The Effect of Processing Condition on the Functionality of Faba Bean Protein

A collaboration between Singapore Institute of Technology/Massey University and Big Idea Ventures

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3rd - 28th August 2020

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1 Introduction

There are many sources of plant proteins, each differs in their physicochemical and structural characteristics which may result in varying performance. Faba bean protein is a common ingredient found in many plant-based products, including meat alternatives, beverages, ice cream, and many more. Till date, little is known on the effect processing conditions such as pH, temperature and pressure treatment on the physicochemical and functional properties of faba bean protein. Therefore, this project aims to study the functional properties of faba bean protein under temperature and pressure conditions, respectively. The knowledge gained could widen the application of faba bean protein in food systems.

1.1 Thermal Processing

Subjecting heat to protein can potentially affect their functionalities in foods. Heating the protein solution above the critical temperature changes the native state to the denatured state. The mechanism of denaturation is largely due to the major noncovalent interactions being destabilized. Exothermic interactions such as hydrogen bonding, electrostatic and van der Waals interactions are not stable at high temperatures and can be stabilized at low temperatures. In contrast, hydrophobic interactions are endothermic and they are not stable at low temperatures and can be stabilized at high temperature. However, the hydrophobic interactions are only stable up to a certain temperature (60 - 90°C) during heating and above this temperature, water structure will break down and interfere with the hydrophobic interactions. Only peptide hydrogen bonds in proteins remain stable over a wide range of temperatures as they are mostly buried in the interior (Damodaran, 1996).

1.2 High Pressure Processing

Pressure-induced denaturation can occur at ambient temperature and temperature as low as 4 °C if the pressure is sufficiently great. At pressure of 100 – 1200 MPa, the protein undergoes a pressure-induced denaturation, as observed from the changes in their spectral properties. Denaturation occurs at a sufficiently high temperature as proteins are flexible and compressible. Void spaces in between the globular proteins lead to compressibility even though

amino acid residues are densely packed in the interior. As a result of pressure-induced denaturation, the volume of the protein will be reduced by about 30 – 100 ml/mol when the protein unfolds and eliminating the void spaces. The decrease in volume is also caused by hydration of the non-polar amino acid residues when the protein unfolds. Pressure processing is able to induce gelation in proteins and can even produce a softer gel than thermally induced gels. 16 % soy protein solution will gel when 100 – 700 MPa hydrostatic pressure for 30 minutes at 25 °C is applied (Damodaran, 1996). High pressure processing has gained much interest in the food industry as it enables food to retain most of the natural flavour, colour, taste, texture and vitamins (Galazka et al., 2000).

2 Material and methods

Faba bean protein (VITESSENCETM Pulse CT 3602, 60 % protein) were obtained from Ingredion Singapore Pte Ltd. All the other chemicals used in this project were of analytical grade and purchased from Sigma Aldrich, St Louis, MO, USA, unless otherwise indicated.

2.1 Sample Preparation and process Conditions

2.1.1 Pressure Treatment of Plant Proteins

10 % (w/w) slurry was prepared by dissolving the protein powder into deionised water and stirred using the magnetic stirrer (400 rpm) for 30 minutes at room temperature. The pH of the solution (pH 3, 5, 9) was adjusted using either 1M HCl or NaOH. Thereafter, the solution was packed into Polyamide/Polyethylene (PA/PE) pouch for HPP treatment (200, 400 and 600 MPa for 10 minutes) using the high pressure processing equipment (Hiperbaric 300i). After which, the samples were stored chilled (~4°C) overnight and 0.02 % (w/w) sodium azide was added the following day.

2.1.2 Thermal Treatment of Plant Proteins

10 % (w/w) slurry was prepared by dissolving the faba bean protein powder into deionised water and stirred using magnetic stirrer (400 rpm) for 30 minutes at room temperature. The pH of the solution (pH 3, 5, 9) was adjusted using either 1M HCl or NaOH. Thereafter, the solution was poured into glass bottles and heated in a thermostatically controlled water bath to 85 °C

and hold for 30 minutes, with constant stirring. The sample was then cooled to room temperature (~25 °C) and 0.02 % (w/w) sodium azide was added.

2.2 Determination of Protein Solubility

The biuret and Lowry method were used to determine the soluble protein content by using the Total Protein Kit (TP0200 and B3934) obtained from Sigma Aldrich. The kit contains protein standard (bovine serum albumin), biuret reagent and Folin and Ciocalteu's Phenol reagent.

2.2.1 Preparation of Protein Standard Curve

0.5 ml of the protein standard, containing 100 mg/ml bovine serum albumin, was pipetted into a 50 ml volumetric flask and topped up to the mark with 0.85% sodium chloride solution to make a concentration of 1000 μg/ml. This solution was further diluted to make protein concentration of 0 μg/ml, 250 μg/ml, 500 μg/ml and 750 μg/ml as indicated in Table 1. 2.2 ml of biuret reagent was added to each solution, mixed and left to stand at room temperature for 10 minutes and following that, 0.1 ml of the Folin and Ciocalteu's Phenol was added to the solution, mixed and left to stand for 30 minutes. The absorbance of the solution was read at 400 – 1000 nm using Spark® Multimode Microplate Reader (Tecan Trading AG, Switzerland) with test tube 1 as a reference. The absorbance values were plotted against protein concentration (Appendix B).

Table 1: Solutions used to obtain a calibration curve.

Test Tube	**Diluted Protein Standard (ml)**	**0.85% Sodium Chloride Solution (ml)**	**Protein (μg/ml)**
1	0	0.20	0
2	0.05	0.15	250
3	0.10	0.10	500
4	0.15	0.05	750
5	0.20	0	1000

2.2.2 Preparation of Sample for Analysis

The sample (10% w/w) was centrifuged at 20,000 × g for 30mins, 20°C and the supernatant was then filtered through a 0.45μm syringe filter (Sartorius, Minisart® Syringe Filter, Germany). The filtered supernatant was diluted with 0.85% sodium chloride to give a final protein concentration of 150 – 1000 μg/ml. 2.2 ml of biuret reagent was added to each solution, mixed and left to stand at room temperature for 10 minutes and following that, 0.1 ml of the Folin and Ciocalteu's Phenol was added to the solution, mixed and left to stand for 30 minutes. The reference used was prepared in the same way as described in 3.4.1. The absorbance of the solution was read at 735 nm using Spark® Multimode Microplate Reader (Tecan Trading AG, Switzerland). Triplicate measurements were taken for each sample.

2.3 Determination of Phase Stability

50ml of the sample was poured into a clear polystyrene tube and stored chilled (~4°C). In order to ensure homogeneity in each tube, the sample was stirred for at least one minute before pouring into the tube. Visual observation and height of sediment were taken after 24 hours and 1 week of storage. The images of the samples were taken in a benchtop photobooth with controlled lighting. Phase stability (%) is calculated using the equation below:

$$\text{Phase stability (\%)} = \frac{\text{Height of solution in tube} - \text{Height of bottom layer in tube}}{\text{Height of solution}} \times 100$$

2.4 Determination of Zeta Potential

Zeta potential of 1% (w/w) protein solutions before and after treatment were determined by electrophoretic light scattering using Horiba SZ-100 zeta potential analyser. The sample was injected into a carbon electrode cell (6mm) and a measurement of the particle electrophoretic mobility results in the calculated zeta potential. Triplicate measurements were taken for each sample using Smoluchowski model at 25°C.

2.5 Rheological Measurements

Viscosity measurement was performed on a controlled stress rheometer (Anton-Paar MCR 102, GmbH, Germany) using the concentric cylinder geometry (CC27, bob radius 13mm and cup radius 14mm) Pre-shearing of the solution was carried out at $1000s^{-1}$ for 20 secs and the sample was allowed to rest for 5 minutes before a shear rate of 0.1 to $1000s^{-1}$ was applied for the viscosity measurement. Duplicate measurements were taken for the viscosity test. Temperature sweep was performed at 0.5% strain and 1 Hz to investigate the gelation temperature by heating the sample from 25°C to 95°C and cooling from 95°C to 25°C at 2°C/min.

2.6 Measurement of Foaming Properties

Foaming properties were determined according to the method described by He et al. (2016), with few modifications. Untreated, pressure and heat treated faba bean solutions were centrifuged at 28,000 × g for 1 hour to remove insoluble particles and protein aggregates. In addition, 2% (w/w) sodium caseinate solution was prepared and included in the foaming experiment as a comparison since sodium caseinate is known to exhibit good foaming properties. The protein concentration in the supernatant was determined using the method described in 3.3.2 and the protein was adjusted to the same concentration across all samples prior to whipping. Volumes of 120 ml (V_1) diluted samples were whipped in a laboratory blender (Waring®) for 2 minutes on 'Low' speed. Immediately after whipping, the mixture of foam and liquid was poured into a 250 ml measuring glass cylinder. The volume of liquid and total volume (V_2) was recorded at 1 minute intervals for the first 5 minutes, then at 3 minutes intervals for the next 20 minutes. FC and FS were calculated using the following equations:

$$\text{FC}\ (\%) = \frac{V_2 - V_1}{V_1} \times 100$$

$$\text{FS}\ (\%) = \frac{V_3}{V_2} \times 100$$

Where V_1 is the initial volume used (120 ml), V_2 is the volume of the mixture after blending and V_3 is the volume after being left for 30mins.

