Influences of Key Components and Surface Modifications on Beer Lacing

Master's Thesis in Food Technology

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Lund 2017

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Abstract

Beer is one of the most favorable alcoholic beverages and has a large consumption in the world. However, there are fewer beer consumers who perceive the lacing character after drinking. Four different concentrations (25 mg/L, 50 mg/L, 75 mg/L and 100 mg/L) of iso-α-acid and two levels (0.3% and 0.15%, w/w) of protein content were investigated towards lacing property on modified hydrophilic and hydrophobic glassware. Lacing area, lacing index and connectivity of laced foam were measured and two-way ANOVA was conducted to each result. There was no linear correlations found in each factor for every indication, but the statistical analysis showed that there was significant difference ($p<0.05$) among iso-α-acid concentrations on hydrophilic surface in terms of lacing area, and it is also the same case on both glass surface properties as for lacing index, in addition protein content influenced the value of lacing index on hydrophobic surface as well. Perimeter Maybe perimeter of lacing fragments was calculated to analyze lacing connectivity. It also showed the significant difference ($p<0.05$) over iso-α-acid concentrations. The interaction effect of protein and iso-α-acid also affect the results significantly. However, the specific interaction between these two factors needs more investigations. Independence of lacing pattern on hydrophilic and hydrophobic glass surface was demonstrated from student t-test according to perimeters. Moreover, the contact angle of each sample on hydrophilic and hydrophobic glass slides was discussed in detail. This thesis provides some new viewpoints for the evaluation of beer from beer lacing property and interesting results obtained in this work are important for comprehensive understanding the nature of foam and lacing connecting to the composition of beer and the glassware surface property.

Key words: Beer; lacing; iso-α-acid; protein; image analysis; hydrophilic and hydrophobic surface

Preface

This degree project work was carried out during 16 January 2017 to 16 June, 2017. All experiment was conducted at the labs of department of food technology in Lund University. In order to study the impact of key components on lacing property, the influence of different concentrations of protein and iso-α-acid on morphological properties of lacing was examined. Image analysis is an emerging technique that has been applied in the food industry to objectively assist the quality and process control. By using Image J software, lots of different parameters of images are possible to be calculated. This is the most fun part, which is the process of translating the information acquired from images into numerical results. Different wettability on vessels was also investigated for studying the effect of hydrophilicity and hydrophobicity on lacing.

There are many people who gave me much help and support to accomplish this wonderful degree project. First of all, I want to thank my supervisor, Yi Lu. She gave me tons of academic and technical support in the very beginning stage to the end of this project. Since she is doing researches in the same area, I have got much useful information from her. She is a very amiable supervisor that trying to fix my problems every time with much patience. I would never forget those nights staying in the lab, dealing with data, making soup noodles in the kitchen and sharing together with her. I had a very nice time spent with her for this project work. Secondly, I would like to thank who gave me the inspiration of image analysis method. Björn Bergenståhl, he could always come up with endless ideas and possible theories behind the phenomenon. I wish I could be a person really into research and knowledge alike him one day. Thirdly, I want to send the acknowledgement to my examiner Lars Nilsson, who involved in discussions during all stages and gave really helpful and feasible suggestions. Also, I am so glad to have a bunch of amazing classmates to spend two-year student life with. At last, I want to say thanks to my parents who support me for this two-year abroad master study financially and mentally.

During the two-year master study of Food Technology and Nutrition, I found myself have improved abilities of communication, scientific way of thinking, tackling with problems and data analysis. I appreciate for choosing to come to Sweden, to Lund University for the advanced study.

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1. Introduction

1.1 Background

Beer is the most favorable alcoholic beverages around the world from the statistics of worldwide consumption by types of alcohols (EpiAnalysis, 2012). As shown in Figure 1.1, the consumption of beer was greater than any other alcoholic beverages such as wine, spirit and premix based on the data of global alcohols consumption per capita from 1997 to 2015. The pronounced difference between beer and other alcoholic beverages presented at the beginning of the statistic, and such difference rose up rapidly year by year. The consumption of beer grew from 13.0 liter per person in 1997 to over 18.0 liter in 2015, and the amount of beer consumption was 6 times of wine, the second consumed drink in the world. It is predicted that the beer consumption will still keep a continuously increasing trend year by year, whereas the consumption of other drinks like wine, spirit and premix has been keeping a constant consumption level since 1997. Therefore, the gap of beer consumption and other alcohol drinks consumption will become wider and wider.

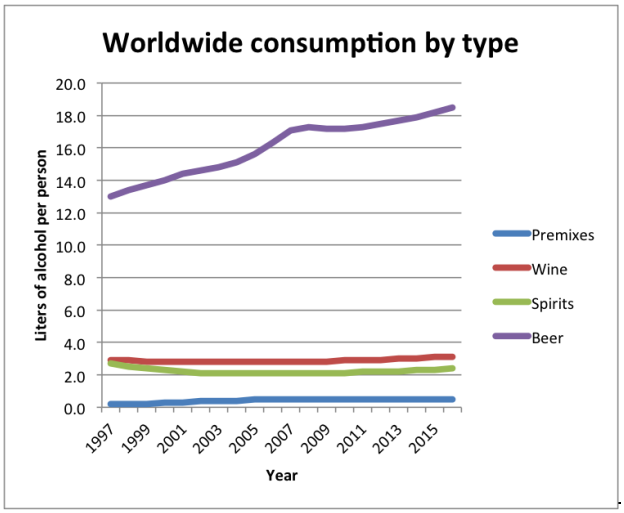

Figure 1.1 Worldwide consumption of alcoholic beverages during the period of 1997-2015. (EpiAnalysis, 2012)

However, the preference and amount of consumption differed in different regions. The biggest contribution to this increasing expenditure of beer is the consumers from Asian countries (Kirinholdings, 2015). Figure 1.2(a) displays the ratio of beer consumption of 7 regions in the world in 2014. People in Asian countries consumed the largest amount of beer and it took up 34.0% over the total amount of these 7 regions in this year, this percentage was followed by an order of Europe (27.0%), Central and South America (16.6%), North America (13.9%), Africa (6.8%), Oceania (1.1%) and Middle East (0.6%). From the Figure 1.2(b), it can be seen that the amount of beer consumption from Asia, Central and South America and Africa kept increasing within 10 years, which was respectively around 65.000 kL, over 30.000 kL and over 10.000 kL of beer consumption within a decade (2005-2014). The value of Europe fluctuated over 10 years, and the amount of beer consumption was larger than Asia in 2005, but it was approximately the same consumption amount in 2014 (50.000 kL), while the beer consumption of Asia increased up to 65.000 kL, which is over 15.000 kL ahead of European. The amount of beer consumed by North America, Oceania and Middle East

countries was almost constant level with very less changes over this period.

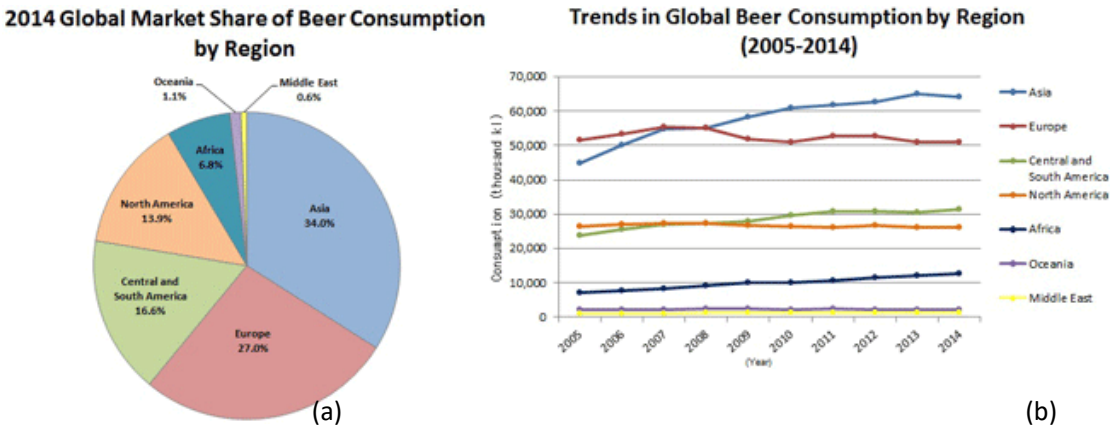

Figure 1.2 (a) Global market share of beer consumption by region in 2014. (b) Trend of global beer consumption by region within a period of 2005-2014. (Kirinholdings, 2015)

1.2 Beer-brewing

Back to the beer beverage itself, commonly it is made of barley, hop, beer brewing yeast and water. (Bamforth, 2008) It is also pervasive to see beer brewed by wheat and oat nowadays. Gluten free option is a bit difficult to find on the regular shelf, but they are available in market which are made of other crops free from gluten cereals such as millet, rice, sorghum, buckwheat and corn (maize) or pseudo cereals. It is also an available way to degrade gluten compounds by yeast fermentation. (Hager et al., 2014) Depending on the top or bottom fermentation types from different strains of yeast, the final product is categorized differently. The main product from top fermentation is called ale and the original Pilsen based bottom fermented beer is called pilsner (also known as lager) (Bamforth, 2008).

