PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

EFFECT OF CONCENTRATION AND PH ON FRACTURE PROPERTIES OF AERATED AND NON AERATED WHEY PROTEIN GELS

TANIA ALEXANDRA ALFARO MOHR

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Master of Science in Engineering

Advisor:

JOSE MIGUEL AGUILERA RADIC

Santiago de Chile, August, 2008

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Members of the Committee:

JOSE MIGUEL AGUILERA RADIC

ELSA MARCELA OLIVARES HINTZE

JAIME ORTIZ VIEDMA

LUIS CONTESSE BECKER

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To my parents, brothers, friends and all who support me in this work.

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RESUMEN

Esta tesis trata sobre la influencia de la concentración y el pH en las propiedades de los geles elaborados a partir de aislado de suero proteico (WPI), y la forma en que la inclusión de burbujas de aire afecta en el esfuerzo deformación y comportamiento de fractura, y el módulo de elasticidad (E). Nueve soluciones de WPI a concentraciones 16, 18 y 20% p/p y tres condiciones de pH (5,5, 5,8 y 6,1) fueron aireados por agitación mecánica y luego llevados a un baño termo regulado a 80 °C durante 60 minutos. Los geles no aireados fueron elaborados a condiciones similares. Las propiedades mecánicas de los geles fueron medidos por compresión en un eje, identificando esfuerzo de fractura, esfuerzo a máxima deformación (90%) y E. Todas las muestras de geles no aireados (NAG) se expandieron lateralmente y se fracturaron durante la compresión, mientras que los geles aireados (AG) se deformaron sin fractura incluso a 90% de deformación. Para todas las condiciones estudiadas, valores de esfuerzo y E resultaron más altos para NAG. El aumento de pH y la disminución de la concentración de WPI redujeron los valores de los parámetros mecánicos de todos los geles. Por otra parte, mientras NAG a pH 6,1 resultaron de color amarillo y translúcido, AG y NAG con pH 5,5 y 5,8 fueron blancos y opacos. La densidad de NAG y AG varió entre 1042 – 1073 and 334 – 475 kg/m^3, respectivamente. La inclusión de burbujas de aire en geles de WPI permite el modelado de muchas propiedades físicas para aplicaciones específicas.

Palabras Claves: geles, proteína de suero, módulo de elasticidad, fractura.

ABSTRACT

This paper deals with the influence of concentration and pH on properties of gels made from whey protein isolate (WPI), and how the inclusion of air bubbles affects their stress–strain and fracture behavior, and modulus of elasticity (*E*). Nine WPI solutions of 16, 18 and 20 % w/w concentration and three pH conditions (5.5, 5.8 and 6.1) were aerated by mechanical stirring to constant volume and set into gels by heating at 80 °C for 60 minutes. The controls were a set of non aerated gels produced under similar conditions. Mechanical properties of gels were measured by uniaxial compression, identifying fracture stress, stress at maximum deformation (90%) and E. All samples of non aerated gels (NAG) expanded laterally and fractured during compression while aerated gels (AG) deformed without fracture even at 90% of deformation. For all the studied conditions, stress and *E* values were higher for NAG. Increasing pH and decreasing WPI concentration reduced the value of mechanical parameters of all gels. Moreover, while NAG with pH 6.1 were yellow and translucent, AG and NAG with pH 5.5 and 5.8 were white and opaque. The density of NAG and AG varied between 1042 – 1073 and 334 – 475 kg/m^3, respectively. The inclusion of air bubbles into WPI gels permits modulation of many physical properties for specific applications.

Key words: Gels, whey protein, bubbles, elasticity modulus, fracture.

1. INTRODUCTION

There is strong evidence that obesity is increasing worldwide at an alarming rate catching the attention of nutritionists and food technologists (WHO, 2000). The interest to consume fewer calories while maintaining the type of foods and nutritional content has prompted a search for analog products with lower calorie density. The total cost of food consumed in the United States was $ 1.14 trillion dollars in 2007, an increase of 5.4% over 2006 (USDA, 2008) and expenditures away from home were about 48.9% of this total. Thus, there is an opportunity to develop new foods for the commercial market.

Gels are of central importance in the category of semisolid, high-moisture foods, imparting important specific properties because gels can contain more that 99% water and still retain the mechanical characteristics of a solid. From the rheological point of view, two central characteristics of dairy products can be distinguished: viscosity and elasticity. Both properties are important for the organoleptic quality of product (Renard et al., 2006).

1.1. Protein Gels

Protein gels have been the subject of detailed study in recent years mainly because these gels are being used not only in the food industry, but also in different areas, including medical applications, such as developing matrices for the formation of artificial organs (Klein, 2003).

Protein gels can be formed by several means, including by application of heat, pressure, acidification, or by changing the solvent. The different mechanisms of gel formation can be classified basically in physically induced (heat, pressure) and chemically induced (acid, ionic, enzymatic) (Aguilera & Rademacher, 2004). These processes allow the formation of microstructures that give rise to foods with different physical and sensory properties, such as cheese, yogurt or tofu. (Renard, van de Velde, Visschers, 2006). There is also another gelation method, cold gelation, where protein aggregates are gelled at (sub) ambient temperatures.

When whey protein molecules are heated, they aggregate, and under suitable conditions gels may be formed. These heat induced whey protein aggregates or their gels can be used

in the food industry to alter the texture and water-binding properties of foods. Under appropriate ionic strength, pH, gelling time, and thermal gelation conditions, whey protein gels are capable of immobilizing large quantities of water. The adjustment of these physical conditions determines the structural network, waterholding capacity, and rheological properties of whey protein gels (Hudson, Daubert & Foegeding, 2000). Tombs (1974) presented two models for heat-induced globular protein gels: random aggregation and aggregation into “strings of beds” structure, resulting in a turbid coarse network or a transparent fine-stranded protein network, respectively. The type of network formed is associated with changes in the balance between attractive and repulsive forces between the aggregating particles (Doi, 1993). Hermansson (2008) studied also two-phase gel systems than can result from the sequence of gel formation. One example is a mixture of whey protein and gelatin: the whey protein forms a gel network on heating and the gelatin gel just fills the pores of the whey protein network on cooling.

1.2. Aerated Protein Gels

Bubbles offer the possibility of novel food structures and textures, attractive appearance and improved volumes, while being cheaper, versatile and non-fattening - the ideal food ingredient. Reasons to create aerated foods vary: to reduce the density of the product, change the texture and rheology, modifying digestibility, a decrease in the intensity of flavours, among others (Campbell & Mougeot, 1999). It is also important to mention the economic factor, since incorporating air, reduces cost per unit volume. Aerated solutions or liquid foams are common examples of complex soft materials: whipped egg white, chocolate mousse, and the head of beer and the froth of shampoo. One attractive alternative may be to entrap abundant amounts of water and/or air in gel matrices, to design products that promote satiety with reduced calories density (Aguilera, 2005) and add mouth-feel and increase digestibility (Skurtys & Aguilera, 2008). Dairy products (mousses, frozen desserts) for example contain typically from 15-60% air, with variations in air content and bubble size distribution giving textural diversity. The positive benefits of aeration of food products have primarily to do with texture: fluid products such as whipped cream and

mousse give smoothness and novelty, while solid products, such as puffed breakfast cereals and snacks, become light and crisp (Campbell & Mougeot, 1999).

Although the final continuous matrix of an aerated food may be liquid (e.g., beer foam), viscoelastic (e.g., marshmallows) or solid (e.g., meringue), bubbles are always initially dispersed in the bulk of a liquid solution or a viscous dispersion. Because aerated liquids are thermodynamically unstable, bubbles must be stabilized at their air-liquid interface usually by surface active agents such as surfactants, proteins, or solid particles (Zuñiga & Aguilera, 2007). Furthermore, drainage and bubble coalescence are retarded by increasing the viscosity of the liquid in lamellae between the bubbles. Hence, when bubbles become physically entrapped in a gel network the food will be stable (Boom, 2008). Niranjan and Silva (2008) mention several conventional methods to incorporate bubbles to foods: mechanical agitation under positive pressure, dispersion under vacuum, steam induced-formation and, chemical reactions.

