FOAM FRACTIONATION AS A MEANS TO RECOVER AND FRACTIONATE BIOACTIVES

Tong Tong Yu[1], Zaid S Saleh[2]

[1]*Department of Chemical and Materials Engineering, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

[2]*Department of Chemical and Materials Engineering, The University of Auckland, Private Bag 92019, Auckland, New Zealand*

Abstract

In this research, the optimum conditions for the separation of polyphenols from apple juice (AJ) and the separation of BSA from a BSA/water solution were found by looking at the enrichment ratio (ER) and recovery. A mixture of the AJ and BSA was also studied to see how the different conditions affect separation of either compound. For AJ only, maximum ER was achieved at a feed concentration of 0.00184mg/mL of polyphenols (10.9˚Brix), air flowrate of 10mL/min, pH of 6.03 and temperature of 4.1˚C. For the BSA only solution, maximum ER was achieved at a feed concentration of 2.5g/L, air flowrate of 7mL/min, pH of 5-6 and temperature either above or below 20˚C. For the mixture, maximum polyphenol ER was at a feed polyphenol concentration of 0.0018mg/mL, feed BSA concentration of 5g/L and a pH of 3.7. Whereas maximum BSA ER was achieved when the feed polyphenol concentration was again 0.00184mg/mL, but a minimum was seen at 5g/L BSA feed concentration and pH of 3.7. These results show that there are interactions between the polyphenol and proteins that affects the separation of compounds. The bubble size distribution and gas hold-up of the AJ and BSA solutions were then compared and the BSA solution had larger bubbles present and a greater gas hold-up, which is attributed to the greater surface activity of BSA protein. Finally basic modelling – linear regression, was performed to link the ER to the different conditions tested. The models were judged by comparing R^2 values, the F-statistic and the p-value and all showed weak relationships between the ER and the independent variables. Further data is required to improve the model as from previous literatures these variables have been seen to contribute to adsorption, which in turn affects the ER.

Keywords: *Foam Fractionation, Polyphenols, BSA, Optimum Conditions*

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1. INTRODUCTION

Foam fractionation is encompassed under the term ‘adsorptive bubble separation techniques’. This term includes several techniques (froth flotation, bubble fractionation, solvent sublation), which all require the use of bubbles to adsorb compounds that are required to be extracted from a liquid medium [1]. Foam fractionation, as the name suggests, forms foam from the bubbles. This foam is collected and collapsed producing foamate, which should contain a high concentration of the compound to be extracted.

For adsorption to occur, the compound to be extracted must be surface active i.e. be able to change the surface tension. Thus, in foam fractionation, adsorption should not only extract the desired compound, but also allow foam to be stabilised [2]. Typically the performance of foam fractionation is quantified by looking at the enrichment ratio, recovery and sometimes the selectivity (when more than one surface active compound present).

Foam fractionation has been applied to a wide range of applications and is most significantly used in the waste water industry. Foam fractionation is widely applicable as it allows the extraction of compounds that are very low in concentration, while still being inexpensive as energy expenditure is low. Foam fractionation has also been commonly used for the extraction of proteins as it is usually ‘gentle’ enough that denaturation would not occur. Another advantage of using foam fractionation is that large quantities of liquid can be processed, saving time. These factors make foam fractionation a possibly better alternative to other separation methods such as chromatography (expensive and time consuming but produces very pure samples), membrane technology (again expensive and would require replacements often due to fouling) and solvent extraction (solvent required may be expensive or unsafe; there may also be difficulties in removing solvent).

There has been very limited use of foam fractionation in the food industry but there is potential for further commercialisation. Apple juice (AJ), the most common non-citrus juice, contains polyphenols that can cause browning and bitter/ astringent tastes [3]. Bioactives in fruits have also been linked to decreased risk of chronic diseases [4]; this includes the polyphenols found in AJ. Polyphenols are also surface active due to amphiphilic properties, so extraction of polyphenols from AJ would increase quality of AJ, while being a possible source of polyphenols, which as a functional food could be used to produce supplements. Therefore the objective of this research was to find the optimum conditions for foam fractionation of polyphenols

from AJ and to mathematically model this process using simple linear regression. This research also compared the aforementioned process with foam fractionation of bovine serum albumin (BSA) (a globular protein) as at times polyphenol-protein complexes can be found in juices.

There are several variables which affect the performance of foam fractionation: gas flow rate, pH, temperature, feed concentration. These parameters were tested to find the optimal conditions i.e. conditions at which the highest enrichment ratio is achieved. Experiments were done on AJ, BSA in solution as well as a mixture of both. Bubble size analysis was also performed to compare the size of bubbles found in AJ and BSA solution. Adsorption is very important in foam fractionation as it not only is the mechanism of extraction, but also is how the foam becomes stabilised as surface tension is lowered [2]. The Gibb's equation allows quantification of the adsorption of a species at the gas liquid interface (γ – surface tension, R – universal gas constant, T – temperature, C – concentration in liquid) [1]and forms the basis for Leonard and Lemlich's model described next:

$$Surface\ excess, \Gamma = -\frac{1}{RT} \cdot \frac{d\gamma}{dlnC}$$

From the literature it was generally seen that because of the many different factors affecting the performance of foam fractionation models are very complicated but in general a material balance was performed initially. Leonard and Lemlich proposed a very extensive model in 1965 that allowed the prediction of the concentration of the outlet streams for different modes of operation, at steady state, by taking into account many parameters involved with the interstitial flow and drainage to form dimensionless numbers, which were combined with material balances [5]. The complexity of this model made it very difficult to apply as several parameters could not be found easily and there was no way of knowing when steady state was achieved.

In 2006, Stevenson proposed a new empirical model, which provided a simpler model of drainage, compared to the work of Leonard and Lemlich [6]. Dimensional analysis was performed to allow predictions of drainage with only information about the liquid hold-up being required and it was seen that a simple power law could be used to relate these two [6].

From the literature it can be seen that modelling involves the use of dimensionless number and mass balances. This research could provide a simple model of predicting the enrichment ratio of polyphenolics when foam fractionation is used as a means of extraction.

The use of foam fractionation in biotechnological applications (generally the recovery of proteins e.g. enzymes) seems to have the longest history as proteins are naturally surface active and other methods of separation are time consuming and economically infeasible for large scale applications e.g. chromatography, ion exchange and liquid-liquid extraction [7]. A number of reports have appeared since the 1930's about the extraction of enzymes: In 1937 Ostwald and Siehr removed albumin from beet and potato juice [7, 8]; in 1945 Bader and Schütz used foam fractionation to extract components of bile [9]; in 1954 London et al. used foam fractionation to purify urease and catalase mixtures under different conditions (varied protein concentration, pH, gas flow rate, column characteristics, bubble size and use of additives) and that under optimal conditions recovery of urease were close to 100% [10]; in 1959 Schnepf and Gaden investigated the effect of pH and concentration on the separation of bovine serum albumin from solution using foam fractionation and it was reported that maximum enrichment was seen at the isoelectric pH (point where solubility of protein is at its minimum) and decreased with increasing protein concentrations [11].

In more recent times, a more comprehensive study has been done on the foam fractionation of BSA, used as a model for potato wastewater, which can contain 0.1wt% to 0.2wt% of proteins [12]. This involved the testing of feed concentration, superficial gas velocity, feed flow rate, bubble size, pH and ionic strength. It was found that enrichments increased with decrease in feed concentration, bubble size and superficial gas velocity and that the pH and ionic strength changes made bubble sizes difficult to control –minimum enrichment was at the isoelectric pH, which does not correlate with the report by Schnepf and Gaden [12]. A model was also developed based on previous work by Narsimhan and Ruckenstein, which should have allowed predictions for the enrichment ratio though quantitative predictions could not be made as there was a distribution of bubble sizes [12].

There has been limited use of foam fractionation in the food industry, but research has shown there is potential for commercial application of this technology as it is an inexpensive way of extracting dilute high value compounds. For example lactoferrin, found in diary processing, is of high value as a functional food and can be made into supplements [13]. In 2001, Saleh and Hossain used foam fractionation to separate BSA from binary and ternary mixtures consisting of BSA, α-lactalbumin and lactoferrin prepared to be comparable to mixtures found in the dairy industry. The foam was used to remove BSA and α-lactalbumin, while the target protein lactoferrin remained in the liquid. In this investigation the feed concentration, bubble size, superficial gas velocity and feed pH were again tested to see how the enrichment ratio and recovery were affected. Again it was found that enrichment and recovery increased with lower feed concentrations, lower gas velocity and smaller bubbles [14]. At the isoelectric point, maximum enrichment and recovery was seen [14], this is concordant with results seen by Schnepf and Gaden [11]. The mass transfer coefficient was found for different feed concentrations and it was observed that this value increases with increasing concentration [14].