From the nutritional value of beer, it contains 0.1%--0.5% of protein, 2%--4% of carbohydrate, 0.0% of fat and other trace amount of minerals and vitamins (Evans and Bamforth, 2009). Protein, as an amphiphilic substance, plays an important role in foam formation and stabilizing foam. Among various proteins in beer, lipid transfer protein 1 (LTP-1) and protein Z groups have been studied in relation to foam properties. And also the most researches were focused on the effects of molecular size, denaturation, and proteolysis on foam properties such as foam stability (Van Nierop et al., 2004)(Kapp and Bamforth, 2002). Kapp and Bamforth studied the foaming properties of albumin and hordein proteins that extracted from barley, and they found that the denaturized forms of albumin and hordein protein increased hydrophobic character on their molecular structures which can enhance the foam stability (Kapp and Bamforth, 2002). Nierop et al. researched about the impact of different wort boiling temperature on lipid transfer protein 1 and the effect on foam stabilizing properties, and they obtained that LTP 1 and protein Z, from the family of hordein protein in barley, are tolerant to high temperature and proteolysis, and the LTP 1 is a foam promoter when it exists in heat-denatured form. (Van Nierop et al., 2004) All the previous studies paid much more attention to the specialized protein, but in present work we take the total amount of protein in beer as a parameter, to discuss its influence on the foam properties.

Hop (*Humulus lupulus L.*) is an indispensable ingredient from beer brewing, which gives out typical

bitterness of beer flavor, and the formation of bitter compounds in beer is intricate. During the boililng process of wort, flower of hop was added and boiled together at the last stage. Hop flower contains key compound, the lupulin, which is a kind of α-acid (Figure 1.3). However, α-acid does not have bitter taste. During the heat treatment of boiling, α-acid was dissolved into the liquid and the further thermal isomerization occurred to form iso-α-acid, which is the compound conferring to bitter taste in beer (Figure 3) (De Keukeleire, 2000). As for the structure of iso-α-acid on Figure 1.3, the hydroxyl groups around double bonds have abilities to give out H^+ and shows moderate acidity (pKa=3) (Bamforth and Kanauchi, 2003). Furthermore, various kinds of carbon chains of R group show hydrophobic property. Therefore, equipped with both hydrophilic (hydroxyl group) side and hydrophobic (carbon chain) side make it an ideal surfactant. Several studies have shown the promoting effect of iso-α-acid on foam stability, and the reduced iso-α-acids (e.g. tetrahydro-iso-α-acid, hexahydro-iso-α-acid and rho- iso-α-acid) were also studied for the foam stability in degassed beer by Kunimune and Shellman (Kunimune and Shellman, 2008). (Caballero et al., 2012)

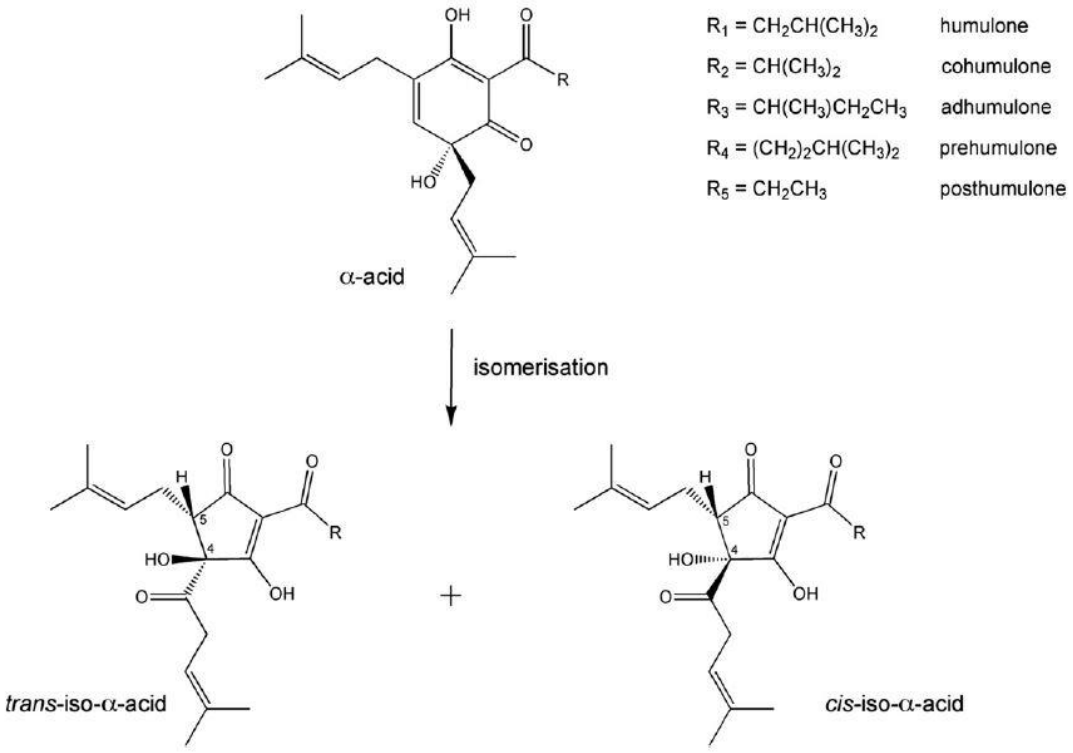

Figure 1.3 Chemical structures of iso-α-acids to trans, cis-iso-α-acids via isomerization. (Caballero et al., 2012)

Iso-α-acid, as a bioactive compound, also acts as a preservative in beer for keeping the shelf-life of beer (Cattoor, 2011). However, it is also unstable and photosensitive. In order to keep flavor in beer, it is packaged in brown bottles for preventing its degradation. (Blanco, Nimubona and Caballero, 2014) The relationship between shelf-life of beer and the degradation of iso-α-acid has been studied in literature, it was reported that the trans-iso-α-acid was relatively more stable than any other forms of iso-α-acids. (Blanco, Nimubona and Caballero, 2014) (Nimubona et al., 2013) As for protecting degradation, three methods were investigated such as addition of phenolic compounds, iso-α-acid or reduced iso-α-acid and use of riboflavin-binding proteins. (Caballero et al., 2012)

1.3 Beer-lacing

Lacing is a unique property of foam and it is an important property for beer evaluation. Lacing refers to the adhesion or cling of the foam on the glass surface after consumption of foaming beverages (Sohrabvandi, Mousavi, Razavi and Mortazavian, 2010). It gives consumers visual perception and it is also one of the meaningful parameters of beer. Not only the foamability or foam stability is of interest, but also the morphological properties are important parameters for the quality of beverages and food products. As for foaming beverage, several specific properties of foam are usually investigated, which are foam stability; foam quality; lacing (adhesion or cling); bubble hazing and bubble gushing. (Sohrabvandi, Mousavi, Razavi and Mortazavian, 2010)(Leiper and Miedl, 2008) Bamforth et al. stated in their publication that "a beer drinker drinks as much with his or her eyes as with their mouth" (Bamforth, Butcher and Cope, 1989) to suggest the important value of appearance especially the foam other than criticizing only by flavor. There is an interesting illustration (Figure 1.4) reported by Evans and Bamforth, they gave an example on how different people would perceive the quality of different beers. The professional beer drinker is able to appreciate beer from the appearance as the beer head, ratio of foam / liquid and lacing pattern. However, the lacing property seems more sophisticated for consumers to realize and to appreciate aesthetically. (Evans and Bamforth, 2009)

Beer drinker 1 (a Belgian?)		
"Lively beer with good head"	"Flat beer"	"Great lacing, top beer!"
Beer drinker 2 (a Londoner?)		
"Ripped off, foam is not beer!"	"That is more like it, a full measure of beer"	"A dirty glass"
Beer drinker 3 (a Lady?)		
"Will this foam stick to my lip and wreck my make-up?"	"Not very lively and appealing beer"	"This glass has not been properly cleaned before filling"

Figure 1.4 The perceptions of three different drinkers of beer foam quality. (Evans and Bamforth, 2009)

1.4 Aim of the project

Lacing is highly related to foam. Therefore, the factors affecting the properties of foam will influence the properties of lacing at the same time. The components which positively correlated with lacing and foam are peptides, glycoproteins, phenolics (e.g. iso-α-acid), dextrin, non-starch polysaccharides with high molecular weight (e.g. β-glucans), minerals, glycerol, sugar alcohols, and so on. Apart from the composition, the temperature, pH, pouring height and angle will affect as well. (Sohrabvandi et al., 2010) Besides, the impact of the diameter of containers and shape of walls could not be ignored and was also investigated (Papara et al., 2009).

However, there are very limited publications concerning the beer lacing properties compared with the foam stability and foamability. Lacing is suggested to categorize into three major types based on its forming patterns: "ring, mesh and powdery" (Kunimune and Shellhammer, 2008). The lacing properties were well studied by Jackson and Bamforth as well. They developed a method named Jackson and Bamforth's method, in which the lacing was quantified as lacing index, the experiments were operated by using a special apparatus as shown in Fig. 1.5, and the value of lacing index was calculated from the UV absorbance of laced foam and beer at 230 nm. The lacing properties hence were compared between different beers. (Jackson and Bamforth, 1982) The influence of vessel wettability on foamability was studied by Hamlett et al. Since it showed a difference in wettability of beer on hydrophilic and hydrophobic containers, the foam retention time was longer on hydrophobic glassware and lacing presented largely different properties accordingly. (Hamlett, Wallis, Pugh and Fairhurst, 2015) By NIBEM Cling Meter (NIBEM-CLM), the cling area and foam retention time were measured for the mixture of unhopped beer and iso-α-acids or reduced iso-α-acids (e.g. tetrahydro-iso-α-acid, hexahydro-iso-α-acid and rho-iso-α-acid), and they found that the amount and kind of iso-α-acid contributed to the amount of lacing and independent characteristics (Kunimune and Shellhammer, 2008).

