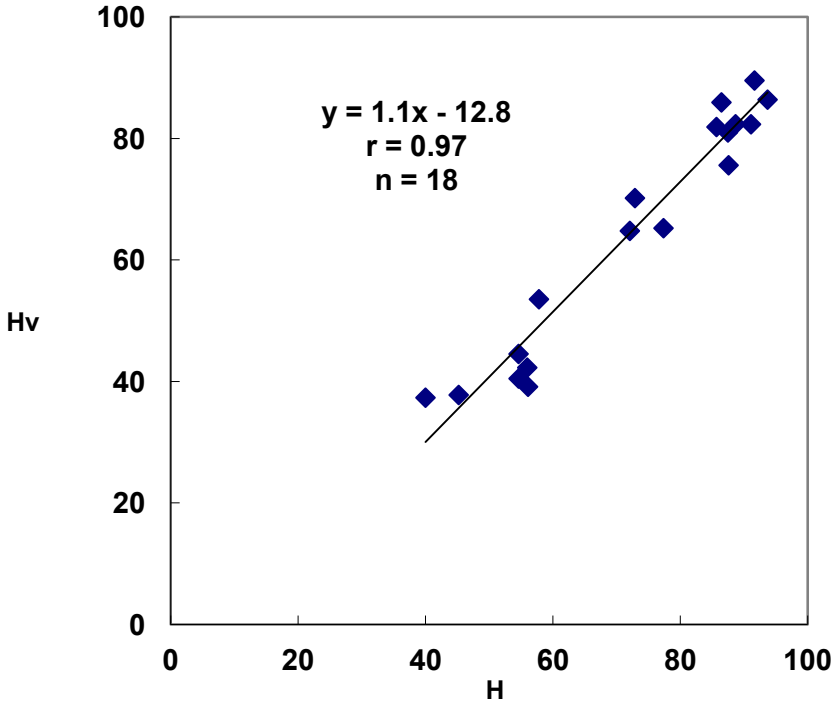

Figure 4.1.1.14 *CIECAM02 descriptor of H measured by TSR plotted as function of visual* H_v.

The SPD (spectral power distribution) data measured were transformed to CIECAM02 Lightness (J), Colourfulness (M) and Hue Composition (H) values, and they have a direct correspondence to the visual lightness, colourfulness and hue, respectively, showing as Figures 4.1.1.12-4.1.1.14. These comparison results were used in the software development for DigiEye imaging system (Colour Science Department, the University of Leeds). As the observer accuracy (Table 4.1.1.4), the correlation coefficient of colourfulness was lower than those of lightness and hue.

B. Translucency Evaluations

A panel of twelve observers took part in this evaluation. As might be expected there was a strong correlation between transparency and opacity, the relationship between the two terms appearing to be linear (Figure 4.1.1.15 and 4.1.1.16). In Figure 4.1.1.15, pseudo-beers containing scatterers potentially applied leverage to the data set of all samples, and therefore, regression were applied again to analyse “clear” samples (all commercial samples in highball glasses and pint glasses, and colorant solutions) as showing in Figure 4.1.1.16. The absolute value of the correlation coefficients of Figure 4.1.1.15 (all samples) is higher than that shown in Figure 4.1.1.16 (“clear” samples), because the “clear” samples show a narrower spread of transparency/opacity characteristics, which is more difficult for observers to distinguish. Besides, in this study, the leverage points (Figure 4.1.1.15) resulted in apparently better correlations (Everitt, 2006), of which the correlation coefficient was -0.99 (n = 30).

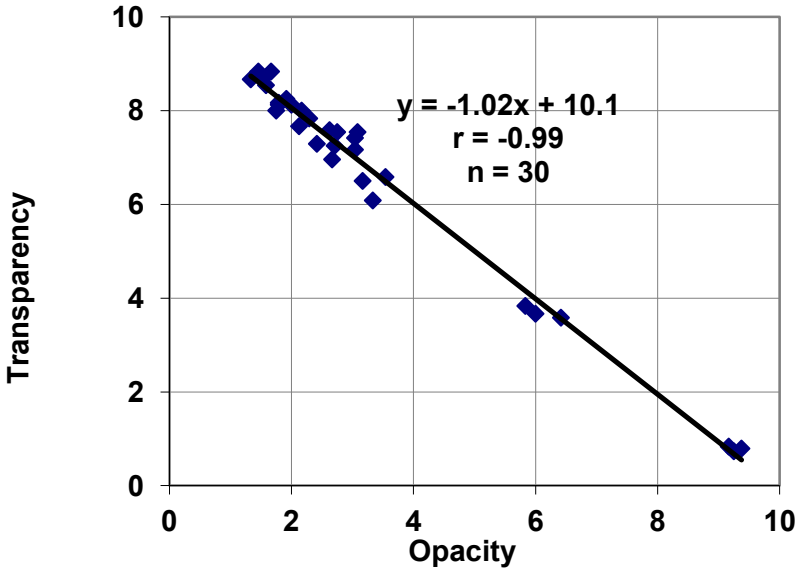

Figure 4.1.1.15 *The correlation between transparency and opacity for commercial beers and pseudo- beers.*

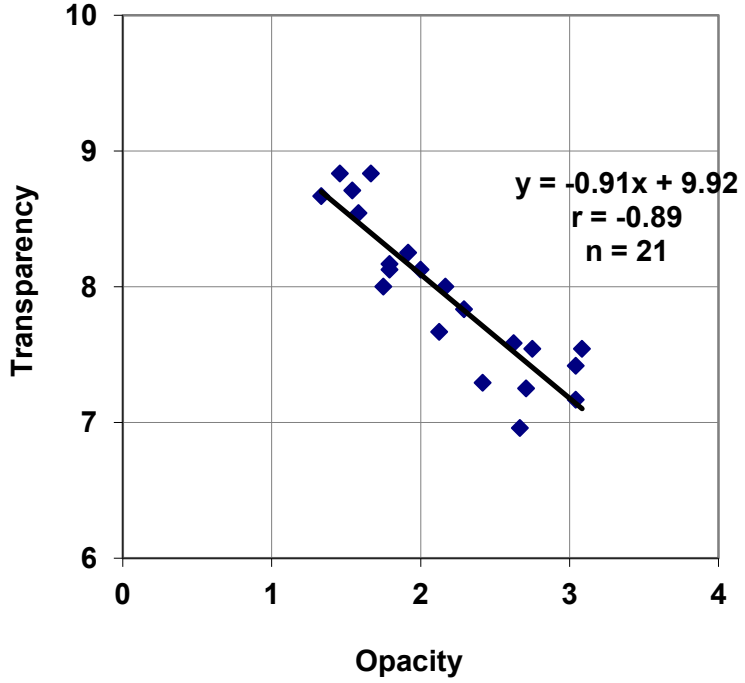

Figure 4.1.1.16 *The correlation between transparency and opacity for commercial beers and pseudo-beers with no scatterers (i.e. Y6S0, Y9S0 and Y10S0).*

Two extremes for transparency and opacity were set, both from 0 to 10. The

relationships demonstrated in Figures 4.1.1.15 and 4.1.1.16 given the intercept and slope of the regression line suggested that the results for each sample would sum close 10. As might be expected, as transparency increases the observer-assessed opacity decreases. The sum of the transparency and opacity was approximately 10 for each sample. When the two sets of data are summed, the sums range from 9.4 to 10.5, (Figures 4.1.1.17 and 4.1.1.18). The panellists were sensitive to the presence of haze in the pseudo-beer samples (Figure 4.1.1.15 and Figure 4.1.1.18). For solutions with same colorant contents (labelled as same Y, Y6 Y9 and Y10), the transparency decreased with the increasing levels of scatterer (labelled as increasing S, S0, S2, S6 and S10) as would be expected.

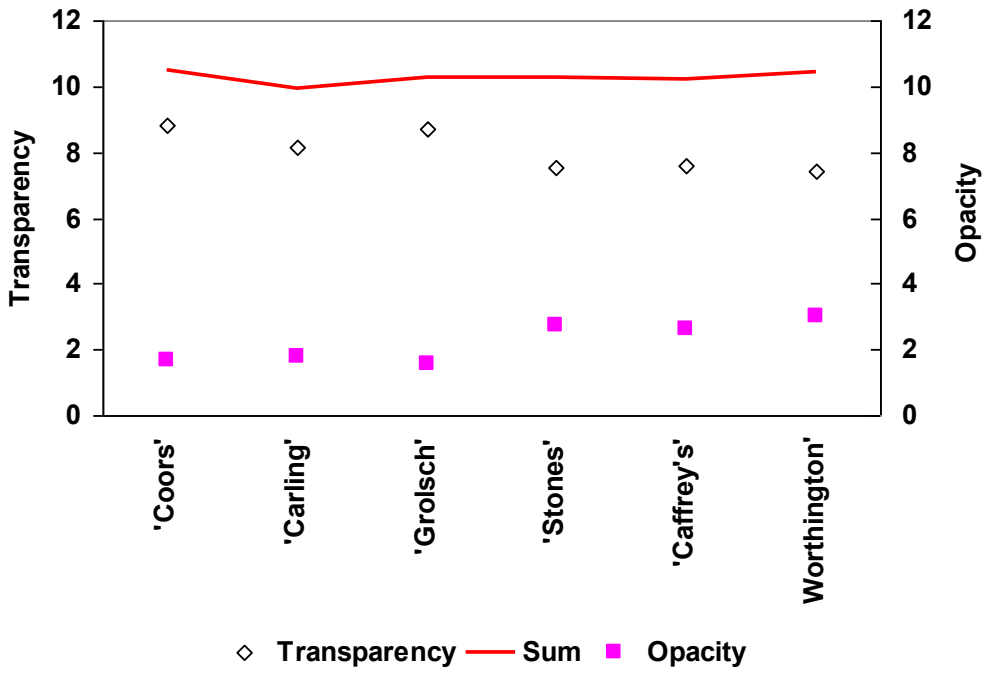

Figure 4.1.1.17 *The complementarity between transparency and opacity for commercial beers.*

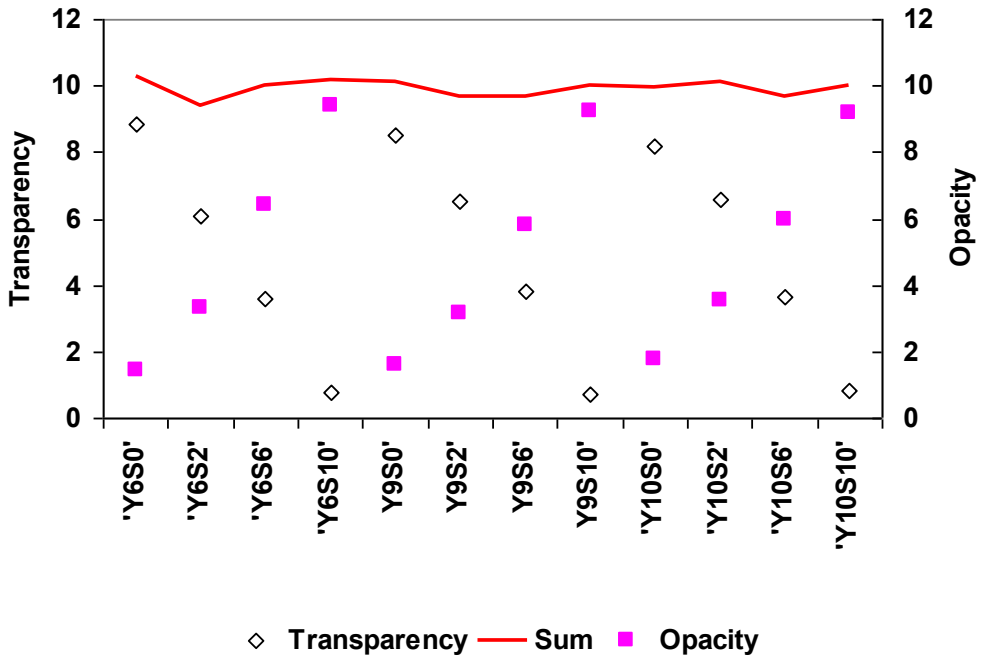

Figure 4.1.1.18 *The complementarity between transparency and opacity for pseudo-beers.*

From Figure 4.1.1.18, it could be seen that the observers gave similar score on translucency characteristics for the samples with same scatterer concentrations, for examples, the observers gave the similar transparency/opacity values to the samples Y6S2, Y9S2 and Y10S2, which were 6.1 and 3.8, respectively.

Transparency is used to describe the passage of light through the liquid, whereas the opacity is used to describe the turbidity (Section 3.1.1). According to the data obtained from the observers, clarity appears to be linearly related to transparency, the clarity increasing when the transparency increased for each sample, whether pseudo-beers or commercial beers are being assessed (Figure 4.1.1.19). Observers clearly distinguished between the pseudo-beers containing particles (the leverage points) and the "clear" samples on both of transparency and clarity.

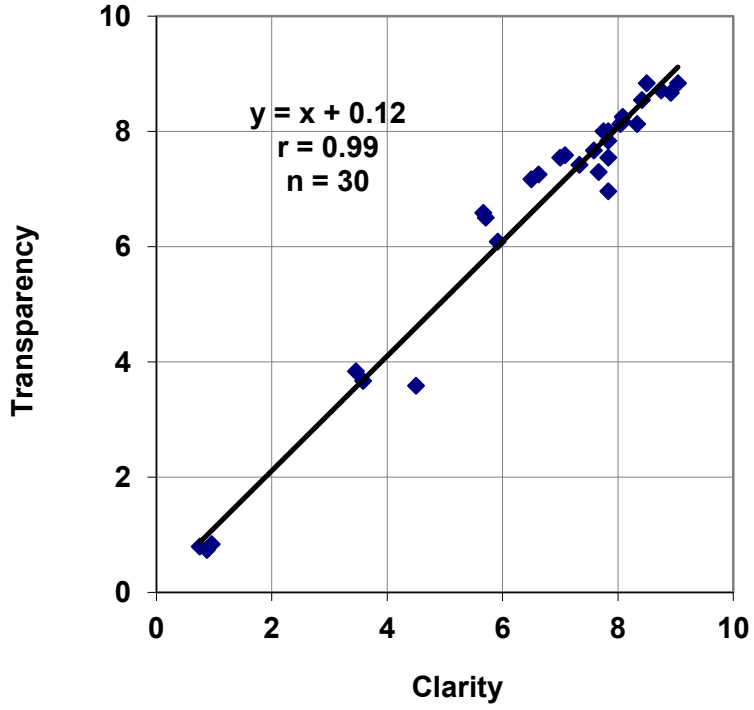

Figure 4.1.1.19 *The correlation between transparency and clarity for commercial beers and pseudo-beers.*

Some industries use five categorical terms to describe whether the liquid food clear or not (Section 3.1.1). In this study, the five-point scale is used to describe the translucent characteristics of liquid products, which is described by the adjectives bright, clear, dull, hazy and cloudy (Section 3.1.1), and in this part of experiment, observers scored one to five to indicate the five terms.

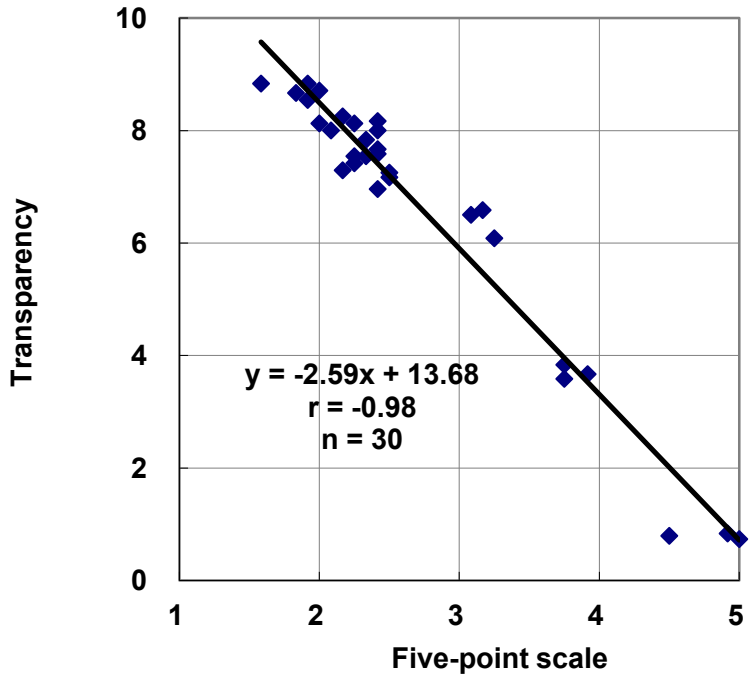

Figure 4.1.1.20 *The correlation between transparency and five-point scale for commercial beers and pseudo-beers.*

The observers gave integers on the five-point scale experiment (e.g. an observer considered one sample was clear, and so he scored this sample two. For another sample, this observer considered it was dull, so he scored it three). Figure 4.1.1.20 shows the correlation results between mean values of visual transparency and mean values of visual five-point scale of the commercial and pseudo-beer samples. The mean values of five-point scale were accurate to 0.01 (e.g. 3.23). But this observation is not sufficient to reflect the relationship between "transparency and "five-point" description, as the latter is a discrete ordinal scale in this experiment. Thus, the average result data (mean values) of the five-point scale were analysed by being rounded up (i.e. a mean value of five-point scale of one sample was 3.23, it would be rounded up to 3; and a mean value was 3.85, it would be rounded up to 4), resulting in the data shown in Figure 4.1.1.21. From this figure, it is clear that observers broadly assigned the correct descriptor to the more quantitative transparency attribute. However for samples with intermediate translucency (e.g. transparency values between six and eight labelled in the dotted box),

the observers could not distinguish/score clearly. This could mean that some confusion existed around the meaning of these terms, or that it is difficult to distinguish the samples in this area.

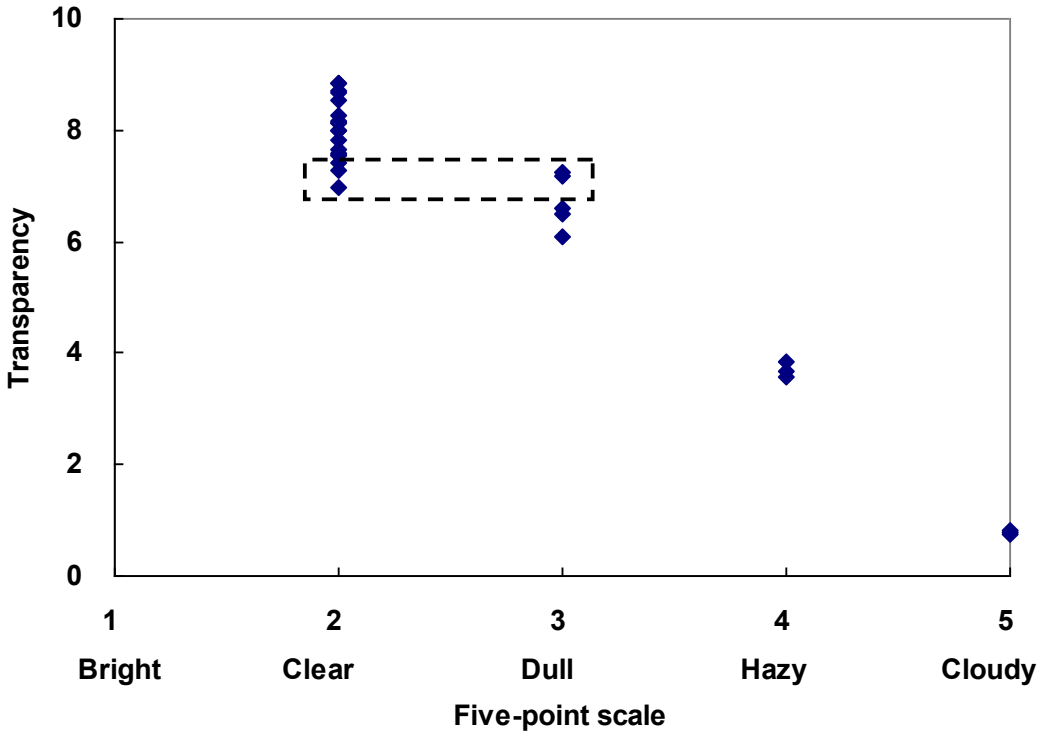

Figure 4.1.1.21 *Visual five-scale and transparency for commercial beers and pseudo-beers. Observers could not distinguish/score clearly for samples which were in dotted box.*

Based on the results reported in Figures 4.1.1.15 to 4.1.1.20, visual transparency is significantly negatively correlated to visual opacity and the five-point scale and significantly positively correlated to visual clarity. As mentioned, the above results and figures were obtained from a panel of twelve observers (Section 3.1.1). The *CV* measures for the industry terms on translucency scaled were calculated between each observer's results and the mean results of the four describing terms of translucency (Table 4.1.1.5). The opacity results indicated the opacity was the most difficult term to scale for observers, which had the higher *CV* value of 27.4, it means the observers had more variability on using "opacity" than "transparency". "Opacity" is more difficult to

control and thus there was bigger variation.

Table 4.1.1.5 *Observer accuracy on industry terms on translucency by beers:*

	Transparency	**Opacity**	**Clarity**	**Five-point scale**
CV%	14.1	27.4	16.0	17.7

4.1.2 Psychophysical Evaluations and Instrumental Measurements on Red Wines

4.1.2.1 Instrumental Measurements

The five red wines were taken from different processing stages (Section 3.1.2). The Dr Lange Haze meter was used to measure the haze for these wine samples (Figure 4.1.2.1), and ten measurements were taken for each sample.

From Figure 4.1.2.1, the five red wine samples that had been taken from tank directly (i.e. without any further processing) had the highest haze values (i.e. samples A1 to E1). The haze content of successive samples decreased due to the processing steps employed (from processing stage 1 to 2 for each sample). The haze content of all of the wines, except type B, was decreased slightly by filtering through the membrane. Each sample was divided into five parts, and the haze content of each part was tested twice. Thus, ten measurements were taken for each sample. The higher haze content of B3 than B2 may be due to the problem of processing. There was the possibility that this result was due to sampling, because the haze content of final product of type B was higher than the other four wines. However, human assessment did not distinguish the translucency difference caused by processing (including type B) visually, see Section 4.1.2.2.

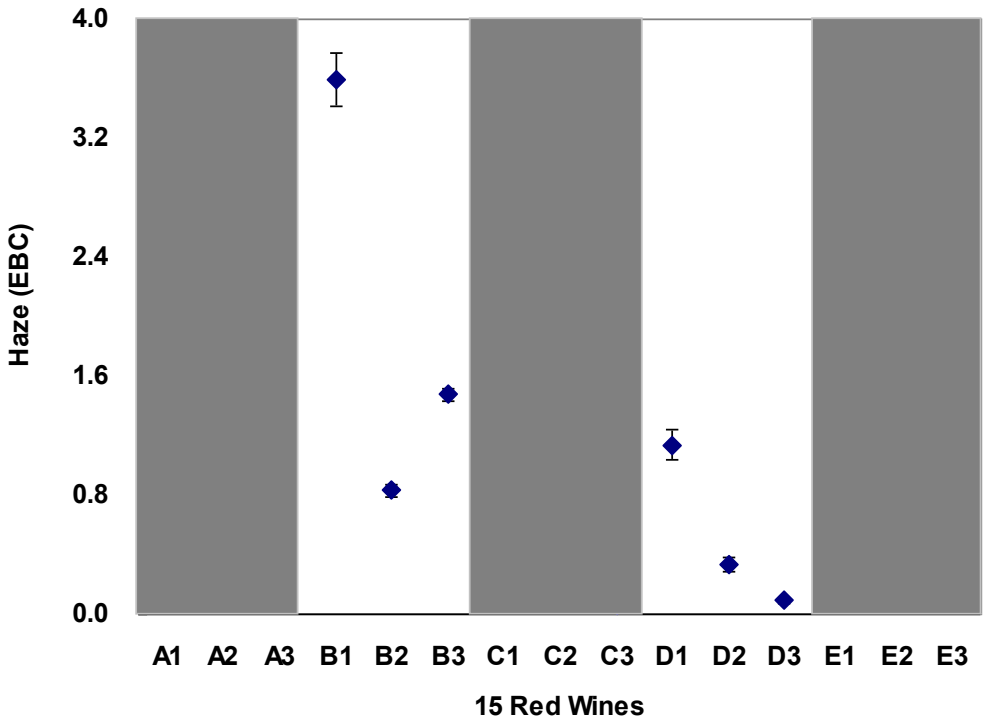

Figure 4.1.2.1 *Haze content in the red wine samples. A to E indicate the five types of red wines, 1 to 3 indicate the samples were taken from different processing stages (as described in Section 3.1.2). Error bars signify standard deviation.*

4.1.2.2 Psychophysical Determinations

A. Colour Appearance Attributes Evaluations

In red wine filtration, two important factors must be considered, the degree of clarity and the amount of colour lost (Section 1.1). In this study, observers were asked to evaluate different wine samples pre-/postfiltration.

The observers were asked to score the fifteen red wine samples on colour appearance, (Figures 4.1.2.2, 4.1.2.3 and 4.1.2.4). As described in Section 3.1.2, wines A (Italian Red) and E (Italian Red), C (Cote Du Rhone) and D (Cote Du Rhone) are the same

products but different batches, thus, strictly speaking, there were three types red wines tested in this part of experiment (wine B was Bordeaux Rouge).

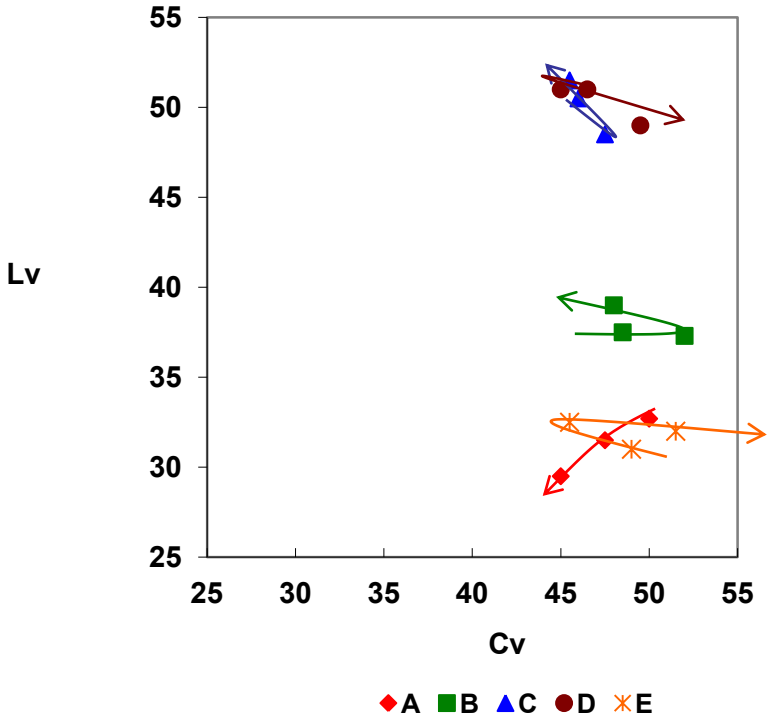

Figure 4.1.2.2 *Visual results on lightness and colourfulness of the red wine samples. A to E indicates the five types of red wines, and samples along the arrow indicating they were taken from different processing stages (as described in Section 2.1.2).*

Compared with the other attributes, observers appeared to be more sensitive to lightness. They could separate the samples into three parts, with C and D being of similar lightness, while A and E were of lower lightness, and the B samples were separated from the other four samples. From this point of view, observers could distinguish the three brands of wine correctly. However, moving along the arrows, the directions were different. It means observers were not able to detect the lightness changes caused by processing.

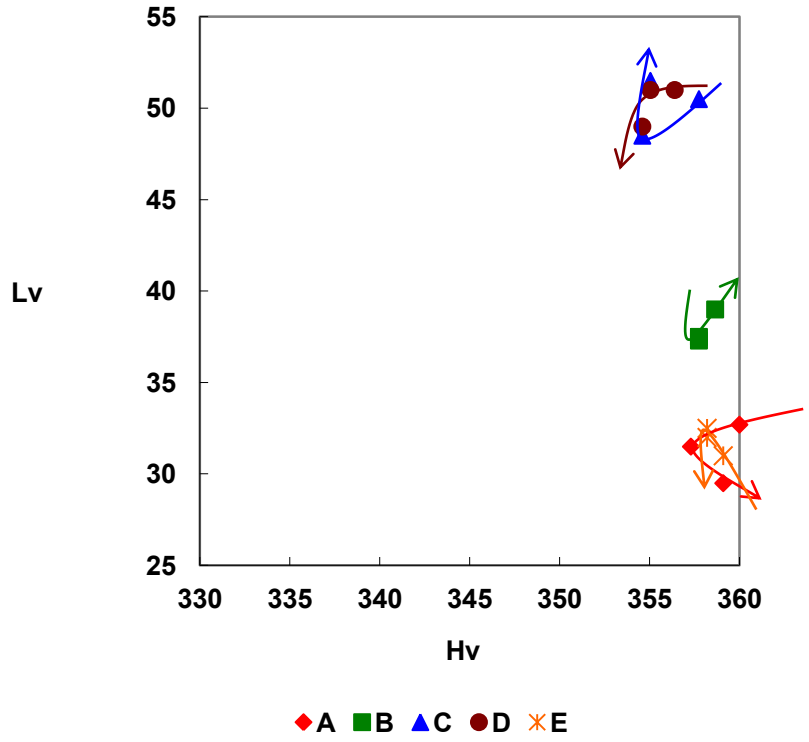

Figure 4.1.2.3 *Visual results on lightness and hue of the red wine samples. A to E indicates the five types of red wines, and samples along the arrow indicating they were taken from different processing stages (as described in Section 3.1.2).*

In this study, the observers were not given an upper limit to the scale of the colourfulness they could use, thus the colourfulness could range from zero to infinity in a sense. But they gave the results distributing from 45 to 52 units. All of the observers reported pure red or bluish red for all the samples ($a_v > 0$, $b_v < 0$). But the observers were not able to distinguish different brands of wines or the wines in different processing stages based on Figures 4.1.2.2 - 4.1.2.4. The different brands of wines were not separated on a_v, b_v, C_v or H_v, and the arrows did not follow the same direction. These reflected the same results with those of beers (Section 4.1.1.2) and some other researches (Section 2.4.2).

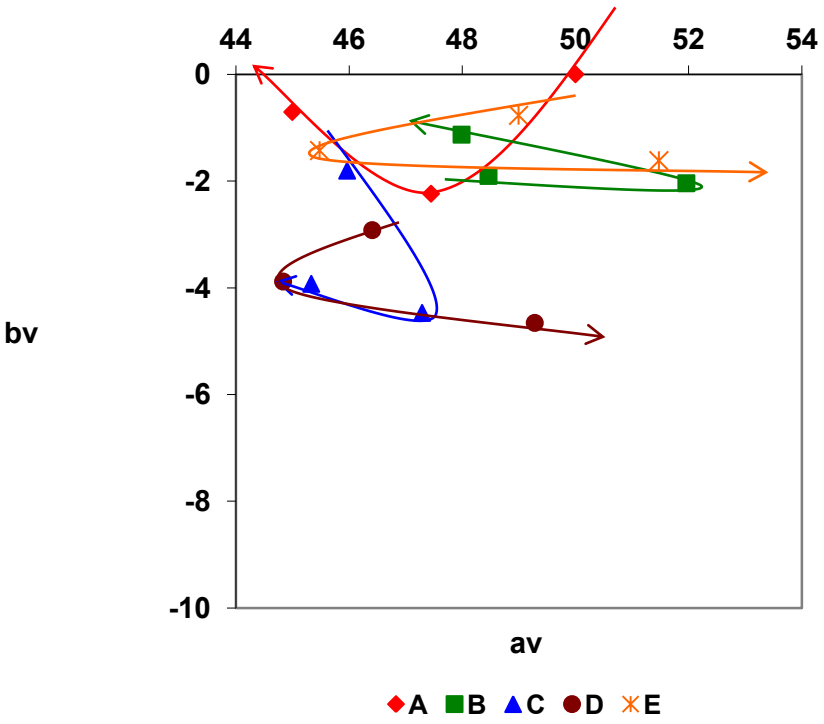

Figure 4.1.2.4 *Visual hue composition a_v and b_v of the red wine samples. A to E indicates the five types of red wines, and samples along the arrow indicating they were taken from different processing stages (as described in Section 3.1.2).*

The observer accuracy on red wine samples are listed in Table 4.1.2.1, based on the 450 observations (see Section 3.1.2). The calculation comparisons were between individual attribute given by observer and the relevant mean values.

Table 4.1.2.1 *Observer accuracy on visual colour appearance attributes for red wines: lightness (L_v), colourfulness (C_v) and hue (H_v).*

	L_v	C_v	H_v
CV%	17.4	27.6	1.7

For the colour appearance attributes, the *CV* values for lightness (17.4) and colourfulness (27.6) were larger than those in beer experiment, but were lower than

reported in previous research of Gonzalez-Miret Martin et al. The *CV* value for hue was much smaller (1.7), because these red wines do not spread greatly on hue, which ranged from 354 to 360, according to Figure 4.1.2.3. Observers performed the most poorly on colourfulness again, in agreement with other research reports of magnitude estimation of colour appearance (Section 2.4.2).

The visual results were also compared with the TSR measurements (CS-1000) in a Petri Dish, and the SPD data measured were transformed to CIECAM02 Lightness (J), Colourfulness (M) and Hue Composition (H) values, and they have a direct correspondence to the visual lightness, colourfulness and hue, respectively, showing as Figures 4.1.2.5-4.1.2.7. These comparison results were used in the software development for DigiEye imaging system (Section 2.5).

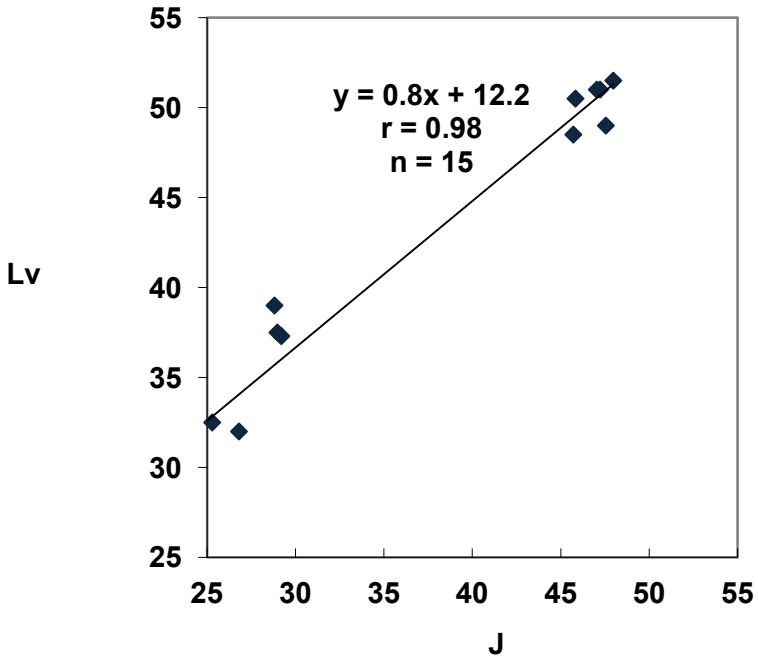

Figure 4.1.2.5 *CIECAM02 descriptor of J measured by TSR plotted as function of visual* L_v.