The same researchers also investigated the extraction of α-lactalbumins from low-value effluent whey permeate solution, again found in the dairy industry [15]. It was seen that the temperature of the system was an important

consideration as this affects the volume of the foam and enrichment, while the air flow rate did not significantly affect enrichment [15]. In 2013, Liu et al. used foam fractionation and acid hydrolysis to recover isofalvone aglycones from soy whey wastewater. This was done as isoflavone aglycones have potential health benefits and foam fractionation was used as chromatography and solvent extraction is infeasible due to the low concentration and large quantity of waste water [16]. In this example, foam fractionation was used as a preliminary way of concentrating soy isoflavones, after which, acidic hydrolyses was used to produce aglycones from glycosides. Another potential use of foam fractionation is in the juice sector particularly AJ production. Apple is one of the most common non-citrus fruits and a large proportion of apples are processed into AJ [17]. Fruit and vegetable juice has accounted for around US$4billion in terms of trade and in 2007 [17], the value of juice imports was about US$1.5billion, a growth from US$1billion in 2006 [17]. Thus, this is a significant industry where foam fractionation could be used in the future.

2. METHODOLOGY

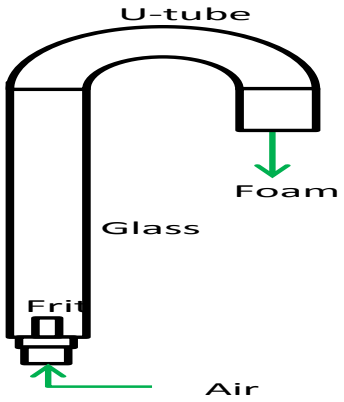


Fig -1: Schematic of foam fractionation column

The foam fractionation column used was a glass column with two metal frits at the bottom connected to a compressed air supply (Fig. 1). These metal frits allowed the generation of small air bubbles and a rotameter was used control the flowrate of the air. Initial experiments conducted allowed optimum condition ranges to be found with regards to the separation of polyphenols from AJ and BSA from a BSA/water solution. Immature AJ concentrate was used to make up juice of varying ˚Brix, which corresponded to different feed polyphenol concentrations.

2.1 Polyphenol Extraction - Optimum Conditions

Firstly the effect of feed phenolic concentration on fractionation performance was determined. This involved the testing of different AJ concentrations (hence different polyphenol concentration) and a Brix refractometer was used to measure concentration in terms of ˚Brix. The concentrations tested were 2.7˚Brix (0.00104mg/mL catechin equivilant = polyphenol conc.) 5.3˚Brix (0.00135mg/mL), 7.1˚Brix (0.00173mg/mL), 10.9˚Brix (0.00184mg/mL), 12.5˚Brix (0.00238mg/mL), 15.4˚Brix (0.00293mg/mL). At an air flowrate of 7mL/min, each run was allowed to foam until foam stopped rising up the column. Samples from the initial feed juice, the foamate and the remaining juice were collected for each run and all samples were analysed using the Folin- Ciocalteu assay for total phenolic content, which analyses each sample in triplicate and provides absorbance values, from which concentration values are obtained using standard curves. The foam fractionation performance was then determined by looking at:

$$Enrichment\ ratio\ (ER) = \frac{Polyphenol\ concentration\ in\ foam}{Polyphenol\ concentration\ in\ feed}$$

$$Recovery = Percentage\ of\ feed\ polyphenols\ recovered\ in\ foam$$

The experiment was repeated to test different parameters each time: air flowrates of 5mL/min, 10mL/min, 12.5mL/min, 15mL/min were tested with the concentration kept at 15˚Brix. The pH values tested were 2.5, 3.75, 4.6, 6.03 and 7.0 and this was varied by adding NaOH or HCl solutions to the juice. The effect of temperature was seen by testing three different temperatures: 4.1˚C, 17.6˚C and 40.9˚C. For all experiments a sample of the initial feed, foamate and remaining feed was collected, the weight of the fomate measured and the Folin-Ciocalteu assay performed.

2.2 BSA Extraction - Optimum Conditions

These experiments were very similar to the separation of polyphenol, except that BSA solutions were used. Again initial feed concentration was tested: 5mg/L, 10 mg/L, 15 mg/L, 20 mg/L. Samples from the initial feed, the foamate and the remaining solution were taken for each run, these samples were analysed using the Bradford protein assay. Performance was still measured using ER and recovery. The same parameters, seen above: air flowrates, pH (3.7, 5.1, 6.2, 7 and 8.3) and temperature were tested to determine the optimum conditions for BSA separation by again comparing ER and recovery values.

2.3 Mixture of Polyphenol and BSA – Optimum Conditions

Firstly the extraction of a mixture containing equal concentrations of BSA and polyphenols was tested but it was seen that the amount of foam produced was not enough to conduct testing so different concentrations were used subsequently. Different parameters were again tested: air flow rates, concentration of the different components (10˚Brix, 5˚Brix, 2.5g/L BSA, 5g/L and 7.5g/L), pH and temperature. Both the Folin- Ciocalteu assay and the Bradford assay were performed and new standard curves that took into account the effect of each component were plotted. The performance was then determined but this time selectivity was also found:

$$Selectivity = \frac{ER\ of\ BSA}{ER\ of\ polyphenol}$$

2.4 Gas Hold-up and Bubble Size Distribution

Gas hold-up was found for each of the solutions at their normal unadjusted conditions, by visually observing the drop in liquid level, five minutes after the rotameter was suddenly closed. The difference in volume was found as both the diameter of the column (3.5cm) and the height of the solution in the column were known. The gas hold-up was then taken as the change in volume divided by the volume after rotameter was closed. To find the bubble size distribution, a Canon EOS55D camera was used to take photos of bubbles passing through the AJ and BSA solutions. A piece of black paper was used as the background and a torch was used to illuminate bubbles. This could not be done on the mixture solution due to its opacity. The photos were then analysed using LensEye, which provided bubble size values. In LensEye, initially a threshold L value was determined by using the 'Show Contours' function. An L value that showed clear definition of bubbles was then chosen as the threshold L value.

Next a binary file was produced, which made the background white and bubbles had an L value higher than the threshold value were coloured black. A section of this binary file was then analysed by firstly setting the 'Blob Analysis' options: Min area to remove blob was set to 2 pixels then using 'Colour Analysis' each black object is numbered and its area (in pixels) was provided in a spread sheet. Then assuming the bubbles were purely circular, the radius in pixels can be found (as $A = \pi R^2$). And because a reference object was included, 1cm could be found in terms of pixels e.g. for AJ solution 1cm = 164pixels. This means that the radius can then be converted into cm values and thus give more useful information with regards to size.

Finally a bubble size distribution graph was plotted. Size ranges were made and frequencies of bubble sizes were found. The frequency was then converted into a frequency density:

$$Frequency\ density = \frac{Frequency}{Total\ no.of\ bubbles\ analysed}$$

3. RESULTS AND DISCUSSION

3.1 Statistical Analysis

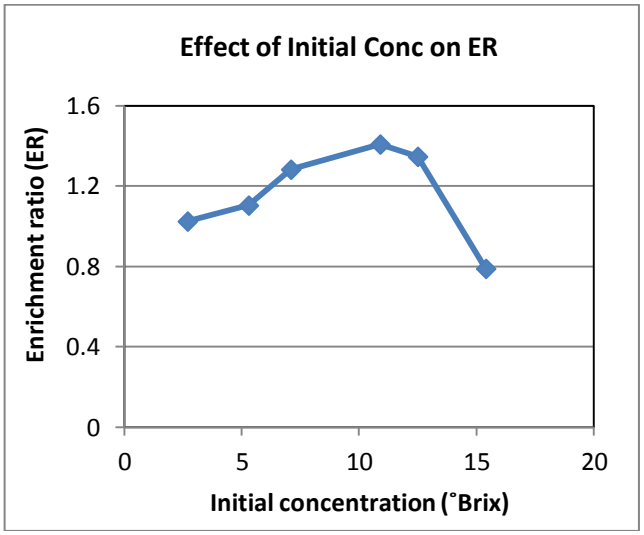
R (free statistical software) was used to analyse raw data values. These raw data values are the concentrations of the feed, foam and after solutions, found from the absorbance values acquired through performing the Folin or Bradford assays. ER and recovery are both quotients used to allow the comparison of separation at different parameters. To work these out, the averages of the foam and feed concentrations were used as each sample was analysed in triplicates. This increased the complexity of statistical analysis as the act of introducing ratios has been see to increase right skewness as well as non-normality (when the coefficient of variance of the denominator is large) [18, 19].