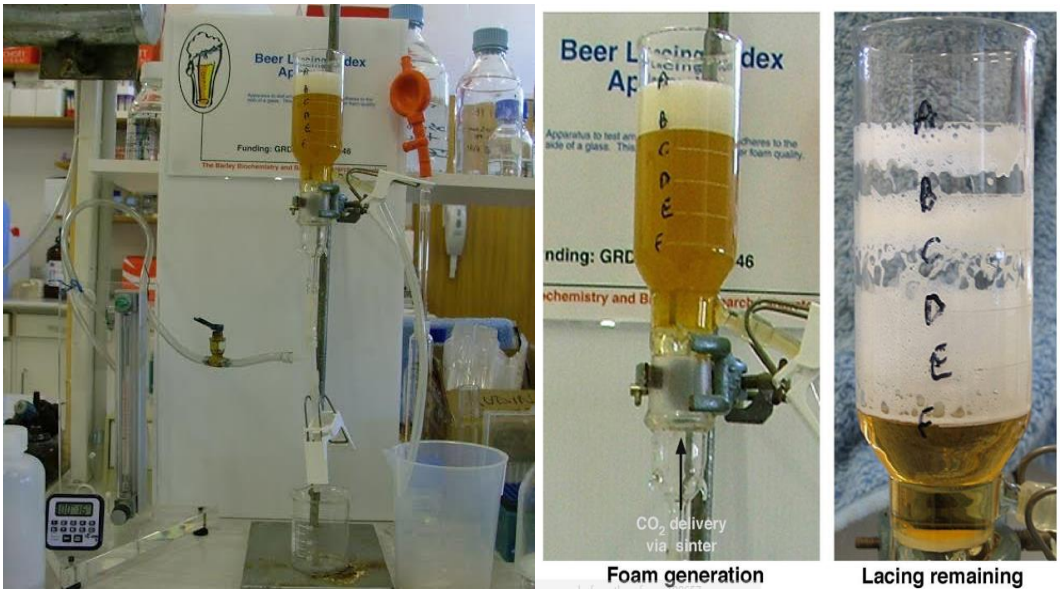

Figure 1.5 Apparatus of measuring lacing by Jackson and Bamforth's method. (Evans, 2016)

Image analysis is a cost-effective method for studying macroscopic characteristics (Broeke, Pérez, and Pascau, 2015), and this investigation method has been becoming more and more popular. It was widely used in investigation of agricultural and food products. Visual inspection is implied regarding to examine the shapes, colors, sizes, surface area and brightness of wide ranges of food products, such as vegetable, fruit, grain and even meat and fish. Therefore the selection from morphological properties of food is highly valued. (Broeke, Pérez, and Pascau, 2015) Moreover, through the modification of traditional method of measuring beer foam head retention instead of counting time by human eyes, it acquired more precise and high correlated result by using image analysis (Cimini et al., 2016). Video recording is also an image analyzing method. Foam lifetime, drainability, foam volume and number and sized of bubble can also be evaluated by acquired video, which was reported in a study of an assessment of foam quality in sparkling wines (Condé et al., 2017). All in all, image analysis is a promising tool to be implied in researches in various fields.

Analysis of variance (ANOVA) and student t-test were conducted for statistical analysis of data aimed to prove the effect of each experimental factor. ANOVA is a statistical method used for comparing over two entities (factors, treatment, equipment and so on). Based on the variance of each treatment and the estimated variance of error calculated, the value of quotient was compared with F distribution according to different degrees of freedom and confidence interval. Two-way ANOVA was used for comparing two factors over different levels of treatment. It is also called blocking as it calculates in ways of different blocks (each block is one certain degree of factor one and factor two). Student t-test is for generally comparison if there is significant difference between two sets of treatments. It calculates the possibility of the significant difference between two treatments at certain confidence interval. These two statistical analyzing tools are used in wide range in scientific researches. (Box, Hunter and Hunter, 2005).

This project is an integral work beginning with extraction of iso-α-acid from hop, design of experiment, set-up equipment, image acquisition and analysis. In this thesis, firstly the research background and the progresses achieved on the research of the foam formation and its properties were shortly reviewed. Secondly the experiment process and method of dealing with image analysis were described clearly. Thirdly, it is the main content of present work, which contains modification of the glassware to hydrophilic and hydrophobic surface, and then comparison of the lacing properties and the lacing amount and patterns between hydrophilic and hydrophobic surfaces of glassware by image analysis. Most importantly, the relationship of lacing index or lacing area with the concentration of iso-a-acid and protein was discussed for the hydrophilic and hydrophobic glass surfaces. Some new insights and interesting results obtained in this work are important for comprehensive understanding the nature of foam and lacing connecting to the composition of beer and the glassware surface property. This thesis provides some new viewpoints for the evaluation of beer from beer lacing property, and it will attract much attention of the readers in the field of food science and technology.

2. Experimental method

2.1 Materials and reagents

2.1.1 Raw materials and chemicals

Beer used for experiment is Spendrups premium lager (2.8% alcohol by volume, ABV) produced by Spendrups Bryggeri AB, Sweden. It is a hopped barley beer without widget. Polaris pellet (16.9% alpha acid, German) was used for iso-α-acid extraction. Acetic acid (99-100%, GPR RECTAPUR® VWR, Germany) and sodium acetate (>99%, Sigma-Aldrich) are used for the preparation of pH 4 buffer. Phosphoric acid (MERCK, Germany) was used to adjust pH value. Isooctane (analytical pure, EMSURE®, Germany) was used to extract iso-α-acid from crude extraction. Methanol (HiperSolv CHROMANORM, VWR, France) was for dissolving iso-α-acid. Ammonia (28%, GPR RECTAPUR® VWR, Germany), hydro peroxide (Laboratory reagent grade, 20 volumes>6%, Fisher Scientific, UK), hydrochloric acid (5 mol/L, AVS TITRINORM VWR, France) and ethanol (96%, GPR RECTAPUR® VWR, Germany) were used for the cleaning of glassware and hydrophilicity modification. Trichloro silane (18C, 99% purity, Sigma-Aldrich) and cyclohexane (100% AnalaR NORMAPUR, VMR, EC) were used for hydrophobicity modification. Maltodextrin (95% purity) was for sampling. Milli-Q water Millipore, France) was used for absorbance measurement.

2.1.2 Equipment

Centrifuge (Beckman Coulter Allegra X-15R); Rotatory evaporator (LABOROTA 4000, Heidolph); Tensiometer (THE TRACKER from TECLIS - I.T.C); UV-visible spectrophotometer (Varian Cary 50 Bio); Oven (Horo F.Nr.035); Foam generator (Grräddsifon 0.5 L, Exxent, MerxTeam AB, Göteborg, Sweden)(Figure 2.1) with N_2O charger (7.5 g, isiGmbH, Austria); pH meter (Manual Metrohm 744 pH-meter); Camera (Nikon D3300 with lens of DX VR AF-S 18-55mm 1:3.5-5.6 GII); Beaker (150 ml, round shape with diameter 50 mm, SCHOTT, Germany).

Figure 2.1 Foam generator (Grräddsifon 0.5 L, exxent).

2.2 Method development

2.2.1 Preparation of iso-α-acid

Hop pellet (16.9% alpha acid, Polaris variety from Germany, Humlegården Sweden) was boiled in pH 4 acetate buffer (46 mM acetic acid and 34 mM sodium acetate). Supernatant was modified to pH 2 with 85% H_3PO_4 and then isooctane was added and kept rotary shaking. Afterwards, upper isooctane phase was collected and removed by reduced pressure at room temperature. The glass was rinsed with acidified methanol (0.1mL 85%H_3PO_4 in 100mL MeOH). Iso-alpha acid amount was quantified by HPLC-DAD (Agilent Technologies 1260 Infinity). ICS-I3 (total iso- 62.3%) was purchased from Labor

Veritas as standard and dissolved in acidified methanol. An isocratic elution with 75% mobile phase A (MeOH), 35% B (0.1M EDTA solution with pH 2 modified by H_3PO_4) at 1.2 mL/min at 40^{O}C on C18 reversed phase column (Agilent Eclipse, XDB-C18, C18 5 µm, 4.6*150 mm Agilent, Waldbronn, Germany) at 275 nm. Figure 2.2 shows the chromatogram for the ICS-I3 standard. The retention time is 6.0 min (co-iso-humulone), 7.5 min (n-iso-humulone) and 8.4 min (ad-iso-humulone). (Jaskula et al., 2007)

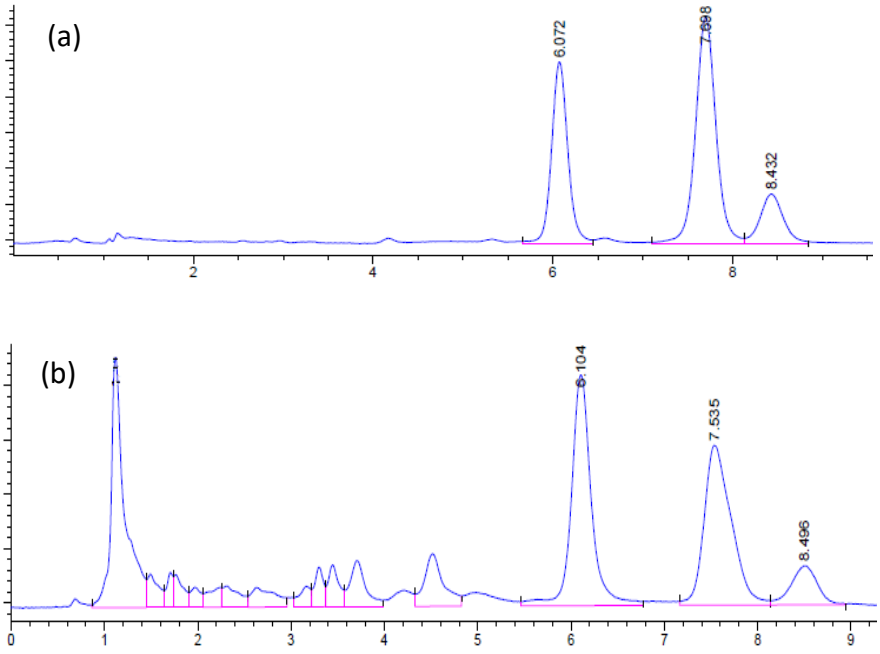

Figure 2.2 Chromatogram of (a) standard iso-α-acids sample (ICS-I3) and (b) the extracted sample from hop generated by LLE

