ABSTRACT

FULLER, GEORGE THOMAS. Improving the Functional Properties of Dried Meat Proteins. (Under the direction of Dr. Tyre C. Lanier.)

Meat proteins are currently not used as functional food ingredients due to significant loss of functionality which occurs during drying. However, a functional dried meat protein ingredient may provide significant quality and cost benefits to food manufacturers. In this study, the effects of alkaline pH adjustment (up to pH 11, to effect greater charge separation of proteins) and trehalose (a known lyoprotectant) during freeze drying were studied on the gelation properties and viscosity of rehydrated freeze-dried chicken breast meat powders. Aqueous dispersions of 3% w/w protein were tested for viscosity by shear rate sweep and mixed protein gels of 6% w/w freeze-dried chicken breast meat protein and 6% w/w raw chicken breast meat protein were tested for held water, cook loss and fracture properties by torsion analysis. These were compared to control gels produced from 12% w/w raw chicken breast meat with the same solution conditions. Inclusion of trehalose before freeze drying increased the gel properties for all pH treatments. Gels prepared from proteins dried at pH 7.2 and 9.0 with freeze-dried chicken breast meat protein actually had superior gel properties compared to those of gels made from raw (non-dried) chicken breast meat under similar conditions. Improved dispersion of the freeze-dried proteins compared to raw, non-dried meat was proposed as the mechanism responsible for the improvement in gel properties. This study indicates that utilizing the combination of added lyoprotectant with a process step to increase protein dispersibility can significantly improve the functionality of dried meat powders.

Improving the Functional Properties of Dried Meat Proteins

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Master of Science

Food Science

Raleigh, North Carolina

2011

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DEDICATION

To Mom, Dad, Matt, Adam and Robert. Thank you for your immutable love and support. It is always with me. To my wife and love, thank you for being you.

BIOGRAPHY

Thomas Fuller has been very lucky. He was born and raised in the Baynes Community in the town of Anderson, NC. He spent much of his youth outdoors in the forests, fields and ponds of his home. His father taught him how to be a perfect person. No other wiser, hardworking, supportive, devoted or fun father has existed. His mother's love and devotion taught him about what is really important in life. Thomas earned an AAS in Culinary Arts from Johnson & Wales University in Charleston, SC. He then earned a BS in Food Science from NC State University. Thomas also completed an internship at the Wizard's Cauldron in Yanceyville, NC. During these periods, Thomas worked continuously as a cook and chef in Charleston, SC and Raleigh, NC. In 2009, he began his MS degree in Food Science under the supervision of Dr. Tyre Lanier. During this time, Thomas completed a summer internship with Frito-Lay. On June 18th 2011, Thomas married Esra Cakir. Next, Thomas will pursue a PhD in Food Science at Massey University in Palmerston-North, New Zealand.

ACKNOWLEDGEMENTS

Penny, thank you for your friendship and support over the past few years. I am very lucky to have been able to spend time with you. Best of luck finishing out your career at NC State. I will miss our time together. Dr. Lanier, thank you for supporting me over the years and giving me a shot as a graduate student. You have given me so many opportunities to excel as a young researcher. I will be forever grateful for your contributions to my life. FBNS faculty and staff, thank you for an amazing 6 years full of awesome experiences and expert education. Chef Bruce, your confidence in me still perplexes me. You always treated me differently from the very beginning and it did not go unnoticed. Thank you for years of support, stories, great times and so many memories. I miss work not because of the work but because of the great people I worked with. My family, thank you for everything. Esra, I love you so much. I will celebrate you for the rest of my life.

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INTRODUCTION

In recent years, protein rich powders produced from milk, legume and egg sources have become staple functional ingredients in many manufactured food products. These ingredients provide food manufacturers with a concentrated source of functional protein that can significantly improve product quality. Although proteins from muscle represent the largest source of protein in the American diet (Smit and others 1999), they are not dried for use as functional ingredients mainly due to poor retention of functionality when dried (Mackie 1994).

For example, surimi is a frozen commodity, traded world-wide, that is comprised mainly of refined fish myofibrillar (muscle) protein and is a key ingredient in many manufactured seafood products as it is the main gelling (heat-induced) component. The cost of surimi is greatly influenced by the requirement to ship and store a product containing ~75% moisture in a frozen state (Carvajal and others 2005). Considerable effort was mounted in Japan beginning in the late 1960s to develop a highly functional surimi powder (Niki and others 1992), but with little commercial success.

