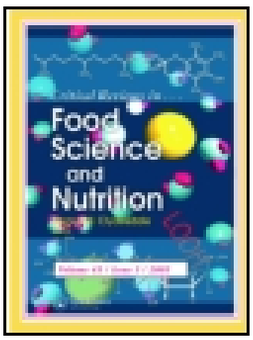

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Solubilization of Myofibrillar Proteins in Water or Low Ionic Strength Media: Classical Techniques, Basic Principles and Novel Functionalities

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ABSTRACT

The qualitative characteristics of meat products are closely related to the functionality of muscle proteins. Myofibrillar proteins (MPs), comprising approximately 50% of total muscle proteins, are generally considered to be insoluble in solutions of low ionic strength (< 0.2 M), requiring high concentrations of salt (> 0.3 M) for solubilization. These soluble proteins are the ones which determine many functional properties of meat products, including emulsification and thermal gelation. In order to increase the utilization of meat and meat products, many studies have investigated the solubilization of MPs in water or low ionic strength media and determining their functionality. However, there still remains a lack of systematic information on the functional properties of MPs solubilized in this manner. Hence, this review will explore some

typical techniques that have been used. The main procedures used for their solubilization, the fundamental principles and their functionalities in water (low ionic strength medium) are comprehensively discussed. In addition, advantages and disadvantages of each technique are summarized. Finally, future considerations are presented to facilitate progress in this new area and to enable water soluble muscle MPs to be utilized as novel meat ingredients in the food industry.

Keywords

Myofibrillar protein; Solubility; Low ionic strength; Water; Functionality.

Abbreviations

AO alginate oligosaccharide

BH beef heart muscle

G′ storage modulus

HEPES N-(2-hydroxyethyl)- piperazine-N¢-2-ethanesulfonic acid

HPP high pressure processing

kDa kilo Daltons

LMM light mero-myosin

MDB mechanically deboned meat

MPs myofibrillar proteins

MHC myosin heavy chain

MSTM mechanically separated turkey meat

pI isoelectric point

pKa acid disassociation constant

PSI possible solubility-inhibiting

SDS-PAGE sodium dodecylsulfate-polyacrylamide gel electrophoresis

PSE-like pale, soft, exudative-like

Introduction

Meat is a nutrient-rich food which can play an important role as a supplementary protein

source for humans in addressing macro- and micro-nutrient deficiencies in diets in many regions of the world (Boland et al., 2013). Compared with proteins from plant pulses, legumes or grains, meat proteins have a higher digestibility and are distinguished by their richness in all the essential amino acids, with none being limiting (Pereira and Vicente, 2013). However, meat proteins have not been fully utilized to the same extent as have milk or soybean products because of the low solubility of myofibrillar proteins (MPs). Only about 50% of meat proteins are solubilized in low ionic strength solutions or water, whereas near complete solubilization is achieved at relatively high concentrations of salt (> 0.3 M NaCl or KCl) (Krishnamurthy et al., 1996).

The solubility of muscle proteins has been the subject of much research and is usually defined as the amount of protein remaining in a solution of defined characteristics, after application of a specified centrifugal force and duration (Hultin et al., 1995). Meat proteins are generally grouped into three categories based on their solubility: sarcoplasmic proteins, which are soluble in water or in solutions of low ionic strength; MPs, which are soluble only in solutions of relatively high concentrations of salt; and stromal or connective tissue proteins that remain insoluble in high-salt solutions (Ito et al., 2003). The latter group represents only about 2-5% of the total muscle proteins (Bailey and Light, 1989).

Classical procedures for isolating the major MPs initially involve their dissolution prior to their separation. Myosin, the major protein fraction, can be precipitated from MPs solubilized in

0.6 M KC1 solution at pH 8.5-9.0 when the solution is diluted to 0.1 M KCl, pH 7.0 (Banga and Szent-Györgyi, 1942). Actin can be isolated by isoelectric precipitation of a water extract of an acetone powder prepared from whole muscle, initially extracted as described above (Straub, 1943). In order to separate myosin from actomyosin, a solution of 0.3 M KCl at pH 6.5 was diluted with water whereby actomyosin could be precipitated, leaving behind a solution of myosin that was then precipitated by further dilution (Szent-Györgyi, 1943). On the basis of these primary and subsequent studies, it is now common practice to solubilize the major proteins by commencing with an extraction of a muscle tissue homogenate using a buffered 0.6 M KC1 solution (pH 6.0). Such extraction of muscle proteins is necessary for the subsequent separation, purification, and isolation operations required for further biochemical studies. Reducing the salt content of muscle protein preparations allows the determination of their properties under more physiological conditions (Stanley et al., 1994).

In addition, solubility directly affects other physico-chemical and functional properties of foods, such as their emulsifying, foaming, gelling properties and viscosity. Therefore, solubility is a key functional property for the development of new formulations and food products. Many functional properties of muscle food proteins are related to the solubilization of the protein, and extraction of muscle proteins is the first step in preparation of these proteins for use as functional ingredients, such as binders and emulsifiers and for thermal gelation in various foods. Ionic strength has long been considered highly important because it influences myofibrillar protein

solubility. Commercially, it is generally accepted that a high concentration (2% to 3%) of salt is required to fully develop the functional properties of muscle tissue foods (Sun and Holley, 2011; Xiong and Brekke, 1991). Assuming that lean skeletal muscle contains 75% water, the final molar concentrations of salt can be calculated to be 0.47 to 0.68 M with the addition of 2 to 3% salt (Ishioroshi et al., 1979). Ionic strength also affects the microstructure of myofibrillar gels. At low ionic strength (0.25 M KCl), fine-stranded gel structures were formed, whereas at high ionic strength (0.6 M KCl), coarsely aggregated gel structures were observed in the pH range 5.5 to 6.0 (Hermansson et al., 1986). However, some reports concluded that solubilization of the MPs was not an absolute necessity for gel formation by MPs (Chang et al., 2001; Feng and Hultin, 2001). A fresh myosin suspension in 0.2 M KC1 at pH 6.0, having low solubility, formed a finer network structure than that of a heat-induced myosin gel in 0.6 M KC1 solution at the same pH (Ishioroshi et al., 1979). The improved network structure obtained at the lower ionic strength was explained by the formation of filaments from myosin monomers (Kaminer and Bell, 1966). Adjustment of pH from 6.4 to neutrality improved the gelling ability and water-holding capacity by two-fold that of water-washed, minced chicken-breast muscle at physiological ionic strengths, where the majority of the MPs, including myosin, were insoluble (Chang et al., 2001; Feng and Hultin, 2001). It seems that either myofibrillar proteins in high salt solutions (> 0.3 M) having high solubility, or in low ionic solutions（< 0.25 M) with low solubility, each possess unique functional properties.

Although the salt-soluble (> 0.3 M) proteins have mainly been equated with the MPs, myosin and actin, there have been various reports that the solubility of these MPs from various species are soluble in solutions of relatively low salt concentrations (Cho et al., 2007; Hayakawa et al., 2009; Ito et al., 2003; Katayama and Saeki, 2007; Saleem et al., 2015). If MPs can indeed be solubilized in water, or in low ionic strength solutions, then there would be good opportunities for enabling innovation of meat products. For example, the fibrous hard textural nature of meat makes eating difficult for people with mastication and swallowing difficulties. Thus producing a soft-textured meat product (together with low salt), or in the form of a liquid diet, would be extremely valuable as an oral nutritional supplement for elderly people and for dysphagic patients to avoid malnutrition (Nieuwenhuizen et al., 2010; Tokifuji et al., 2013).

With this preface, this review discusses the current understanding of the solubilization of MPs in low ionic strength solutions (< 0.2 M) and then their functional properties are analyzed. Later, areas for future research have been identified to enable the use of water soluble MPs as novel meat products for application in the food industry.

Myofibrillar structures and solubilities

Muscle consists of three groups of proteins classified by their solubility and by their location in muscle tissue. They are represented by myofibrillar, sarcoplasmic and stromal fractions (Lee et al., 2010). The MPs are responsible for the contractile properties of muscle and in pre-rigor meat (Goll et al., 2008). As shown in Fig. 1A, muscle myofibrils are comprised of thin and thick

myofilaments (Pearce et al., 2011). The backbone of the thin filaments consists of the protein actin while the largest component of the thick filament is the protein myosin. Myosin is a large protein made up of two heavy chains (Mw 220 kDa) and two pairs of small subunits referred to as light chains (Mw 130 kDa) (Bandman, 1999). Myosin consists of a tail or rod region forming the backbone of the thick filament and a globular head region that extends from the thick filament and interacts with actin in the thin filament. The rigor complex formed by the interaction of myosin and actin is referred to as actomyosin (Huff-Lonergan and Lonergan, 2005). Actin exists as double helical filaments composed of polymerized globular monomers each with a molecular weight of approximately 43 kDa (Xiong, 1994). While the fibrous proteins myosin and actin together form the myofibrillar structure, it also contains many regulatory proteins including the tropomyosin-troponin complex, α-actinin, M-protein and C-protein, and also the scaffold proteins, such as titin (connectin), nebulin and desmin, which provide support for the whole myofibrillar structure. Tropomyosin and troponin are the two key proteins associated with actin to form thin filaments. α-Actinin is the major component located in the Z-line (discs) that connect the actin filaments (Takahashi and Hattori, 1989). C-Protein is a component of the thick filaments which is bound to the assembly of myosin tails that forms the filament backbone (Moos et al., 1978). M-Protein is a prominent structural protein of sarcomeric M bands. Together with titin, M-protein forms structures that cross-link myosin thick filaments in the central clear zone (Obermann et al., 1998). Titin is a massive protein with a molecular weight of about 1

million Daltons (Tornberg, 2005) and along its length are binding sites for other sarcomere proteins and the carboxyl terminal of titin is reported to bind to myosin (Maruyama, 1997). Titin is crucial for the orderly organization of the sarcomere (Labeit and Kolmerer, 1995). Desmin connects the Z-line proteins between myofibrils (Taylor et al., 1995) and contributes to the maintenance of sarcomere structure.

The terms *"solubility"* and *"extractability"* are frequently interchanged, assuming that once the protein is solubilized, it can be readily extracted from muscle fibers or myofibrils into a solution. Sodium chloride (NaCl) and potassium chloride (KCl) affect muscle protein solubility (Nayak et al., 1996). Salt alters the electrostatic, hydration, and water structuring effects of MPs, resulting in enhanced solubility (salting-in effect) or insolubility (salting-out) (Chang, 1997). As shown in Fig. 1B, the major component of thick filaments is myosin, an elongated, two-headed molecule consisting of two identical heavy chains and two pairs of light chains (Craig and Woodhead, 2006). Myosin filaments disaggregate into myosin monomers in high ionic strength buffers (> 0.3 M) and monomeric forms can subsequently re-aggregate back into filaments when the ionic strength is lowered (< 0.3 M) (Bandman et al., 1997; Sinard et al., 1989). The myosin rod is responsible for the self-association and formation of the thick filament backbone. The use of light mero-myosin (LMM), the C-terminal two-thirds of the myosin rod, has shown that myosin can associate only through certain specified functional portions of LMM (Craig and Woodhead, 2006). Using electron microscopy or X-ray diffraction patterns of para-crystals of LMM,

Tsunashima and Akutagawa (2004) have shown that the assembled form of the myosin rod appears as axial staggers of 14 nm, as observed by a helical repeat of 43 nm. This formation can be explained by electrostatic attractions between charged clusters in periodic distributions of the myosin-rod amino acid sequences (Nakasawa et al., 2005; Sohn et al., 1997).

Several research groups have suggested that Z-band disruption is possibly caused by release of α-actinin, which then loosens the myofibrillar structure (Dutson et al., 1974; Hay et al., 1973). Presumably, such ultra-structural alterations are conducive to protein extraction and solubilization. The existence of various isoforms of several other structural proteins (M-protein, C-protein, H-protein, and X-protein) may also contribute to increasing its solubility during extraction by affecting the stability of the thick filaments. M-protein provides transverse structural constraints to the extraction of myosin and its dissociation is essential for extraction of total myofibrillar proteins (Offer and Trinick, 1983; Parsons and Knight, 1990).

Therefore, myosin, the major protein in myofibrillar structures, has been proposed to be responsible for the overall solubility of myofibrillar proteins. The different rates and degrees of extraction of MPs are also related to the ultra-structures of the Z-bands as well as the release of α-actinin and certain subsidiary structural proteins including M-protein.

Processing methods for solubilizing MPs in water or low ionic strength solutions and their resulting functionality

In order to accomplish the solubilization of MPs in low ionic strength solutions (<0.2 M),

attempts have been made by employing both chemical and physical methods. The main processes, advantages and drawbacks of the various techniques used for solubilization of MPs under these conditions are summarized in Table 1. The solubilization procedures, basic solubilization principles and the functionality of processed MPs are discussed in subsequent sections.