2.2.2 Modification of glass beaker surface

Hydrophilization: Glassware was immersed in 30% HCl overnight. Rinsed with copious water and dried at 80 ℃ for 4 hours. (Hamlett, Wallis, Pugh and Fairhurst, 2015)

Hydrophobization: Boiled the glassware with a solution of NH_4OH:H_2O_2:H_2O (1:1:5) (v/v/v) for 10 min, followed by a solution of HCl:H_2O_2:H_2O (1:1:5) (v/v/v) for 10 min. Then it was washed by ethanol and treated with a cyclohexane solution containing 0,1% of Trichloro silane (99% purity, Sigma-Aldrich) at room temperature for 1 hour, finally washed with ethanol and dried with nitrogen gas at 40 ℃ for 24 hr. (Wahlgren and Arnebrant, 1990) (Marczak, Kargol, Pssarski and Celichowski, 2016)

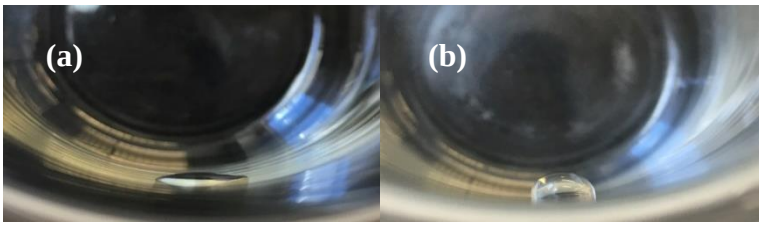

Figure 2.3 Images for the glassware with hydrophilic and hydrophobic surfaces. (a) hydrophilic surface cleaned with HCl, (b) hydrophobic surface modified with $C_{18}H_{37}Cl_3Si$

2.2.3 Contact angle measurement

Glass microscopic slides were modified based on method described in "2.2.2 Modification of glass beaker surface". 5 μL each sample solution was dropped on the edge of glass slide. The recording camera of tensiometer (THE TRACKER from TECLIS - I.T.C) was focused to acquire a clear image of liquid drop. Software (WINDROP_2008_PXR) was used to calculate the contact angles.

2.2.4 Experimental design

Experiment was designed based on single-control method to investigate the influence of each factor on lacing property. Solutions were prepared based on Table 2.1 below. Low level of protein was achieved by diluting beer with pH 4 buffer and adding maltodextrin in order to decrease protein content and keep the same carbohydrate level. Each solution was spiked with ethanol to simulate beer with 3% alcohol.

Table 2.1 Design of experiment

Factors	level
Surface property	Hydrophobic, hydrophilic
Protein	0.15%, 0.3%
Iso-α-acid	25mg/L,50 mg/L,75 mg/L,100 mg/L

2.2.5 Lacing test

Based on the designed experiments, the samples were prepared in foam generator. 7.5 g N_2O charger was injected and the whole bottle was kept in cooling room (5 ℃) for 16 hours. The foam was delivered when pressed the trigger of output. Then the liquid part was drained out carefully from the bottom using a capillary tube (diameter: 0.5×10^{-3} m; length: 1.36 m) and needle tubing. It was given an absorbing force from the other side of capillary tube by needle tubing and liquid was drained out constantly according to potential differences between beaker and volumetric cylinder on the ground. The weight of beaker was measured initially and after the lacing test in order to calculate the quantity of lacing. The set up apparatus is shown in Figure 2.4.

Figure 2.4 Image of setting up experimental apparatus.

2.2.6 Lacing index

All the remaining laced foams were collected by 10 ml water. The absorbance of lacing solution and the diluted beer sample was measured by UV-spectrometer at 230 nm. The lacing index was calculated by the equation (1) below.

$$\boldsymbol{Lacing\ Index(LI)} = \frac{\boldsymbol{Absorbance\ \ of\ recovered\ material\ at\ 230\ nm \times volume\ of\ water}}{\boldsymbol{Absorbance\ of\ beer\ at\ 230\ nm \times dilution \times volume\ of\ beer\ used}} \times 1000 \qquad \textbf{(1)}$$

2.2.7 Image acquisition

To take a clear picture is crucial for image analysis. Digital photograph was taken immediately after drained all liquid. A piece of black board was inserted halfway into glass beaker in order to make only one side of laced foam visible. A digital camera was set up with a stand in front and adjusted the mode and setting for taking sharp photos of lacing on the surface of beaker. The beaker was seated on the platform in the equipment of measuring surface tension. Lighting in the surface tension closet was the only one available illumination and the beaker was lied down horizontally in order to acquire light from the top of beaker to bottom hence avoided intense light only on one side of beaker.

2.2.8 Lacing area analysis

The process of analyzing lacing area was conducted as follows: Firstly, pictures were applied with "Cylindrical Projection Transformer" plugin. This step is for transforming the cylindrical surface into a planar surface. Secondly, half of the upper part of the picture is selected to adjust the range of brightness and contrast value. Thirdly, transform the whole picture into "8-bit" color form. The crucial part of image analysis process is to define the best threshold in order to distinguish all laced foam from black background. Finally, middle part of the beaker was chosen to measure shadow area shown as percentage. Figure 2.5 shows color photo and picture after processing by Image J (Schneider et al., 2012). Macro was used to measure the foam lacing area and deal with pictures in loops, as shown in Figure 2.5. The lacing area was estimated based on the equation (2).

$$\boldsymbol{Lacing\ Area}(\%) = \frac{\boldsymbol{area\ of\ shadow\ in\ selected\ field}}{\boldsymbol{area\ of\ selected\ field}} \times 100\% \quad (2)$$

Figure 2.5 Example of image processing and Macros.

2.2.9 Lacing pattern analysis

The images were converted to white/black and then analyzed. Black parts displayed the laced foam on glass surface. The diameter of beaker was set as 5 cm long. By selecting and labeling every separated fragment of laced foam, perimeter was measured according to the length of outlines. The values of perimeter from an image were summed up to represent as one parameter of lacing. (Appendices-Figure 7.3)

3. Results

3.1 Modification of glassware surface

Two glass slides were modified and their hydrophilicity and hydrophobicity was confirmed by contact angle as described above in method 2.2. 5 μL Milli-Q water was dropped on the surface of glass-slide to observe the contact angle and images were captured by tensiometer (THE TRACKER from TECLIS - I.T.C). It showed that contact angles for hydrophobized surface and hydrophilized glass are 90 ° and 33.1 ° respectively, which confirms the hydrophobization and hydrophilization process. Therefore, the chloride acid and trichloro silane (18C) were selected for the following tests.

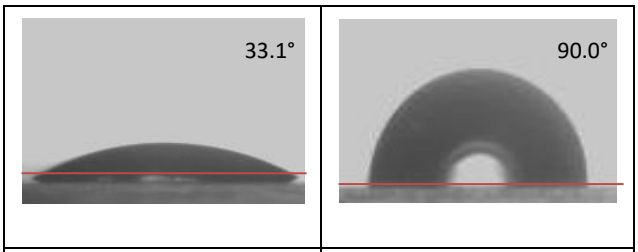

Figure 3.1 Contact angles for modified glass-slide with HCl and $C_{18}H_{37}Cl_3Si$

		Iso-α-acid 25mg/L	Iso-α-acid 50mg/L	Iso-α-acid 75mg/L	Iso-α-acid 100mg/L
Hydrophobic	Protein 0.3%	59.2°	54.8°	53.3°	47.2°
	Protein 0.15%	72.2°	72.1°	70.1°	65.9°
Hydrophilic	Protein 0.3%	46.0°	41.2°	29.6°	29.4°
	Protein 0.15%	41.4°	34.6°	33.3°	37.6°

Figure 3.2 Contact angle images for different samples.

Subsequently, contact angles for different concentrations of protein and iso-α-acids were measured, as shown in Figure 3.2. It is clear that the samples on the hydrophobic glass surface have higher contact angle than those on the hydrophilic glass surfaces. In addition, contact angle decreased along with the increase of the amount of iso-α-acid, except for the sample of 0.15% protein content at 100 mg/L iso-α-acid concentration. It is found that series changes of contact angle for 0.3% protein concentration

were relatively higher than those with 0.15% protein concentration. For example, on the hydrophobic glass surface, the contact angles differed from 59.2 °to 47.2 °for the sample with 0.3% protein content; whereas for 0.15% of protein content it varied from 72.2 °to 65.9 °. For the case of hydrophilic surface, it also illustrated the same phenomena, which are 46.0 ° to 29.4 ° and 41.4 ° to 37.6 ° respectively. Moreover, it was also found that on the hydrophobic surface, the higher protein concentration tends to have larger contact angle compared to the lower one; but on contrary, on the hydrophilic glass surface the lower protein concentration presented the higher contact angle.