The most widely utilized functionality of meat proteins is their ability to gel when heated. Chopping meats with salt causes breakdown of the intricate structure in muscle and results in dispersion of individual proteins which then more freely associate upon heating to form strong and deformable gel structures. Alternately, the muscle proteins can be liberated from the muscle structure by chopping and introducing high net charge, by shifting the pH well away from the pI.

The loss of gelling functionality that can accompany drying of proteins is generally attributed to aggregation induced by protein denaturation. This is of course exactly why heating, under the appropriate conditions (dispersed proteins), leads to gelation, but in the case of drying the aggregation occurs prematurely and is undesirable. Denaturation (unfolding) of a protein exposes hydrophobic groups, previously buried in the interior of the folded protein. Hydrophobic association of neighboring protein molecules drives the initial formation of aggregates (Walstra 2003a).

Now standard practice, the addition of sugars and sugar alcohols to surimi was introduced in the early 1960s (Okada 1992) for the purpose of preserving functional properties during freezing (cryoprotection). Essentially, cryoprotectants function to maintain the proteins in a more native, folded state, such that they are less reactive in protein-protein bonding. Disaccharides, which are good cryoprotectants, also can function as lyoprotectants during freeze-drying, but by a different mechanism than in cryoprotection.

While cryo-/lyoprotectants act to stabilize the less reactive folded state of proteins, electrostatic repulsion can also be introduced to further inhibit aggregation. For example, extremes in pH induce high net charge which inhibit protein association, and thus minimize aggregation.

The goal of this study was to investigate how elevated pH might affect the heat-induced gelling functionality of chicken meat proteins when freeze-dried with and without trehalose as a cryo/lyoprotectant. Our hypothesis was that protein unfolding during freezing and drying could possibly be minimized by trehalose addition, while protein association leading to aggregation might be minimized by inducing high charge separation of proteins.

Chicken, rather than fish, meat was chosen as the test material since the proteins of muscle from homeotherms is more stable than that from poikilotherms, thus enhancing our chance of success in producing functional dried meat protein. Likewise freeze-drying was chosen because no heating of proteins is required for drying to occur. If the stabilizing principles being applied in this study proved useful in freeze-drying meat proteins, perhaps they might also be helpful when using drying methods that employ heating.

REVIEW OF LITERATURE

1. Comminuted meat products

Comminuted meat products like sausages and hotdogs are prepared from raw meat by first chopping with salt and water to make a paste, then cooking to form a gel. Here, salt functions as the protein 'activating' agent as the cations and anions associate with the charged groups of meat proteins (Gordon and Barbut 1992). This increases the electrostatic repulsion between proteins resulting in 'swelling' of the myofibrils caused by the uptake of water (Offer and Trinick 1983). The swelled myofibrils fragment to some extent during chopping resulting in liberation of individual myosin/actomyosin molecules from the myofibril and increased protein-water interactions. Upon heating, myosin and to a lesser extent actin contribute to the formation of a gel matrix that entraps water and fat. The gel forming ability is regulated by many factors including the protein structure, protein concentration, muscle type and animal source as well as processing conditions like pH, ionic strength and heating rate (Xiong and others 1999).

1.1. Effects of salts and phosphates

Gel properties such as fracture stress, fracture strain and water/fat holding capacity of heat-set protein gels are significantly influenced by the microstructure. Fine-stranded gels are characterized by a smooth appearance, high water holding capacity, and high deformability/strength whereas particle gels exhibit a grainy appearance, lower water holding capacity, and lower deformability/strength (Langton and Hermansson 1992). Fine-stranded gels generally form far from the isoelectric point (pI) at low ionic strength while particulate gels form near the pI at high ionic strength (Walstra 2003a). The dependence of the gel properties on microstructure has been well described for globular proteins like whey

(Foegeding and others 1995) but not for comminuted meat gels. Only the microstructures of gels prepared from purified myosin have been described (Hermansson and others 1986; Hermansson and Langton 1988).

Typical conditions for the study of comminuted meat gels roughly correspond to industrial practice, employing a pH range of 5.5-7.5, salt content (typically NaCl) of 1-3% w/w and phosphate addition up to 0.5% w/w (US legal limit). Some discrepancy exists in the literature, however, as to the effects of pH and ionic strength on meat protein gelation. For example, when pH is held constant comminuted poultry meat gels (frankfurters) exhibit higher strength and deformability values with increasing salt content (Amato and other 1989; Barbut and others 1988). At constant salt content, gel properties also improve when pH is increased in comminuted turkey breast meat gels (Barbut 1997; Daum-Thunberg and others 1992). However, if gels are prepared from washed, minced chicken breast meat (WMCBM), the effects of pH and ionic strength change. Chang and others (2001a) demonstrated that fracture stress and strain values of gels made at neutral pH from WMCBM changed relatively little when NaCl content was 0.15%, 0.88% or 2.5%. However, WMCBM gels prepared with 0.88% NaCl were higher in fracture stress and strain when prepared at neutral pH compared to those at pH 6.0-6.5. Feng and Hultin (2001) also showed that fracture stress and strain of gels increased as pH increased from 6.4-7.0 at 0.15 M NaCl for gels prepared from WMCBM.