2.7 Statistical Analysis

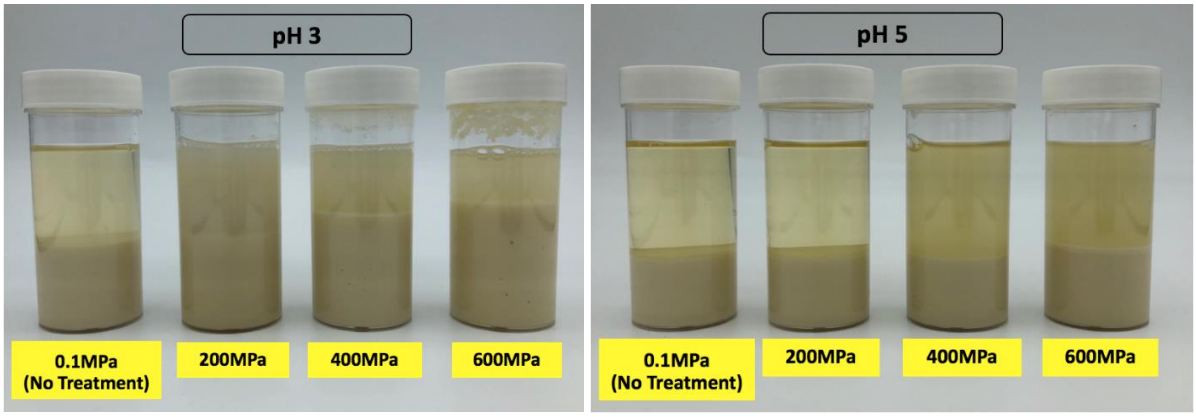
Analysis of variance was conducted with the ANOVA function of Minitab 18 Statistical Software (Minitab®, United States). Significant differences between sample means were analysed using Tukey's range test and at a significance level of 0.05 (95% confidence interval). The mean and error bar using the standard deviation of the results were presented. Different lower-case letters indicate that there is a significant difference between the mean values.

3 Result and discussion

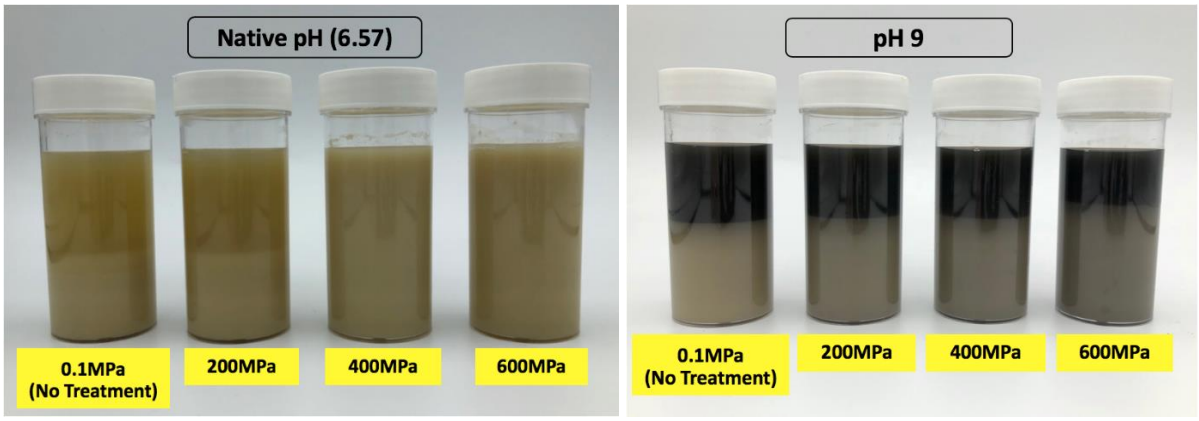
3.1 Effect of Varying pH and HPP Treatment on Faba Bean Protein

3.1.1 Phase Stability

The effect of different degree of HPP treatment on phase stability of faba bean protein at pH 3, 5, native and 9 are presented in Figure 1 (a), (b), (c) and (d) respectively. With the exception at native pH, 400MPa and 600MPa, no stable suspension was formed at other conditions. However, gel formation was observed at pH 3, 400MPa and 600MPa and this would be further discussed in later section.



(a) **(b)**

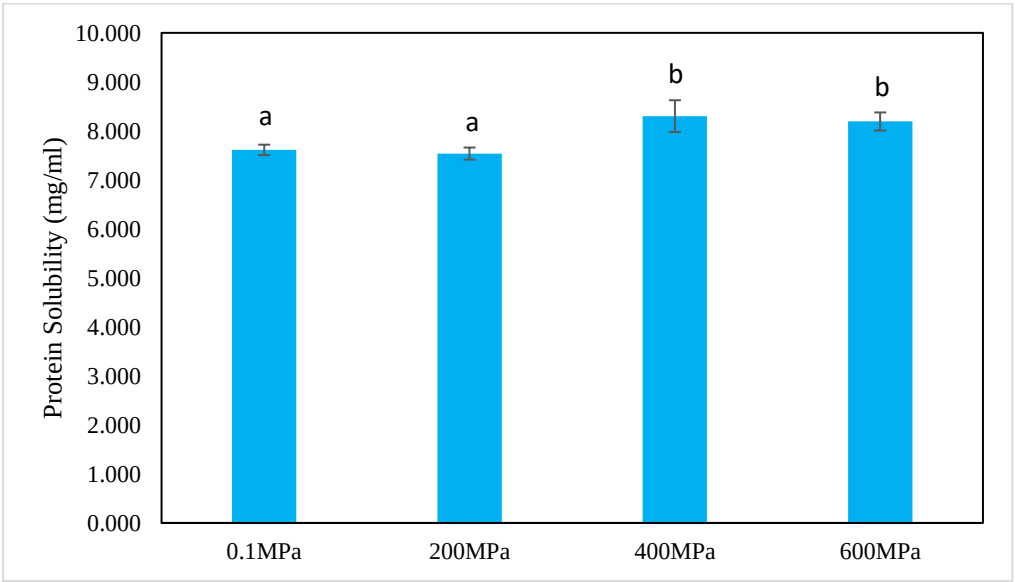


(c) **(d)**

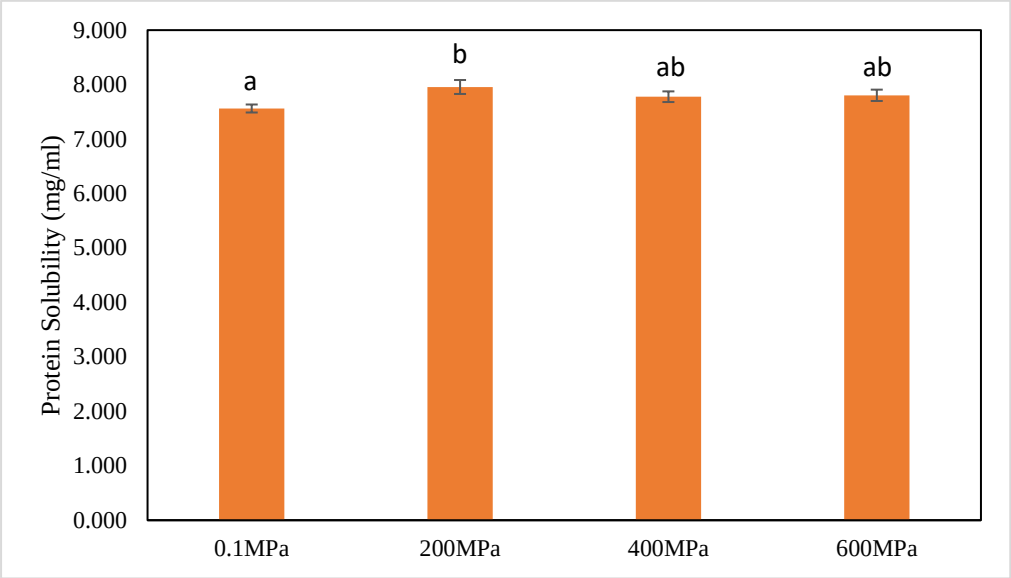
Figure 1: Effect of different levels of HPP on phase stability of 10% w/w faba bean protein at (a) pH 3, (b) pH 5, (c) native pH 6.57, (d) pH 9.

3.1.2 Solubility

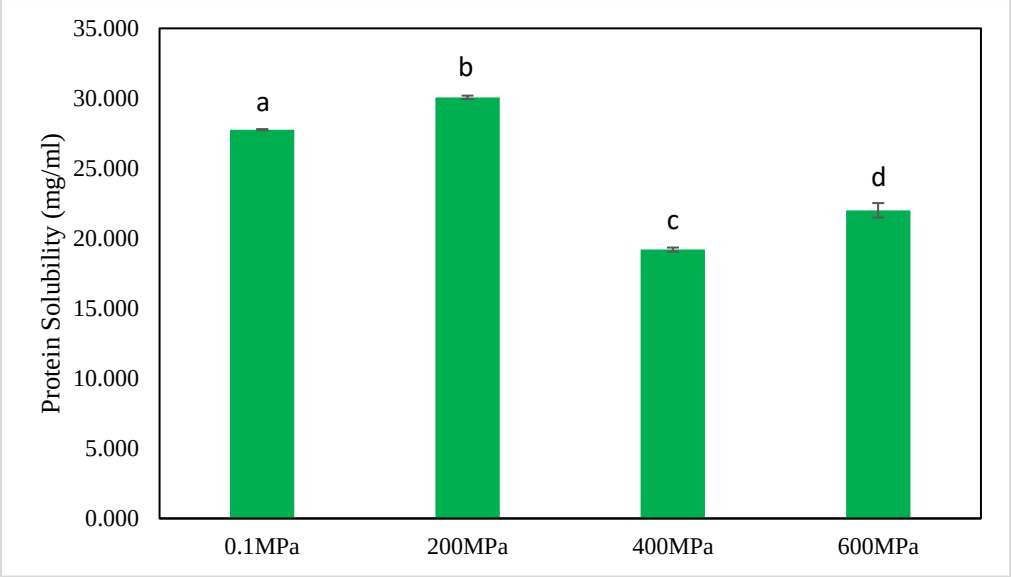
The effect of varying HPP treatment on the solubility of faba bean protein at pH 3, 5, native pH and pH 9 are presented in Figure 2 (a), (b), (c) and (d) respectively. Protein solubility differed significantly at native pH across varying HPP conditions. However at other pH conditions, only slight or no significant differences were observed. The results suggested that protein conformation near neutral pH had a greater tendency to be modified by pressure and it could be due to larger degree of protein denaturation (larger aggregate formed) at higher pressure conditions (>200 MPa) which leads to a decrease in solubility but better suspension stability as seem from Figure 1(c).