Water washing and dilution

Method description and MPs solubility Hultin and his collaborators developed a procedure for the preparation of water soluble MPs from cod. When minced cod muscle was washed 3 times in water (1/3: weight/volume) and then extracted with 50 volumes of water (water reduced the ionic strength below 0.3 mM), all the proteins of the washed minced muscle tissue became soluble (Stefansson and Hultin, 1994). SDS-PAGE of whole muscle extracts and soluble proteins demonstrated that all the major MPs had been solubilized at this ionic strength. (Stefansson and Hultin, 1994) also noted that solubilization was both prevented, and reversed, by low concentrations of salt. This indicated that no extensive proteolysis had occurred during the process. As washing is a dilution process, gradually reducing the ionic strength, successive low volume washes caused the minced muscle tissue to become swollen and difficult to de-water. A final extraction with 50 volumes of water was necessary to lower the ionic strength sufficiently for solubilization to occur. It should be noted that the solubility through this process was sensitive to both ionic strength and pH (Hultin et al., 1995). Kelleher et al. (2004) used this

process to solubilize MPs of white fleshed fish including haddock, monkfish, red hake, silver hake, winter flounder in water. However, when chicken breast muscle was treated similarly, no solubilization of MPs occurred, even after lowering the ionic strength to values well below that achieved with fish muscle (Hultin et al., 1995; Krishnamurthy et al., 1996). In addition, the water solubility of mackerel light muscle proteins was less than 40% when using a similar approach (Kelleher et al., 2004).

Basic principle Since NaCl at concentrations from 0.3 to 1.0 M induces a salting-in effect of MPs, it is thought that Cl^- ions bind to the filaments and increase the electrostatic repulsive force between the filaments, allowing the filament lattice to expand (Offer and Trinick, 1983). However, Stefansson and Hultin (1994) found that cod muscle MPs were soluble in water at neutral pH with a very low ionic strength (approximately 0.3 mM or less). Typical solubility patterns of the proteins of washed cod mince produced via the water washing and dilution procedure, as a function of ionic strength, are shown in Fig. 2. At an ionic strength of 0.2 mM, 90% of muscle proteins were soluble, while only 15% of the proteins were soluble at an ionic strength of 1 mM. It is therefore clear that reducing the ionic strength of the extracting solution to a value sufficiently low permits the solubilization of the cod muscle proteins. A hypothesis to explain the solubilization process at very low ionic strength has been proposed as follows. MPs carry a net negative charge at neutral pH. After successive water washings and dilution, the ions in the muscle are removed and the ionic strength decreases to a low value. The repulsive forces

from these negatively charged side chains of MPs are sufficient to drive the individual protein molecules apart when a large amount of water is made available, resulting in their high solubility in water (Hultin et al., 1995; Stefansson and Hultin, 1994). Lowering the pH, or increasing the salt concentration to shield these negative charges, can reduce the repulsive forces, leading to a decreased solubility. Possibly resulting from the salting-out phenomenon, with increasing salt concentrations higher than 1 M, the solubility begins to decrease (Fig. 2). This is generally ascribed to the loss of a stable hydrophilic surface, causing the exposed hydrophobic areas of proteins to interact, inducing aggregation. As stated, this water washing and dilution procedure was not suitable for the solubilization of MPs from chicken breast or mackerel light muscle MPs. However, the reason for these differences may relate to differences in the initial pH of the postmortem muscle. It has been observed that those species of fish which had higher solubility had an initial flesh pH of approximately 6.6 or higher when extracted, while those with low solubility were below pH 6.6 (Kelleher et al., 2004). Both the chicken breast muscle and the mackerel light muscle had initial pH values that were relatively low (pH 6.2). When cod muscle (generally having a pH 7.0 or higher), was initially exposed to low pH (5.8) extracting solutions and then washed twice with water 1:3, and finally extracted into 50 volumes of water, the solubility of its MPs decreased substantially (Kelleher et al., 2004). Compared to cod muscle, both mackerel light muscles and chicken breast muscles have higher contents of both α-actinin in the Z-disk and the cytoskeletal protein desmin (56 kDa), a protein which connects adjacent

Z-disks both longitudinally and horizontally (Kelleher et al., 2004; Xiong, 1994). These types of structural proteins have been suggested as "possible solubility-inhibiting" (PSI) polypeptides, exerting a negative effect on the solubilization process. Hence removal of these PSI is required for complete solubilization of the MPs (Hultin et al., 1995; Ito et al., 2003; Krishnamurthy et al., 1996; Stefansson and Hultin, 1994).

Functionality Again, it should be noted that this water washing procedure is species dependent. By using the water washing and dilution procedure, most of MPs from fish species such as cod can be solubilized in water. Large volumes of water are necessary to disperse the proteins at neutral pH. Careful attention should also be paid to the ionic strength, whether it is suitably low enough to achieve solubilization. However, while the extraction of MPs at low ionic strength may be useful for separation of the different MPs its application in food technology might be limited, since the ionic strength of a food cannot be adjusted to such a low value. To the best of our knowledge, no studies have been published on the functional properties of water soluble MPs prepared with this procedure.

Washing with low salt, pH adjusted solutions and water dilution

Method description and MPs solubility When minced chicken breast muscle was washed with water to an ionic strength lower than 0.3 mM, solubilization of MPs was less than 2% (Krishnamurthy et al., 1996). Myofibrillar proteins of mackerel light muscle also had limited solubility when treated under these conditions (Feng and Hultin, 1997). However, an alternative

method has been developed to achieve solubilization. This procedure was accomplished by treating homogenized chicken breast muscle with a succession of washes in moderate concentrations of NaCl (25-150 mM) together with a sufficiently high pH (7.0-7.2), followed by extracting with 50 volumes of water (Feng and Hultin, 1997; Krishnamurthy et al., 1996; Stanley et al., 1994). With this approach, over 90% of the MPs were soluble in physiological concentrations (150 mM) of NaCl solutions, or less, and a neutral pH. SDS-PAGE patterns showed that the proteins solubilized in water were essentially identical to the major MPs and the solubilization of the final washed, minced muscle could be prevented, or reversed, by the addition of low concentrations (0.3-0.6 mM) of NaCl (Krishnamurthy et al., 1996), suggesting that proteolysis of the MPs was not an explanation for their high solubilization. Protein extractability by this method varied with muscle type. A positive linear relationship was observed when the proportion (%) of white fibers (IIb) in beef or chicken muscles were plotted against the protein yield (%) in the homogenate (Stanley et al., 1994), indicating that much more protein might be extracted from muscles with higher contents of white fibers when using this procedure. Salt and pH adjustment was reported as essential to achieve high solubilization of the chicken breast muscle proteins (Krishnamurthy et al., 1996). However, some of the differences observed between the solubility of cod and chicken breast muscle proteins may have been related to the different centrifugal forces used for solubility measurement. A lower centrifugal force was used for chicken breast muscle proteins (18000 g for 20 min) compared with those used (5000 g

to 41000g for 10-60 min) to define solubility of muscle proteins (Hultin et al., 1995).

Basic principle It would be expected that a procedure using higher pH values (around pH 7.2), low ionic strength（≤ 30 mM) and removal of PSI-polypeptides would result in greater extraction of MPs from chicken breast and mackerel light muscle. When using this procedure for chicken breast muscle, 95% of the proteins were solubilized when the NaCl washes were buffered with 5 mM histidine at pH 7.4. This was significantly higher than that observed when using NaCl solutions alone without any pH adjustment, where the solubility was only 29% (Krishnamurthy et al., 1996). Thus the presence of histidine in the low-salt washing solution plays a critical role in increasing the solubility of MPs in water. This enhancing effect might result from the higher pH of the buffered histidine or by a unique characteristic of histidine itself. However, when N-(2-hydroxyethyl)- piperazine-N¢-2-ethanesulfonic acid (HEPES) was used instead of histidine for pH adjustment, a similar solubility was found (Krishnamurthy et al., 1996). Therefore, it is likely that the increased solubilization effect was attributable to higher pH rather than a specific role of histidine. However, the role of histidine is still debatable (see next Section). Maintenance of the washing solution at pH 7 or above has been shown to be essential for effective solubilization of mackerel light muscle proteins in water (Feng and Hultin, 1997) as muscle proteins are above their isoelectric point (IEP) and carry a net negative charge, thus inducing electrostatic repulsive forces between them. Given their structures, ionic strength would be expected to have a strong influence on the myofibrillar matrix. When using a low salt-pH

adjusted washing solution and 50 volumes of water for extraction, a relative low ionic strength would be achieved. Under these conditions it is possible that repulsive protein-protein interactions lead to myofibrillar swelling. When myofibrillar pellets were washed by a succession of moderate concentrations of NaCl at pH 7.4, they showed a swollen appearance, and it was assumed that the lower protein content of the pellet indicated a higher water content with greater myofibrillar swelling (Chang et al., 2001; Offer and Trinick, 1983). A significant negative correlation was obtained between the solubility and protein content of the washed pellet, suggesting that the enhanced solubility was highly associated with myofibrillar swelling (Stanley et al., 1994). In fact, swollen myofibrillar proteins have been observed with phase-contrast microscopy after washing with NaCl and histidine solutions (Ito et al., 2003).

Low concentrations (50 mM) of calcium ions form insoluble complexes with myosin and actomyosin thereby reducing the solubility of MPs in water. For effective solubilization of MPs from chicken breast muscle Krishnamurthy, et al., (1996) showed that it was necessary to remove calcium ions by repeated washings. Thus, the effect of washing to remove ions enables the proteins to swell and bind water. The higher the pH (the further from the IEP) and the lower the ionic strength, the greater the swelling. Xiong (2005) found that transverse expansion of myofibrils with a simultaneous extraction of myosin from the sarcomere resulted in substantial swelling of muscle fibers. Once swollen the structure would be readily dispersed or solubilized in the final 50 volumes of water, thus resulting in its enhanced solubility. Moreover, the addition

of 25 mM NaCl solution, adjusted to neutral pH would preferentially remove any PSI-polypeptides responsible for preventing MPs from being solubilized in water (Feng and Hultin, 1997; Krishnamurthy et al., 1996). Initial removal of these particular proteins, by raising the pH of a 25 mM NaCl solution from 6.3 to 7.0, is critical for their dissolution and separation, thus allowing subsequent solubilization of the remaining muscle proteins in water (Feng and Hultin, 1997; Kelleher et al., 2004). For chicken breast muscle, several PSI-polypeptides, including X-protein and C-protein of the thick filaments and α-actinin and amorphin of the Z-disk, are removed by neutral salt solutions (Krishnamurthy et al., 1996). Based on their molecular masses and relative abundance, M-protein (166 kDa), α-actinin (95 kDa) and desmin (56 kDa) might be involved in preventing the solubilization of MPs from mackerel light muscle in water (Feng and Hultin, 1997). However, only α-actinin has been identified in both chicken breast muscle and mackerel light muscle as being a PSI-polypeptides. Since α-actinin is an important component of the Z-disk, binding thin filaments from opposing directions into that structure, its release by washing with a neutral pH, moderate salt solution (25-150 mM NaCl) might loosen the structure of myofibrils, thus promoting the solubilization of MPs with water extraction. It is therefore not unreasonable to expect that the removal of α-actinin in this manner is critical in determining the high solubility of MPs in water. A proposed mechanism for solubilization of MPs in water using this procedure is depicted in Fig. 3. Washing with moderate concentrations of salt (25-150 mM NaCl) at neutral pH might remove the endogenous ions from

muscle, increase the pH to induce repulsive forces among MPs, leading to the release of certain PSI-polypeptides, which loosen the myofibrillar structure, resulting in a swollen condition. Dilution with 50 volumes of water then enables extraction and solubilization of most of the remaining MPs.

Functionality Despite these promising results, the main limitation of this method is the difficulty in lowering the ionic strength sufficiently with repeated washings and dilution steps. Following removal of the inhibitory proteins, the final extraction must be carried out with relatively large volumes of water. However, Ito et al. (2003) reported that it was difficult to achieve the solubilization of chicken breast muscle proteins in water according to this method. However, the solubilized muscle proteins in low ionic strength solutions at neutral pH produced by this method might be useful for the isolation of proteins for research purposes or for commercial use. The functionality of the final product remains to be elucidated.