1.3. Textural Properties

The versatility to build functional gels matrices is greatly extended when mixed and "filled" gels matrices are considered. Often a blend of gelling polymers provides improved properties than those of a single component gel and sometimes synergistic effects may be found (Zuñiga & Aguilera, 2007). Nussinovitch et al. (1992) were able to alter the textural properties of hydrocolloid gels by means of the incorporation of CO_2 bubbles produced through a chemical reaction.

Fracture properties of gels are typically more highly correlated with sensory texture than small-strain rheological properties (Montejano, Hamann, & Lanier, 1985). According to van Vliet and Walstra (1995) fracture in materials generally occurs when "bonds between the structural elements of a material in a certain macroscopic plane break, resulting in a breakdown of the structure over length scales much larger than the structural elements, and ultimately a falling apart of the material".

For viscoelastic materials, such as whey protein gels, a significant amount of energy can be stored or dissipated when deformed. Whey protein gels may be stabilized by chemical (disulfide bonds) and physical cross-links (electrostatic interactions, hydrogen bonds and

hydrophobic interactions). During the deformation of physically cross-linked gels the process of bending, breaking and reforming of low energy bonds results in the dissipation of energy (Foegeding, Gonzalez, Hamann, & Case, 1994; Van Vliet & Walstra, 1995; Walstra, 1996). In addition, such processes require a finite time to occur, resulting in a rate- or time-dependent behavior (van Vliet & Walstra, 1995).

The typical shape of compressive force-deformation curves for aerated foods or foams, when converted into an engineering stress- strain relationship, has three zones (Corradini & Peleg, 2006). These zones are: I-small deformation of the intact structure; II- buckling and fracture of cell walls, and; III-compaction of what is increasingly collapsed cell wall material. In the first zone it is possible to define a 'modulus of elasticity' (*E*). Corradini and Peleg (2006) defined *E* as the slope of the stress-strain curve for very low deformations (elastic range).

The main objetive of this research was to study the effect of air inclusion into WPI solutions, at different concentrations and pHs, the formation of gels by heating, and the mechanical properties of aerated gels.

2. MATERIALS AND METHODS

2.1 Whey protein isolate solutions

A protein solution was prepared using whey protein isolate (Instantized BiPro, Davisco, Foods International, Le Sueur, USA) (Appendix A), containing 95% protein, which is characterized by a low content of lactose, salt and fat, dispersed in distilled water. WPI solutions at concentration of 16, 18 and 20% by weight were stirred at room temperature until complete dissolution for 8 minutes. Each type of WPI solution was adjusted to three different pH values (5.5, 5.8 and 6.1) with careful incorporation of 1N of HCL under agitation while measuring with a digital pH-meter (Hanna® model P-200, Hanna Instruments, Woonsocket, RI, USA) previously calibrated with buffer solutions.

2.2 Formation of aerated protein gels

WPI solutions (20mL) were placed in a 100mL glass beaker and prehetead at 60ºC under agitation. To aerate the solution a magnetic stirrer (Nuova II Stir Plate, Thermolyne, Dubuque, Iowa, USA), was used with a micro magnet (35x6mm). The stirring time was 15 minutes. The speed range of the micro magnet was between 972 to 1028 rpm for the highest and lowest viscosity of WPI solution, respectively. The velocity of the micro magnet was obtained experimentally by a sequence of photos took with a high-speed video camera Pulnix TM-6740GE (Pulnix, Inc., San Jose, CA, USA) with a pixel resolution of 640×480. The beaker containing the aerated solution was immediately placed into a thermo-regulated water bath (SW-22, Julabo, Seelbach/Black Forest, Germany) at 80 °C and kept for 60 minutes. To corroborate that the geometrical centre of the solutions reached the desired temperature, heat penetration measurements were made (Appendix B). Gels were removed from the bath and left to cool at room temperature for 180 minutes.

2.3 Formation of no aerated gels

For each sample, 20mL of solution where placed in a similar glass beaker used for aerated gels. The dissolved air in the solution was extracted in a vacuum chamber (26.33 kPa absolute) for 40 minutes to ensure that an ungasified solution remained to form the gel

without any microscopic bubbles that can affect the results. Then, WPI solutions were placed into the same thermoregulated bath at 80°C for 60 minutes. Gels were removed from the bath and left at room temperature for 180 minutes.

2.4 Rheological measurement of WPI solutions

The rheological behavior of WPI solutions was determined in a Reolab MC20 rheometer (Physica Inc., Springs, TX, USA) at 30°C. Measurements of four samples were performed.

2.5 Optical microscopy images of aerated gels

The microstructure of aerated WPI gels was observed by selected cuts (3 mm thickness) of the gels with a light microscope image (Olympus CKK440, Optical Co. LTD., Tokyo, Japan), connected to a digital camera (Cool Snap Pro Color, Photometrics Roper Division, Inc., Tucson, AZ, USA) to acquire the images and using magnifications of 4x. In this way, each image (1392x1040 pixels) was saved as TIFF image file of approximately 1.38 MB, without compression.

2.6 Magnetic resonance imaging (MRI) of WPI gels

MRI was done in a 1.5 Tesla Intera Philips Medical System (Intera, Gyroscan PMS/DICOM 2.0). A head coil was chosen due to the size of the samples. 10 cm x 10 cm proton density scanning sequence images were acquired with a resolution of 256x256 pixels. Slice thickness was set to 2 mm resulting in a voxel size of 0.390625 x 0.390625 x 2 mm. The echo time was set to 20 ms and the repetition time to 1500 ms. Number of sampled averages was set to 8 to increase the signal to noise ratio. The total time for the acquisition was 17 minutes and 34 seconds. Images were observed by means of Philips DICOM Viewer R1.1V1L1-SP01 software (Philips Medical Systems Nederland B.V., Best, The Nederlands).

2.7 Uniaxial compression measurement

The applied uniaxial compression test was the same for aerated and non-aerated gels. Samples of aerated and non aerated gel cylinders made with a mold (35 mm diameter, 20 mm height) were compressed with a 35 mm diameter plate using a TAXT2i Texture Analyzer (Stable Micro Systems, Godalming, UK). Six replications for each treatment were performed. The measurements were carried out at 30 °C at a constant deformation speed of 0.2 mm/s and up to a compression strain of 90%. From the compression measurements, small and large deformation properties were determined.

The specimen's absolute deformation was expressed as the Hencky´s or true strain (ε_H), which is preferred for calculating strain resulting for large deformations (Hamman *et* al., 2006; Steffe, 1996; Corradini & Peleg, 2000):

$$e_H = -\ln\left(\frac{H}{H_0}\right) \qquad (2.1)$$

where H_0 is the initial specimen height and H is the final height after deformation. The overall stress acting on the sample during compression was expressed as the so-called true normal stress (σ_t):

$$s_t = \frac{F}{A_0} \qquad (2.2)$$

where F is the force measured during compression and A_0 is the initial cross-sectional area of the sample. The dense compact structure of a non aerated gel may offer a larger resistance to deformation than that of an aerated gel structure, thus, changes in cross-sectional area are much smaller. At low strains, according to several studies (Ashby, 1983; Gibson & Ashby, 1999), the specimen's deformability can be characterized by a 'modulus of elasticity', E:

$$E = \frac{s_t}{e_H} \qquad (2.3)$$

E was calculated from the linear part of the first region of the force-displacement curve for a typical cellular solid (Corradini & Peleg, 2000) where a small deformation of the intact structure is induced (e.g., ε<0.1) (Fig. 2-1).

Measurements were performed at 30 ºC and repeated 6 times. For the calculations the volume of the samples was assumed to be constant and any release of water from the samples was ignored.