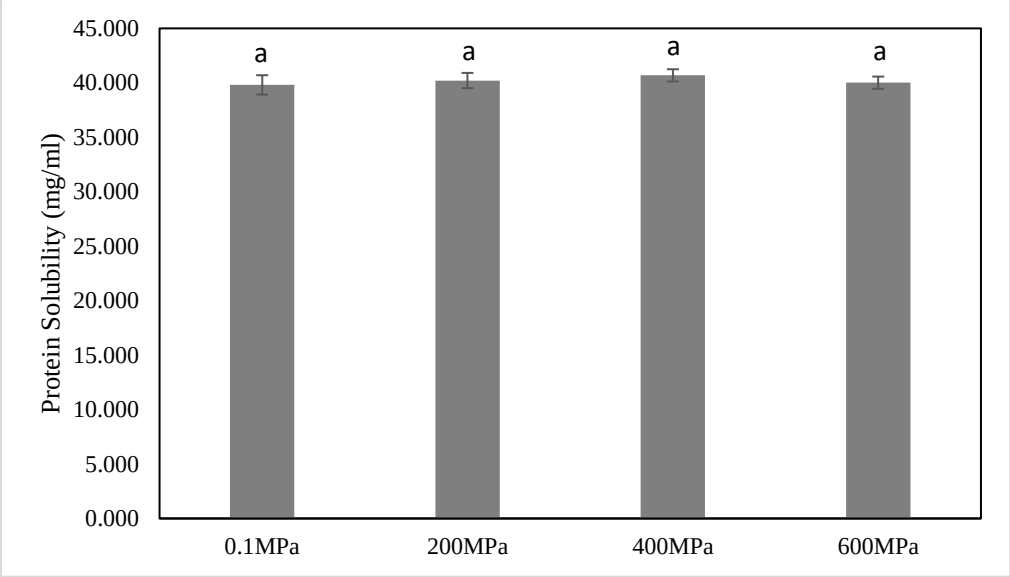
(a)


9.000
8.000
7.000
6.000
5.000
4.000
3.000
2.000
1.000
0.000
Protein Solubility (mg/ml)
a
b
ab
ab
0.1MPa
200MPa
400MPa
600MPa

(b)


35.000
30.000
25.000
20.000
15.000
10.000
5.000
0.000
Protein Solubility (mg/ml)
a
b
c
d
0.1MPa
200MPa
400MPa
600MPa

(c)

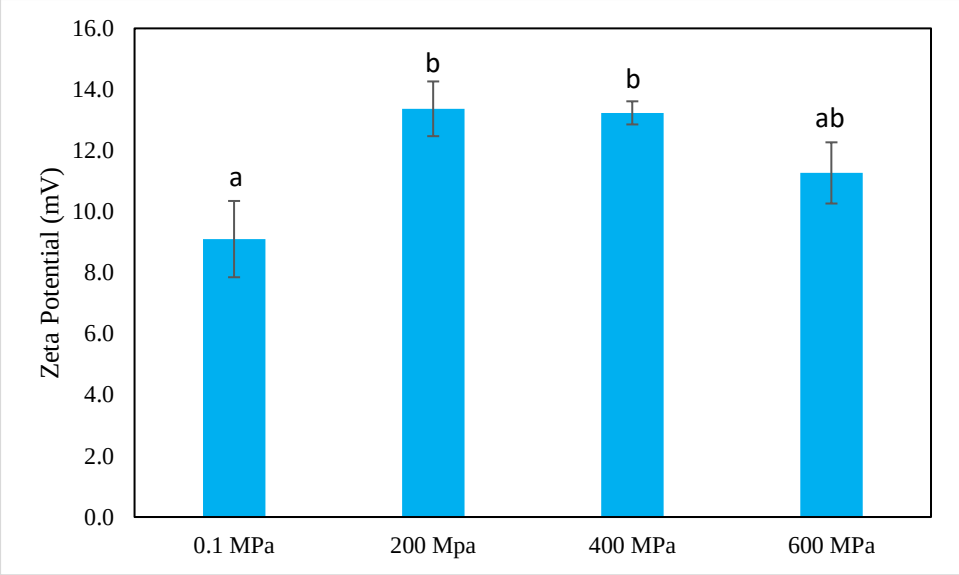


(d)

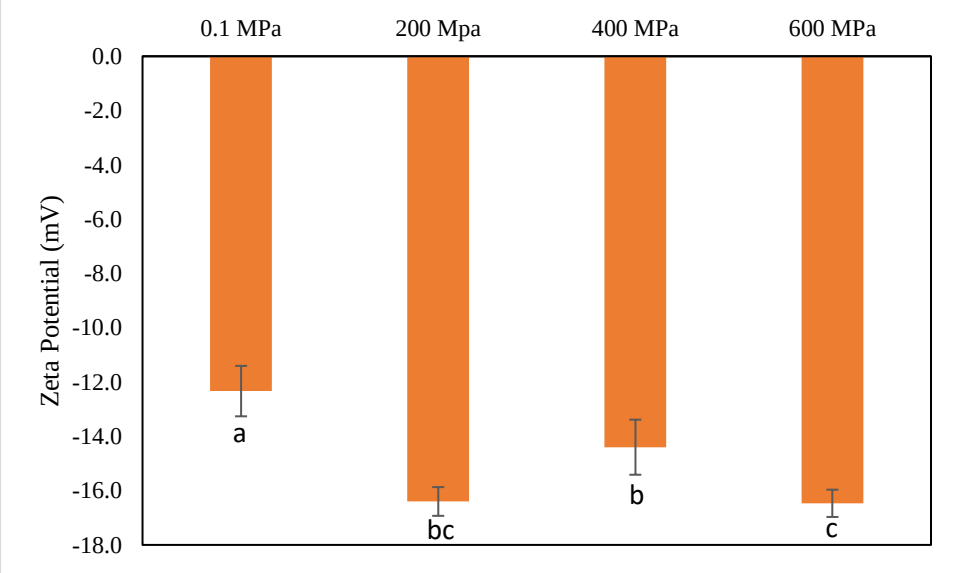
Figure 2: Effect of different levels of HPP on solubility of 10% w/w faba bean protein at (a) pH 3 (■), (b) pH 5 (■), (c) native pH = 6.57 (■), (d) pH 9 (■). Columns with different letters indicate significant differences ($P < 0.05$).

3.1.3 Zeta Potential

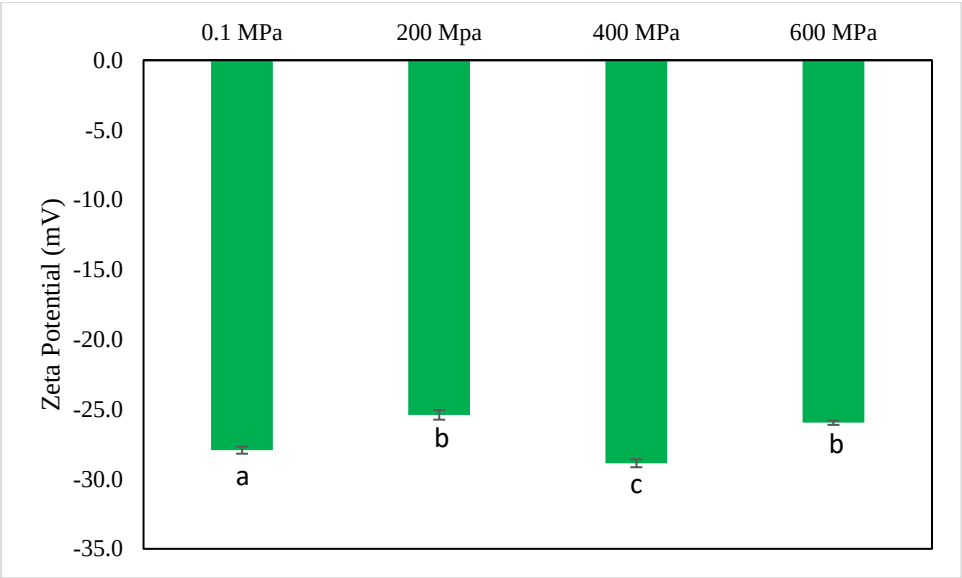
The effect of different levels of pressure treatment on zeta potential of faba bean protein at pH 3, 5, native pH and pH 9 are presented in Figure 3 (a), (b), (c) and (d) respectively. Based on Figure 3 (a) and (b), the net charges were low at pH 3 and pH 5, indicating that proteins were near the isoelectric point. The reported value for isoelectric point of faba bean protein is pH 4.4-4.5 (Rahma, 1988). Due to the low surface charge, protein-protein interactions dominated and larger aggregates formed resulted in a lower solubility. Although changes in zeta potential could indicate that the protein has been modified by processes such as HPP, the results were not drastic to make a conclusive deduction on the effect of HPP on protein conformation.


