COVER SHEET

TITLE: Effects of sucrose, sugar alcohols and whey protein concentration on the foaming properties of Native Whey Protein concentrate and Whey Protein Isolate.

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ABSTRACT

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Abstract

Whey proteins are utilized in the food industry as foaming agents and consequently whey protein foams are an integral component of many foods such as meringue and whipped cream. The aim of the study was to investigate the effects of carbohydrates (sucrose, maltitol and sorbitol) on the foaming properties of native whey proteins (Native WPC-80). Additions of sucrose, maltitol and sorbitol (10 % (w/w)) to a solution containing 5 % native whey proteins resulted in significantly ($P<0.05$) lowered foam overrun. In contrast, the foam stability of native whey proteins was significantly enhanced when sucrose, maltitol and sorbitol were separately incorporated into 5 % native WPC-80 solution. An increase in solution viscosity due to carbohydrate addition was disadvantageous for air incorporation but significantly increased the strength of foam lamella wall surrounding foam air bubbles. The net effect of this increase in solution viscosity was poorer foaming ability but better foam stability of native whey proteins.

The effect of various whey protein concentrations was evaluated at 2 %, 5% and 8% using two commercial WPI and native WPC-80. An increase in whey protein concentration increased both foam overrun and foam stability. This could be attributed to increased concentration of proteins that were absorbed onto air-water interface of the foam system and thicker, more rigid interfacial lamellar films which in turn retarded serum drainage.

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Abstract

Whey proteins are utilized in the food industry as foaming agents and consequently whey protein foams are an integral component of many foods such as meringue and whipped cream. The aim of the study was to investigate the effects of carbohydrates (sucrose, maltitol and sorbitol) on the foaming properties of native whey proteins (Native WPC-80). Additions of sucrose, maltitol and sorbitol (10 % (w/w)) to a solution containing 5 % native whey proteins resulted in significantly ($P<0.05$) lowered foam overrun. In contrast, the foam stability of native whey proteins was significantly enhanced when sucrose, maltitol and sorbitol were separately incorporated into 5 % native WPC-80 solution. An increase in solution viscosity due to carbohydrate addition was disadvantageous for air incorporation but significantly increased the strength of foam lamella wall surrounding foam air bubbles. The net effect of this increase in solution viscosity was poorer foaming ability but better foam stability of native whey proteins.

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introduction

Proteins are widely used as functional ingredients in the manufacture of processed foods. They are well known for their ability to provide texture and physical stability to food systems. Among many functional roles played by proteins in processed foods, foaming is of particular interest to food scientists and processors alike as they have numerous applications in the manufacture of processed foods. Foams can be described as a two-phase system consisting of air bubbles surrounded by protein films which are usually in the form of thin liquid lamellar phase (Britten and Lavoie, 1992). Whey protein concentrates (WPC) are often used as food ingredients for foaming agent in foods because of its unique foaming properties. These include but are not limited to marshmallow, meringue-type products and whipped cream/toppings type of products. As foodstuffs, they are applied not only because of their functional properties but also because of their high nutritive value (Daubert & Foegeding, 2000). Various proteins can be used as foaming agents in foods but WPC, especially in their native form, exhibit the greatest foaming properties relative to many other food proteins which can be used as foaming agents. This is because native WPC has a more superior surface properties (e.g. surface hydrophobicity,

surface tension) and form foams with higher yield stress at lower protein concentration and less whipping time relative to most other food protein foams (Pernell, Foegeding, Luck and Davis, 2001)

Protein forms and stabilizes foams by rapidly absorbing and unfolding at the air-water interface to form a cohesive film around air bubbles which promotes foam development. The viscoelastic film at the interface is attributed to protein-protein interactions such as electrostatic and hydrophobic interactions (Liang and Kristinsson, 2005). WPC is a complex mixture of proteins and each protein component possesses a different isoelectric point (pI). Therefore, it is expected that the inherent structure of Native Whey Protein (NWP) can only be kept intact within a very narrow range of various processing conditions. The most dominant protein in WPC is β-Lactoglobulin (50-55 % of total whey protein) with pI of 5.3 (Harnsilawat, Pongsawatmanit & McClements, 1989). Many factors affect the foaming properties of food proteins such as WPC. These include, but are not limited to, physicochemical properties, environmental conditions (such as pH and temperature), presence of additives (such as sucrose), protein concentration (Patel and Fry, 1987), ionic strength and composition (Phillips et al., 1990). Many studies have been done on relating WPC foaming performance and processing parameters (pH, temperature, pressure etc) and compositional and physicochemical variables to their surface properties (Liao and Mangino, 1987; Lee et al., 1992). For example, Lau and Dickinson (2005) showed that the addition of sucrose reduces the foaming ability (degree of foam development) of WPC by increasing the continuous phase viscosity of the foam which in turn allows less air to be incorporated. Guang Wang and Tong Wang (2008) also showed that detrimental effect of yolk contamination on foaming properties of egg white proteins (EWP) arises from protein-lipid interactions at the air-liquid interface. Like EWP, the foaming properties of WPC are also greatly affected by lipids. Lipids, especially phospholipids, are good surfactants and therefore reduce foaming properties of WPC by competitive adsorption and disturbing protein-protein hydrophobic interaction at the interface.

The foaming properties of WPC are often defined by the foam overrun (degree of foam development/ foaming ability) and foam stability (measure of how long foams can stay intact before it collapses). The degree of WPC foam development is mainly dependent on protein surface properties while foam stability depends on the mechanical properties of the liquid lamella surrounding the air bubbles (Nakai, 1983; German et al, 1985). The effect(s) of protein concentration in solution, addition of sugars, lipids on the foaming properties of WPC were some of the main factors that were investigated in studies involving foaming properties of WPC. These factors affect foaming properties of WPC mainly by enhancing or limiting the ability of whey proteins to be absorbed and unfold at the interface through physical and/or chemical means. In this study, we wish to investigate the effect(s) of whey protein concentration and addition of sucrose and sugar alcohols (sorbitol and maltitol) on the foaming properties of WPC and WPI using standard conditions. The hypotheses of this study were two-fold. First, we would like to know whether increasing protein concentration alters the foaming properties of whey proteins and secondly, we want to test whether sugar alcohols as an additive, have similar effects on foaming properties of whey protein compared to that of sucrose. The objective of

the study was to evaluate the effects of protein concentration and sugar additives on the foaming properties of native and traditional whey proteins.

Material and Methods

Materials

Investigations were conducted using powdered Native Whey Protein Concentrate (Native WPC-80) that was produced by means of membrane filtration of milk done on a laboratory scale. The powder contains 78.18 % protein by composition. Two powdered Commercial Whey Protein Isolate (WPI) samples produced by Hilmar ingredients and Glanbia ingredients, containing 90.13 % and 90.07 % protein by composition respectively were used for protein concentration study only. Sucrose (ACS), obtained from Fisher Scientific Inc was used as an additive. Sorbogen 712 crystalline sorbitol and Roquette P 200 maltitol were used as sugar alcohol additives. Deionized water (18.2 MΩcm @25 C) used for both rinsing and hydration purposes were obtained from MilliQ (Millipore) deionized water dispenser.

Preparation of protein solutions

Preparing a 5 % (wt/wt) native WPC-80 /WPI solution involved dispersing an appropriate amount of the powder in an appropriate volume of distilled water (DI water). DI water was added to the powder to yield approximately 125 g of whey protein solution. The water was first added incrementally to the powder with stirring using a glass rod until a smooth paste was formed. Then, the balance of the water was added to bring the total weight of the mixture to 125 g. The protein dispersion was then stirred at room temperature using magnetic stirrer for abcut 2 hours and then refrigerated at 4°C for 16 hours to complete the hydration process. At the end of the hydration process, the pH of the solution was measured and subsequently adjusted to 7.00 with 1.0 N HCl and/or NaOH solution as necessary. At this pH, whey proteins are soluble and consistently formed adequate foams suitable for measurements of overrun and stability (Foegeding et al, 1990). The temperature of the solution was adjusted to about 25°C using a water bath set digitally at 25°C prior to use.

Protein concentration study

The standardized method for preparing 5 % (wt/wt) native WPC-80/WPI solution was used to prepare approximately 375 g of its 8 % (wt/wt) counterpart. Protein concentration study was conducted at 2 %, 5 % and 8 % protein concentration in solution by preparing about 125 g of whey protein solution at each concentration. 5 % and 2 % NWP solutions were prepared by means of dilution of the 8 % stock solution.

Addition of Sucrose, Maltitol and Sorbitol

The standardized method for preparing 5 % NWP solutions was used to incorporate various carbohydrate additives into native WPC-80/WPI solution. Sucrose was added to an appropriate amount of the 5 % NWP solution to yield 125 g of whey protein solution containing 10 % or 15 % sucrose by weight. The latter was used in sucrose concentration studies The same treatment was used for preparing separate 10 % solution of maltitol and sorbitol.

Foam Formation

Foams were formed by whipping NWP solution in a Kitchenaid® Mixer (model no: KSMC50S). Before the sample was whipped, about 118 g of native WPC-80/WPI solution was transferred to fill a tared weighing boat (volume approximately 118 ml). The weight of the solution in the boat was recorded to facilitate overrun measurements. The whey protein solution in the weighing boat was then transferred back into the mixing bowl and was whipped at the highest speed (5) continuously for 10 minutes.

Foam Overrun Test

Immediately following the 10 minute whipping period, the mixer was stopped and the beater was carefully lifted to minimize the disturbance of the foam. Samples of foam were gently scooped out with a metal spatula and used to quickly fill two tared weighing boats using small scoops each time so as to avoid creating entrapped air pockets. The excess foam was then scraped off the top of the weighing boat using the same spatula to level the top of the foam to obtain constant volume for each measurement (Foegeding et al, 1990). This whole period of analysis was limited to approximately 2-3 minutes to minimize changes in the foam.

The overrun was calculated by the following equation:

$$\%\ Overrun = \frac{(Weight\ of\ 118\ mL\ protein\ solution) - (Weight\ of\ 118\ mL\ foam)}{(Weight\ of\ 118\ mL\ foam)} \times 100$$

Foam Stability Test

The same foam that was used for overrun test was used for this test. After the overrun test, samples of foam were gently scooped out with a metal spatula in the same manner as in the overrun test and used to quickly fill two identical funnels that have been tared separately with the aid of a 100 ml measuring cylinder. Foam stability was measured by monitoring the time taken to reach a liquid drainage weight equivalent to 50 % of the weight of the foam that was placed and leveled to a fixed volume in a plastic funnel. The liquid drainage was collected in a 100 ml beakers positioned on a weighing balance. Next, the foam-filled funnel was placed in a ring stand vertically above a tared 100 ml glass beaker on a weighing balance. A stopwatch was then started to measure the time required for 50 % drainage. The time to attain 50 % drainage was used as an index of foam stability (Halling, 1981).