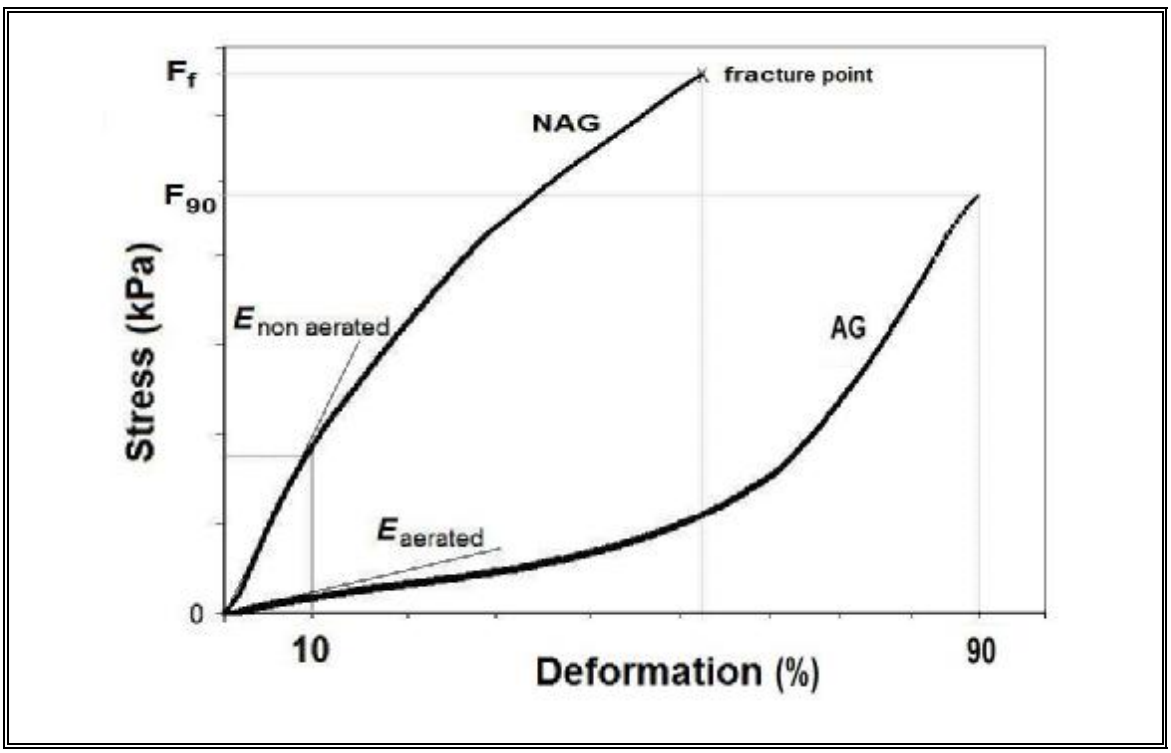

Figure 2-1: Stress deformation relationship of aerated and non aerated gels. The line defining the models of elasticity is shown in each case.

2.8 Density

Density of aerated and non aerated gels was determined in duplicate by weighting cylindrical samples of known dimensions (20mm height x 35mm diam.). Density was calculated using Eq. 2.4:

$$r = \frac{m}{V} \qquad (2.4)$$

where m is the mass (g) and V is the volume of the gel sample (19.24 cm^3).

Samples were weighted in a precision balance (HF-3000, A&D Company, Nerima-ku, Tokyo, Japan) to the second decimal point at room temperature (25 °C) and values averaged.

3. RESULTS AND DISCUSSION

All AG were white and opaque. Non aerated gels (NAG) at pH 6.1 were dark yellow and translucent compared with those formed at pH 5.8 and 5.5 that were white and opaque (Fig. 3-1). These results are in accordance with studies by Ju and Kilara (1998) who reported that a gel made from 18% whey protein solution (WPS) at pH 7.0 was brown and transparent. They also stated that the turbidity of the WPS gel did not change until the pH approached 6.0. Boye et al. (2000) also reported that gels made of β-lactoglobulin, α-lactalbumin, bovine serum albumin (BSA) and their mixtures were opaque at pH 3.0 and translucent at pH 8.6.

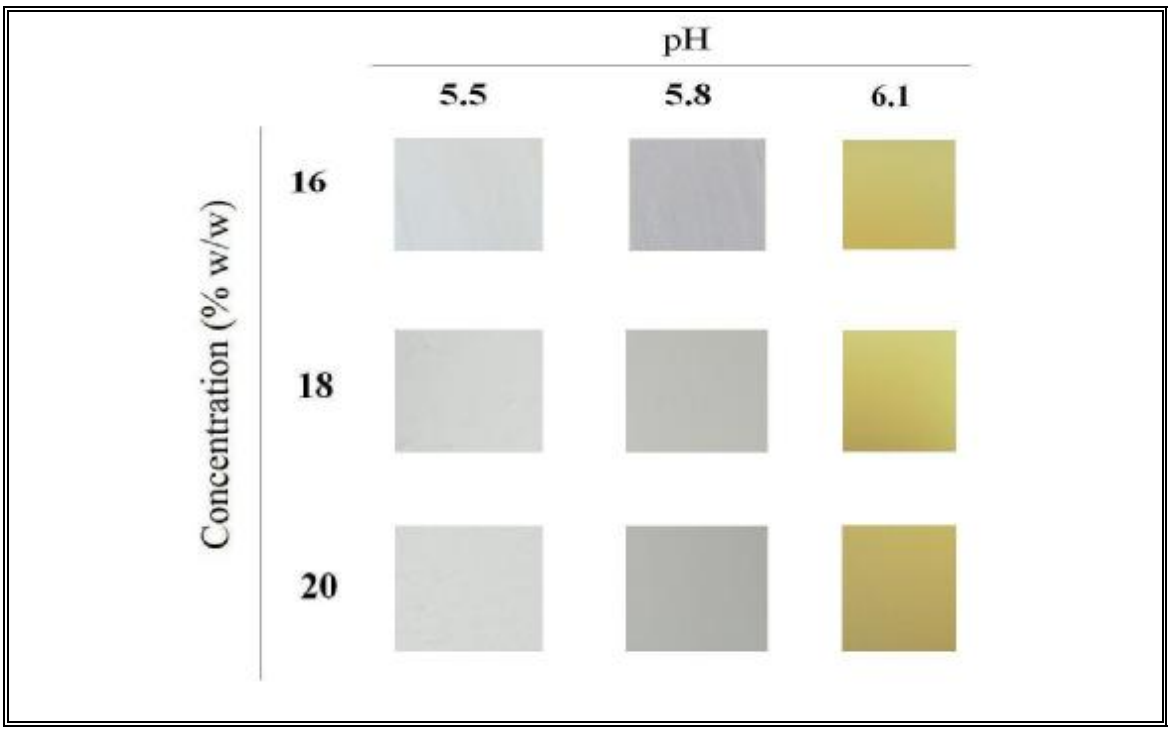

Figure 3-1: Color images of the nine non aerated WPI gels samples. Whiteness does not reproduce well in printed version.

3.1 Rheological measurements of WPI solutions

The change in viscosity of WPI solutions depended on the concentration and pH. The lowest viscosity, 3.31 mPa×s, was found at a concentration of 16 % (w/w) and pH 6.1. In general, viscosity increased as pH decreased for constant concentration while for constant pH viscosity increased as concentration augmented (Table 1). An earlier study showed that changes in viscosity can affect directly the gas holding properties of WP solutions; the higher the viscosity of the continuous phase, the more gas can be held in the foamed product (Bals & Kulosik, 2003). Viscosity is also important to control the drainage of liquids in foams.

Table 1: Viscosity of WPI solutions at 30°C. Mean value and standard deviation.

Viscosity (mPa×s)		**pH**		
		5.5	**5.8**	**6.1**
Concentration (% w/w)	**16**	3.69 ± 0.02	3.56 ± 0.02	3.31 ± 0.12
	18	4.22 ± 0.01	4.09 ± 0.01	3.95 ± 0.03
	20	5.68 ± 0.05	5.18 ± 0.06	5.08 ± 0.01

3.2 Fracture and deformation properties of gels

All NAG fractured before they reached 90% compression while aerated gels (AG) did not fracture probably because the presence of air cells allowed to accommodate the deformations of the matrix, or they acted as restriction points for crack propagation. In order to analyze the behaviour of NAG and AG, values obtained from compression to 90% (F_{90}) for AG are listed alongside with fracture stress for NAG (F_f) in Table 2. NAG fractured at strains much lower than those equivalents to 90% deformation and with higher stress values than those corresponding to the maximum stress (F_{90}) of AG with similar concentration and pH.

Table 2. Stress at 90% deformation (F_{90}), stress at fracture (F_f) and modulus of elasticity (E) of aerated and non aerated whey protein gels. Mean values and Standard deviation.

