International Journal of Science, Environment
and Technology, Vol. 4, No 1, 2015, 96 – 100

ISSN 2278-3687 (O)

Review Article

ACID/ALKALINE SOLUBLIZATION METHOD OF PROCESSING PROTEIN

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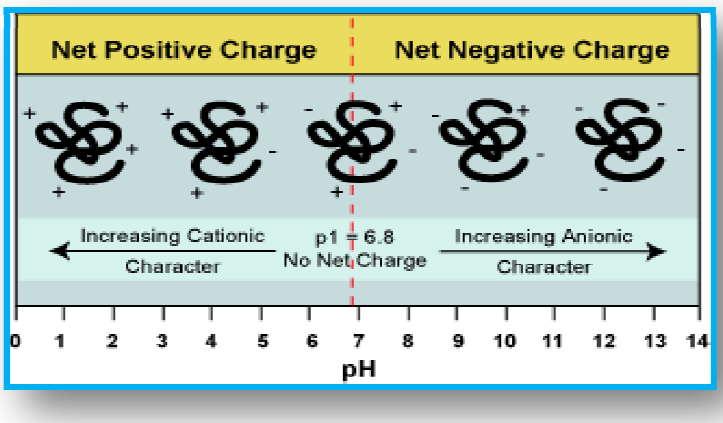
In 1999, there was a major technology breakthrough regarding isolation of muscle proteins from low value raw materials. Hultin and Kelleher patented the acid solubilisation process as a way of improving yield and stability of muscle protein isolates. A few years later, a similar process, but based on alkaline solubilization was patented. The acidic/alkaline solubilization process was developed at the University of Massachusetts Marine Station, Gloucester, MA, USA.

Principle: The process utilizes the principle that the solubility of a comminute protein-containing material homogenized in water is affected by the pH of the mixture.

Mechanism: At extreme acid or alkaline conditions, strong positive and negative changes, respectively, on the myofibrillar and cytoskeletal proteins drive them apart by repulsion whereby, interactions with water can take place, and thereby solubilisation. At neutral pH, myofibrillar proteins are primarily bound in myofibrillar segments and myofibrillar bundles. The degree of protein solubility in an aqueous medium is the result of electrostatic and hydrophobic interactions between protein molecules, and proteins are extracted when electrostatic repulsion between proteins is greater than hydrophobic interactions (Zayas 1997). When the homogenate was adjusted to pH 2 or 12, protein solubility increased. At extremes

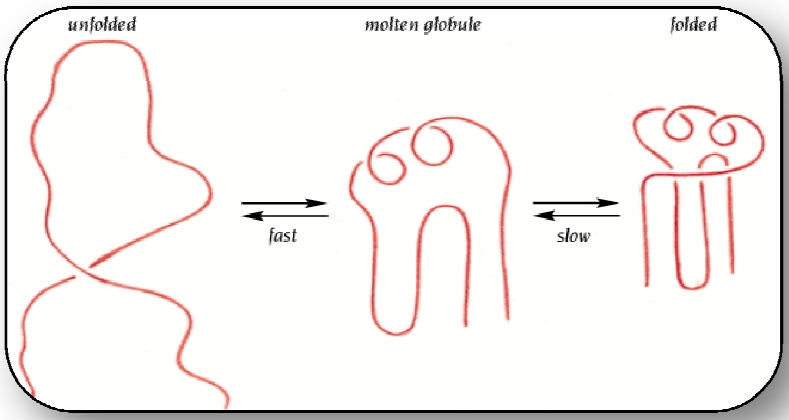


of pH, solubility increased to almost five times that of the original (pH 6.3). The increased solubility at this extreme pH could be due to the co-extraction of the restraining proteins which could not be extracted at neutral pH (Kristinsson and