Firstly raw data for each parameter of interest was analysed for equal variance using the Levene test, and for normality using the Shapiro-Wilk test. It was seen that most of the populations could be considered normally distributed and those that could not be considered normal can appeal to the central limit theorem (CLT). Even if sample sizes were quite small, further experimentation could be performed to increase sample sizes to allow the CLT to apply.

With regards to equal variance, most of the groups had evidence against the null hypothesis (that variance is equal), thus this means that transformations need to be further done. Only natural log transformations can be done to still produce results, where the confidence interval can be interpreted, as this maintains the ordering of the numbers (based on size). But even if all raw data was seen to have equal variance and be normal, the ratio variables are still likely to be right skewed so this has not been pursued due to time constraints. Due to limited time, repeat experiments have only been done on a few of the parameters to be tested. Only experiments that have been repeated have more two ER and recovery values, thus ANOVA can only performed on these experiments. But due to the statistical changes discussed previously and limited data available, ANOVA results obtained would not be particularly significant. Thus error bars have not been included in ER and recovery graphs and so to improve statistical analysis more repeat experiments would need to be undertaken, with further research into the effect of ratio on statistics.

3.2 AJ Optimum Conditions

3.2.1 Effect of Initial Concentration



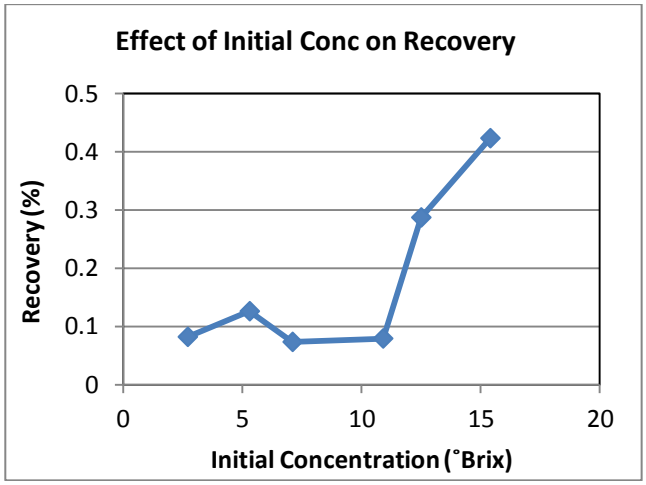


Chart -1: Graphs showing the effect of initial AJ conc. on ER (above) and recovery (below) (pH = 3.7, flowrate = 7mL/min, temp = 20˚C)

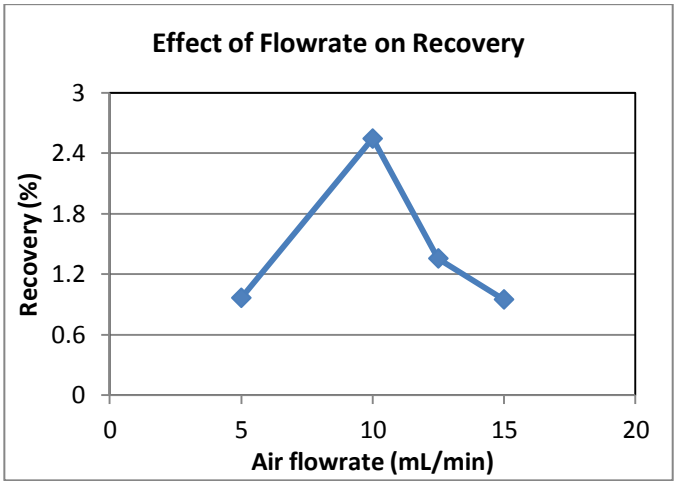
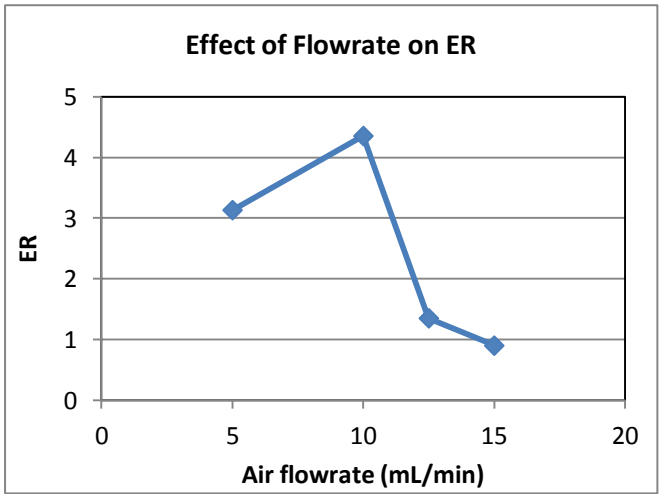


Chart -2: Graphs showing the effect of air flowrate on ER and recovery (initial conc. = 15.9˚Brix, pH = 3.7, temp = 20˚C)

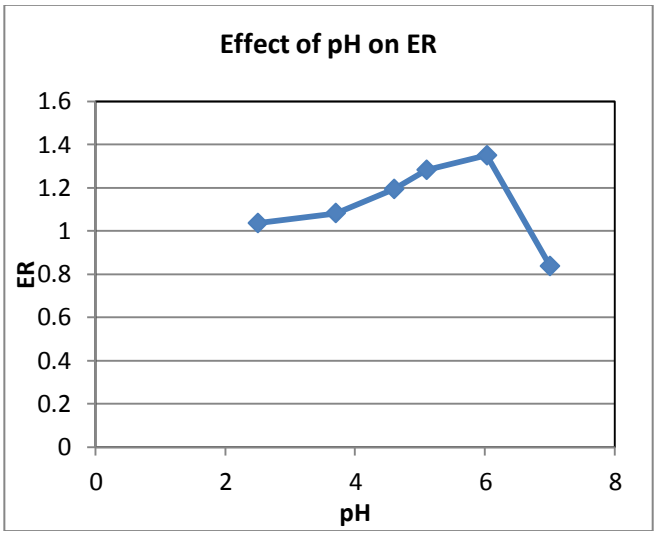
In chart 1 it can be seen that the ideal initial concentration to obtain maximum ER was around 12˚Brix (0.001g/L of catechin equivilant), with ER on both sides of this value decreasing. This shows that there is an optimal amount of polyphenols that must be present, below which there is less significant interaction between air bubbles and polyphenols due to limited amounts. ER was also seen to decrease with further increases in AJ concentration: this is because with increased concentration, the proportion of water decreased producing a decrease in the surface tension of the bubbles. This in turn produced a wetter foam i.e. higher proportion of water in foam, which decreased the concentration of polyphenols in the foam and so decreased the ER. A decrease in surface tension will also affect the adsorption of other compounds, for example sugar adsorption may have increased, which further contributed to the decrease in foam polyphenol concentration and to decreased foam formation.
Recovery on the other hand was seen to increase with initial concentration as with increased amount of polyphenols in the initial solution means increased amount of polyphenols separated out by the foam. This result correlates with literature results [20].

3.2.2 Effect of Flowrate



Air flow rates used were in the range of 5mL/min to 15mL/min and from Chart. 2 it can be seen that both ER and recovery peaked when flowrate was at 10mL/min. This is because lower flowrates meant less air passed into the frits, which decreased the amount of bubbles generated. This then led to decreased absorption surfaces, so less polyphenols separated and thus a lower ER and recovery at 5mL/min. Above 10mL/min, the increased superficial gas velocity decreased the residence time of the bubbles [20], so there was less time for adsorption to occur and less time for the foam to drain. The increased flowrate also causes greater entrainment of liquid in the foam, which effectively diluted the foam, but increased foam volume. Thus as less polyphenols had adsorbed onto the air bubbles and the foam was wetter, ER decreased at higher flowrates. Generally recovery should increase with greater air flowrate due to the increased entrainment of liquid but here it was seen to decrease and this may also be due to the decreased adsorption that in turn decreased foam stability leading to decreased amounts of foam.