Foam Drainage Kinetics Test

The kinetics of foam drainage was investigated at five minutes interval. At the start of the foam stability test, the time taken for the first drop of drainage to enter an empty 100 ml beaker was recorded. The weight of the empty beaker was noted prior to the start of the foam stability test and the increase in the weight of the beaker at every five minutes interval following the time needed to produce first drop of drainage was monitored using a WEIGHING balance. The increase in drainage weight at every five minutes interval was used to determine measurement for the foam drainage kinetics.

Results & Discussion

Effect of sucrose, maltitol and sorbitol on foaming properties of native whey proteins

Foam overrun

The addition of sucrose decreased foaming ability of native WPC-80 as indicated by the lower overrun compared to the control (Figure 1) ($P < 0.05$). Similarly, the % overrun decreased with the addition of maltitol. Like sucrose and maltitol, the addition of sorbitol also decreased the foaming ability of native WPC-80. The degree of the reduction in overrun level relative to the control upon addition of maltitol and sorbitol was lower than that of sucrose. Statistical test conducted on the results of these two sets of results indicated that there was no significant difference between the effect of the addition of sorbitol and maltitol on % overrun of native whey protein ($P > 0.05$).

Statistical analysis indicated that there was a significant difference in the % overrun values obtained for the control with that of sucrose, sorbitol and maltitol ($P<0.05$). However, there was no significant difference ($P>0.05$) between the NWP samples treated with sorbitol and maltitol. A recent study conducted by Foegeding and Yang (2009) have showed that there are significant ($P<0.05$) differences in the foam overrun of WPC and WPI when sucrose was used as an additive relative to the case where no additive is present. The study also discussed possible explanations behind these differences.

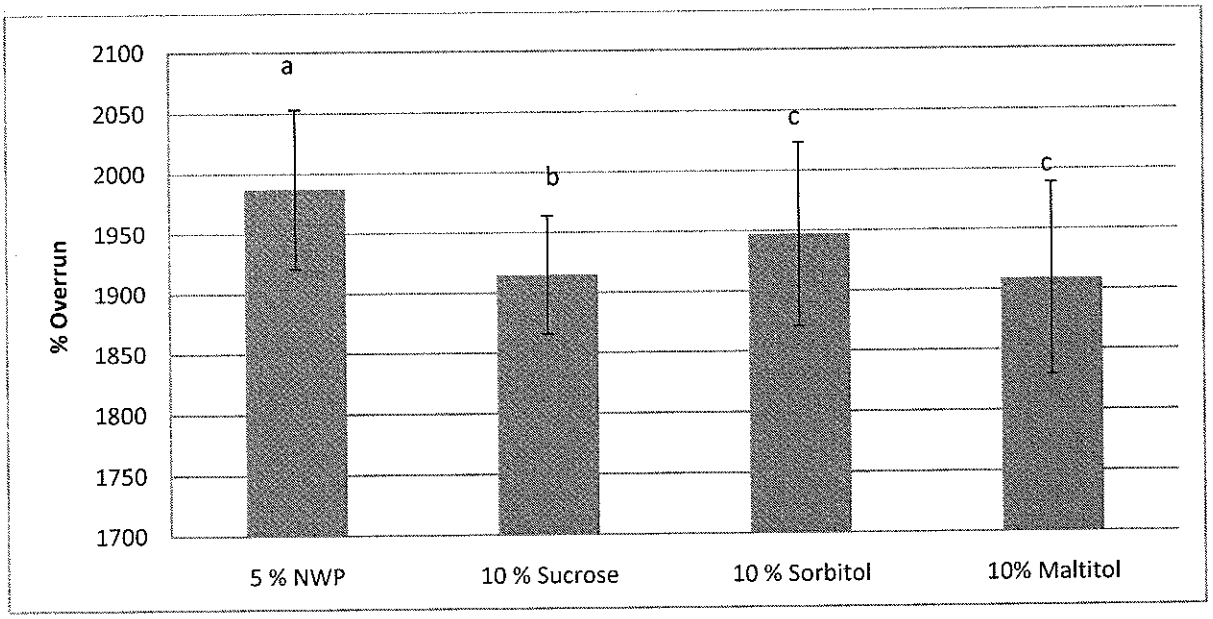

Fig. 1. Effect of sucrose, maltitol, sorbitol on % overrun of NWP made from 5 % native WPC-80 solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from at least three replicates.

Foegeding and Yang (2009) proposed that sucrose changes the foaming properties of whey proteins mainly by altering the bulk phase viscosity of the protein solution and the interfacial properties of whey proteins. Foaming ability of whey proteins is highly dependent on the ability

of the proteins to be adsorbed onto the liquid-air interface. Whey protein adsorption occurs typically in three stages: 1) diffusion of proteins to the interface, 2) initial adsorption at the interface, 3) unfolding and rearrangement of the adsorbed whey proteins at the interface. The decrease in overrun of the foams formed when sucrose was added to native WPC-80 solution may be attributed to an increase in the protein solution viscosity which allows less air to be incorporated into the interfacial liquid lamellae (Raikos et al, 2006). Increased viscosity of the native WPC-80 solution is disadvantageous for air incorporation and the rapid diffusion and unfolding of whey proteins at the air-water interface (Raikos et al, 2006). The increase in solution viscosity may slow diffusion rate of the protein molecules toward the interface, and thereby reducing the amount of foam formed in a given period of time. Consequently, there is reduced surface hydrophobicity of the whey proteins which in turn leads to decreased surface activity of whey proteins (Lau and Dickinson, 2005). Another important factor that may affect the overrun level of whey protein foams is the size of air bubbles in the foam (Raikos, et al., 2007) .The effect of sugar alcohols on the foaming properties of whey proteins has not been studied as extensively as that of sucrose. However, I believe that these concepts may also be used to explain the effect of addition of maltitol and sorbitol on the foaming ability of native whey proteins as addition of sugars into protein solution results in a change in the solution viscosity. Addition of maltitol and sorbitol may lead to an increase in the viscosity of the native whey protein solution which enables lower volume of air to be incorporated in the same amount of time as the control sample. This may cause less unfolding of the native whey proteins (NWP) to occur at the interface which in turn decreases surface activity of NWP.

The addition of sucrose can affect foaming ability of whey proteins by limiting protein unfolding and the development of protein-protein interactions at the liquid-air interface as sucrose is known to create a less favorable thermodynamic environment for protein unfolding. However, it is difficult to isolate the complicated interrelated effects of electrostatic interactions, hydrogen bonding and hydrophobic forces on molecular level (Dickinson & Merino, 2002). In this discussion, we will focus on the effect of hydrogen bonding between sucrose/sugar alcohols and whey proteins molecules on the foaming ability of NWP. Whey proteins are made up of many different kinds of proteins. Therefore, it is difficult to attribute the effect of sucrose to any one particular protein in NWP. The main protein present in NWP is β-lactoglobulin (constitutes ~ 55 % of NWP) and therefore is often used as the representative protein to explain the effects of hydrogen bond formation with sucrose on foaming ability of whey proteins. Foegeding et al. (2009) proposed that the adsorption of β-lactoglobulin decreases in the presence of sucrose possibly because β-lactoglobulin forms hydrogen bonds with the sucrose molecules, which results in poor unfolding activity at the interface and culminating in reduced surface activity. The β-lactoglobulin molecules which participate in hydrogen bonding formation with sucrose preferentially remain in solution rather than adsorb to the air-water interface (Antipova, Semenova and Belyakova, 1999). The effect of hydrogen bonding formation of β-lactoglobulin with sugar alcohols (maltitol and sorbitol) on the foaming ability of NWP has not been extensively studied. However, we believe that it may also be used to describe their roles in the increase of % overrun of NWP foams.

Maltitol is a sugar alcohol derivative of maltose, a disaccharide. Its molecular formula reveals that it has 9 hydroxyl (OH) groups available for hydrogen bonding with other entities as opposed to sucrose which has 8 OH groups available for hydrogen bonding. The difference in the effect of maltitol and sucrose on the foaming ability of NWP may not be dependent on their inherent hydrogen bonding capabilities but in their molecular structures. About 99 % of sucrose molecules are in their cyclic form when in aqueous solution. This arrangement (two cyclic rings made up of fructose and glucose joined by α,β(1,2) linkages) exposes all of its hydroxyl groups to the exterior of the molecule. This means intramolecular hydrogen bonding between OH groups of sucrose are unlikely as the OH groups are too distant between adjacent OH groups which in turn maximizes the effective hydrogen bonding potential of a sucrose molecule with β-lactoglobulin. Maltitol is made by hydrogenation of maltose molecules which only has one cyclic ring in its structure. The other portion of the molecule is made up of aliphatic polyols carbon chain. The OH groups in this portion of the molecule are within close proximity to each other and may have a higher tendency to form hydrogen bonds with each other rather than with β-lactoglobulin in NWP. This could reduce the effective hydrogen bonding potential of maltitol with β-lactoglobulin which in turn allows more NWP to be adsorbed to the air-liquid interface compared to sucrose. This helps to minimize the reduction in the foaming ability of NWP as measured by the % overrun.

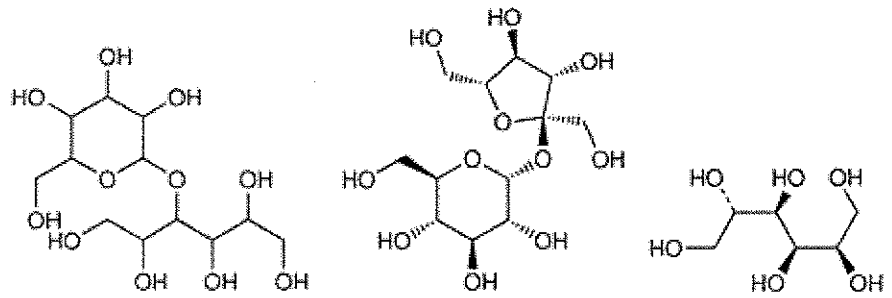

Fig . 2. Chemical structures of maltitol, sucrose and sorbitol (from left to right) (Sources: Wikimedia commons and aisonschem.en.chemnet.com)

Sorbitol, the sugar alcohol derivative of glucose, also increases the % overrun of NWP. This may also be attributed to the hydrogen bonding effect of the sugar alcohol. Relative to sucrose, the molecular structure of sorbitol has fewer OH groups (6 per molecule as opposed to 8 in sucrose). This means that the effective hydrogen bonding potential of sorbitol with β-lactoglobulin molecules is less than that of sucrose which in turn allows more β-lactoglobulin to be adsorbed onto the air-water interface relative to that of sucrose. This contributes to better foaming ability of NWP relative to that of sucrose.