16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Zeta Potential (mV)
a
b
b
ab
0.1 MPa
200 Mpa
400 MPa
600 MPa

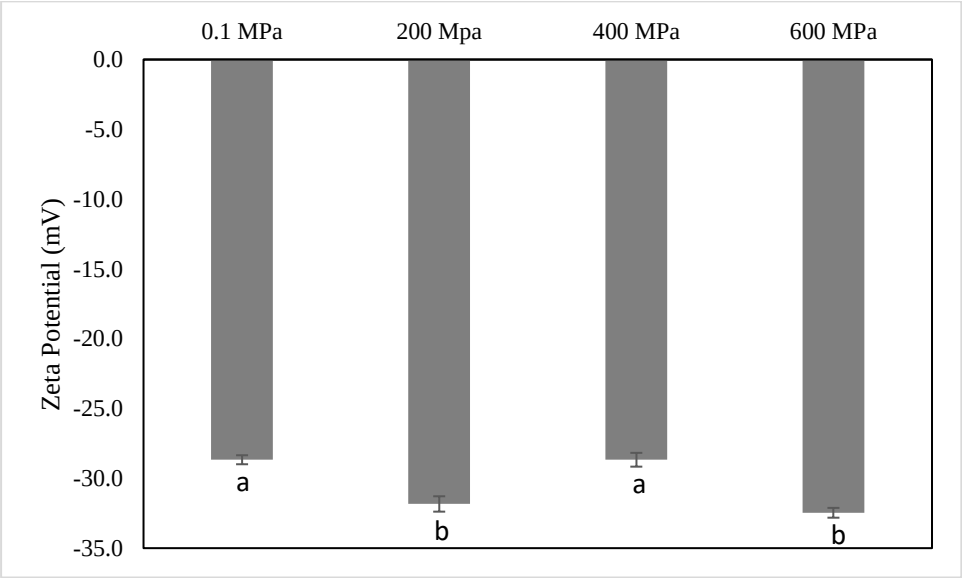
(a)


0.1 MPa
200 Mpa
400 MPa
600 MPa
0.0
-2.0
-4.0
-6.0
-8.0
-10.0
-12.0
-14.0
-16.0
-18.0
Zeta Potential (mV)
a
bc
b
c

(b)



(c)



(d)

Figure 3: Effect of different levels of HPP on zeta potential of 1% w/w faba bean protein at (a) pH 3 (■), (b) pH 5 (■), (c) native pH = 6.57 (■), (d) pH 9 (■). Columns with different letters indicate significant differences ($P < 0.05$).

3.1.4 Rheological Properties

The effect of different levels of pressure treatment on the viscosity of faba bean protein at pH 3, 5, native pH and pH 9 are presented in Figure 4 (a), (b), (c) and (d) respectively. The samples that formed a gel at pH 3, under the process condition of 400MPa and 600MPa, were pre-sheared using Silverson mixer before carrying out the viscosity measurement.

The viscosity of the samples without treatment and at 200MPa were low at pH 3, 5 and native pH. A significant increase in viscosity was observed in samples after being subjected to HPP conditions at 400MPa and 600MPa, at native pH as seen from Figure 4 (c). An increase in viscosity is attributed to protein aggregation and the interaction of denatured protein (Ho et al., 2018). As 600 MPa resulted in a larger degree of denaturation, the viscosity of the solution was the highest. This can be correlated to the results from the phase stability test (Figure 1 (c)); higher viscosity of the continuous phase had a higher impact on phase stability. The degree of protein-protein interaction might have resulted in a weak gel formation which helps to entrap water and increase the bulk viscosity with no phase separation.

It was also observed that all the faba bean protein solutions at higher viscosity (Approximately more than 80 mPa.s at $0.1s^{-1}$) exhibit a shear thinning behaviour as the protein molecules tend to align in the direction of flow.

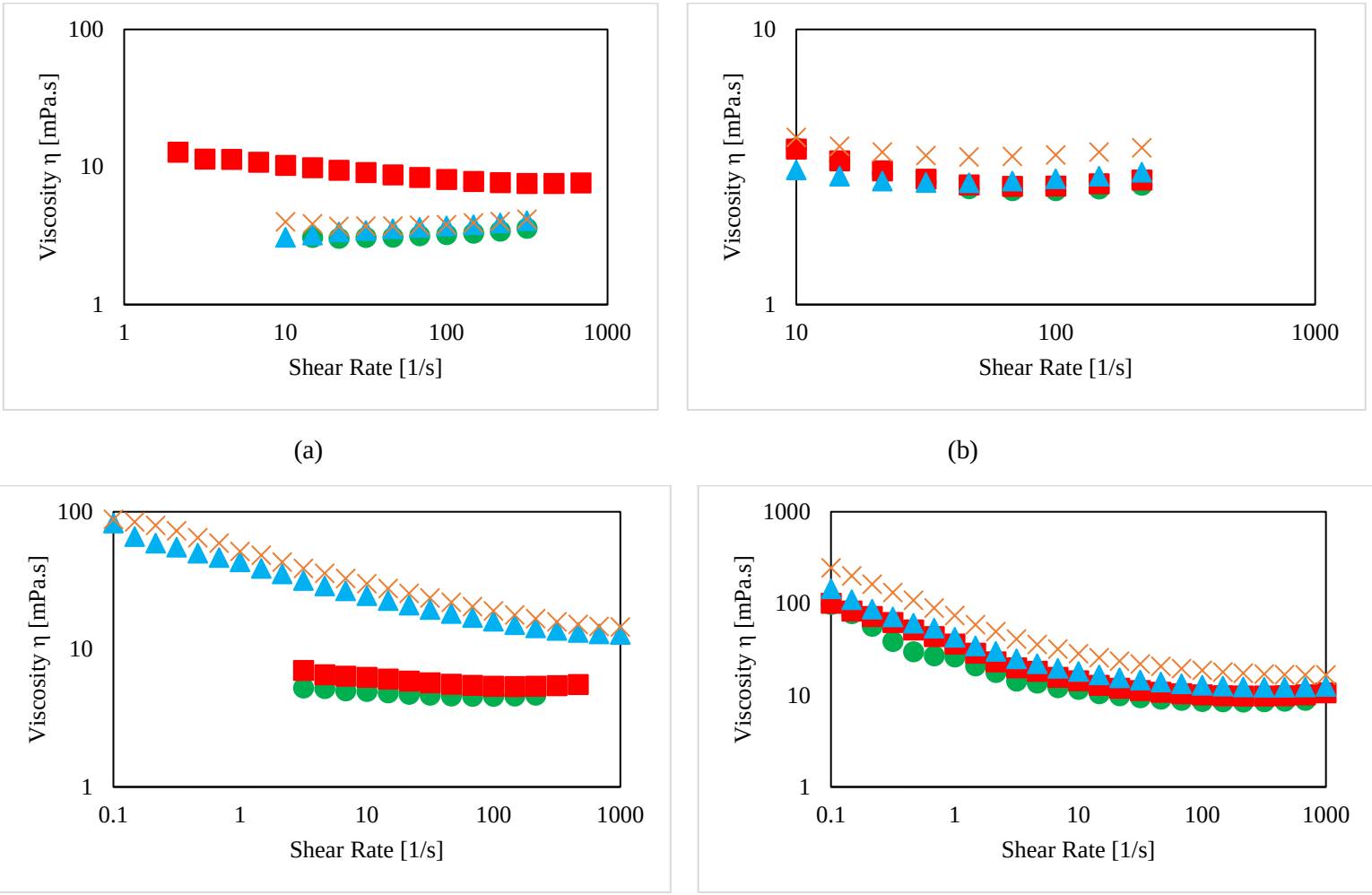
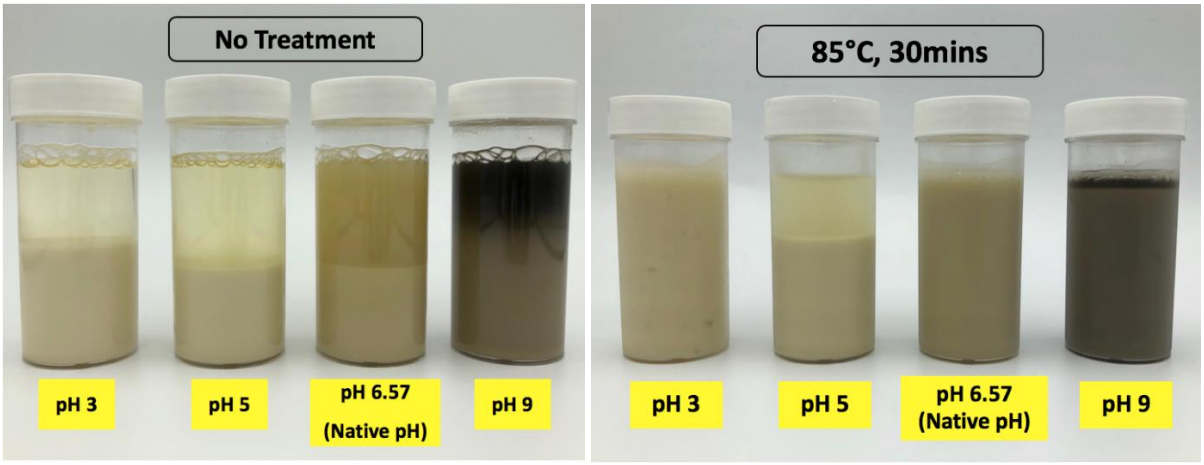


Figure 4: Viscosity profile of 10% w/w faba bean protein at (a) pH 3, (b) pH 5, (c) Native pH = 6.57, (d) pH 9 subjected to no treatment (●), 200MPa, 10mins (■), 400MPa, 10mins (▲) and 600MPa, 10mins (×). Results are an average of duplicate measurements.

3.2 Effect of Thermal Treatment on Faba Bean Protein at Different pH

3.2.1 Phase Stability

The phase stability of faba bean protein in deionised water at varying pH is presented in the figures below. Figure 5 (a) were samples that did not go through any heat treatment while Figure 5 (b) shows samples subjected to heat treatment at 85°C for 30 minutes. A stable suspension was observed in both pH 3 and native pH after heat treatment. Gel formation was observed at pH 3, 85°C and this would be further discussed in later section. As previously discussed, pH 3 and 5 is near the isoelectric point of faba bean protein and therefore, the greater separation was observed. A stable suspension seen in pH 3, 85°C may be attributed to the gel formation, which helps to entrap water. Similar to the HPP treated sample at native pH, heat treatment at 85°C also resulted in a stable suspension due to heat denaturation and modified the protein conformation.