Washing with low salt-L-histidine solution combined with ultrasonication

Method description and MPs solubility Recent reports have suggested that ultrasonication (used as a pure physical force) is effective in increasing solubilization of MPs in low ionic strength solutions from various vertebrate muscles (Cho et al., 2007; Ito et al., 2003; Saleem et al., 2015). A combination of extractions based on washing with a low salt-pH adjusted solution containing L-histidine and with water dilution followed by ultrasonication has proved beneficial (Ito et al., 2003). The initial step involved homogenization of comminuted muscles with a cold

solution containing 25 mM NaCl and 5 mM L-histidine (pH 7.5) followed by washing with 2.5 mM NaCl and 5 mM L-histidine (pH 7.5) solution. This was repeated three times and the resulting precipitate was termed 'washed' myofibrils. After suspending the washed myofibrils in three to five volumes of water, the suspension was subjected to ultrasonication at 50 watts and 20 kHz (Ito et al., 2003). The suspension was then centrifuged for 20 min at 37 000 g. By using this procedure, more than 80% of chicken breast muscle MPs were solubilized in water. All identified MPs were soluble in water and no fragmentation of the main MPs was evident from ultrasonication (Ito et al., 2003; Saleem et al., 2015). This procedure was shown to be effective for MPs from chicken leg muscle, pork loin, beef shoulder loin, and lamb muscle (Ito et al., 2003).

As discussed above, washing with a combination of low salt and L-histidine solution might be effective in removing the PSI-polypeptides. Ultrasonication was essential for additional disruption of the highly-ordered structure of the myofibrils, leading to their solubilization (Ito et al., 2003; Saleem et al., 2015). Other studies have demonstrated that sonication without L-histidine, gave solubilization of myofibrillar proteins in a physiological ionic medium (0.1 M NaCl ionic strength) (Cho et al., 2007; Saleem et al., 2015). Solubilization of myofibrillar protein containing 0.1 M NaCl at pH 8.0 after sonication was greater than 90% (Cho et al., 2007). It is clear that ultrasonication is a useful aid in preparing water-soluble MPs.

Basic principle Several reasons have been proposed to explain why more than 80% of MPs

were solubilized by using the ultrasonication procedure. One of these is the pH of the washed myofibrillar suspension. As the pH of post-rigor meat is generally in the range of 5.5-6.0 (Warriss, 2000), near the isoelectric point of MPs, washing with NaCl and histidine solutions at pH 7.0 increases the pH of myofibrils and increased the solubility of the MPs. In the absence of histidine (without pH adjustment), only 60% solubility was achieved (Ito et al., 2003). This is presumably because the PSI-polypeptides were not removed by washing with NaCl and histidine solutions as we discussed. However, release of α-actinin from myofibrils by washing with NaCl and L-histidine solution has been observed (Ito et al., 2003; Krishnamurthy et al., 1996). As we discussed above, α-actinin is one of the major components of myofibrils and constitutes the Z-disk; its release may facilitate the solubilization of MPs during ultrasonication. Despite increasing the pH to approximately 7 and removing α-actinin by the washing procedure, the washed myofibrils, when homogenized in distilled water did swell and still retained some high-ordered structures. However the resulting high viscosity (Ito et al., 2003), made it difficult for total solubilization.

Ultrasound has been used to physically or chemically alter food properties (Jambrak et al., 2008). Cavitation bubbles resulting from sonication, are rapidly formed and then violently collapse, leading to extreme local temperatures and pressures. It is these changes that can produce very high shear energy waves and turbulence in the cavitation zone (Gülseren et al., 2007; Hu et al., 2013). With ultrasonic treatment, the highly-ordered structures of myofibrils can

be completely disrupted, and most MPs are solubilized. It has been concluded that the disassociation of myosin from titin and the Z-disc leads to the solubilization of the main MPs. (Cho et al., 2007; Ito et al., 2003; Saleem et al., 2015; Fürst et al., 1988; Ito et al., 2003). Furthermore, ultrasonication directly affects the solubility of myosin through disassociation of the filaments from the actomyosin complex and depolymerizes the fibrous structure in a time-dependent manner (Saleem et al., 2015). However, although the physical force applied by ultrasonication enables MPs to be solubilized in water, it does cause partial damage to their structures. Several groups (Cho et al., 2007; Saleem et al., 2015) have shown that Ca^{2+}, Mg^{2+}-ATPase activities of myofibrillar proteins are partially inactivated by exposure to ultrasound, probably resulting from conformational changes in their structures.

A further consideration on the function of L-histidine during the washing procedure has been investigated. Previous works had established that myosin is essentially insoluble in aqueous solutions at low ionic strengths due to the formation of filamentous myosin aggregates *in vitro* (Guo et al., 2015; Hayakawa et al., 2009, 2010; Lin and Park, 1998; Sinard et al., 1989; Takai et al., 2013). However, myosin was solubilized in neutral and low ionic strength solutions when dialysed into 5 mM L-histidine solution, without any change in pH, (Hayakawa et al., 2009). This indicates that L-histidine indeed has a specific effect in solubilizing MPs in water rather than specifically increasing the pH as we mentioned above. This also suggests that solubilization of myosin is not caused by the shortening of any polypeptides of myosin (Hayakawa et al., 2009).

To clarify the role of L-histidine in the solubilization of myosin at a low ionic strength, the effects of L-histidine on filament formation, the conformational characteristics and the morphology of myosin in a low ionic strength solution (1 mM KCl) were investigated by a number of groups (Guo et al., 2015; Hayakawa et al., 2009, 2010). The introduction of L-histidine caused unfolding of myosin, resulting in loss of α-helical structure and exposed buried hydrophobic and sulphydryl groups to the myosin surface (Guo et al., 2015). This induced elongation of the LMM region of myosin, contributed to weakening of the myosin filament and the dissociation of myosin in a low ionic strength solution (Hayakawa et al., 2009, 2010), which ultimately increased the solubility of myosin (Fig. 4). However, further investigations are needed to determine how L-histidine induces the conformational changes of myosin.

L-Histidine is not the only amino acid to exert an effect on solubilization. Takai et al. (2013) reported that addition of 50 mM arginine increased the equilibrium solubility and activation energy of self-association of monomeric myosin in a physiological salt solution (100-200 mM NaCl) without altering the structure of myosin. A net positive charge (or positively charged side chain) of arginine at neutral pH appears to play a role in their interaction with myosin, affecting its solubility. Consequently, it seems that both the conformational effects of L-histidine and the physical disruption effects of ultrasonication on MPs results in their solubilization in water.

Functionality The physicochemical properties of proteins are responsible for their functional properties which strongly impacts how they can be used in food systems. The functionality of the

water soluble MPs produced by the procedures described here should be considered for their potential in new meat products. Heating is usually an essential process in manufacturing and for consumption of meat products. It was found that when heating at temperatures above 60 °C, 90% of the water-soluble MPs remained soluble and showed very low viscosity. However, a decrease in protein solubility was observed by heating at temperatures below 50 °C (Ito et al., 2004). The low solubility and high viscosity of water-soluble MPs heated at 40 °C or 50 °C appeared to be caused by a reversible thermal denaturation and aggregation of the proteins at those temperatures. It seems reasonable to suggest that heating at a temperature above 60°C induces unfolding, re-solubilization, and stabilization of the water-soluble MPs. Therefore for practical use of water-soluble MPs, heating at the higher temperature is an essential process. With the aim of preserving water soluble MPs until use, freeze-dried protein powder has been prepared and investigated. However, it is well documented that freezing often causes denaturation of proteins, resulting in their insolubility (Chou and Lin, 2010; Korzeniowska et al., 2013). Cryo-protectants, such as trehalose, usually function to protect proteins from denaturing and aggregating upon freezing and during frozen storage (Korzeniowska et al., 2013). Following freeze drying, both heating and addition of trehalose are essential to achieve solubilization of more than 80% of water-soluble MPs (Ito et al., 2004).

Given that this material may be used as a protein supplement, it is important to evaluate its nutritional value. Amino acid analysis showed that the amino acid composition of water-soluble

MPs was essentially the same as that of whole meat (Ito et al., 2004). Thus, water-soluble MPs produced by this procedure could be used as pure and high-quality protein supplements. However, as the preparation procedure for water-soluble MPs is complicated, a simple procedure for large-scale production needs to be developed for the manufacture of protein supplement products. Another drawback of the sonication method is that the acoustic energy produced can be absorbed into the solution, giving rise to elevated temperatures due to cavitation, resulting in possible thermal damage of the food (Alarcon-Rojo et al., 2015). Moreover, ultrasound can generate highly reactive free radicals, leading to protein oxidation that can change their physical and chemical properties (Hu et al., 2013). Therefore, the use of ultrasound in solubilizing myofibrillar proteins in water by industry should consider a method of radical control in order to prevent unwanted oxidation reactions.

On the other hand, as we discussed above, myosin molecules disperse as monomers and are solubilized in low ionic strength solutions containing L-histidine. In order to clarify its processing characteristics, the properties and structures of heat-induced myosin gels solubilized by this procedure have been investigated. The heat-induced myosin gels in a low ionic strength solution containing L-histidine have different properties from those of myosin in high ionic strength solutions. Myosin, in a low ionic strength solution with L-histidine, was shown to have a longer rod segment than did myosin in a high ionic strength solution (Hayakawa et al., 2009). It has been suggested that LMM of the rod plays an important role in the formation of gel network

when heating (Reed and Park, 2011). The structural changes in LMM might cause changes in the electrostatic and/or hydrophobic properties of myosin molecules, affecting the interaction between the rods during heating, resulting in a fine network of myosin and a transparent gel (Hayakawa et al., 2012). It would therefore be of interest to test the use of L-histidine in processing meat products based on its ability to increase the solubility of monomeric myosin.

Acid or alkaline solubilization

Method description and MPs solubility Regarding isolation of muscle proteins from low value raw materials, the acidic/alkaline solubilization process was developed at the University of Massachusetts Marine Station, Gloucester, MA, USA by Hultin and Kelleher (Huhin and Kelleher, 1999; Hultin and Kelleher, 2002; Hultin and Kelleher, 2000). Briefly, the process utilized the principle that the solubility of comminuted protein-containing material homogenized in water is affected by the pH. At extreme acid (2.5–3.5) or alkaline (10.8–11.5) conditions, the strong positive or negative net charges on the MPs drive them apart by repulsion, whereby interactions with water can take place, and thereby solubilization (Nolsøe and Undeland, 2009). During the initial pH-shift, meat proteins dissolve in water at either acidic or alkaline pH (step 2 in Fig. 5), and this approach is typically used to separate lipids by flotation (light fraction) and other insoluble materials (bones, skin, and stromal proteins) by sedimentation (heavy fraction) from the dissolved meat proteins (middle fraction). This water soluble protein solution is then adjusted to pH 5.5, the isoelectric point (pI) of meat proteins (step 4 in Fig.5), for their recovery

(Matak et al., 2015). For fish muscle proteins, shifting the pH from the isoelectric point resulted in increased solubility at low ionic strength. Most species investigated achieved complete solubilization of MPs at low ionic strength, pH 3.0 (Kelleher, 2000). The effect of pH on the solubility of cod proteins at low ionic strength (10 mM) was very dependent on alkaline pH. There was a large change in solubility between pH 8.9 (20% soluble) and pH 9.2 (93% soluble) (Dagher et al., 2000). A sharp increase in solubility of Pacific whiting muscle MPs occurred at alkaline pH between 9.5 and 11.0 and at acidic pH between 3.0 and 1.5 (Choi and Park, 2002). High-intensity ultrasound combined with an alkaline solubilization process also significantly improved the consistency of alkaline muscle homogenate, especially at less extreme pH (pH 10.5), where protein solubility increased (Tian et al., 2015). Acidification was slightly more effective in increasing the total protein solubility of herring light muscle proteins than alkaline treatment (92% vs 89%, respectively), yielding greater protein recovery during precipitation at pH 5.5 (Undeland et al., 2002). The process can certainly be applied to other meat processing areas, for example in the poultry industry. The maximum solubility of chicken thigh and chicken breast muscle proteins was found to be at the extremes of pH (in the acidic and alkaline range) (Ke and Hultin, 2005; Omana et al., 2010). An increase in solubility of mechanically separated turkey meat (MSTM) and spent duck meat protein was observed with both acidification and alkalization (Hrynets et al., 2011; Nurkhoeriyati et al., 2011). It is general thought that the acid process gives a higher protein solubility compared to the alkaline method, thus yielding the

higher protein recovery (Nolsøe and Undeland, 2009; Özyurt et al., 2015). This might be attributed to a greater number of ionizable groups having pKa values between 2.5 and 7.0 than between 7.0 and 11.0 (Hrynets et al., 2011). Although SDS-PAGE patterns of the acid recovered protein isolates from MSTM did not show any sign of hydrolysis (Khiari et al., 2014), many published works have suggested that myosin heavy chain (MHC) and actin are degraded during acid processing. The reduction in amount of MHC might result from the degradation of myosin by acidic proteases (Özyurt et al., 2015; Thawornchinsombut and Park, 2004; Undeland et al., 2002). With alkaline homogenates, hydrolysis was negligible (Omana et al., 2010; Undeland et al., 2002). It should be noted that addition of acid and base during acid or alkaline solubilization alters ionic strength. The highest ionic strength of nearly 0.2 mM was recorded for pH 1.50 (pH adjusted from 1.5 to 12) (Chen and Jaczynski, 2007). One of the advantages of using such a system as in Fig. 5 is the ability for water recycling. However, as a consequence of water re-cycling, accumulation of salt occurs, increasing the ionic strength, thus affecting the pI of muscle proteins. The pI of muscle proteins is an important parameter because as the charges on a protein's surface diminish, so do the protein-water interactions and hence, protein water solubility. As a consequence of increased ionic strength, the MPs precipitate at a lower pH, meaning that the pI had shifted in the acidic direction (Chen and Jaczynski, 2007; Thawornchinsombut and Park, 2004). Therefore, the pH used must reflect the changing pI so as to achieve maximum protein solubility (recovery efficiency solubilization) during continuous

processing.