Foam stability

The effect of sucrose, maltitol and sorbitol on the half-life (50 % drainage time) of NWP foams is shown in Figure 3. The addition of sucrose increased foam stability of the egg white proteins and the foams exhibited significantly longer half-life compared to that of the control ($P < 0.05$). Similarly, the half-life of NWP foams significantly increased with the addition of maltitol relative to that of the control ($P < 0.05$). Addition of sorbitol to native WPC-80 solution also increased the half-life of the NWP foams relative to that of the control. Statistical analysis indicated that there was a significant difference in the % overrun values obtained for the control with that of sucrose, sorbitol and maltitol ($P<0.05$). However, there was no significant difference ($P>0.05$) between the drainage time of the NWP samples treated with sorbitol and maltitol. A recent study conducted by Foegeding et al (2009) and Herceg et al (2009) have discussed possible explanations behind the differences seen in the foaming ability of NWP when sucrose was present as an additive.

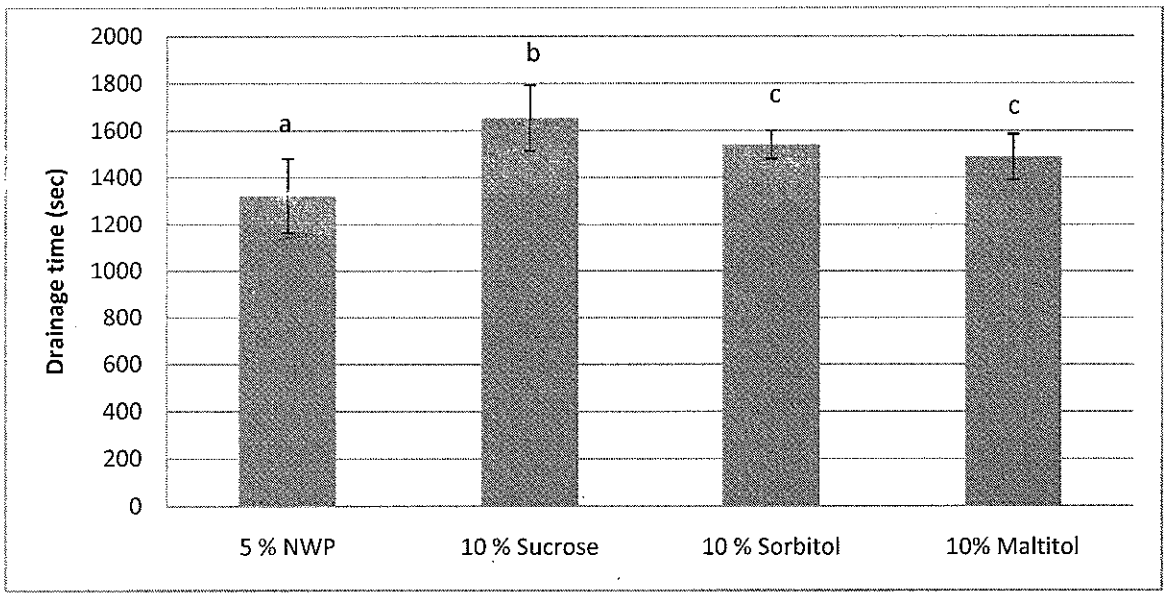

Fig. 3. Effect of sucrose, maltitol and sorbitol on half-life (50 % drainage time) of native whey protein foams made from 5 % native WPC-80 solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from at least three replicates.

The increase in foam stability of NWP with addition of sucrose might be attributed to an increase in the viscosity of the medium in which the sucrose in being incorporated (Native WPC-80 solution). Sucrose contributes to foam stability by increasing the viscosity of lamella and thereby retarding drainage (Davis and Foegeding, 2004). This in turn results in a stronger foam microstructure (individual air bubbles surrounded by liquid lamella phase) which collectively contributes to better foam stability. Protein foam stability is influenced by the fluid continuous phase (Halling, 1981). The increase in viscosity due to addition of sucrose could impede the movement of liquid through the network of protein lamellae, thereby slowing the drainage rate. The increase in foam stability due to addition of sucrose might also be attributed to increased intermolecular interactions between native whey proteins molecules which cause

them to remain in aggregates in the foam structure thereby contributing to the overall stability of the foam microstructure. The increased protein-protein interactions led to development of multilayer cohesive protein film at the interface which results in an increase in lamella film thickness which was thought to slow drainage rate and prevent foam collapse, thereby increasing overall foam stability. Light microscopy revealed that smaller air bubbles size (Figure 5) was observed after addition of sucrose relative to that of the control (Figure 4). This finding was consistent with that of Raikos et al. (2007). The increase in foam stability with sucrose addition might be attributed to the reduction of air bubbles size. Smaller air bubbles would form stiffer foams and consequently bubble size in protein foam was inversely related to foam stability (Kinsella, 1981). To date, there is no satisfactory explanation to the effect of smaller air bubbles size of protein foam on foam stability.

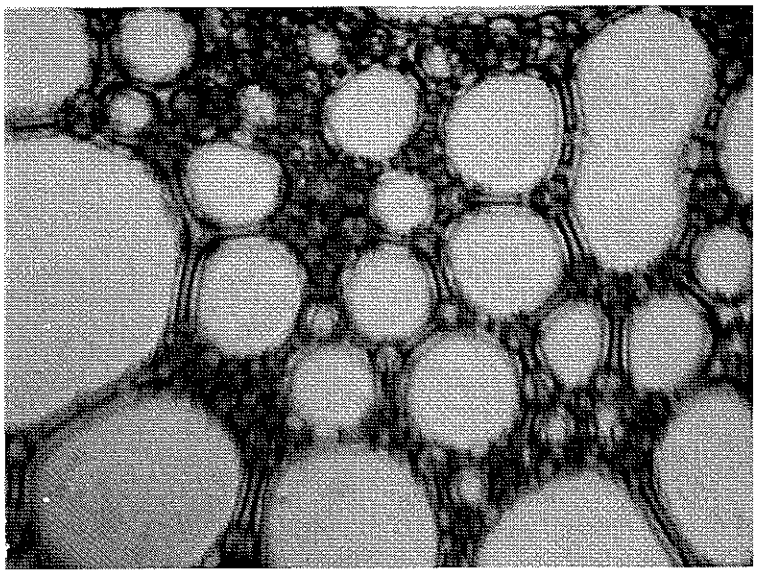

Fig. 4. Light microscopy of NWP foams made from 5 % native WPC-80 solution at pH 7.00 and ambient temperature.

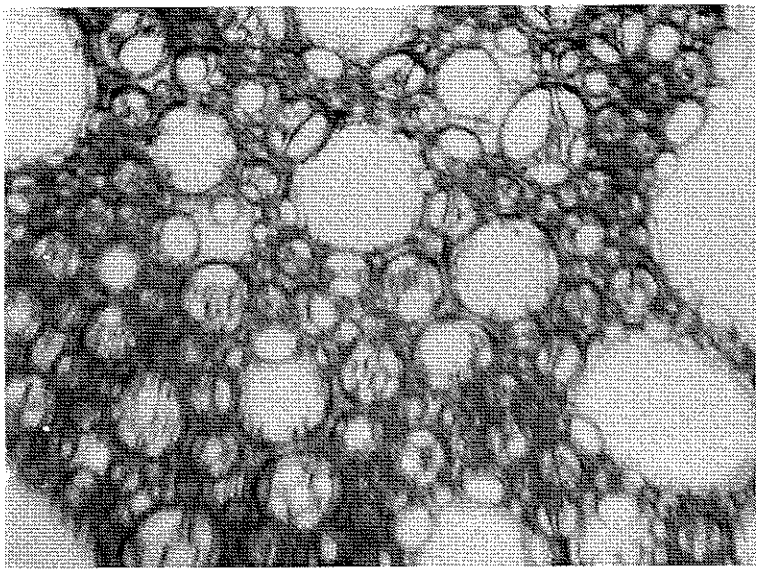

Fig. 5. Light microscopy of NWP foams made from 5 % native WPC-80 and 10 % sucrose solution at pH 7.00 and ambient temperature.

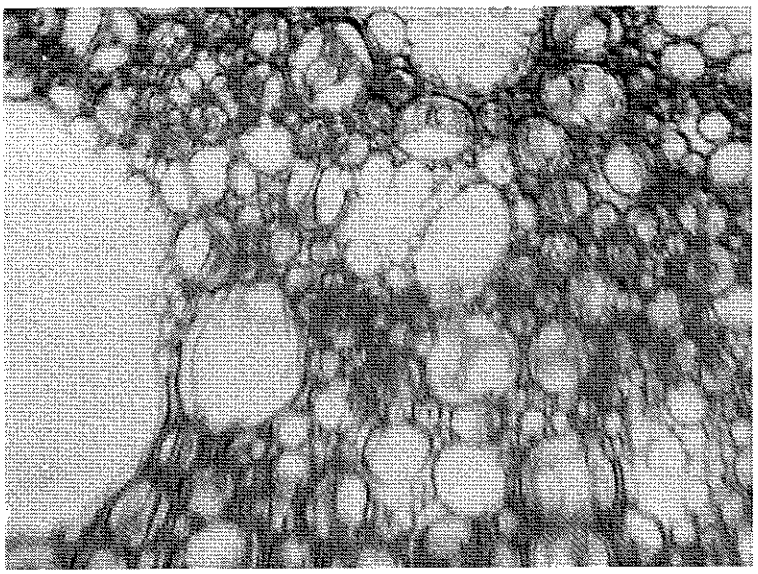

Fig. 6. Light microscopy of NWP foams made from 5 % native WPC-80 and 10 % maltitol solution at pH 7.00 and ambient temperature

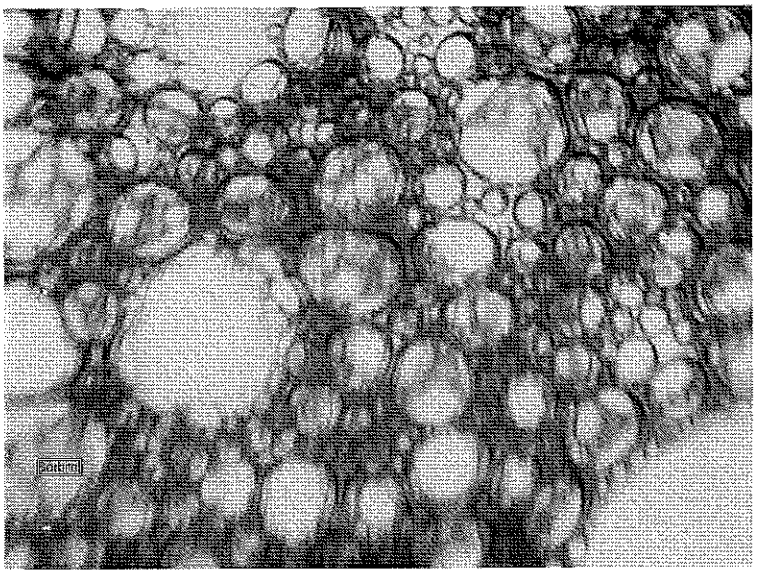

Fig. 6b. Light microscopy of NWP foams made from 5 % native WPC-80 and 10 % sorbitol solution at pH 7.00 and ambient temperature.