Fish isolates produced by isoelectric precipitation also exhibit an altered response to ionic strength compared to comminuted meat gels. For example, Tadpitchayangkoon and Yongsawatdigul (2009) found that the addition of NaCl improved deformation but lowered breaking force of fish protein isolate gels. This study and others have speculated that an

altered response to NaCl may result from protein modification induced by the pH changes during processing, removal of sarcoplasmic proteins, breakdown of the z-line to release myofibrillar proteins into better dispersion or concentration of myofibrillar proteins by the process (Davenport and Kristinsson 2011; Tadpitchayangkoon and Yongsawatdigul 2009; Kim and Park 2008; Wright 2007).

Knipe and others (1985) found that phosphates improved the water holding capacity of meat pastes made at low salt concentrations, implying that added phosphate could promote meat protein solubilization/dispersion even when salt levels were present at levels typically insufficient to be effective. Since most phosphates are alkaline, they raise the pH of meat gels further from the isoelectric point to enhance charge separation of the proteins and thereby contribute to formation of a fine-stranded structure (Knipe 2004). Yasui and others (1964) also indicated that pyrophosphate promotes dissociation of actomyosin and attributed this effect to its ability to improve meat gelation. Polyphosphates are also effective, but apparently only dissociate actomyosin upon their hydrolysis by meat phosphatase enzymes to pyrophosphate.

Few studies have tested the effects of phosphates at concentrations higher than 0.5%. Kijowski and Mast (1988) studied the effects of 1-4% NaCl and/or 0.25-1% pyrophosphate (PP) or tripolyphosphate (TPP) on the thermal stability of water-washed myofibrils and finely cut chicken breast muscle. They reported that both phosphates enhanced thermal stability of myosin at 0.25 and 0.5% but destabilized it at greater concentrations. Shiau and Pan (1981) studied leached, minced mackerel gels with added 2.5% salt, 10% cassava starch and 0-10% added pyrophosphate. The greatest breaking force for gels was found when

pyrophosphate concentration was between 0.3-0.5% at pH ~6.2. Increasing pyrophosphate with increasing pH decreased breaking force values. For example, 10% added pyrophosphate at pH 8.0 reduced breaking force by roughly half compared to the highest value. Without added pyrophosphate, the breaking force was highest at pH 6.6 and decreased with increasing pH.

1.2. Effects of protein dispersion

Historically most researchers have contended that strong and deformable comminuted meat gels are produced with the addition of 0.3-0.6 M NaCl due to increased solubilization of the myofibrillar proteins. However, research by Chang and others (2001a, 2001b) indicated that good quality gels can be formed at low ionic strength despite low myofibrillar protein solubility when prepared using a method involving homogenization of the muscle in 10 parts water. Other studies (Hayakawa and others 2010; Ito and others 2003; Krishnamurthy and others 1996; Stefansson and Hultin 1994) showed that significant amounts of myofibrillar proteins can even be solubilized in low ionic strength solutions when prepared under certain conditions. Together, these studies provide evidence that the effect of salt on meat gels changes depending on the gel preparation method. Fish isolate gels prepared by isoelectric precipitation have also been shown to produce gels of similar or better quality when prepared without added salt (Kim and Park 2008; Davenport and Kristinsson 2011). The major commonality between studies that show an altered effect of ionic strength on gel forming ability is significant dilution and dispersion of the myofibrillar proteins in water before gelation. The effect of better myofibrillar protein dispersion has been found to be beneficial for comminuted and diluted/dispersed meat gels. Using electron microscopy, Sato and

Tsuchiya (1992) showed that dispersion and uniform distribution of myofibrillar proteins results in improved gelling quality of comminuted surimi gels. Wright (2007) studied two methods of protein dispersion on diluted and dispersed meat gels and confirmed that improved dispersion of myofibrillar proteins prior to gelation resulted in improved gel forming ability.