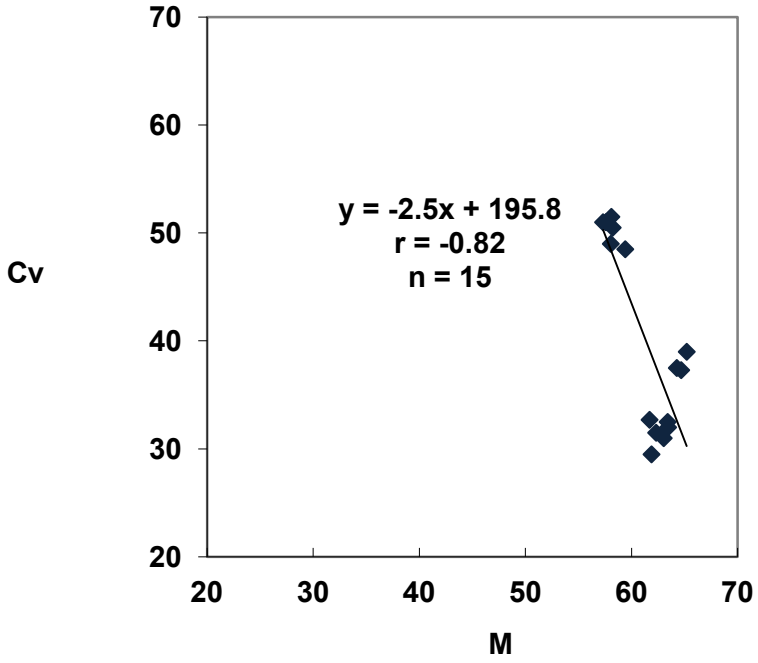

Figure 4.1.2.6 *CIECAM02 descriptor of M measured by TSR plotted as function of visual* C_v.

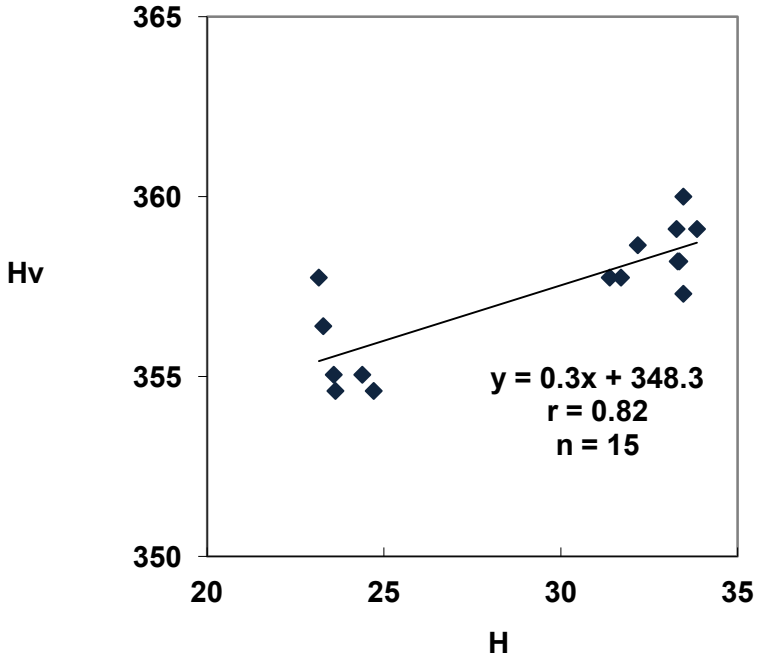

Figure 4.1.2.7 *CIECAM02 descriptor of H measured by TSR plotted as function of visual* H_v.

B. Translucency Evaluations

Analogous to the beer experiment, for the wine experiment, the observers showed a significant correlation between opacity and transparency for the samples (Figure 4.1.2.8), with these two terms appearing to be linearly related ($p < 0.05$). As expected, as transparency increases, observed opacity decreases. Red wine types C and D were thought being different from the other three types of wine samples on these two attributes, labeled in the circle. When the two sets of data are summed, the sums ranged from 9.7 to 10.7 (Figure 4.1.2.9).

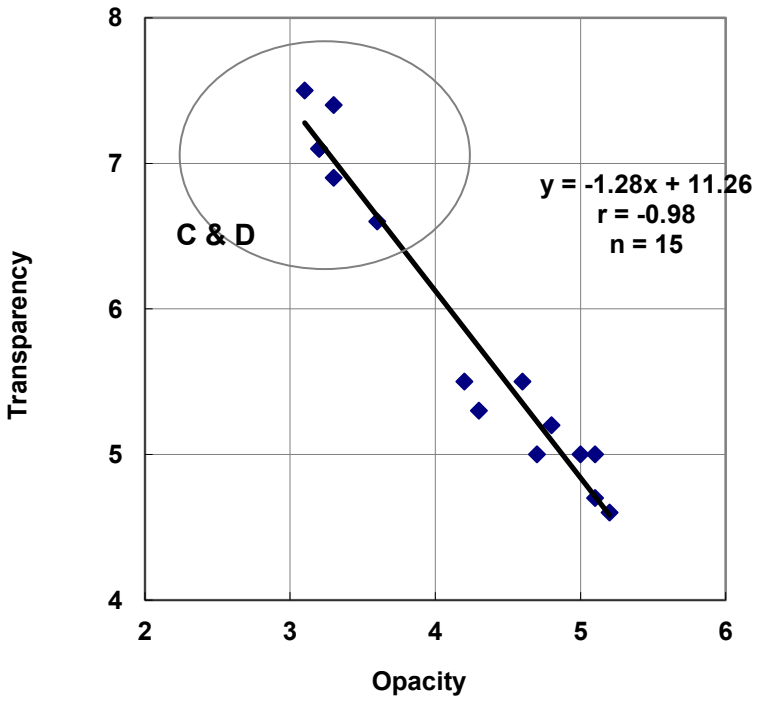

Figure 4.1.2.8 *The correlation between transparency and opacity of red wine samples. Red wine C and D were labelled in dotted circle.*

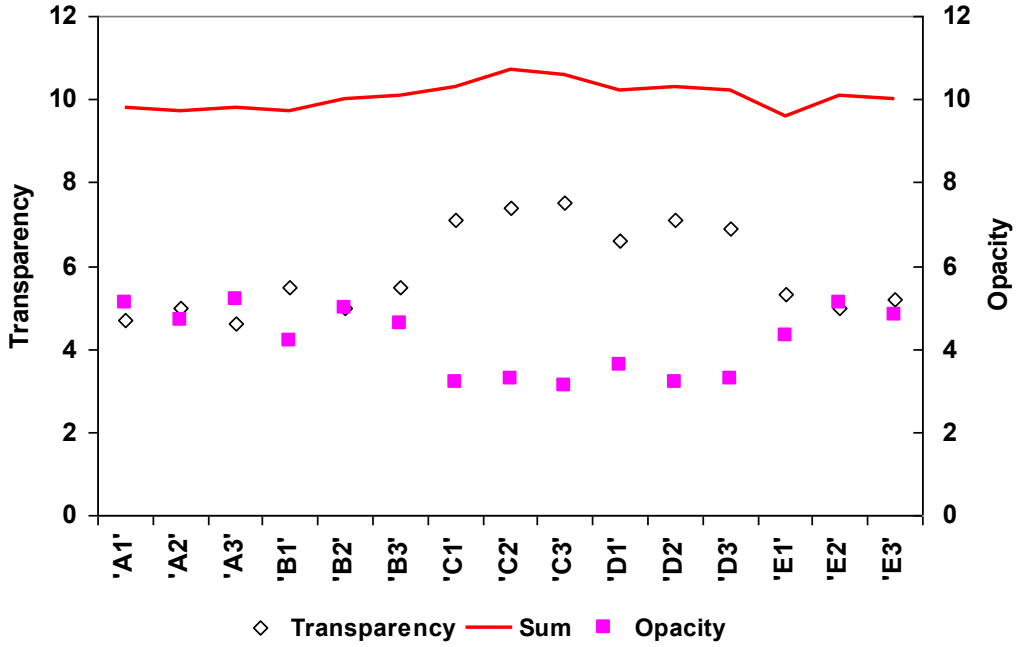

Figure 4.1.2.9 *The complementarity between transparency and opacity of red wine samples.*

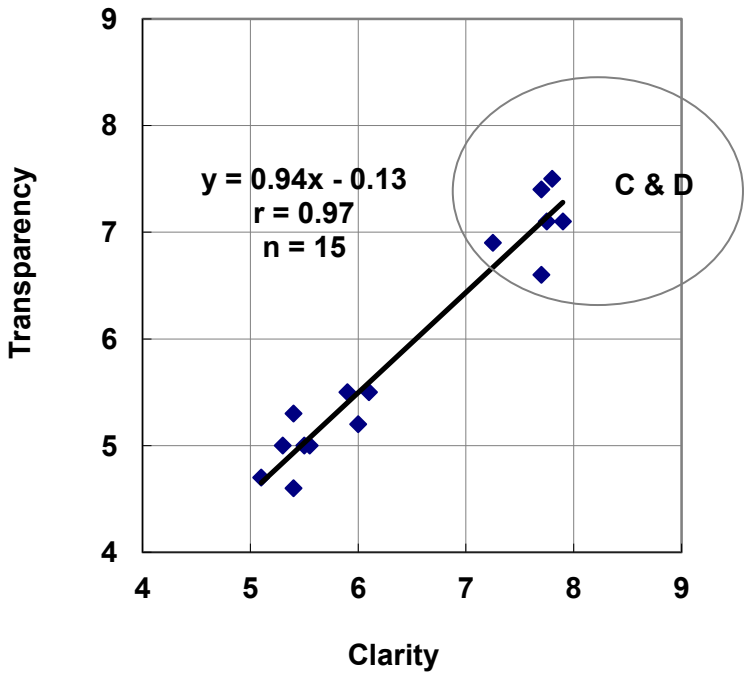

Figure 4.1.2.10 *The correlation between transparency and clarity of red wine samples. Red wine C and D were labelled in dotted circle.*

According to the data obtained from the observers, clarity appeared to be linearly related to transparency, i.e. the clarity increased when the transparency increased for each wine sample (Figure 4.1.2.10). The observers separated types C and D (in the circle) from the other three types on transparency and clarity attributes, as being shown in this figure.

From Figure 4.1.2.11, all the red wine samples were rated using the five-point scale between 2 to 3 units, which were described between clear and dull. But actually, the haze content of these three types (five batches) samples was different due to production processing. These visual characters were affected by the dark colour of red wine rather than the haze contents.

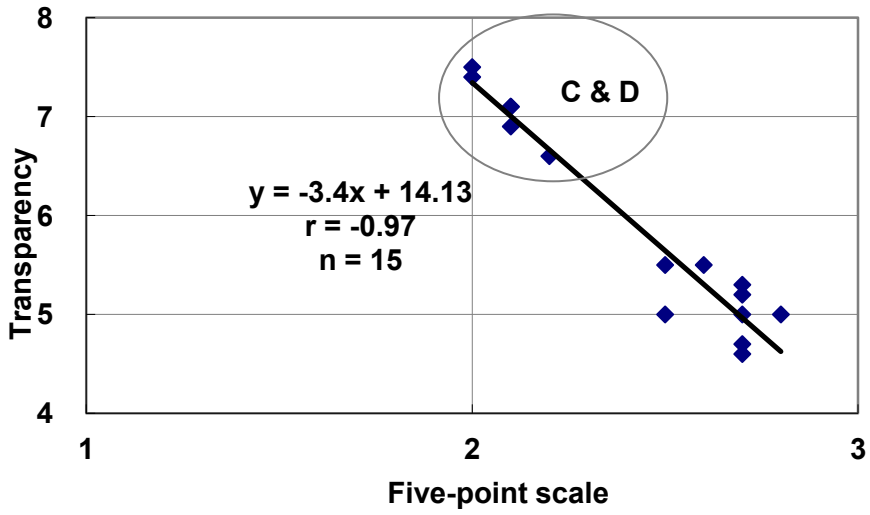

Figure 4.1.2.11 *The correlation between transparency and five-point scale of red wine samples. Red wine C and D were labelled in dotted circle.*

The calculated mean values of five-point scale of red wines presented in Figure 4.1.2.11 were accurate to 0.1. But this result is not sufficiently accurate to reflect the "five-point scale", which is discrete. Thus, the average result data of the five-point scale were rounded up (i.e. a sample with five-scale 3.2, it would be rounded up as 3.0; whereas if a sample with five-point scale 3.9, it would be rounded up as 4.0), and the correlation

between transparency and five-point scale of red wine samples was plotted as Figure 4.1.2.12. Red wines A and E, C and D are the same products sampling from different batches, see Section 3.1.2. From Figure 4.1.2.12, observers could generally separate the five batches red wines into groups on transparency correctly, and they scored the samples clear and dull. In summary, for transparency characteristics, panellists could distinguish different products but could not distinguish the effects of processing stages. From the view of the correlation between transparency and five-point scale, observers correctly correlated higher transparency (6.5 to 7.5 units) to "clear" and lower transparencies (4.5 to 5.5) to "dull".

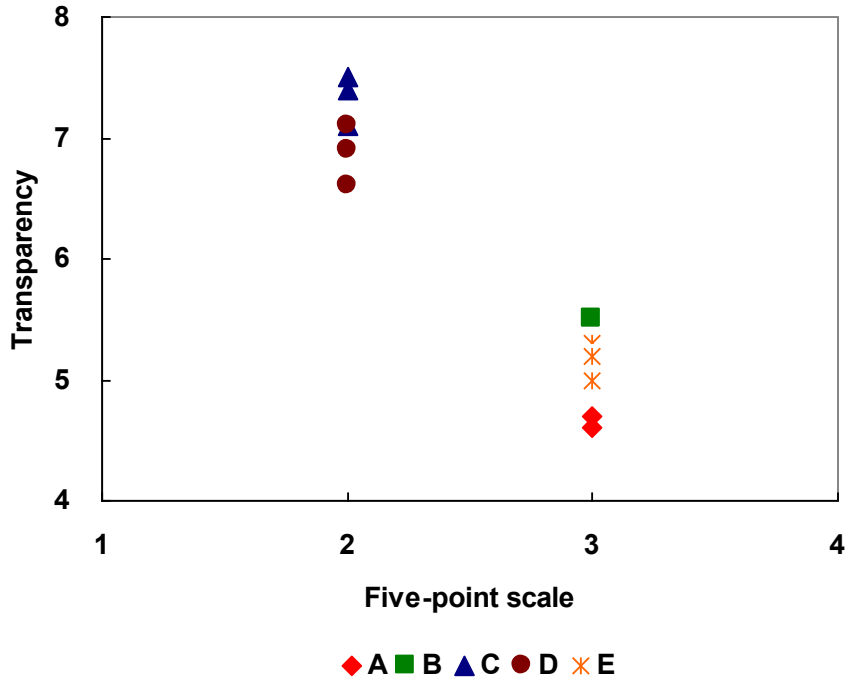

Figure 4.1.2.12 *Visual five-point scale and transparency of red wine samples.*

As can be seen (Figures 4.1.2.8-4.1.2.12), as with the beer samples, for the wine samples there were similar correlations between transparency and opacity, clarity and five-point scale. But for each characteristic, the panellists could not make distinguish between the different processing stages. The haze contents of the each batch wine (Figure 4.1.2.1) were different, but the panellists could not identify that visually.

There were two reasons for this: firstly, the difference of the haze content may be below threshold for human to detect. Secondly, the differences in levels of the haze were obscured by the low lightness (dark look) and similar hue values. No matter which reason, it is difficult or impossible for human to sense the haze content differences or even rank it following up the processing stages for the haze levels in these samples.

Table 4.1.2.2 shows the visual performance on the industry terms on translucency characteristics of red wine samples. The observers performed better on these translucency characteristics of red wine samples than beer samples (Table 4.1.1.5), because it is difficult for panellists to distinguish the translucency characteristics for these samples. The panellists scaled their results in relatively narrow ranges (Figures 4.1.2.8 to 4.1.2.12) on each translucency term, and thus, the variation was small. Especially on "five-point scale", the observers gave same description to the samples, so the observer performance on five-point scale showed a mere 10.8% variation.

Table 4.1.2.2 *Observer accuracy on industry terms on red wine samples:*

	Transparency	**Opacity**	**Clarity**	**Five-point scale**
CV%	12.6	15.6	10.8	10.8

Summary

- For the six commercial beer samples, their colour was considered to be stable over three days for the purposes of this study, with the indication of ΔE_{00} values are all less than 0.8 units. The haze values of the six beers tested were below 0.9 after they had been opened for three days; therefore, this was established the maximum time from sample preparation to experimental sensory assessment.

- The six commercial beers on colour appearance attributes were visually tested in

highball glasses and pint glasses, which varied significantly in their visual performance when they were presented in highball glasses and pint glasses.

- In this study, the observer performance was indicated by CV. It indicated that the lightness and hue results were more consistent, and the colourfulness results indicated the colourfulness was the most difficult attribute to scale. The CV measures for the industry terms on translucency scaled indicated that the opacity was the most difficult term to scale for observers, which had the higher CV value. It means the observers had more variability on using "opacity" than "transparency". "Opacity" is more difficult to control and thus there was bigger variation.

- Visual transparency is significantly negatively correlated to visual opacity and the five-point scale and significantly positively correlated to visual clarity. For five points scale, observers broadly assigned the correct descriptors to the more quantitative transparency attribute. However for samples with intermediate translucency, the observers could not distinguish/score properly. It means some confusion existed on these terms, or it is difficult to distinguish the samples in this area.

- Panellists could distinguish different red wine products but could not follow up the processing stages on colour appearance attributes and translucency characteristics.

4.2 Identification of Brewing Processing Control Points

Brewing is a complex process, which typically takes 1-4 weeks. Many variable factors of the process can affect the quality of final products. This study attempts to identify the

processing stages where changes of colour and translucency might provide additional information for process control during beer production. In this study they are referred to as critical control points (CCPs).

For these three brews (Section 3.2.1), the colour appearance attributes and the haze levels were analysed. The samples were taken following the consecutive processing stages in brewing as detailed previously (Section 3.2.1). The samples of mashing end, mash filtration/lautering, boiling, whirlpool end and cooling end were collected in the brewing day, whereas the samples of fermentation and conditioning were collected every twenty-four hours. Therefore, each brewing took approximate fourteen days.

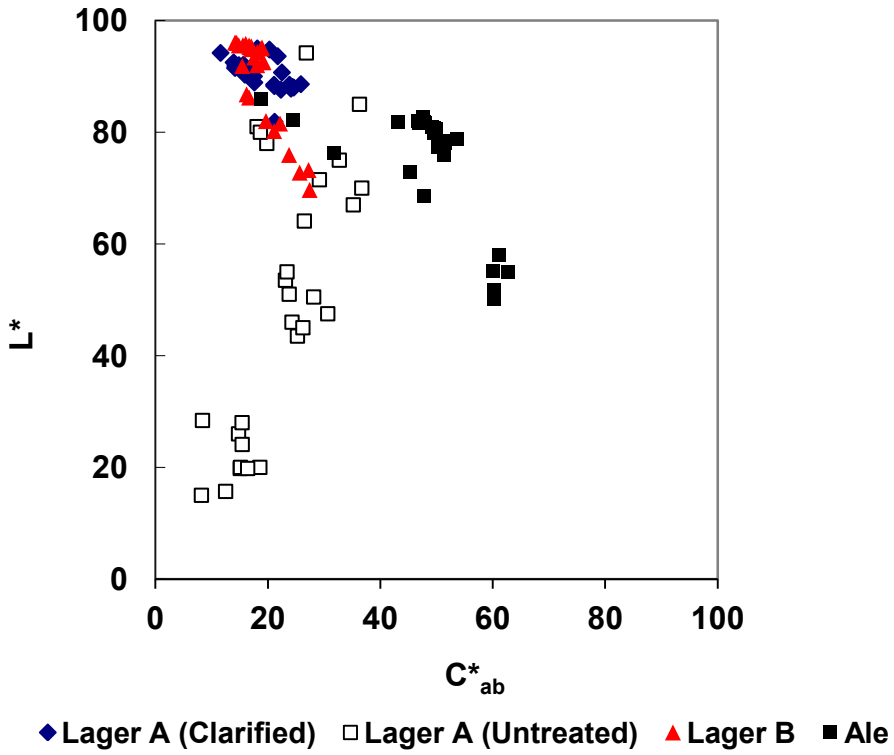

Figure 4.2.1 *Samples taken following the consecutive processing stages plotted in CIELAB $L^*C^*_{ab}$ colour plates.*

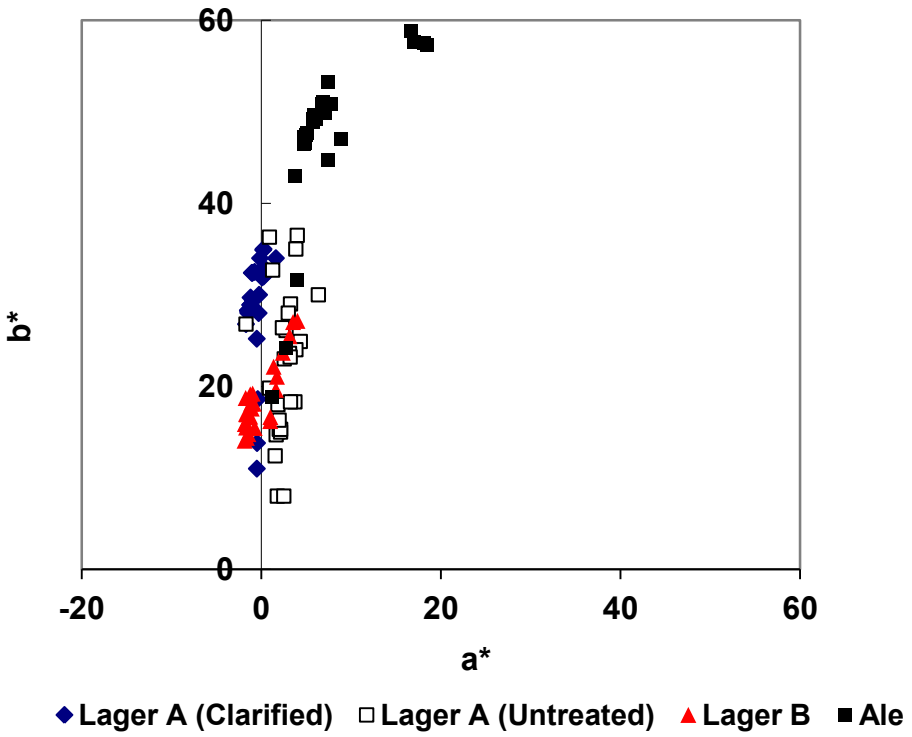

Figure 4.2.2 *Samples taken following the consecutive processing stages plotted in CIELAB a^*b^* colour plates.*

Figures 4.2.1 and 4.2.2 generally show the colour appearance results of all samples on $L^*C^*_{ab}$ and a^*b^*colour plates. In the following figures, the consecutive samples taken following processing stages are marked with different background, their CIELAB values were tested (e.g. Figures 4.2.1.1—4.2.1.4). Thus, the points by the first legend respond samples taken from the end of mashing (labeled as mashing end in the figures) stage. In all cases, filtration and centrifugation of the samples resulted in higher L^* values (i.e. the samples were lighter).

4.2.1 Lager Brewing A

4.2.1.1 L* a* b* Colour Analysis

As mentioned in Section 2.2, beer samples should be free from particles when their colour characteristics were evaluated by spectrophotometric analysis, otherwise light scattering will existed affect the accuracy of measurements. In this experiment, samples with/without particles were prepared (Section 3.2.2.1), and the results were compared.

CIELAB L^*, a^*, and b^* parameters were determined for each consecutive sample (obtained from processing line) from the corresponding tristimulus values. Figure 4.2.1.1 shows the L^* parameters of the samples collected during lager A production. We can see from this figure that, comparing to the filtered and centrifuged samples (clarified samples), the L^* of untreated samples ranged widely (10 to 90 units), whereas that of the clarified samples ranged between 80 and 96 units. This is presumably due to the random particles within the original samples, which have various light scattering and presence of absorbing properties.

From this figure, there was an elevation of L^* (lightness) caused by mash filtration—the difference between the sample collected in mashing end and the first sample in mash filtration was significant. During mash filtration, the wort became increasingly light, as being shown by the L^* increases in Figure 4.2.1.1, and there was an increase in L^* from 88.5 to 90.7 because of the onset of sparging, by which the wort, and therefore the colorants, are diluted (Sections 2.1 and 2.2.4). After the addition of hops, the lightness of wort decreased and there was further decrease after boiling. Then after whirlpool and cooling, the L* trended upwards until the final filtration.

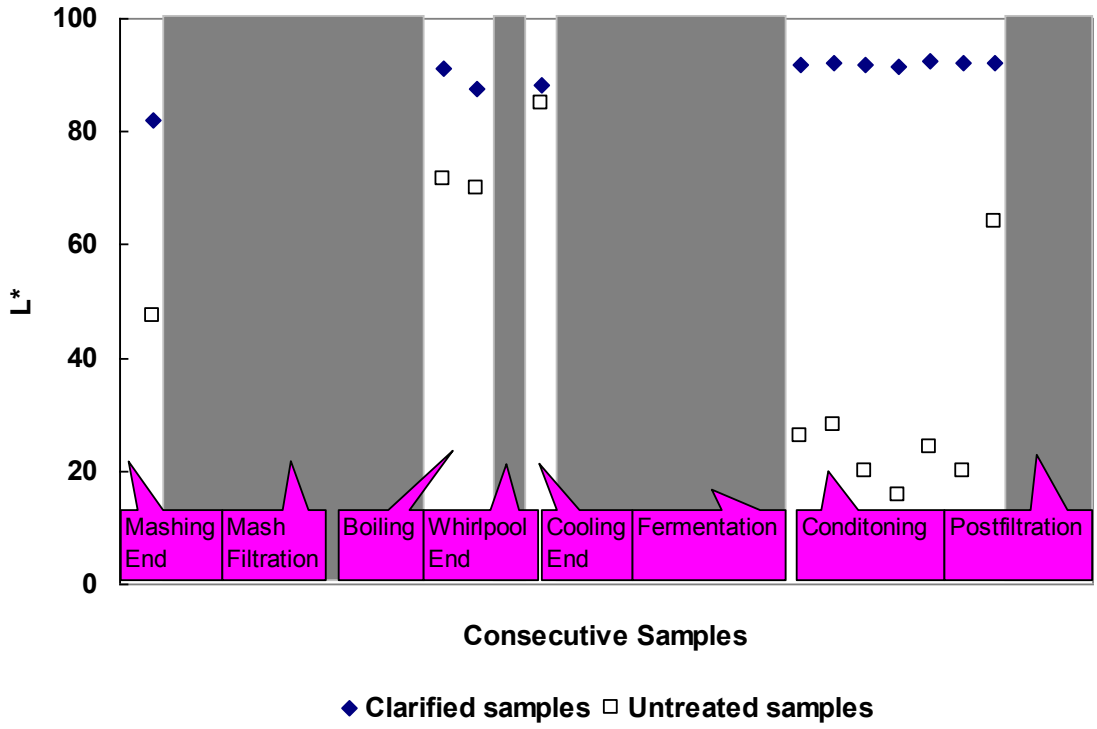

Figure 4.2.1.1 *CIELAB L^* parameter (lightness) of the clarified and untreated samples of lager A production.*

Figure 4.2.1.2 shows the a^* parameter (hue composition a value) of the samples collected during the lager A production. The corresponding a* values indicates a predominantly red ($a^* > 0$) or green ($a^* < 0$). Similarly as for the L^* results (Figure 4.2.1.1), the a^* results of the untreated samples covered a wider range (0 to 7) comparing with those of clarified samples (-2 to 2), due to the existing particles' scattering characteristics. We can see from this figure that the corresponding a* values of the filtered and centrifuged samples indicated a small green hue ($a^* < 0$), whereas those of untreated samples indicated a small red hue ($a^* > 0$). In particular in the conditioning stage, the untreated samples became increasingly red, whereas the filtered and centrifuged samples became increasingly green.

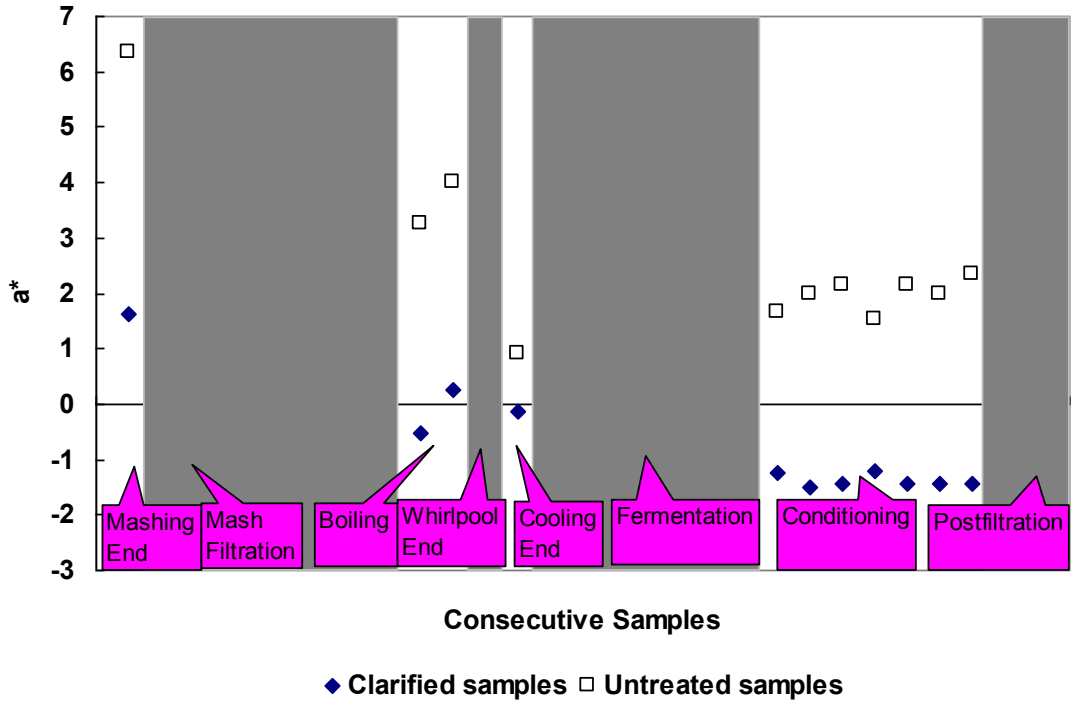

Figure 4.2.1.2 *CIELAB a* parameter of the clarified and untreated samples of lager A production.*

From the mashing end, the a* decreased until the wort had been boiled, especially after sparging onset (from the fourth sample in mash filtration), the a* values were near zero (neutral), and then decreased from 0.08 to -0.3. The likely reason for the increasing by boiling was the progression of the Maillard browning reaction (Nursten, 2005) and polyphenol oxidation (McMurrough et al., 1984), which contributes to yellow-red colour. After that stage, the a* value trended downwards towards a green colour.

Figure 4.2.1.3 shows the b* parameter (hue composition b value) of the samples collected in the lager A production. We can see that, the corresponding b* values of the samples indicated a yellow hue ($b^* > 0$).

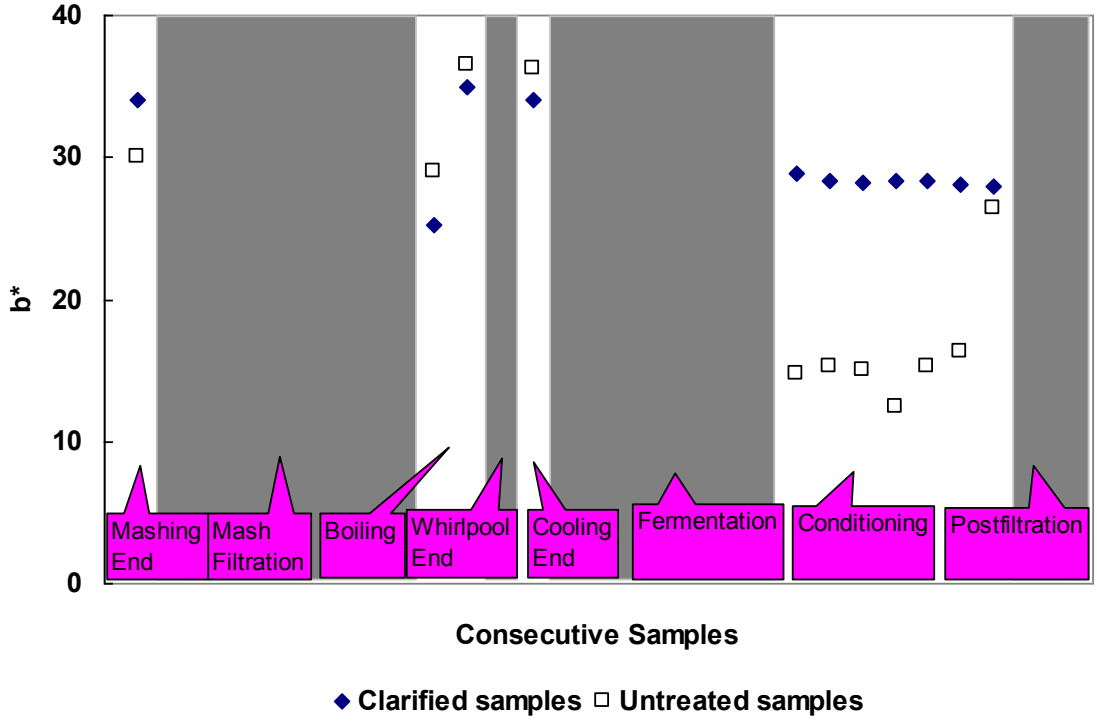

Figure 4.2.1.3 *CIE b^* parameter of the clarified and untreated samples of lager A production.*

From the onset of mash filtration, the b^* value decreased towards to 0 (neutral). The reason for causing this is sparging during the mash filtration stage, which diluted the liquor (Sections 2.1 and 2.2.4), thus the colorants were diluted to lower concentration which was downwards towards to neutral. During boiling, the colour was increasingly in yellow due to Maillard browning reactions (Sections 2.1 and 2.2.4) and polyphenol oxidation (Section 2.1), which contribute to yellow-red colour. After that stage, the b^* value trended downwards for the filtered and centrifuged samples.