(a) **(b)**

Figure 5: Phase stability of (a) untreated and (b) heat treated, 85 °C, 30 mins 10% w/w faba bean protein at different pH.

3.2.2 Solubility

The effect of heat treatment on the solubility of faba bean protein at varying pH is presented in Figure 6. There was no significant difference in solubility at pH 3 and pH 5, whereas at native pH and pH 9, there was a marked decreased in solubility. The solubility of protein is influenced by temperature and typically has an increasing trend when heated up to 50 °C. Above this temperature, the solubility will be lower than the native proteins (Zayas, 1997). The occurrence of decreased solubility is primarily caused by denaturation (unfolding), and the exposure of non-polar groups lead to aggregation and precipitation (Damodaran, 1996). Heat-treated sample at pH 3 although had a stable suspension as seen from Figure 5 (b), the solubility was low. This may suggest that water was entrapped in the gel network and a stable suspension was formed.

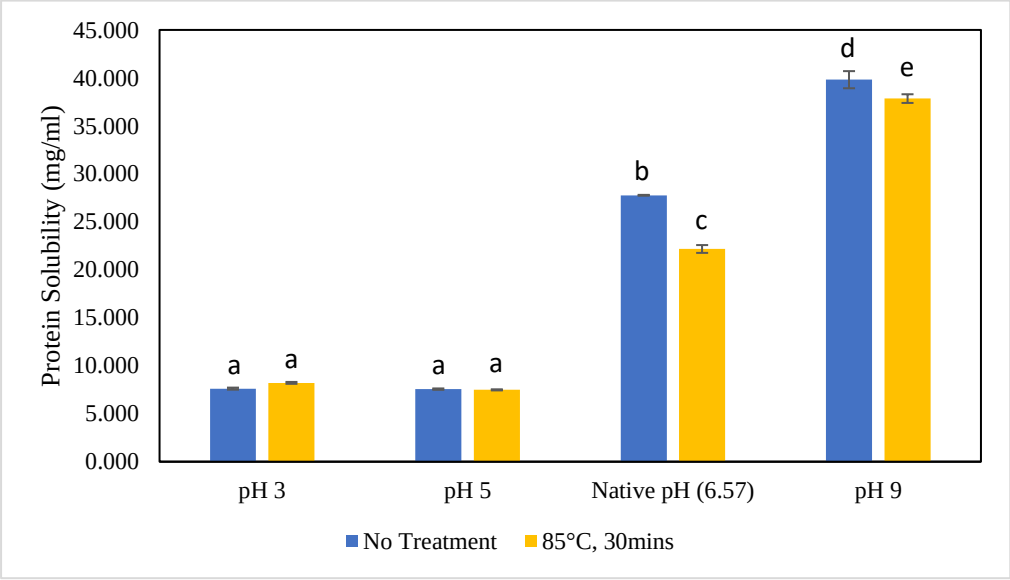


Figure 6: Effect of heat treatment, 85°C, 30mins and no treatment on 10% w/w faba bean protein solubility at different pH. Columns with different letters indicate significant differences ($P < 0.05$).

3.2.3 Zeta Potential

The effect of heat treatment on the zeta potential of faba bean protein at varying pH is presented in Figure 7. Heat treatment decreased the surface charge of faba bean protein at pH 5 and native pH. Although changes in zeta potential could indicate that the protein has been modified by processes such as heat treatment, the results were not drastic to make a conclusive deduction on the effect of heat on protein conformation.

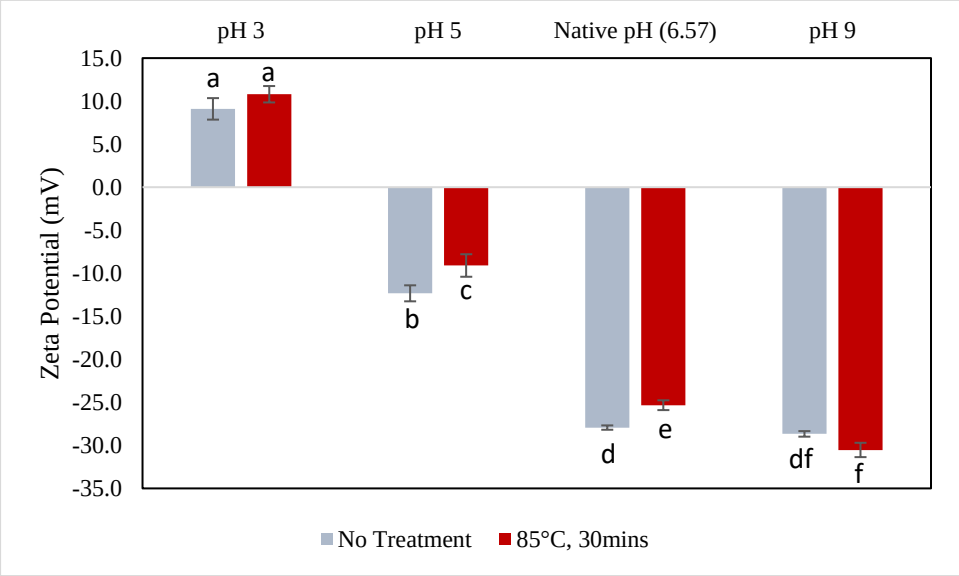


Figure 7: Effect of heat treatment, 85°C, 30mins and no treatment on zeta potential of 1% w/w faba bean protein at different pH. Columns with different letters indicate significant differences ($P < 0.05$).

3.2.4 Rheological Properties

The effect of heat treatment on viscosity of faba bean protein at varying pH is presented in Figure 8.

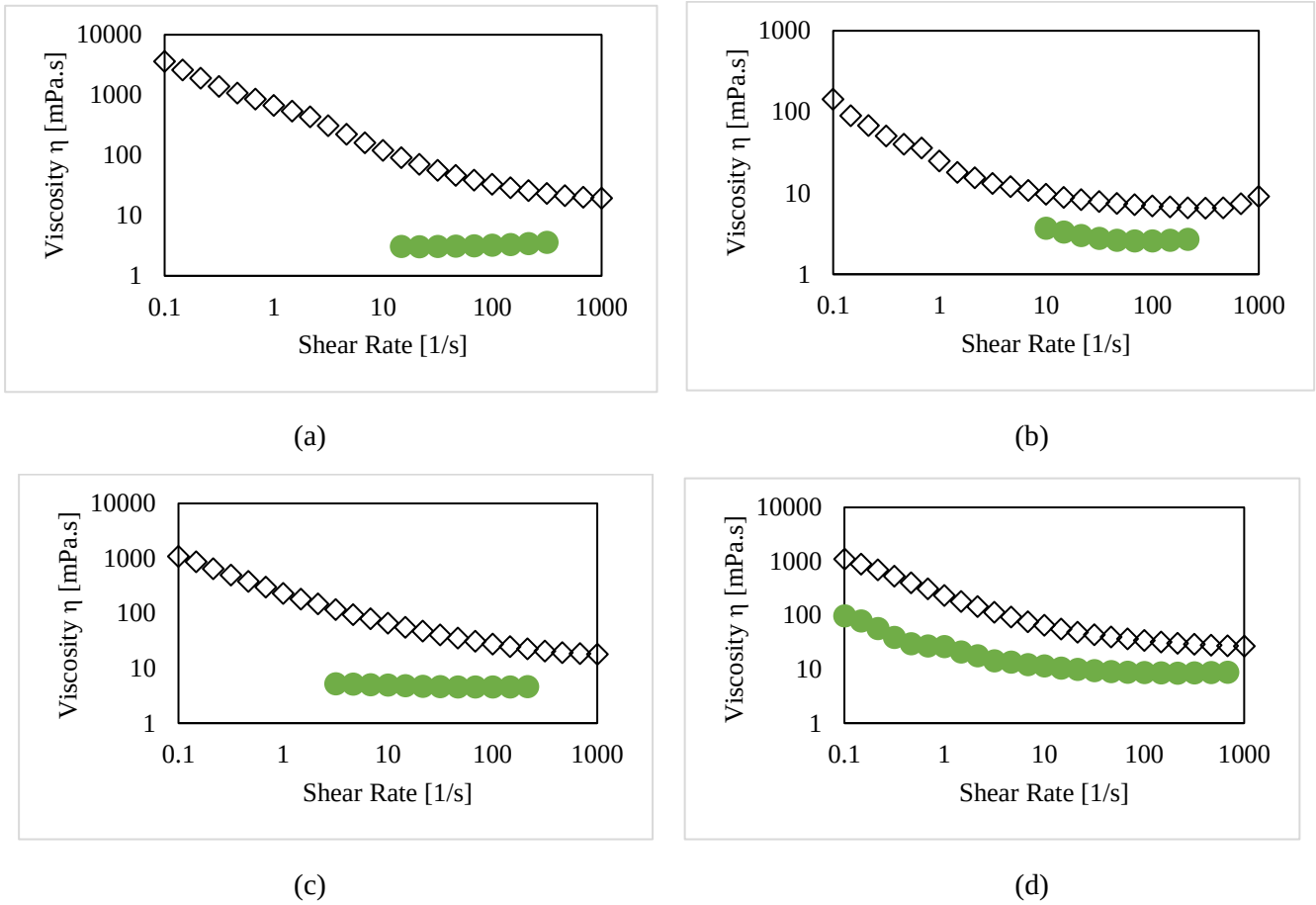


Figure 8: Viscosity profile of 10% w/w faba bean protein at (a) pH 3, (b) pH 5, (c) Native pH = 6.57, (d) pH 9 subjected to no treatment (●) and 85 °C, 30 mins (◇). Results are an average of duplicate measurements.