Basic principle The behavior of muscle MPs during the acid or alkaline solubilization process has been discussed and comprehensively reviewed (Gehring et al., 2011; Matak et al., 2015; Tahergorabi and Jaczynski, 2014; Tahergorabi et al., 2014). As shown in Fig.6, the pH of muscle homogenates is usually near the pI (5.5), where the protein molecule assumes a zero net electrostatic charge. At this pH, protein-water interactions are at their minimum, while MPs are present as aggregates that are held together by weak protein-protein hydrophobic interactions, causing protein precipitation (Tahergorabi et al., 2014). However, it is possible to adjust the conditions that the muscle proteins are subjected to so that the protein side chains can assume different electrostatic charges. This suggests that the solubility of muscle proteins can be switched on or off by providing conditions that either favor or disfavor protein solubility. When acid is added to a solution, it dissociates yielding hydronium ions (H_3O^+). Protonation of negatively charged side chains on glutamyl or aspartyl residues leads to an increased net positive surface charge. Similarly, when a base (OH^-) is added to a solution, deprotonation of the side chains on tyrosyl, cysteinyl, or lysyl residues contributes to an increased net negative surface charge. Consequently, the solubilization of muscle proteins is ascribed to the protonation of aspartyl and glutamyl (pKa = 3.8 and 4.2, respectively) residues at acidic pH and deprotonation of lysyl, tyrosyl, and cysteinyl (pKa = 9.5–10.5, 9.1–10.8, and 9.1–10.8, respectively) residues at basic pH. When the charge equilibrium is reached and a protein solution reaches homeostasis,

the final status of a protein surface electrostatic charge at a given pH is referred to as the 'net' charge. The accumulation of a net positive or negative charge induces protein–protein electrostatic repulsion and an increased hydrodynamic volume due to expansion and swelling (Gehring et al., 2011; Tahergorabi and Jaczynski, 2014). As proteins present a more positive or negative net charge, they gradually commence electrostatic interactions with water (i.e., protein–water interactions). As a result of these increased protein–water interactions, the protein–protein hydrophobic interactions decrease. Therefore, with the protein molecules becoming more polar (charged), more water associates on and around the protein surface and the proteins become water-soluble. The viscosity decreases sharply as soon as the proteins become water soluble. As mentioned, the pH-mediated acid or alkaline solubilization process is also influenced by the ionic strength. Increased ionic strength shifts the minimum solubility of muscle proteins toward a more acidic pH (Chen and Jaczynski, 2007). Addition of salt to obtain an ionic strength of 600 mM shifts the pI of fish muscle proteins by about 2 pH units (Thawornchinsombut and Park, 2004). Salt ions can interact with oppositely charged groups on the protein to form a double layer of ionic groups, enhancing the solubility by a salting-in effect. It is suggested that Cl^- binds positively charged amino acids to a greater extent than does Na^+ to negatively charged amino acids (Tahergorabi and Jaczynski, 2014). Thus, more H_3O^+ must be introduced into the system to neutralize the increased negative charges to an extent that the net electrostatic charge becomes zero and the pI is reached. Therefore, in an acid or alkaline solubilization continuous system,

protein solubility curves (or profiles), as a function of pH and ionic strength, are often experimentally constructed in order to achieve the maximum solubility.

The pH-induced changes in the 3-dimensional structure (un-folding, re-folding, and mis-folding phenomena) that myosin and particularly MHC undergo during acid or alkaline solubilization, have an effect on the functional properties of the recovered proteins, affecting solubility (Kristinsson and Hultin, 2003a; Mohan et al., 2007; Raghavan and Kristinsson, 2008). When muscle proteins are subjected to extreme pH values during the acid or alkaline processes, the proteins are partly unfolded. The electrostatic repulsion results from an increased net charge, favoring protein un-folding. The un-folding is more entropically favorable and therefore, stabilizes the system (Gehring et al., 2011). This partial unfolding leads to substantial changes in the conformation and structure of the proteins (Fig. 7) which in turn leads to different properties of the proteins after refolding (Kristinsson and Hultin, 2003a). Regarding acid unfolding, the myosin rod may fully dissociate due to electrostatic repulsion within the coiled-coil, while it does not dissociate at alkaline pH. At both high and low pH values, the tertiary structure is lost, suggesting the "molten globular" configuration of the head. Large parts of the myosin light chains are lost with either pH treatments (Fig. 7). The head group of myosin is more affected by acidification and alkalization than the rod segment (Mohan et al., 2007). On pH readjustment to neutrality for the purpose of recovery, the heavy chains take on a structural form similar to the native state with the coiled-coil rod re-associating from acid pH while leaving the globular head

less packed, more hydrophobic and structurally less stable. The misfolded head region is thermally more unstable (Nolsøe and Undeland, 2009). Myosin molecules are not only affected by acid or alkali *per se*, but also by the type of acid/bases used to achieve the extreme pH. The relationship between conformation and storage modulus (G′) of acid-treated myosin has been studied by (Raghavan and Kristinsson, 2007). The G′ of myosin increased with an increase in myosin denaturation. The lower the pH, the more the myosin was denatured. From testing different acids during acidification, it was found that the G′ of acid-treated myosin decreased in the order $Cl^- > SO_4^{2-} > PO_4^{3-}$. In another study, two types of bases, NaOH and KOH were used for unfolding myosin under three different alkaline pH conditions (Raghavan and Kristinsson, 2008). Treatment of myosin with KOH resulted in a greater denaturation and a higher gelling ability compared to NaOH. It was also shown that salt (NaCl) stabilized the conformation of myosin against acid or alkali unfolding, and thus, denaturation (Raghavan and Kristinsson, 2007, 2008). Therefore, optimizing the conditions is necessary for a particular muscle type during acid or alkaline processing.

Functionality Proteins solubilized by acid or alkali treatment are usually isolated by precipitation at their isoelectric pH for use as a source of muscle protein. This process has been used for the efficient recovery of high quality, functional protein isolates from sources such as fish, krill, chicken, and beef by-products (James and DeWitt, 2004; Tahergorabi et al., 2012; Tian et al., 2015; Wang et al., 2015). Gels made from silver carp protein isolates recovered under

alkaline conditions with organic acids had improved textures and color properties compared with those made from protein recovered under acidic conditions (Paker et al., 2015). With, or without NaCl, acid treated proteins from washed tilapia muscle had significantly lower gel quality compared to alkaline treated proteins (Ingadottir and Kristinsson, 2010). In protein isolates from tilapia muscles, both acid and alkali-aided processes led to a reduction of lipids. A greater lipid reduction was also observed at alkali pH. The alkali-aided processes exhibited appreciable levels of protein solubility, emulsifying capacity, oil-holding capacity and viscosity (Foh et al., 2012). The acid treated proteins of tilapia muscle exhibited poorer gelling ability compared to alkaline-treated proteins which formed strong and elastic gels upon heating. Enhanced gel strength was observed with acid- and alkali-treated myosin compared to native myosin prepared by conventional salting methods, but there was no significant improvement in the gel strength of acid- and alkali-treated MPs. The greatest improvement in functionality was from the alkali treatment. Acid and alkali treatment improved the emulsification properties of myosin and MPs, which was correlated with an increase in surface hydrophobicity and surface/interfacial activity (Kristinsson and Hultin, 2003b). Firmer protein gels ($P < 0.05$) were obtained from rainbow trout processed by-products when solubilized at pH 12.0 compared with those treated at acid pH (pH 2.5), possibly resulting from less proteolysis and denaturation at the basic pH. However, proteins recovered from acidic treatments had higher ($P < 0.05$) lipid contents than those from alkali treatments, which may account for the greater whiteness of the acid-treated proteins (Chen and

Jaczynski, 2007). Another factor to consider with acid treatment of fish muscle is that unless a protease inhibitor is added, it is likely that a partial proteolysis of recovered MPs will occur, resulting in poor gelling properties (Chen and Jaczynski, 2007). However, these hydrolytic changes seen during acid processing can be minimized by using minimum times at low pH (Undeland et al., 2002). Considering the proximate and amino acid compositions, alkaline treatment of Klunzinger's ponyfish created a more desirable amino acid composition than the acidic process (Özyurt et al., 2015). For krill protein isolates, a continuous mode acid or alkali solubilization process has been applied to recover functional muscle protein. As found for fish, lipids were retained to a higher degree and more krill protein was solubilized at acidic rather than basic pH. Possibly because of higher proteolysis and denaturation at acidic pH, MPs gels prepared from krill, using alkali conditions, had better texture than the acidic counterparts.

Regarding protein isolates from poultry, it is possible to remove most of the fat, collagen and calcium that was originally present in mechanically deboned meat (MDB) from turkey using the acid or alkaline solubilization processes. For spent duck protein isolation, the alkaline process resulted in the lowest lipid content, whereas acid treatment significantly increased myoglobin removal (Nurkhoeriyati et al., 2011). Gels made from these alkaline solubilized-protein isolates showed good textural properties and water retention ability and were lighter in color than the original MDB turkey (Liang and Hultin, 2003). Both alkaline and acid extractions resulted in significant decreases in cooking and water losses compared to raw MSTM. Acid treatments were

more effective for removal of total heme pigments from MSTM. Overall, the color characteristics of protein isolates were markedly improved compared to the initial material and were also lighter in color when subjected to acid extraction (Hrynets et al., 2010). Emulsion-forming and foaming properties were found to be improved in alkali-extracted proteins from under-utilized chicken dark meat (thigh) and from MSTM compared to those from acid extractions (Hrynets et al., 2010; Omana et al., 2010). As some of the dark pigment components are likely to be extracted from meat products containing bones, skin, etc, when using the acid or alkaline solubilization processes, the color properties of the recovered proteins will be poor. In such cases, TiO_2 can be added to the recovered proteins as a whitening agent. Addition of TiO_2 to proteins recovered from these by-products allows development of heat-set gels with color and texture comparable to gels prepared from chicken breast (Tahergorabi et al., 2011; Tahergorabi et al., 2012).

For beef heart (BH) muscle, the acid-solubilization process, without salt addition, exhibited improved gel attributes (water-holding capacity, cook yield, texture profile analysis) compared with untreated BH or surimi-type BH without salt addition (James and DeWitt, 2004).

In summary, improvements in the functionality of acid or alkaline treated muscle proteins were directly linked to the extent of partial unfolding and refolding of myosin. A general observation was that the functionality (thermally induced gelation and texture properties) of both myosin and MPs was slightly improved using the alkaline treatment compared with the acid

process, probably as a consequence of less hydrolysis during alkaline treatment (Nolsøe and Undeland, 2009). Alkaline solubilization resulted in increased gel strength compared to acid solubilization, possibly because of the different pH-induced conformational changes and the mis-folding of protein structures (Fig. 8) as a result of increased surface hydrophobicity and content of -SH groups. With alkali-induced un-folding and re-folding, myosin retained more primary amines and glutamyl-carboxyamide groups in closer proximity than did proteins recovered under acidic conditions. During heating, the content of total –SH groups decreased in proteins prepared at alkaline pH (Pérez-Mateos et al., 2004). The loss of –SH groups indicated an increase of –SH/–SS– interchange reactions and/or oxidation of –SH groups resulting in the formation of –SS– linkages. The –SS– linkages are strong covalent bonds that greatly contribute to thermally induced gelation and texture development (Gehring et al., 2011). In addition, lipids were also removed to a greater extent from the recovered muscle proteins when solubilization was conducted at alkaline pH. Depending on the effectiveness of the removal of heme proteins, and the lipid content after acid or alkaline processing, different muscle species require different processes to achieve isolates/gels that are as lightly colored as possible.