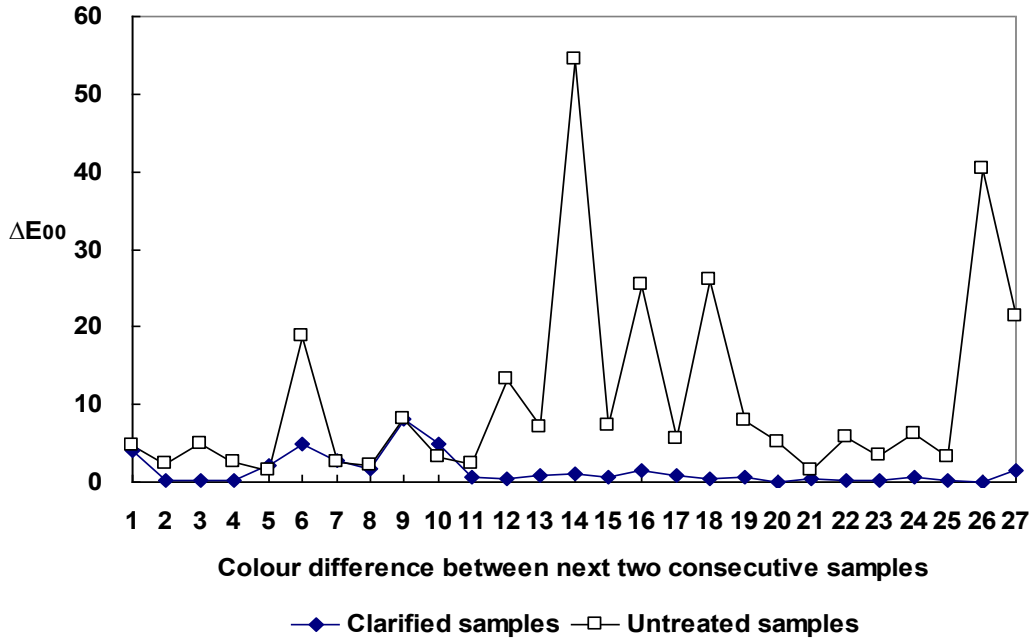

Figure 4.2.1.4(a) *Change in colour ΔE_{00} between adjacent sampling points during the production of lager A.*

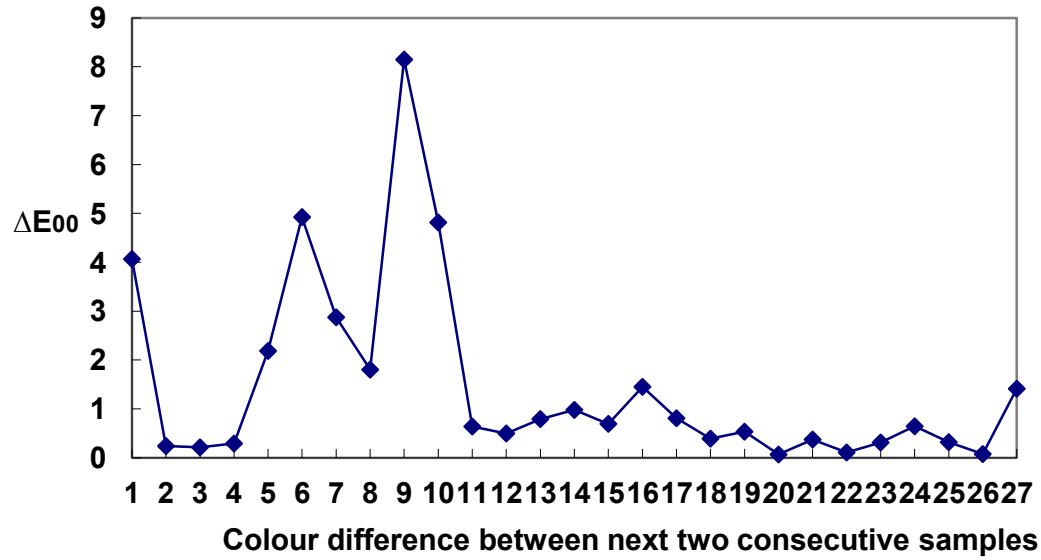

Figure 4.2.1.4(b) *Change in colour ΔE_{00} between consecutive sampling points during the production of lager A (clarified samples illustrated in Figure 4.2.1.6(a).*

(The colour difference between the samples collected by: 1: mashing and the first mash filtration sample; 2-8: mash filtration; 9: last one of mash filtration and hopped wort; 10: boil start and boil end; 11: boil end and whirlpool end; 12: whirlpool end and cooling end; 13: cooling end and fermentation start (yeast was added); 14-20: fermentation; 21: last one of fermentation and first one of conditioning; 22-26: conditioning; 27: last one of conditioning and final filtration.)

Figure 4.2.1.4(a) shows the differences in colour between the next two consecutive samples of lager A for filtered and centrifuged (clarified) and untreated samples. By comparing these curves, the colour change was more apparent for the untreated samples (ranging from 0 to 55), whereas for the filtered and centrifuged (clarified) samples, ranged from 0 to 9. There were two reasons to produce these two curves rather than one: Firstly, the particles contained in the samples affected colour appearance attributes significantly, and therefore, samples contained or not contained particle appear different colour (and also ΔE_{00}). Another possibility was the colorants in the samples were separated during clarification.

In Figure 4.2.1.4(b), there were several peaks and points of ΔE_{00} higher than 1.0, which could be distinguishable visually. These higher values corroborated the stages in Figures 4.2.1.1-4.2.1.3 at which the colour changed obviously (i.e. mash filtration, boiling, final filtration).

4.2.1.2 Translucency Analysis

The translucency characteristics of the samples were assessed on the turbidity of the samples by turbidimeter. The untreated samples were used for this investigation. Figure 4.2.1.5 shows the turbidity of the lager A samples from the mash filtration stage to postfiltration. During the mash filtration stage, the turbidity decreased, as would be expected, and after boiling, the turbidity increased, presumably due to the hops added, and some protein and polyphenol coagulation occurring. After adding yeast, the turbidity increased immediately, but there was an obvious change when the samples had been fermented for twenty-four hours (the second point in fermentation), presumably because of yeast growth. During fermentation and conditioning, turbidity decreased due to natural sedimentation of polypeptides and polyphenol complexes which are then

separated. Filtration of beer involves the removal of yeast and the sedimented protein and polyphenol and other haze material, which gives beer its brilliance. After filtration, the turbidity decreased to < 0.8 EBC.

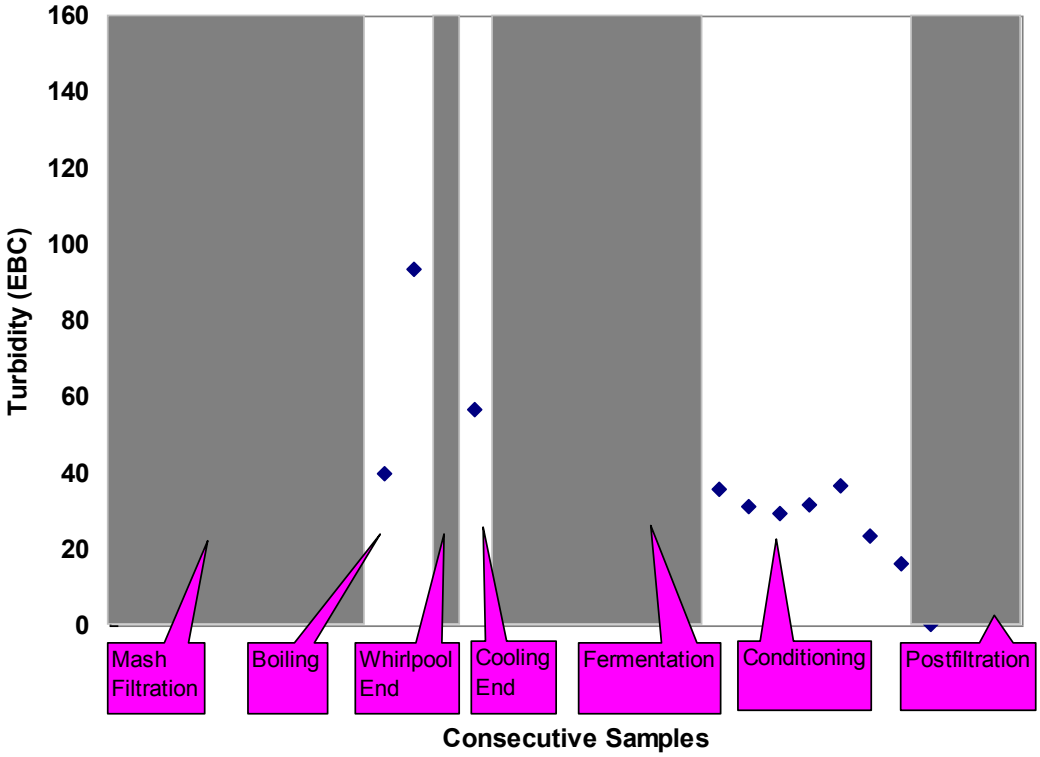

Figure 4.2.1.5 *The turbidity of samples for the lager A production.*

4.2.2 Lager Brewing B

4.2.2.1 L* a* b* Colour Analysis

Analogous to the investigation made for lager A, CIELAB L^*, a^*, and b^* parameters were determined for each consecutive sample (obtained from processing line) from the corresponding tristimulus values for lager B. Only clarified (filtered and centrifuged) samples were used for test, because the particles existed in the untreated samples scattered light and affected colour appearance attributes significantly according to the results of Lager Brewing A (Section 4.2.1.1).

Figure 4.2.2.1 shows the detail of L^* parameter of samples of lager B. We can see that the L^* parameter increased throughout the process.

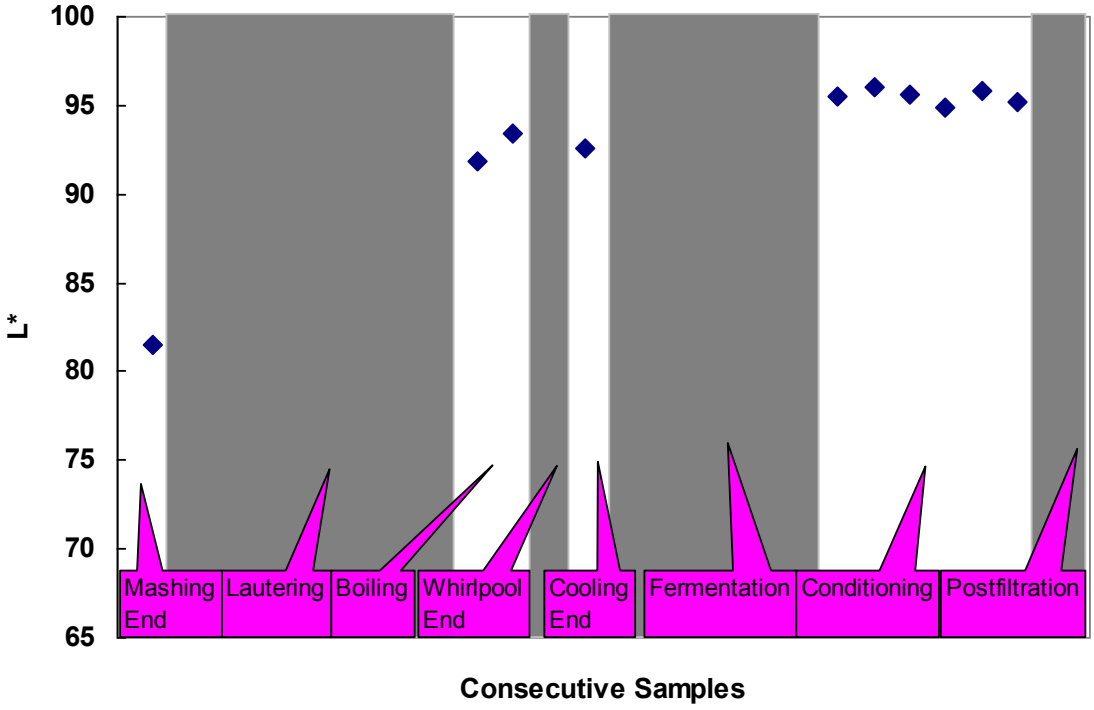

Figure 4.2.2.1 *CIELAB L^* parameter of the samples of lager B production.*

The major differences of processing operation between lagers A and B were mash clarification; mash filtration was used in lager A brewing whereas lautering in lager B brewing. Similar to lager A production, there was a clear increase in L^* during mash clarification. During lautering, the wort became increasing lighter, as indicated by increasing in L^* from 69 to 94 (Figure 4.2.2.1). After hops addition, the L^* increased slightly in the following stages. During fermentation and conditioning stages, presumably some colorants were adsorbed on the surface of the yeast and were removed with the settling yeasts (Section 2.1). Therefore, the beers became lighter until the postfiltration stage.

Figure 4.2.2.2 shows the a^* parameter of the samples collected in the lager B production. We can see from the figure that, the corresponding a^* values samples indicated the

samples covered a small red hue ($a^* > 0$) to a small green hue ($a^* < 0$). Unsurprisingly the final product appeared slightly green.

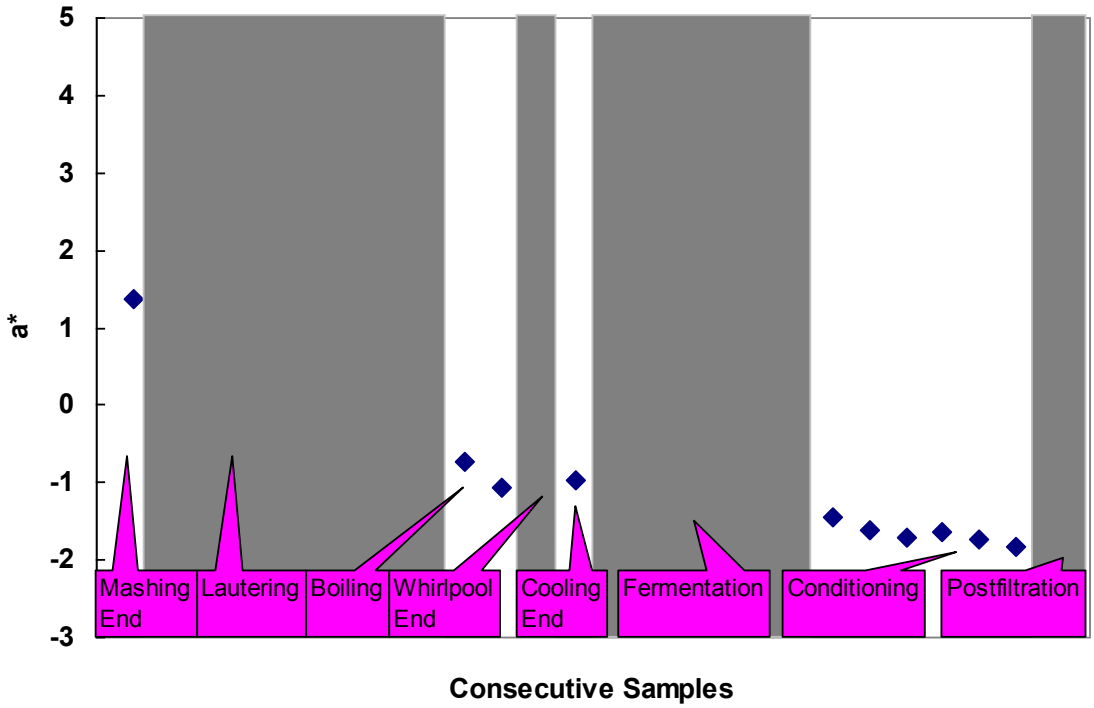

Figure 4.2.2.2 *CIELAB a^* parameter of the samples of lager B production.*

Through the whole process, the a^* varied in a range between -1.74 to 4.03, with the major variation occurring during lautering, in which there was a steep change towards neutral colour ($a^* = 0$, Section 2.2.1), because sparge liquor diluted the wort, the colorants were diluted, too, (Section 2.1). After boiling stage until postfiltration, the hue component a* values of wort/beer varied over a narrower range, of about 1.5 units.

Figure 4.2.2.3 shows the b^* parameter of the samples collected in the lager B production. We can see that the corresponding b^* values of the samples indicated a yellow hue ($b^* > 0$) for all the samples.

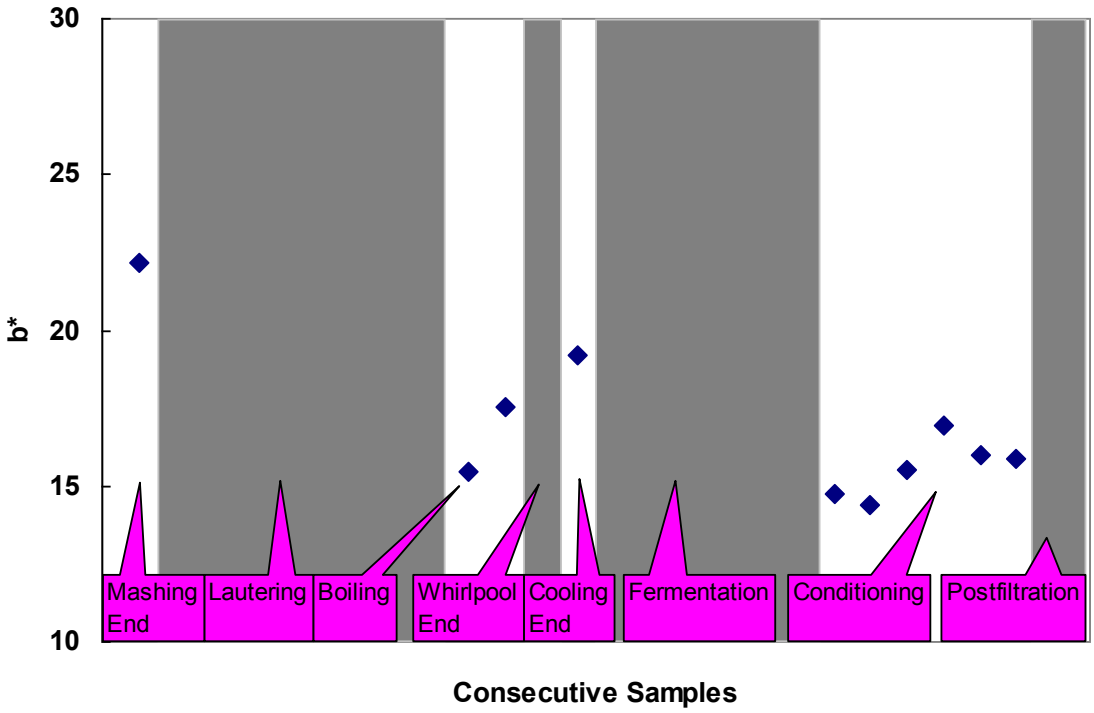

Figure 4.2.2.3 *CIELAB b* parameter of the samples of lager B production.*

The value of b* decreased towards to 0 (neutral colour) during lautering due to sparging. During boiling and whirlpool separation, the wort liquid becomes yellower, probably contributed by Maillard reactions and polyphenol oxidation

Figure 4.2.2.4 shows the differences in colour between the next two consecutive samples of lager B.

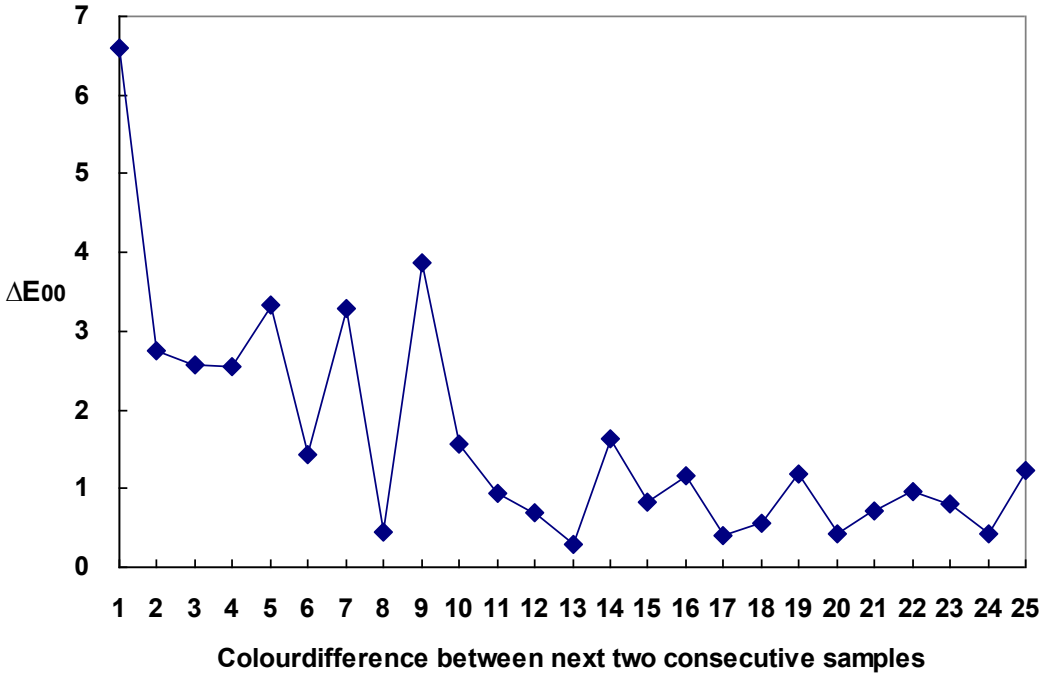

Figure 4.2.2.4 *Change in colour ΔE_{00} between adjacent sampling points during the production of lager B. Samples were centrifuged and filtered.*

(The colour difference between the samples collected by: 1: mashing and the first lautering sample; 2-8: lautering; 9: last lautering sample and boil start; 10: boil start and boil end; 11: boil end and whirlpool end; 12: whirlpool end and cooling end; 13: cooling end and fermentation start (yeast was added); 14-18: fermentation;19: last one of fermentation and first one of conditioning; 20-24: conditioning; 25: last one of conditioning and final filtration.)

The colour change between adjacent samples ranged from 0 to 6.6. From this figure, most colour changes occurred at the stages of lautering, due to sparging, in which the colorants were diluted. Similar to lager A production, there was a large difference between the samples collected before (the difference between last lautering sample and boil start sample, labeled as 9) and after boiling (the difference between boil start and boil end sample, labeled as 10). There were relatively higher colour changes during fermentation and conditioning stages, due to complexity and variability of the reactions and the removed colorants adsorbed on the yeasts and particles (Sections 2.1 and 2.2.4).

4.2.2.2 Translucency Analysis

The translucency characteristics were assessed on the turbidity of the samples by turbidimeter, as for lager A. For the turbidity measurement, the samples collected from mashing were not tested, due to the presence of grist particles, which is meaningless for either transparency or haze attributes. Thus testing began with the lautering samples.

Figure 4.2.2.5 shows the turbidity of the lager B samples from the lautering stage to postfiltration.

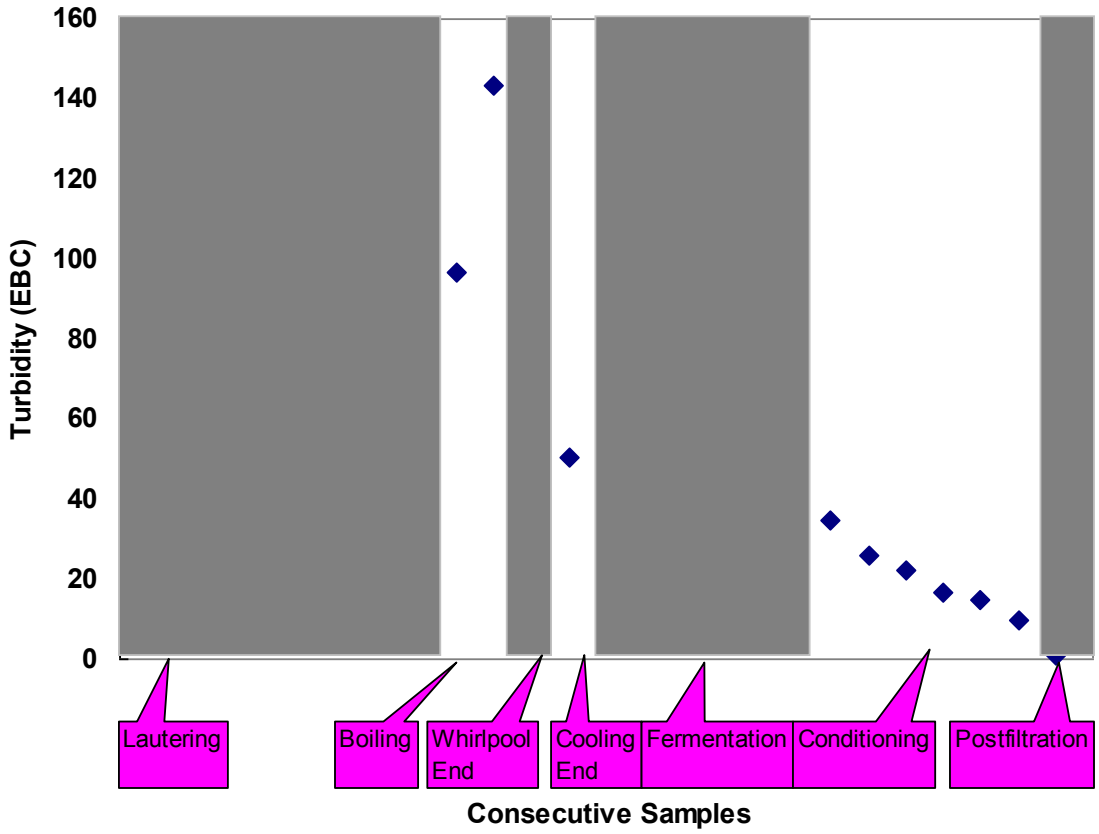

Figure 4.2.2.5 *The turbidity (EBC) of samples for the lager B production.*

The trends of this curve were similar to those of lager A production (Figure 4.2.1.5) at each processing stage (under different background in these figures).During the lautering stage, the turbidity decreased, as would be expected. After boiling, the turbidity

increased, due to the hops added and some protein and polyphenol coagulation. Some of the haze was separated by whirlpool separation, so that the turbidity decreased. After yeast addition, the turbidity increased after twenty-four hours (the second point in fermentation stage), presumably due to the yeast growth. During fermentation and conditioning, turbidity generally decreased. After filtration, turbidity decreased to < 1.0 EBC.

4.2.3 Ale Brewing

4.2.3.1 CIELAB L^* a^* b^* Colour analysis

Figure 4.2.3.1 shows the detail of L^* parameter of samples of ale. We can see that, the trend of the L^* parameter during the whole process was upwards, indicating that the consecutive samples taken from this pilot brewing were lighter.

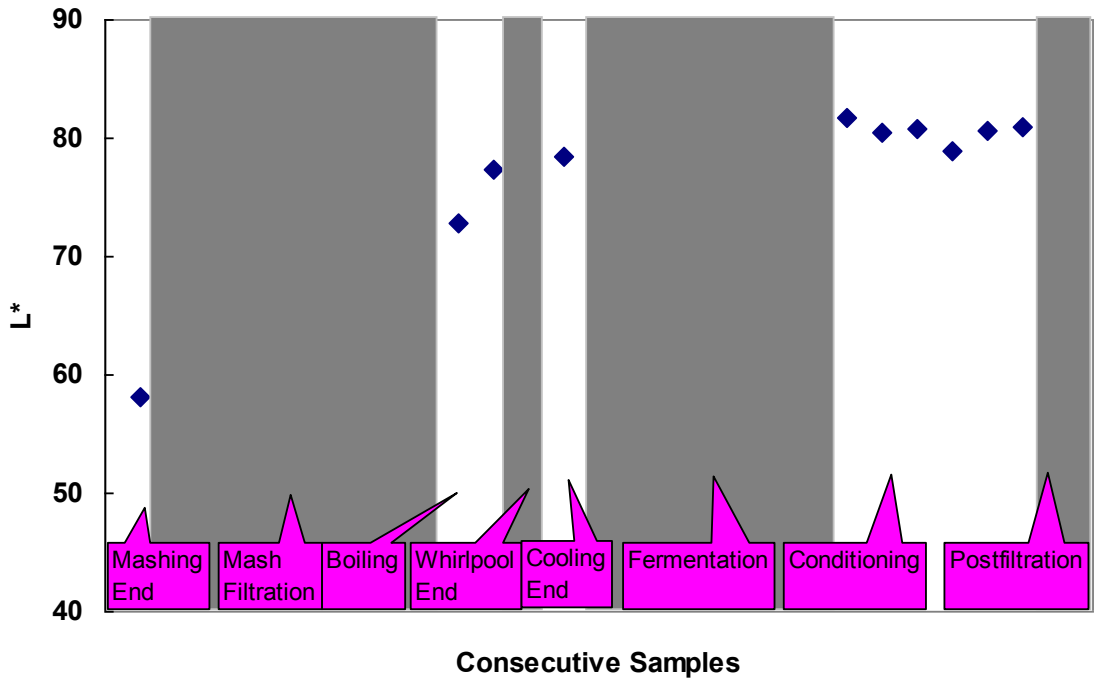

Figure 4.2.3.1 *CIELAB L^* parameter of the samples of ale production.*

During mash filtration, the wort became lighter, as can be seen from the increasing L^* values in Figure 4.2.3.1, particularly after sparging onset (from the fourth sample in mash filtration), the lightness increased from 55 to 86. After adding hops, the extracted hop components caused lightness decreased. Subsequently, the L^* value increased in relative narrower range (compared with that in mash filtration) until the postfiltration.

Figures 4.2.3.2 and 4.3.3.3 show the a^* and b^* parameters of the samples collected in the ale production. The corresponding values indicated a predominantly yellow hue ($b^* > 0$), with a red contribution ($a^* > 0$). From these two figures, both a^* and b^* decreased steeply after sparging onset (from the fourth sample in mash filtration stage). The wort increased in red and yellow hue by boiling, indicated by the increased a^* and b^* values in Figures 4.2.3.2 and 4.2.3.3. From the whirlpool separation stage, the values a^* and b^* varied over a relatively low range (comparing with the variation over the whole process) until the final products were obtained, as for the two lager productions.

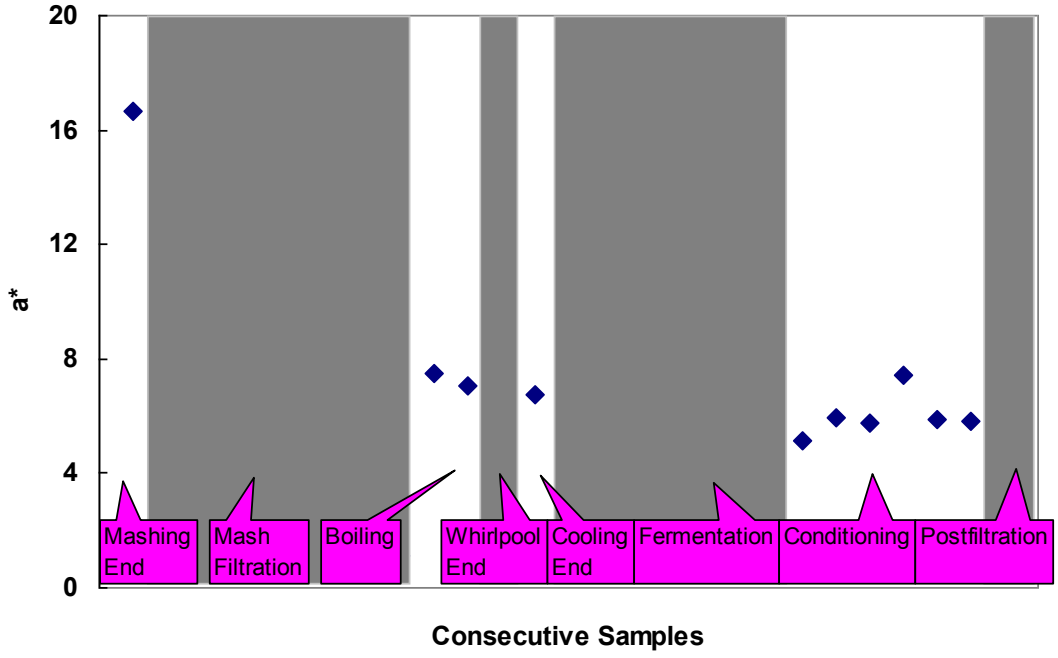

Figure 4.2.3.2 *CIELAB a^* parameter of the samples of ale production.*

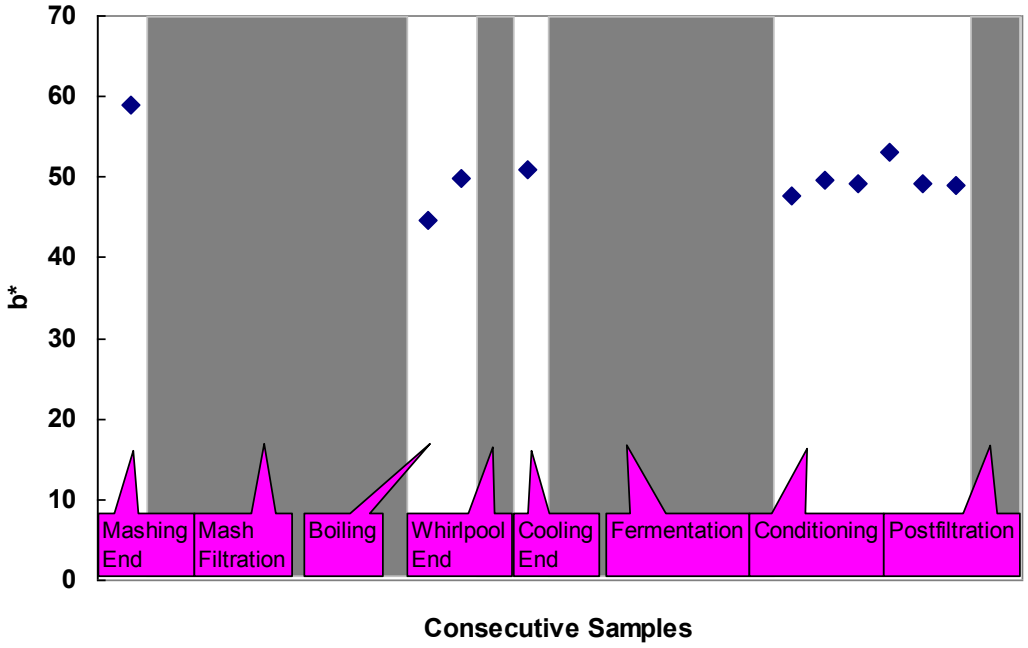

Figure 4.2.3.3 *CIELAB b^* parameter of the samples of ale production.*

Figures 4.2.3.4 shows the differences in colour between the next two consecutive samples of ale. The colour change ranged from 0 to 14.4. From this figure, most colour change occurred at the stage of mash filtration, and the biggest changes occurred by boiling (labeld as 9 and 10 in figure), as obtained for lager A and B. For mash filtration, the greatest effect was caused by sparging, whereas for the boil stage, the major factor may be that components are extracted from hops.

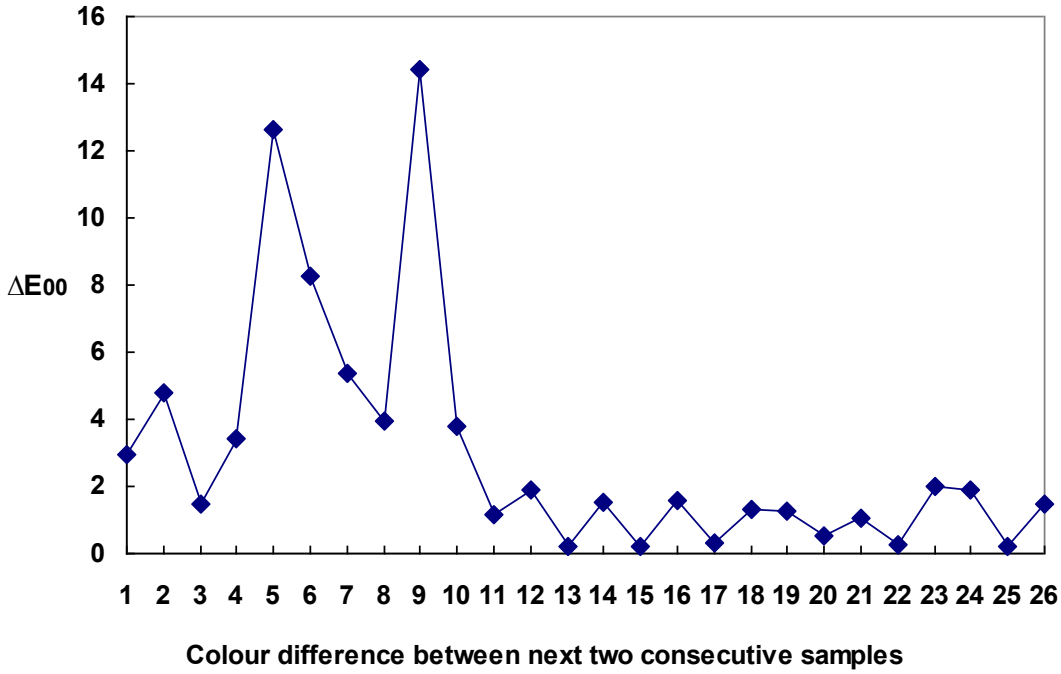

Figure 4.2.3.4 *Change in colour ΔE_{00} between adjacent sampling points during the ale production. Samples were centrifuged and filtered.*

(The colour difference between the samples collected by: 1: mashing and the first mash filtration sample; 2-8: mash filtration; 9: last mash filtration sample and boil start; 10: boil start and boil end; 11: boil end and whirlpool end; 12: whirlpool end and cooling end; 13: cooling end and fermentation start (yeast was added); 14-19: fermentation; 20: last one of fermentation and first one of conditioning; 21-25: conditioning; 26: last one of conditioning and final filtration).