An increase in the foam stability of NWP when maltitol and sorbitol were added to the protein solution was thought to be attributed to an increase in the viscosity of the NWP solution. An increase in viscosity could positively affect the foam microstructure due to an increase in the strength of the lamellar wall surrounding the air bubbles (Davis and Foegeding, 2004). According to the present data, the extent of increase in foam stability with maltitol and sorbitol addition was lesser that that of sucrose. Light microscopy revealed NWP foams with sorbitol and maltitol as additives were composed of air bubbles that were larger in size relative to that of sucrose (Figure 5 &6). According to Kinsella (1981), this means that the protein foam formed in the presence of sugar alcohols is probably less stiff than that of sucrose. Therefore, in agreement with the present data, NWP foams show lesser increase in the overall stability of the foam microstructure when maltitol and sorbitol were used as additives. This comparison was made relative to that of sucrose. Consequently, NWP foams treated with sugar alcohol have a higher drainage rate (loss of liquid from foam microstructure per unit time) and decreased the overall stability of the foam relative to the sucrose sample.

Effect of whey protein concentration in solution on foaming properties of Native Whey Protein (NWP) and Whey Protein Isolate (WPI)

Foam overrun

The effect of protein concentration on the foaming properties of whey proteins was conducted at 2%, 5% and 8% protein concentration levels using three different kinds of whey protein powders namely Native WPC-80 and two commercial samples of WPI (Hilmar and Glanbia WPI). Increasing whey protein concentration resulted in an increase in the foaming ability of whey proteins as indicated by their % overrun values in Figures 7, 8 and 9. The results indicated that increasing whey protein concentration from 2 to 5 % resulted in a significant increase ($P<0.05$) in % overrun and hence foaming capacity of both native and traditional whey

foams. This was also true for increase in protein concentration from 5 to 8 % ($P<0.05$). These observations were valid for all the three whey protein samples that were being investigated in the study. Britten and Lavoie (1992) stated that maximal foaming capacity of whey proteins occur around 8 % protein concentration in solution. Increasing whey protein concentration beyond this level could greatly decrease the solubility of whey proteins in solution and a loss of solubility could adversely affect the foaming ability of whey proteins as reflected by decreasing % overrun level.

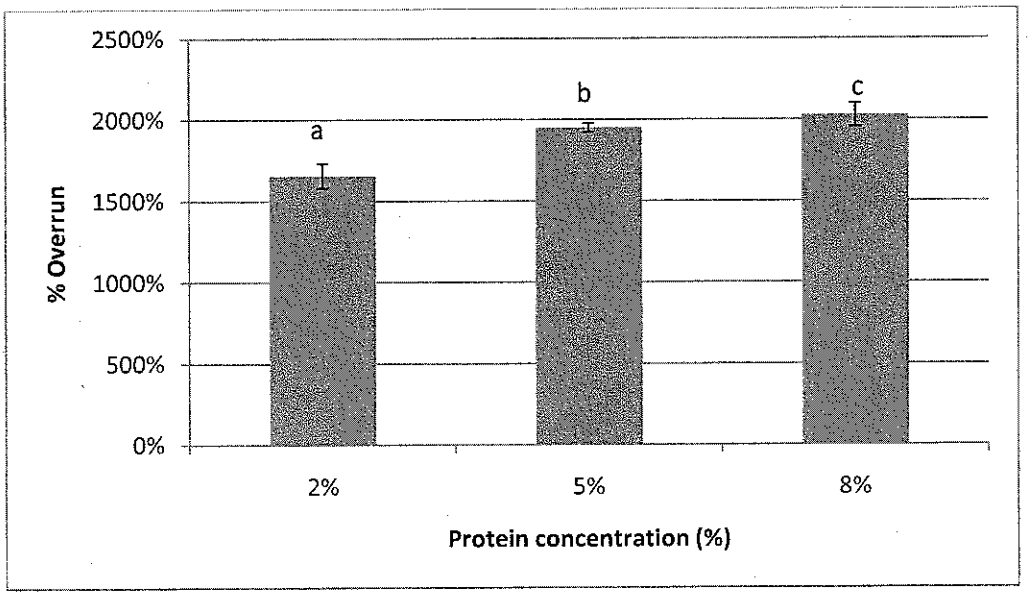

Fig. 7. Effect of whey protein concentration in solution on % overrun of native whey protein foams made from native WPC-80 solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from two replicates.

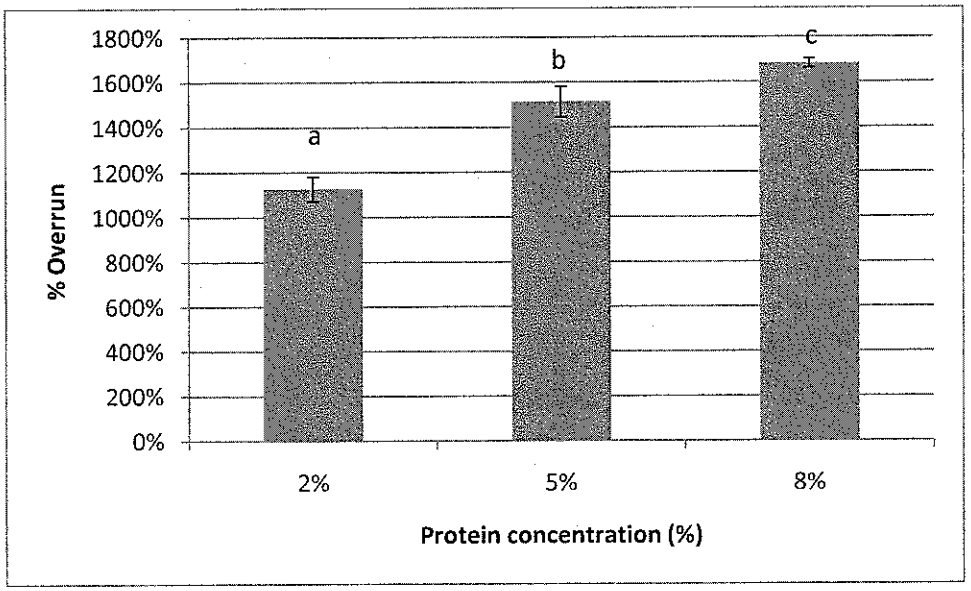

Fig. 8. Effect of whey protein concentration in solution on % overrun of whey protein foams made from Glanbia WPI solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from two replicates.

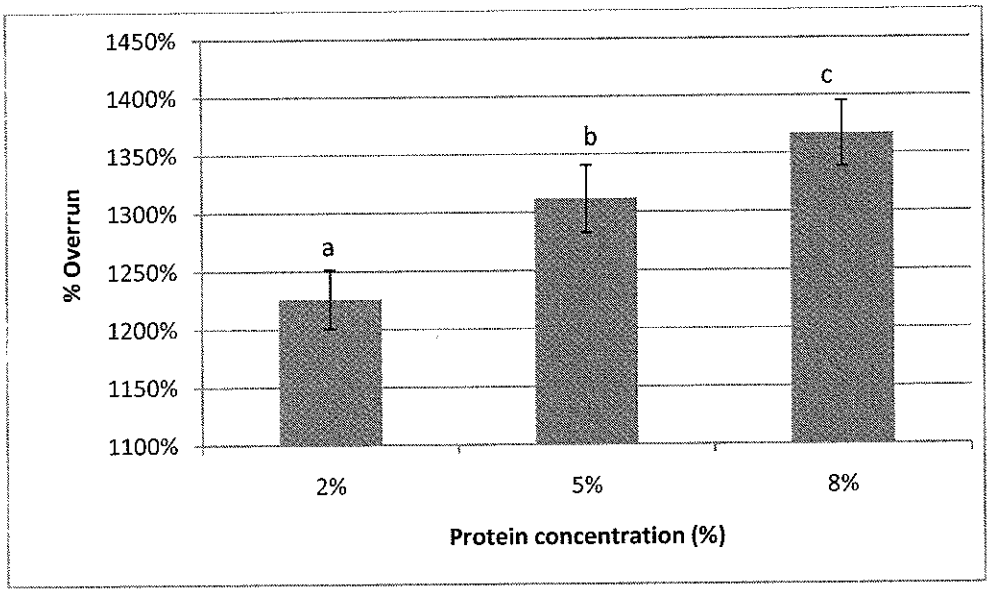

Fig.9. Effect of whey protein concentration in solution on % overrun of whey protein foams made from Hilmar WPI solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from two replicates.

Increase in the % overrun with increasing concentration of whey protein could be attributed to the increased amount of proteins available for adsorption onto the air-water interface. Increasing the protein concentration resulted in greater of surface concentration of whey proteins at the interface which in turn increased foaming ability of whey proteins (Raikos et al, 2006). Foam expansion is another parameter that can be used to explain the effects of increasing protein concentration on the foaming capacity of whey proteins. An increase in the concentration of whey proteins leads to better solubility of whey protein solutions and this enhances the surface activity of whey proteins which in turn helps to expand the volume of foam produced (Britten and Lavoie, 1992). However, at very high protein concentration level (> 8 %), the solubility of the whey protein solutions is reduced and consequently the degree of foam expansion maybe reduced. Britten and Lavoie (1992) proposed that in the concentration range where whey proteins were fully soluble, there is a direct relationship between foam expansion and protein concentration.