	Concentration (% w/w)	**F_{90} (kPa)**	**F_f (kPa)**	***E* (kPa)**	
		Aerated	*Non Aerated*	*Aerated*	*Non Aerated*
pH 5.5	**16**	165.9 ± 5.3	214.2 ± 9.0	17.1 ± 1.7	196.0 ± 8.5
	18	207.8 ± 8.6	361.7 ± 0.9	24.9 ± 1.4	310.7 ± 7.3
	20	293.6 ± 11.1	488.3 ± 2.3	27.2 ± 1.4	445.5 ± 8.7
pH 5.8	**16**	119.8 ± 9.8	134.8 ± 8.6	12.2 ± 2.3	145.5 ± 6.7
	18	174.1 ± 4.9	264.7 ± 9.8	18.5 ± 1.9	218.8 ± 9.8
	20	262.1 ± 3.1	395.8 ± 7.9	22.1 ± 2.7	354.2 ± 7.3
pH 6.1	**16**	99.9 ± 9.2	113.7 ± 4.6	6.7 ± 1.1	103.2 ± 2.9
	18	127.3 ± 3.9	227.7 ± 2.4	10.0 ± 3.4	155.0 ± 6.8
	20	195.0 ± 6.6	384.4 ± 1.8	14.9 ± 1.9	258.8 ± 9.3

A two-way Anova statistical test was applied to values in Table I-2, with the two independent variables or factors being concentration and pH. The results showed significant differences between the set of samples ($p<0.05$).

In response to the stress applied aerated samples accommodated a large longitudinal deformation before the test stopped. For NAG, energy was stored during deformation and released as fracture when a critical stress was achieved. Images from the compression of gel samples, intact and at two levels of compressive deformation, are presented in Fig. 3-2. As AG were deformed, the cross sectional area augmented slightly; the material collapsed on itself and hence the sample did not expand laterally to a great extent. Supposedly, the collapsed solid matrix primarily filled the spaces occupied by air cells. This is consistent with the bread crumb experiment conducted by Corradini and Peleg (2000). On the contrary, at progressively higher compressive deformations the NAG exhibited lateral expansion until they fractured. Since the volume probably remained quite constant (except

for the liquid released) lateral expansion accommodated the change in height. As could be expected, NAG offered a stronger resistance to deformation than AG at similar strains.

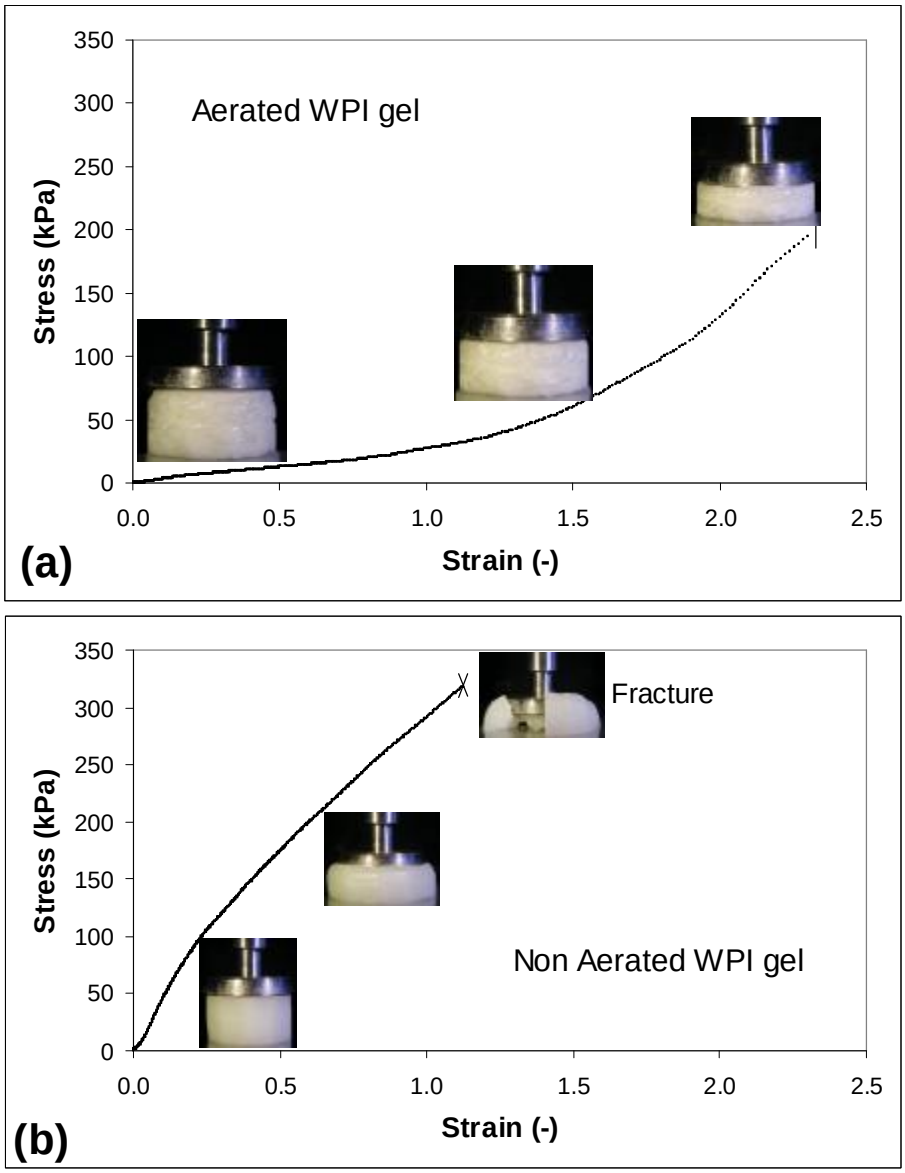

Figure 3-2. (a) Representative compression curve to fracture point aerated gel of 20% WPI (w/w), pH = 6.1. (b) Representative compression curve up to 90% the initial height for non aerated gel of 20% (w/w) WPI, pH = 6.1.

As protein concentration increased F_{90} of AG, F_f of NAG and E for both type of gels increased (Table 2). These results are in agreement with those of fracture stress of fine stranded whey protein isolate gels (Lowe, Foegeding & Daubert, 2002).

The stress-strain curves of gels containing less protein were always below that those having higher protein concentration (Fig. 3-3). Foegeding (2006) made a similar observation, this is, that increasing network concentration results in increased fracture stress. The logical interpretation is that as a fracture plane proceeds through the material, there is higher density of network strands that must be ruptured. Since pH affects molecular conformation and intermolecular interactions, it is not surprising that it influences gel network structure and mechanical properties.

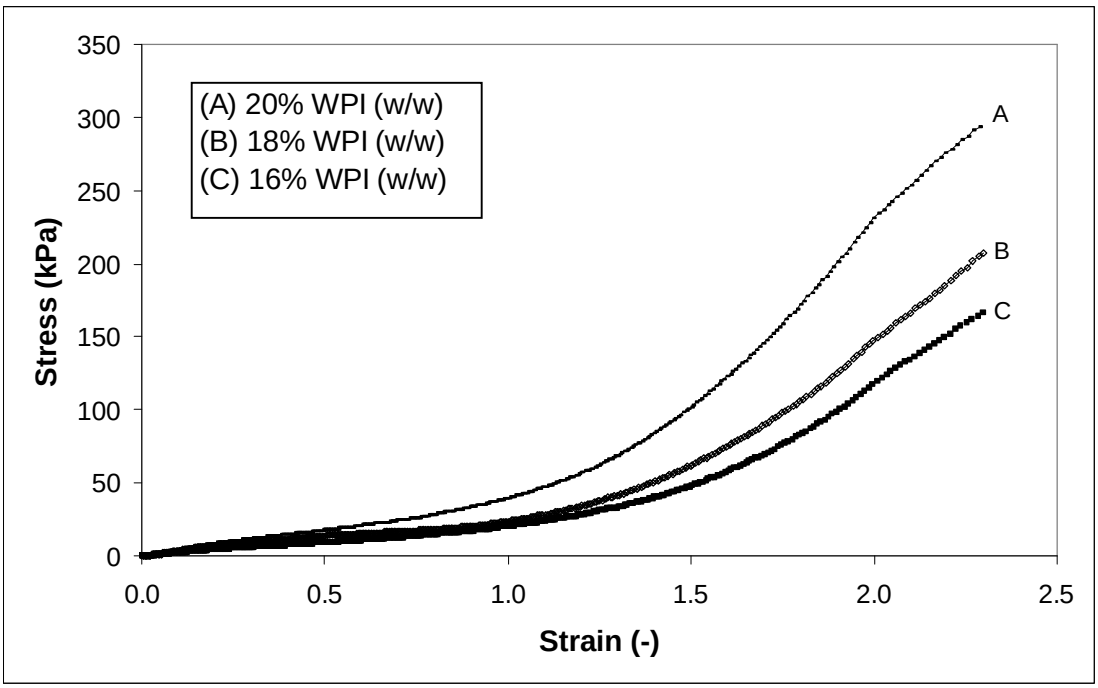

Figure 3-3. Stress–strain curves of aerated WPI gels (pH 6.1) for three different concentrations studied.

