

# Thermo mechanical analysis of heat- induced denaturation and gelation of whey prot...

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## **Introduction to proteins**

Proteins are polypeptides made up of amino acids joined together by polypeptide bonds. The number of amino acids in a protein molecule can vary from several amino acids to thousands of amino acids. Proteins are made of a combination of several of the 20 naturally occurring amino acids as well as other minor amino acids. Proteins are very important molecules in the body . They form the structural components of animal and human tissue (Yildiz, 2009).

The primary structure of proteins is determined by the sequence of the amino acids in the protein. A functional amino acid must contain the correct sequence of amino acids. A Change of even one amino acid in the amino acid sequence can make a protein non functional. The property of the amino acids in a protein determines its function and proteins interactions with other molecules. The amino acids in a protein molecule can form interactions with other molecules by forming temporary dipoles, ionic interactions between the charged groups of a protein and other molecules, forming attractions between opposite charge groups and through van Der Waals forces (Yildiz, 2009).

Proteins perform many functions in the body like regulation of the replication of the genetic code and regulation of the cellular machinery that form the phenotype of organisms. Proteins are also involved in many chemical reactions which are essential for cell growth and development. Proteins perform their major tasks through interaction of their three dimensional

tertiary and quaternary structures with other molecules . Proteins functional properties are determined on their three dimensional structures, Polypeptide chains and structure of their compact domains. Protein compact domains that are folded serve as the modules used for larger assembly of proteins or act as either catalytic or binding sites of other molecules (Yildiz, 2009).

## **Whey protein isolate**

Whey protein isolate is a dietary supplement obtained from filtering milk protein. It is made up of a mixture of globular proteins . Whey proteins have the highest amount of pure protein containing up to 95 percent of pure protein and a very high value of biological availability. Whey is obtained by filtering whey: the byproduct in the process of casein and cheese production (Fox & Mcswweeney, 2009).

Whey protein isolate mainly consists of proteins inorganic salts vitamins and a small fraction of other components. Whey proteins are also important for their gelling, emulsifying and foaming properties that make them essential in food production. Whey isolate is isolated from milk through ultra filtration, microfiltration and other ionic methods. Whey proteins MAKE of 20 percent of the protein found in milk. The concentration of protein in whey is 0. 6 percent weight by volume (Bongkosh et al, 2000).

The composition of individual proteins in whey proteins consist of Beta lactolbulmin, alpha lactolbumin, bovine serum albumin and immunoglobulins. Beta lactolbumin and alpha lactolbumin are the most abundant proteins found in whey (Fox & Mcswweeney, 2009).

## **Beta lactolbulmin**

It accounts for 70 percent of whey proteins. It is a globular protein. It is made up of 162 amino acids and has about 20 genetic variants. The most predominant of the genetic variants include beta lactolbumin A and Beta lactolbumin B . These two variants are present in almost equal frequencies in whey proteins. The only difference between these two lactolbumin A and B is lactobulmin A contains aspartate and valine amino acids in position 64 and 118 respectively while B contains glycine and alanine in these positions (Leksrisompong et al, 2011).

B lactolbulmin has a diameter of 2 nm and also has nine anti parallel strands of beta strands. The interiors of these strands are hydrophobic while the exteriors are made up of hydrophilic amino acids. The monomer of beta lactolbumin consists of two disulfide bonds made of cysteine and one thiol group. It has a molecular weight of 18 kilodaltons and it is a dimer globular structure with an isometric point of 5. 2(Leksrisompong et al, 2011).

## **Alpha lactolbumin**

It is the second most important protein found in whey. It is a globular protein that has 162 amino acids of molecular weight 14 kilodaltons. It has an ellipsoid shape with two distinct lobes. One lobe has four helices while the other lobe has two beta strands having a loop like chain. It also has four intermolecular disulfide bonds. It naturally binds one calcium ions and has no SH bonds (Leksrisompong et al, 2011).

## **Bovine serum albumin**

It is a minor component of the whey protein accounting for 0.3 to one percent of whey protein. It is made up of 582 amino acids. It has a molecular weight of 69 kilodaltons and an iso-electric point of 4.7. It is globular and elliptical it has 17 disulfide bonds and only one free sulfhydryl bonds (Leksrisompong et al, 2011).

## **Lactoferrin**

It accounts for less than one percent of whey isolate. It is a globular glycoprotein and has a molecular weight of 80 kilodaltons. It is made of one polypeptide chain of 700 amino acids it has two globular domains connected by a short alpha helix. It has an isometric point of 8.7. It has binding sites for iron copper and other metals localized within the two protein globules (Yildtiz, 2009).

## **Immunoglobulins**

They also make a very little quantity of whey proteins. They are large shaped proteins produced by the beta cells and are used by the immune system against pathogens. They are glycoproteins made of heavy and light chains of amino acids. A small tip of the protein is highly variable allowing the existence of varieties of different immunoglobulin's used as targets of antigens (Fox & Mcsweeney, 2009).

## **Denaturation of whey proteins**

Three of the four proteins found in whey are very sensitive to heat and PH. They include alpha and beta lactolbumin and serum albumin. Denaturation

of whey proteins entails the unfolding of proteins by heat and PH induction. The denaturation of whey proteins is a cooperative process that has a large thermal coefficient. The denaturation rate of whey proteins of individual proteins differs. B lactolbumin have a reaction order of 1.5 whereas alpha lactobulmin has a first order denaturation step (Bongkosh et al, 2000).