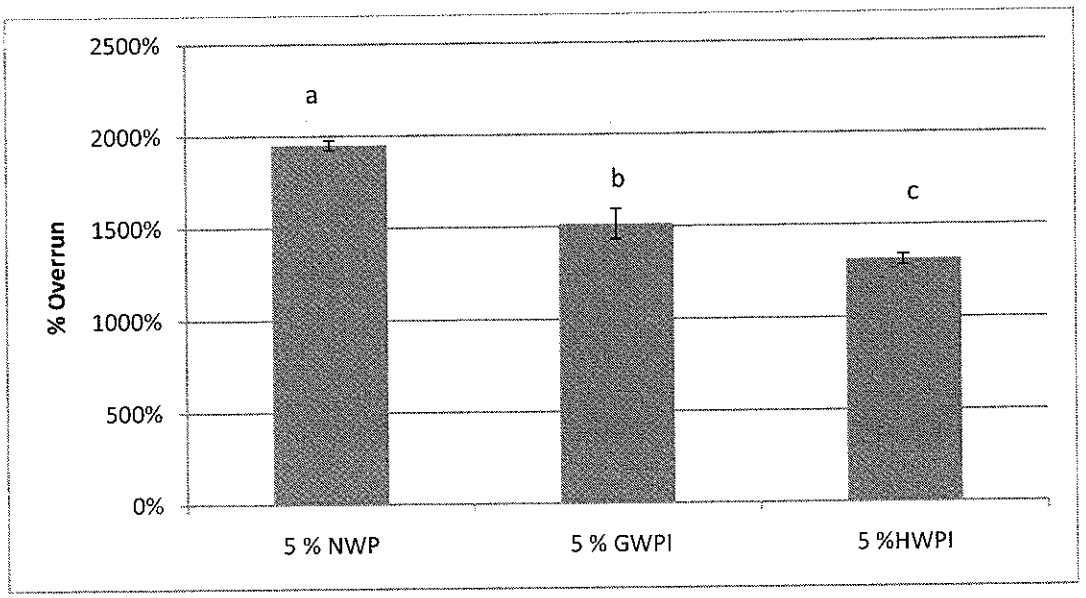

Fig. 10. % overrun of whey protein foams made from 5 % native WPC-80, Glanbia WPI and Hilmar WPI solutions at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from two replicates.

The results presented in Figure 10 showed that at similar protein concentration level, native whey proteins has better foaming ability than WPI. The differences in the % overrun were statistically significant ($P<0.05$). Heino et al (2007) postulated that an important reason for excellent foaming ability of native whey protein relative to traditional whey protein was due to differences in the composition of powders being investigated. Native whey protein powders (e.g. Native WPC-80) typically have a much lower fat content than cheese whey protein powders (e.g. Glanbia and Hilmar WPI). Although a detailed determination of the composition of the various whey protein powders used in the study was not carried out, many studies (Vaghela and Kilara, 1996; Joseph and Mangino 1988; Muller 1976) have confirmed that the high fat content in WPC and WPI powders reduces the foaming ability of whey proteins in these powders (Vaghela and Kilara, 1996). Joseph and Mangino (1988) reported that WPI made of traditional whey contains high levels of phospholipids and lipoproteins which are foam depressing agents. Fat displaces proteins in the air/water interface which weakens foaming ability of whey proteins (Karleskind *et al.* 1995). Native WPC-80 powder possibly has a much lower total content of fats and lower proportion of phospholipids and lipoproteins percentages of total fat and therefore exhibited a much better foaming ability (higher foam overrun) compared to WPI powders (Glanbia and Hilmar).

Foam stability

Increasing whey protein concentration generally resulted in an increase in the foam stability of whey proteins as indicated by their drainage time in Figure 11, 12 and 13.

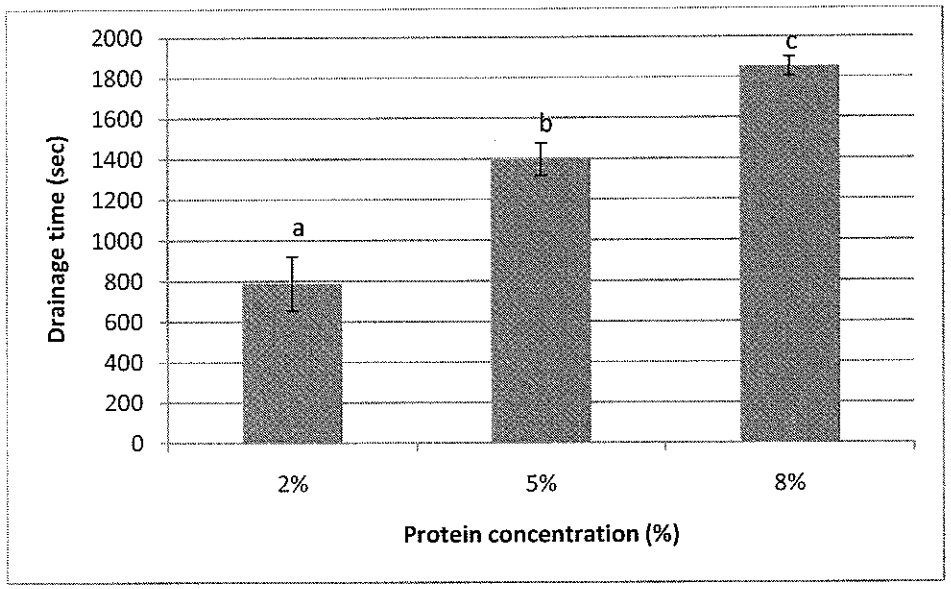

Fig. 11. Effect of whey protein concentration in solution on half-life (50 % drainage time) of native whey protein foams made from native WPC-80 solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from at least three replicates.

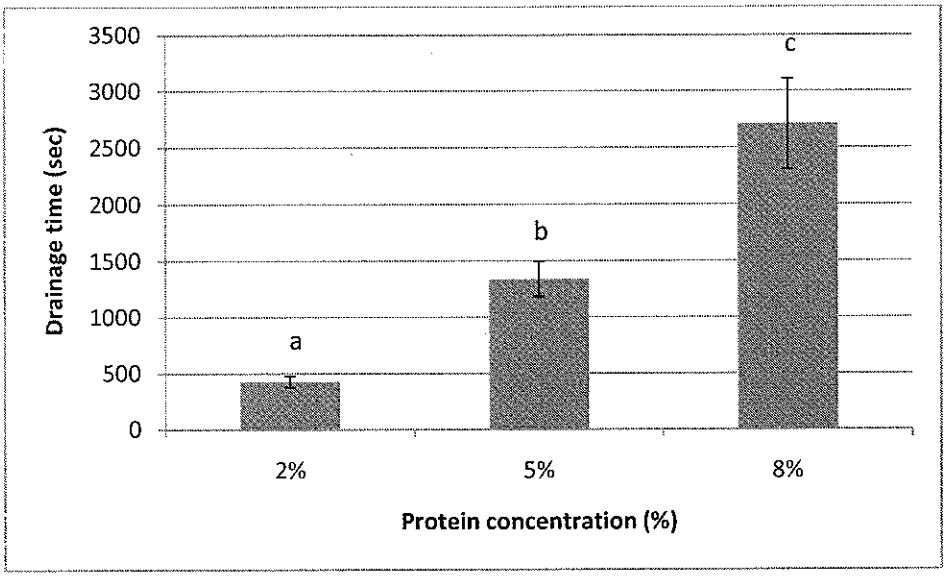

Fig. 12. Effect of whey protein concentration in solution on half-life (50 % drainage time) of whey protein foams made from Glanbia WPI solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from two replicates.

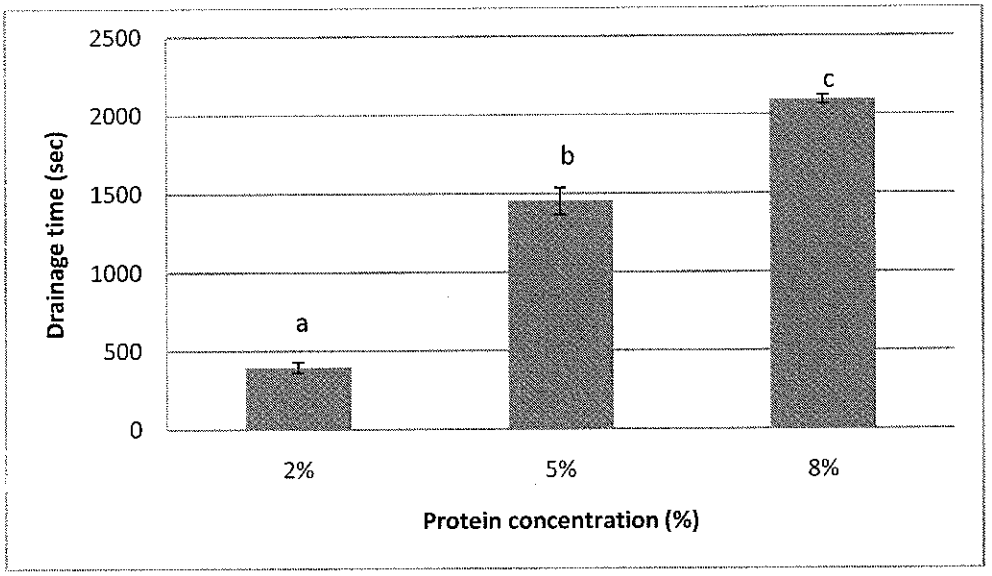

Fig. 13. Effect of whey protein concentration in solution on half-life (50 % drainage time) of whey protein foams made from Hilmar WPI solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from two replicates.

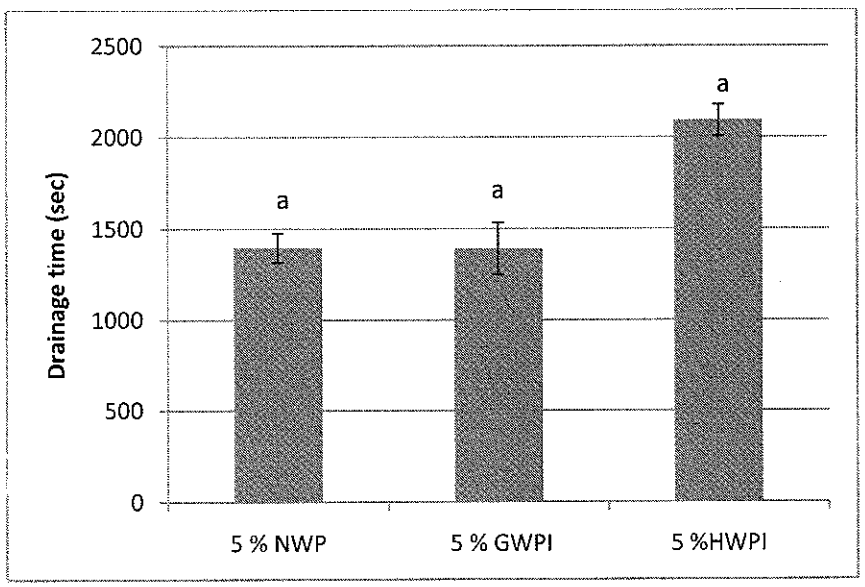

Fig. 14. half-lives (50% drainage times) of whey protein foams made from 5 % native WPC-80, Glanbia WPI and Hilmar WPI solutions at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from two replicates.