The effect of heat treatment had a significant impact on the viscosity of the faba bean protein; where heat treatment increases the viscosity of the samples at all the pH value. With reference to the viscosity at a fixed shear rate of $100s^{-1}$, the highest increased in viscosity as compared to the sample with no treatment was seen in pH 3 sample and the difference was 29.78 mPa.s. This may be because a gel network has been formed which resulted in a drastic increase in viscosity of the bulk solution. As pH 5 is near to the isoelectric point of the protein, phase separation occurred (Figure 5) and therefore, the viscosity was the lowest at 100 s^{-1}.

Protein solubility of the sample at native pH and pH 9 decreased after heat treatment (Figure 30) but showed an increase in viscosity. Proteins are denatured by heat and protein solubility will be decreased. Viscosity of a protein solution may also be increased when partial denaturation increases the hydrodynamic size of proteins (Damodaran, 1997).

3.3 Comparison of the Effect of HPP and Thermal Treatment on Faba Bean Protein

3.3.1 Effect on the Phase Stability, Solubility and Viscosity of Faba Bean Protein

The results of phase stability, solubility and viscosity were compared between HPP and thermal-treated samples, as shown in Figure 9, 10 and 11 respectively. Based on Figure 9, the heat-treated sample had the best phase stability at all pH as compared to pressure treated and untreated samples. Except at native pH, HPP treated (400 MPa and 600 MPa) samples exhibited similar stability to thermal treated sample. This showed a high potential for the usage of HPP in place of heat treatment for protein modification.

HPP treated sample at 200 MPa had the highest solubility (Figure 10) at native pH. The rest of the samples (400 MPa, 600 MPa and 85 °C) resulted in a decrease in solubility as compared to the sample with no treatment. Despite that, no phase separation was observed for these samples while there was phase separation in the sample treated at 200MPa. Hence, it could be deduced that solubility was not a good indicator of phase stability.

Lastly, for the viscosity results (Figure 11), it was also shown that the heat-treated sample had the highest viscosity at all pH as compared to pressure treated and untreated samples. Pressure treatment at 600 MPa was also a potential method to increase the bulk viscosity of the solution. Each of these observations may be advantageous when it comes to food application and

depending on the type of application and functionality, this study may serve as a guide on choosing the optimum processing conditions. For example, if the application is a high protein beverage, a pressure treatment of 400 MPa may be potential to achieve a stable suspension and increase the mouthfeel of the beverage because of an increased in viscosity. It is also necessary to conduct further research using a food model system as protein is affected by many other factors such as protein concentration, ionic strength and presence of other bio-polymers.

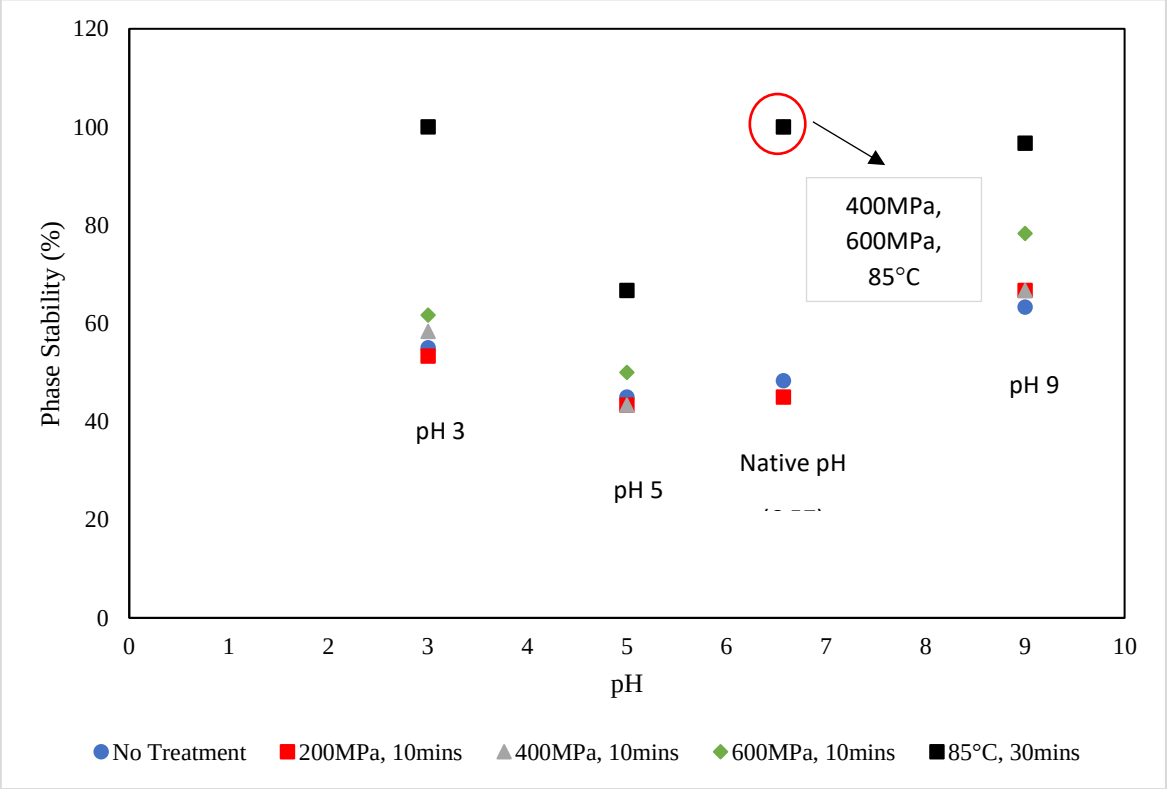


Figure 9: Comparison of phase stability between pressure and heat treated faba bean protein (10% w/w) at varying pH.

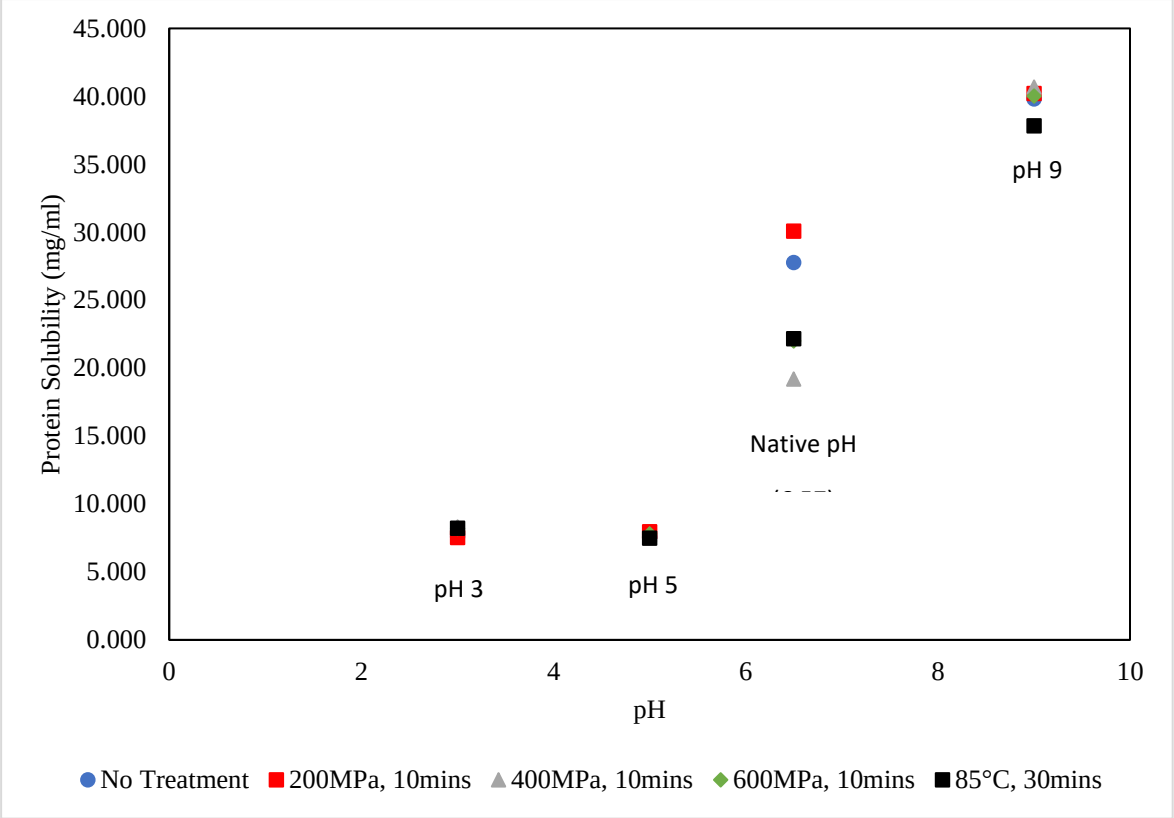


Figure 10: Comparison of solubility between pressure and heat treated faba bean protein (10% w/w) at varying pH.