3.2.3 Effect of pH



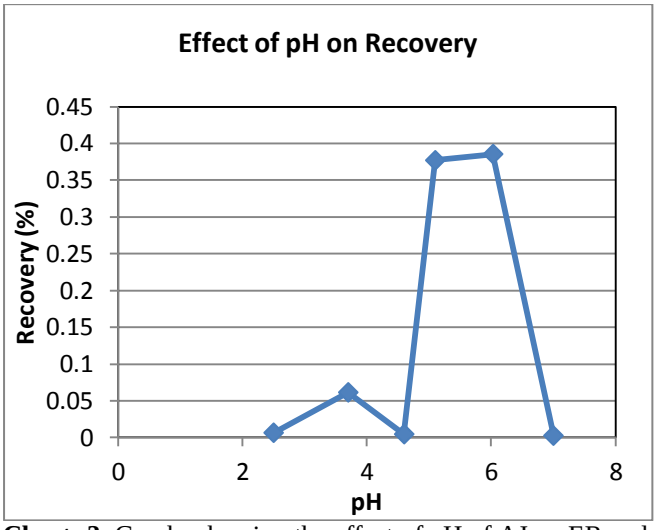
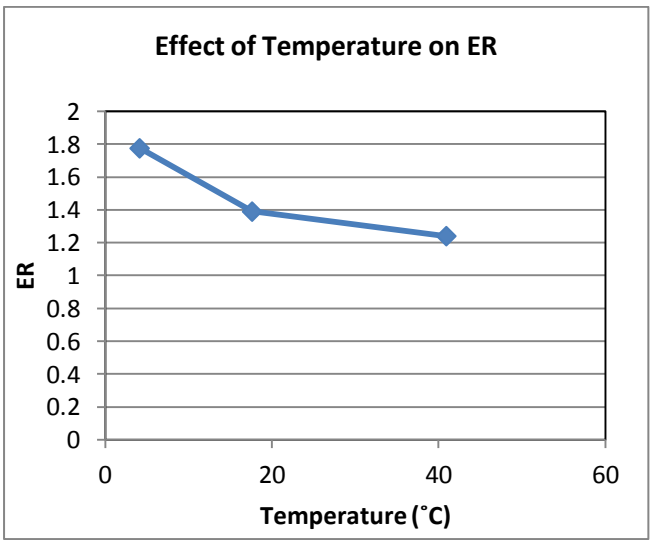


Chart -3: Graphs showing the effect of pH of AJ on ER and recovery (initial conc. = 10.4˚Brix, flowrate = 7mL/min, temp = 20˚C)

The pH of the feed AJ solution affects the surface tension of the air bubbles, which in turn affects adsorption [21]. Looking at Chart 3, both ER and recovery showed maximal values at around a pH of 5 to 6 suggesting that the isoelectric point, of the polyphenolic compounds present, is around this point. The isoelectric point is the pH at which a molecule has no net electrical charge. So at a pH of around 5 – 6 the polyphenolic compounds interacted less with water (a polar molecule) i.e. solubility decreased and more with the air bubbles, thus increased adsorption. Looking at the recovery graph, pH values above and below pH = 5 to 6 were very small (all less than 0.01% except when pH=3.7) and is due to the instability of the foam. This in turn was caused by poorer adsorption due to change in surface tension and is also due to the fact that the polyphenolic compounds had increased interaction with water molecules due to a possible electrical charge.

3.2.4 Effect of Temperature



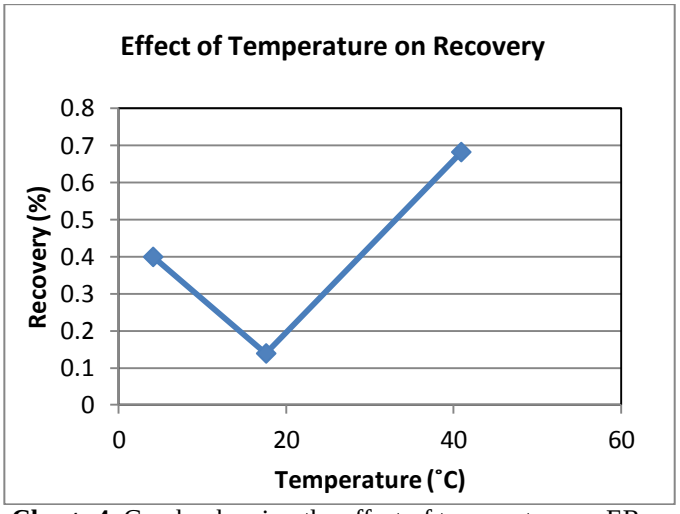


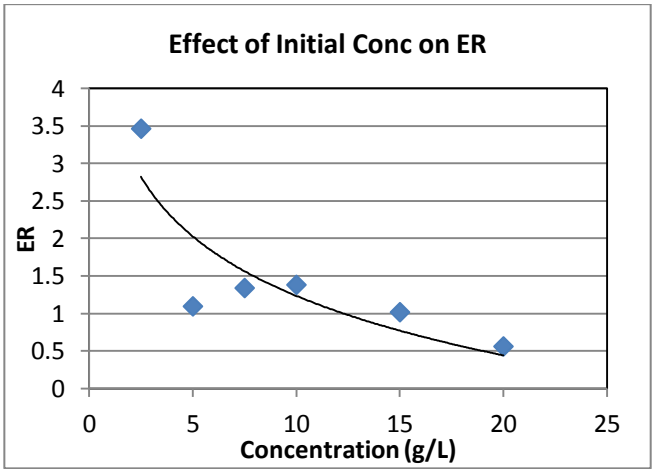
Chart -4: Graphs showing the effect of temperature on ER and recovery (initial conc. = 11˚Brix, flowrate = 7mL/min, pH = 3.7)

It can be observed in Chart 4 that ER decreased with increasing temperature, while recovery initially decreased but then increased going from 20˚C to 40˚C. The ER decreased with increasing temperature as with lower temperature there is greater surface tension of the air bubbles, which in turn increased adsorption and so there was more foam stability. The surface tension changes as with increasing temperature the solution viscosity decreases. Due to this there was also increased foam drainage, which decreased the wetness of the foam and thus increased the foam polyphenol concentration. This then contributed to the higher ER values seen at lower temperatures.

The recovery graph seen is quite unusual as recovery is expected to decrease with increasing temperature, similar to the ER graph, again because of the increased surface tension at lower temperatures. The unusually high recovery at 40˚C may be due to experimental error such as loss of heat due to inadequate insulation and inaccurate measurement of foam volumes as these values were very small (in the µL to mL range).

3.3 BSA Solution Optimum Conditions

3.3.1 Effect of Initial Concentration



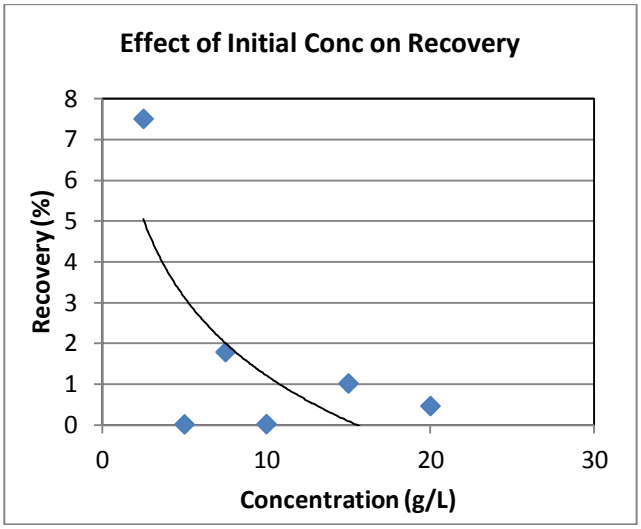
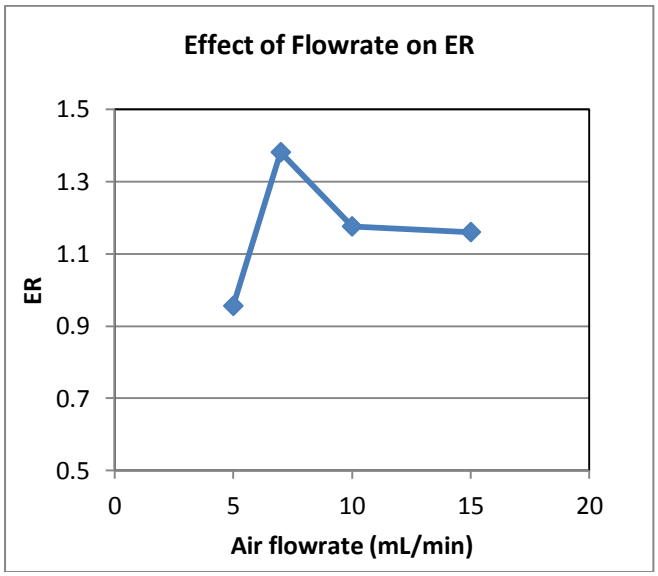


Chart -5: Graphs showing effect of initial BSA solution concentration on ER and recovery (pH = 7, flowrate = 7mL/min, temp = 20˚C)

Looking at Chart 5 it can be noted that both ER and recovery decrease with increasing feed BSA solution concentration. ER and recovery decreases with increasing initial BSA concentration and can be explained in a similar manner as seen in 2.1 Effect of initial concentration (AJ). Lower BSA concentration corresponded to a higher proportion of water, which increased surface tension of bubbles and so enhanced adsorption. This generated foam that had a high concentration of BSA, which contributed to the higher ER and recovery observed. These trends seen are different to the trends observed when AJ was used as no peaks are seen in ER and recovery decreases due to BSA interacting differently with the air bubbles due to its larger size compared to polyphenolic compounds.