The foam stability of whey proteins in both Glanbia and Hilmar WPI was significantly greater ($P<0.05$) than native WPC-80 at 8 % concentration level but not at the 2 % concentration level ($P>0.05$). The foam stability of NWP showed no significant difference ($P>0.05$) relative to its WPI counterparts at 5 % protein concentration level. This finding was in contrast with the findings of studies that investigate differences in the foaming properties of native and traditional whey proteins. Heino et al (2007) proposed that an important reason for excellent foaming stability

of native whey protein relative to traditional whey protein was due to differences in the composition of powders being investigated. Native whey protein powders (e.g. Native WPC-80) typically have a much lower fat content than traditional whey protein powders (e.g. Glanbia and Hilmar WPI). Determination of the composition of the various whey protein powders used in the study was not carried out so we do not know whether the better foam stability of WPI relative to native WPC-80 is due to differences in the fat content of the two powders. The reason why the foaming stability of the traditional WPI is comparable to that of NWP in this case might be attributed to the type of interfacial lamella films that is formed in the foam structure. Both the native and traditional whey protein might form foams that consist of interfacial films that are similar in thickness and rigidity. This might be due to similarity in the type of protein network that were formed in both cases which gave rise to similarity in the strength of intermolecular bonds that held the proteins together in their lamellar structure. Alternatively, the fat content of the Native WPC-80 powder may be higher than that of typical NWP powders. This weakens the foam stability of the NWP such that its foam stability is at the same level with that of traditional whey proteins in WPI and hence there was no significant difference ($P<0.05$) between the foam stability of native whey protein and that of the traditional whey protein.

An increase in whey protein concentration caused a corresponding increase in the foam stability of both native and traditional whey proteins although the extent of this increase was significantly different ($P<0.05$). This may be attributed to the formation of greater number of interfacial films in the foam microstructure which resulted in greater strength of the lamellar wall of these bubbles. Increased intermolecular interactions between the protein molecules may also have contributed to the strength of the lamellar walls. As protein concentration increased, thicker and more rigid interfacial films were produced which in turn increased apparent viscosity of whey protein solutions and reduced the rate of foam drainage by allowing more water to remain in the foam matrix. (Britten and Lavoie, 1992)

Effect of sucrose concentration in solution on foaming properties of egg white proteins

The effect of sucrose concentration study on the foaming properties of NWP was conducted at 0 %, 10% and 15% sucrose concentration levels in 5 % (wt/wt) NWP solution. Increasing sucrose concentration significantly decreased ($P<0.05$) the foaming ability of NWP as indicated by their % overrun values in Figure 15. In contrast, a significant increase ($P<0.05$) in the foam stability of NWP as indicated by its half-life was observed when sucrose concentration level was increased from 0 to 15 % (Figure 10). The decrease in overrun with increasing sucrose concentration might be attributed to increasing hydrogen bonding formation effects of sucrose as well as increasing viscosity of NWP solution. As more sucrose was added to NWP solution, there were more sucrose molecules that were available to form hydrogen bonds with β-lactoglobulin and other proteins in NWP. As a result, there were more protein molecules which participate in hydrogen bonding formation with sucrose and they preferentially remain in solution rather than adsorb to the air-water interface (Antipova, Semenova and Belyakova, 1999). In addition, increasing levels of sucrose incorporation into NWP solution resulted in increasing viscosity of the NWP solution. This allows less air to be incorporated into the interfacial liquid lamellae which is disadvantageous for rapid diffusion and unfolding of NWP at

the air-water interface (Raikos et al, 2006). As a result, the foaming ability of NWP decreased with increasing level of sucrose.

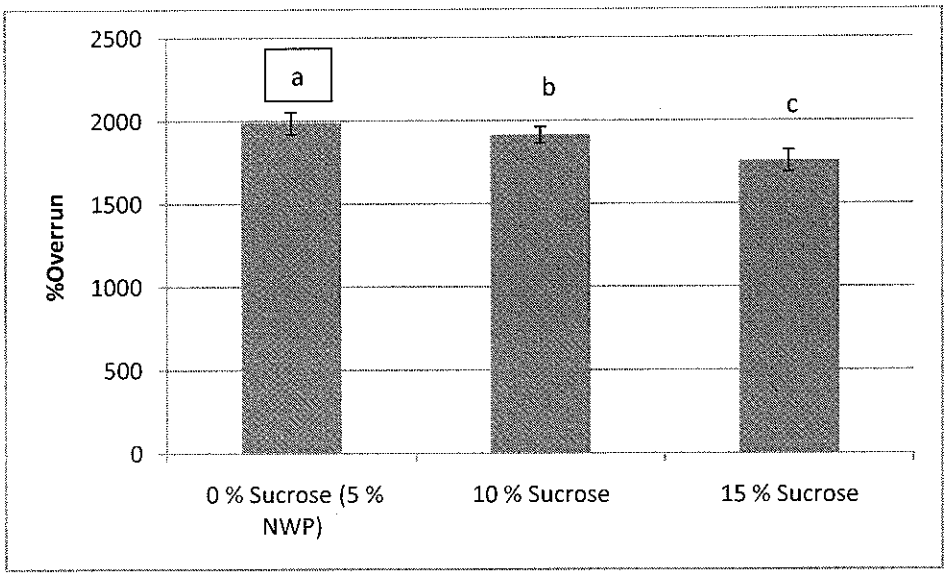

Fig. 15. Effect of sucrose concentration in NWP solution on % overrun of Native whey protein foams made from 5 % NWP solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from at least three replicates.

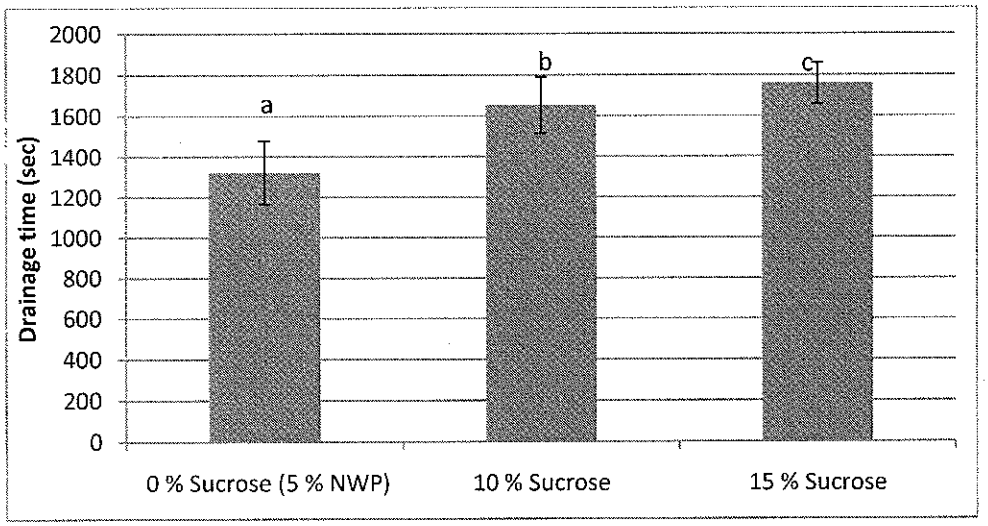

Fig. 16. Effect of sucrose concentration in NWP solution on half-life (50 % drainage time) of Native whey protein foams made from 5 % NWP solution at pH 7.00 and ambient temperature. Data are the arithmetic mean of readings obtained from at least there replicates.

Increasing levels of sucrose incorporation caused a corresponding increase in foam stability of NWP. This might be attributed to an increase in the viscosity of the NWP solution. Sucrose contributes to foam stability by increasing the strength of lamella and thereby retarding drainage (Davis and Foegeding, 2004). This in turn resulted in a stronger overall foam

microstructure that was endowed by stronger lamellar wall surrounding the air bubbles which collectively contributed to better foam stability.

Foam Drainage Kinetics

The effect of sucrose, sorbitol and maltitol on the foam stability of NWP foams can also be described by the kinetics of their foam drainage. One of the most recent models that were used for describing the kinetics of foam drainage was postulated by Elizalde et al (2001). They described the kinetics of liquid drainage from foams stabilized by proteins of different origins including whey protein concentrate by means of the following two parameter equation: $v(t) = \frac{Vt}{(B+t)}$ where v (t) refers to the volume of drained liquid at time t, V refers to the maximum volume of drained liquid and B refers to the time needed to reach the half-life of the foam in terms of drainage. Whether this kinetic model is a good fit to the present drainage kinetics data obtained for the various samples may be known if the volume of the drainage instead of the weight of the drainage was recorded as foams collapse over time. It might be possible to determine the density of drainage that was produced as the foam samples collapse to determine the volume of a given mass of drainage. However, the density of the drainage produced by the foams was also not determined in this study. Furthermore, the maximum volume/mass of the drained liquid was not determined in this study Therefore, it is not possible to determine whether the present data fits the kinetic model.

Linear regression model could be applied to the present data in order to approximate the effects of sucrose, sorbitol and maltitol on the foam stability. In this study, the drainage kinetics data of NWP foams both in the presence and absence of sucrose and sugar alcohol additives was intended to be used to support the half-life data of the protein foams used in the study. This was done by comparing the values of the slope of the linear regression line for the data of the various NWP samples. The values of the slope of the lines would represent the approximate rate of drainage of liquid from the NWP foam samples being investigated in the study. This can be done if the linear regression model can satisfactorily fits the actual data. The coefficient of determination (R^2) value for each data set under consideration needs to be at least 0.9 in order for the model to satisfactorily fit the actual data. The rate and extent of liquid drainage from lamellae are the principal factors determining the stability of protein foams (Halling, 1981).

Drainage kinetics of native whey protein foams with sucrose, sorbitol and maltitol as additives.

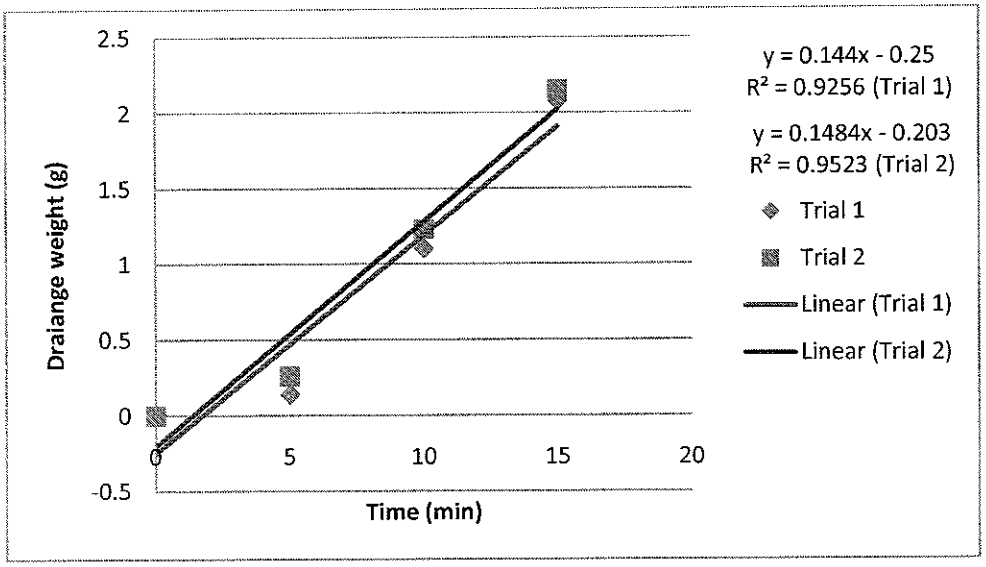

Fig. 17. Linear regression analysis of the drainage of liquid as a function of time data of Native whey protein foams made from 5 % NWP solution at pH 7.00 and ambient temperature. Data consist of two replicates from a single experiment. Squares and diamonds on the graph represent actual data.