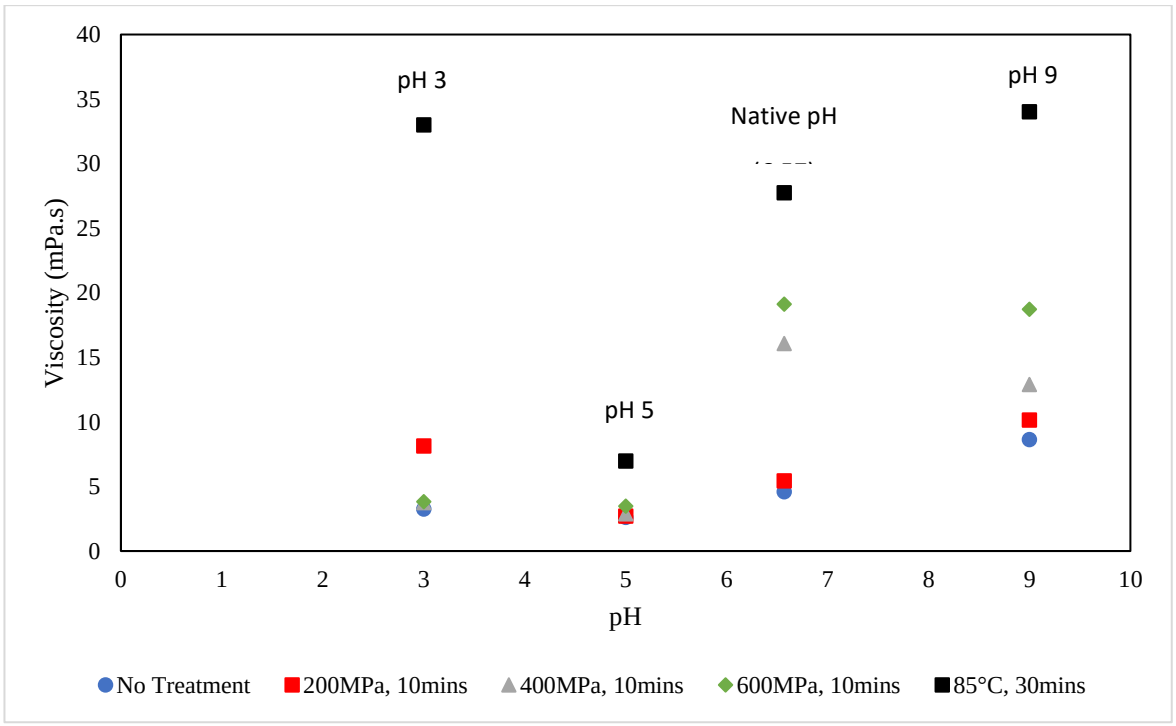
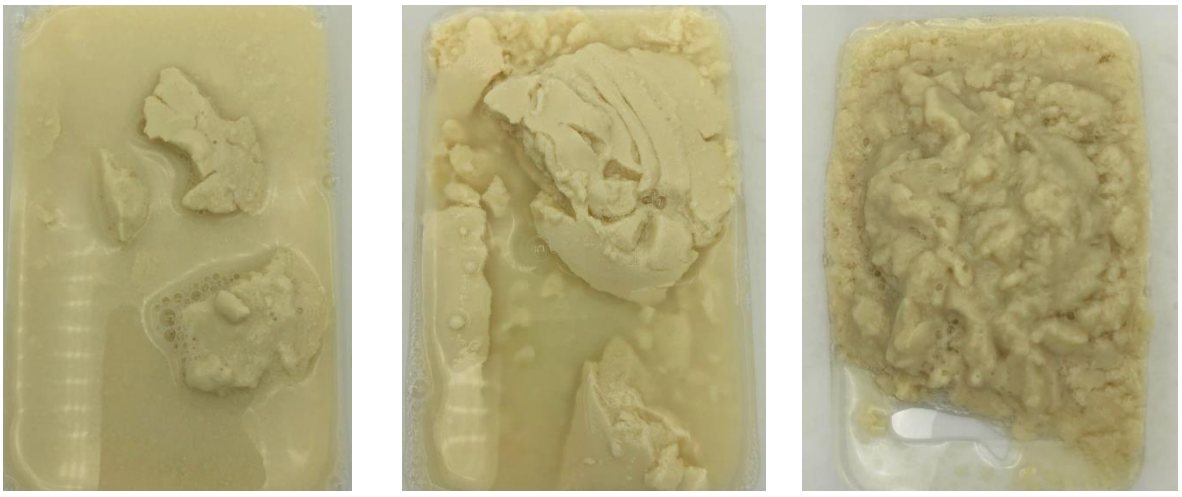


Figure 11: Comparison of viscosity (at 100 s^{-1}) between pressure and heat treated faba bean protein (10% w/w) at varying pH.

3.3.2 Visual Observations of Heat and Pressure-induced Gels

Soft gels were observed in 10% w/w faba bean protein subjected to heat treatment (85°C, 30mins) and pressure treatment (400MPa and 600MPa, 10mins) at pH 3. The appearance of the gels was different and this signifies the different degree of denaturation that resulted in this network formation. At sufficiently high pressure and concentration, proteins will be denatured and form gels in a similar way to that induced by heat. However, the appearance is different as heat primarily affect hydrogen-bonded networks and pressure affects hydrophobic and electrostatic interactions (Molina et al., 2002). The gelation process in faba bean protein is thermo-irreversible. Under sufficient heat, protein undergoes denaturation when disulphide bonds are formed and hydrophobic amino acid residues are exposed. Further heating results in aggregation and interaction between proteins and a gel is formed (Ehsanzamir, 2018).

Analytical techniques such as texture profile analysis and water holding capacity may be employed to investigate textural properties and modification of proteins may be further understood through electrophoresis and differential scanning calorimetry. Ahmed, et al. (2007a) had conducted a study on soy protein and it was found that above 300MPa, the gel was formed by rupturing non-covalent interactions within protein molecule, and this gel was softer than thermal induced gel.



(a) (b) (c)

Figure 12: Appearance of 10% w/w faba bean pressure and heat induced gels at pH 3, (a) 400MPa, 10mins, (b) 600MPa, 10mins, (c) 85°C, 30mins.

3.3.3 Foaming Properties

The foam capacity and stability were evaluated for untreated, HPP treated (200MPa, 400MPa and 600MPa, 10mins) and thermal treated (85°C, 30mins) faba bean protein, sodium caseinate was also included as a comparison. Figure 13 shows the graph of foam volume against a time period of 30 minutes, Table 2 is a summary of the foam capacity and stability and lastly, Figure 14 are pictures taken during the foam experiment.

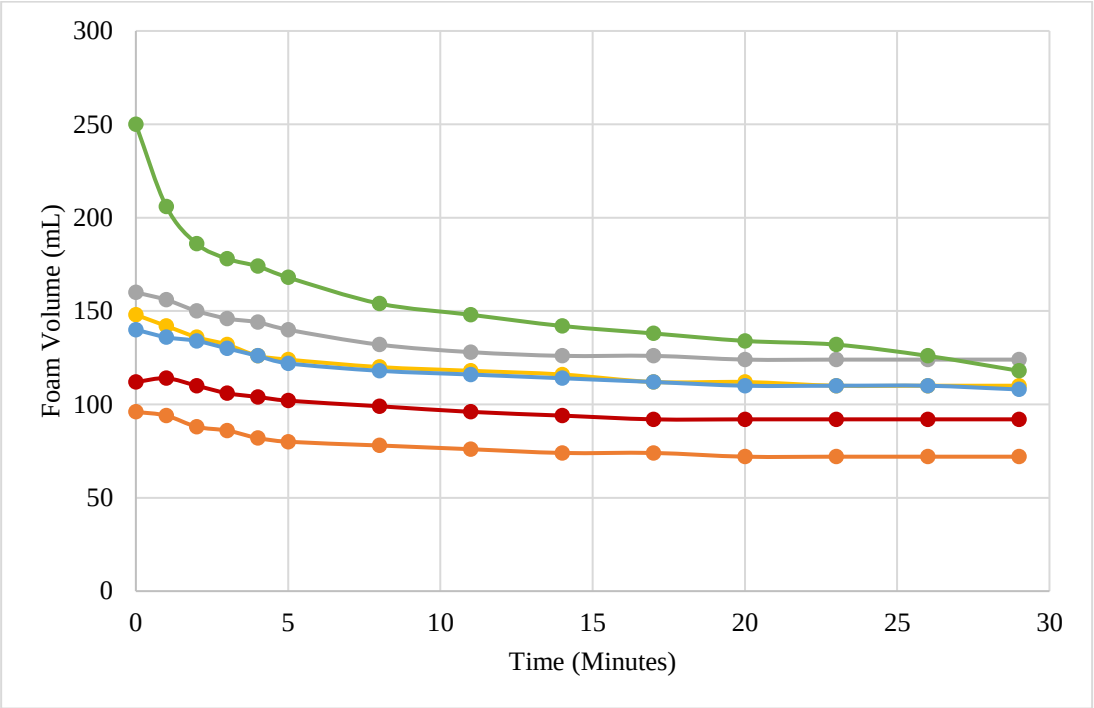


Figure 13: Graph of foam volume over time for faba bean protein: 0.1MPa (—●—), 200MPa, 10mins (—●—), 400MPa, 10mins (—●—), 600MPa, 10mins (—●—), 85°C, 30mins (—●—) and sodium caseinate (—●—).

Table 2: Foam capacity and stability of faba bean protein and sodium caseinate.

	0.1MPa	**200MPa**	**400MPa**	**600MPa**	**85°C**	**Sodium Caseinate**
Foam Capacity (%)	55	68	92	82	75	108
Foam Stability (%)	96	98	99	98	100	90