3.2 Lacing area analysis

Table 3.1 Results of the lacing area estimated from triplicate test (%)

Surface property	Protein (w/w)	Iso-α-acid			
		25 mg/L	50 mg/L	75 mg/L	100 mg/L
Hydrophobic	0.3%	21.0±1.3	20.1±0.8	22.0±1.7	22.1±1.2
	0.15%	19.8±1.4	18.9±1.3	18.6±1.7	19.8±2.0
Hydrophilic	0.3%	7.8±1.8	16.8±1.1	21.3±0.3	27.0±0.5
	0.15%	3.0±1.2	19.6±1.6	25.4±1.0	20.8±3.5

Lacing area was analyzed and plot in Figure 3.3. The average value was used to discuss the effects of surface property, and the concentration of iso-α-acid and protein. On the hydrophobic glass surface (Figure 3.3(a)), at 0.3% protein concentration, values of lacing area fluctuated at a range of 19.6% to 22.1%; at 0.15% protein concentration, values were a slightly lower and changed from 19.8% to 18.6% with increasing of the concentration of iso-α-acid from 25 to 100 mg/L. For the hydrophilic glass surface (Figure 3.3(b)), at 0.3% protein concentration, the value of area increased from 7.8% to 27.0% continuously with increasing of iso-α-acid concentration from 25 to 100 mg/L; at 0.15% protein concentration, the area increased at the beginning from 3.0% to 25.4% with increasing the iso-α-acid concentration from 25 to 75 mg/L, and then it decreased to 20.8% when the concentration raised to 100%. Therefore, the lacing area was less dependence on the concentration of iso-α-acid on the hydrophobic surface. While on the hydrophilic surface, it is largely correlated to the concentration of iso-α-acid. In addition, the lacing area depended largely on the concentration of iso-α-acid, but less changed with the variation of protein concentration, irrespective to the hydrophilic or hydrophobic surface.

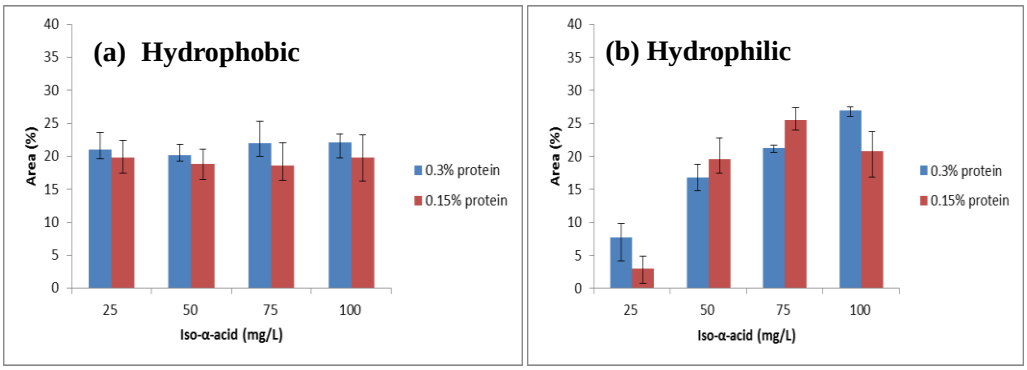

Figure 3.3 Analyzed lacing area for different samples.

By a two-way ANOVA analysis among all triplicate data, it was observed that iso-α-acids had a significant difference as well as the pro-iso interaction effect on hydrophilic surface ($p<0.05$). Nevertheless, none of the factors showed significant difference on hydrophobic surface ($p<0.05$). Furthermore, no significant difference was observed for lacing area on hydrophobic and hydrophilic surface by student t-test ($p<0.05$).

3.3 Lacing Index analysis

The value of lacing index was calculated based on the absorbance of laced foam and original solutions. Table 3.2 listed all the calculated data. The lacing indices were compared in Figure 3.4, it is clear that the same variation tendency presented for both the hydrophobic and hydrophilic glass surfaces as seen in Figure 3.4(a) and (b). The results showed that the lacing index increased with increasing concentration of iso-α-acid, and it reached maximum at 75 mg/, then dropped at 100 mg/L for the sample at 0.15% protein; While, for the sample at 0.3% protein the lacing index increased linearly (R^2=0.97) with increasing concentration of iso-a-acid. Moreover, the values of lacing index are larger for the samples with 0.15% protein compared with the sample at 0.3% protein when the concentration of iso-a-acid below 75 mg/L, and it is reversed at 100 mg/L, irrespective to the hydrophobic or hydrophilic surfaces. However, lacing index from hydrophilic surface varies much more than that from hydrophobic surface, the former changed between 15 and 41, and the latter changed between 25 and 37.

Table 3.2 Lacing Index calculated from triplicate tests.

Surface property	Protein (w/w)	Iso-α-acid			
		25 mg/L	50 mg/L	75 mg/L	100 mg/L
Hydrophobic	0.3%	18.8±0.5	28.2±2.6	31.5±1.4	32.8±1.5
	0.15%	25.5±1.1	28.4±1.0	37.3±1.2	30.4±1.9
Hydrophilic	0.3%	15.6±1.8	20.6±0.5	31.2±0.8	35.8±2.3
	0.15%	15.5±2.6	25.5±1.2	41.2±3.5	31.2±4.1

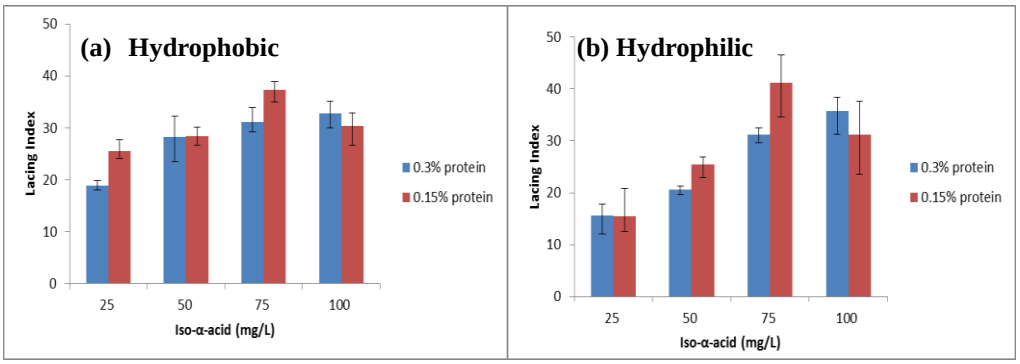

Figure 3.4 Lacing Index for different samples.

By a two-way ANOVA analysis among all triplicate data, it was observed that iso-α-acids and interaction between protein and iso-α-acid had a significant difference on both glasses ($p<0.05$). Besides, protein was observed a significant difference on hydrophobic glass. Furthermore, student t-test showed no difference on LI on both glass ($p<0.05$).

3.4 Lacing pattern analysis

In order to demonstrate the distribution of lacing, perimeters from every fragment were analyzed. All the data estimated are shown in Table 3.3 and the analyzed perimeters are displayed in Figure 3.5.

Table 3.3 Perimeters (cm) of lacing analyzed from images of triplicate test

Surface property	Protein (w/w)	Iso-α-acid			
		25 mg/L	50 mg/L	75 mg/L	100 mg/L
Hydrophobic	0.3%	171.3±9.8	271.9±7.2	319.5±18.3	279.6±24.5
	0.15%	243.0±18.6	281.6±15.3	331.9±17.6	191.4±13.8
Hydrophilic	0.3%	71.5±5.8	209.9±9.4	255.8±24.7	195.4±16.4
	0.15%	40.9±15.8	243.7±6.4	280.2±7.7	195.7±6.1

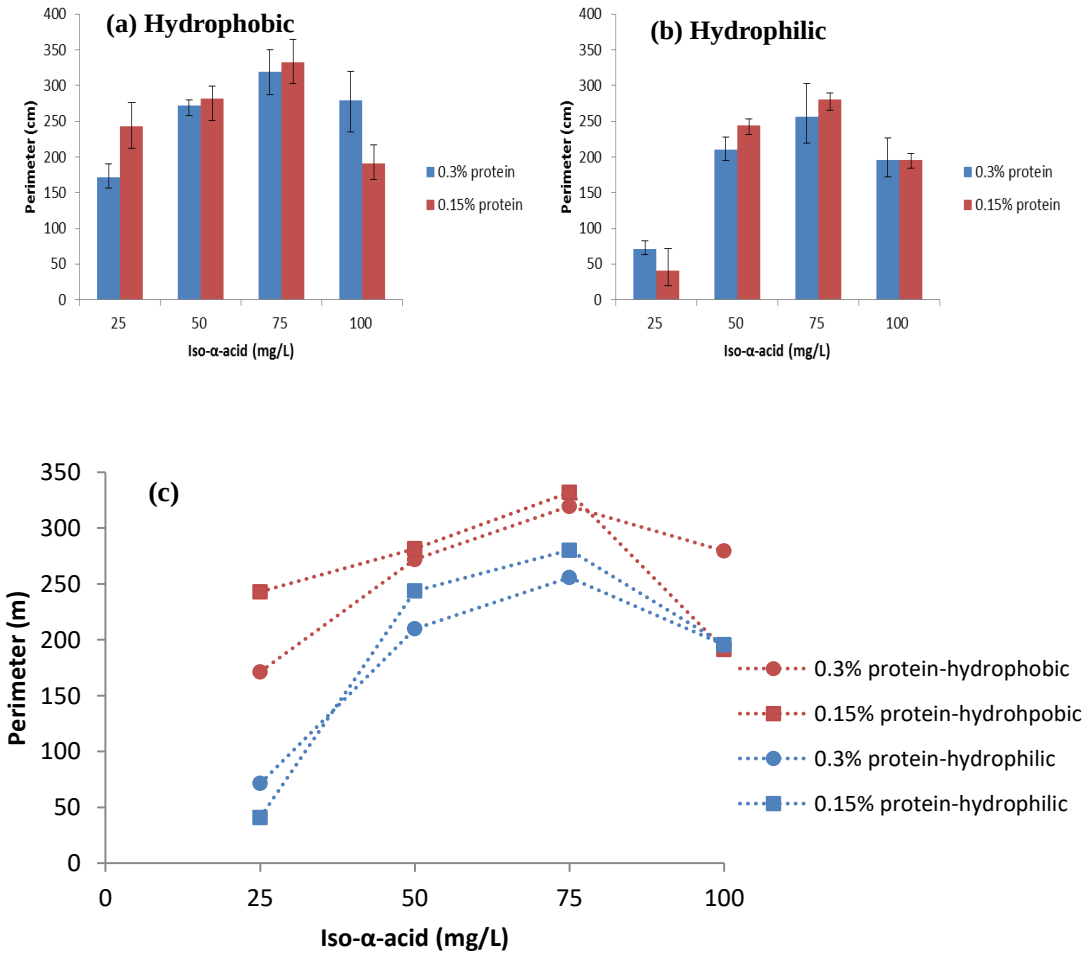

Figure 3.5 Perimeter of lacing for different samples.