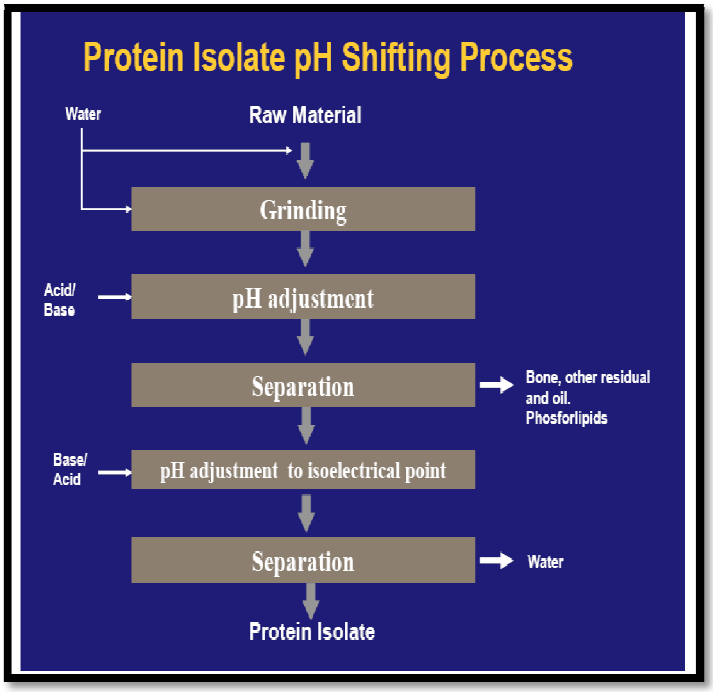
*Received Jan 21, 2015 * Published Feb 2, 2015 * www.ijset.net*

Hultin, 2003a). Proteins separated at physiological pH include sarcoplasmic proteins, dissociated actin, troponin and desmin (Kristinsson and Ingadottir, 2006). At pH above or below isoelectric pH (pI), the proteins become negatively or positively charged depending on the pH, resulting in electrostatic repulsions between molecules and hydration of charged residues, contributing to the solubility of proteins. Strong electrostatic repulsions among protein molecules and the increased protein–solvent interaction at this pH could have contributed to this increased solubility (Kristinsson and Hultin 2003b).

Conformational changes taking place in protein during the acid and alkaline process:



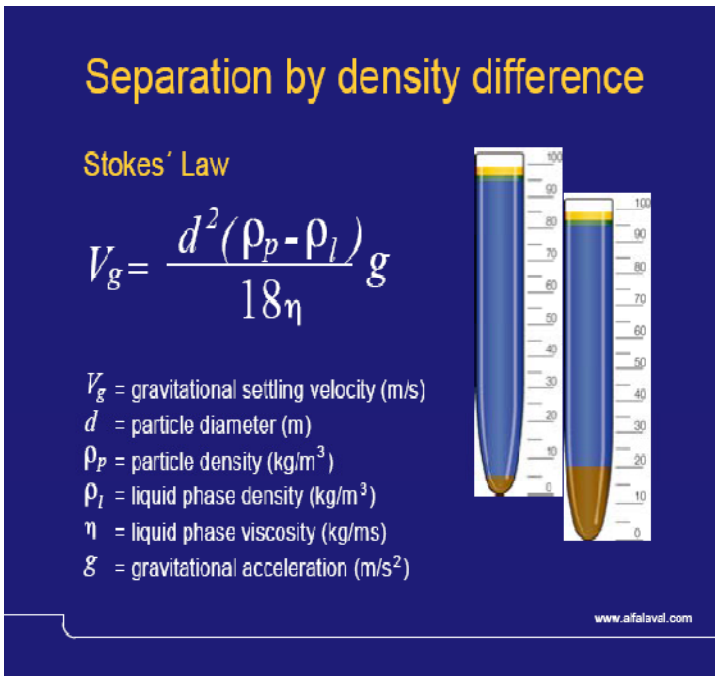
Contrary to earlier findings that the exposure of proteins to low and high pH transforms it to a random coil form, recent works indicated that many proteins would only be partially unfolded into a compact state called 'molten globule', which retains most of the secondary structure while losing its tertiary structure (Goto and Fink, 1989; Goto et al., 1990). An important feature with the acid and alkaline processes is that when muscle proteins are subjected to the extreme pH values, the proteins are partly unfolding. This partial unfolding leads to substantial changes in the conformation and structure of the proteins which in turn leads to different properties of the proteins after refolding (Kristinsson and Hultin, 2003a and b). At both pHs, the tertiary structure was lost, suggesting the "molten globular" configuration. The unfolding and



refolding on pH alteration made the proteins transform into a molten-globule conformation that is considered to enhance the functional properties of value added products. Different conformational changes imparted to muscle proteins can greatly contribute to alterations and enhancement in functional properties of proteins and hence their use in the development of value added products (Mohan *et al.,* 2007).