3.3.2 Effect of Air Flow-rate



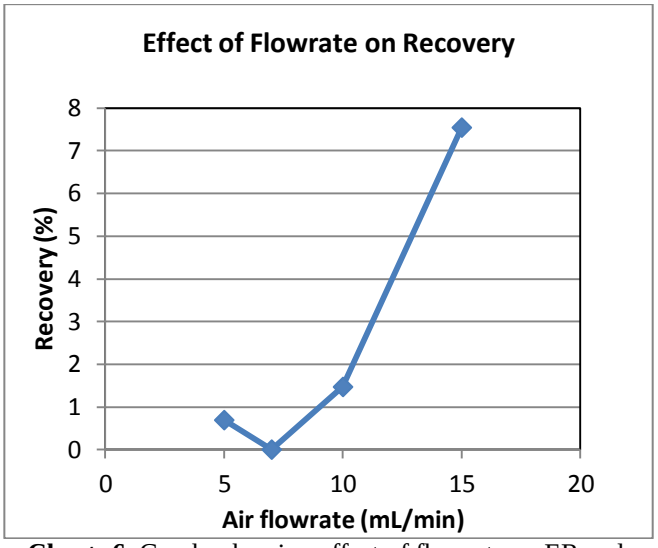
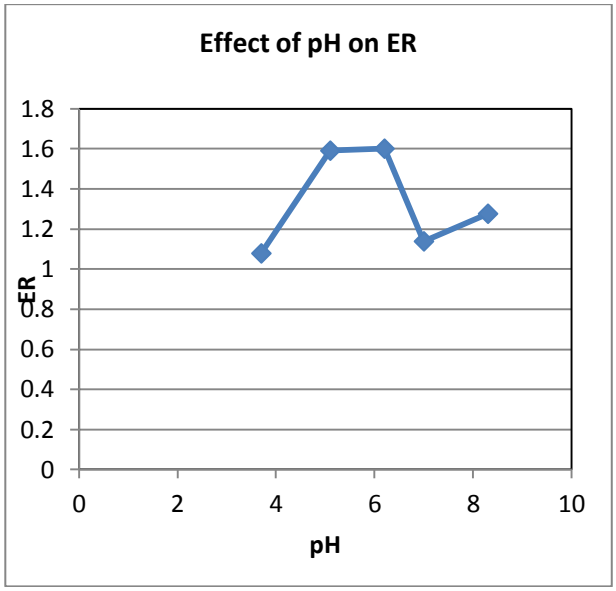


Chart -6: Graphs showing effect of flowrate on ER and recovery (initial conc. = 10g/L, pH = 7, temp = 20˚C)

ER decreases with increasing flowrate (from 7mL/min onwards) and recovery increases with increasing flowrate (Chart. 6). The optimum flowrate is 7mL/min as this produced the maximum ER showing that below this flow not enough bubbles are formed, this decrease in adsorption surface area reduces the BSA adsorbed, which in turn decreases the ER as foam BSA concentration is less. The amount of foam formed is also dependent upon flowrate due to increased flowrate increasing the amount liquid entrained in the foam (foam volume increases) hence increased recovery [12]. The larger flowrates also meant larger superficial gas velocities, and so produced not only a reduced residence time but also a reduced drainage time. Again this diluted the foam BSA concentration but increase volume.

3.3.3 Effect of pH

Foam fractionation of proteins (which are amphoteric compounds i.e. able to act as an acid or a base) is highly dependent upon the pH as this affects molecular charge, surface tension as well as bubble size. The optimum pH in terms of peak ER is around 5 – 6, whereas in terms of recovery it is around 7. The ER peak is at around pH = 5 to 6 as this is close to the isoelectric point of BSA at pH= 4.8. At its isoelectric point, the BSA proteins had no net charge, thus were able to interact more readily with air bubbles as water molecules are polar. This along with a decrease in intermolecular repulsion increased the adsorption of BSA so foam protein concentration increased also, producing the ER peak.



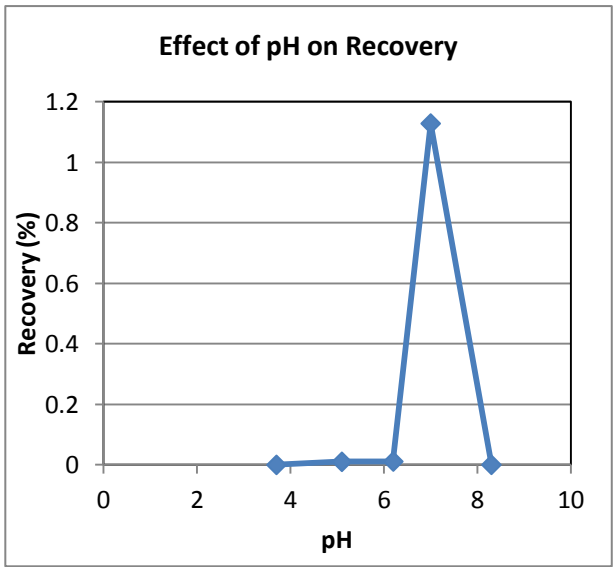
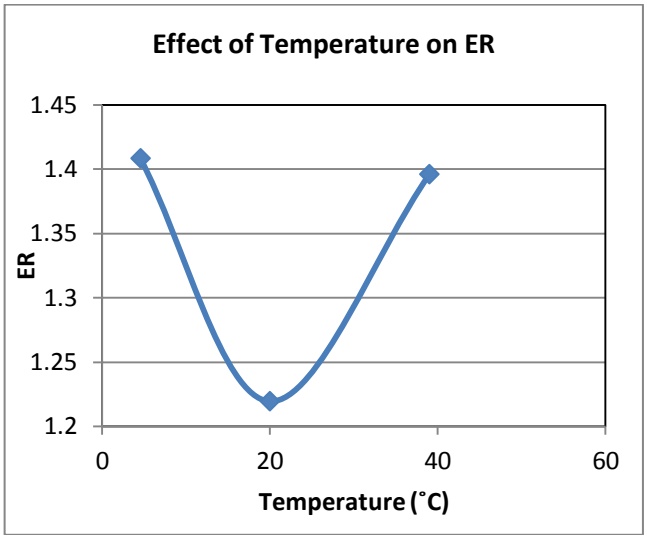


Chart -7: Graphs showing effect of pH ER and recovery (initial conc. = 5g/L, flowrate = 7mL/min, temp = 20˚C)

The maximum recovery of 1.1% was achieved at a pH of 7, which was the normal pH of the solution i.e. no HCl or NaOH solutions were added (Chart .7). This pH is above the isoelectric point of BSA, thus the proteins would have exhibited surface charge, meaning increased interaction with water and so decreased adsorption. But with regards to recovery a pH of 7 meant that proteins were not denatured by pH extremes. Proteins are compounds that have a specific structure as discussed previously and are condition sensitive; this means that any extreme conditions can cause protein denaturation. Adjustment of the pH may have caused structure loss of the BSA, which would then reduce adsorption. Hence decreased recoveries are seen at pH values above and below 7 due to there being fewer proteins present.

3.3.4 Effect of Temperature

Looking at chart 8, ER reaches a minimum at 20˚C, while the highest ER value obtained (=1.4) was at 4.6˚C. This again is due to the lower temperature increasing viscosity, which in turn increased the surface tension that then increased adsorption. ER increase from 20˚C to 40˚C, this shows that to obtain higher ER values, temperature must be either below or above 20˚C. ER is larger at the higher temperature as viscosity is reduced and though this decreases surface tension, foam drainage is improved. This meant that a drier foam was collected that had a higher concentration of BSA. The increased temperature also amplified thermal motion increasing the interaction between BSA and bubbles, thus a higher ER value was seen at 40˚C, but not as high as the ER seen at 4.6˚C due to the difference in viscosity.