Alpha lactolbumin A denatures faster than lactolbumin B. The rate of denaturation is as follows in decreasing order, Alpha lactolbumin A > Alphalactobulmin B > Bovine serum albumin > immunoglobulin. The thermal denaturation rates of whey proteins are also influenced by PH greatly. The denaturation of beta lactolbumin occurs at high PH. Bovine serum albumin is resistant to denaturation at high temperatures. The thermal denaturation of Lactoferin is independent of PH (Bongkosh et al, 2000).

The thermal precipitation of whey proteins is determined by electrostatic charge. These electrostatic charges are also determined by the increased rates of precipitation at high PH. At low temperature ranges, the rate determining step in the reaction is the unfolding of the proteins tertiary structure. Denatured whey proteins are easily digested and easily absorbed in the body unlike denatured whey proteins (Bongkosh et al, 2000).

Whey proteins are easily denatured by high temperatures. Heat denatures whey proteins denaturation of whey proteins does not change the nutritional value of whey proteins. Denaturation just alters the physical structure of whey proteins. Alpha lactolbumin is the only heat stable protein in whey.

Denaturation of whey proteins increases when PH is increased

(Leksrisompong et al, 2011).

High thermal stability of whey proteins is observed at temperatures below 3.5 percent. Both alpha and beta lactolbumin undergo changes at a PH of below 4 percent. The dimer configuration of beta lactobulmin dissociates at low PH because of electrostatic repulsion and the monomers that result are resistant to coagulation (Leksrisompong et al, 2011).

## **Gelation of whey proteins**

Gelation of whey proteins gelation is an important property of whey proteins. The gelation property of whey proteins makes them useful in food processing industries as gelation agents. Beta lactolbumin is the primary gelling agent of whey proteins while alpha lactolbumin does not have good gelling properties. The gelling properties of proteins are influenced by heat, concentration, the extent of denaturation, PH, presence of ions and ionic strength (Leksrisompong et al, 2011).

Gelation is generated by the action of pressure and heat. The process of thermal gelation of whey proteins is a two step process. The first step entails the initial unfolding or denaturation of proteins. The second step entails the rearrangement of whey proteins and the aggregation of the functional groups of whey proteins. These functional groups then become available to form many intermolecular interactions forming three dimensional gel network (Navarra, 2008).

The denaturation rate and the aggregation rate of whey proteins is determine the microstructure of the gel formed. Ie . whether the gel formed is course or fine stranded. This microstructure is also influenced the macroscopic properties of gels like appearance and the ability of gels to bind water. Fine stranded gel structures are formed when the repulsive processes in the gel are bigger than attractive forces. A balance of the attractive and repulsive forces in polypeptide chains is very critical for gel network formation (Leksrisompong et al, 2011).

## **Cold gelation**

Cold gelation of whey protein requires the pretreatment of whey protein with temperatures of about 80-90 degrees centigrade for 30 minutes. Heating is necessary to cause the globular structure of whey proteins to unfold and form some linear proteins. Mixing denatured proteins with salt like sodium chloride makes the electrostatic forces to be shielded to form gels.

Electrostatic repulsion of heated whey proteins prevents gel formation . This force is overcome by the addition of salts like sodium chloride (Fox & Mcsweeney, 2009).

The major driving force of gelation during the cooling process of gels is the hydrophobic effect. The gelation rate increases up to a certain temperature. The non hydrophobic forces like hydrogen bonding, configuration entropy, Van der Waals and disulphide bond formation are essential in the determination the final gel rigidity (Fox & Mcsweeney, 2009).



## **TAXT-2 Texture Profile Analyzer (TPA) machine**

A textual profile analyzer is a machine involved in the analysis of the mechanical properties of food or the sensual properties of food that are detected by humans. Textual analyzers perform the test of texture and sensual properties on food through applying some controlled forces on the food product. They then record the response of the food product in the form of force, time and deformation the parameters that TAXT 2 measures include hardness, springiness index, resilience, cohesiveness, adhesiveness, gumminess and chewiness (Yildtz, 2009).

## **The thermo hake rs50 machine**

The THERMO HAKE RS50 machine is a machine used in the measurement of the rheological properties of a product like visco-elasticity. Rheology is used as one of the most useful technique in the characterization of polymers. Gels, due to their viscosity are analyzed for their mechanical response to the oscillatory shear under conditions of deformation (Yildtz, 2009).

## **Dynamic oscillations measurements**

Dynamic oscillations measurements are the most commonly used method of oscillatory testing because they show the viscous and the elastic behavior of material measured. They measure oscillations through recording stress and strain frequency. The frequency sweeps of the dynamic oscillation measurements are useful in the comparison of food products through comparing the effects of various ingredients and their visco- elasticity (Yildtz, 2009).

## **The effect of changes in pH and concentration on the structural properties of heat-induced whey protein gels.**

The gelation of whey proteins is dependent on the PH and the concentration of gels. The structure of proteins denatured by heat is highly dependent on PH of the solution of proteins.

When the PH of a protein is less than the iso-electric point of proteins, Proteins have a brittle -weak structure. This brittle structure is caused by the presence of only very few amino groups along the protein chains . The overall positive charges in proteins are few in the protein when the PH is close to the isoelectric point of whey protein (5. 4). The net charge of a protein is near zero at the isoelectric point meaning there is little electrostatic repulsion in denatured proteins (Monahan et al, 1995).

When the PH of a protein is higher than the isoelectric point, there are many negatively charged groups in a denatured protein. These negative charges make the protein to have a strong and elastic structure. Increase in PH results in many net charges and higher electrostatic repulsive forces in proteins meaning the gels are elastic and strong. More surface charges result from higher PH that result in an increase in electrostatic repulsion and higher sensitivity to PH by whey proteins (Monahan et al, 1995).

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