At constant concentration, gels of higher pH were softer that those made at lower pH (Fig. 3-4). These results compare well with those from Errington and Foegeding (1998) who reported that whey protein gels formed at pH 6.5 and 7.0 were strong (fracture stress of 59-

75 kPa), while those formed at pH 2.5 and 3.0 were weak (fracture stress of 18-19 kPa). Renkema (2004) obtained similar results with soy protein isolate (SPI) gels, in which the stress at pH 7.6 was only 25% that of gels of pH 5.2. The reason why the fracture stress at pH 5.5 is highest could be due to the fact that this pH is the closest to the isoelectric point, hence, protein–protein interactions were strongest (Renkema, 2004; Aguilera, 1995; Boye et al., 2000). It was observed that NAG at pH 5.5 had poorer water holding properties than those at pH 6.1. Serum was released as soon as the gels were pressed.

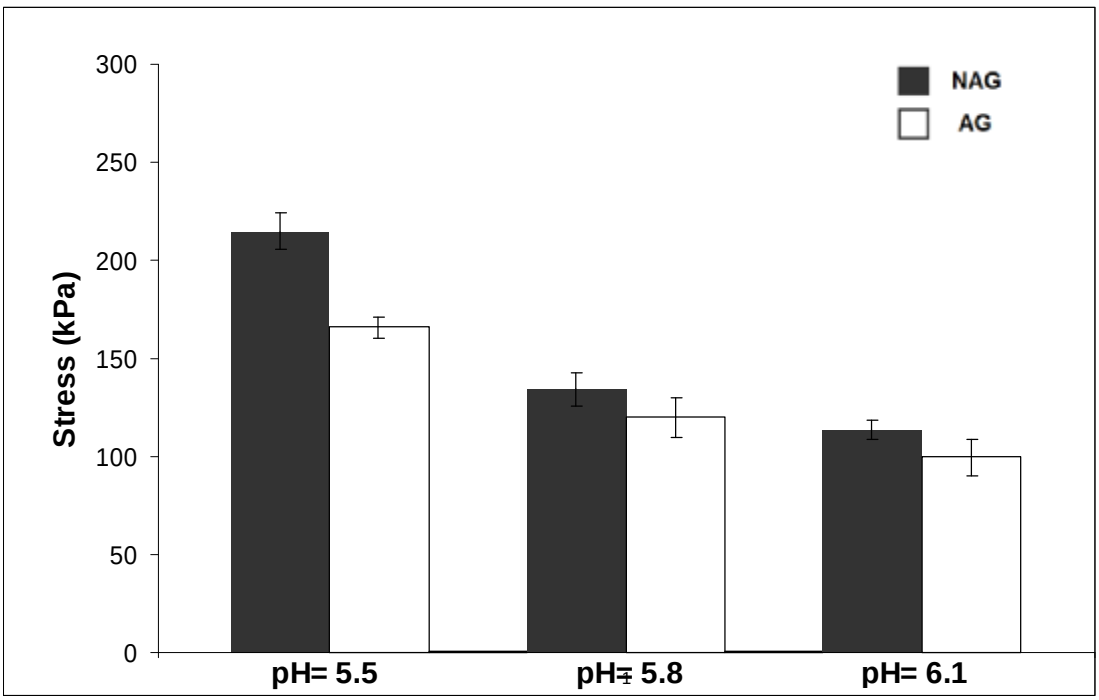

Figure 3-4. Fracture stress values for NAG and compression stress achieved at 90% deformation (without fracture) for AG, both at 16% WPI concentration (w/w) and pH: 5.5; 5.8; 6.1.

The average stress difference between AG (F_{90}) and NAG (F_f) expressed as percentage of the latter varied between 51% and 89%. This means that incorporation of air bubbles not only suppressed fracture but made gels more deformable and softer. The weaker behavior was expected at constant volume, AG had less protein in the matrix than NAG.

To better analyze the effect of the inclusion of air bubbles in WPI gels, the difference in F_{90} and F_f of the extreme values of concentration and pH was considered (e.g, 16 and 20%

WPI and pH 5.5 and 6.1). Average difference values for the pH effect were 81.9 kPa in gels with air and 112.8 kPa in those without air. The same analysis for the concentration difference gave averages of 121.7 kPa for aerated and 268.6 kPa for NAG. This means that under conditions of this study a change in concentration had a more pronounced effect on the hardness of gels than a change in pH.

In their study Bordenave-Juchereau et al. (2004) found that only protein concentration and pH had a statistically significant effect on gel hardness. An increase in protein concentration resulted in an increase in the gel hardness, in agreement with results obtained in this study.

3.3 Modulus of elasticity (E)

The modulus of elasticity is the slope of the initial linear-elastic part of the stress-strain diagram or the first discernible region that represents a small deformation of the intact structure (Corradini & Peleg, 2000). Thus, E was calculated from the first linear part of the stress-strain profile where the minimum R^2 was 0.9521. All curves in Fig. 3-5 describe the elastic response (strain < 0.045) of samples at 16% WPI (w/w) showing that NAG for all 3 pH (curves A, B, C) have larger values than those for AG. At pH 5.5 the highest values was achieved for both AG and NAG. Values of the elasticity modulus (E) were for curves A, B, C; 196.0 ± 8.5, 145.5 ± 6.7, 103.2 ± 2.9, respectively, and for curves D, E, F; 17.13 ± 1.7, 12.17 ± 2.3, 6.7 ± 1.1, respectively. The incorporation of air affects significantly the E value of the material and that could be because the air presents less resistance to the compression. Visually is possible to corroborate that when the compression was done, no radial expansion occurred. In average, the modulus of elasticity of the AG is 7.2% of the respective NAG, ranging from 6.1 to 8.7%.

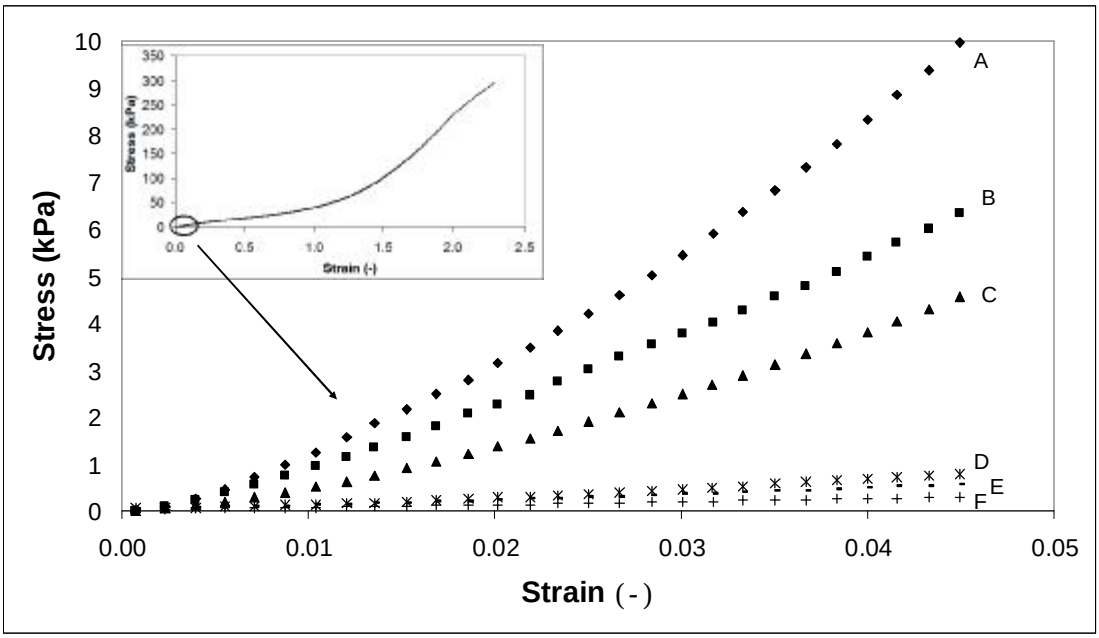

Figure 3-5. Stress–strain curves for non aerated WPI gels at three different pH [5.5 (A), 5.8 (B) and 6.1 (C)] and aerated WPI gels at the same pHs [(5.5 (D), 5.8 (E) and 6.1 (F)]. The mean R^2 for all curves is 0.9863 (values between 0.9798-0.9937).