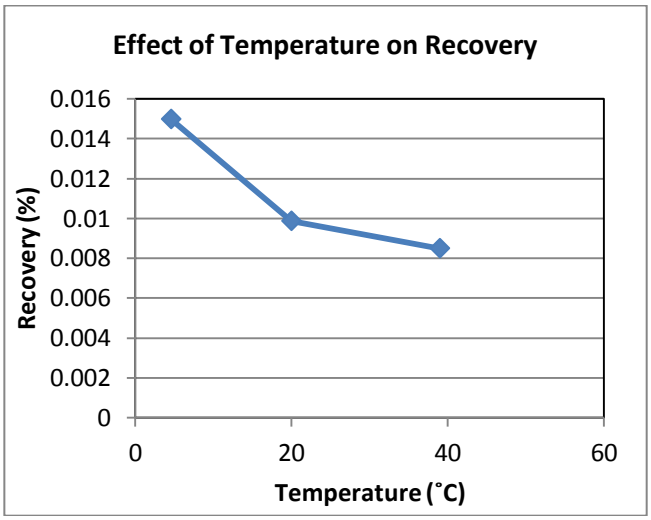


Chart -8: Graphs showing effect of temperature on ER and recovery (initial conc. = 5g/L, flowrate = 7mL/min, pH = 7)

Recovery decreases with increasing temperature (chart 8) and is due to decreased stability of foam formation due to the decreased viscosity that comes with higher temperatures. As it was less stable for foam to form, less foam was collected and so a smaller amount of proteins were recovered.

3.4 MIXTRUE OPTIMUM COONDITIONS

3.4.1 Effect of pH on Polyphenol Separation

Maximum ER and recovery, of polyphenols, were obtained at a pH of 3.7, which is the unadjusted pH for the mixture solution. This is because between pH = 4 and pH = 6, BSA molecules are preferentially adsorbed due to the isoelectric point of BSA (pH = 4.8) being found in this region (see selectivity graph below). At pH = 3.7, the BSA molecules probably have a surface charge and so interact more with the water molecules than the polyphenolic compounds do and so this pH polyphenolic compounds are preferentially adsorbed. This is different compared to the AJ only graphs, where highest ER's were seen between pH = 5 and pH = 6.

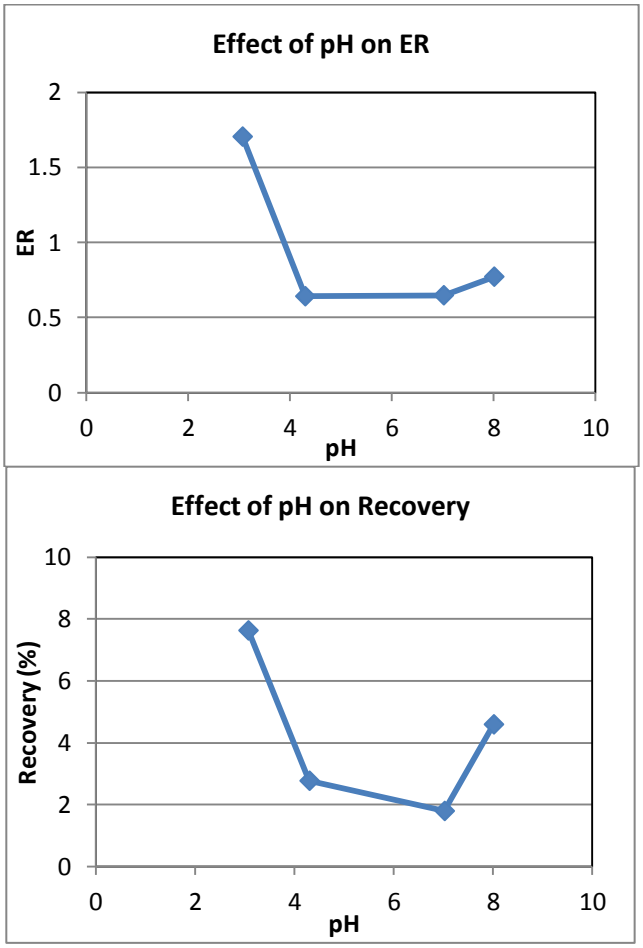


Chart -9: Graphs showing effect of AJ/BSA mixture pH on ER and recovery (initial conc. = 5g/L BSA and 10˚Brix, flowrate = 7mL/min, temp = 20˚C)

There are also protein–polyphenol interactions and one aspect affected by these interactions is foam drainage. The presence of protein increased the viscosity of the solution (compared to an AJ only solution) and the presence of protein-polyphenol interactions would further increase viscosity as the interactions produce larger molecules. The increased viscosity decreased the foam drainage, and so increased the volume of wet foam obtained from foam fractionation and explains the greater increased recovery values seen in Chart. 9 (max recovery of 7.6%).

Both ER and recovery were seen to begin increasing from pH = 7 onwards. This is due to the increased pH denaturing the proteins, which in turn decreased the protein-polyphenol interaction and so increased amount of polyphenols present.

3.4.2 Effect of Polyphenol and BSA Concentration on Polyphenol Separation

From chart 10 it can be seen 10˚Brix has higher ER values than 5˚Brix for all BSA concentrations. This shows that increasing initial concentration of the AJ increases the polyphenol ER as increasing ˚Brix corresponds to an increased initial concentration of polyphenols. More polyphenols present initially meant more polyphenols were separated out in the foam, producing more concentrated foam and hence higher ER values.

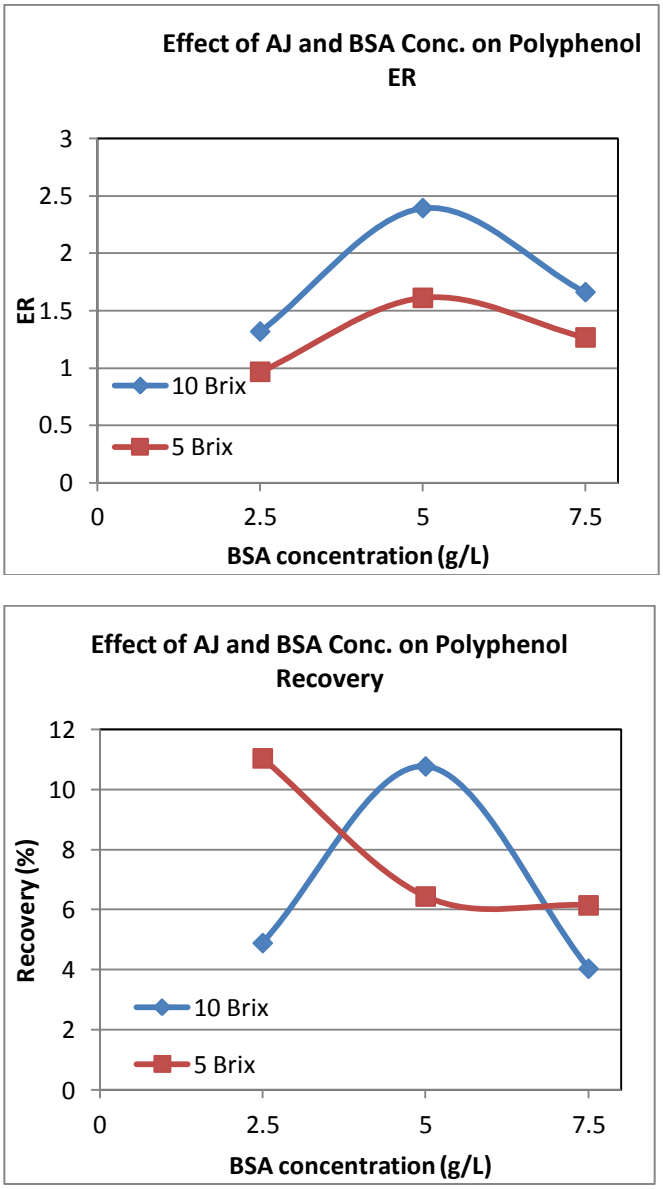


Chart -10: Graphs showing effect of AJ and BSA conc. on ER and recovery (flowrate = 7mL/min, pH = 3.7, temp = 20˚C)

ER was optimum when the BSA concentration was 5g/L, as at 2.5g/L there was less protein-polyphenol interaction. These protein-polyphenol complexes are able to stabilise foam but too much protein can cause increases in viscosity, which decreases foam drainage and dilutes foam, hence a decreased ER at BSA concentration of 7.5g/L. The increased viscosity also increases surface tension, promoting foam formation which increases absorption so there is a balance between foam drainage and increased surface tension. This also explains the recovery seen for the 10˚Brix solution.

The recovery for the 5˚Brix solution shows a decreasing trend, with respect to BSA concentration. This may be due to insufficient polyphenol content so decreased protein-polyphenol interactions and less stable foam formation.