As mentioned earlier (Sections 4.2.1.1 and 4.2.2.1, CIEDE2000 (ΔE_{00}) has been used here to express the colour difference between two adjacent samples collected in brewing, as Figures 4.2.1.4, 4.2.2.4 and 4.2.3.4, thus we can conclude which processing stage affects colour change during processing. According to these figures, obtained from different brews, we observe:

- The peak ΔE_{00} values appear between the end of mash clarification (lautering or mash filtration) and boil start. During wort production, gravity is an important parameter governing colour change. Especially in sparging, the components

contributing colour in mash liquor are diluted, thus the colour changes significantly.

- Another clear colour difference (ΔE_{00}) occurs during boiling, between the start and the end. The main contributions to beer colour are made by melanoidins produced (Sections 2.1 and 2.2.4) and oxidized polyphenols ((Section 2.1) during boiling.
- Oxidized polyphenols are other colour contributors of beer, especially in the presence of trace metals (Section 2.2.4). But there is not an obvious colour change due to oxygen introducing from the ΔE values, compared with the other processing stages.

4.2.3.2 Translucency Analysis

The transluency characteristics were assessed on the turbidity of the samples by turbidimeter, as for lager A and B.

Figure 4.2.3.5 shows the turbidity of the ale samples from the mash filtration stage to final filtration. During mash filtration, the turbidity decreased, and after boiling, the turbidity increased, due to the hops added and some ongoing protein and polyphenol coagulation. Some of the haze was separated by whirlpool separation, resulting in a corresponding decrease in turbidity. After adding yeast, the turbidity increased after twenty-four hours, similar to lagers A and B. During fermentation and conditioning, turbidity generally decreased. After filtration, the turbidity decreased to < 0.8 EBC.

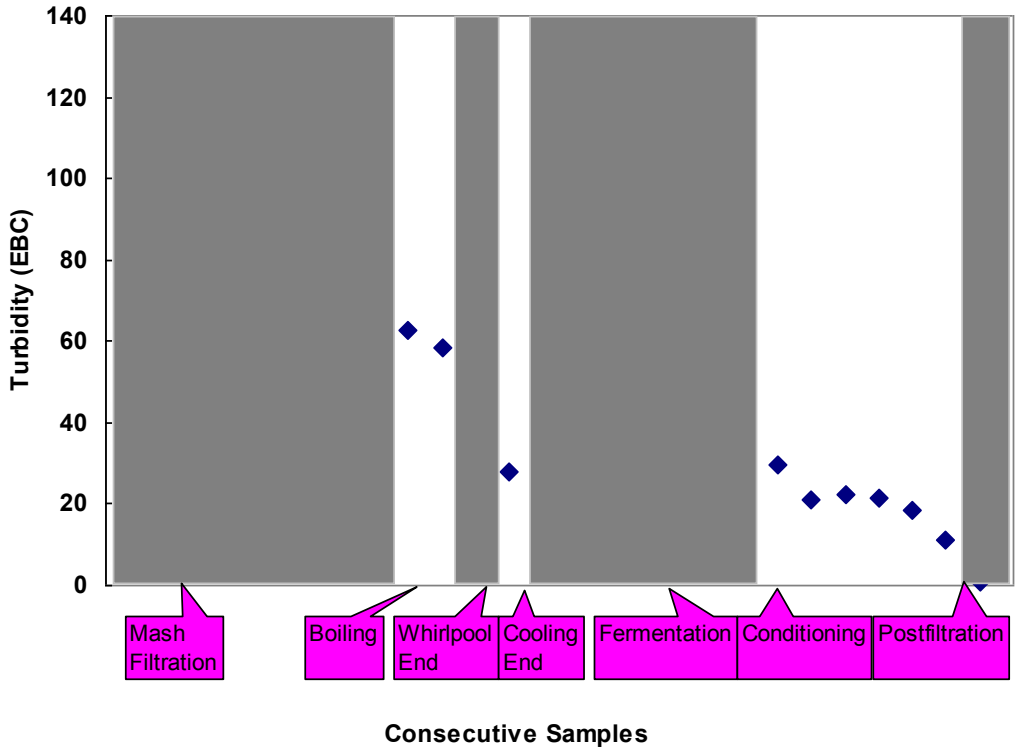

Figure 4.2.3.5 *The turbidity (EBC) of samples for the ale production.*

Figures 4.2.1.5, 4.2.2.5 and 4.2.3.5 show the translucency characteristics of beer process samples from the mash clarification stage to final filtration. In contrast to the colour analyses, the turbidity results are rather variable. However we still can conclude some adjacent stages where the haze content changes significantly:

- Mash clarification end
- Boiling start and end
- The whole fermentation periods
- Prefiltration of the final beer products
- Postfiltration of the final beer products.

Summary

- Beer colour and haze content are affected by many factors during brewing processing, e.g. the chemical reactions of ingredients and residual particles in the liquid. Clarified and untreated wort/beer samples show different trends on colour appearance attributes and translucency characteristics. Therefore, liquid samples were clarified for the following research in this study.

- The magnitudes of colour changes between any two of the consecutive samples suggest the control points for colour monitoring of brewing. The peak ΔE_{00} values appear between the end of mash clarification (lautering or mash filtration) and boiling start. During wort production, gravity (i.e. density) is an important parameter governing colour change. Especially in sparging, the components contributing colour in mash liquor are diluted, thus the colour changes significantly. Another clear colour difference (ΔE_{00}) occurs during boiling, between the start and the end. The main contributions to beer colour are made by melanoidins produced and oxidized polyphenols during boiling. Oxidized polyphenols are other colour contributors of beer, especially in the presence of trace metals. But there is not obvious colour change due to oxygen introducing from the ΔE values, comparing with the other processing stages.

- Turbidity results are rather variable and complex between batch to batch in brewing processing, because of reactions between ingredients, processing operations and the settled particles.

- Considering the magnitudes of colour and turbidity changes, five Critical Control Points (CCPs) of brewing process monitoring on colour and translucency were defined as: 1: Mash clarification (mash filtration or lautering) end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

4.3 Instrument Testing in Beer Production and Products

4.3.1 System Repeatability Testing

4.3.1.1 Fixed Aperture Lens *vs* Zoom Lens Testing

Pilot brewed ale was used as sample to test the repeatability of the digital imaging system by both fixed aperture lens and zoom lens. Colour appearance attributes (lightness, colourfulness and hue) and translucency characteristics (transparency and opacity) were analysed.

Figures 4.3.1.1, 4.3.1.2 and 4.3.1.3 show the colour appearance attributes (lightness, colourfulness and hue) and translucency characteristics (transparency and opacity) results of the pilot brewed ale by the digital imaging system incorporating a fixed aperture lens and its consistency over four hours was tested.

Over the four hour test period, the lightness, colourfulness and hue ranged between 24.7 and 26.0 (lightness), 74.6 and 76.0 (colourfulness), 48 and 50 (hue), respectively. This is not wide comparing with the full range of lightness (0, 100), colourfulness (0, infinity) and hue (0, 400). From the software output, the hue is expressed as a composition percentage for the beer samples, which is then converted into the hue expression in CIE colour space. For example, the hue of one beer sample obtained from the software is 83Y17R, indicating that the hue of this sample is composed of 83% yellow and 17% red, which is then converted in hue expressed in CIE colour space, as 83. The hue composition obtained from the software is an integer, thus, the hue results expressed in CIE colour space are all integers (Figure 4.3.1.2).

Colour difference CIELAB ΔE^*_{ab} and CIEDE2000 (ΔE_{00}) can be used to distinguish between beers/worts having small colour difference. For most beers, the threshold values for ΔE^*_{ab} range from 0.7 to 4.0 (Section 2.2.1). From Figures 4.3.1.1 and 4.3.1.2,

each test was compared with the first test, and CIELAB ΔE^*_{ab} was calculated ranging from 0.39 to 2.37 and CIEDE2000 ΔE_{00} was ranging 0.24 to 1.3 by the fixed aperture lens, respectively (Table 4.3.1.1.1). Those indicated that it is difficult for most humans to observe colour changes visually during the four hours testing, no matter which calculation was used.

Table 4.3.1.1.1 *Colour difference (ΔE^*_{ab} and ΔE_{00}) of pilot brewed ale measured by digital imaging system (fixed aperture lens) in four hours. There were 16 comparison between 17samples.*

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
ΔE^*_{ab}	0.71	1.06	.1.02	1.66	2.13	2.27	1.73	1.70	1.67	2.08	2.09	2.29	2.07	2.37	2.30	2.06
ΔE_{00}	0.24	0.41	0.58	0.63	0.84	1.00	0.91	0.96	0.97	0.95	0.95	1.12	1.1	1.30	1.15	1.08

The transparency and opacity ranged between 1.3 and 1.9, and 7.9 and 8.6, respectively. The transparency was clearly inversely correlated with opacity (Figure 4.3.1.3). There were 17 samples, some of which presented the same translucency results (e.g. four samples have transparency/opacity with 1.7/8.1), thus, only eight points could be seen on Figure 4.3.1.3.

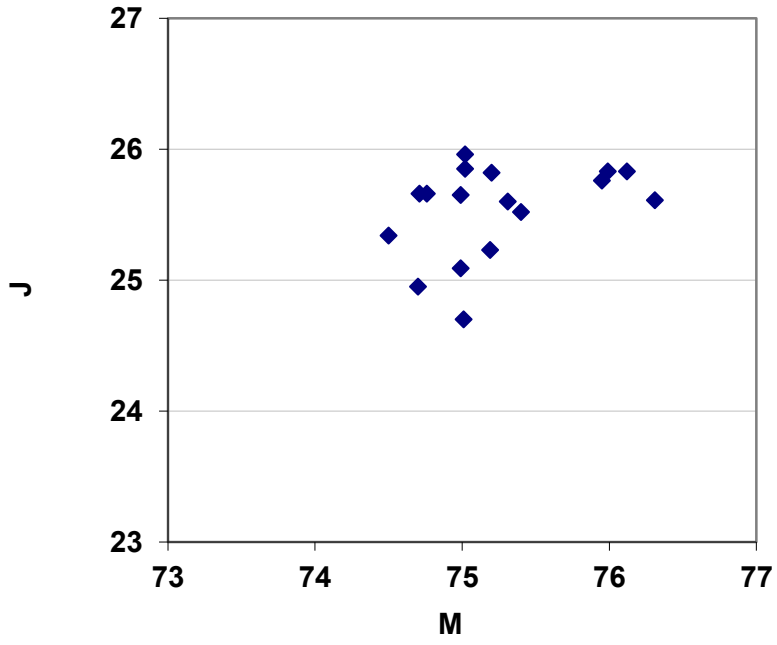

Figure 4.3.1.1 *The digital imaging system (fixed aperture lens) repeatibility test on lightness (J) and colourfulness (M). The pilot brewed ale was set as the test sample.*

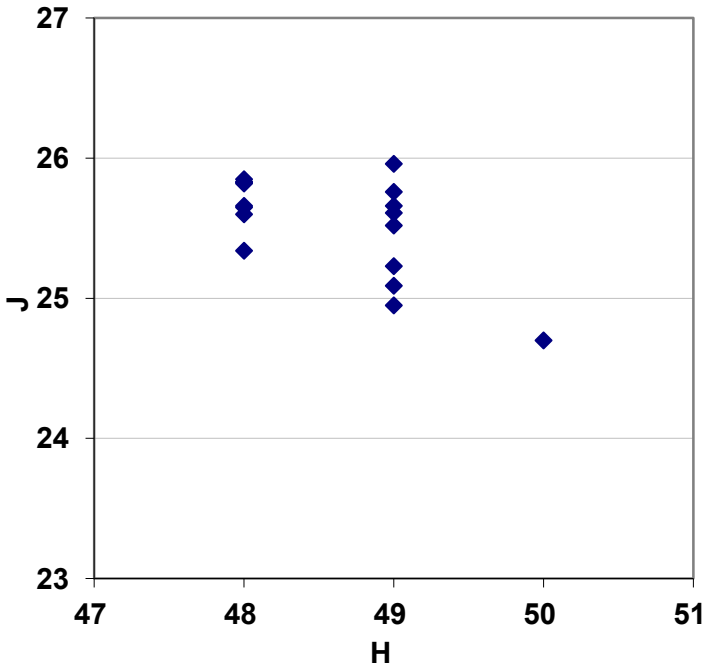

Figure 4.3.1.2 *The digital imaging system (fixed aperture lens) repeatability test on lightness (J) and hue (H) in integers. The pilot brewed ale was set as the test sample.*

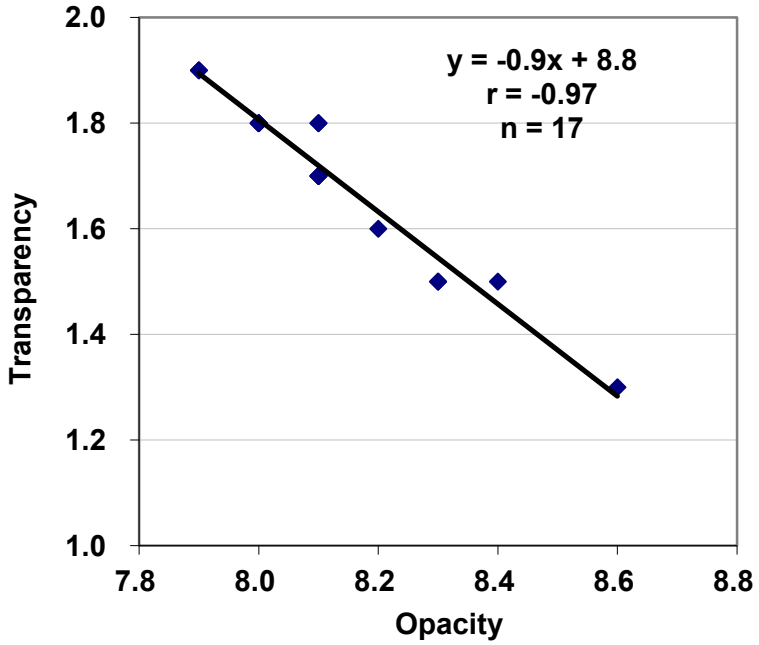

Figure 4.3.1.3 *The digital imaging system (fixed aperture lens) consistency test on transparency and opacity. The pilot brewed ale was set as the test sample.*

The same pilot brewed ale was also used as sample to test the repeatablity of the digital

imaging system assessed using a zoom lens. Colour appearance attributes (lightness, colourfulness and hue) and translucency characteristics (transparency and opacity) were analysed. Figures 4.3.1.4, 4.3.1.5 and 4.3.1.6 show the colour appearance attributes (lightness, colourfulness and hue) and translucency characteristics (transparency and opacity) results of the pilot brewed ale by the digital imaging system consisting zoom lens on repeatability in four hours.

From these figures, over the four hours, the lightness, colourfulness and hue ranged between 27.2 and 27.9 (lightness), 70.0 and 72.0 (colourfulness), 44 and 46 (hue), respectively. It is not wide compared with the full range of these attributes. From Figures 4.3.1.4 and 4.3.1.5, CIELAB ΔE^*_{ab} was calculated (the same calculation as those for fixed aperture lens Table 4.3.1.1.1), which ranged from 0.15 to 1.01 by the zoom lens, and ΔE_{00} ranged from 0.12 to 0.60. In these colour change range, human is almost impossible to distinguish colour difference for the samples visually (Section 2.2.1).

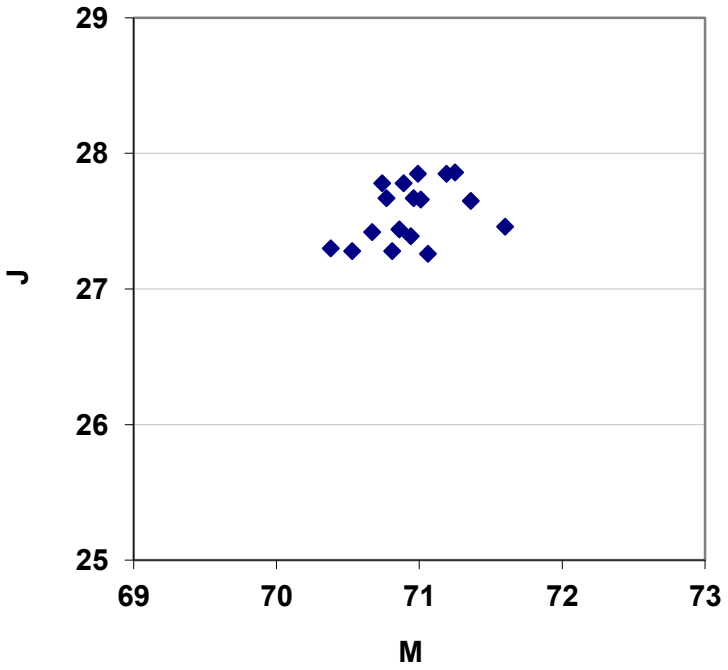

Figure 4.3.1.4 *The digital imaging system (zoom lens) consistency test on lightness and colourfulness. The pilot brewed ale was set as the test sample.*

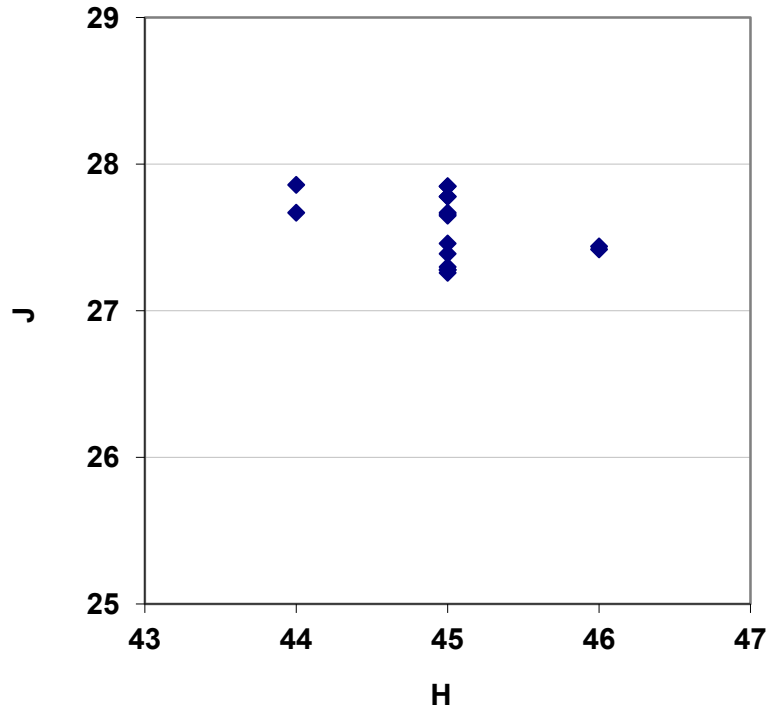

Figure 4.3.1.5 *The digital imaging system (zoom lens) consistency test on lightness and hue. The pilot brewed ale was set as the test sample.*

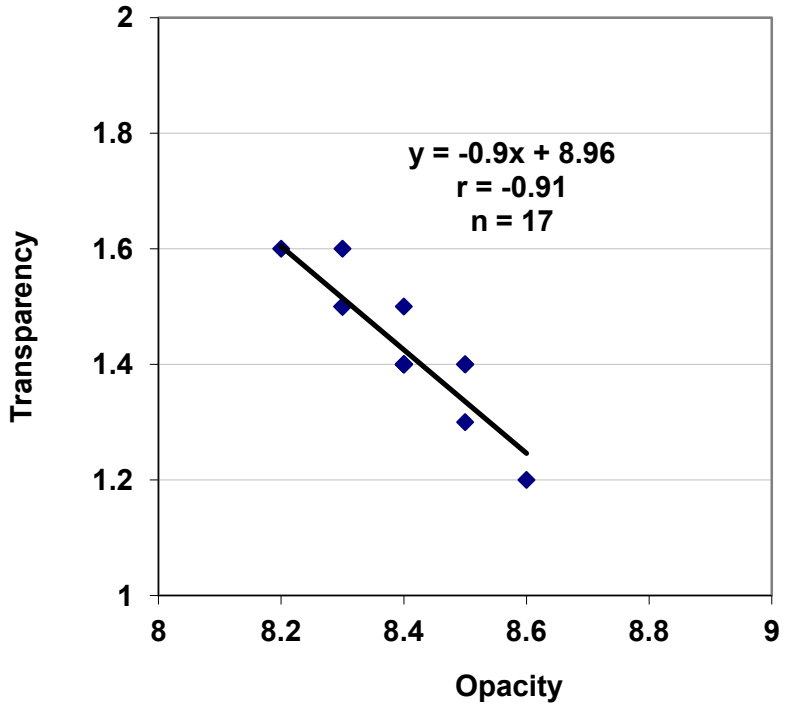

Figure 4.3.1.6 *The digital imaging system (zoom lens) consistency test on transparency and opacity. The pilot brewed ale was set as the test sample.*

The transparency and opacity ranged between 1.2 and 1.6, and 8.2 and 8.6, respectively, both being inversely correlated.

Since it is not possible to know the "true" values of the colour appearance or translucency characteristics of the samples by the imaging system, the absolute accuracy can not be assessed. Thus, we analyse the consistency/repeatability of the digital imaging system on the basis of precision, which can be regarded as reproducibility and is a statistical measure of a variation in samples on repeat determinations of the same sample. The coefficient of variation (CV), as determined by the ratio of standard deviation to the mean of the replicates, is the best quantitative measure of the precision (Section 4.1.1.2; Kotz, 2008). According to Figures 4.3.1.1 to 4.3.1.6, the system consistency results can be summarised and we obtain the mean and CV values (Table 4.3.1.1).

Table 4.3.1.1 *Fixed aperture lens vs zoom lens on digital imaging system consistency about colour attributes and translucency characteristics. 17 samples were tested.*

	Fixed aperture lens		**Zoom lens**	
	Mean	**CV%**	**Mean**	**CV%**
Lightness	25.5	1.4	27.6	0.8
Colourfulness	75.2	0.7	70.9	0.4
Hue	48.6	1.3	45.0	1.1
Transparency	1.7	9.6	1.4	7.0
Opacity	8.1	2.3	8.4	1.2

All the CV values of consistency results by the zoom lens were smaller than those by fixed aperture lens, so that there was less variability of colour and translucency measurements. Therefore, for the further measurements, all the experiments were based on the zoom lens for higher precision.

Colour differences were calculated for the two aperture lens evaluations as mentioned above. Over a four hour testing period, CIELAB ΔE^*_{ab} ranges between 0.39 to 2.37 for the fixed aperture lens, and 0.15 to 1.01 for the zoom lens, whereas for CIEDE ΔE_{00} ranged between 0.24 to 1.30 and 0.12 to 0.60, respectively. This also indicates that the system is more consistent with the zoom lens.

4.3.1.2 Pilot Brewed Samples Testing

The zoom lens was selected for further tests and research on the digital imaging system. The consistency of the digital imaging system was evaluated by one pilot brewed lager and one pilot brewed ale, and the same evaluations were repeated three times to ascertain repeatability and precision.

Figures 4.3.1.7, 4.3.1.8 and Table 4.3.1.2 show the mean values of colour appearance attributes (lightness, colourfulness and hue) and translucency characteristics (transparency and opacity) distribution results of the pilot brewed lager by the digital imaging system on its consistency. Each point in these figures was obtained in every fifteen minutes over four hours; therefore seventeen points were obtained for each test. The test was repeated three times, so totally fifty-one points were got in each of the following figures.

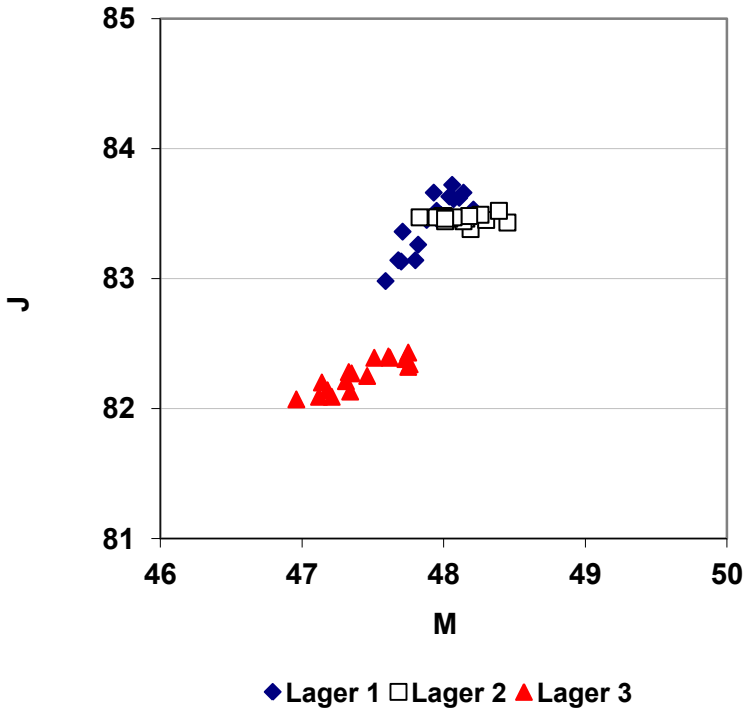

Figure 4.3.1.7 *The digital imaging system consistency tests on lightness (J) and colourfulness (M). The pilot brewed lager was set as the test sample.*
The tests were repeated three times on the same lager: Lager 1: The first test; Lager 2: the second test; Lager 3: the third test.

From Figure 4.3.1.7, the lightness of the lager sample ranged between 82 and 84 units for the three analyses, whereas the range was smaller for each individual test, which was less than 1 unit. A similar distribution was observed for colourfulness: the whole range was between 46.5 and 48.5, whereas it was less than 1.0 unit for each individual test.

From Figure 4.3.1.8, the hue values distributed for three groups for all analyses, as 81, 82 and 83, which are all integers calculated by the software of the digital imaging system.

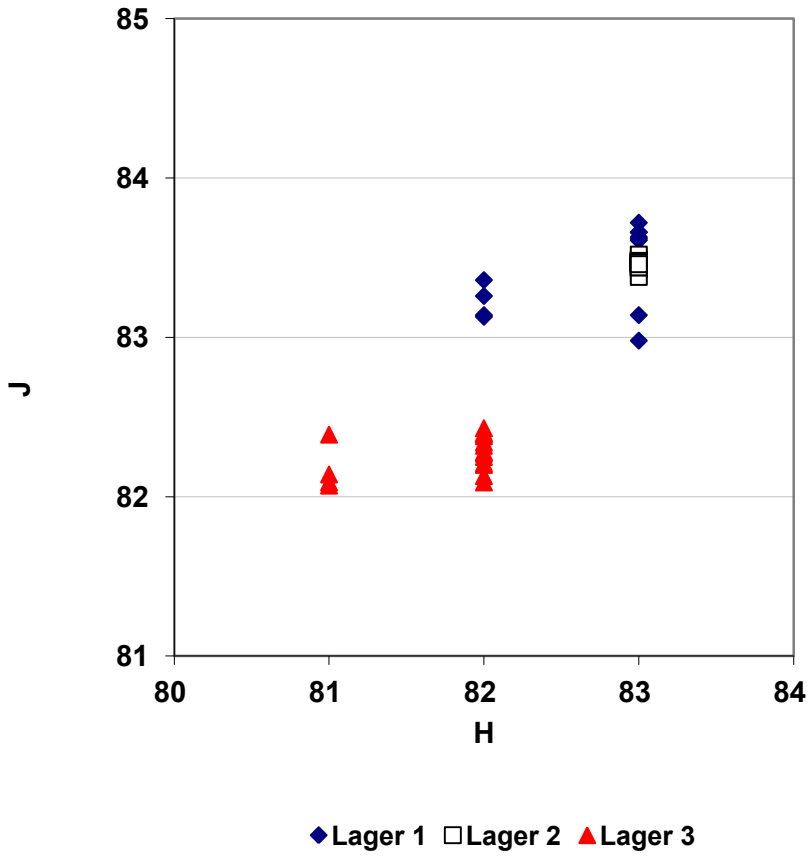

Figure 4.3.1.8 *The digital imaging system consistency tests on lightness (J) and hue (H). The pilot brewed lager was set as the test sample.*
The tests were repeated three times on the same lager: Lager 1: The first test; Lager 2: the second test; Lager 3: the third test.

The colour difference (CIELAB ΔE^*_{ab}) was calculated (as Section 4.3.1.1) for the three lager tests, which ranged from 0.02 to 1.38, and CIEDE2000 ΔE_{00} ranged from 0.005 to

1.20. Humans would be expected to be barely able to tell the colour difference between the most of the samples visually (Section 2.2.1). In contrast the new digital imaging system distinguishes the colour appearance attributes of the samples, indicating that this system could be more sensitive than the human eye.

Table 4.3.1.2 shows the transparency and opacity results for the pilot lager. The variation of the transparency/opacity measurement (0.1 units) was not high compared with the full range of transparency and opacity (0 to 10).

Table 4.3.1.2 *The digital imaging system consistency tests on transparency and opacity, pilot brewed lager was set as the test sample.*

	Transparency	**Opacity**	**Sample size**
Lager 1	8.2	1.9	9
	8.1	2.0	8
Lager 2	8.1	2.0	11
	8.2	1.9	1
	8.1	1.9	5
Lager 3	8.1	2.0	16
	8.1	1.9	1

The tests were repeated three times by the same lager: Lager 1: The first test; Lager 2: the second test; Lager 3: the third test.

For one pilot brewed ale, we carried out the same tests on colour appearance attributes and translucency characteristics (Figures 4.3.1.9, 4.3.1.10 and 4.3.1.11).

From Figure 4.3.1.9, the lightness of the ale sample ranged between 47.2 and 48.0 for the three analyses, which was less than 1 unit. We can also get the similar distribution for colourfulness; the whole range was between 75 and 77.5, whereas it was smaller for each individual test. From Figure 4.3.1.10, the hue values were distributed at two groups for all analyses, as 55 and 56.

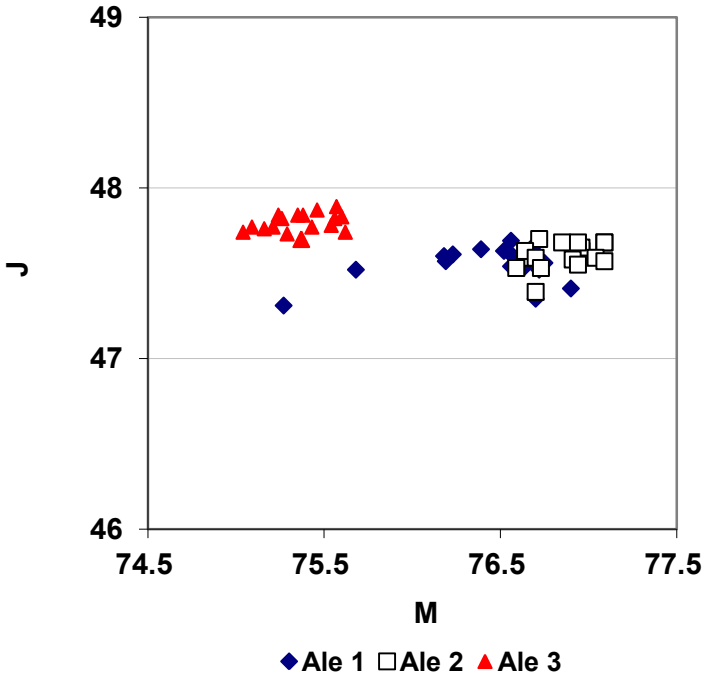

Figure 4.3.1.9 *The digital imaging system consistency tests on lightness (J) and colourfulness (M). The pilot brewed ale was set as the test sample.*
The tests were repeated three times by the same ale: Ale 1: The first test; Ale 2: the second test; Ale 3: the third test.

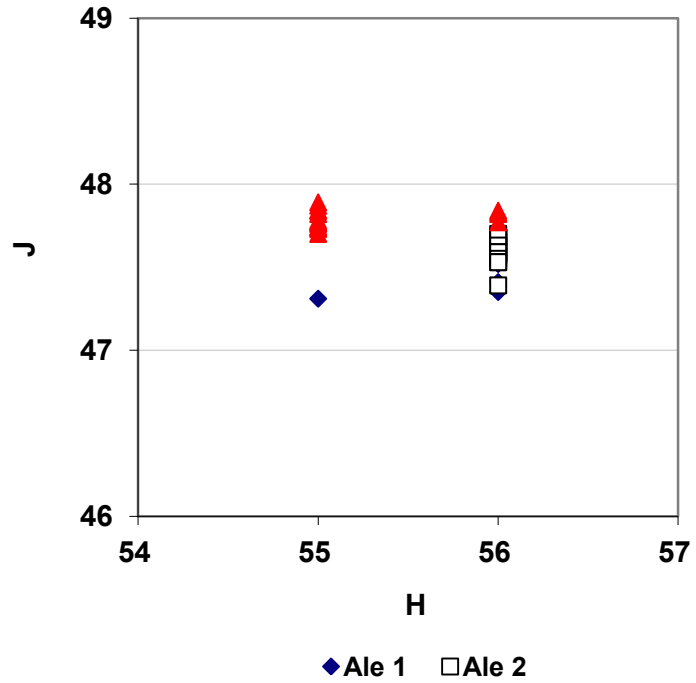

Figure 4.3.1.10 *The digital imaging system consistency tests on lightness (J) and hue (H). The pilot brewed ale was set as the test sample.*
The tests were repeated three times by the same ale: Ale 1: The first test; Ale 2: the second test; Ale 3: the third test.

Colour differences CIELAB ΔE^*_{ab} was calculated (as Section 4.3.1.1) for the three ale tests, which range from 0.10 to 1.31, and CIEDE2000 ΔE_{00} ranged from 0.05 to 0.34, which were lower than human visual threshold on beer samples.