E depended on the pH and the concentration of protein solution much in the same way as F_{90} and F_f i.e., it increased with protein concentration at constant pH and decreased when pH increased for a constant concentration.

The increment in E with concentration and as the IP was approached may be explained by a larger number and stronger crosslinks holding the protein network.

3.4 Density

There was a small effect of the protein concentration in AG and even smaller effect in NAG but not of pH on the density of samples. As expected, a higher protein concentration caused an increase in density, both for AG and NAG. Increased concentration resulted also in higher viscosity of the liquid matrix separating bubbles, hence, the drainage of solution during the gelling stage was retarded and for the same volume more protein was occluded in the gels (Table 3). Halling (1981) established that overrun is influenced by drainage rates, i.e., decreased liquid drainage leads to lower overrun and higher foam density. Davis

and Foegeding (2004) found that the rate of liquid drainage through foams decreased when increasing WPI content.

Table 3: Density (kg/m^3) of aerated and non aerated WPI gels.

Concentration (% w/w)	Aerated gels			Non aerated gel		
	pH					
	5.5	5.8	6.1	5.5	5.8	6.1
16	405 ± 17	345 ± 31	334 ± 6	1056 ± 4	1045 ± 8	1042 ± 9
18	445 ± 18	412 ± 12	342 ± 3	1060 ± 2	1058 ± 4	1056 ± 2
20	475 ± 10	426 ± 7	358 ± 12	1073 ± 4	1070 ± 2	1068 ± 5

()[a]: Means within rows followed by the same letter are not significantly different ($p < 0.05$)

The average differences in density between concentrations at the same pH for both types of gels were rather small but it was larger for AG than NAG (6.6 and 1.1%, respectively). No statistical evidence exists to conclude that changes in pH (at constant concentration) led to density variation although in the case of NAG a trend clearly exist that at higher pH the density of gels was lower. When all samples were compared for equal characteristics (pH and concentration) but differing only in whether they contained or not air it was found that on average the density of AG corresponded to 37% of NAG. This means that aeration of gels was successfully accomplished by the method used.

Most real life cellular solids are random and disordered and this irregularity in morphology significantly affects the mechanical properties of the cellular solids (Zhu, Hobdell & Windle, 2001). The modulus of elasticity of cellular solids has been related to the modulus and density of the cell wall material (Corradini & Peleg, 2000) (Fig. 3-6). Data in Table 4 was empirically adjusted to a model relating the actual density of the aerated gels and their modulus according to (Eq. 3.1):

$$E = k(r)^n \qquad (3.1)$$

where E is the modulus of elasticity, ρ is the density of the gel, and n and k are constants.

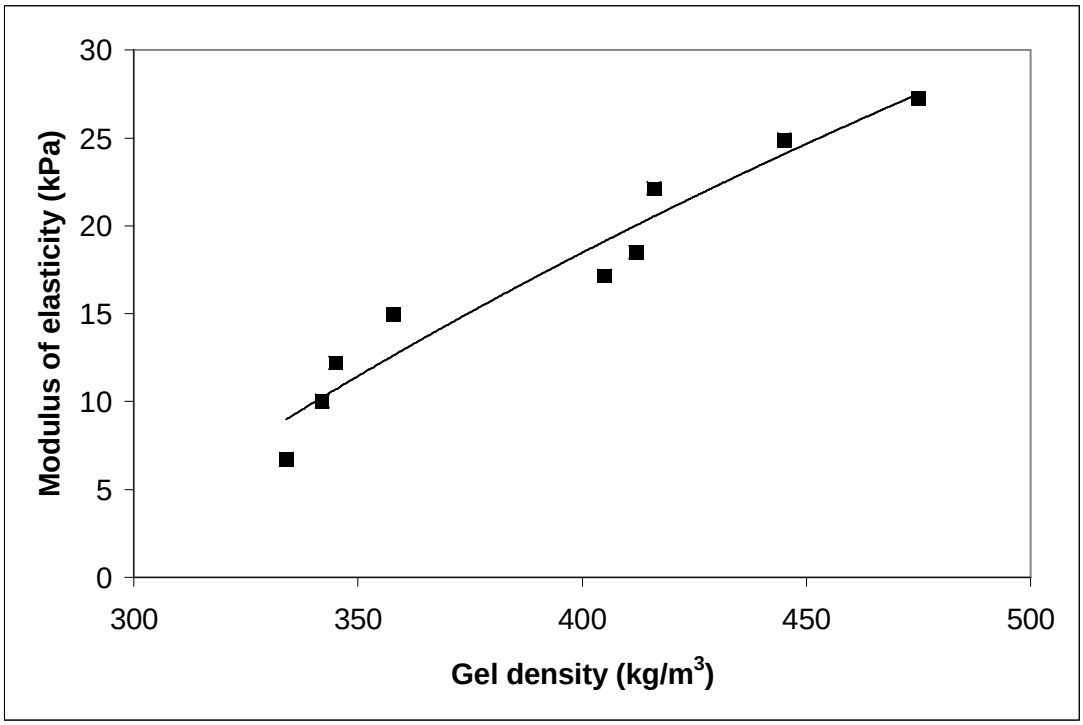

Figure 3-6. The relationship between density and modulus of elasticity from the nine aerated gels.

Values for the parameters of Eq. 3.1 were obtained through a non-linear regression as shown in Eq. 3.2 ($R^2 = 0.94$):

$$E = 328.8(r)^{3.349} \qquad (3.2)$$

The value of *n* generally lies in the range $1 < n < 4$, giving a wide spectrum of properties at a given density (Gibson & Ashby, 1999), and n = 3.349 falls in the proposed range of values.

3.5 Light microscopy images of WPI

The microstructure of WPI aerated gels was observed using light microscopy (LM). LM images (Fig. 3-7) showed in some cases a difference in matrix structure as a function of pH and almost no differences between concentrations. Gray areas in the pictures represent protein, while white areas represent the pores within the gels structures containing air. At

pH 5.5, a coarse matrix with a clear contrast between strands and pores were found at 16%. A finer structure was observed at pH 6.1.

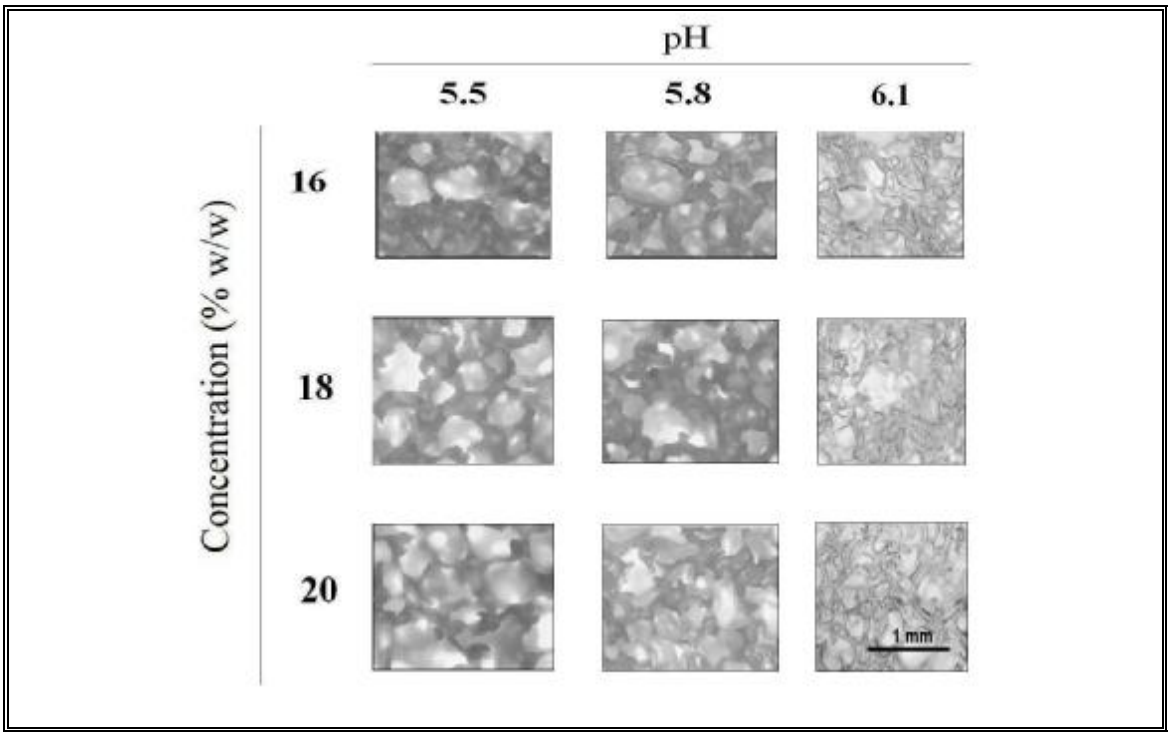

Figure 3-7. Images from the nine aerated WPI gels samples.