Protein solubility in salt solutions has also been used as an indirect measure of a protein's functionality. Several studies have reported that the pH shift process drastically lowers salt solubility of the isolated proteins (Marmon et al., 2012; Marmon and Undeland, 2013). It is presumed that the reduced water-protein interaction when acid- or alkali-solubilized proteins

precipitate at pH 5.5 (compared to pH 6.5) allows for further protein-protein interactions via both hydrophobic interactions and hydrogen bonding, due to a closer proximity and less repulsion between the proteins. Once formed, neither neutralization of the pH nor high salt concentrations can break these interactions. Thus, the bonds formed restrict the salt and water to interact with the proteins, thereby enabling lower solubility at salt solution. Precipitation at pH 6.5 showed great promise for proteins solubilized by acid or alkaline processes, as it did not influence the protein yield, but enabled higher solubility in salt (Marmon et al., 2012). As an alternative approach, where there is a need for enhanced protein functionality, this type of extracted functional protein isolate from poultry may potentially substitute for lean meat for use in chicken patty formulations (Khiari et al., 2014). The incorporation of acid or alkaline solubilized protein isolates into whole muscle by injection has also been evaluated and found to improve textural and sensory properties (Vann and Mireles DeWitt, 2007). Functional muscle proteins have thus far been recovered on a laboratory scale using a batch mode (Kristinsson and Hultin, 2003a) and pilot scale (Mireles DeWitt et al., 2007). However, it must be stressed that there are practical limitations regarding the amount of water that can be used in the acid and alkaline processes. A continuous bioreactor system for processing by-products and muscle has been proposed (Tahergorabi and Jaczynski, 2014) where there is reuse of water, thus reducing processing costs. However, the purity of the process water, and thus its reuse, greatly depend on the processing parameters. Attempts for up scaling these processes are currently being carried out. Nevertheless,

extreme care needs be paid to safety precautions as, for example, the use of acids may cause metal corrosion.

Overall, these findings provide additional understanding to the behavior of muscle MPs, and their acid or alkaline solubilized counterparts, in food systems. This then allows for the development of this technology at a commercial scale for the efficient processing of novel food products at low ionic strength.

Protein glycation

Method description and MPs solubility Covalent bonding of proteins to polysaccharides, and smaller reducing sugars via the Maillard reaction, has been shown to alter the functionality of proteins under mild and safe conditions without requiring the addition of toxic chemical reagents (Liu et al., 2012; Oliveira et al., 2014). Preparation of neo-glycoprotein from myofibrillar proteins by the Maillard reaction with reducing carbohydrates has been established as a promising method for dissolving MPs in solutions of low ionic strength (about 0.05-0.2 M) (Saeki and Inoue, 1997; Saeki and Tanabe, 1999). As summarized in Table 2, MPs can be conjugated with various reducing sugars, such as glucose, ribose, maltose, malto-triose and alginate oligosaccharide (AO) through the Maillard reaction. Such glycation of fish and chicken proteins can improve their functional properties, including solubility in low ionic strength solutions, emulsifying properties and antioxidant functions. The procedure involves suspending meat proteins in 10 to 50 mM NaCl, mixing with reducing sugars, and then lyophilisation. To

react the proteins with sugars through the Maillard reaction, the lyophilised protein-sugar mixtures are incubated at 30 to 60°C under controlled relative humidity using a temperature- and humidity-controlled cabinet. The covalent linkage to carbohydrates changes the net charge on proteins and introduces hydrophilic residues, resulting in a product having improved solubility in low ionic strength solutions (Sanmartín et al., 2009). By glycosylation, isolated myosin and actin became solubilized in low ionic strength media. The total solubility of MPs in 0.01-0.16 M NaCl increased with the progress of glycosylation, and at its optimum, was almost equal to the solubility of MPs without glycosylation in 0.5 M NaCl (Saeki and Inoue, 1997). Specifically, the solubility of myosin in 0.1 M NaCl increased with extent of glycosylation and reached the same level as in 0.5 M NaCl as for non-glycosylated myosin (Tanabe and Saeki, 2001). Where ribose was used for glycosylation at 30°C for 2 h, 72% of myosin and 80% of actin were solubilized in 0.16 M NaCl (Saeki and Tanabe, 1999). During glycosylation, the available lysine content of the MPs usually decreased. An improvement in the solubility of MPs by conjugation with glucose occurred when 17% of the available lysine residues had been glycated (Saeki and Inoue, 1997). For glycosylation of scallop MPs, a 60% decrease in reactive lysine was required to achieve solubility improvement (Katayama et al., 2002). As there was a decrease in lysine availability, the nutritional value of these glycosylated proteins may be reduced (Oliveira et al., 2014). However, when conjugated with AO, the solubility of fish MPs in a low ionic strength medium was markedly improved without any significant loss of available lysine (< 10%) (Sato et al.,

2003; Sato et al., 2000; Takeda et al., 2007). Myosin became highly solubilized at lower NaCl concentrations when conjugated with AO (Maitena et al., 2004). In the case of myosin rod from carp, high solubility (> 80%) was obtained when 10%, 15%, and 45% of the available lysine had reacted with maltotriose, maltose and glucose, respectively (Katayama et al., 2004). When chicken myofibrillar protein was glycosylated with glucose and maltose, the solubility of each type of glycosylated chicken protein in 0.1 M NaCl solution was in excess of about 60% (Nishimura et al., 2010). Possibly as a result of the greater thermal stability of MPs from homo-thermic animals, chicken MPs only required a small amount of glucose compared to carp myofibrillar proteins for their solubilization (Nishimura et al., 2010).

As discussed above, most studies have used the dry-heating method for conjugate synthesis. However, this method is not feasible for large scale production, since the reaction requires the material to be dried prior to treatment and requires long reaction times, controlled humidity and temperatures (Oliveira et al., 2014). In order to further increase the efficiency of glyco-conjugation at lower temperatures, an enzymatic glycosylation of chicken natural actomyosin with glucosamine using transglutaminase has been found to be effective (Hrynets et al., 2014). Such glycosylation methods involving enzymes in liquid systems may be a promising alternative, having a positive effect on protein solubility. Moreover, because of the covalent attachment of reducing sugars to the myosin heavy chain, there is a gradual decrease in the electrophoretic mobility of myosin heavy chain on SDS-PAGE with increasing glycosylation

(Maitena et al., 2004; Takeda et al., 2007). It has been suggested that by regulating the Maillard reaction during the early stages of glycosylation there would be an improvement in protein solubility (Saeki, 2010). No protein degradation was detected in these glycosylated muscle proteins, which retained their functionality. In summary, the solubility of MPs in water (in a low-ionic-strength medium) can be effectively improved by glycosylation without protein degradation. Further, relative humidity, reaction time and also the mixing ratio of MPs to sugars are important factors in achieving a higher solubility in a low-ionic-strength medium (Katayama and Saeki, 2004).

Basic principle The Maillard reaction refers to a complex group of reactions which occur spontaneously during the processing of some foods. Generally, the reactions can be separated into three stages; "early," "advanced," and "final" (Sanmartín et al., 2009). The early stage of the Maillard reaction is characterized by an initial glycosylation reaction between a carbonyl group of a reducing sugar with an available amine group. Upon protein glycosylation, there is a loss of available lysine and a simultaneous production of fructosamine (Saeki, 2010). When lyophilized proteins were mixed with sorbitol (a non-reducing sugar) and incubated under suitable conditions for conjugation, the available lysine content remained unchanged, and no production of fructosamine was observed (Katayama et al., 2002). These results indicate that the formation of the conjugates of MPs-carbohydrate is based on the Amadori rearrangement that occurs during the first step of this reaction when an available amino group from lysine is linked with a carbonyl

group of a reducing sugar (Fig. 8). It has been reported that no protein polymerisation or brown discoloration was observed during glycosylation (Maitena et al., 2004; Saeki, 2010), hence the glycosylation occurred at the early stage of the Maillard reaction. The reason for the dependence of MP solubility on NaCl concentration is largely known at the molecular level, however there is a need to understand why glycosylation results in improved solubility of MPs in low ionic strength solutions. This has been extensively studied over the last decade with major emphasis on effects of glycosylation on the dynamic behavior and filament forming ability of myosin and myosin rod (Katayama et al., 2004; Katayama and Saeki, 2004; Katayama et al., 2002; Maitena et al., 2004; Saeki, 2010; Takeda et al., 2007; Tanabe and Saeki, 2001). Although the head region of myosin is water-soluble, myosin is a water-insoluble protein due to the insolubility of the rod region. When myosin is present in low-ionic-strength media at neutral pH, the molecules aggregate and assemble into insoluble filaments via the rod region. When myosin is glycosylated in 0.1 M NaCl, the rods lose their filament-forming ability and their solubility is enhanced to that observed in 0.5 M NaCl with non-glycosylated myosin (Tanabe and Saeki, 2001), reflecting functional changes in the rod region. Thus, the increase in the solubility of myosin at low ionic strength results from its solubility improvement in the rod region, where in 0.1 M NaCl, it transforms to a monomer and becomes solubilized (Katayama et al., 2004; Maitena et al., 2004; Takeda et al., 2007; Tanabe and Saeki, 2001). As glycation progresses the rod filaments gradually shorten and the filaments collapse enabling the rods to exist in a monomeric state. This

is necessary to achieve water solubilization of myosin rod (Katayama and Saeki, 2007). On the other hand, the pI shift to a more acidic value by conjugation with AO might also contribute to the solubility improvement of myosin at pH values above 5.0 (Maitena et al., 2004). It has been estimated that the pI of the myosin rod decreases from 4.98 to 4.17 (carp) and from 5.03 to 4.29 (scallop) when 69% and 73% of the available lysine has reacted with glucose resulting in an improved solubility of 97% and 83%, respectively (Katayama et al., 2004). The self-assembly of the myosin rod is accelerated by a decrease in negative charge repulsion (Katayama et al., 2004). The negative net charge of the myosin rod increases with the progress of the glycosylation because the positively charged lysine residues (ε-amino groups) are lost as a result of the reaction with glucose, which might in turn, affect the pI and hence filament formation. Therefore, it is possible that the loss of the filament formation of glycosylated myosin was related to an increase in the negative charge repulsion among myosin rod regions (Saeki, 2010). No change was observed in the α-helix content of the glycosylated myosin rod (Katayama et al., 2004; Tanabe and Saeki, 2001), whereas the filament-forming ability was lost. It is apparent that the dissociation of myosin rod filaments is achieved without substantial conformational changes in the rod region. It is then speculated that reducing sugars bound to lysine residues would be located on the surface of the rod molecule and inhibit the filament formation of myosin rod. To confirm the hypothesis, effect of glycosyl-unit size on solubility improvement of the myosin rod was also examined. The results of Katayama et al. (2004) indicate that glycosyl units having

larger molecular sizes effectively interfere with the interaction between the myosin rods, giving improved solubility. The carbohydrate moiety of a glycosylated amino acid may have imposed a steric hindrance, giving rise to increased protein solubility by inhibiting the filament formation of the rod regions (Hrynets et al., 2014; Sanmartín et al., 2009). Through the Maillard reaction, a variable total number of hydroxyl groups from reducing sugars can be conjugated to the myosin surface. Such an increase in hydration would improve the solubility (Nishimura et al., 2011). In summary, a molecular mechanism for the solubilization of myosin in water by glycosylation is proposed in Fig. 8. With the progress of glycation between the positively charged lysine residues in myosin with reducing sugars through the Maillard reaction, the positive net charge of the myosin rods was diminished, thus increasing the negative charge repulsion among myosin molecules. As a result of enhanced negative charge repulsion among myosin molecules and the introduction of hydrophilic sugars onto the surface of the myosin rod region as a physical barrier, the filament forming ability of myosin rod is impaired, and myosin becomes water soluble in low ionic strength solutions. It is assumed that solubilization of myosin by glycation occurs through a similar molecular mechanism regardless of the species (Katayama and Saeki, 2007).