On the hydrophobic glass surface (Figure 3.5(a)), for the case of 0.15% protein concentration, values of perimeter are greater than the one at 0.3% of protein content at the concentration of iso-α-acid below 100 mg/L. Later on, it dropped down sharply with the 0.15% protein concentration at 100 mg/L iso-α-acid and the value was exceeded by the samples with 0.3% protein concentration, whereas the

error increased with the increase of iso-α-acid concentration. On the hydrophilic glass surface (Figure 3.5(b)), for the case of 0.3% protein concentration, perimeter increased from 71.5 cm to 255.8 cm initially with the increase of iso-α-acid concentration from 25 to 75 mg/L, while it decreased to 195.4 cm at the concentration of 100%. The same trend was also observed for the case of 0.15% concentration. It increased from 40.9 cm to 280.2 cm, and then decreased to 195.7 cm. Therefore, the influence of lacing connectivity did not depend on the surface property of glass, irrespective to its hydrophilicity or hydrophobicity. (Figure 3.5(c)). It is interesting that the perimeter presented the highest values at 75 mg/L iso-α-acid for all the experiments with different protein concentration on different surface properties, and they showed the same value around 195 cm at 100 mg/L iso-α-acid, except for the case of 0.15% protein on the hydrophilic surface.

By a two-way ANOVA analysis among all triplicate data, it was observed that iso-α-acids and interaction between pro-iso had a significant difference on hydrophobic glasses ($p<0.05$). For the case of hydrophilic one a significant difference presented within different levels of iso-α-acid. Student t-test between hydrophobic and hydrophilic two sets of data was also conducted by using perimeter as parameter. *T.* test showed the values of hydrophobic and hydrophilic glass surfaces are independent and a significant difference presented at 95% confidence level.

Table 3.4 Summary of factors with significant difference ($p<0.05$)

	Lacing area	Lacing index	Perimeter
Hydrophilic	iso-α-acid pro-iso interaction	iso-α-acid pro-iso interaction	iso-α-acid
Hydrophobic		protein iso-α-acid pro-iso interaction	iso-α-acid pro-iso interaction

4. Discussion

4.1. Foam generation

Jackson and Bamforth's method of measuring lacing is based on their original established apparatus. Carbon dioxide was injected through special sinter into degassed beer in cylinder. Specific gas flow and certain height of generated foam were regulated as well. The CO_2 sparging process was repeated several times until the liquid interface lowered to labeled line. (Jackson and Bamforth, 1982) However this CO_2 bubbling method also has some drawbacks, e.g. the generated foam is unstable and collapse quickly due to too big gas bubbles among foams and the foam itself is too wet. These shortcomings lead to scarce lacing clinging on glass surface. It was also reported that the quality of foams generated by CO_2 sparging methods were different from what consumer perceived beer head in daily life. (Evans et al., 2011)

So as to approach a feasible method to generate foam, which is able to lace from prepared degassed beer solutions, several foam generation ways were examined. Kapp and Bamforth (Kapp and Bamforth, 2002) used a simplified shaking procedure to measure foam stability. It was conducted by protein solution in addition with hop extracted iso-α-acids and shook it 10 times within 3 seconds in enclosed testing tube. The cap was removed immediately after shaking. This simple method is suitable for an

easy examination of the foam stability but it didn't give an ideal amount of lacing to the sides. The reason for scarce lacing can be considered as the entire container surface was wetted during the shaking procedure which made foams difficult to adhere to the inner surface of container. This is the reason why a clean and dry glass showing pleasant lacing of beer other than a watery glass. Thus, the previous work was focused on establishing a method to measure foam stability and foamability but exempt lacing property of the foam.

In this work a foam generation procedure was designed, that is to use foam generator to produce homogenized ideal volume of foam. Degassed samples were injected with 7.5 g pure N_2O charger and kept in cooling room for overnight (16 hours). The output of foam generator is controllable, so the volume of foam can be regulated for each experiment. The advantages of foam generator are: (1) it has a controllable output of foam, which is possible to regulate a constant volume of foam in experiment; (2) generated foams are homogeneous with uniform air bubbles dispersed in foams so that the lacing property is only affected by its composition and glass surface hydrophobic/hydrophilic property.

4.2 Lacing area analysis

Kunimune and Shellhammer reported the effects of iso-α-acid and several kinds of reduced iso-α-acids on foam retention time and cling area (same term as lacing area) measured by NIBEM equipment. Linear correlations showed in each reduced form of iso-α-acid over its concentrations in terms of foam retention time, but no correlation was observed for cling area. The changes of cling area over iso-α-acid concentrations in their study had a similar trend as shown of the lacing area changes at 0.15% protein content on the hydrophilic glass surface from this study. While, it was found in this work that the lacing area is not continual increasing with the addition of iso-α-acid, which is in accordance to the results reported in literature (Kunimune and Shellman, 2008). Besides, they proposed a technological limitation of the measuring machine NIBEM-CLM, difficulty in preparing the standard glass, and also the inherent variation in foam collapse and cling phenomena. (Kunimune and Shellman, 2008) Herein, a hypothesis was given that the viscosity of generated beer foam increased along with the increase of iso-α-acids, which possibly results in the stronger interaction between foams. Hence the stability of foam system structured by protein and iso-α-acid was stronger than the strength of adherence on glass surface, which leads to a decrease of lacing area at a higher concentration (100 mg/L) of iso-α-acid. For the lab experiment of measuring lacing, difference of viscosity and texture of varied samples was able to distinguish to the human eye. However, this theoretical assumption needs to be further proved by practical trials.

When look through the data overall (in Appendices Table 7.1), the standard deviations for some samples are relatively large. This is ascribed to the shortcomings of Image J in analyzing lacing area of pictures. Firstly, although the lighting source was improved a lot, it still did not reach the best ideal illumination; glass beaker was lain down on the platform in tensiometer closet so that lighting was irradiated from the top of beaker, hence avoiding a bright lighting spot on the glass wall. In spite of the improvement the distribution of light is still not equal and it needs to adjust brightness of pictures manually in Image J analyzing tool. Although it takes time to figure out best threshold for analyzing laced foams, once the macros were established, all pictures can be dealt with instruction. Secondly, it has a limitation on revealing the foam thickness. As it is observed during the experiment, viscosity and stability were enhanced along with the increasing amount of iso-α-acid due to high concentration of

solutes and the possibility of aggregation of protein and iso-α-acid. Therefore, lacing load on glass surface varied at the same time. At high concentration of iso-α-acid, a thicker layer of the laced foam was approximately 2.0 mm which is different from a bubble clear lacing (less than 1.0 mm). It is a cake of fluffy foams without clearly observable air bubbles seen from outside of beaker. Hence in this case, lacing area analysis by Image J is not sufficient enough to describe lacing. Finally, lacing is uncertain and unique, which makes it hard to control and maintain a good reproducibility. There is no identical lacing pattern can be found in practice even if under the completely same experimental conditions, and thus the results fluctuate at a relatively larger range.

Nevertheless, from two-way ANOVA focusing on protein concentration and iso-α-acid concentration, the results proved statistically iso-α-acid has a domain effect on lacing area on hydrophilic glass surface. However, there is no statistical difference on the hydrophobic glass surface, and the average values at varied levels of iso-α-acid are changed in a narrow range. Based on these results, it is deduced that the iso-a-acid has much stronger affinity with the hydrophilic surface compared to the hydrophobic surface due to the result of contact angle, which is further explained in the "4.5 Contact angle and assumptions of molecular interaction" section, thus the concentration of iso-α-acid presented larger effect on the area of lacing on the hydrophilic surface, but not on the hydrophobic one.

4.3 Lacing Index analysis

The lacing index did not follow the same tendency as lacing area with the changes of concentrations of iso-α-acid and protein. This indicates the lacing index and the foam area are different parameters and they represent the different properties of lacing, even though they showed a similar trend in some cases. Figure 4.1 shows correlations of lacing area and lacing index. It is clear that results of the hydrophobic surface were relatively distributed in a narrow area comparing to these results of hydrophilic glass surface, which were spread in a wider range. It suggests that the impact of the concentrations of protein and iso-α-acid on hydrophobic glass surface is unapparent or less relevant. Moreover, the less change of lacing on hydrophobic glass surface can be ascribed to the limitation of the capacity of lacing on hydrophobic surface. Even though at a lower concentration of iso-α-acid and protein content, it gave a decent amount of lacing area on the hydrophobic surface and this makes it have a small variable space to display even more lacing. In other word, at two dimensions, lacing was saturated on the hydrophobic glass surface hence it changed slightly and fluctuated at a small range.