There were differences in the aerated gel microstructure at pH 5.5 and 5.8 against 6.1. AG at 5.5 and 5.8 were both characterized by more rounded porous and more homogenous structure than AG at pH 6.1. Van Camp and Huyghebaert (1995) reported similar findings for whey proteins and indicated that there are marked intermolecular network bondings between adjacent polypeptide side chains and that the hollow spacing (diameter 1.5–4.5 μm) form separate entities capable of maintaining the liquid enclosed during deformation.
In general discerning the type of gel matrix was difficult due to superposition of bubbles at different depth and a bad contrast between the matrix and the bubbles. In previous studies of WPI gels at 8% (w/w) by CLSM it was shown that they were grainy and that gels formed at a pH near isoelectric point resulted in particulate gels with large pores in the polymeric network (Brink et al., 2006).

3.6 Magnetic resonance imaging (MRI)

MRI is based on the magnetic field generated by the spin of the hydrogen atom. An advantage of MRI in this study is the ability to image at any plane inside the gels non-invasively and non-destructively. In Fig. 3-8 (right) is an image of a NAG, where whiter means that the density of hydrogen atoms is higher than in the image of the left (AG). Despite it was not possible to calculate the density with this resonator, the image from a non aerated gel formed at pH 5.8 and concentration 18% gel was used as reference to compare with pixel intensity of the nine aerated gels. The ratio of the average pixel intensity level of the non aerated control gel over the average intensity levels of aerated gels are shown in Table 4. Values where obtained from a voxel average taken from a 17x17mm^2 area with 3mm thickness each sample showed in Fig. 3-9. The values show that at pH 6.1 the average fractional intensity level was significantly higher than at pH 5.8 or 5.5. No differences were found for the effect of concentration.

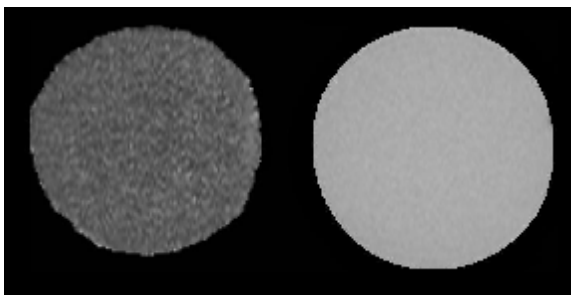

Figure 3-8. MRI from an aerated gel (left) and non aerated gel (right) at 18% WPI (w/w) and pH 5.8.

Table 4: Fraction of the mean intensity level.

Fraction of the mean intensity level (-)		**pH**		
		5.5	**5.8**	**6.1**
Concentration (% w/w)	**16**	0.375 [a]	0.243 [a]	0.419 [b]
	18	0.245 [a]	0.223 [a]	0.346 [b]
	20	0.209 [a]	0.159 [a]	0.252 [b]

()[a]: Means within rows followed by the same letter are not significantly different ($p < 0.05$)

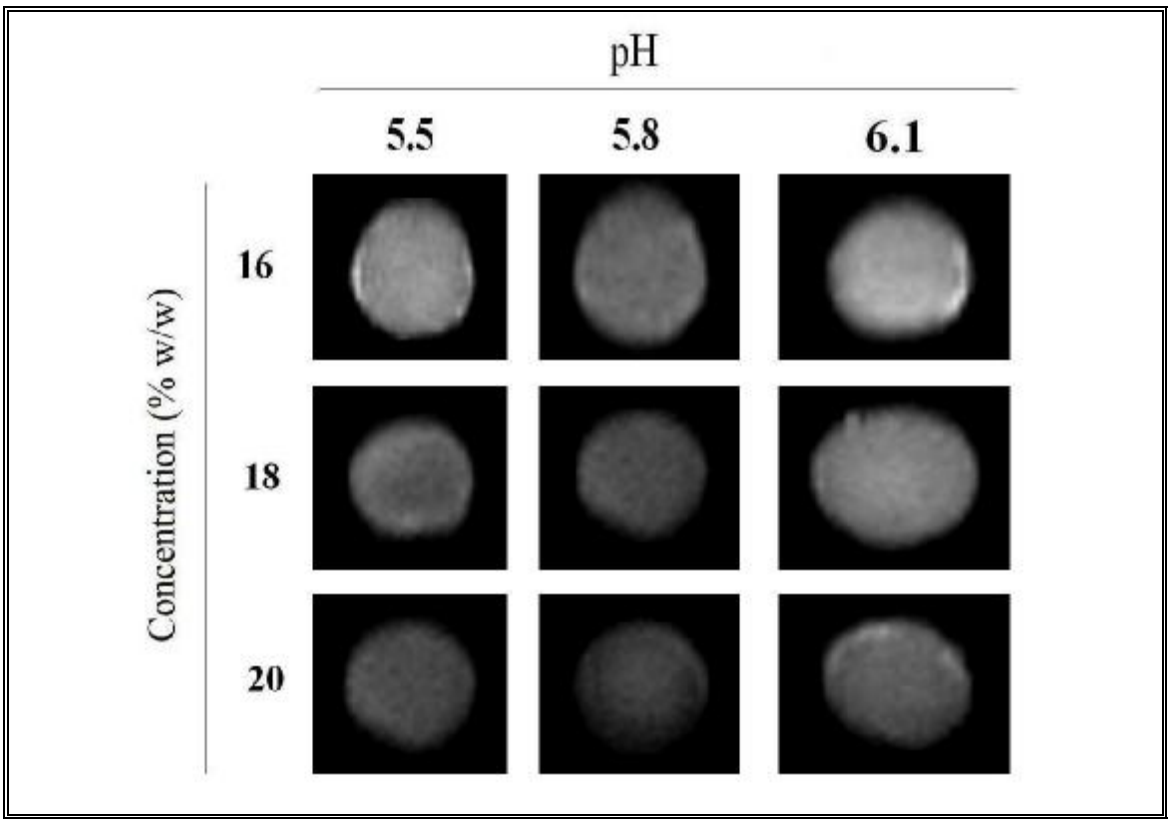

Figure 3-9. MRI from the nine aerated WPI gels

4. CONCLUSSION

Incorporation of air bubbles into whey protein gels appreciably altered their optical, physical (e.g., density) and mechanical properties. The shape of the stress-strain curves of each type of gel revealed differences in modulus of elasticity, fracture stress (F_f) and stress at maximum compression of 90% (F_{90}). NAG fractured at strains much lower than those equivalents to F_{90} of AG and with higher stress values than the F_{90} of AG of similar concentration and pH.

Both, concentration and pH affected considerably the modulus of elasticity and stress-strain properties of AG and NAG. Higher concentration resulted in higher E, F_{90}, and F_f while higher pH gave the opposite behavior.

Although no conclusive structural data was obtained from the light microscopy images some differences in gel structures were observed that may have affected the mechanical properties. Non-invasive MRI is a promising technique to probe into the internal structure of gels but the low resolution of the equipment used did not permit detection of the porous structure of aerated gels. These gels had only a fraction of the average pixel intensity of images of non-aerated gels due to their lower water content and the blurring effect or air bubbles.

Interestingly, aerated gels had a density lower than water while non aerated gels were denser than water. This property may be exploited in some gastronomic applications.