Functionality In terms of functionality, water soluble conjugates of MPs-carbohydrate have excellent thermal stabilities and improved emulsifying properties. When MPs are conjugated with AO, a water soluble MPs-AO conjugate having excellent heat tolerance is obtained. They had a greater stability in a wide range of NaCl concentrations, pH levels, and thermal conditions

compared with the native MPs (Sato et al., 2003). Similar results have been observed for carp myosin (Maitena et al., 2004; Sato et al., 2005) and in spawned-out salmon meat (Takeda et al., 2007). Thermal stability of chicken MPs was also improved by conjugation with glucose and maltose (Nishimura et al., 2010). This improvement might result from suppression of the thermal aggregation temperature of myosin by attachment of sugars and increase hydration due to the presence of additional hydroxyl groups (Nishimura et al., 2010). It has been suggested that glycosylation exhibits a protective effect on actomyosin during heat-induced gelation. Protein aggregation induced by heating was possibly prevented by stronger steric repulsions between actomyosin molecules due to the presence of higher amounts of carbohydrate moieties on its surface, acting as molecular spacers (Hrynets et al., 2014). When neo-glycoprotein was prepared from carp myofibrillar protein using the Maillard reaction, the emulsifying properties of the myofibrillar protein were improved by glycosylation during the early stage of the Maillard reaction. Further progress of the Maillard reaction would likely result in loss of solubility (Saeki, 1997).

Conjugation with AO is an effective way of improving the emulsion-forming ability of MPs. The MPs-AO conjugate was solubilized in wide range of NaCl concentrations and pH values resulting in high stability and with excellent emulsion-forming ability (Sato et al., 2003). The enzymatic conjugation product actomyosin-glucosamine prepared at 37 °C improved the emulsifying activity and stability of actomyosin, particularly at the protein's pI (Hrynets et al.,

2014). The ability of glyco-conjugates to form emulsions with higher stability may be attributed to the improvement in protein solubility and the formation of a sugar steric layer at the interface, providing stabilization against coalescence (Wong et al., 2011). Also, some unique functional properties were obtained by glycation. Nishimura et al. (2011) showed that glycosylated chicken breast MPs when conjugated with maltose (1:4) exhibited an antioxidative function against superoxide anion radicals. However, despite substantial evidence that glycation improves functionality, this beneficial effect is diminished at higher levels of glycosylation. It has been suggested that in order to suppress denaturation, glycosylation of fish muscle protein should take place at relatively low humidity and in the presence of sorbitol, or a large quantity of reducing sugars (Saeki, 2010, 2012).

It has been mentioned that the nutritional value of proteins may be reduced during the early stages of the Maillard reaction because of the decrease in available lysine residues. In terms of food safety, Maillard reaction products frequently result in the formation of anti-nutritional and toxic substances. Glycation under controlled conditions, in terms of incubation time, pH, a_w, and temperature, may prevent the Maillard reaction from progressing to the more advanced stages, thus preventing the formation of harmful components. MPs-carbohydrate conjugates formed by Maillard reaction may be deemed as “novel food”, but it should be subjected to safety testing before being consumed by humans (Oliveira et al., 2014).

High pressure processing

Method description and MPs solubility For completeness, this final section briefly addresses the possible use of high pressure processing (HPP) or high hydrostatic pressure, which has been demonstrated to affect muscle protein solubility in whole muscle and meat batter systems. HPP is a technique largely used in the food industry for inactivation of micro-organisms, thus extending product shelf life, but it has also been shown to have beneficial effects on certain food qualities, especially for meat.

For muscle foods, changes induced by HPP have been shown to result in improved tenderization (Bouton et al., 1977; Sikes et al., 2010), improved gelling properties (Macfarlane and McKenzie, 1976) enhanced binding and reduced cooking losses in meat emulsions (Sikes et al., 2009). Some of the beneficial properties mentioned here specifically result from the increased solubility of certain MPs in homogenized meat systems. For this reason we considered that HPP should be included in this review, although it may prove unlikely to contribute to our ultimate aim of solubilizing MPs for the uses in food systems stated in the Introduction.

Macfarlane and colleagues McKenzie (1976) were first to demonstrate and effect of HPP on MPs solubility. They showed that when muscle homogenates, or washed myofibrils, were suspended in various 0.5 M salt solutions (NaCl, KCl or KI) at pH 6.5, and subjected to HPP (150 MPa) and then returned to ambient (0.1 MPa), there was a three-fold increase in the amount of protein solubilized, with 60 to 70% of the MPs being solubilized (Macfarlane, 1974; Macfarlane and McKenzie, 1976). For KCl and KI a significant increase was observed even at

0.1 and 0.2 M.

Since then, there is substantial evidence that MPs and certain structural elements show an increase in solubility with HPP, both at low ionic strength, 0.1 M KCl (Ma et al., 2011) or 20 mM Tris, pH 7.6 (Marcos and Mullen, 2014) and at high ionic strength, 0.5 M KCl or 0.6 M NaCl (Sikes et al., 2010). However, this is clearly dependent upon conditions used as aggregation can also occur at higher pressures (Cheftel and Culioli, 1997; Gross and Jaenicke, 1994).

The appearance of specific MPs solubilized following HPP of whole meat or meat homogenates has been shown in various reports (Cheah and Ledward, 1996; Jung et al., 2000; Sikes et al., 2009). Ma et al. (2011) investigated the solubilization of isolated MPs from beef muscle when using HPP (0.1 to 600 MPa) at 20, 40 and 60°C and found that, amongst other proteins, the solubility of the Z-line component α-actinin increased with HPP at 100 MPa but decreased at higher pressures. It has been well documented that F-actin depolymerizes to G-actin under similar pressures (Ikkai and Ooi, 1966). Recently Marcos and Mullen (2014) demonstrated an increase in the solubility of beef myofibrillar proteins after pressure treatment at 200 to 600 MPa at 20°C. The treated and control muscles were homogenized in 20 mM Tris, 2 mM EDTA, 4 mM $MgCl_2$, pH 7.6, in presence of protease inhibitors and solubilized MPs were obtained in the supernatant following centrifugation at 14,000 rpm for 20 min. Using 2D-electrophoresis of the supernatant they showed that the appearance of myosin light chain 1, tropomyosin α-1 chain,

tropomyosin β chain and troponin T increased with increasing pressure. Protein solubilization increased with increasing temperature and with pressures increasing up to 400 MPa.

Basic principle Depending on the conditions used (pressure, temperature and time), it is possible to disrupt membrane structures and modify the conformational structures of proteins. Such alterations may result in proteins having different functional properties as a consequence of their aggregation, depolymerization, solubilization and/or denaturation (Buckow et al., 2013; Marcos and Mullen, 2014).

Regarding underlying reasons for HPP resulting in solubilization of individual proteins, under specific treatment conditions, most of the investigations and theoretical analyses have centered the role of water-protein relationships during the early stages of events leading to denaturation of globular proteins (Ghosh et al., 2001; Harano and Kinoshita, 2006; Harano et al., 2008; Silva et al., 2014). Apart from experimental observations, there is little information on the actual mechanisms for the solubilization of fibrous proteins.

When high pressure is applied, it has been proposed (Harano et al., 2008) that the native structure of a protein initially becomes slightly more compact before swelling as a result of water ingress into the 3-D structure. The final stage is that of a denatured protein state having a random coil structure. The swelling and ingress of water into a protein's interior is thought to result from weakened interactions between hydrophobic amino acid side chains that were originally in close proximity and a strengthening of their interactions with water (Hummer et al., 1998; Weber,

1992). This is thought to result in overall swelling, perhaps with some weakening, but without disruption of the overall conformational structure of the protein (Ghosh et al., 2001). However, Silva et al. (2014) stated that forces other than hydration, such as electrostatic interactions, are also likely to contribute.

In summary, disruption of the native state of proteins under pressure has been stated by Silva et al. (2014) to be the result of "contributions from 1) the loss of free volume arising from packing defects in the completely folded structure; 2) hydration of exposed nonpolar amino-acid residues; 3) electrostriction of exposed charges." All these factors and including the water penetration, loss of hydrophobic protein-protein interaction, protein unfolding, loss of free volume, and increase of protein hydration might result in enhanced water solubility of proteins after HPP treatment under defined conditions. However, the knowledge of the mechanistic behavior of pressure on fibrous muscle proteins is less understood.

Functionality Depending on the conditions used, HPP may offer some benefit for the extraction of MPs, particularly when used in combination with low molarities of salt. Consideration should also be given to the use of this technique in combination with some of the treatments described in this review.

Summary and future considerations

The functionality of muscle MPs plays an important role in routine applications in the food industry. However, attempts to solubilize these proteins whilst retaining functionality have

focused on the use of high ionic strength salt solutions (> 0.3 M). Few studies have investigated their solubility and techno-functional properties in water or in low ionic strength solutions. This review summarized the recent advances achieved in solubilizing MPs in low ionic strength solutions（< 0.2 M). The main procedures described, included water washing and dilution, washing with low salt-pH adjusted solution and water dilution, washing with low salt-L-histidine solution combined with ultrasonication, acid or alkaline solubilization, protein glycation and high pressure processing. Comprehensive discussions of the basic principles and on the functional properties of MPs prepared by each method are presented.

Solubilization of MPs in water is a topic of interest for food scientists and technologists since, as it would enable recovery of MPs with unique and enhanced functional properties without the use of high content salts. Therefore, this is an area of research with potential use in the food industry for production of low-salt, high value products from red meat, poultry and fish muscle, or their muscle by-products.

Future considerations are described as followings:

▪ Further fundamental information on the behavior of MPs solubilized in water, or at low ionic strength, together with their functionality in food systems is required to gain a thorough understanding of the relationship between the structure of MPs and their functional properties, thereby enabling their use for the food industry as ingredients to improve functional properties of food products. Of particular interest is the application of acid-alkaline solubilization method and

the glycation method to prepare water soluble MPs from poultry and fish by-products or quality defect meat (e.g. PSE-like meat) so as to create food ingredients with excellent functionality and great value addition in different food systems.

- The recovery of MPs from poultry and fish in low ionic strength solutions for their utilization as food ingredients is not yet industrially available or even possible. To overcome this, further optimization of the process conditions is required to make it feasible on an industrial scale.

- Additionally, in view of the drawbacks of the current procedures in solubilizing MPs in water or low ionic strength solutions, new techniques which are simple, safe, eco-friendly, easily to control, and having high efficiency should be developed. These processes need to consider not only their water solubility but the MPs should have high stability together with improved functional properties and with minimum protein degradation. Recent novel and emerging technologies in food science may provide the necessary guidance.

Regarding functionality, production of water soluble MPs would open up a promising area of research for development of a wide range of meat products. For example, the water-soluble MPs could be used for developing new food items such as protein-rich beverages, nutritional emulsifiers, and provide an excellent source of proteins for dysphagia patients and for those with difficulty to masticate foods.

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Table 1 Methods used for solubilizing myofibrillar proteins in water or low ionic solution.

Method	Source of	Main process	Advantages	Disadvantages
Water washing and dilution	Fish	Three times water washing and water dilution (< 0.3 mM) at neutral pH	High protein solubility No additives like salt required. Inexpensive process	Difficulty in low ionic strength adjustment process Large amount of water required
Washing with low salt-pH adjusted solution and water dilution	Beef, chicken and mackerel light muscle	Four times washing with 2.5-25 mM NaCl, pH 7.4 solution and water dilution (< 30 mM) at neutral	Relative high protein solubility No catalyst is required Low environmental impact	Difficulty in low ionic strength adjustment process Large amount of water required
Washing with low salt-L-histidine solution and ultrasonication	Chicken, pork, beef and lamb	Repeated washing with 2.5 mM NaCl and 5 mmol/L L-histidine solution and ultrasonication in	Relative high protein solubility Solubilization effect regardless of the species	Complicated process Equipment complexity Non-uniform treatment Possible thermal
Acid or alkaline solubilization	Fish, chicken, pork, beef and lamb	Solubilizing muscle proteins under low pH (2.5-3.5) or high pH (10.5-11.5) conditions	High protein solubility Removal of membrane lipids	Necessity of isoelectric precipitation to recover the protein Metal corrosion caused
Protein glycation	Fish, chicken	Protein modification by covalent attachment of carbohydrates through the Maillard reaction in dry-state conditions or	Increased protein solubility Improvement of thermal stability and emulsion-forming ability	Difficulty in controlling the reaction Possible protein denaturation Undesirable color changes Possible formation of antinutritional
High pressure of muscle, homogenates, batters or isolated myofibrils	Beef and lamb (also reported for pork, turkey and chicken but not included here)	Application of pressure at 100 to 300 MPa (10 to 20°C) followed by extraction with low and high ionic strength salts	Moderate protein solubility in low salt solutions Improved gelling quality Can be used in combination with other extraction methods	Expensive process Specific pressures, temperatures and times required

Table 2 Summary of effect of glycation on solubility of myofibrillar proteins and other functional properties.