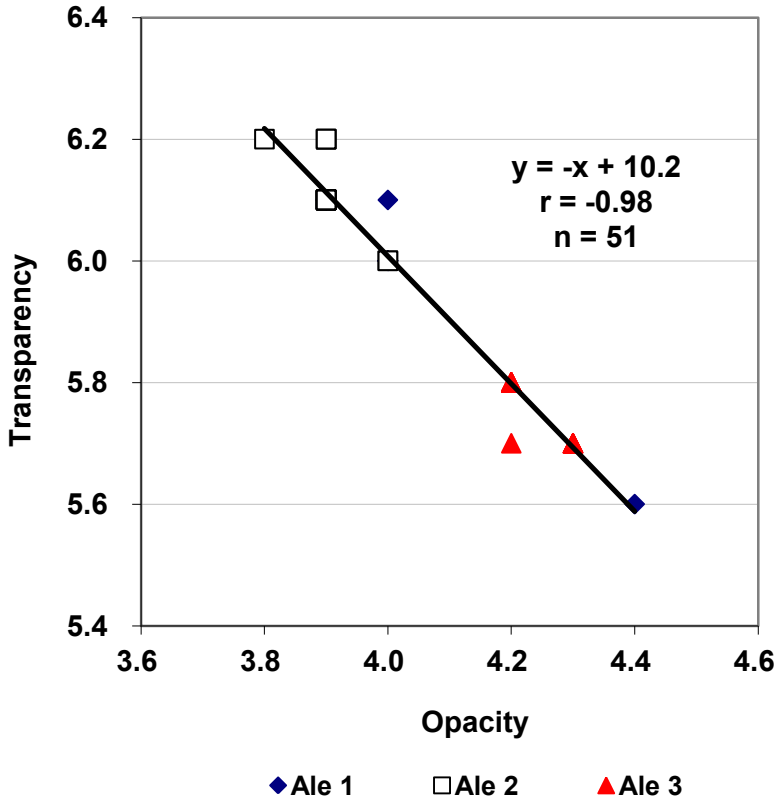

Figure 4.3.1.11 *The digital imaging system consistency tests on transparency and opacity. The pilot brewed ale was set as the test sample.*
The tests were repeated three times by the same ale: Ale 1: The first test; Ale 2: the second test; Ale 3: the third test.

Figure 4.3.1.11 shows transparency and opacity results of the pilot ale. Both of the parameters spanned 0.6 units for all the analysis in the three tests, and the range was not high relative to the full range. It indicates that the digital imaging system shows high precision on translucency characteristics by these three tests.

4.3.2 Pilot Brewing Testing

The digital imaging system was introduced into fifteen lager pilot brews and fifteen ale pilot brews (thirteen pale ales and two dark ales) as a near line colour/translucency measurement instrument. All of the liquid samples collected at CCPs (Critical Control Points) throughout the brewing process were free of large grist/yeast particles or gas.

Figures 4.3.2.1 and 4.3.2.2 show the colour appearance attributes (lightness, colourfulness and hue) distribution of all the pilot brewed samples collected at the CCPs decided (Section 4.2).

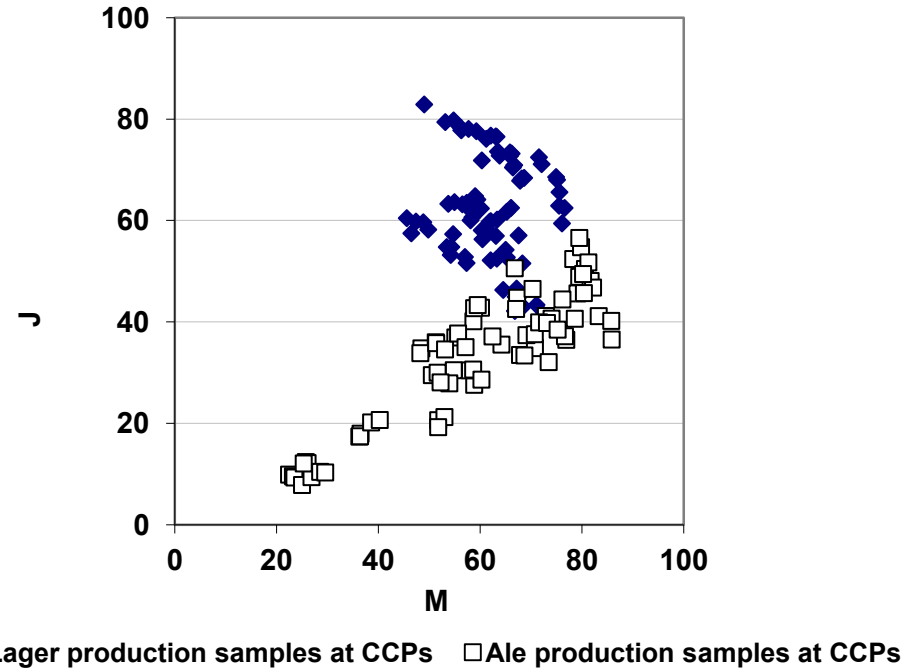

Figure 4.3.2.1 *Lightness (J) and colourfulness (M) of pilot brewed lagers and ales production at CCPs by the digital imaging system.*

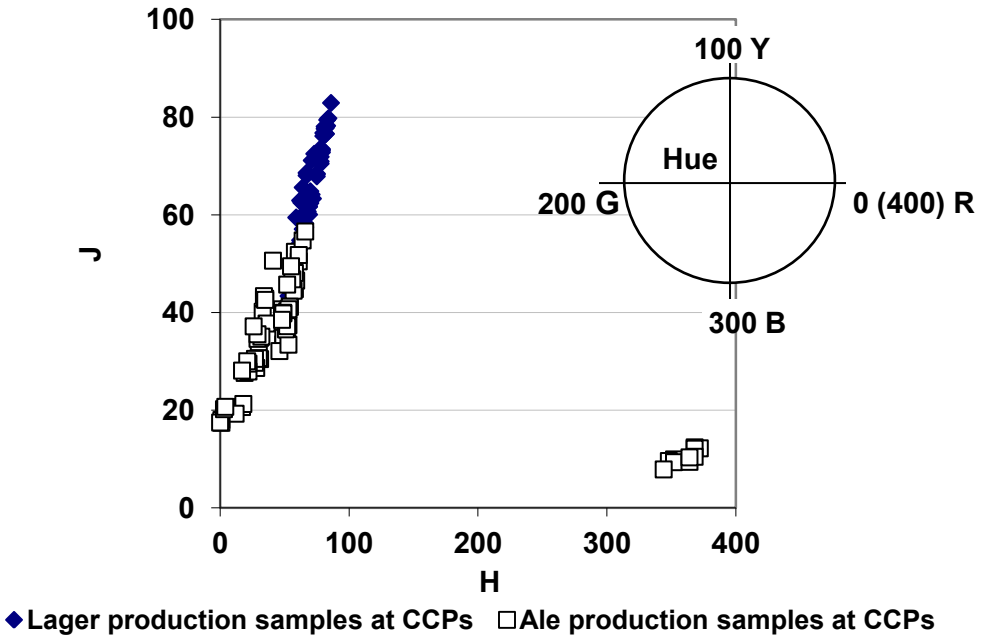

Figure 4.3.2.2 *Lightness and hue of pilot brewed lagers and ales production at CCPs by the digital imaging system.*

As expected, these two figures indicate that lager product samples appeared lighter, less colourful and yellower than most of ale product samples by the digital imaging system. Some samples had a relatively low lightness (Figures 4.3.2.1 and 4.3.2.2), low colourfulness, and were clearly distinct from the other lager or paler ale production samples on hue, which showed the bluish red. The hue values of all pilot brewed lager production samples and most ale production samples ranged between 30 and 90, which were in red-yellow hue. These darks samples were analysed by modified software by the digital imaging system (Section 4.3.2.2).

4.3.2.1 Lager Pilot Brewing Testing

Figures 4.3.2.3, 4.3.2.4 and 4.3.2.5 illustrate the colour appearance attributes of the fifteen pilot brewed lagers during production at the five CCPs as defined earlier (Section 4.2). It is clear that all of the three lagers showed similar colour appearance

attributes at each of the CCPs.

From Figure 4.3.2.3, for the wort production (samples 1s to 3s), the lightness (J) of all the fifteen pilot brewed lagers changed slightly (from 0.1 to 7.0 units for each pilot brewed lager). For all the lager samples, sample 4s (prefiltration) and sample 5s (postfiltration) were with higher values on lightness values, which indicated the samples were lighter, probably because some colour substances were absorbed on yeast and removed by centrifuging and final filtration (Section 2.1). These results are consistent with conventional instrument results (i.e. Figures 4.2.1.1 and 4.2.2.1).

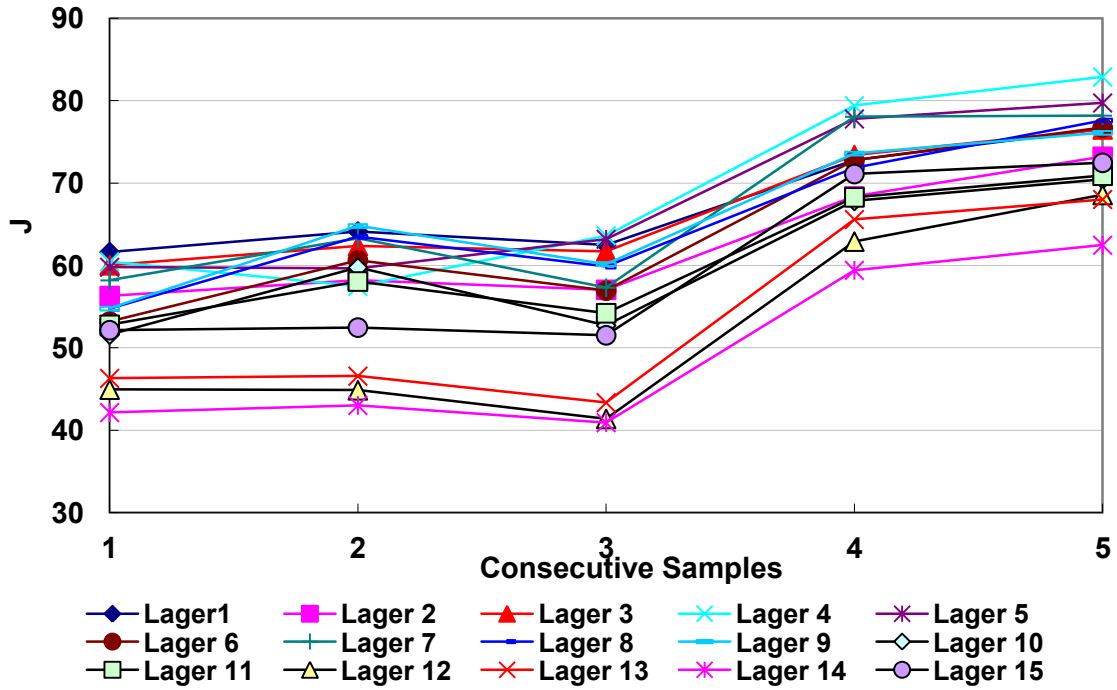

Figure 4.3.2.3 *Lightness (J) of pilot brewed lagers production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

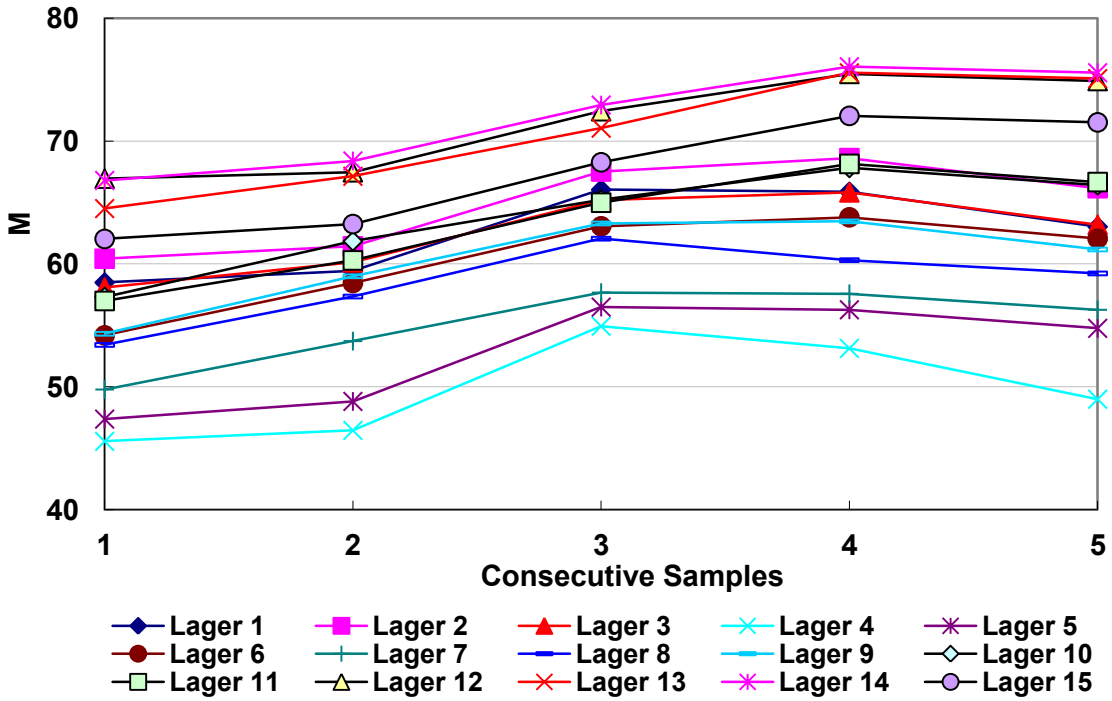

Figure 4.3.2.4 *Colourfulness (M) of pilot brewed lagers production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

The colourfulness (M) increased during wort production (sample 1s to sample 3s) for all of the lagers, and these pilot brewed lagers could be divided into two groups on the basis of changes in colourfulness between sample 3s and sample 4s (Figure 4.3.2.4). The paler lagers (lagers 1, 3, 4, 5, 7 and 8) decreased in colourfulness after fermentation and conditioning, while the other darker lagers increased colourfulness. After final filtration, sample 5s of all the brewed lagers decreased in colourfulness.

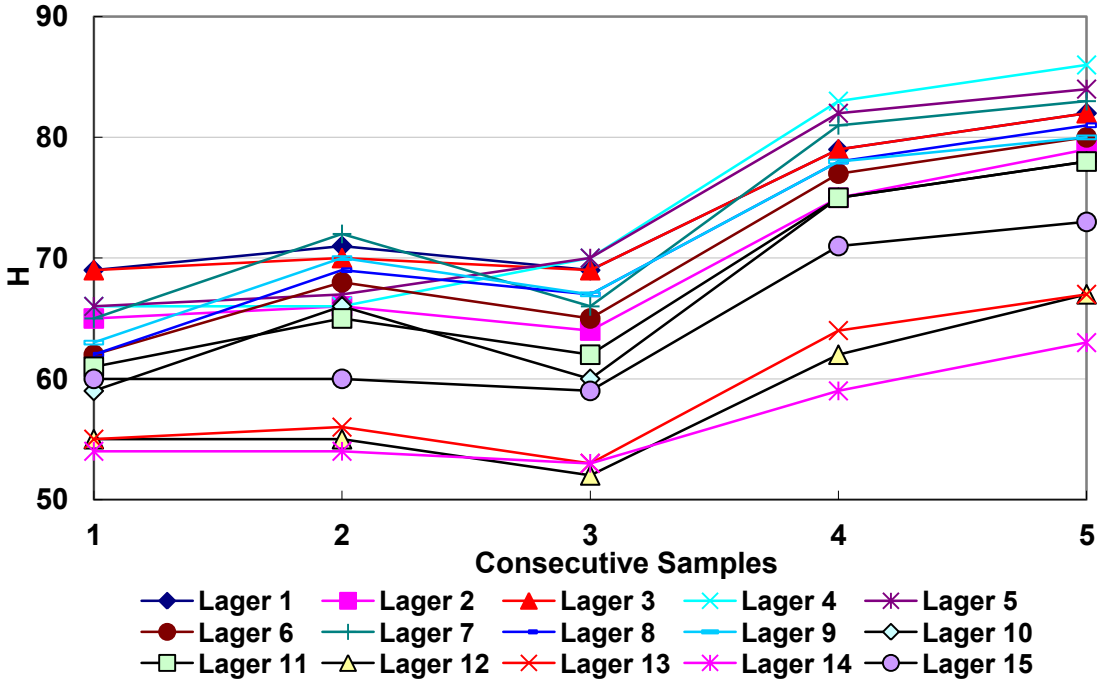

Figure 4.3.2.5 *Hue (H) of pilot brewed lagers production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

The corresponding hue values (H) indicated a predominantly yellow hue (50 to 90 units). For the wort production (the sample 1s to sample 3s), the colour of the liquid became more colourful and redder, presumably because of the Maillard reaction (Nursten, 2005). After fermentation, conditioning and filtration, the final lager products appeared more yellow.

The EBC colours of pilot brewed lagers final products (Figure 4.3.2.6) were approximate 5.0, 7.5, 10.5 and 19.0. These paler lagers (i.e. lagers 1 to 9) had higher lightness characteristic (70 to 83 units), and they ranged between 4.5 to 8.4 EBC colours based on the EBC Recommended method, whereby light absorbance is measured at 430 nm in a 10 mm quartz cuvette against a reference of distilled water (Section 2.2.3). However there was no obvious difference visually between these samples on EBC colours by Lovibond discs (all the three samples approximated around 5.0 or 7.5 EBC colour). It indicates that it is relatively difficult for human to distinguish the colour

difference between most of these samples visually. The EBC colour of the darker lagers (lagers 12, 13 and 14) were approximately 18.8 to 20.1 by the EBC Recommended method, whereas there was not a visually significant difference by Lovibond discs.

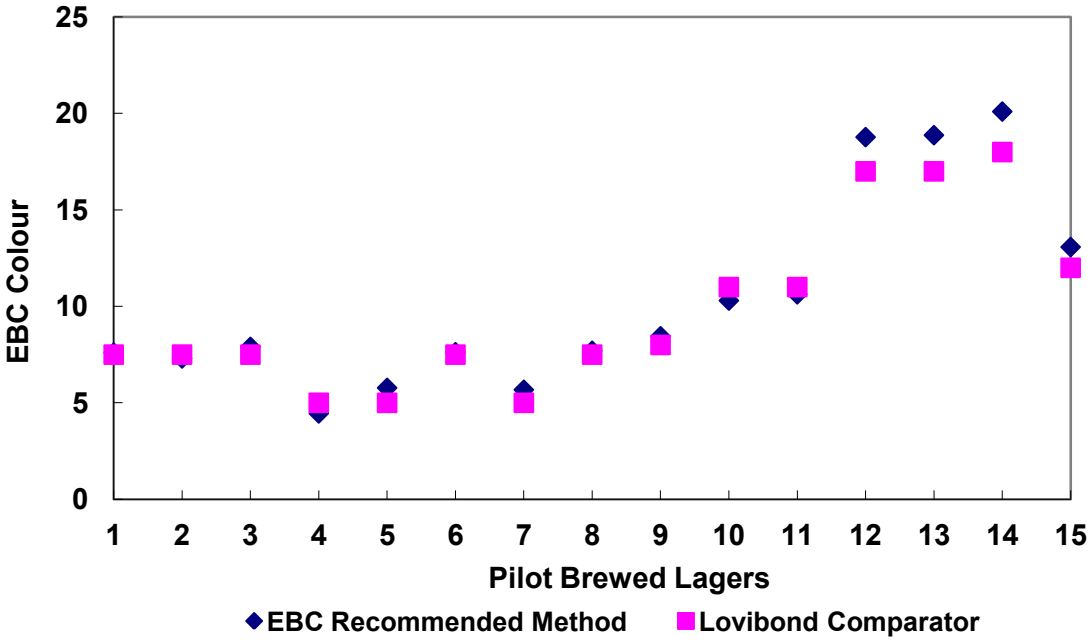

Figure 4.3.2.6 *EBC colour of pilot brewed lager final products.*

The lightness component gives an approximate correlation to the EBC colours of the pilot brewed lager final products (Figure 4.3.2.7) as determined by the EBC Recommended Method. Lagers with low EBC values have high lightness values, which decrease as the EBC colour of the lagers increased, and Smedley (1995) also got the same result in his research. We may say that lightness of beer could be used to predict the EBC colour, but further work is needed to set up their correlations, e.g. different types of beers/worts may vary the correlation coefficients, thus more research and evaluations are needed to set up database and standards for different types of beers/worts.

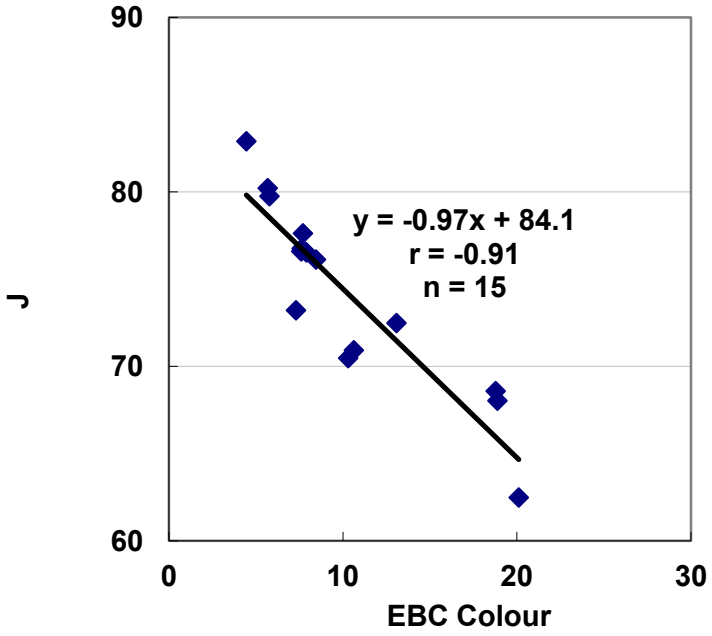

Figure 4.3.2.7 *Correlation between lightness (J) and the EBC colour for pilot brewed lager final products.*

Figures 4.3.2.8 and 4.3.2.9 show the translucency characteristics of the fifteen pilot brewed lagers productions across the five CCPs. All the liquid samples (except the final products) were centrifuged before being tested, thus some of haze content may have been be separated with large grist or yeast particles. The turbidity values of the sample 1s to 4s were between 2.5 to 9.7 EBC units. After the final filtration stage, the final products had turbidity values between 0.54 to 1.12 EBC units, which were reflected in Figures 4.3.2.8 and 4.3.2.9 as higher transparency and therefore lower opacity scores. While every effort was made to ensure the consistency of sample preparation, there were still some relatively extreme samples (Figures 4.3.2.8 and 4.3.2.9), i.e. lager 7 at points 2 (sample 2) and 3 (sample 3). These extreme values may be caused by the natural brewing processing, which could be affected by parameters and other factors, like processing conditions, materials, etc.

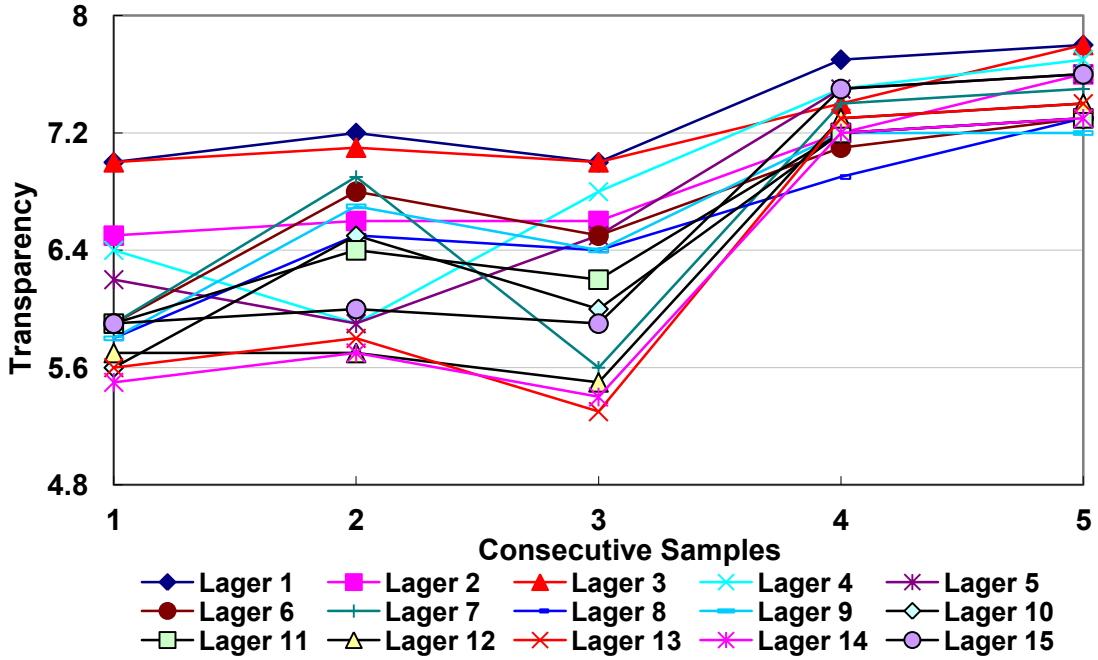

Figure 4.3.2.8 *Transparency of pilot brewed lagers production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

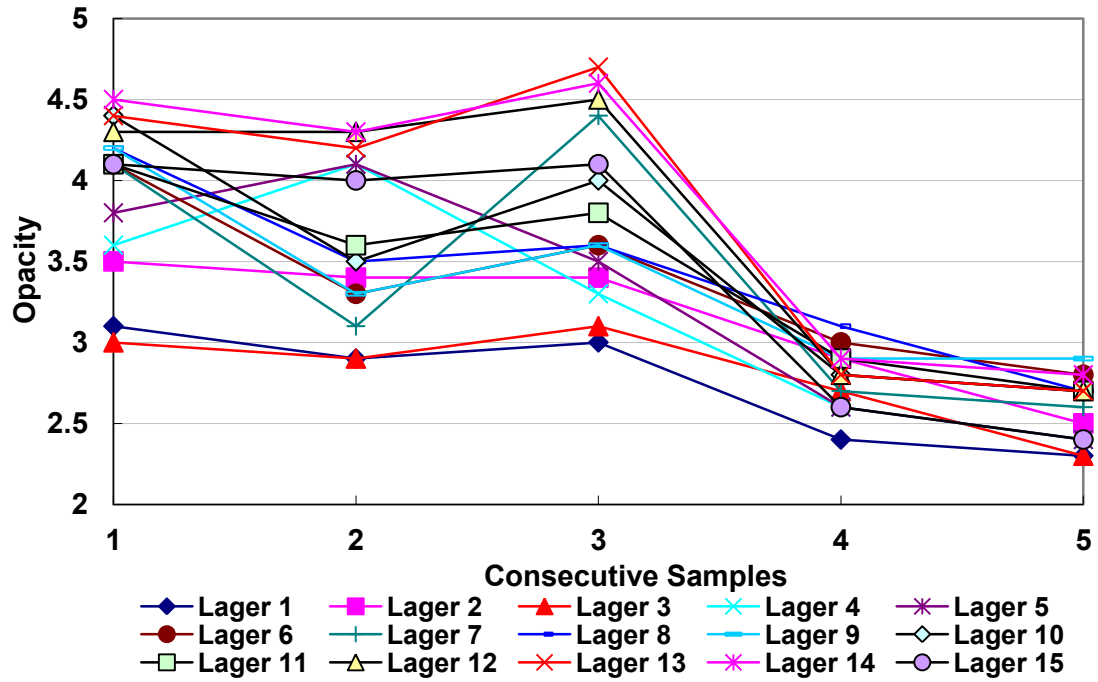

Figure 4.3.2.9 *Opacity of pilot brewed lagers production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

4.3.2.2 Ale Pilot Brewing Testing

In an analogous study on thirteen pilot brewed paler ales (Figures 4.3.2.10, 4.3.2.11, 4.3.2.12), colour performance was broadly similar in colour appearance attributes.

During wort production (sample 1s to 3s), the colour of the liquid became darker, more colourful and moved towards a red hue, presumably because of Maillard reaction. After fermentation, conditioning and filtration, all final ale products appeared lighter and more colourful (relative to boiled wort) and more yellow, similar to those characteristics of pilot brewed lagers.

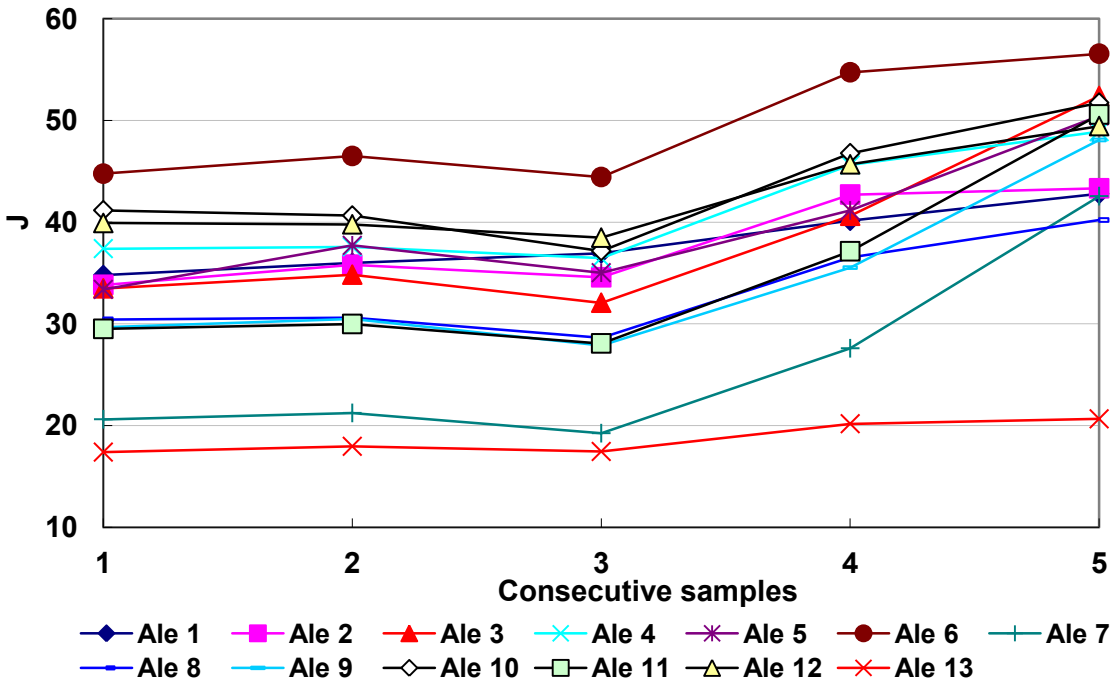

Figure 4.3.2.10 *Lightness (J) of pilot brewed ales production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

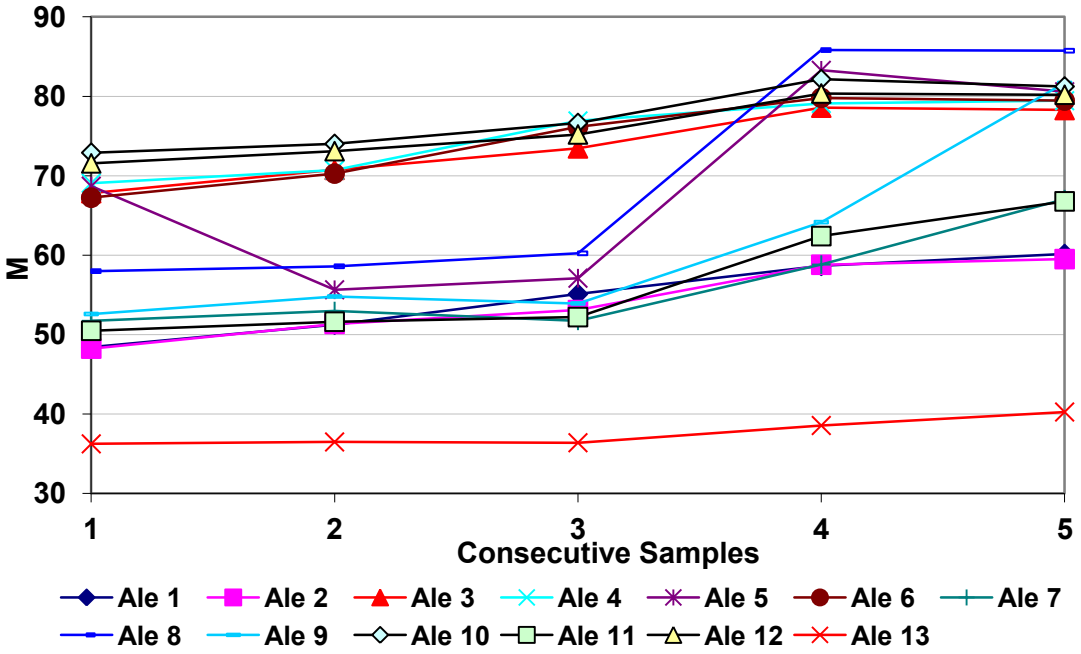

Figure 4.3.2.11 *Colourfulness (M) of pilot brewed ales production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

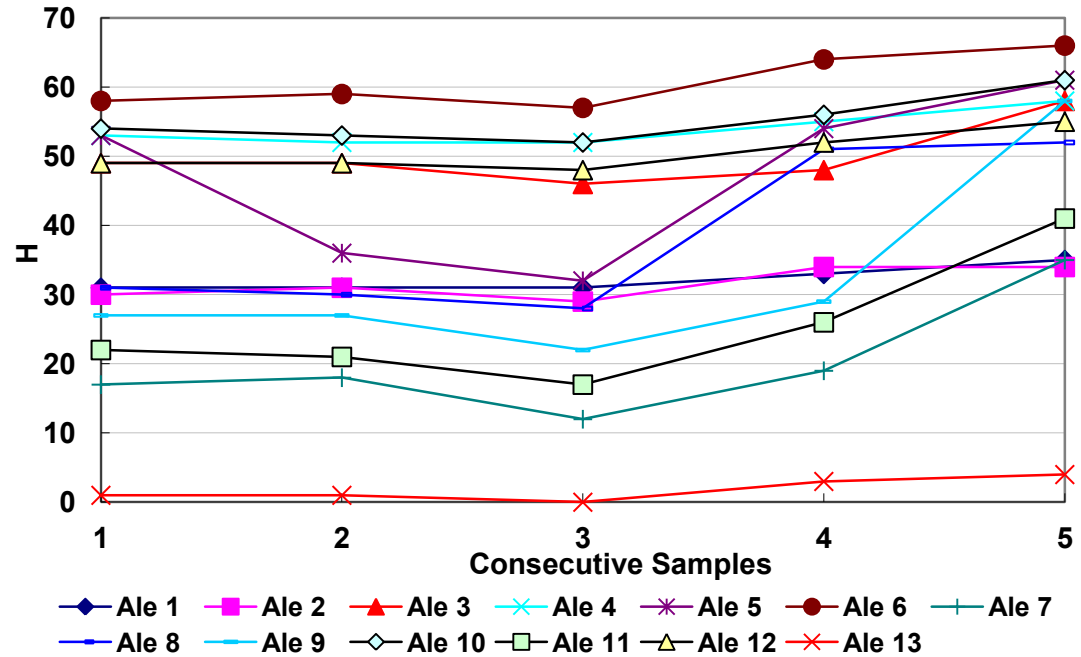

Figure 4.3.2.12 *Hue (H) of pilot brewed ales production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

EBC colours were measured on final products of the pilot brewed ale samples according to the EBC Recommended Method and also assessed visually using a Lovibond Comparator. From Figure 4.3.2.13, EBC colours of these pilot brewed lagers were approximate 33 (ales 1, 2, 3, 7, 8), 20 (ales 4, 5, 6), 18 (ales 9, 10, 11, 12) and 70 (ale 13).