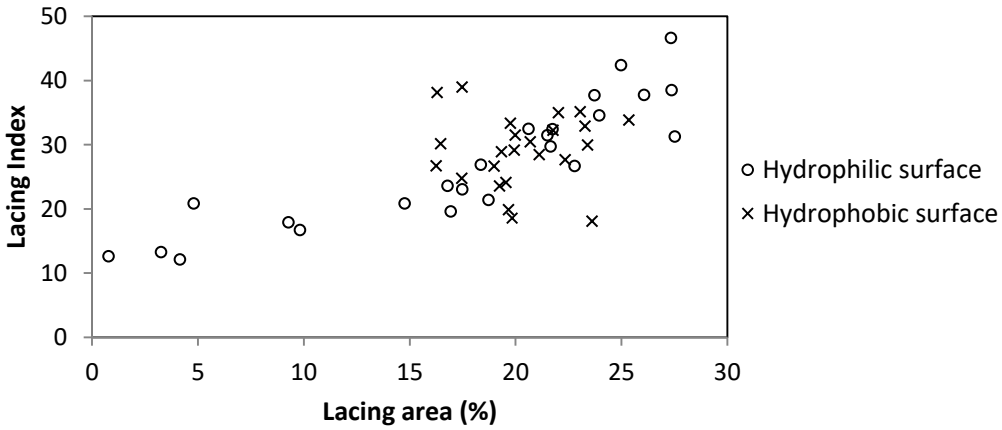

Figure 4.1 Correlations between lacing area and lacing index on different surface qualities.

As for statistical analysis, it showed significant differences over concentrations of iso-α-acid at 95% confidence interval from two-way ANOVA analysis irrespective to the hydrophobic or hydrophilic glass surface. It is also noticed that the effect of protein concentration on lacing was also statistically proved to be significantly different on hydrophobic glass surface. It indicates that lacing index is an important parameter sensitive to the protein concentration. While between the two factors, iso-α-acid is the main effect of lacing (Appendices 7.4&7.5). In all, there are several substantial parameters to indicate the lacing formation and its properties, thus it is not reasonable or convincible to draw a conclusion simply from one or two indices.

4.4 Lacing connectivity analysis

The study from Kunimune and Shellhammer has reported inspiring results that they discovered the lacing patterns varied with the concentration and the type of reduced forms of iso-α-acid. Based on distributions and characteristics of laced foams, they categorized lacings into three groups: ring, mesh and powdery. But unfortunately this morphological property cannot be quantified or described numerically. (Kunimune and Shellman, 2008) From the former study of individual advanced course, which is a small project to investigate beer lacing patterns of commercial beer on hydrophilic and hydrophobic glass surface by pouring method. Hydrophobic and hydrophilic glass showed apparent different lacing patterns (Figure 4.2). Besides, hydrophilic glass has intact lacing with good continuity, and hydrophobic glass displayed more dispersed lacing with many small lacing fragments. This is a very interesting result that showed on different surface properties. Therefore, the number of perimeters of each independent lacing fragment was measured by image dealing software “Image J”. The concept of perimeter measured from lacing fragments is that it tends to have a greater value when there are more independent small fragments and laced foams are highly dispersed instead of bigger area of lacing fragments.

The result of statistical T test for the difference between hydrophobic glass surface and hydrophilic glass surface by the parameter of perimeter, and it showed a significant difference at 95% confidence interval. In this study, three parameters of lacing area, lacing index and perimeter were investigated and compared simultaneously. Among these parameters, only the statistical analysis of perimeter demonstrated a significant difference between two kinds of glass surfaces. Therefore, perimeter can be used as one parameter to describe and characterize lacing pattern quantitatively.

Figure 4.2 Lacing from beer by pouring method on hydrophilic (left) and hydrophobic (right) beakers

4.5 Contact angle and assumptions of molecular interaction

Figure 4.3 displays the result of contact angle with concentration of iso-α-acid. It is obvious that the contact angles reduced along with the increasing concentrations of iso-α-acid. From microscopic point of view, on hydrophilic glass slide, the surface is possibly displaying a positive charge with cations as a result of immersion in 30% hydrochloric acid, and the negative charge of iso-α-acid (pKa=3), the interaction of cation and anion enhanced the affiliation of solution on glass slide by raising up the amount of iso-α-acid, thus the liquid drop extended for wider interact surface on glass accordingly. On hydrophobic glass slide, the hydrophobic part of hydrocarbon chain in iso-α-acid and polypeptides should contributed to interact with hydrophobic surface of glass slide, thus contact angles decreased with the increase of hydrophobic groups. The hydrophobicity of proteins from barley was researched and it has shown that the foam stability was enhanced with the more hydrophobic side group of peptides. (Bamforth and Kanauchi, 2003) In this case, it is also reasonable to elucidate that on the hydrophobic surface of glass slide, contact angle of 0.15% protein concentration is lower than with 0.3% concentration of protein. And in turn on hydrophilic surface of glass slide, hydrophobic peptides are resistant to stay on surface hence the lower contact angle was observed for the 15% protein concentration.

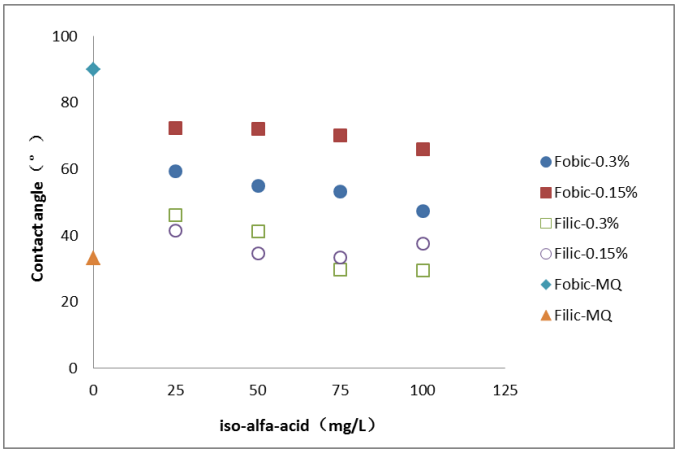

Figure 4.4 Contact angle of Milli-Q water and each sample on modified hydrophilic and hydrophobic glass slides.

However, the foam system in beer and the interaction between proteins and iso-α-acid are more intricate. Theoretically, both the polypeptides (proteins) and iso-α-acid both have amphiphilic structures with both hydrophilic and hydrophobic sides, but polypeptides show more hydrophobic property in beer system. In the study from Simpson and Hughes, it is assumed that the binding force by ion-dipole effect between iso-α-acid and polypeptides as well as the hydrophobic interactions help to stabilize foams (Simpson and Hughes, 1994). This is in an agreement with the reported literature that the more hydrophobic groups can benefit for strengthening the foam stability. (Kunimune and Shellman, 2008)(Evans et al., 2008). The conclusion was also observed in during the present study, which the generated laced foams on glassware surface with higher iso-α-acid concentration stayed longer time and it slowly dried up or dissipated compared with samples with lower concentration of iso-α-acid. Nevertheless, the stability and retention time of laced foam on glass surface can be an interesting topic for future studies.

5. Conclusion

From the contact angle measurement, it indicated that the proteins in beer were of hydrophobic property and iso-α-acid is an ideal surfactant and it can help beer to wet on glass surface as its concentration increasing, on both the hydrophilic/hydrophobic glass surface. But the interaction between protein and iso-α-acid is complex and needs more evaluations. Through the analysis of lacing area, it shows lacing on hydrophilic glass surface was more sensible to the concentration of iso-α-acid. The results of lacing index showed that both protein and iso-α-acid concentrations significantly ($p<0.05$) influenced the value of lacing index on hydrophobic glass surface. Interaction effect of protein and iso-α-acid was significant by statistical analysis at 95% confidence interval. As for connectivity of laced foam, it was more fractured and independent lacing on hydrophobic glass surface. While lacing on hydrophilic glass surface was more intact and continued. This is persuasive to prove the difference in morphological characteristic between lacing generated on hydrophobic and hydrophilic surface of container.