Conjugate(s)	Common name (species)	Reaction conditions	Solubility in low ionic strength solution	Other functionality	Reference
Myofibrillar protein- glucose	Carp (*Cyprinus carpio*)	1:9, 40-60 °C, a_w 0.65, 0-48 h	Increase in solubility in 0.01-0.16 M NaCl , 63% in 0.1 M NaCl, pH 7.5	Improvement of Emulsifying Properties	(Saeki, 1997; Saeki and Inoue, 1997)
Myofibrillar protein-ribose	Carp (*Cyprinus carpio*)	1:15, 30 °C, a_w 0.65, 0-8 h	Increased solubility in 0.05-0.16 M NaCl, 73% in 0.16 M NaCl, pH 7.5	-	(Saeki and Tanabe, 1999)
Myofibrillar protein- alginate oligosaccharide	Carp (*Cyprinus carpio*)	Ratio 1:0.4, 1:1,1:4, and 1:9 , 40-50 °C, a_w 0.35-0.65, 0-120 h	Increase in solubility in low-ionic-strength media, more than 80% in 0.05 M NaCl	Improvement of thermal stability and emulsifying	(Sato et al., 2003; Sato et al., 2000; Sato et
Myosin-glucose	Carp (*Cyprinus carpio*)	1:36, 30-40 °C, a_w 0.65, 0-50 h	96% in 0.1 M NaCl, pH 7.5	-	(Tanabe and Saeki, 2001)
Myosin-ribose	Carp (*Cyprinus carpio*)	1:15, 30-40 °C, a_w 0.65, 0-10 h	91% in 0.1 M NaCl, pH 7.5	-	(Tanabe and Saeki, 2001)
Myofibrillar protein-glucose	Scallop (*Pecten yessoensis*)	1:18, 40-60 °C, a_w 0.05, 0.35,0.65, 0.95, 0-50 h	More than 80% in 0.1 M NaCl, pH 7.5	-	(Katayama and Saeki, 2004; Katayama et
Myosin-alginate oligosaccharide	Carp (*Cyprinus carpio*)	1:1, 40 °C, aw 0.35, 0-48 h	More than 90% in 0.1 M NaCl, pH 7.5	Improvement of thermal stability	(Maitena et al., 2004)
Myosin-glucose, maltose, and maltotriose	Carp (*Cyprinus carpio*) Scallop (*Pecten yessoensis*)	-, 50 °C, aw 0.35, 0-72 h	Improved solubility in 0.1 M NaCl, pH 7.5	-	(Katayama et al., 2004)

Myofibrillar protein- alginate oligosaccharide	Salmon (*Oncorhynchus keta)*	1:1, 60 °C, aw 0.05, 0.35,0.65, 0.95, 0-3 h	Improved solubility in 0.1 M NaCl, pH 7.5	High stability during storage	(Takeda et al., 2007)
Myofibrillar protein-glucose or maltose	Chicken breast	1:4, 1:5, 1:6, 1:9, and 1:18 for glucose, 1:3, 1:4, 1:5, and 1:6 for maltose, 60 °C, aw 0.35, 0-6 h	More than 60% in 0.1 M NaCl, pH 7.5	Improvement of thermal stability Antioxidative	(Nishimura et al., 2010, 2011)
Actomyosin-glucosamine	Chicken breast	1:1, 1:3, 25 or 37 °C, 6 h in liquid mixtures by transglutaminase-catalyzed glycosylation	increased from 8.7 to 34% in water at isoelectric point (pI)	Improvement of emulsifying activity and	(Hrynets et al., 2014)

Not mentioned

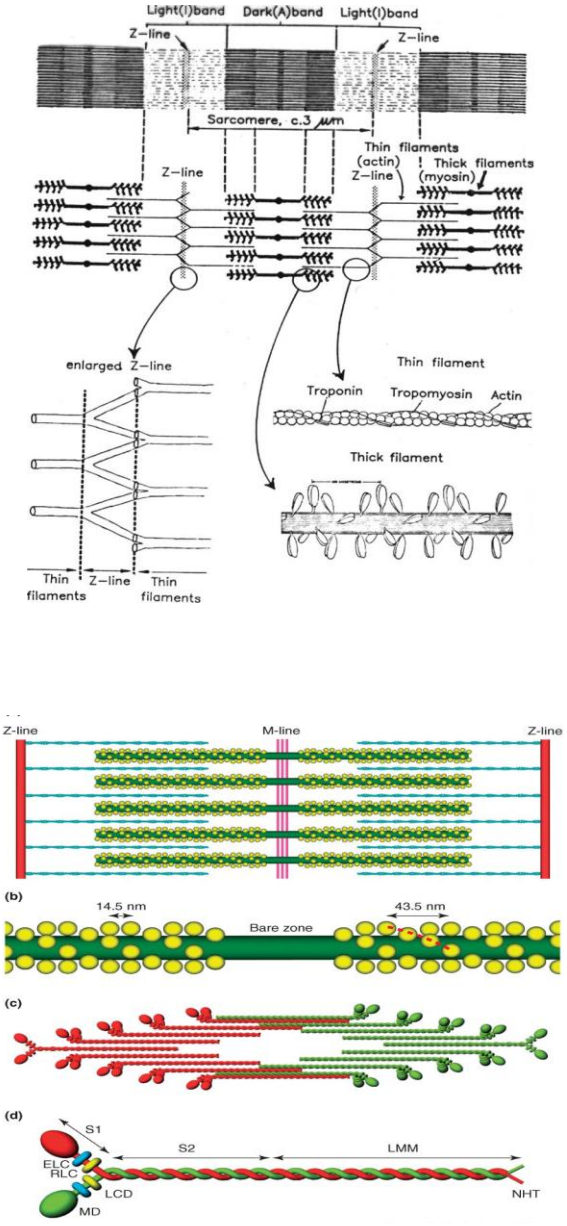

Fig. 1 (A): Myofibrillar structures showing thin and thick filaments. Source: adapted from (Tornberg, 2005). (B): Myosin and thick filaments. Source: adapted from (Craig & Woodhead, 2006).

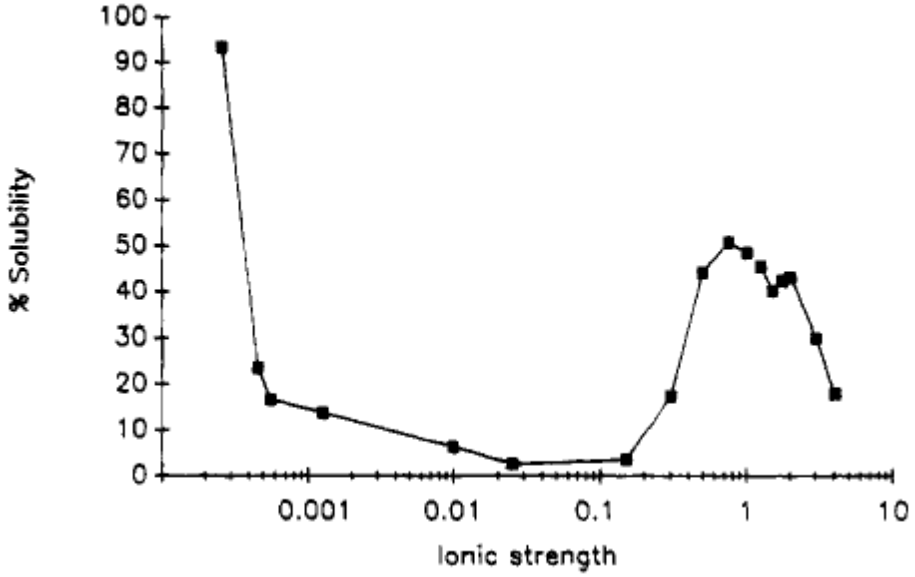

Fig. 2 Effect of ionic strength on the solubility of myofibrillar proteins from cod muscle.

Source: adapted from (Stefansson & Hultin, 1994).

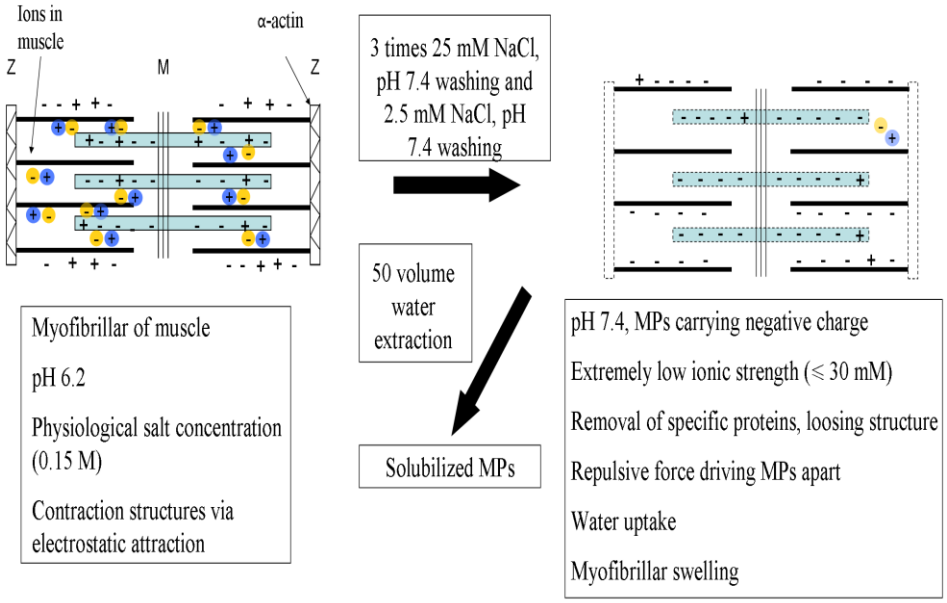

Fig. 3 Proposed mechanism of the solubilization of MPs in water by washing with low salt-pH adjusted solution followed by dilution in water.

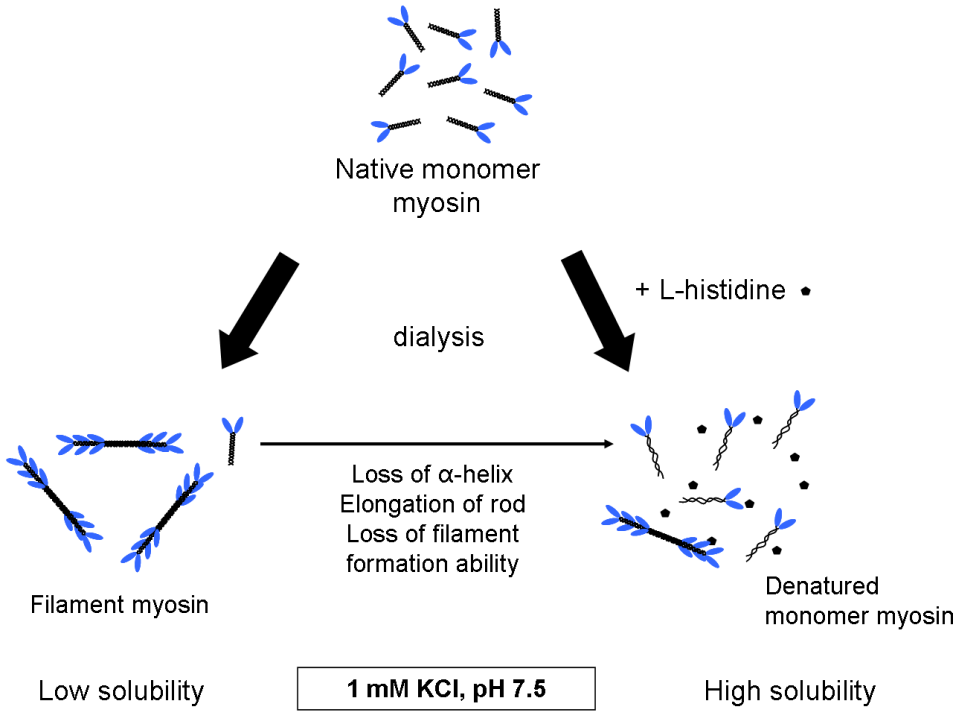

Fig. 4 Proposed mechanism for the solubilization of myosin in solutions of low ionic strength (1 mM KCl, pH 7.5) in the presence of 5 mM L-histidine