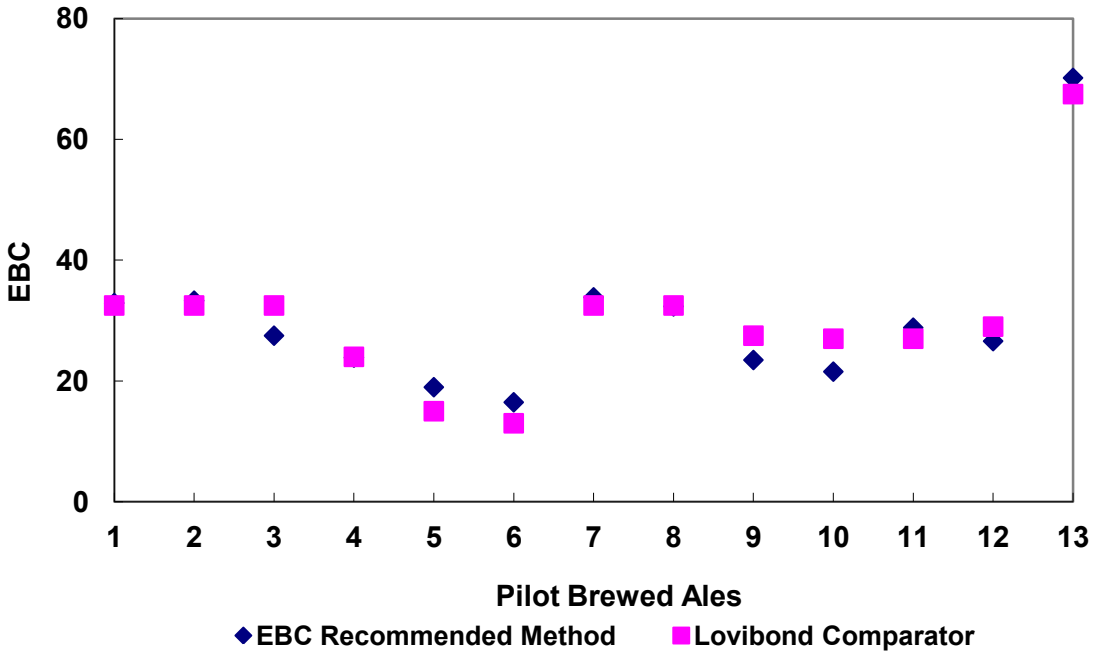

Figure 4.3.2.13 *EBC colour of pilot brewed ale final products.*

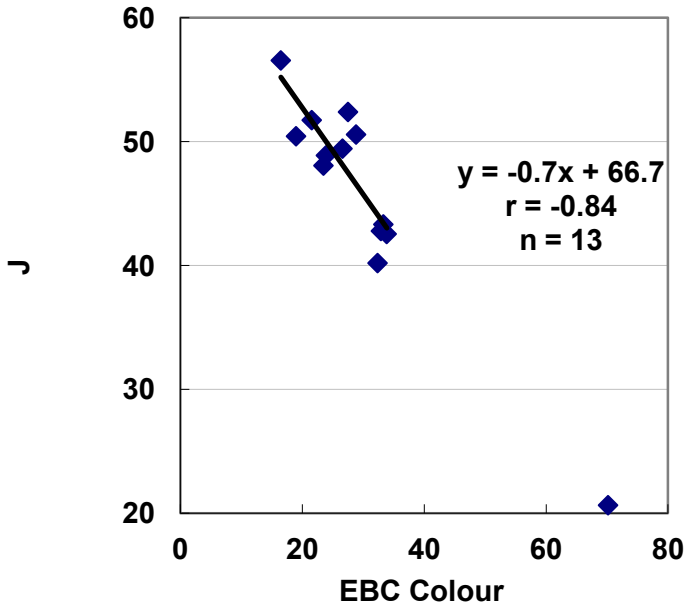

Figure 4.3.2.14 *Correlation between lightness and the EBC colour for pilot brewed ale final products.*

Figure 4.3.2.14 shows the correlation between lightness and EBC colours of the pilot brewed ale final products. As the lightness decreases, the EBC values increase.

Figures 4.3.2.15 and 4.3.2.16 show the translucency characteristics of the thirteen pilot brewed ales across the CCPs. All of the liquid samples (except the final products) were centrifuged before being tested, thus some of haze content may be separated with large grist or yeast particles.

During wort production, all of the samples collected at boil end showed lowest transparency and highest opacity for a given product and after being final filtered, the final products had higher transparency and lower opacity scores as would be expected.

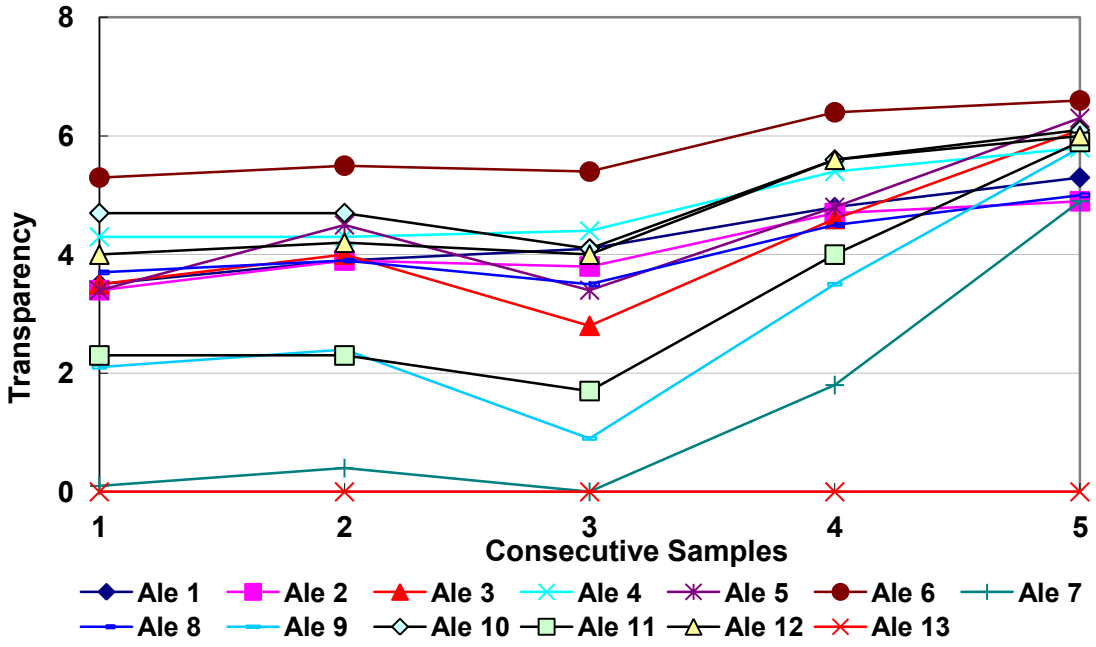

Figure 4.3.2.15 *Transparency of pilot brewed ales production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

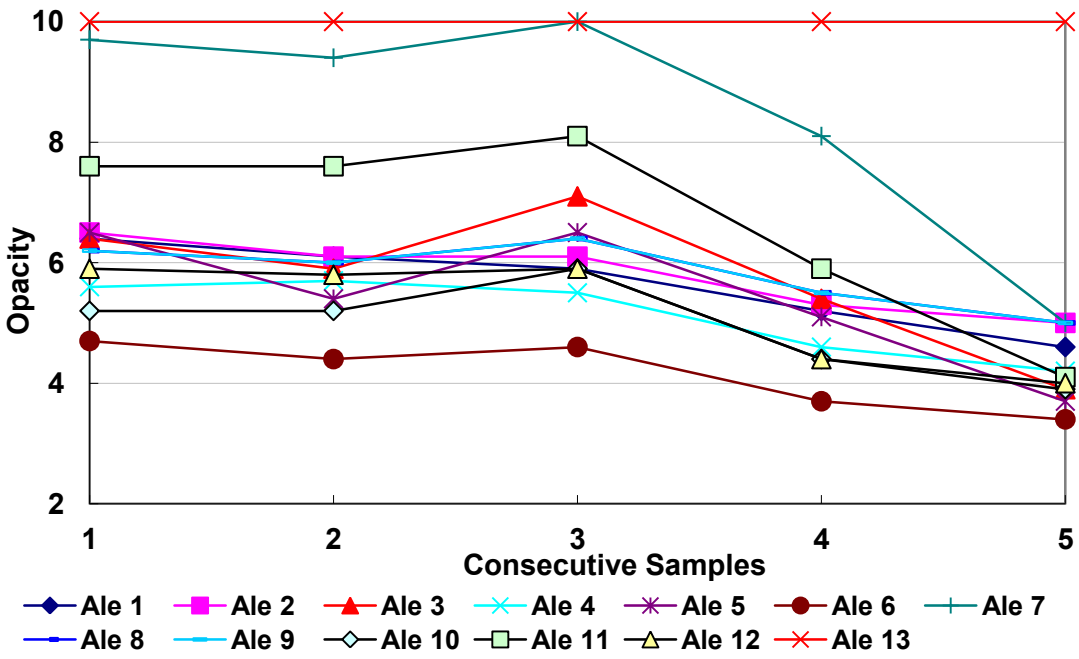

Figure 4.3.2.16 *Opacity of pilot brewed ales production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

The wort production samples (1 to 3) of ale 7, sample 3 of ale 11 and all samples of ale 13 were darker than the other samples, which had the lightness close to or lower than 20 units. These four samples performed differently from the other samples on transparency/opacity (Figures 4.3.2.15 and 4.3.2.16), which close to or at the extreme values set for transparency/opacity (0, 10). The haze contents for the samples 1s to 4s were similar, but these four dark samples seemed distinct from the other samples on transparency/opacity characteristics obtained from the digital imaging system.

From the above pilot ale results, we observed that some darker ale samples seemed to respond differently when evaluated by the digital imaging system. Therefore some extreme dark samples were used for additional system testing. Two dark ales (75 - 78 EBC) were pilot brewed and analysed using modified software. Figures 4.3.2.17, 4.3.2.18 and 4.3.2.19 show the colour appearance attributes of these two dark ales.

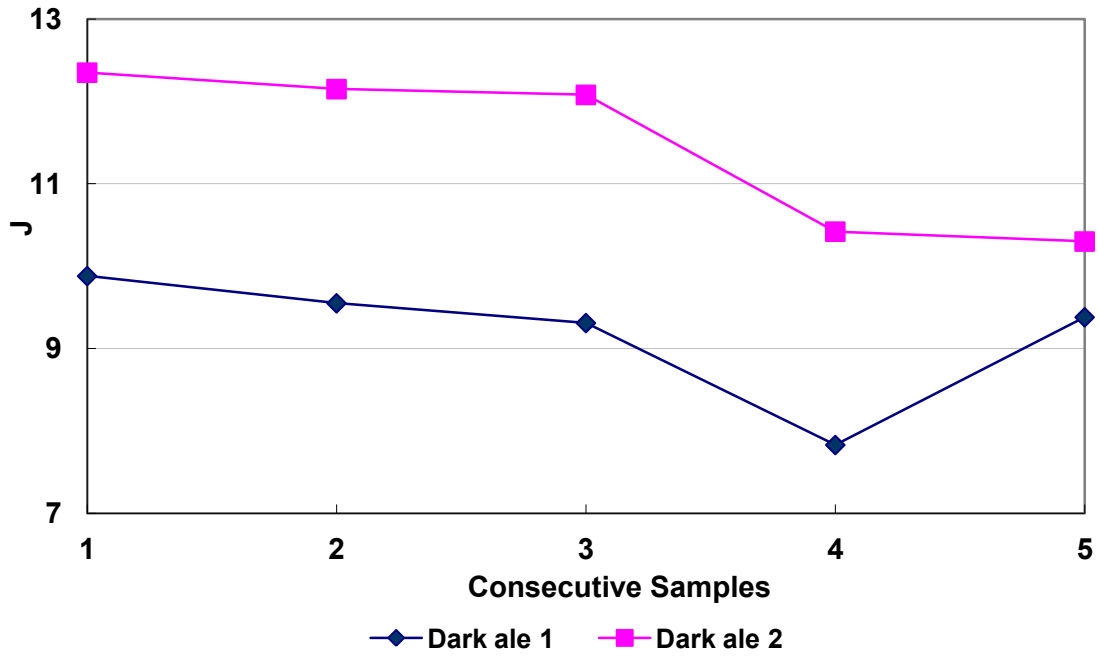

Figure 4.3.2.17 *Lightness of pilot brewed dark ales production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

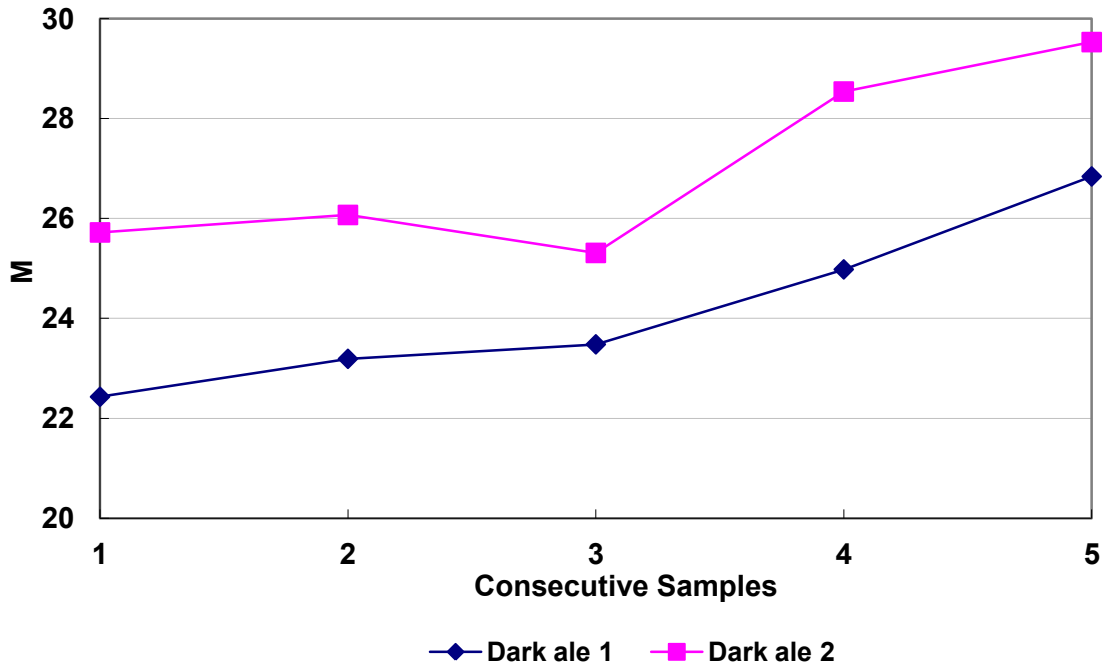

Figure 4.3.2.18 *Colourfulness of pilot brewed dark ales production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

Comparing Figures 4.3.2.19 and 4.3.2.3, 4.3.2.10, we can observe that the two dark ales performed different on lightness from the other paler ales and lagers across the five CCPs. During fermentation, the colour of beer becomes lighter, as mentioned before (Section 2.1). According to this theory, in our case, samples 4 and 5 should be lighter than the other first three samples in each brew, and in fact, all lagers and paler ales we brewed followed this pattern. But both of the two pilot brewed dark ales do not follow this theory by the new digital technology. More modification for the system is needed, such as redesigning the metal cell to add more depth, so that dark liquids can be distinguished more readily. Additionally, the software will need to be updated to integrate the new designed cell.

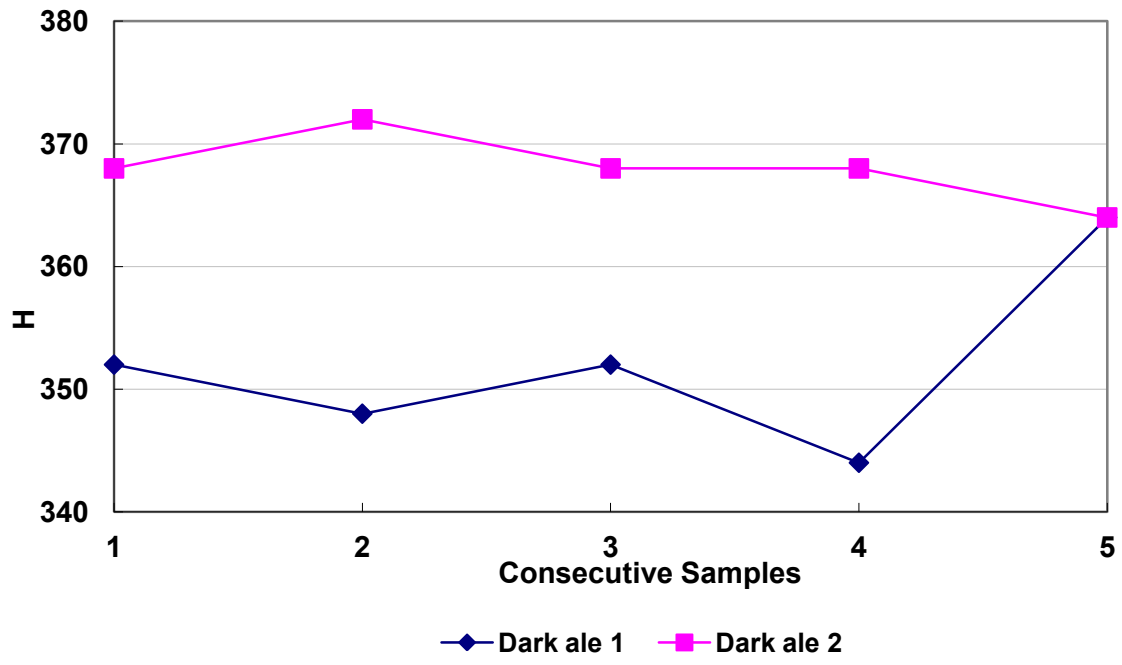

Figure 4.3.2.19 *Hue of pilot brewed dark ales production at CCPs by the digital imaging system.*

The samples were collected at critical control points (CCPs): 1: Mash clarification end; 2: Boil start; 3: Boil end; 4: Prefiltration; 5: Postfiltration.

The translucency characteristics for the two dark ale samples along CCPs were analysed. Using the modified software for dark samples, the transparency for all the ten samples of the two products was 0, whereas opacity was 10.

For these two pilot brewed dark beers, the test procedures were the same as those for lagers and paler ales. We centrifuged samples 1 to 4 for each production, and the turbidity values of the samples were between 2.5—9.7 EBC units. The results obtained by turbidimetry showed that the samples were low in haze, whereas they appeared opaque from the digital imaging system. This means that the transparency/opacity results for dark liquids relate to colour (lightness) more than haze characteristics by the digital imaging system.

4.3.3 Commercial Beers Testing

Figures 4.3.3.1 and 4.3.3.2 generally show the colour appearance attributes (lightness, colourfulness and hue) distribution of all the commercial lagers and ales, and the points can be treated in four sections.

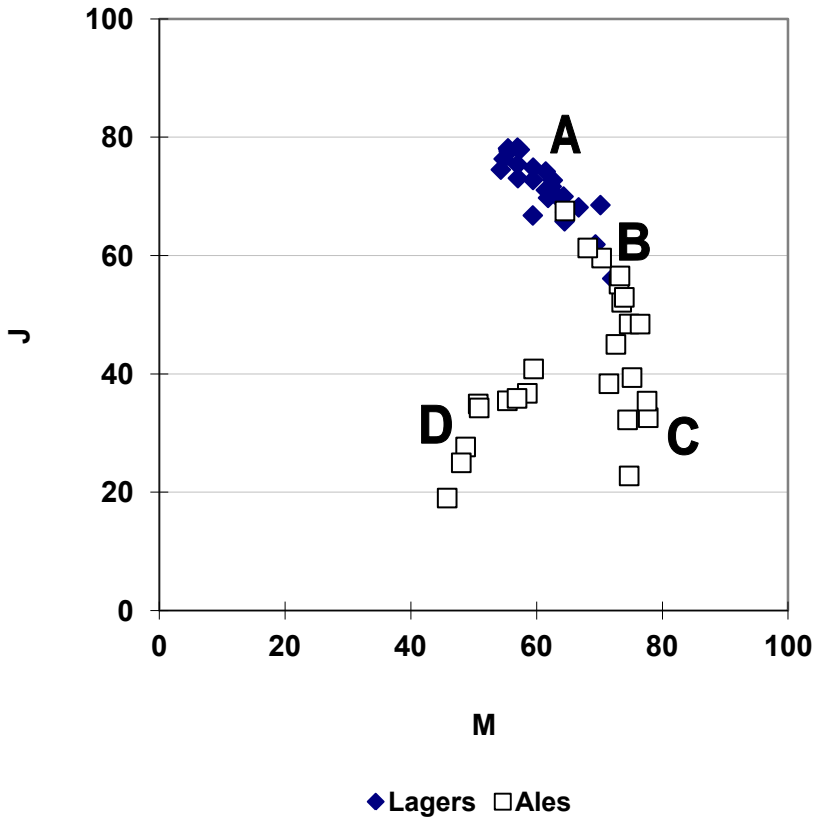

Figure 4.3.3.1 *Lightnes (J) and colourfulness (M) of commercial beer products by digital imaging system. A-D were four sections to describe colour.*

Here we have divided the commercial samples into four groups on the basis of their colour performance. In group **A**, the paler lagers had high lightness values. The lagers were less vividly coloured; that is, their colourfulness values were lower. The corresponding hue values indicated a predominantly yellow hue (70 – 78 units). In group **B**, colourfulness increased steadily while lightness decreased. The hue of the beers continued to move from yellow towards yellow-red. The majority of beers were found along this section namely darker lagers and paler ales. Group **C** represents an area where lightness continued to decrease, and colourfulness continued to increase to reach the highest values (78 units). The darkest beers, corresponding to brown ales and stouts, were found in group **D**. Lightness continued to fall, even close to 20 units. The colourfulness of these beers decreased with decreasing lightness value, and the hue moved further towards to red region (towards to 0).

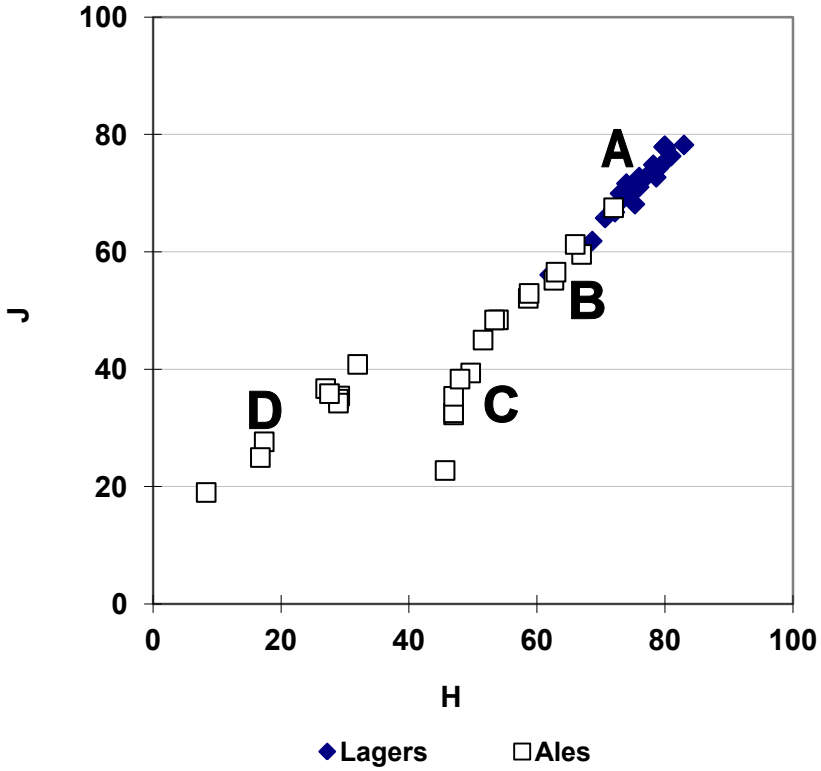

Figure 4.3.3.2 *Lightness and hue of commercial beer products by digital imaging system. A-D were four sections to describe colour.*

The lightness component was closely correlated to the EBC colours of the commercial beers (Figure 4.3.3.3) as determined by the EBC Recommended Method (Section 2.2.3). Beers with low EBC values have high lightness values, which steadily decrease as the EBC colour of the beers increased. The correlation was the similar as the previous results of pilot brewed lagers (Figure 4.3.2.7) and ales (Figure 4.3.2.14).

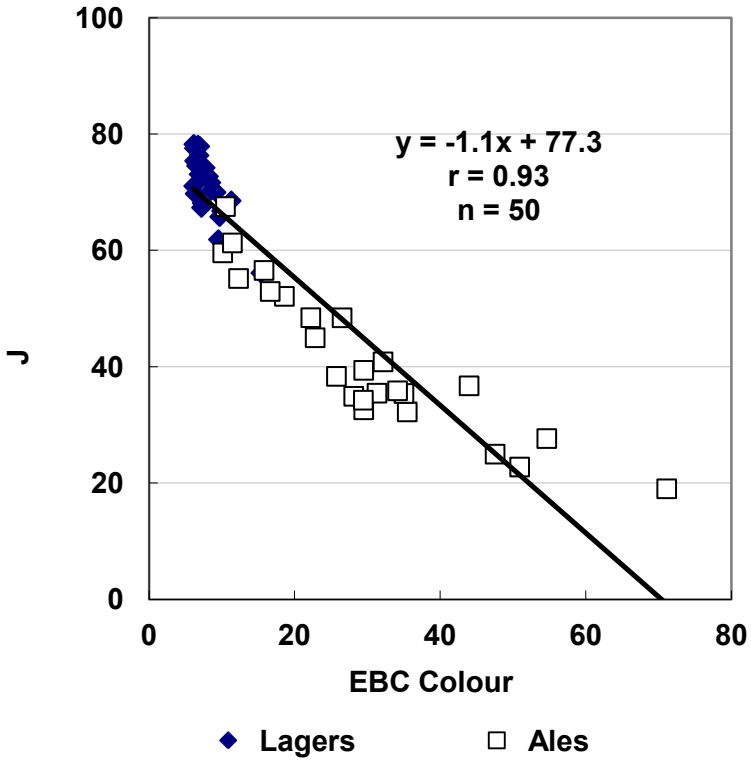

Figure 4.3.3.3 *Correlation between lightness and the EBC colour for commercial beers.*

Figures 4.3.3.4 and 4.3.3.5 show the translucency characteristics (transparency and opacity) results of the twenty-five commercial lagers and ales by the digital imaging system. The transparency gives a linear correlation to the opacity of the beers.

The same transparency against opacity characteristic analysis was taken for both commercial lagers and ales, and we obtained similar linear correlation between these characteristics. The lagers which are lighter had transparency between 6.5 and 8.2 units (a range of less than 2.0 units) and opacity between 2.0 and 3.5 units. The ales had

wider transparency/opacity distribution comparing with lagers, which ranged from 0 and 7.3 in transparency and 2.8 to 10 in opacity.

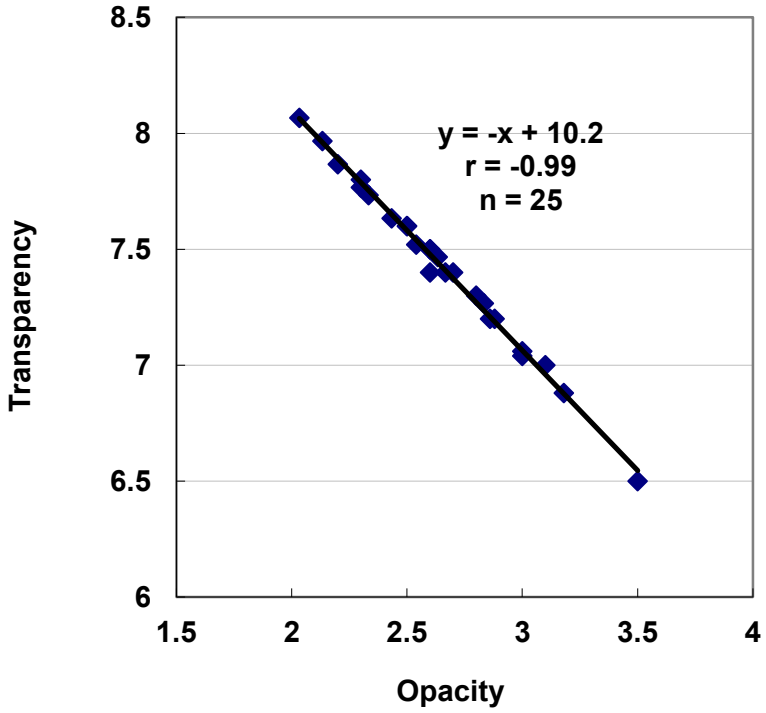

Figure 4.3.3.4 *Transparency and opacity of commercial lagers by digital imaging system.*

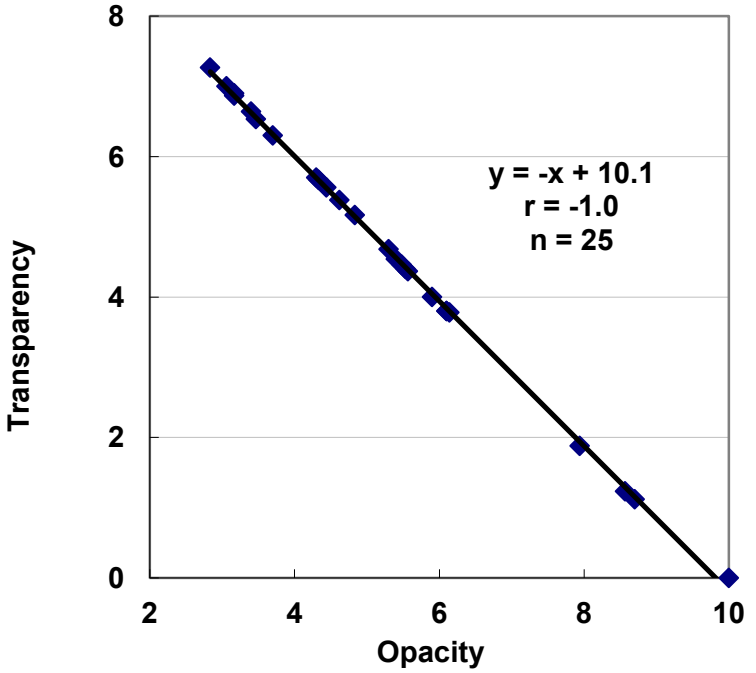

Figure 4.3.3.5 *Transparency and opacity of commercial ales by digital imaging system.*

The turbidity of all samples was measured by a turbidimeter. The measurement results were ranged from 0.34 to 0.84 EBC (see Sections 2.3.1 and 3.2.1) units, indicating commercial lagers and most ales had similar turbidity. However, the transparency values of ales were much wider than those of lagers measured by the digital imaging system. In other words, transparency results of the beers obtained from digital imaging system do not correlate wuth the turbidity (EBC) results. This implies that the translucency characteristics are related to colour (lightness) more than haze characteristics by this system. This effect was also shown in Figures 4.3.3.6 and 4.3.3.7.

The lightness gives an approximate linear correlation to the transparency of the commercial lagers and ales (Figure 4.3.3.7). Beers with low transparency values have low lightness values, which steadily increase as the transparency of the beers increases. Thus, all the lagers had higher lightness and transparency values, and the darkest ale had transparency 0 units.

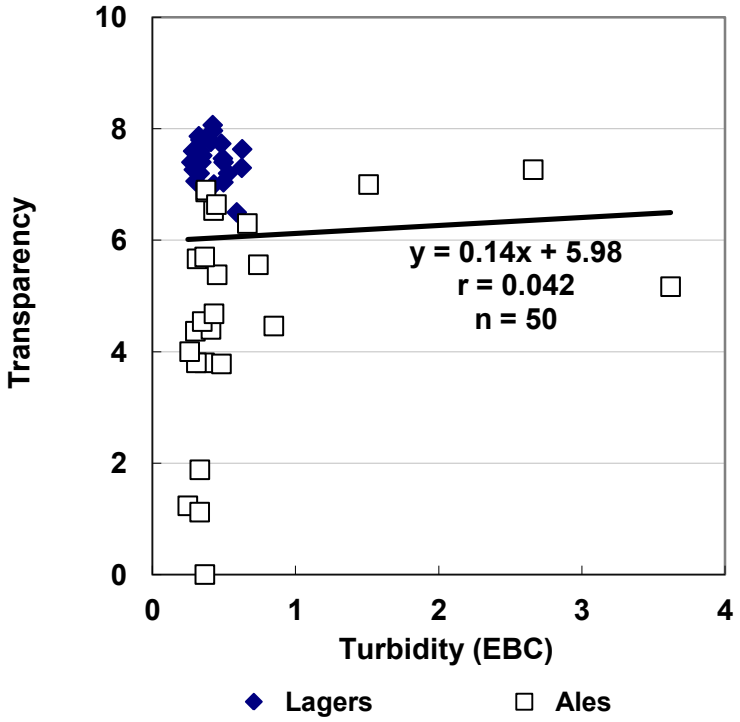

Figure 4.3.3.6 *Transparency* vs *turbidity of commercial beers.*

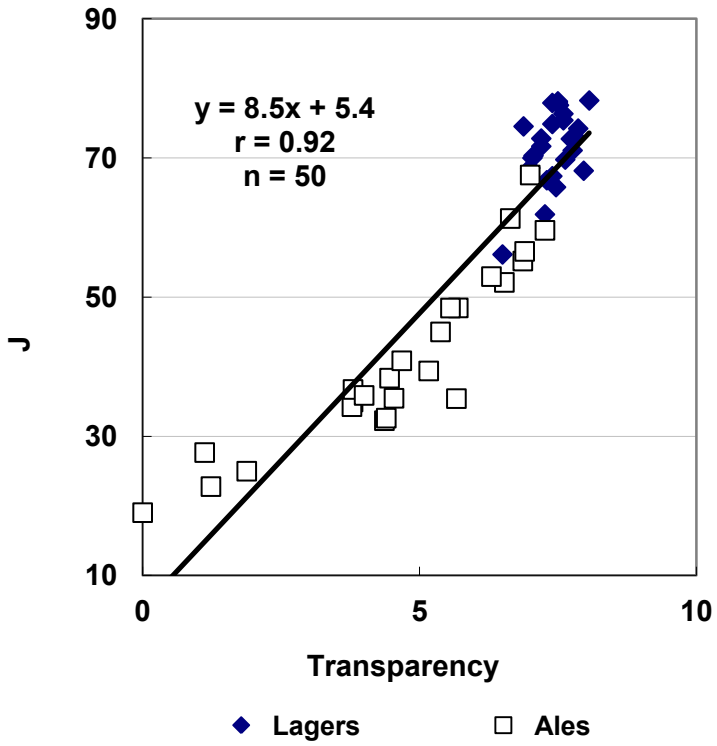

Figure 4.3.3.7 *Lightness (J)* vs *transparency of commercial beers by digital imaging system.*

4.3.4 Correlation between the Colour Appearance Measuring Methods

The correlations of colour appearance measured by digital imaging system (with multiple path-length), tele-spectroradiometry (TSR) and sensory visual method (with highball glass) were investigated in this research. Six commercial beers were tested. The output results of digital imaging system and SPD (spectral power distribution) data measured were transformed to CIECAM02 Lightness (J), Colourfulness (M) and Hue Composition (H) values, and they have a direct correspondence to the visual lightness, colourfulness and hue, respectively, showing as Figures 4.3.4.1-4.3.4.9.

Figures 4.3.4.1 to 4.3.4.3 show the CIECAM02 predictions of J, M and H measured by digital imaging system plotted against those measured by TSR (with highball glasses)

for the commercial beers, respectively. It is obtained that the lightness and hue attributes highly correlate measured by digital imaging system and TSR (Figure 4.3.4.1 and 4.3.4.3).