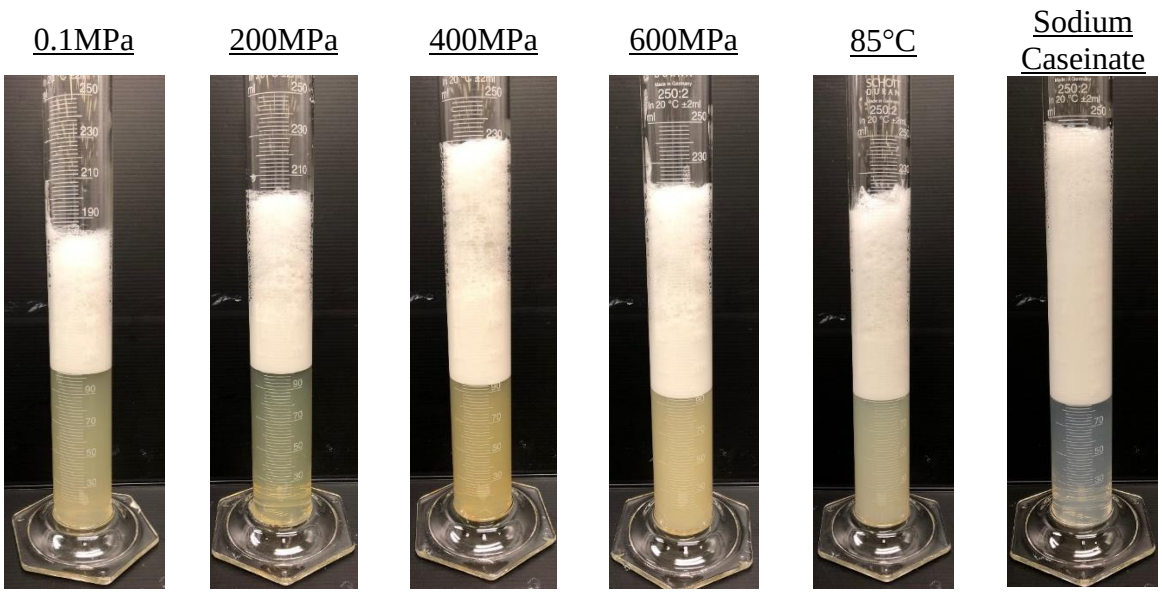


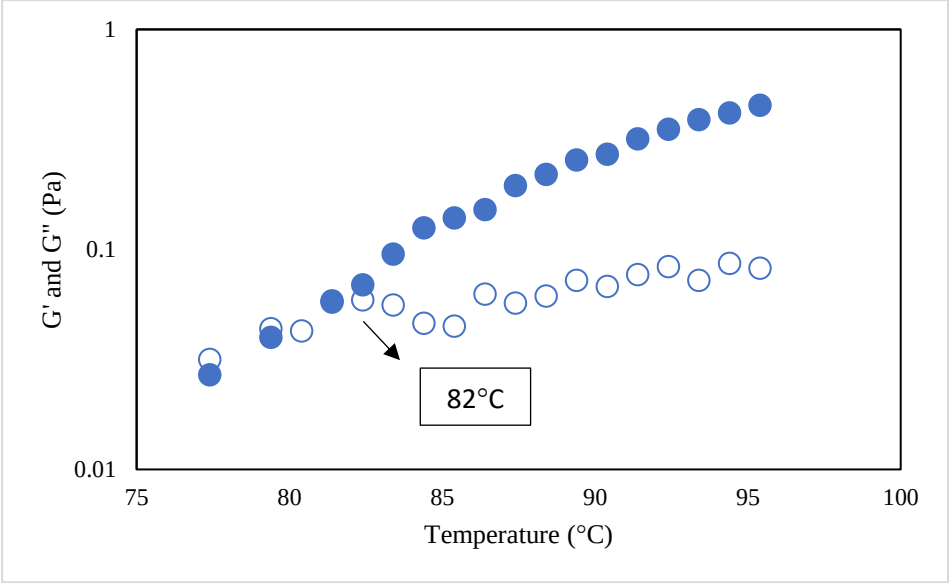
Figure 14: Pictures of foam volume taken 5 minutes after whipping. (a) 0.1MPa, (b) 200MPa, (c) 400MPa, (d) 600MPa, (e) 85°C, (f) sodium caseinate.

The results above (Table 1) showed that both process treatments resulted in better foam capacity compared to sample without treatment. Foam capacity is a measure of the protein ability to form an interfacial membrane that incorporates air bubbles while foam stability is a measure of the interfacial membrane strength against coalescence between two foams (Chao et al., 2018). The presence of adsorbed protein at the interface between aqueous and gaseous phase stabilises the structure of the foam and faba bean protein was capable of producing a stable foam. The results showed that both process treatments can be used to improve the interfacial properties of faba bean protein. This is beneficial to the industry for cost reduction as lesser protein is required for the same performance (Ibanoglu & Karatas, 2001). The difference in the foaming capacity at varying process conditions is due to the varying degree of protein denaturation. Unfolding and increased in surface hydrophobicity may lead to increased protein adsorption at the interface and improving foam formation (Baier & Knorr, 2015). Based on this study, faba bean protein subjected to 400 MPa for 10 minutes produced the highest foam volume and good stability. The foam capacity was improved from 55% to 92%. However, as pressure was increased to 600MPa, the foam capacity decreased by 10% to 82%. This trend was also seen in a study by He et al. (2016) on bovine lactoferrin, whereby the highest foam capacity was observed when the protein was subjected to HPP at 400MPa and decreased with further increase in pressure. On the other hand, thermal treatment of 85°C for

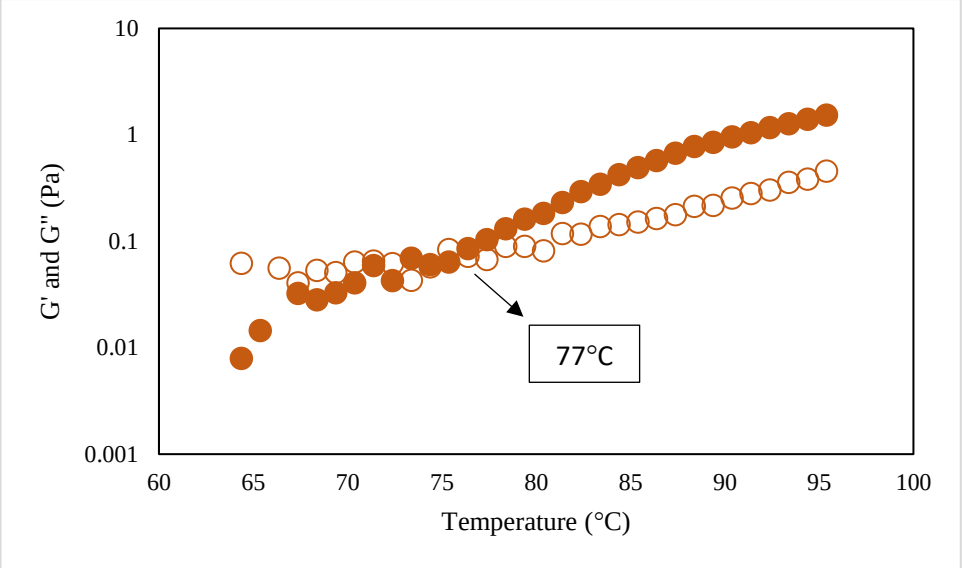
30 minutes led to a lower foam capacity as compared to the pressure treatment of 400MPa and 600MPa. Therefore, this showed a high potential for the usage of HPP in place of heat treatment for protein modification.

3.3.4 Effect of Faba Bean Concentration on Rheological Properties

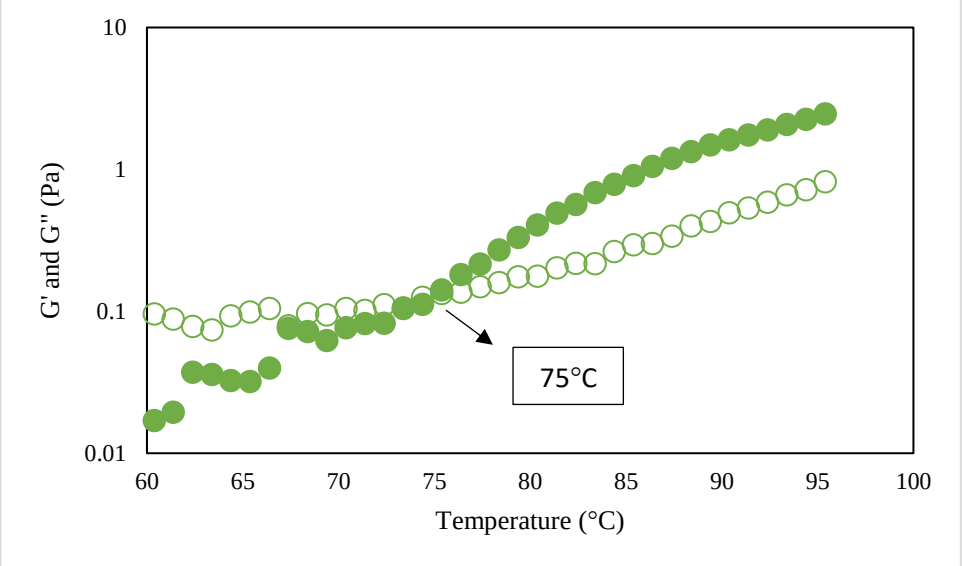
The gelation temperature of faba bean protein at native pH decreased with higher protein concentration (Figure 15). 8% (w/w) protein concentration was tested as well but no visible cross-over of the storage moduli (G') and loss moduli (G") was observed and therefore, the minimum concentration for soft gel formation was at 10% w/w. As protein concentration increases, there was a greater degree of protein-protein interactions and thus, a lower temperature was needed for gel formation.



(a)



(b)



(c)

Figure 15: Gelation profile of (a) 10% w/w faba bean protein (G': ● and G": ○), (b) 12% w/w faba bean protein (G': ● and G": ○), (c) 14% w/w faba bean protein (G': ● and G": ○) at native pH, using 0.5% strain and 1 Hz.

4 Conclusion

In overall, it was found that HPP treatment of faba bean protein at 400MPa and 600MPa resulted in a stable suspension. This signifies that HPP is a potential method to modify the conformation and properties of faba bean protein. Results from solubility also showed that it has the highest solubility at all pH compared to the other plant proteins and this may be an indicator of potential functional properties.

The use of legume as an ingredient to produce food products that are acceptable to consumers is still challenging for the food industry. The potential use of faba bean protein is in the manufacture of gluten-free food such as pasta, bread, snacks, etc. Legume proteins have also been used in meat products as binders and extenders and this resulted in a product with lower fat content and enhanced nutritional value. Faba bean is rich in protein, fibre and other non-nutrient compounds that are beneficial for health. Hence, it can help to meet the increasing worldwide demand for proteins by partial replacement of meat and dairy products in the human diet (Multari et al., 2015). The findings of faba bean protein can potentially be applied to BIV portfolio company that develops plant-based egg or dairy alternative products.