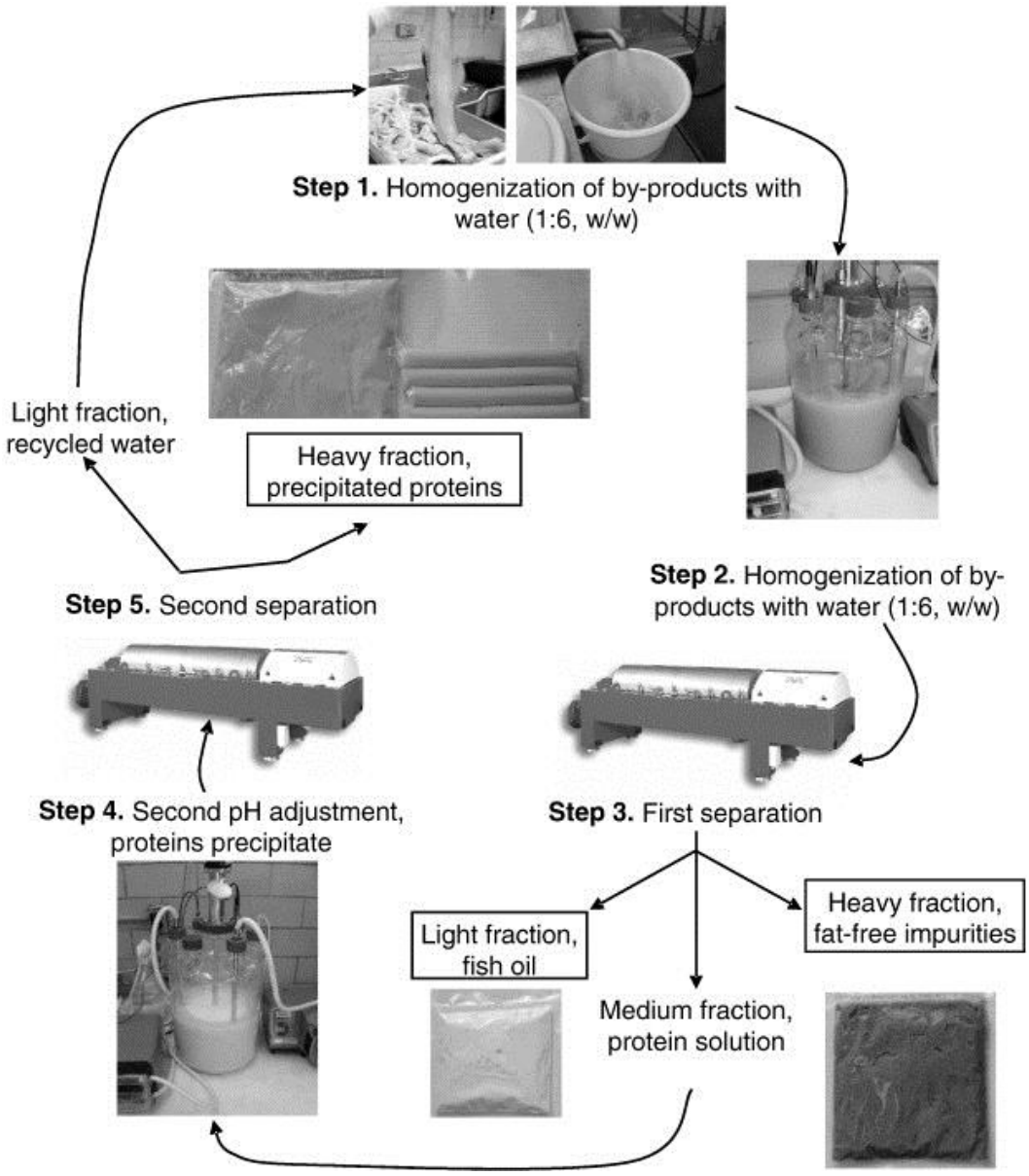

Fig. 5 Schematic of the acid or alkaline solubilization/precipitation technology proposed for fish processing by-products.Source: adapted from(Tahergorabi & Jaczynski, 2014)

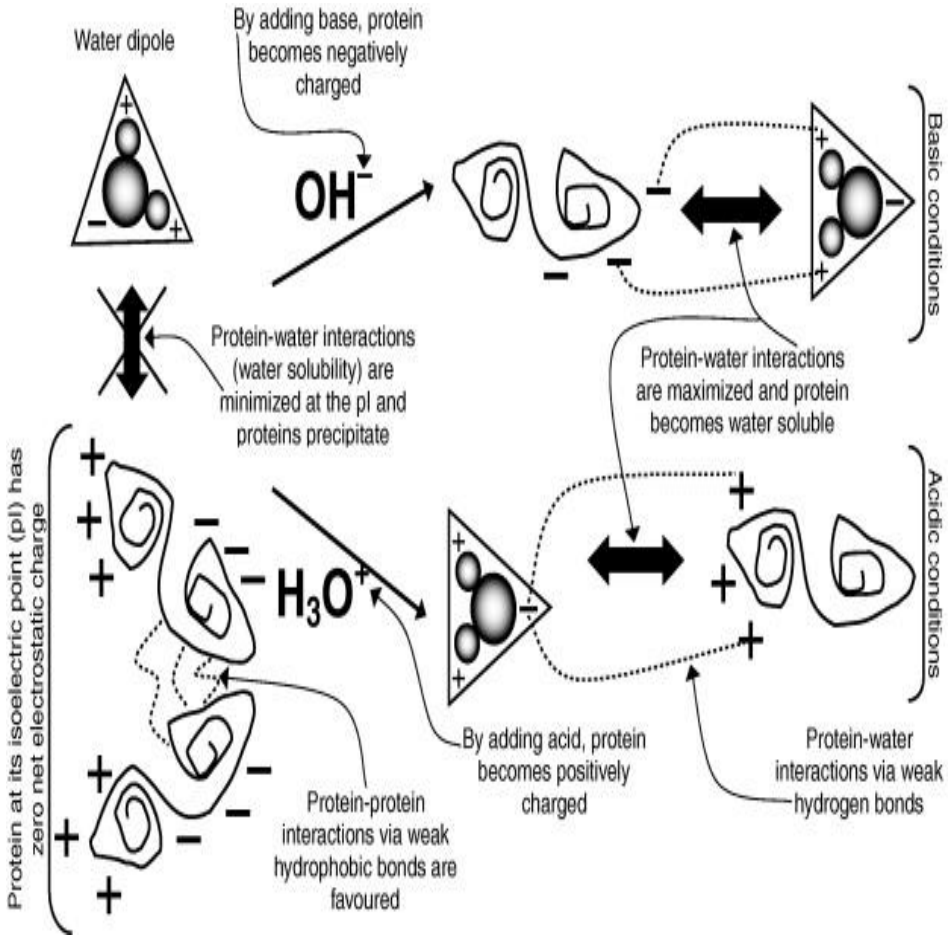

Fig. 6 Schematic showing the biochemical basis for acid or alkaline solubilization. Protein-water interactions prevail under acidic or basic conditions distant from the pI, resulting in protein solubility in water.Source: adapted from (Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2011)

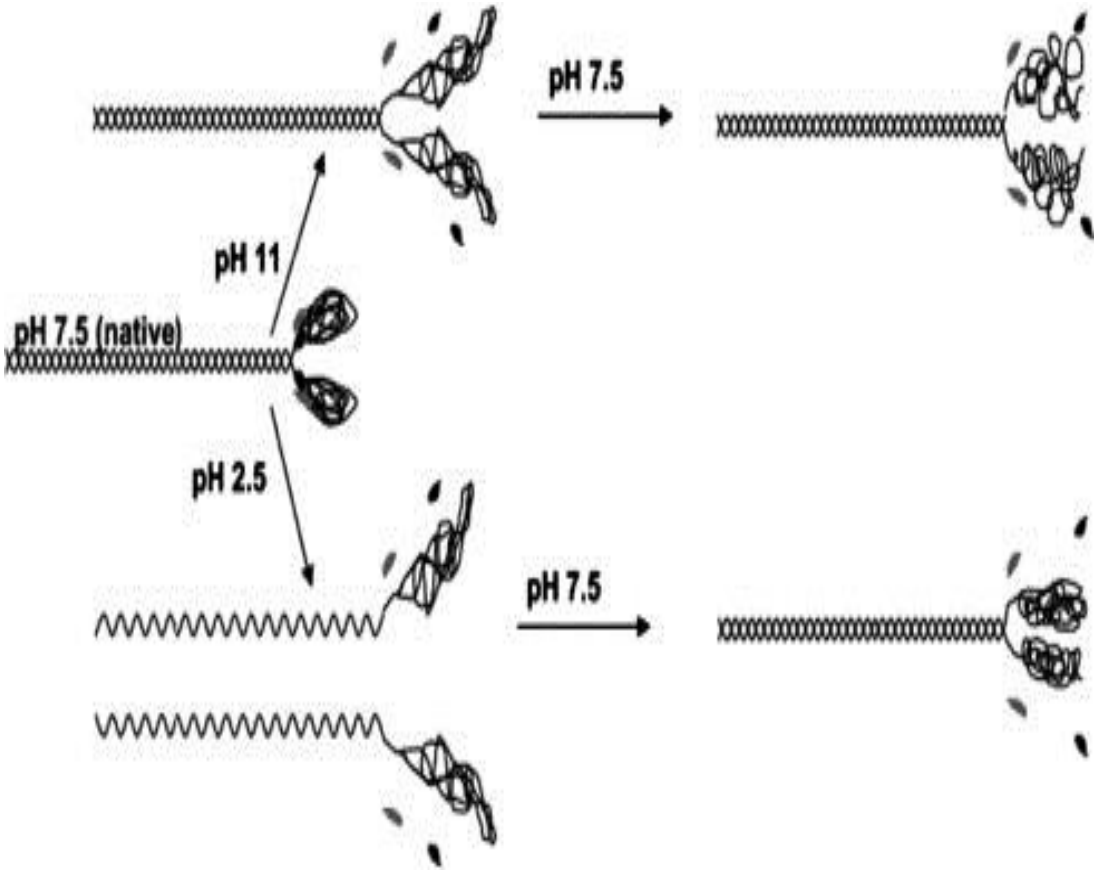

Fig. 7 The pH-induced changes in the 3-dimensional structure of fish myosin during acid or alkaline solubilization. Source: adapted from (Kristinsson & Hultin, 2003)

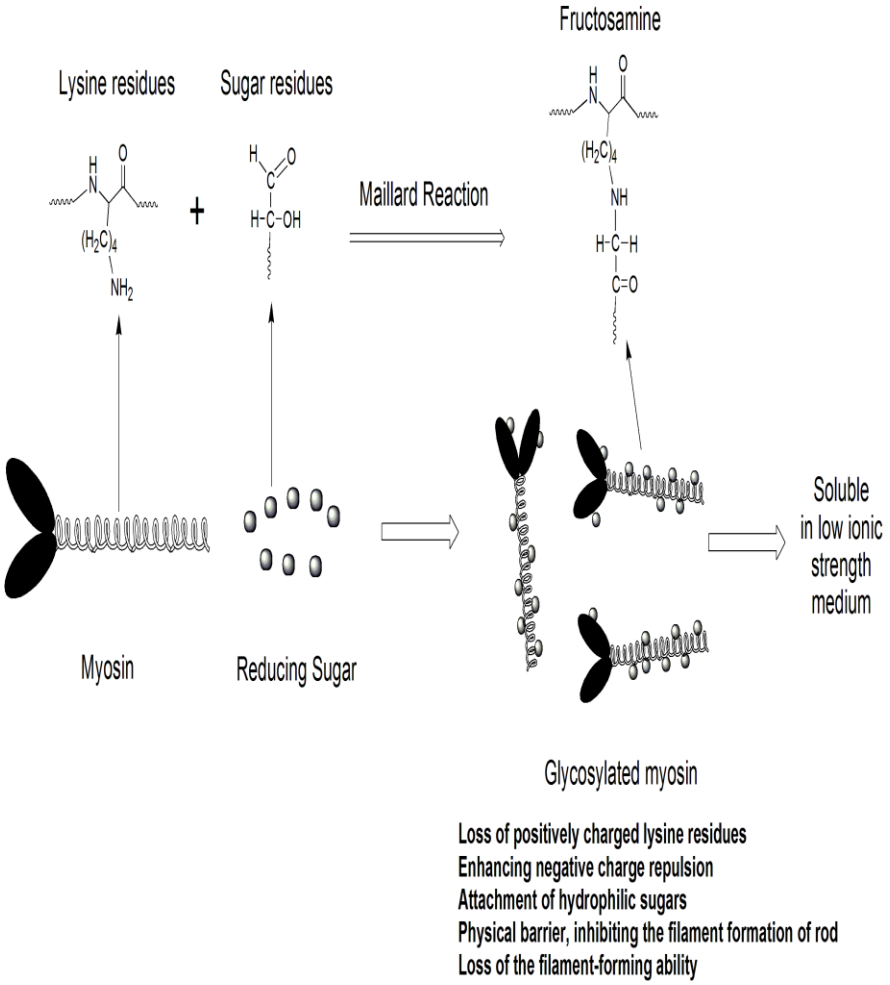

Fig. 8 Proposed mechanism for the solubilization of myosin at low ionic strength medium by glycation.