The addition of air to gels opens the opportunity of transforming brittle and breakable NAG into pliable and softer gels AG. Future work may include varying the level of aeration to better mapping the behavior of AG. As changes in concentration and pH affected the mechanical behavior of WPI gels they could be used to tailor make gel structures for specific uses (e.g., softer gels at constant protein concentration).

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A P P E N D I C E S

APPENDIX A: INSTANTIZED BIPRO PROFILE

DAVISCO
FOODS INTERNATIONAL, INC.*

Instantized *Bi*PRO®

Nutrient Information
Expressed per 100 grams product, as is.

Calories	379	
Calories from fat	15	
Total Fat	1.7	g
Saturated Fat	1.0	g
Trans Fat	0.0	g
Cholesterol	20.0	mg
Sodium	600.0	mg
Potassium	65.0	mg
Total Carbohydrates	0.0	g
Dietary Fiber	0.0	g
Sugars	0.0	g
Protein (N x 6.38)	91.0	g
Vitamin A	<100	IU
Vitamin C	<2	mg
Vitamin D	<8	IU
Iron	2.0	mg
Calcium	125.0	mg
Phosphorus	95.0	mg
Magnesium	25.0	mg
Moisture	5.6	g
Ash	1.7	g

Amino Acid Profile
Expressed per grams on a protein basis

Alanine	4.7	g
Arginine	2.3	g
Aspartic Acid	11.1	g
Cysteine	3.0	g
Glutamic Acid	16.5	g
Glycine	1.6	g
Histidine*	2.2	g
Isoleucine*‡	5.1	g
Leucine*‡	11.9	g
Lysine*	11.1	g
Methionine*	2.3	g
Phenyladanine*	3.5	g
Proline	4.4	g
Serine	2.7	g
Threonine*	4.4	g
Tryptophan*	2.7	g
Tyrosine	3.5	g
Valine*‡	5.5	g

* Essential Amino Acid
‡ Branched Chain Amino Acid

Figure A1. Complete profile of Instantized BiPro

APPENDIX B: TEMPERATURE PROFILE

To check the temperatures to which is subjected the gel, the temperature was registered in intervals of one minute with thermocuples (type T) in three gel positions: center, half of the radio and outer edge at the same time for both gels, aerated and the non aerated. The data was recorded by means of a data logger OMEGA OMB-DAQ-3000 (OMEGA Engineering Inc., Stamford, USA) and the program DaqView32 version 9.03. Results showed that the real bath temperature was around 85°C instead 80°C (set temperature). From this experiments it is possible to corroborates that the minimum time that the gel should be left in bath is 40 minutes, that time is necessary for the solution centre to form a gel on studies conditions (Figures B1, B2 and B3).

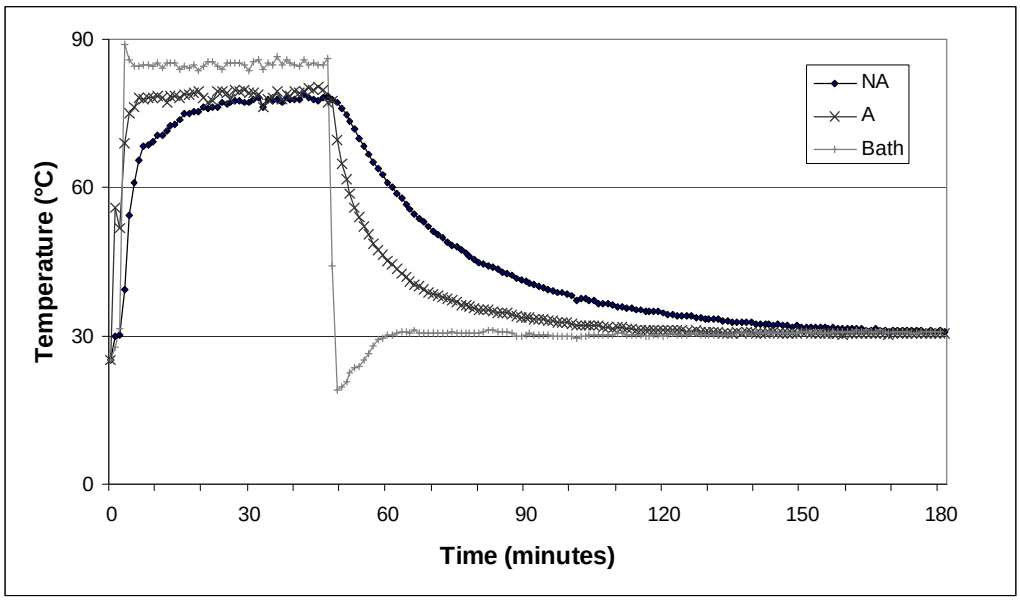

Figure B1. Temperature profile of WPI solutions of no aerated (NA) and aerated (A), measured in geometrical centre of the gel.

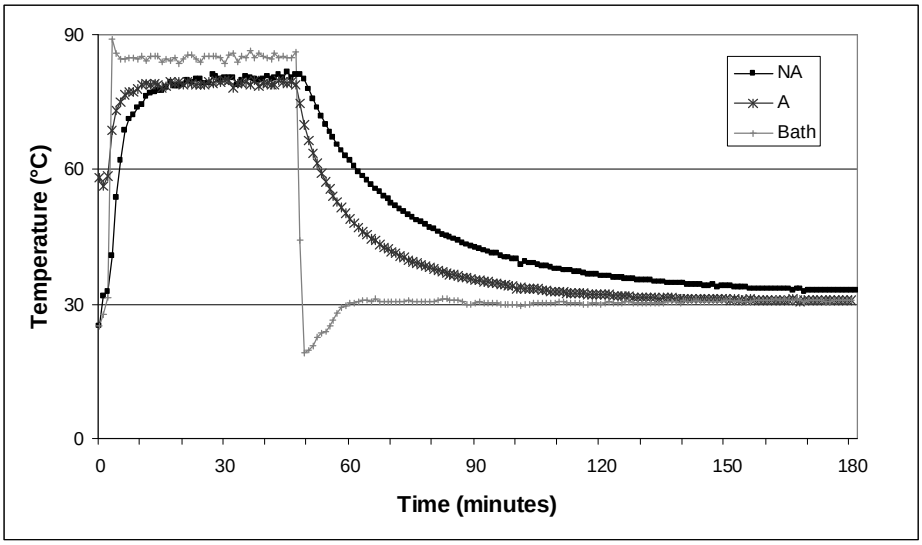

Figure B2. Temperature profile of WPI solutions of no aerated (NA) and aerated (A), measured in the half radio of the gel.

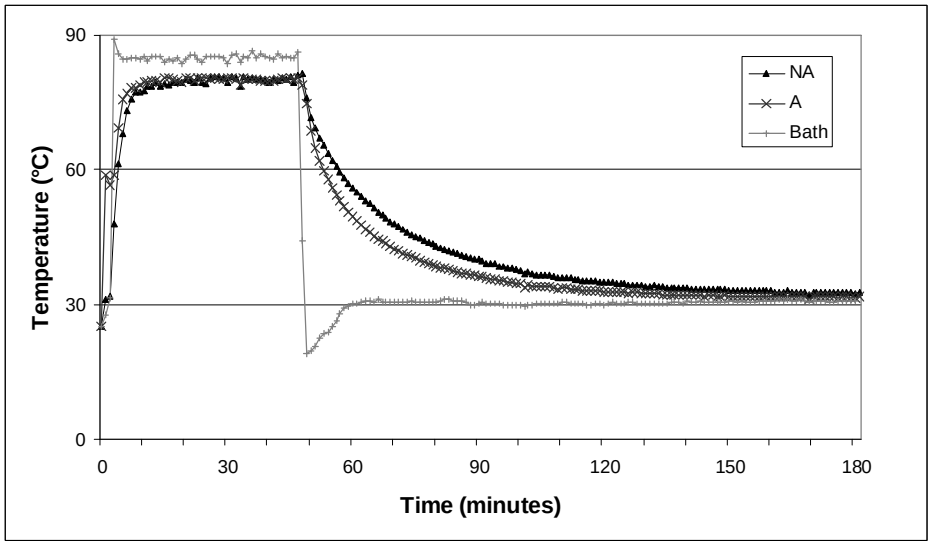

Figure B3. Temperature profile of WPI solutions of no aerated (NA) and aerated (A), measured at the border of the gel.