3.4.3 Effect of pH on BSA Separation

No significant peak seen with regards to ER of BSA (Chart 11), different compared to when BSA only solution used (peak seen around the isoelectric point of BSA). This is due to the interactions between the BSA and the polyphenols affecting adsorption. Recovery of BSA was at a minimum at pH = 7, whereas previously this produced the highest recovery.

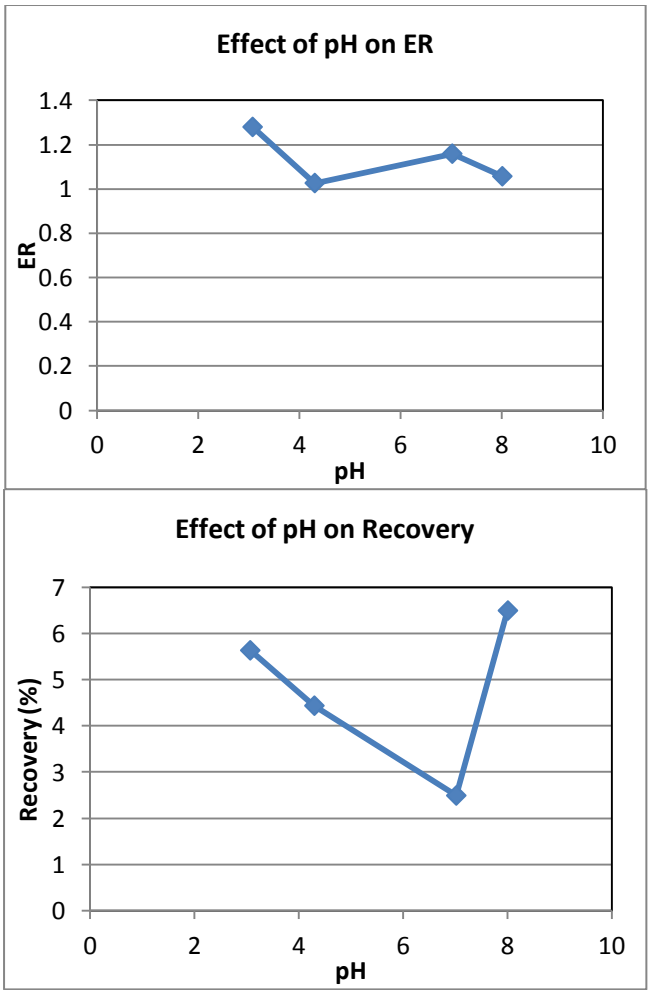


Chart -11: Graphs showing effect of pH on ER and recovery of BSA (initial conc. =5g/L BSA and 10˚Brix, flowrate = 7mL/min, temp = 20˚C)

3.4.4 Effect of Polyphenol and BSA Concentration on BSA Separation

ER was greater at higher AJ concentration (higher polyphenol concentrations), in Chart 12 it can be seen that 10˚Brix had higher ER concentrations than the 5˚Brix solution, except at higher BSA concentrations. This can again be explained by the protein-polyphenol interactions stabilising the foam. BSA ER was seen to have a minimum point for the 5˚Brix solution at 5g/L of BSA, whereas for the 10˚Brix solution ER decreases with increasing BSA concentration, which is unusual. The 10˚Brix solution should have a curve similar to the 5˚Brix solution trend as at 5g/L of BSA, polyphenol ER was seen to peak. Recovery of BSA shows similar trends as seen in recovery of polyphenol (chart 12).

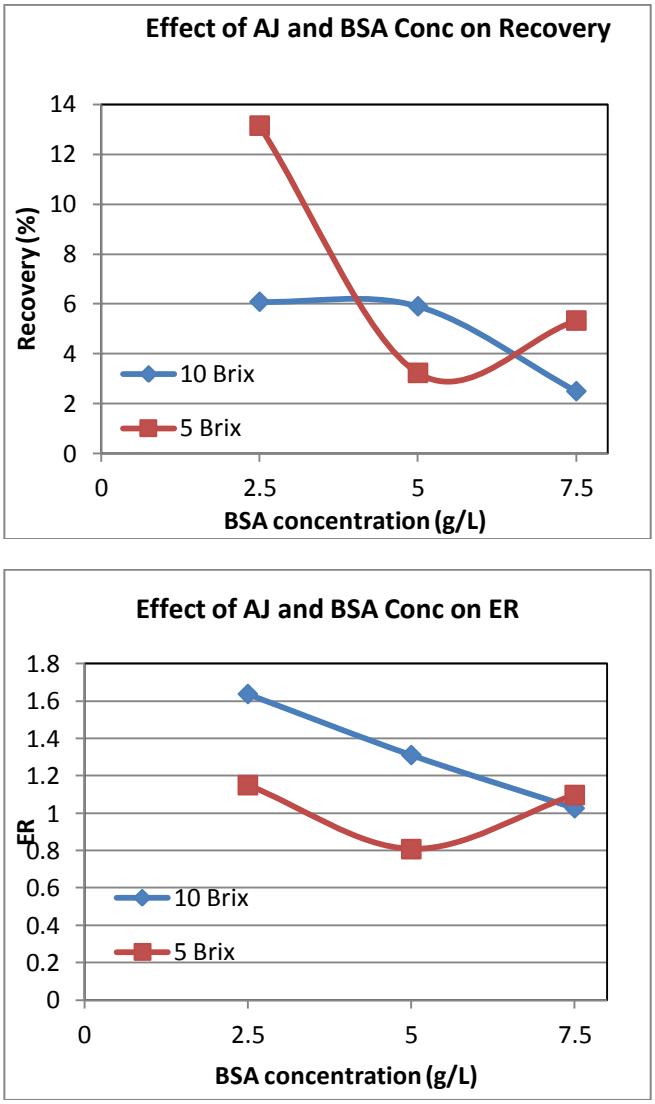
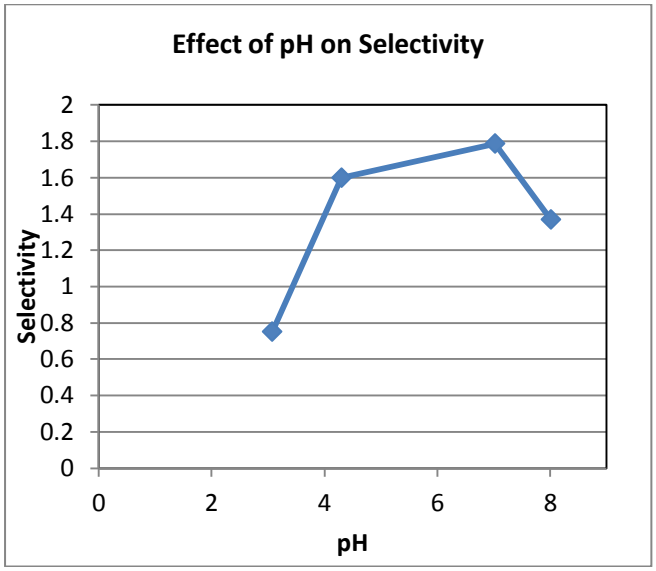


Chart -12: Graphs showing effect of AJ and BSA conc. on ER and recovery of BSA (flowrate = 7mL/min, pH = 3.7, temp = 20˚C)

3.4.5 Selectivity

At pH values above 3.8, selectivity is greater than one (chart 13A), showing preferential adsorption of BSA compared to polyphenols. The selectivity then begins to decrease from a pH of 7 onwards. Again this is due to the isoelectric point of BSA being at pH = 4.8, which increased BSA adsorption as it decreased BSA interaction with water. Above pH of 7, the proteins begin to denature decreasing amount of proteins present. AJ concentration does not significantly affect selectivity (chart 13B) as both 10˚Brix and 5˚Brix solutions showed similar selectivity values. Selectivity is greater than one only when the BSA concentration was 2.5g/L, so lower BSA concentrations mean greater adsorption of BSA (in preference to polyphenols). At the BSA concentration of 5g/L selectivity was around 0.5, showing that concentration of polyphenols was greater than the concentration of BSA in the foam (preferential adsorption of polyphenols).