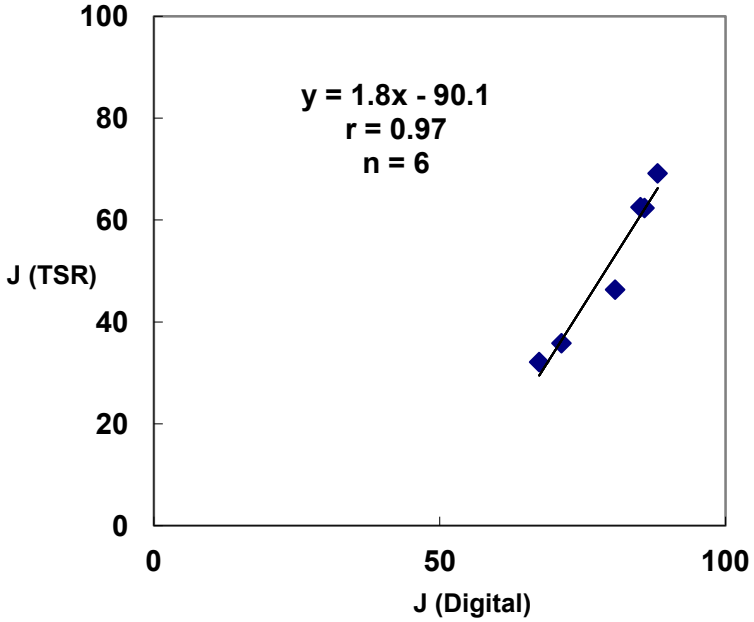

Figure 4.3.4.1 *CIECAM02 descriptor of J (lightness) measured by digital imaging system plotted as function of J (lightness) measured by TSR.*

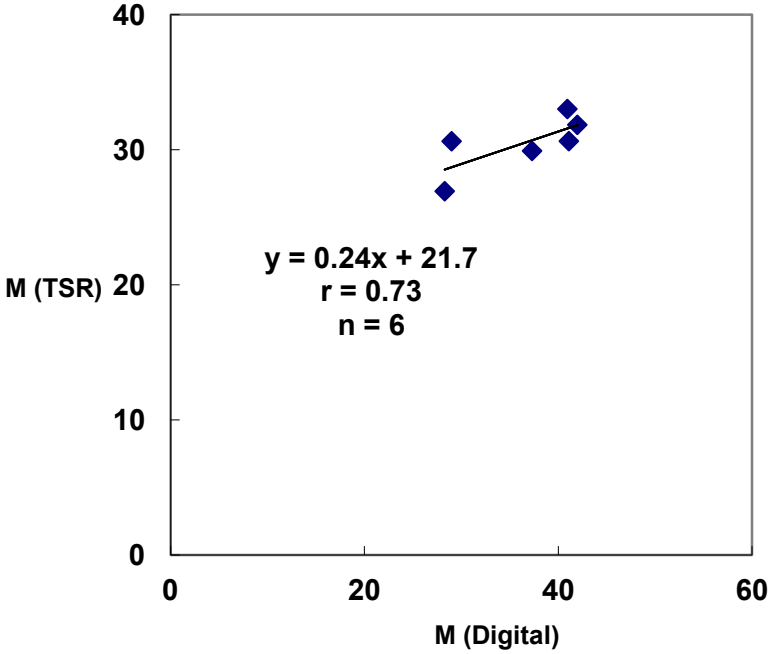

Figure 4.3.4.2 *CIECAM02 descriptor of M (colourfulness) measured by digital imaging system plotted as function of M (colourfulness) measured by TSR.*

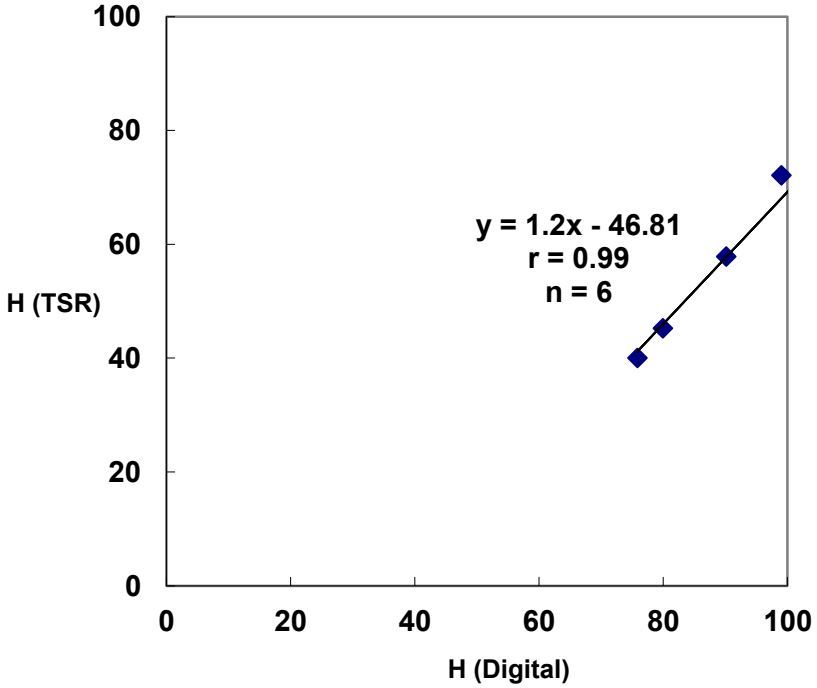

Figure 4.3.4.3 *CIECAM02 descriptor of H (hue) measured by digital imaging system plotted as function of H (hue) measured by TSR.*

The instrumental results are found to have reasonable agreement with the visual results. Figures 4.3.4.4-4.3.4.9 show the correlations between each colour appearance attribute evaluated by psychophysical assessments and physical measurements (digital imaging system and TSR), respectively.

These results obtained in this investigations show high correlations between the sensory visual assessments and digital imaging system (with multiple path-length), and the sensory visual assessments and TSR (with highball glasses), especially on lightness and hue with higher correlation coefficients. But similarly as previous studies (Section 4.1.1.2), the correlations on colourfulness (Figures 4.3.4.5 and 4.3.4.8) were lower, as the coefficients are smaller comparing with those of lightness and hue. This indicated to improve the characterization of the colour appearance model associating with the truly psychophysical perception of the colour appearance by the human eyes.

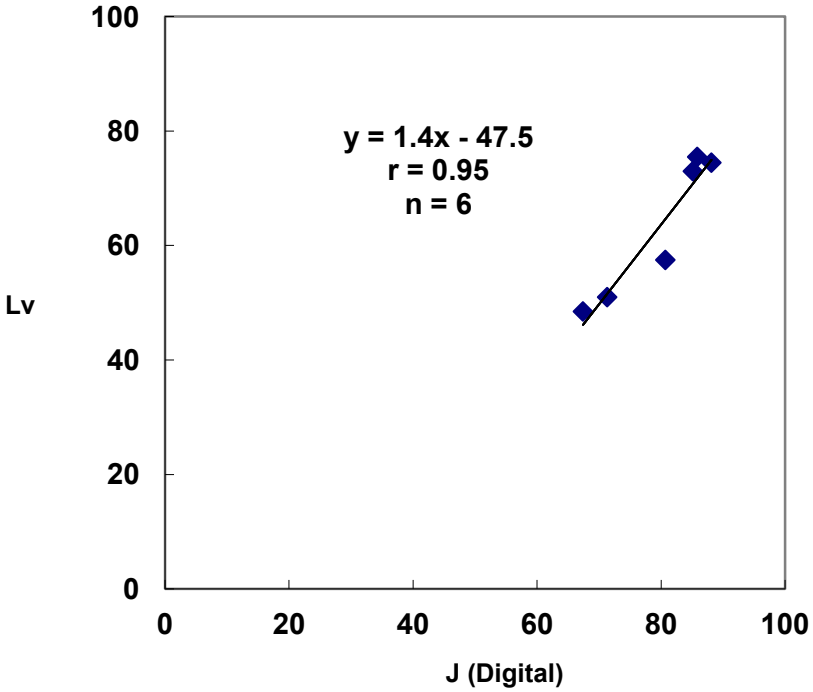

Figure 4.3.4.4 *CIECAM02 descriptor of J (lightness) measured by digital imaging system plotted as function of visual L_v (lightness).*

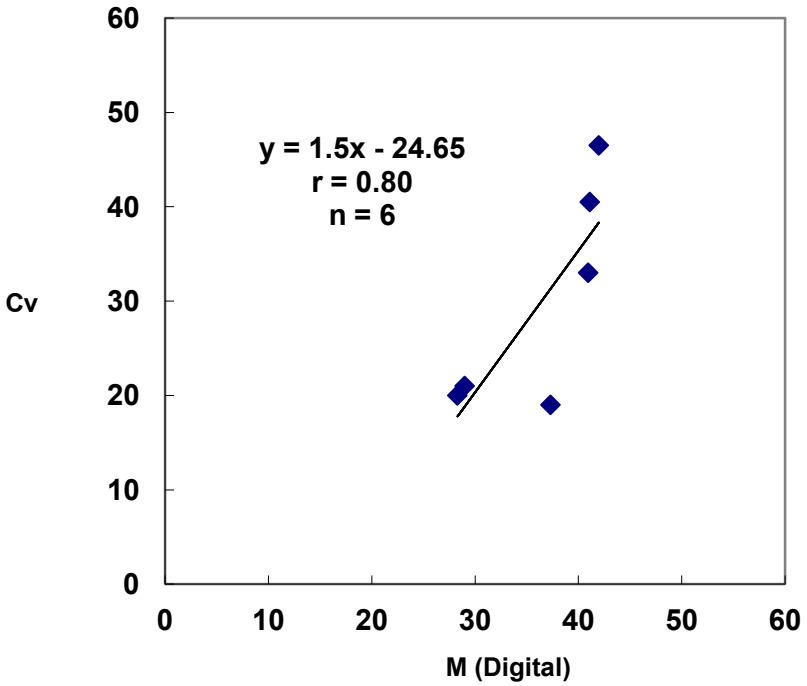

Figure 4.3.4.5 *CIECAM02 descriptor of M (colourfulness) measured by digital imaging system plotted as function of visual C_v.*

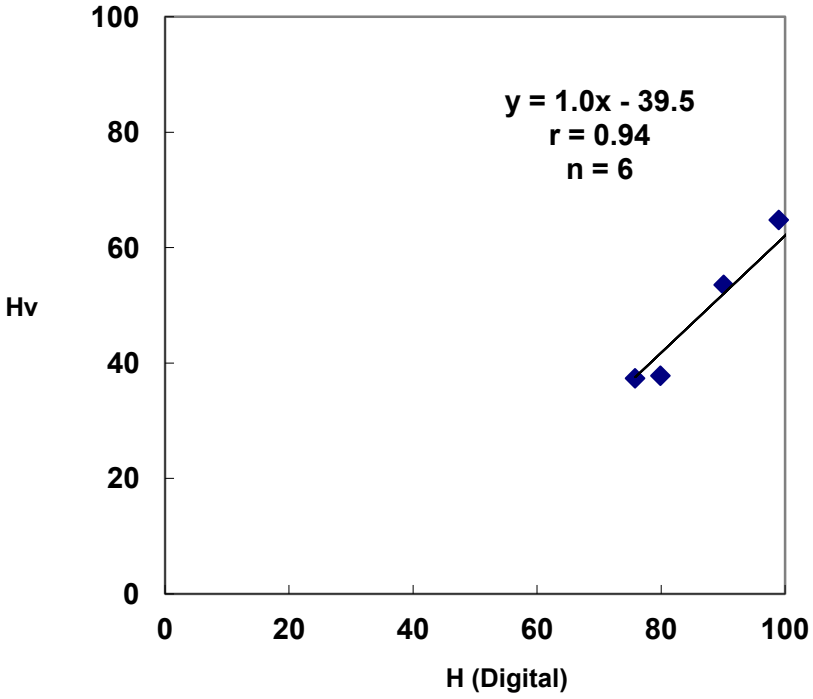

Figure 4.3.4.6 *CIECAM02 descriptor of H (hue) measured by digital imaging system plotted as function of visual H_v.*

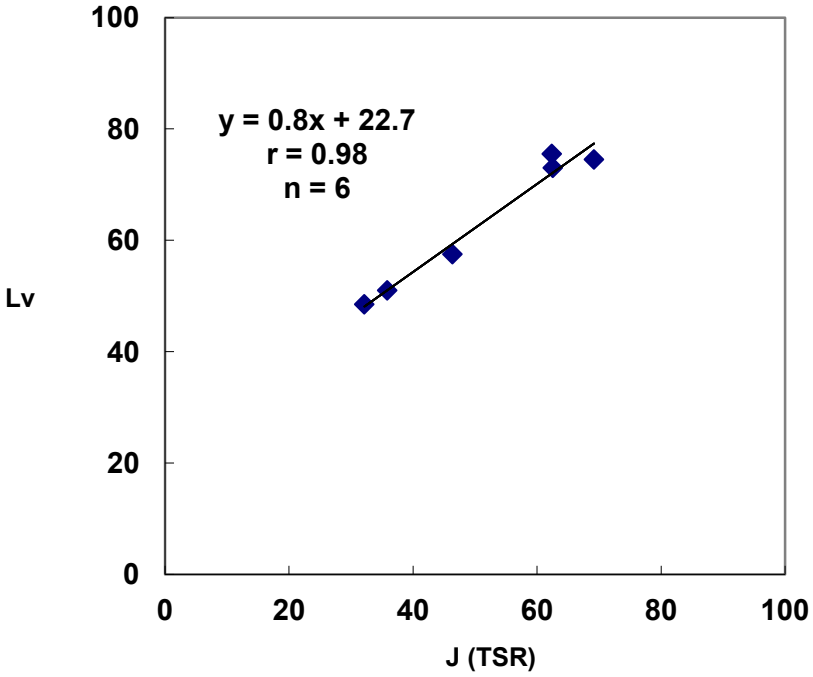

Figure 4.3.4.7 *CIECAM02 descriptor of J (lightness) measured by TSR plotted as function of visual L_v.*

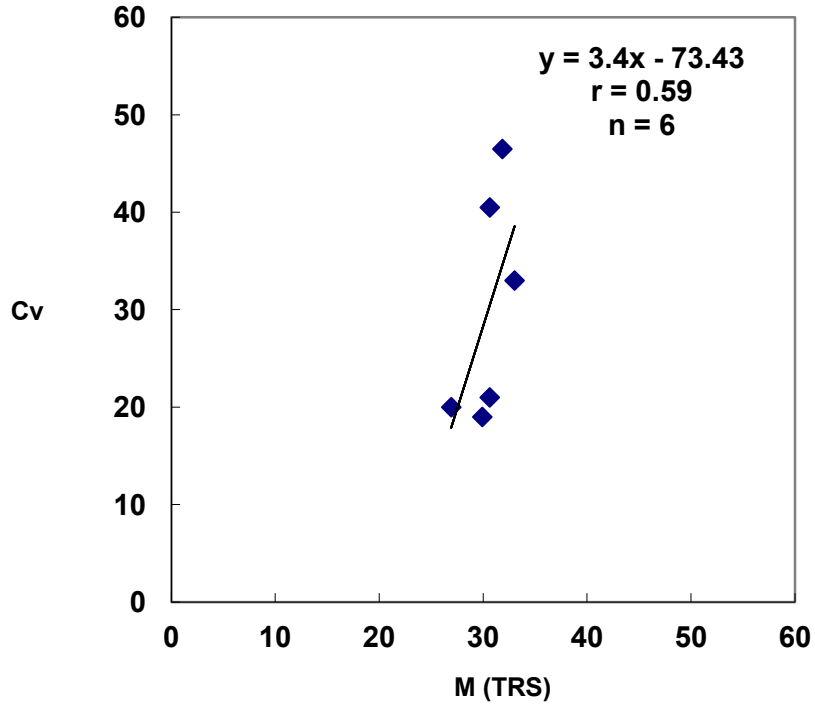

Figure 4.3.4.8 *CIECAM02 descriptor of M (colourfulness) measured by TSR plotted as function of visual* C_v.

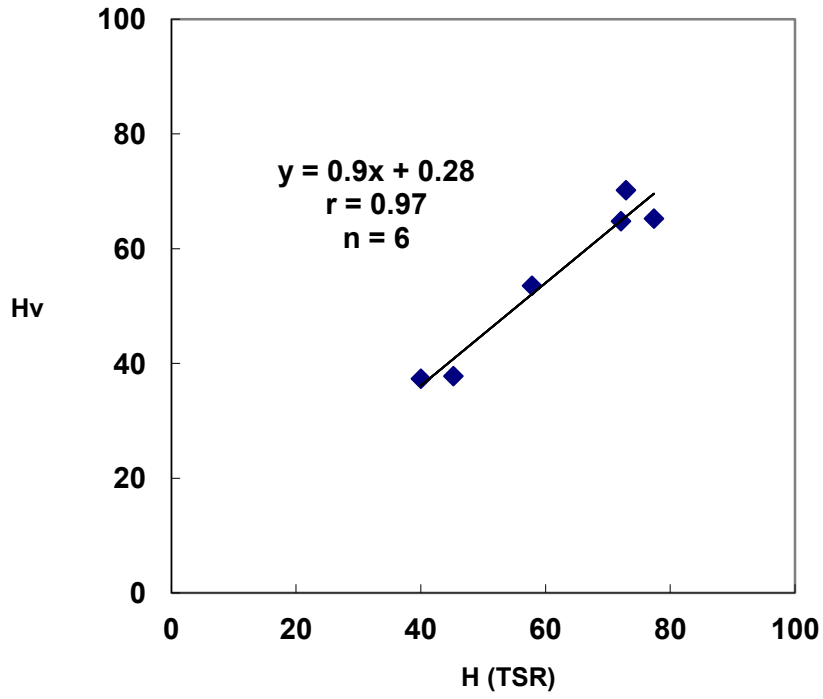

Figure 4.3.4.9 *CIECAM02 descriptor of H (lightness) measured by TSR plotted as function of visual* H_v.

4.3.5 The System at the Brewing Processing Line

The Coors standard method for clarity emphasises that haze measurement is sensitive to measurement angle, thus visual assessments must also be made of all samples. Within Coors, sensory words used to express clarity include cloudy, hazy, bitty, medium clarity, good clarity, and bar bright. These terms are used as adjectives rather than as reference points on a formal scale. So, we could see the disadvantages in this case, the conventional instruments may mislead the measurement results, which are then confirmed by human sensory test. But at present, there is not a standard sensory evaluation system on such requirements of psychophysical tests.

What are industries needs for their quality control on colour and translucency of the products? “We would like a single machine which mimics the human eye and covers all elements of visual perception of products” (Coors Brewers Ltd), and “Our business would welcome developments in the colour of whisky, the haze and colour of specific conditions, like pre-fermentation worts (65-95°C)” (Chivas Brothers). But, neither the current theoretical nor instrumental approaches the industry needs on the quality/quantity measurements.

According to the chapters above, the new digital system could be used online as a colour/translucency monitoring instrument in brewing processing, as Figure 4.3.5.1. The cell could be set at the critical control points (CCPs) decided (Section 4.2), at mash clarification end, boil start, boil end, pre-filtration; post-filtration.

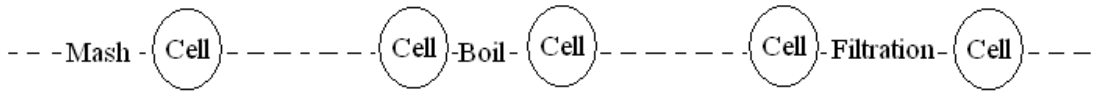

Figure 4.3.5.1 *Introducing the cell of the digital system into brewing processing line.*

Introducing the system on brewing processing line could implement process monitoring online on colour and translucency of beer products on time. Thus, the brewers could

take remedial measures on time if something goes wrong.

Some modification and improvements of the cell are needed, for example, pumps must be included next to the cell, and also the filters to clarify the grist/particles and degas, as Figure 4.3.5.2.

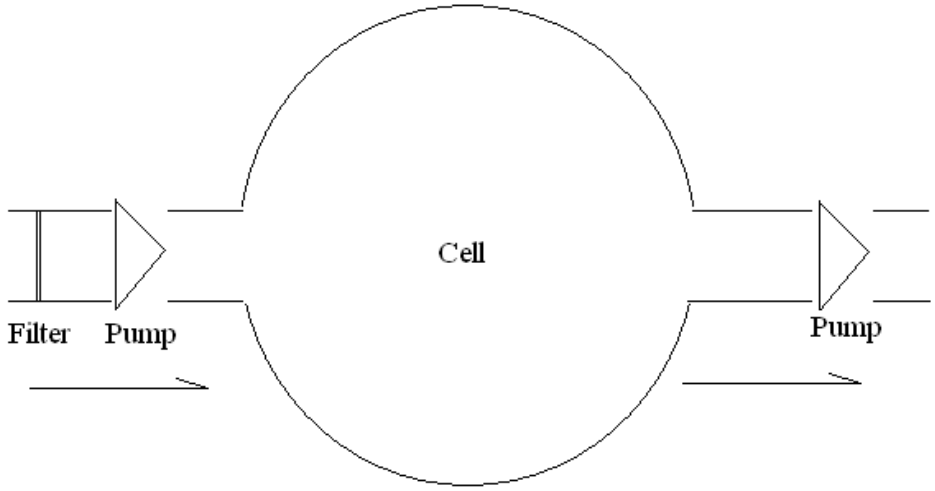

Figure 4.3.5.2 *Flow chart of the cell as part of the digital system into the processing line.*

The system as tested here has not performed adequately for darker liquids. A new cell with more depth rather than the six-depth would be required for such products, together with updated software.

Summary

- A stepping cell was optimised designed for variety of liquid food products. This cell can be further modified incorporation a pump for continuous or stepped in line sampling. The accompanying software was also developed to automatically evaluate transparency (T), lightness (J), colourfulness (M), and hue composition (red (R), yellow (Y), green (G) and blue (B)) data for each sample.

- When comparing a fixed aperture lens with a zoom lens for four hours, all the CV values of consistency results by the zoom lens were smaller than those by fixed aperture lens, so that there was less variability of colour and translucency measurements. Colour difference calculations show the same results in the same time period. Thus, zoom lens was selected for the following research in this study.

- The system repeatability test was carried out using both pilot brewed lager and ale samples. The colour difference ΔE_{00} ranged from 0.02 to 1.38 (lager) and 0.10 to 1.31 (ale), by which humans visual system almost cannot tell the colour differences of the liquid samples.

- The digital imaging system indicates that lager product samples appeared lighter, less colourful and yellower than most of ale product samples at CCPs. This is consistent with conventional instrument results and human observations. In the pilot brewing processing, the digital imaging system was introduced as a near line colour/translucency checking instrument at each of the CCPs. The colour/translucency changes in similar trends could be obtained by this system, and thus, this system could be set at the CCPs in real industry productions as monitoring instrument and technology.

- Comparing to the EBC Recommended method and Lovibond discs, the digital imaging system is more sensitive to distinguish colours. Lightness obtained through digital imaging system was correlated to EBC colour, therefore, EBC colour could be predicted by lightness obtained through digital imaging system.

- Some darker ale samples seemed to respond differently when evaluated by the digital imaging system. Additional system testing with some extreme dark samples show different trend. More modification for the system is needed, such as redesigning the metal cell to add more depth (i.e. < 2mm), so that dark liquids

can be distinguished more readily. Additionally, the software will be updated to integrate the new designed cell.

- The results obtained by turbidimetry in EBC unit showed that the extreme dark samples were low in haze (with low EBC values), whereas they appeared opaque from the digital imaging system. This means that the transparency/opacity results for dark liquids relate to colour (lightness) more than haze characteristics (e.g. EBC values) by the digital imaging system.

- Colour and translucency performance of commercial lagers and ales were evaluated by the digital imaging system. It is also obtained that, for beers, the lightness attributes were correlated to EBC colour, and the translucency characteristic is highly correlated to lightness rather than turbidity (EBC value).

- Colour appearance attributes were assessed by psychophysical methods (visual methods) and instrumental methods (digital imaging system and TSR). From the comparison results between these three methods, lightness and hue were highly linear correlated between these three methods. Correlations of colourfulness between these three methods were weaker

Chapter 5. CONCLUSIONS

In food and beverage products, the consumers often assess the initial quality of product by their colour and appearance, e.g. clarity. At present there are limitations in our understanding of the theoretical and technological aspects of the measurement of both colour and translucency of liquid food products, including beers and other alcoholic beverages.

Novel methods for determination colour appearance and translucency of alcoholic beverages are introduced and applied in this research. Comparing with different standard conventional methods existing to determine colour in beers, these novel methods including psychophysical assessment (sensory visual tests) and physical assessments (DigiEye digital imaging system), consider both characteristics of colour and translucency of beers. Advantages over conventional measuring techniques for beer colour/translucency were obtained by applying these novel methods in terms of colour appearance attributes, which are expressed as lightness, colourfulness and hue composition, transparency of the beer subjects. Comparing DigiEye digital imaging system with some other physical methods, the advantages and disadvantages are listed:

Advantages:

- Providing appearance attributes values
- Capturing the appearance of the whole object
- Instant measurement and analysis
- No-contact with object
- No limit to shape or form of object
- Considering luminance-dependents effects
- High repeatability

Disadvantages:

- Not accurate as spectral based system

- Need calibration target
- Limiting to illumination and environment
- More improvements for dark samples
- More improvements for fitting translucency characteristics (i.e. transparency or opacity values) with haze content (i.e. EBC values).

5.1 Psychophysical determinations

Psychophysical experiments were conducted using model samples developed with different colorants and scatterers, and also using real industrial samples of beers and red wines. Observers performed similarly on commercial beer samples comparing with experiment results using model samples. The overall observer performances indicated that a group of ten observers can successfully assess the colour appearance attributes for lightness, hue, industry terms on translucency, with slightly larger variation for the colourfulness attribute.

As red wines increase in redness, observers perceived that they become darker and more colourful. Although the red wine samples were taken from different stages in processing, the observers scored the colour appearance in a narrow range for each type of red wine sample, especially on hue values.

All the panellists that joined in psychophysical experiments on colour appearance attributes were experts in this area, who were trained and could be expected to be sensitive to these colour apprearance attributes. In any future study, the inclusion of 'untrained' panellists in visual assessments to facilitate the quantification of training on the magnitude and variance of such experiments may be considered.

Each industry has its own visual appearance attributes, and some of the terms are used as adjectives rather than as reference points on a formal scale. In this study, it was shown that some of the terms may cause an observer some confusion. Based on beer and red wine samples, observers linearly correlated opacity, clarity and the five-point scale with transparency. Opacity was clearly opposite in meaning to transparency, although these two tests were taken separately. When the values of opacity and transparency were added together, a value close to ten was obtained. As the correlation between these terms had been established, 'transparency' may be used as a common term in industries to describe clarity of beverage and any other liquid products. But some more experiments are needed to extend the samples database to prove this hypothesis.

5.2 Brewing Processing Control Points

During beer production, particularly in wort clarification and boiling, the CIELAB colour parameters show distinct changes, whereas in the fermentation and conditioning stages, the changes are less obvious. This may be caused by fluctuation of solids during fermentation and conditioning, in which absorption of colour subjects occurred, and thus both colour and translucency were affected. Representative measurements and analyses are difficult, because of the complexity of reactions and operations in these processing stages and variation of raw materials.

As translucent materials, alcoholic beverages have a range of particle sizes, which cause difficulties for quality control on their colour attributes, not only on final products, but also the whole processing line. The particles contained in the processed product for beers have large effects on the colour attributes. The CIELAB test results show that there are significant differences between the liquids containing particles or not. The

trends of L^*, a^* and b^* values of the liquids without any particles were clear, whereas those of the liquids containing particles are more varied. In particular during the operations concerning larger particles (i.e. fermentation and conditioning), the L^*, a^* and b^* values are spread widely, due to their presence. Thus, beer or wort samples must be clarified for their colour measurements by conventional methods, like tristimulus measurements based on spectrophotometer, which means the particles or haze components should be separated.

So "Where should the monitoring points in the whole processing be?" The aim here was to determine the critical control points governing colour and translucency development in specific liquid processes for beer during processing. Based on the background theory and practical experiments in this study, we set the critical control points in brewing process at the end of mash clarification, boil start and end points, and pre-filtration and post-filtration of the final beer products. At these stages, there are magnitude changes of colour/translucency, which are caused by operations, chemical reactions, etc.

5.3 Instrument Testing in Beer Production and Products

The digital imaging system was evaluated both on pilot brewed beers (off-line and final products) and commercial products. The camera with fixed aperture and zoom lens both resulted in a system that showed high precision on image capture. It was presumed that the fixed lens would perform better on precision, because there was no need to adjust the focus on each testing. Unexpectedly, the fixed aperture lens does not show an enhanced performance compared to the zoom one. Therefore, the zoom lens was used in the system for further evaluation in this study. The system showed desirable consistency over four hours from six tests on two pilot brewed samples.

In the pilot brewing processing, the digital imaging system was introduced as an off-line

colour/translucency checking instrument at each of the CCPs. For all pilot brewed lagers and most ales, the colour appearance attributes and translucency characteristics followed a similar trend, which tracked known changes during brewing (Section 2.1). However the system did not perform adequately on extreme dark product samples, by which the dark ales were distinct from the other samples. In other words, more evaluation and improvement are needed for the system development, such as software modification which could be used to analyse special liquids (i.e. extremely dark liquid), and a cell designed to accommodate more depth (i.e. < 2mm). For the commercial products, there is a continuum from lager to ale on colour appearance based on the novel technology. But it seems that the translucency characteristics obtained from the digital system relies on colour (lightness) more than haze characteristics.

Some of final products of pilot brewed lagers and ales and some commercial beers which are assigned similar EBC colour values have been distinguished by the new novel digital imaging system. The lightness component gave an approximate correlation to the EBC colours of the pilot brewed beer final products and commercial beers as determined by the EBC Recommended Method. Beers with low EBC values have high lightness values, which steadily decrease as the EBC colour of the lagers increased.

This novel digital imaging system might solve the problem that there are limitations in the measurement of the colour/translucency of beverages. Comparing with conventional instruments and methods used for beer colour/translucency analysing, this novel system is more effective and could be set online directly. Furthermore, the two characteritics of colour and translucency could be output at the same time.

Regarding the measurement of colour appearance, DigiEye digital imaging system showed good agreement with the colour appearance parameters on lightness and hue compositions assessed by TSR and by psychophysical assessments. Some improvement on the correlation of colourfulness is still needed.

In summary, this novel technology has the potential to be a powerful tool for analysing beverage colour appearance, and probably be the method of choice in the future of the brewing industry. But some improvements are needed to model between translucency characteristics (i.e. the output transparency and opacity) with the turbidity (i.e. in EBC unit).

Chapter 6. FUTURE WORK

Set up database as standards for industrial beer products at Critical Control Points (CCPs) of colour/translucency. The monitoring colour/translucency quality by digital imaging system should regard the database for beer products.

Modification and improvement of the digital imaging system on colour appearance are needed to correlate better with human perception, especially on colourfulness attribute.

Fitting translucency output ‘Transparency’/ ‘Opacity’ with turbidity EBC unit.

The DigiEye digital imaging system has been used as an off-line colour/translucency monitoring instrument in the ICBD pilot brewery. More modification and improvements are needed if the system is to be used as an online instrument for beer industry:

- Pumps should be designed for setting up the cell into the processing line
- The liquid tested in the cell must be free from grist or gas. All the pilot brewed samples needed to be centrifuged or filtered to remove grist/yeast or degassed. Thus a method for solids removal is needed before the liquid being pump-in the cell
- The system has not performed adequately for darker liquids. A new cell with more depth rather than the six-depth is going to be designed, together with updated software.

REFERENCES

Andrews, J.M.H., 1992. External Wort Boiling—Latest Developments. *Proc. 22nd Conv. Inst. Of Brewing (Australia and New Zealand Section),* Melbourne. pp. 65-68.

Antrobus, C.J. and Large, P.J., 1997. Changes in the Cationic Isoenzymes of Peroxidase during the malting of Barley. I: Tissue Location Studies. *J. Inst. Brew.* 103, 4, 227-231.

Apperson, K., Leiper, K.A., McKeown, I.P. and Birch, D.J.S., 2002. Beer Fluorescence and the Isolation, Characterisation and Silica Adsorption of Haze-Active Beer Proteins. *J. Inst. Brew.* 108, 2, 193-199.

Asano, K., Shinagawa, K. and Hasimoto, N., 1982. Characterization of Haze-Forming Proteins of Beer and Their Roles in Chill Haze Formation. *J. Am. Soc. Brew. Chem.* 40, 147-154.

ASBC (American Society of Brewing Chemists), Beer-10A 2004. Spectrophotometric Colour Method. *Methods of Analysis, 9th Ed.* American Society of Brewing Chemists: St. Paul, MN.

ASTM., 1982. Committee D1. Standard Practice for Visual Evaluation of Colour Differences of Opaque Materials. ASTM D, 1684.

ASTM., 1997. D. 1003-61. Standard Test Method for Haze and Luminous Transmittance of Transparent Plastics. American Society for Testing and Materials. Philadelphia, PA.

ASTM., 2001. E. 301-01. Standard Practice for Computing the Colors of Objects by Using the CIE System, Subcommittee E12.04 on Color and Appearance Analysis.

ASTM., 2008. E. 1697-05. Standard Test method for Unipolar Magnitude Estimation of Sensory Attributes, *in: Annual Book of ASTM Standards, Vol. 15.08, End Use Products.* American Society for Testing and Materials, Conshohocken, PA. pp. 122-131.

Bamforth, C.W., 1985. Biochemical Approaches to Beer Quality. *J. Inst. Brew.* 91, 154-160.

Bamforth, C.W., 1994. β-Glucan and β-Glucanases in Malting and Brewing: Practical Aspects. *Brew. Dig.* 5, 12-16.

Bamforth, C., 1998. *Beer, Tap into the Art and Science of Brewing.* Insight Books. New York. pp. 5-33.

Bamforth, C.W., 1999. Beer Haze. *J. Am. Soc. Brew. Chem.* 57, 81-90.

Bamforth, C., 2003. *Beer, Tap into the Art and Science of Brewing, 2nd Ed.* Oxford University Press. New York. pp. 7-64, 141-158, 171-186.

Barron, V. and Torrent J., 1986. Use of the Kubelka-Munk Theory to Study the Influence of Iron Oxides on Soil Colour. *J. Soil Sci.* 37, 499-510.

Bartleson, C.J., 1978. A Review of Chromatic Adaptation. *AIC Proceedings, Color.* 77, 63-96.

Bentham, 1997. *A Guide to Spectroradiometry.* Bentham Instruments Ltd.Berkshire, UK.

Billmeyer, F.W. Jr and Chen Y., 1985. On the Measurement of Haze. *Color Res. Appl.* 10, 219-224.

Boring, E.G., 1942. Sensation and Perception, *in: The History of Experimental Psychology.* Appleton-Century-Crofts, New York. pp 36-44.

Boulton, C. and Quain, D., 2001. *Brewing Yeast and Fermentation.* Wiley-Blackwell, London. pp. 19-63.

Brainard, D.H., 2003. Color Appearance and Color Difference Specification, *in: The Science of Color, 2nd Ed* (Ed: Shevell, S.K.). Optical Society of America, Oxford, UK. pp. 191-216.

Bretthauer, G., Müller, J.U. and Ruchter, M., 2009. Automation, *in: Handbook of Brewing Processes, Technology, Markets* (Ed: Eßlinger, H.M.). Wiley-Vch Verlag GmbH & Co. KGaA, Weinheim. pp. 531-554.

Briggs, D.E., Hough, J.S., Stevens, R. and Young, T.W., 1981. *Malting and Brewing Science, Vol 1: Malt and Sweet Wort, 2nd Ed.* Chapman & Hall, London. pp. 1-14, 254-303.