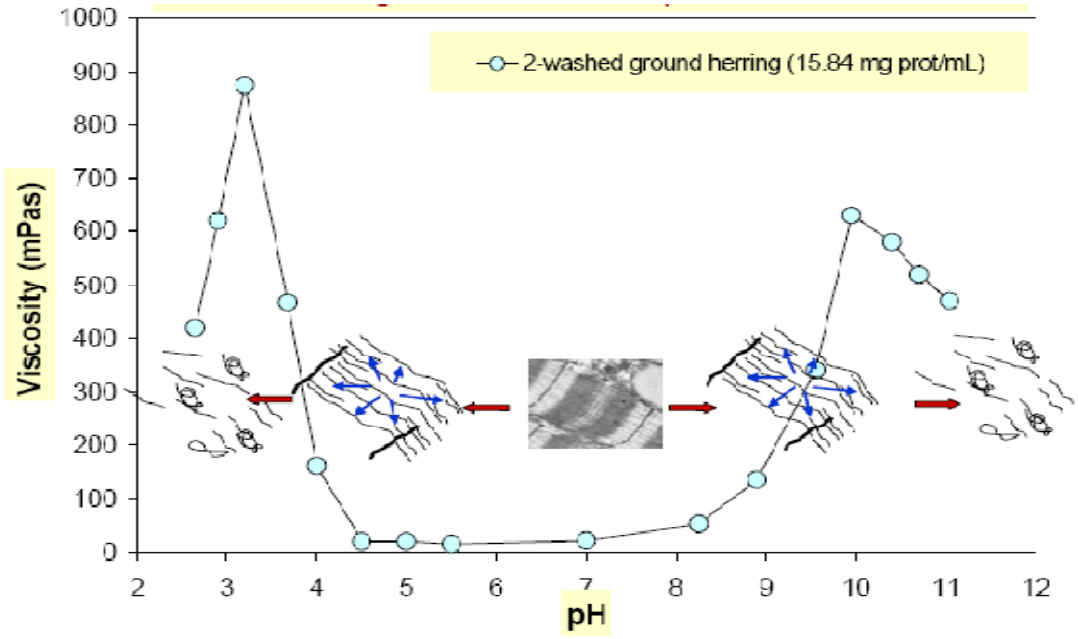
Steps in extraction process: (Hultin and Kelleher 2000)

- **Homogenization:** Minced (ground) muscle is mixed with 6–9 parts of water and homogenized. The objective of this process step is to finely grind the meat protein and add the adequate amount of water into the process to maintain low viscosity.
- **pH shifting:** Homogenate is solubilized by adding a base or an acid. The objective of this process step is to reach, in a controlled way, the desired pH that will solubilize the protein fibres (~pH 3.0 in acid process and pH~12 in alkali process).
- **High-speed centrifugation and separation:** The homogenate is centrifuged and undissolved material is separated from dissolved material. The objective of this process step was to separate away all the none-soluble proteins and bone material. For this step it was decided to use a high speed separator that utilizes centrifugal force for separation of particles from a liquid phase. When using separation by density difference, the Stokes law applies, the separation of the product depends on four factors: Particle size, density of particles and liquid plus the viscosity. In this specific process the particle size is very small and therefore density and viscosity play a significant part in the separation process. The only factor that is related to separation is the gravity acceleration.



However, just when the protein solubilization starts around pH 3–4 and 10–11, the viscosity of the fish homogenate can in certain cases be very high, which has two major disadvantages. The first is that removal of impurities through centrifugation becomes more difficult (step 3),

and secondly, large “extra sediments” are formed during the centrifugation which entraps a lot of the proteins and thus reduces the protein yields.



The high viscosity is thought to arise from expansion and partial solvation of protein aggregates. Below pH 3 and above pH 10.5–11, the aggregates however usually dissociate into smaller units, and the viscosity declines dramatically (Undeland *et al.,* 2002). The picture illustrates the solublization of muscle protein as a function of pH.

Isoelectric precipitation: The pH of the dissolved material is adjusted to the isoelectric point. The objective of this process step is to adjust the pH back to around isoelectric point (pH~5.5 -6.5) to form the protein flock. The “proteins” are then precipitated by adjusting the pH of the solution to the isoelectric point. The refolding of proteins resulted in greater protein–protein interaction.

- **Recovery:** The precipitated proteins are recovered. The separation was done in a decanter centrifuge that again utilizes Stoke law for separation of particles from a liquid phase. In this stage the size of particles is of most concern as the density of the protein flock and the water are very close. If the particles are too small when they enter into the centrifugal force they cannot be separated in time and will follow the liquid stream out of the decanter. This will be regarded as a loss in yield.

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