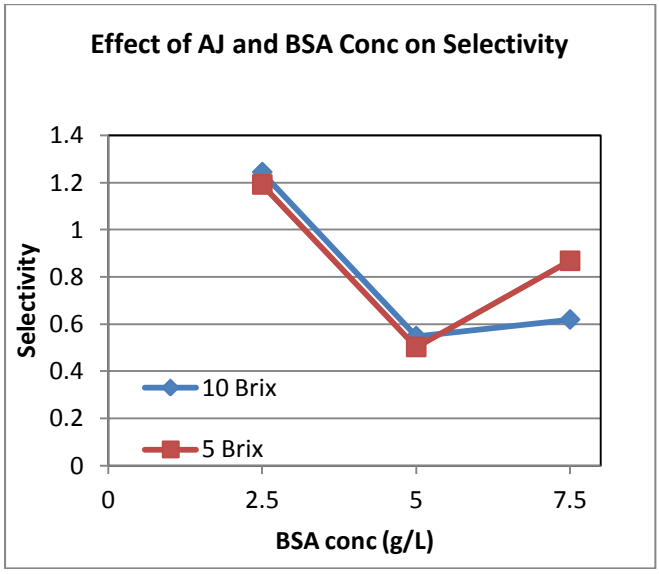
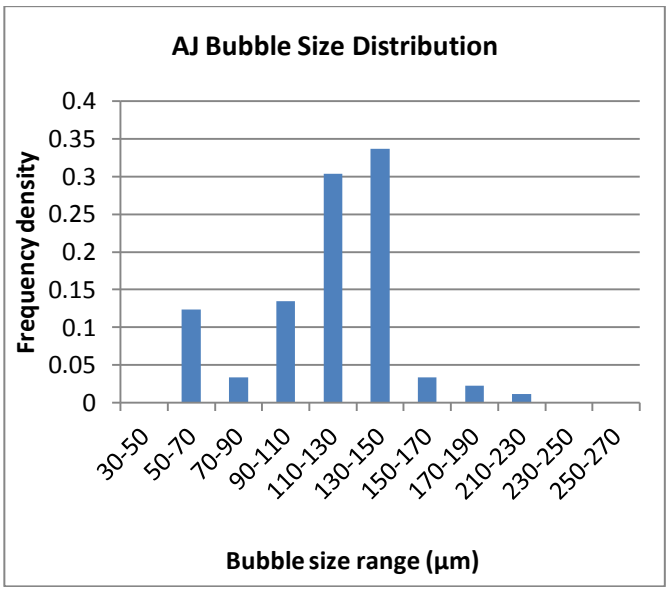


Chart -13: Graph showing effect of pH on selectivity (above) Graph showing effect of AJ and BSA conc. on selectivity (below)

3.5 BUBBLE SIZE AND GAS HOLD-UP

3.5.1 Bubble Size Distribution

Most common bubble size is between 130-150µm in the AJ solution, while in the BSA solution, the most common size is 70-90µm (Chart 14). This is due to the greater effect BSA has on surface tension as it is a more surface active molecule compared to polyphenols, which means a greater proportion of smaller bubbles were formed. The bubble size is more varied for BSA (70µm to 330µm) compared to AJ (50µm to 230µm), this may be due to more bubbles coalescing in the BSA solution due to increased surface tension stability.



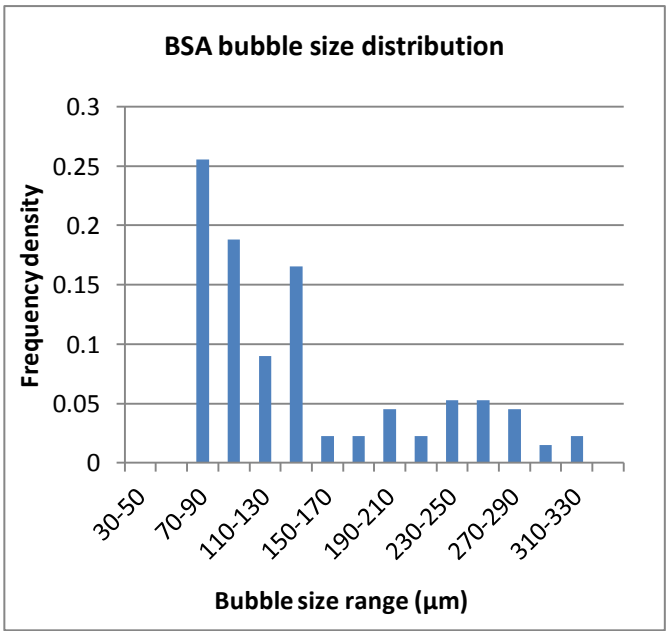


Chart -14: Comparison of the bubble size distribution of AJ, above (conc. = 10˚Brix, flow = 7mL/min, pH = 3.7, temp = 20˚C) and B) BSA, below (conc. = 5g/L, flow = 7mL/min, pH = 7, temp = 20˚C) solutions, 140mm from the frits

3.5.2 Gas Hold-up

From Table 1, it can be seen that BSA had the largest gas hold up i.e. there were more bubbles that formed in the BSA solution than in the AJ and mixture solutions. This is due to BSA providing improved bubble formation due to their surface activity increasing the surface tension of the bubbles (stabilisation).

Table -1: Percentage gas hold-up of different solutions (AJ conc. = 10.3˚Brix, flow = 7mL/min, pH = 3.7, temp = 20˚C; BSA conc. = 2.5g/L, flow = 7mL./min, pH = 7, temp = 20˚C; Mixture conc. = 5g/LBSA and 10˚Brix, flow = 7mL/min, pH = 3.6, temp = 20˚C)

Solution	Gas hold-up (%)
AJ	0.3289
BSA	1.0417
Mixture	0.6536

The AJ only solution had the lowest gas hold up, showing that polyphenols are not as surface active as BSA, so are not able to increase the surface tension as much.

3.6 Modelling of AJ Solution

3.6.1 Linear Regression – Multiple Variables

A simple linear regression using multiple independent variables (initial concentration ($conc_i$), air flowrate, pH and temperature) was performed to model ER using R. These models were then judged by looking at different statistical values – the R^2 value, the F-statistic and the p-value. Looking at Table 2 it can be seen that the full model had a very small R^2 value and F-statistic showing that there is only a weak relationship between ER and the dependent variables. The p-value at 0.44946 (> 0.05) shows that there is no evidence against the null hypothesis (that the independent variables collectively have no effect on ER) so together these independent variables do not seem to contribute significantly to ER. Reduced models were fitted to try to improve R^2, F-statistic and p-value, here different independent variables were removed but the R^2 and F-statistic values were still small and p-value still large.

In reality, these variables do contribute to ER therefore to improve the model more data is required. The lack of data accounts for the weak relationship between the dependent and independent variables. Thus to improve model, more experiments are required to generate more ER data values.

Table -2: Model equations determined using R $ER = \alpha + \beta_1 conc_i + \beta_2 flow + \beta_3 pH + \beta_4 temp$

Model Equation	R^2 value	F-statistic	p-value
Full model: $\boldsymbol{ER = 1.60624 + 0.09715 conc_i + (-0.08016) flow + (-0.08572) pH + (-0.01695) temp}$	0.0127	0.8729	0.4495
Reduced 1: $\boldsymbol{ER = 1.26101 + 0.09719 conc_i + (-0.07991) flow + (-0.08514) pH}$	0.1195	1.13	0.3558
Reduced 2: $\boldsymbol{ER = 1.20038 + 0.09942 conc_i + (-0.07670) flow + (-0.01681) temp}$	0.118	1.115	0.3618

4. CONCLUSION

Several optimum conditions for the separation of polyphenols from AJ, BSA from a BSA/water solution and both components from an AJ/BSA mixture have been identified and recommended for large scale industrial production.

From bubble size and gas hold-up analysis, larger bubbles and greater hold-up were found in the BSA solution due to their greater surface activity compared to polyphenols.

Further experimentation should be done to increase data available for statistical analysis (error bar generation and ANOVA) and for validation. Further data generated can also be used to improve the mathematical model and can be applied to other mathematical models found in literature to see if this fits trends found previously e.g. the mass transfer model found by Hossain and Saleh [14]. Modelling based on mass balances may also provide more accuracy in terms of predictive functions.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Dr Zaid Saleh for the support and guidance provided throughout this research. I would also like to extend my appreciation to the following people:

Professor Murat Balaban, Dr Wei Yu, Sam Ramani, Rick Coetzer, Laura Liang, Raymond Hoffman, Peter Buchnan, Kate Graham, Clara Phan and Anna Lawrence.

Without help from these people at the University of Auckland, this research would not have been possible. Finally I would like to thank my friends and colleagues for their continued moral support.

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BIOGRAPHIES

Tong Tong Yu is currently a fourth year student at the University of Auckland studying towards a Bachelor of Engineering (Chemical and Materials) and Bachelor of Science Conjoint Degree.

Dr Zaid Saleh is a Senior Lecturer in Chemical and Materials Engineering at the University of Auckland, New Zealand. He works in the areas of Separation and Purification Technologies, Food Process Engineering and Energy and Environment. He has published more than 30 peer reviewed papers in high quality journals and refereed international conferences, one patent and two book chapters