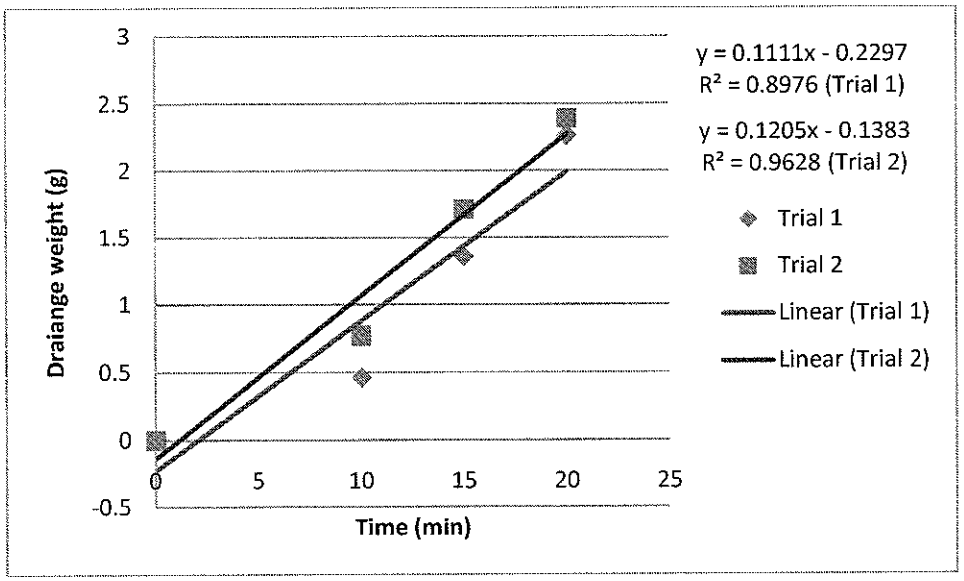

Fig. 18. Linear regression analysis of the drainage of liquid as a function of time data of Native whey protein foams made from a solution containing 5% NWP and 10% sucrose at pH 7.00 and ambient temperature. Data consist of two replicates from a single experiment. Squares and diamonds on the graph represent actual data.

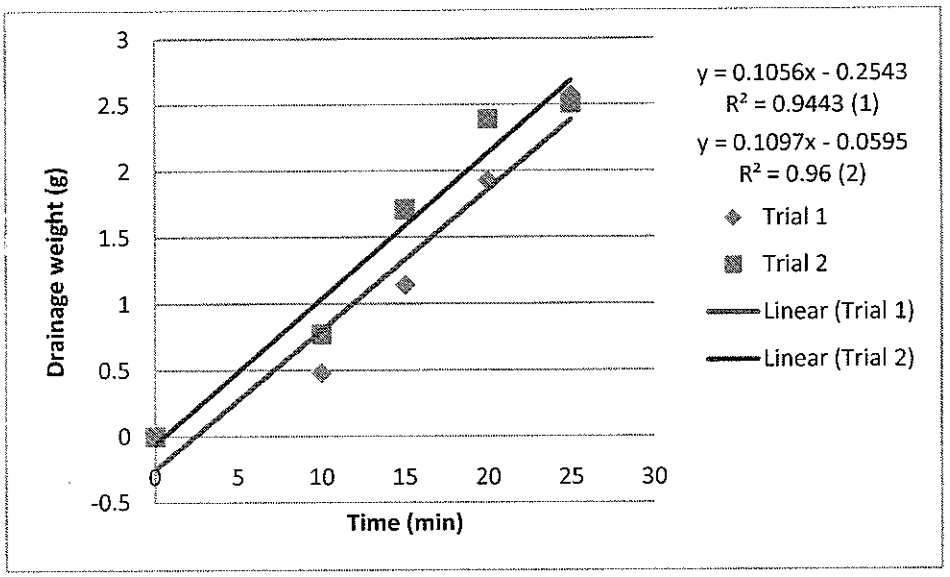

Fig. 19. Linear regression analysis of the drainage of liquid as a function of time data of Native whey protein foams made from a solution containing 5% NWP and 15% sucrose at pH 7.00 and ambient temperature. Data consist of two replicates from a single experiment. Squares and diamonds on the graph represent actual data.

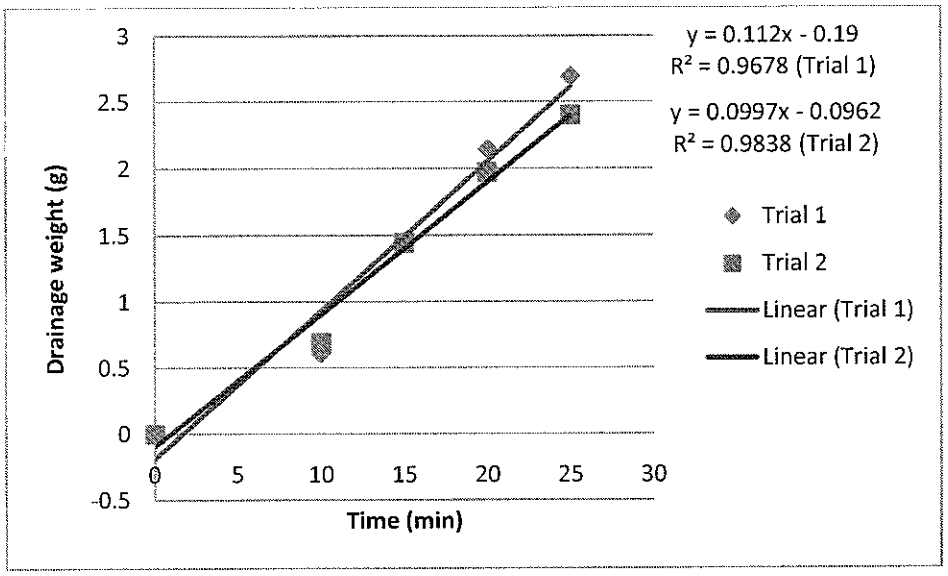

Fig. 20. Linear regression analysis of the drainage of liquid as a function of time data of Native whey protein foams made from a solution containing 5% NWP and 10% sorbitol at pH 7.00 and ambient temperature. Data consist of two replicates from a single experiment. Squares and diamonds on the graph represent actual data.

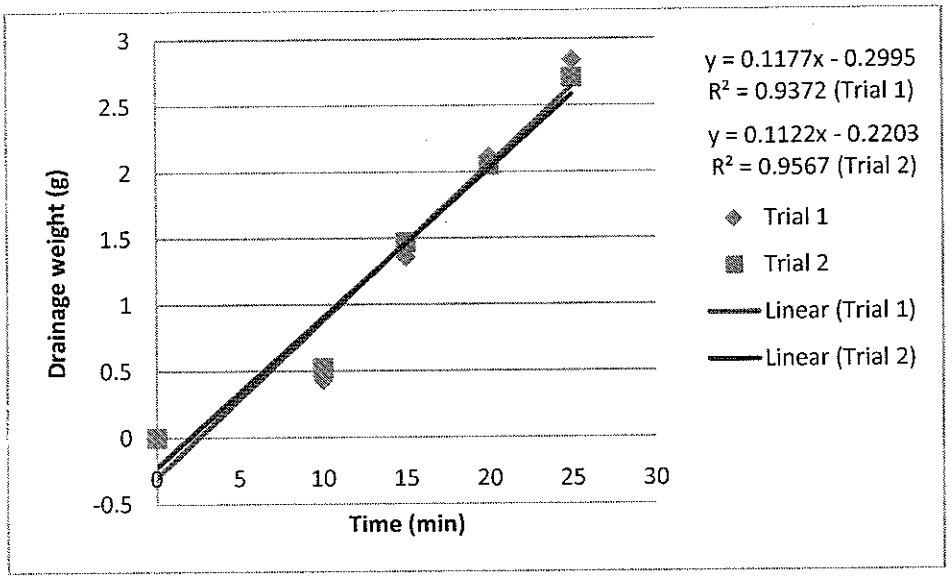

Fig. 21. Linear regression analysis of the drainage of liquid as a function of time data of Native whey protein foams made from a solution containing 5% NWP and 10% maltitol at pH 7.00 and ambient temperature. Data consist of two replicates from a single experiment. Squares and diamonds on the graph represent actual data.

The slope of the linear regression line for each sample of NWP foam represents the rate at which liquid is drained from the foam. The higher the value of the slope of the linear regression line for a particular foam sample, the higher the rate at which liquid was drained from the foam and the shorter the drainage half-life for that foam sample. The value of the slope of the linear regression line for the NWP foam sample containing no additive (Figure 16) was the highest compared to that of the NWP foam samples that contain sucrose, sorbitol and maltitol as an additive. This means that the rate of drainage of liquid from the lamellae of the NWP foam sample containing no additive was the highest and therefore it experienced the highest extent of lamellae rupture within a given amount of time relative to the foam samples with additive and this was the reason why it had the least stable foam among the foam samples. This was consistent with the half-lives data for the NWP foam samples which indicated that there was a significant difference ($P<0.05$) between the half-lives of the NWP foam in the absence and presence of additives. The values of the slope of the linear regression lines for the NWP foam samples containing sucrose, sorbitol and maltitol were fairly similar to each other (within +/- 5 % of each other). Nonetheless, The foam samples with 10 % sucrose and maltitol as additives (Figures 18 & 21) had similar average slope values and a higher average slope value compared to that of sorbitol at the same additive level (Figure 20). This indicated the foam samples with sucrose and sorbitol as additive experienced a greater degree of lamellae rupture within a given period of time relative to that of sorbitol. Comparison of the slope values of the linear regression lines for the various NWP samples indicated that the half-life of samples containing sorbitol as additive was longer than that of sucrose and maltitol. This was not in agreement with the drainage half-lives results obtained in the study. A possible reason could be that the drainage rate data was based on replicates from a single experiment while the half-lives data was based on the average of a number of replicates.