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7. Appendices

		Iso-α-acid			
		25 mg/L	50 mg/L	75 mg/L	100 mg/L
Hydrophilic	0.3% Protein				
	0.15% Protein				
Hydrophobic	0.3% Protein				
	0.15% Protein				

Figure 7.1 Representative lacing photos acquired from each sample

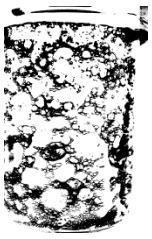
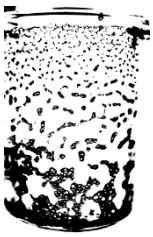
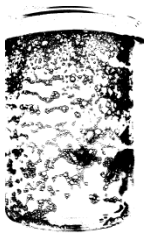
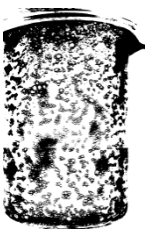
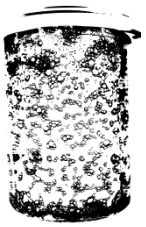
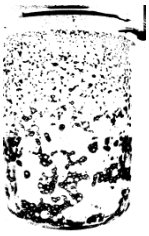
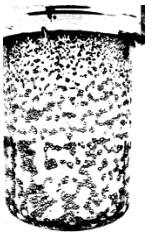
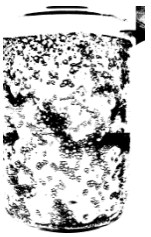
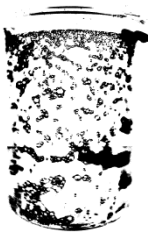
		Iso-α-acid			
		25 mg/L	50 mg/L	75 mg/L	100 mg/L
Hydrophilic	0.3% Protein				
	0.15% Protein				
Hydrophobic	0.3% Protein				
	0.15% Protein				

Figure 7.2 Images of lacing photos dealt with Image J

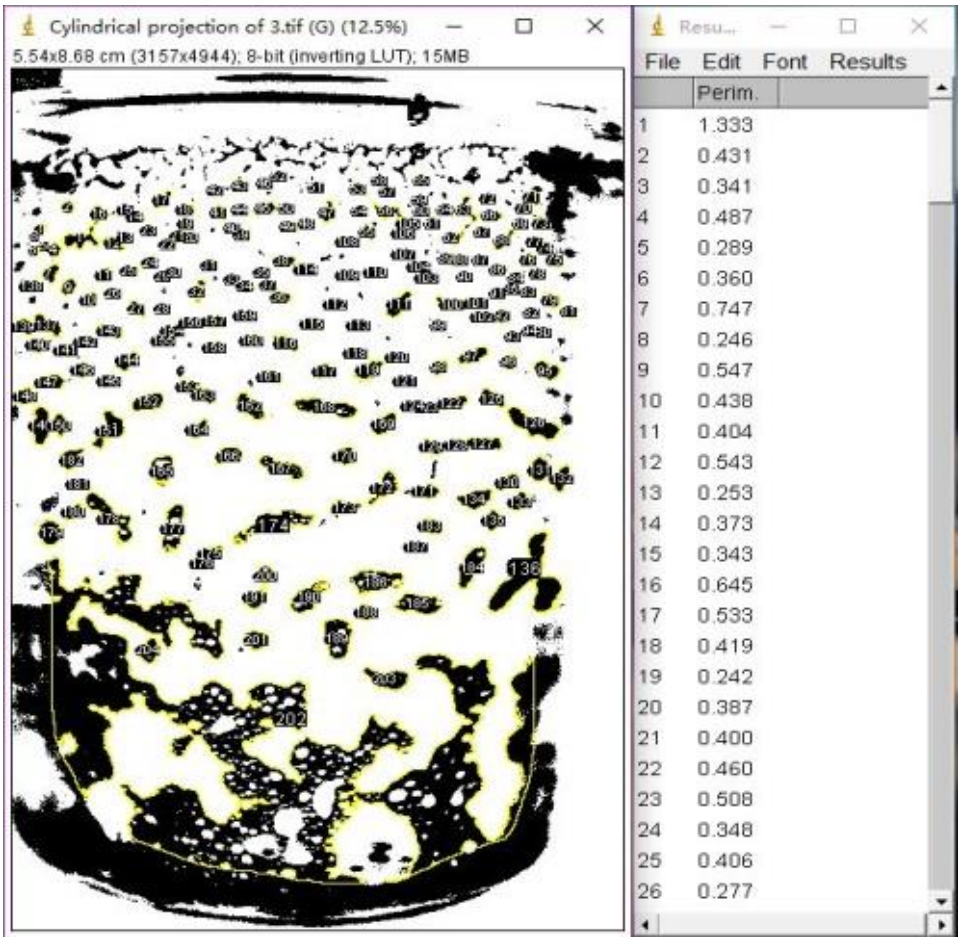


Figure 7.3 Example of measuring perimeter (0.3% protein, 25 mg/L iso-α-acid on hydrophobized glassware)

Table 7.1 All data with average (AVG) and standard deviation (SD)

	Area(%)				Lacing index				Perimeter(cm)			
iso-α-acid	Hydrophilic		Hydrophobic		Hydrophilic		Hydrophobic		Hydrophilic		Hydrophobic	
(mg/L)	0.30%	0.15%	0.30%	0.15%	0.30%	0.15%	0.30%	0.15%	0.30%	0.15%	0.30%	0.15%
	4.16	4.81	23.61	17.46	12.11	20.82	18.08	24.76	69.20	31.13	156.46	275.87
25	9.83	0.80	19.83	22.35	16.69	12.59	18.57	27.65	62.85	19.80	167.83	241.58
	9.29	3.26	19.67	19.54	17.86	13.24	19.86	24.11	82.38	71.84	189.72	211.46
AVG	7.76	2.95	21.04	19.79	15.55	15.55	18.83	25.51	71.47	40.92	171.34	242.97
SD	3.13	2.02	2.23	2.45	3.04	4.58	0.92	1.88	9.96	27.36	16.91	32.23
	14.77	22.80	19.25	18.99	20.84	26.63	23.54	26.63	207.24	247.28	257.53	294.73
50	18.73	18.38	19.34	21.13	21.36	26.83	28.91	28.44	227.35	252.62	279.93	298.89
	16.95	17.50	21.78	16.47	19.59	23.00	32.25	30.12	195.23	231.30	278.39	251.09
AVG	16.82	19.56	20.12	18.86	20.60	25.49	28.23	28.40	209.94	243.73	271.95	281.57
SD	1.99	2.84	1.43	2.33	0.91	2.16	4.40	1.74	16.23	11.09	12.51	26.48
	21.66	23.97	19.94	22.03	29.67	34.55	29.16	34.97	302.57	286.42	321.14	363.98
75	20.62	27.35	20.70	16.30	32.44	46.61	30.44	38.11	218.64	264.81	350.43	328.57
	21.51	25.00	25.35	17.48	31.44	42.34	33.84	38.94	246.21	289.37	286.98	303.28
AVG	21.26	25.44	22.00	18.60	31.18	41.16	31.15	37.34	255.81	280.20	319.52	331.94
SD	0.56	1.73	2.93	3.02	1.40	6.12	2.42	2.09	42.78	13.41	31.76	30.49
	26.08	16.80	23.40	23.29	37.74	23.58	29.95	32.89	172.21	184.13	283.28	189.29
100	27.39	23.73	19.76	16.25	38.46	37.65	33.35	26.68	227.03	205.00	235.46	216.28
	27.54	21.76	23.05	19.99	31.24	32.38	35.10	31.50	187.11	198.09	319.98	168.53
AVG	27.00	20.76	22.07	19.85	35.81	31.20	32.80	30.36	195.45	195.74	279.57	191.36
SD	0.80	3.57	2.01	3.52	3.98	7.11	2.62	3.26	28.34	10.63	42.38	23.94

Table 7.2 Two-way ANOVA with different variances of lacing area on hydrophobic glass surface

	Sum off squares	Degrees of freedom	Mean square	F-value	P-value	F crit
Protein	24.82	1	24.82	3.77	0.07	4.49
Iso-α-acid	6.59	3	2.20	0.33	0.80	3.24
Interaction	4.63	3	1.54	0.23	0.87	3.24
Error	105.32	16	6.58			
Total						

Table 7.3 Two-way ANOVA with different variances of lacing area on hydrophilic glass surface

	Sum off squares	Degrees of freedom	Mean square	F-value	P-value	F crit
Protein	6.39	1	6.39	1.20	0.29	4.49
Iso-α-acid	1336.40	3	445.47	83.62	0.00	3.24
Interaction	124.05	3	41.35	7.76	0.00	3.24
Error	85.24	16	5.33			
Total	1552.09	23				

Table 7.4 Two-way ANOVA with different variances of lacing index on hydrophobic glass surface

	Sum off squares	Degrees of freedom	Mean square	F-value	P-value	F crit
Protein	42.02	1	42.02	6.17	0.02	4.49
Iso-α-acid	487.32	3	162.44	23.85	0.00	3.24
Interaction	91.28	3	30.43	4.47	0.02	3.24
Error	108.98	16	6.81			
Total	729.60	23				

Table 7.5 Two-way ANOVA with different variances of lacing index on hydrophilic glass surface

	Sum off squares	Degrees of freedom	Mean square	F-value	P-value	F crit
Protein	39.43	1	39.43	2.23	0.15	4.49
Iso-α-acid	1639.31	3	546.44	30.93	0.00	3.24
Interaction	177.80	3	59.27	3.35	0.05	3.24
Error	282.70	16	17.67			
Total	2139.23	23				

Table 7.6 Two-way ANOVA with different variances of perimeter on hydrophobic glass surface

	Sum off squares	Degrees of freedom	Mean square	F-value	P-value	F crit
Protein	11.25	1	11.25	0.01	0.91	4.49
Iso-α-acid	47936.63	3	15978.88	19.70	0.00	3.24
Interaction	19726.21	3	6575.40	8.11	0.00	3.24
Error	12979.25	16	811.20			
Total	80653.35	23				

Table 7.7 Two-way ANOVA with different variances of perimeter on hydrophilic glass surface

	Sum off squares	Degrees of freedom	Mean square	F-value	P-value	F crit
Protein	292.36	1	292.36	0.56	0.46	4.49
Iso-α-acid	151984.12	3	50661.37	97.40	0.00	3.24
Interaction	3712.89	3	1237.63	2.38	0.11	3.24
Error	8322.25	16	520.14			
Total	164311.63	23				

Table 7.8 The results of student t-test of perimeters on hydrophobic and hydrophilic glass surface

	Hydrophobic	Hydrophilic
Average	261.28	186.66
Variance	3506.67	7143.98
Observations	24	24
Pearson Correlation	0.62	
Hypothesized Mean Difference	0	
df	23	
t Stat	5.47	
P(T<=t) one-tail	7.38E-06	
t Critical one-tail	1.71	
P(T<=t) two-tail	1.48E-05	
t Critical two-tail	2.07	