Briggs, D.E., 1998. *Malts and Malting.* Blackie Academic, London. pp. 1-34, 113-228.

Briggs, D.E., Boulton, C.A., Brookers, P.A. and Stevens, R., 2004. *Brewing Science and Practice.* Woodhead, Cambridge. pp. 85-170, 189-226, 306-362, 662-715.

Broch, J., 2002. Optical and Appearance Properties, *in: Handbook of Physical Testing of Paper, Vol 2, 2nd Ed., Revised and Expanded* (Ed: Borch, J., Lyne, M.B., Mark, R.E. and Habeger, C.C.Jr.). Marcel Dekker Inc, New York. pp. 90-149.

Buckee, G.K., Morris, T.M. and Bailey, T.P., 1986. Calibration and Evaluation of Haze Meters. *J. Inst. Brew.* 92, 475-482.

Burger, M. and Becker, K., 1949. Oxalate Studies on Beer. *Proc. Am. Soc. Brew. Chem.* 102-115.

Burger, M., Glenister, P.R. and Becker, K., 1956. Oxalate Studies in Beer. II. Supplementary Work and Observations on Calcium-Oxalate Relationships. *Proc. Am. Soc. Brew. Chem.* 169-178.

Chandra, G.S., Proudlove, M.J. and Baxter, E.D., 1999. The Structure of Barley Endosperm-An Important Determinant of Malt Modification. *J. Food Sci. Agric.* 79, 37-46.

Chapon, L., 1994. The Mechanics of Beer Stabilization. *Brew. Guard.* 123, 46-50.

Christie, R.M., 2001. *Colour Chemistry.* Royal Society of Chemistry, Cambridge. pp.12-20.

CIE (Commission Internationale de l' Éclairage), 1971, 1986. *CIE Publication 15: Colorimetry.* Vienna: CIE Central Bureau.

CIE (Commission Internationale de l' Éclairage), 1995. *CIE Publication 116-1995. Industrial Colour-difference Evaluation.* Vienna: CIE Central Bureau.

CIE (Commission Internationale de l' Éclairage), 2004. *CIE Publication 15: Colorimetry 3rd Ed.* Vienna. CIE Central Bureau.

Clarkson, S.P., Large, P.J. and Bamforth, C.W., 1992. Oxygen-Scavenging Enzymes in Barley and Malt and Their Effects during Mashing. *J. Inst. Brew.* 98, 111-115.

Coghe, S., Vanderhaegen, B., Pelgrims, B., Basteyne, A. and Delvaux, F.R., 2003. Characterization of Dark Specialty Malts: New Insights in Colour Evaluation and Pro- and Antioxidative Activity. *J. Am. Soc. Brew. Chem.* 61, 3, 125-132.

Coote, N. and Kirsop, B.H., 1976. Factors Responsible for the Decrease in pH during Beer Fermentation. *J. Inst. Brew.* 82, 149-153.

Corby Bottlers. Clarity Analysis Methodologies of Beverages.

Cui, G., Luo, M.R., Rigg, B., Butterworth, M., Maplesden, N. and Dakin, J., 2004. Grading Textile Fastness. Part 4: An Inter-laboratory Trial Using DigiEye System. *Coloration Technology.* 120, 5, 231-235.

Datamonitor London, 1995. British Beer Market. England.

De Schutter, D.P., Saison, D., Delvaux, F., Derdelinckx, G. and Delvaux F.R., 2008. The Chemistry of Aging Beer, *in: Beer in Health and Disease Prevention* (Ed: Preedy, V.R.). Academic Press Inc, New York. pp. 375-388.

Del Pilar Burera, M., Chrife, J., Resnik, S.L. and Lozano, R.D., 1987. Nonenzymatic Browning in Liquid Model Systems of High Water Activity: Kinetics of Color Changes due to Caramelization of Various Single Sugars. *J. Food Sci.* 52, 4, 1059-1062, 1073.

Delgado-Vargas, F. and Paredes-López, O., 2003. *Natural Colorants for Food and Nutraceutical Uses.* CRC Press, Florida, USA. pp. 7-26, 325.

Depraetere, S.A., Delvaus, F., Schutter, D., Williams, I.S., Windericks, J. and Delvaus, F.R., 2007. The Influence of Wort Aeration and Yeast Preoxygenation on Beer Staling Processes. *Food Chem.* 107, 242-249.

Dornbusch, H., 2000. Bavarian Helles. *Classic Beer Style Series No. 17. Brewers Publications.* Boulder, Colorado. pp. 59.

DuBose, C.N., Cardello, A.V. and Maller, O., 1980. Effects of Colorants and Flavorants on Identification, Perceived Flavor Intensity and Hedonic Quality of Fruit-flavored Beverages and Cake. *J. Food Sci.* 45, 1393-1399, 1415.

Durand, G.A., Corazza, M.L., Blanco, A.M. and Corazza, F.C., 2009. Dynamic Optimization of the Mashing Process. *Food Control.* 20, 12, 1127-1140.

Eaton, B., 2006. An Overview of Brewing, *in: Handbook of Brewing* (Ed: Priest, F.G. and Stewart, G.G.). CRC Press, Florida. pp. 77-90.

EBC (European Brewery Convention), 1998. *Analytica-EBC.* Verlag Hans Carl Gentränke-Fachverlag. Grundwerk.

EBC (European Brewery Convention), 2004. *Analytica-EBC.* Verlag Hans Carl Gentränke-Fachverlag. Nurnburg.

Eßlinger, H.M., 2009. Fermentation, Maturation and Storage, *in: Handbook of Brewing Processes, Technology, Markets* (Ed: Eßlinger, H.M.). Wiley-Vch Verlag GmbH & Co. KGaA, Weinheim. pp. 207-224.

Enevoldsen, B.S., 1970. Application of Debranching Enzyme, Pullulanase in Brewing from Unmalted Cereals. I. Conditions of Mashing and Carbohydrate Composition of the Wort. *J. Inst. Brew.* 76, 546-554.

Enevoldsen, B.S. 1975. Debranching Enzymes in Brewing. *Proc. Eur. Brew. Conv. Congr. 15th,* Nice. 683-697.

EPA, 1999. Guidance Manual for Compliance with the Interim Enhanced Surface Water Treatment Rule: Turbidity Provisions. EPA 815-R-99-010. US Environmental Protection Agency, Office of Water, Washington.

Everitt, B.S., 2006. *The Cambridge Dictionary of Statistics,* 3rd *Ed.* Cambridge University Press, Cambridge. pp. 228.

Fairchild, M.D., 2005. *Colour Appearance Models, 2nd Ed.* John Wesley & Sons Ltd. West Sussex, England. pp.146-165.

Fayle, S.E. and Gerrard, J.A., 2002. *The Maillard Reaction.* Royal Society of Chemistry, London. pp. 1-8.

Fengxia, S., Yuwen, C., Zhanming, Z. and Yifeng, Y., 2004. Determination of Beer Colour Using Image Analysis. *J. Am. Soc. Brew. Chem.* 62, 163-167.

Fisher, C. and Thomas, R. S., 1997. *Food Flavours: Biology and Chemistry.* Royal Society of Chemistry, Cambridge. pp. 99-124.

Francis, F.J., 1995. Quality as Influenced by Color. *Food Quality and Preference.* 6, 3, 149-155.

Francis, F.J. and Clydesdale, F.M., 1975. *Food Colorimetry: Theory and Applications*. AVI Publishing, Westport, CT.

Gan, Q., Howell, A., Field, R. W., England, R., Bird, M. R., O'Shaughnessy, C. L. and Mekechinie, M. T., 2001. Beer Clarification by Microfiltration — Product Quality Control and Fractionation of Particles and Macromolecules. *J. Membrane Sci.* 194, 185-196.

Gescheider, G.A., 1997. *Psychophysics: the Fundamentals, 3rd Ed.* New Jersey, Lawrence Erlbaum Associates, Inc, Mahwah. pp. 207-230.

Gilbert, P. and Haeberli, W., 2008. *Physics in the Arts.* Elsevier Academic Press, Burlington. pp. 82-98.

Gilchrist, A. and Nobbs, J.H., 1997. Dyeing Machine Control Using In-line Colour Measurement. Part 1: In-line Monitoring of Colour in Dye Liquors. *J. Soc. Dyers Colourists.* 113, 327-332.

Goldammer, T., 2008. Hops, *in: the Brewers' Handbook—The Complete Book to Brewing Beer.* Apex Publisher. pp. 49-72.

Goldstein, E.B., 2001. Cross-talk between Psychophysics and Physiology in the Study of Perception, *in: Blackwell Handbook of Perception* (Ed: Goldstein, E.B.). Blackwell Publishers, Oxford. pp. 1-23.

Goldstein, E.B., 2002. *Sensation and Perception, 6th Ed.* Wadsworth. pp.1-34, 185-224.

Gonnet, J.F., 1998. *Colour Effects of Co-pigmentation of Anthocyanins Revisited— 1. A Colorimetric Definition Using CIELAB Scale. Food Chem.* 63, 3, 409-415.

Gonzalez-Miret Martin, M.L., Ji, W., Luo, R., Hutchings, J.B. and Francisco, J.H., 2007. *Measuring Colour Appearance of Red Wines. Food Quality Preference.* 18, 6, 862-871.

Grainger, K., 2009. Wine Quality: Tasting and Selection. Blackwell publishing. pp. 21-34.

Green, B.G., Shaffer, G.S. and Gilmore, M.M., 1993. Derivation and Evaluation of a Semantic Scale of Oral Sensation Magnitude with Apparent Ratio Properties. *Chemical Senses.* 18, 683-702.

Gretenhart, K.E., 1997. Specialty Malts. *MBAA Tech. Quart.* 34, 2, 102-106.

Hari, R.K., Patel, T.R. and Marting, A.M., 1994. An Overview of Pigment Production in Biological System: Functions, Biosynthesis and Applications in Food Industry. *Food Reviews International.* 10, 49-70.

Hind, H.L., 1950. *Brewing Science and Practice, Vol. 2.* Chapman & Hall, London. pp. 400-475.

Hodge, J.E., 1953. Dehydrated Food, Chemistry of Browning Reactions in Model Systems. *J. Agric. Food Chem.* 1, 15, 928-943.

Hoggs, C.S., Nobel, F.R., 1979. A Kubelka-Munk Analysis of the influence of Iron and Titanium Oxides on the Optical Properties of Hard Porcelain. *Sci. Ceram.* 10, 703-710.

Hong, G., Luo, M.R. and Rhodes, P.A., 2000. A Study of Digital Camera Colorimetric Characterization Based on Polynomial Modeling. Colour Res. Appl. 26, 76-84.

Hornsey, I.S., 1999. *Brewing.* The Royal Society of Chemistry, Cambridge. pp 1-14, 85-98.

Hough, J.S., Briggs, D.E., Stevens, R. and Young, T.W., 1982. *Malting and Brewing Science II.* Chapman and Hall, London. pp. 499-526, 776-838.

Howling, D., 1979. The General Science and Technology of Glucose Syrups', *in: Sugar: Science and Technology* (Ed: Birch, G.G. and Parker, K.J.). Applied Science Publishers,

London. pp. 259-285.

Hudston, H.R., 1969. Measurement of Colour in Wort and Beer. *Tech. Quart. Master Brewers Assoc. Americas*. 6, 3, 164.

Hughes, P.S. and Baxter, E.D., 2001. *Beer Quality, Safety and Nutritional Aspects,* Royal Society of Chemistry, Cambridge. pp. 14-39.

Hunt, R.W.G., 1998. *Colour Vision in Measuring Colour 3rd Ed.* Foundation Press. pp. 17-31.

Hutchings, J.B., 1999. *Food Colour and Appearance 2nd Ed.* An Aspen Publication, England. pp. 1-30, 61-90.

Hutchings, J.B., 2003. *Expectations and The Food Industry: The Impact of Colour and Appearance.* Kluwer Academic/Plenum Publishers, New York. pp. 141-174.

IOB (Institute of Brewing), 1986. *Recommended Methods of Analysis, Revision, Method* 2.5, 18.

Ishihara, S., 2004. *Ishihara's Test Chart for Colour Deficiency, 38-plate Ed.* Tokyo, Japan, Kanehara Trading Inc.

Jackson, M., 1996. A Pint of Cloudy Please. *Independent.* 25, May, 41.

Ji, W., Luo, R., Hutchings, J.B. and Dakin, J., 2005. Scaling Transparency, Opacity, Apparent Flavour Strength and Preference of Orange Juice. The 10th AIC, Granada, Spain. 729-732.

Jurado, J., 2002a. Prevailing Pilsner. *The Brewer International.* 2, 2, 20-26.

Jurado, J., 2002b. The Renaissance of Red. *The Brewer International.* 2, 4, 30-37.

Kaersgaard, P. and Hejgaard, J., 1979. Antigenic Beer Macromolecules: An Experimental Survey of Purification Methods. *J. Inst. Brew.* 85, 2, 103-111.

Kołtuniewicz, A. and Drioli, E., 2008. *Membranes in Clean Technologies: Theory and Practice.* Wiley-Vch, Weinheim. pp. 17-30.

Kotschevar, L.H. and Luciani, V., 2006. *Presenting Service: The Ultimate guide for the Foodservice Professional, 2nd Ed.* John Wiley & Sons, Inc., Hoboken, new Jersey. pp. 152-187.

Kotz, J.C., Treichel, P. and Townsend, J.R., 2008. *Chemistry and Chemical Reactivity, 7th Ed.* Cengage Learning. pp. 30-32.

Kramer, P., 2006. Barley, Malt, and Malting, *in: Raw Materials and Brewhouse Operation, Vol 1* (Ed: Ockert, K.). Minnesota: Master Brewers Association of the Americas. pp. 15-54.

Kreder, G.C., 1999. Yeast Assimilation of Trub-bound Zinc. *J. Am. Soc. Brew. Chem.* 57, 4, 129-132.

Kubelka, P. and Munk, F., 1931. A Contribution to the Optics of Colorant Layers. *Z. Tech. Physik,* 12, 11a, 593-601 (in German).

Kühbeck, F., Back, W. and Krottenthaler, M., 2006. Influence of Lauter Turbidity on Wort Composition, Fermentation Performance and Beer Quality in Large-Scale Trials. *J. Inst. Brew.* 112, 3, 222-231.

Kühbeck, F., Back, W. and Krottenthaler, M., 2006. Release of Long-chain Fatty Acids and Zinc from Hot Trub to Wort. *Mschr. Brauwiss.* 59, 3/4, 67-77.

Kühbeck, F., Krottenthaler, M. and Back, W., 2006. Influence of Lauter Turbidity and Hot Trub on Wort Composition, Fermentation, and Beer Quality. *J. Am. Soc. Brew. Chem.* 64, 1, 16-28.

Laing, D., and Hendra, J., 1977. *Beer and Brewing.* Macdonald Guidelines, London. pp. 4-24.

Lalor, E., 2002. *Creating the Future in Brewing, Version One.* Quest International, Cork, Ireland. pp. 2-53.

Lawless, H.T. and Heymann, H., 1998. *Sensory Evaluation of Food.* Kluwer Academic/Plenum Publishers, New York. pp. 28-82, 289.

Lawless, H.T. and Heymann, h., 2010. *Sensory Evaluation of Food: Principles and Practices 2nd Ed.* Springer, New York. pp. 149-177.

Leach, R. and Pierce, R., 1993. Colour and Colour Matching, *in: The Printing Ink Manual, 5th Ed.* Springer, New York. pp. 86-139.

Leiper, K.A. and Miedl, M., 2008. Colloidal Stability of Beer, *in: Beer: A Quality Perspective* (Ed: Bamforth, C.W., Russell, I and Stewart, G.). Academic Press, New York. pp. 111-162.

Leiper, K.A., Stewart, G.G. and McKeown, I.P., 2003. Beer Polypeptides and Silica Gel, Part I. Polypeptides Involved in Haze Formation. *J. Inst. Brew.* 109, 1, 57-72.

Leiper, K.A., Stewart, G.G., McKeown, I.P., Nock, A. and Thompson, M.J., 2005.

Optimising Beer Stabilisation by the Selective Removal of Tannoids and Sensitive Proteins. *J. Inst. Brew.* 111, 2, 118-127.

Lewis. M.J. and Bamforth, C.W., 2006. *Essays in Brewing Science.* Springer, New York. pp. 20-27, 43-57.

Lewis, M.J. and Young, T.W., 1995. *Brewing.* Chapman and Hall, London. pp. 8-35.

Lewis, M.J. and Young, T.W., 2001. *Brewing 2nd Ed.* Chapman and Hall, London. pp. 3-10.

Li, C.J., Luo, M.R. and Hunt, R.W.G., 2000. A Revision of the CIECAM97s Model. *Color Res. Appl.* 25, 260-266.

Loof, H., 1967. Definition, Measurement and Valuation of the White Color of Paper. *Das Papier*. 21, 6, 297-309 (in German).

Lopez, M. and Edens, L., 2005. Effective Prevention of Chill-haze in Beer Using An Acid Proline-Specific Endoprotease from Aspergillus Niger. *J. Agric. Food Chem.* 53, 7944-7949.

Luo, M.R., Clarke, A.A., Rhodes, P.A., Schappo, A., Scrivener, S.A.R. and Tait, C.J., 1991. Quantifying Colour Appearance. Part I. LUTCHI Colour Appearance Data. *Colour Res. Appl.* 16, 166-180.

Luo, M.R., Cui, C.G. and Li C., 2003. *British Partent (Application No. 0124683.4) entitled Apparatus and Method for Measuring Colour (DigiEye ® System), Derby University Enterprise Limited, 4 October.*

Luo, M.R., Cui, G.H., Li, C., Jiang, W. and Dakin, J., 2002. Applying Digital Cameras

for Measuring Colours, *in: Proc. AIC Interim Meet.* Maribor. pp. 121-127.

Luo, M.R., Cui, G.H., and Rigg, B., 2001. The Development of the CIE 2000 Colour-Difference Formula: CIEDE2000. *Color Res. Appl.* 26, 340-350.

MacDougall, D.B., 2002. Colour Measurement of Food: Principles and Practice *in: Colour in Food Improving Quality* (Ed: MacDougall, D.B.). Woodhead Publishing Ltd, Cambridge. pp. 33-63.

Makris, D.P. and Rossiter, J.T., 2000. Heat-Induced, Metal-Catalyzed Oxidative Degradation of Quercetin and Rutin (Quercetin 3-O-Rhamnosylglucoside) in Aqueous Model Systems. *J. Agric. Food Chem.* 48, 9, 3830-3838.

Mayer, H., Marconi, O., Floridi, S., Montanari, L. and Fantozzi, P., 2003. Determination of Cu(II) in Beer by Derivative Potentiometric Stripping Analysis. *J. Inst. Brew.* 109, 4, 332-336.

McMurrough, I. and Delcour, J.A., 1994. Wort Polyphenols. *Ferment.,* 7, 175-182.

McMurrough, I. and O'Rourke, T., 1997. New Insight into the Mechanism of Achieving Colloidal Stability. *Tech. Quar. Master Brewers Assoc. Americas.* 34, 271-277.

McMurrough, I., Roche, G.P. and Cleary, K.G., 1984. Phenolic Acids in Beers and Worts. *J. Inst. Brew.* 90, 181-187.

Melgosa, M., Rivas, M.J., Hita, E. and Viénot, F., 2000. Are We Able to Distinguish Colour Attributes? *Colour Res. Appl.* 25, 356-367.

Mikyška, A., Hrabák, M., Hašková, D. and Šrogl, J., 2002. The Roal of Malt and Hop Polyphenols in Beer Quality, Flavour and Haze Stability. *J. Inst. Brew.* 108, 1, 78-85.

Minolta CS1000, 2005. *Minolta CS1000 Instruction Manual.*

Moll, M., 1987. *Colloidal Stability of Beer in Brewing Science, Vol 3* (Ed: Pollock, J.R.A.). Academic Press, London. pp. 1-327.

Moroney, N., Fairchild, M.D., Hunt, R.W.G., Luo, M.R. and Newman, T., 2002. The CIECAM02 Color Appearance Model. Proc. IS&T/SID 13th Color Imaging Conference (IS&T, Springfield, VA, 2005) pp. 217–223.

Morris, T.M., 1987. The Relationship between Haze and the Size of Particles in Beer. *J. Inst. Brew.* 93, 13-17.

Mudambi, S.R., Rao, S.M. and Rajagopal, M.V., 2006. Food Acceptance and Sensory Evaluation of Foods *in: Food Science Revised 2nd*. New Age International Publishers, New Delhi. pp. 69-74.

Mundy, A.P. and Boley, N., 1999. A Survey of Instrumentation Used for the Determination of Haze in Beer. *J. Inst. Brew.* 105, 2, 75-78.

Nevid, J.S. 2009. Psychology: *Concepts and Applications 4th Ed.* Cengage Learning, Wadsworth. pp. 90-135.

Nobbs, J.H., 1997. Colour-Match Prediction for Pigmented Material *in: Colour Physics for Industry, 2nd Ed* (Ed: MacDonald, R.). Society of Dyers and Colourists, Cambridge. pp. 292-372.

Nursten, H.E., 2005. *The Maillard Reaction, Chemistry, Biochemistry and Implication.* The Royal Society of Chemistry, Cambridge. pp 52-61, 214.

Odhay, B., 2004. Bacterial Contaminants Mycotoxins in Beer and Control Strategies *in: Reviews in Food and Nutrition Toxicity Vol 2* (Ed: Preedy, V.R. and Watson, R.R.). CRC Press, London. pp 1-18.

O'Brien, C.M., 2006. *Fermenting Revolution: How to Drink Beer and Save the World.* New Society Publishers. pp. 53-60.

Oszmianski, J., Chynier, V. and Moutounet, M., 1996. Iron-Catalyzed Oxidation of (+)-Catechin in Model Systems. *J. Agric. Food Chem.* 44, 7, 1712-1715.

Palmer, G.H., 1989. Cereals in malting and Brewing *in: Cereal Science and Technology* (Ed: Palmer, G.H.). Aberdeen University Press, Aberdeen. pp. 61-242.

Palmer, G.H., 1999. *Achieving Homogeneity in Malting.* European Brewery Congress, Nice. pp. 323-363.

Palmer, G.H., 2000. Malt Performance is more Related to Inhomogeneity of Protein and β-Glucan Breakdown than to Standard Analyses. *J. Inst. Brew.* 106, 189-192.

Palmer, G.H., 2006. *Barley and Malt, in Handbook of Brewing* (Ed: Priest, F.G. and Stewart, G.G.). CRC Press, Florida.. pp. 139-160.

Palmer, J.J., 2002. *How to Brew, Ingredients, Methods, Recipes, and Equipment for Brewing Beer at Home.* Defenestrative Publishing Co, New York. pp. 67-88.

Papazian, C., 2006. Beer Styles: Their Origins and Classificaitions *in: Handbook of Brewing 2nd Ed* (Ed: Priest, F.G. and Stewart, G.G.). Taylor & Francis Group. Boca Raton. Florida. pp. 56-73.

Park, C.W., Kang, K.O. and Kim, W.J., 1998. Effects of Reaction Conditions for

Improvement of Caramelization Rate. Korean. *J. Food Sci. Tech.* 30, 4, 983-987.

Penner, M.H, 2010. Basic Principles of Spectroscopy, *in: Food Analysis, Food Science Text Series 4th Ed* (Ed: Nielsen, S.S.). Springer, New York. pp 376-470.

Posada, J., 1987. Filtration of Beer, *in: Brewing Science Vol 3* (Ed: Pollock, J.R.A.). Academic Press, London. pp. 379-437.

Pozdrik, R., Roddick, F.A., Rogers, P.J. and Nguyen, T, 2006. Spectrophotometric Method for Exploring 3-Methyl-2-butene-1-thiol (MBT) Formation in Lager. *J. Agric. Food Chem.* 5, 17, 6123-6129.

Quaglia, G.B. and Gennaro, L., 2003. Enzymes: Uses in Food Processing, *in: Encyclopedia of Food Sciences and Nutrition* (Ed: Caballero, B., Trugo, L and Finglas, P.M.). Academic Press, London. pp. 2125-2139.

Roberts, T.R. and Wilson, R.J.H., 2006. Hops, *in Handbook of Brewing* (Ed: Priest, F.G. and Stewart, G.G.). CRC Press, Florida. pp. 181-279.

Ryder, D.S. and Power, J., 2006. Miscellaneous Ingredients in Aid of the Process, *in: Handbook of Brewing* (Ed: Priest, F.G. and Stewart, G.G.). CRC Press, Florida. pp. 333-382.

Scorpio, R., 2000. *Fundamentals of Acids, Bases, Buffers & Their Application to Biochemical Systems.* Kendall/Hunt Pub Co, Dubuque.

Shankar, M.U., Levitan, C.A., Prescott, J. and Spence, C., 2009. The Influence of Color and Label Information on Flavor Perception. *Chemosensory Perception.* 2, 53-58.

Sharpe, F.R., Garvey, T.B. and Pyne, N.S., 1992. The Measurement of Beer and Wort

Colour—A New Approach. *J. Inst. Brew.* 98, 321-324.

Shellhammer, T.H., 2008. *Beer Colour in Beer: A Quality Perspective* (Ed: Bamforth, C.W., Russell, I and Stewart, G.). Academic Press, London. pp. 213-228.

Shellhammer, T.H. and Bamforth, C.W., 2008. Accessing Color Quality of Beer, *in: Color Quality of Fresh and Processed Food* (Ed: Culver, C.A. and Wrolstad, R.E.). American Chemical Society. pp. 192-202.

Shen, S. and Berns, R., 2011. Color-Difference Formula Perfomance for Several Datasets of Small Color Differences Based on Visual Uncertainty. *Color Res. Appl.* 36,1, 15-26.

Siebert, K., 2009. Haze in Beverages. Advances in Food and Nutrition Research, 57, 53-86.

Siebert, K.J., Carrasco, A. and Lynn, P.Y., 1996. Formation of Protein-Polyphenol Haze in Beverages. *J. Agric. Food Chem.* 44, 1997-2005.

Siebert, K.J., Troukhanova, N.V. and Lynn, P.Y., 1996. Nature of Polyphenol Protein Interactions. *J. Agric. Food Chem.* 44, 80-85.

Siebert, K.J. and Lynn, P.Y., 1997. Haze-active Protein and Polyphenols in Apple Juice Assessed by Turbidimetry. *J. Food Sci.* 62, 79-84.

Siebert, K.J. and Lynn, P.Y., 1998. Comparison of Polyphenol Interactions with Polyvinylpolypyrrolidone and Haze-active Protein. *J. Am. Soc. Brew. Chem.* 56, 24-31.

Smedley, S.M., 1992. Colour Determination of Beer Using Tristimulus Values. *J. Inst. Brew.* 98, 497-504.

Smedley, S.M., 1995 (a). Towards Closer Colour Control in the Brewery. *Brew. Guard.*, Oct., 22-25.

Smedley, S.M., 1995 (b). Discrimination between Beers with Small Colour Differences Using the CIELAB Colour Space. *J. Inst. Brew.* 101, 195-201.

Smythe, J.E. and Bamforth, C.W., 2000. Shortcomings of Standard Instrumental Methods for Assessing Beer Colour. *J. Am. Soc. Brew. Chem.* 58, 4, 165-166.

Speers, R.A., Jin, Y., Paulson, A.T. and Stewart, R.J., 2003. Effects of β-Glucan, Shearing and Environmental Factors on the Turbidity of Wort and Beer. *J. Inst. Brew.* 109, 236-244.

Spooner, M., 1996. Making Sense of Sensory Analysis. *Food Manufacture.* 71, 32-33.

Spooner, M., 1997. Sensory Analysis in the Late 20th Century. *Food Manufacture.* 72, 34-36.

Steiner, E., Becker, T. and Gastl, M., 2010. Turbidity and Haze Formation in Beer Insights and Overview. *J. Inst. Brew.* 116, 4, 360-368.

Stevens, S.S., 1956. The Direct Estimation of Sensory Magnitude—Loudness. *American J. Psychology.* 69, 1-25.

Stevens, S.S., 1957. On the Psychophysical Law. *Psychological Review.* 64, 153-181.

Stewart, G.G, 2004. The Chemistry of Beer Instability. *J. Chem. Educ.* 81, 7, 963.

Stewart, G.G. and Russell, I., 1985. Modern Brewing Biotechnology, *in: Comprehensive Biotechnology Vol. 3, Chapter 17* (Ed: Blanch, H.W., Drew, S. and Wang, D.I.C.). Pergramon, Oxford. pp. 335-382.

Taidi, B., Kennedy, A.I. and Hodgson, J.A., 2002. Wort Substitutes and Yeast Nutrition, *in: Brewing Yeast and Fermentation Performance 2nd Ed* (Ed: Smart, K.). Wiley-Blackwell, New York. pp. 86-95.

Ugliano, M. and Henschke, P.A., 2009. Yeast and Wine Flavour, *in: Wine Chemistry and Biochemistry* (Ed: Moreno-Arribas, M.V., and Polo, M.C.). Springer, New York. pp. 249-437.

van der Maarel, M.J.E.C., van der Veen, B., Uitdehaag, J.C.M., Leemhuis, H. and Dijkhuizen, L., 2002. Properties and Applications of Starch-Converting Enzymes of the α-Amylase Family. *Journal of Biotechnology.* 94, 2, 137-155.

van Roon, J. and Craig, H., 2010. Environmental Imapact of Using Proline-specific Endo-proteinase Technology which Shows Better Stabilization Times at Higher Temperatures. Proceedings of The Institute of Brewing and Distilling, Asia Pacific Section Convention, Gold Coast Australia, presentation 28. The Institute of Brewing and Distilling: Asia Pacific Section.

Vine, R.P., Harkness, E.M. and Linton, S.J., 2002. *Winemaking From Grape Growing to Marketplace, 2nd Ed.* Kluwer Academic/Plenum Publishers, New York. pp 102-153.

Westland, S., Ripamonti, C. and Cheung, V., 2012. Computational Colour Science Using MATLAB, 2nd Ed. John Wiley & Sons, West Sussex. Ltd. pp. 75-92.

Wilson, R.J.H., 1978. The Influence of Oxygen on Fermentation and Storage, *in: European Brewery Convention Monograph.* 17-27.

Xu, H. and Yaguchi, H., 2005. Visual Evaluation at Scale of Threshold to Suprathreshold Color Difference. *Color Res. Appl.* 30, 3, 198-208.

Yang, L. and Kruse, B., 2004. Revised Kubelka-Munk Theory. I. Theory and Application. *J. Opt. Soc. Am. A.* 21, 10, 1933-1941.

Yoshida, S., Imoto, J., Minato, T., Oouchi, R., Sugihara, M., Imai, T., Ishiguro, T., Mizutani, S., Tomita, M., Soga, T. and Yoshimoto, H., 2008. Development of Bottom-Fermenting Saccharomyces Strains That Produce High SO_2 Levels, Using Integrated Metabolome and Transcriptome Analysis. *Appl. Environmental Micro.* 74, 2787-2796.

APPENDIX

Guideline for Assessing Colour and Translucency in Brewing CCPs

1. Purpose

The purpose of this guideline is to ensure correct colour and translucency are assessed by DigiEye viewing system and digital imaging system at CCPs of brewing processing.

2. Procedure of measuring colour and translucency by DigiEye viewing system

2.1 Preparing

- Degas all of the final beer products before testing by filtration through filter paper (Whatman No. 1).
- Separate the grist or yeast contents of all the samples collected at the CCPs apart from final products by centrifugation (e.g. 27504×g (RCF) twice).
- Take tests at room temperature (approximately 20°C).
- Ensure the highball glasses are clean and remain free of dust and other contaminants such as fingerprints.
- Each observer must be pre-screened to determine his or her suitability by the initial colour blindness test (e.g. the Ishihara colour vision test).
- Each observer should be given the same training and instruction relating to the experimental requirements (see Figures 3.1.1.4 and 3.1.1.6).

2.2 Sensory Testing

- For beer products, forward viewing position is used. The observers are seated in a chair 600 mm from the sample placed in the viewing cabinet and facing the central area at the back of the cabinet.
- Ask the observers to score Lightness, Colourfulness and Hue composition of an

area of a sample against a white background. References of lightness and colourfulness should be given (see Figure 3.1.1.3).

- Ask the observers to score Transparency. Opacity, Clarity and Ordinal 5-point scale may be used for comparison. Note, Transparency and Opacity should be tested separately. Two references should be given, i.e. the water reference is assigned a transparency score of 10 and opacity score of 0, and a black card is assigned a transparency score of 0 and opacity score of 10.

2.3 Data Analysis

The observer performance can be quantified by the coefficient of variation (CV) as the following equations:

$$CV = 100\times \frac{\sqrt{\frac{1}{N}\sum_{i=1}^{N}(y_i - x_i)^2}}{\overline{Y}}$$

$$\text{and } \overline{Y} = \frac{1}{N}\sum_{i=1}^{N} y_i$$

Calculate the observer performance by comparing each score given by observers to the relevant mean value.

2.4 Expression of Results

- Translucency is expressed as Transparency Value, and/or Opacity Value, and/or Clarity Scores and/or Ordinal 5-point Scale.
- Colour appearance is expressed as Lightness (L_v), Colourfulness (C_v) and Hue compositions (h_v).

3. Procedure of measuring colour and translucency by DigiEye digital imaging system

3.1 Preparing

- Degas all of the final beer products before testing by filtration through filter paper (Whatman No. 1).
- Separate the grist or yeast contents of all the samples collected at the CCPs apart from final products by centrifugation (e.g. 27504×g (RCF) twice).
- Take tests at room temperature (approximately 20°C).
- Ensure the inside of the multiple path length cell and the sheet of optically flat glass are clean and remain free of dust and other contaminants such as fingerprints.

3.2 Measurement

Put the DigiEye digital imaging system as an off-line instrument measuring colour and translucency at CCPs in brewing processing line.

The measurement of the prepared liquid samples in the cell proceeded as follows:

- Turn on the D65 illuminants in the DigiEye viewing cabinet and warm up for thirty minutes.
- Set up camera (see Figure 1.5.1) and calibrate against a GretagMacbeth® DC chart (see Figure 1.5.2.2). Ensure the chart remains free of dust and other contaminants. The performance of the camera is assessed through colour difference analysis by CIE ΔE_{00} colour difference formulae and the desired median value is less than one.
- Put the multiple path length cell into the DigiEye viewing cabinet (see Figure 1.5.1). The cell position is pre-defined so that the mirror image of the cabinet viewing window is eliminated (see Figure 1.5.2.1).
- Pour he samples carefully into the cell until surface tension raises the liquid surface above the cell edge.
- Use a sheet of optically flat glass to cover the liquid. This is to ensure a parallel top and bottom surface liquid layer. Ensure no air bubbles are caught underneath the glass, as they will introduce random light scattering.
- Take an image of the sample in the cell and automatically import into a named

software package. After defining the measuring area, the software automatically allocates 12 measuring area in squares, as 6 squares over white and other 6 over black, with the depths of 2 mm, 10 mm, 20 mm, 30 mm, 40 mm and 50 mm (see Figure 1.5.2.3).

- The software then reports relevant appearance attributes of transparency, lightness, colourfulness, hue composition as redness, yellowness, greenness and blueness (see Figure 1.5.2.3).

3.3 Data Analysis

Compare the assessed results with the standards made (or relevant references). If the assessed data are within tolerances, the products may be accepted and the subsequent production may be continued. Otherwise, some relevant actions should be taken to search the problems.

3.4 Expression of Results

- Translucency is expressed as Transparency Value (T).
- Colour appearance is expressed as Lightness (J), Colourfulness (M) and Hue compositions (percentage of opponent hues).