The average value of the slope of the linear regression line for the NWP foam sample with 15 % sucrose concentration level (Figure 21) was lower than that of the NWP foam sample with 10 % sucrose concentration level. This set of observation indicated that sucrose conferred greater stability to NWP foams at higher sucrose concentration level. Increasing sucrose concentration level in the formulation of NWP foam increased the viscosity of the NWP solution. This increase in viscosity helped to strengthen the lamellae that surround air bubbles which in turn helped to slow down the rate of drainage of liquid from the lamellae and therefore retarded the extent of lamellae rupture. The overall effect of these events was to increase the stability of the NWP foam as indicated by its drainage half-life. This finding was consistent with the drainage half-lives data of NWP foams at 10 % and 15 sucrose concentration level and therefore can be used to support our observation that the foam stability of NWP foam was greater at higher sucrose concentration.

Drainage kinetics of native whey protein foams at different protein concentration levels.

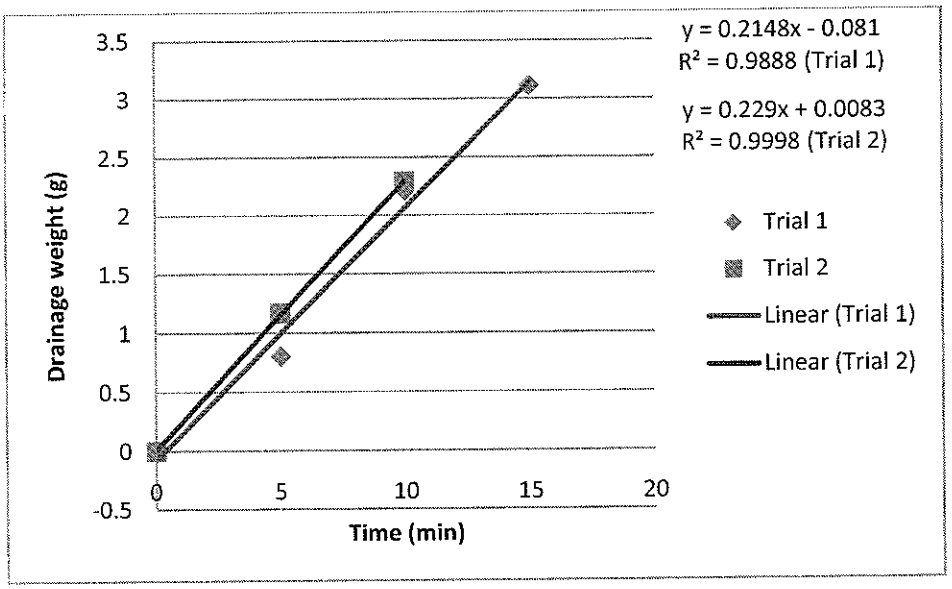

Fig. 22. Linear regression analysis of the drainage of liquid as a function of time data of Native whey protein foams made from 2 % NWP solution at pH 7.00 and ambient temperature. Data consist of two replicates from a single experiment. Squares and diamonds on the graph represent actual data.

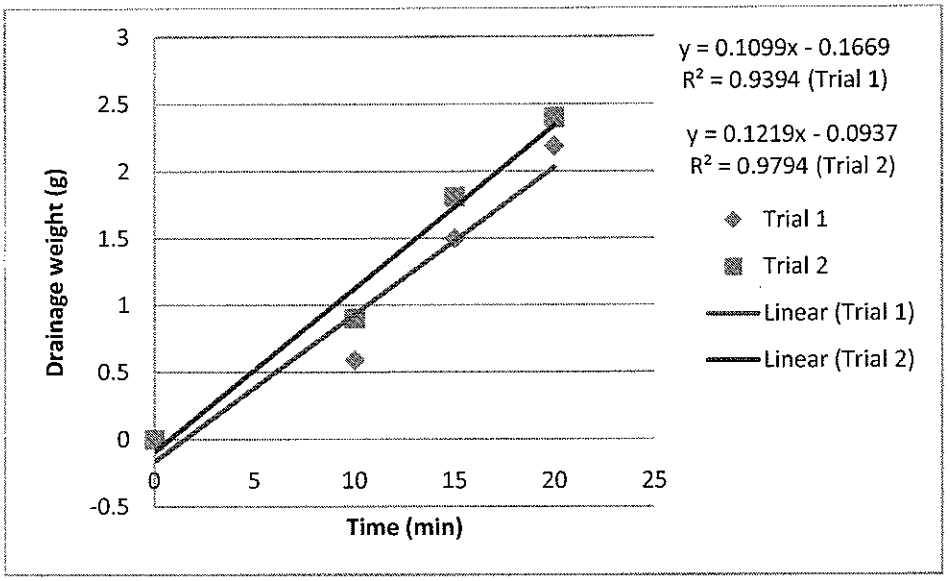

Fig.23. Linear regression analysis of the drainage of liquid as a function of time data of Native whey protein foams made from 5 % NWP solution at pH 7.00 and ambient temperature. Data consist of two replicates from a single experiment. Squares and diamonds on the graph represent actual data.

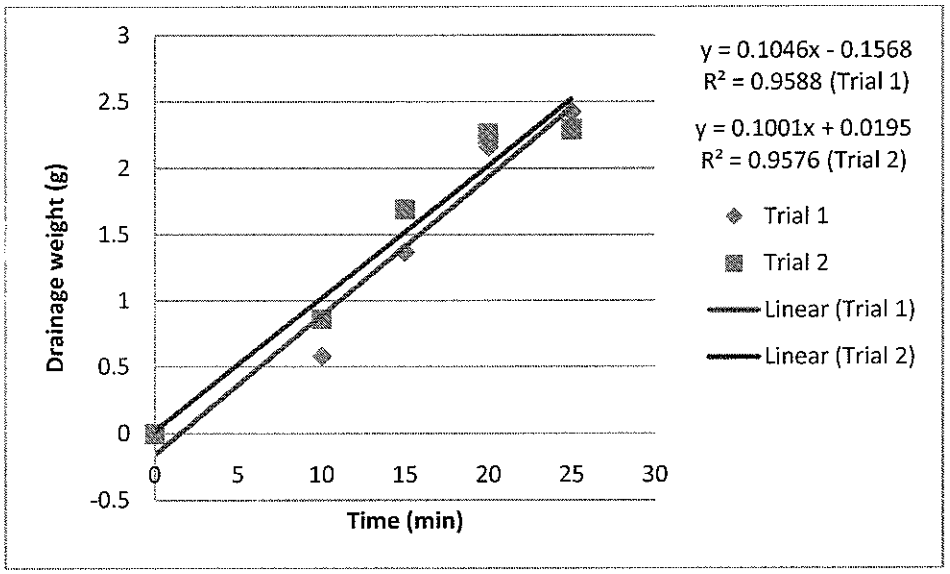

Fig.24. Linear regression analysis of the drainage of liquid as a function of time data of Native whey protein foams made from 8 % NWP solution at pH 7.00 and ambient temperature. Data consist of two replicates from a single experiment. Squares and diamonds on the graph represent actual data.

The average value of the slope of the linear regression line for the NWP at 2 % protein concentration level (Figure 21) was the highest among the three protein concentration levels used in the experiment. The slope of the linear NWP foam sample at 5 % protein concentration level (Figure 22) had an average value that was lower than that of the sample at 2 % protein concentration level but higher than that of the NWF foam sample at 8 % protein concentration level (Figure 23). These observations indicated that the rate of drainage of liquid from the

lamellae of the NWP foam sample at 2 % protein concentration level was the highest and therefore it experienced the highest extent of lamellae rupture within a given amount of time relative to the foam samples at 5 and 8 % protein concentration level and this was the reason why it has the least stable foam among the foam samples. The rate of drainage of liquid for the 5 % protein NWP foam sample was lower than that of the 2 % protein NWP foam sample but higher than that of the 8 % protein sample. This finding was consistent with the drainage half-lives data for the NWP foam samples at 2 %, 5 % and 8 % protein concentration level obtained in this study. Therefore, at protein concentration range where NWP was completely soluble, the higher the protein concentration level for a NWP foam sample, the lower the rate of drainage of liquid from the foam and the more stable the foam was as indicated by its longer half-life.

General Discussion

In this study, the effect of addition of co-solutes and whey protein concentration on the foaming properties of whey proteins, as defined by its foaming ability and foam stability were investigated. Although the addition of various types of carbohydrates (sucrose, maltitol and sorbitol) had different effects on the foaming properties of NWP, they were postulated to work through similar mechanism when exerting these effects. For example, the change in the viscosity of native WPC-80 solution upon addition of these carbohydrates altered the foaming ability and foam stability of NWP relative to control solutions. The same can be said for the proposed hydrogen bonding effect on the foaming ability of NWP when carbohydrates are incorporated into the whey protein solutions. Changes in foam stability of NWP due to addition of sugars may also be attributed to changes in the degree of intermolecular interactions between whey protein molecules which either causes them to remain as aggregates in the foam structure (if there was an increased degree of protein interactions) and a dispersion of the protein molecules with a decreased degree of protein interactions. This effect helped to determine the overall stability of the foam microstructure.

To expand on this study of the foaming properties of NWP, some rheological and physicochemical data could be collected. This could include analysis of yield stress data of NWP foams by using the Vane rheometry method (Pernell, Foegeding and Daubert, 2000) which may provide an insight into the influence of physical properties (such as yield stress) of foams on foaming properties of NWP.

Conclusion

All the factors involved in this study (addition of sucrose, maltitol, sorbitol and protein concentration) have a significant impact ($P<0.05$) on the foaming characteristics of whey protein dispersions as indicated by their foaming properties. The different effects of the various additives on foaming properties of whey proteins may be exploited to improve their functionality of whey proteins as foaming agents in processed foods. In this study, we found that addition of carbohydrates in native whey protein solution confers stability to the NWP foam. Sucrose, maltitol and sorbitol increases stability of NWP by increasing viscosity of the solution and reducing the size of air bubbles that make up the foam. Maltitol, sorbitol and

sucrose may be used in the recipes of meringues and formulation of marshmallows to improve the organoleptic properties of the whey protein containing product (better texture conferred by greater foam stability and better flavor imparted by carbohydrate addition). In addition, sorbitol and maltitol may be used as low-calorie alternative sweetener in these products

Increasing whey protein concentration in both native WPC-80 and WPI solution enhanced both the foaming ability and foam stability of whey proteins due to higher availability of proteins to be adsorbed to the interface. We found that native whey proteins exhibited greater foam overrun than traditional whey proteins at the same concentration level possibly due to differences in fat content of the whey protein powders used. The foam stability of native whey proteins, however, was found to be at the same level to that of the traditional whey protein. This may be due to high fat content of the NWP powder used in the study. Further investigations on the structure-function relationship with regard to the foaming properties of whey proteins and on the interfacial film compositions are required to verify findings of the present study.

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