Taken together, the aforementioned research seems to indicate that dilution and dispersion of myofibrillar proteins in water prior to gelation influences the effect of salt. However, no research to date has studied diluted and dispersed meat proteins at different ionic strengths. If dilution and dispersion of meat proteins significantly changes the effect of salt on meat gels, then these proteins could provide novel functionality in many meat products.

2. Drying of meat proteins

Dehydration is obviously very beneficial to cost control and ease of use when it results in a shelf-stable product with reduced weight and volume (Brennan 1989). Powdered meat extracts are widely used by the food industry to provide flavor to snacks, bouillon cubes and instant sauces (Varnam and Sutherland 1995). However, the gelling functionality of meat proteins is generally regarded to be greatly reduced after dehydration (Mackie 1994). Yet if some acceptable level of gelling functionality could be achieved in meat powders, however diminished from the raw wet state, the cost saving benefits could still make meat powders attractive to the food industry as a substitute for raw meat ingredients.

2.1. Surimi drying

Surimi is fish meat protein that has been minced, water-washed, stabilized with cryoprotectants and frozen (Okada 1992), primarily for use as the key gelling ingredient in the production of surimi seafoods like Asian kamaboko and Western crab analog products. The expense associated with freezing, shipping and frozen storage of a product containing over 70% water led to studies by Japanese researchers in the late 1970's and 80's with the goal of producing a functional dried surimi. Niki and others (1992) highlighted research whereby surimi might be spray-dried (SD) or freeze-dried (FD) into a functional powder. Consistently, they found that FD powders retained greater functionality compared to SD powders. Niki and Igarashi (1982) showed that sucrose, sorbitol and glucose all increased functionality of the dried powder when added up to 25% on a dry weight basis prior to drying. It was also reported that neutral pH fish meat produced powder with greater functionality compared to more alkaline or acidic pH values in the range of approximately 6.0-9.0. In these studies, however, the use of empirical methods for functionality testing prevents comparison with contemporary research which favors the use of fundamental testing methods, especially for the measurement of gelling ability.

One possible problem in interpreting these reports is that gelling functionality was often inferred from measurements of myosin ATPase activity alone. For surimi manufactured by a conventional method, myosin ATPase activity has been shown to be predictive of gelling ability (Haard 1992). However, Kristinsson and Hultin (2003b) noted that it is also quite possible to measure excellent gelling properties when little ATPase activity is evident.

Niki and others (1983) measured water holding capacity (WHC) of rehydrated dried surimi by a centrifugal method (10,000 x g) over a pH range of 6.0-9.0 and found that WHC of the resulting cooked gels increased with increasing pH. Montejano and others (1994) indicated that cooked gels produced from FD tilapia surimi exhibited equivalent fracture shear stress and shear strain as did those made from frozen tilapia surimi. However, surimi gels made from FD trout were significantly less strong and deformable as compared to those made from frozen trout surimi. Research on the use of freeze-dried surimi as a functional ingredient in meat products is limited, although Leyva-Mayorga and others (2002) reported that freeze-dried surimi does improve the gelling properties of low-fat meat emulsions.

2.2. Fish powder

Results from studies on the production of fish powders parallel those conducted on surimi. Yoo and Lee (1993) demonstrated that the addition of sorbitol prior to drying resulted in nearly twice the gelling ability of FD red hake mince. Shaviklo and others (2010) reported that lyoprotectants significantly improved dried saithe protein and that freeze drying produced powders superior in gelling ability to spray drying. Chung and others (2000) tested the use of FD mackerel proteins as a binder for restructured meat and found that 0.1% reducing agents improved their binding ability as measured by tensile strength.

3. Freeze drying

Freeze drying (FD) is considered to be the least damaging method for drying proteins; for example, it is typically the method of choice for drying pharmaceutical proteins. The FD process begins by lowering the product temperature until the solvent (water in foods) freezes

and the product becomes hard. Next, frozen water is converted directly to vapor by lowering the atmospheric pressure around the product and supplying energy in the form of heat. The atmospheric pressure is lowered by a vacuum pump while water vapor is removed from the drying chamber using a low temperature condenser. Water vapor condenses on the condenser (<-60°C) as energy is removed from the water vapor. The sublimation process removes the solvent and leaves behind the desired solute.

Sublimation during FD is typically divided into two stages. Primary drying removes most of the ice present in a product and is characterized by a high sublimation rate and occurs until about 20-50% of the original water content remains. Secondary drying removes non-frozen water which is intimately bonded with the solute, thus requiring more time and/or energy to sublimate. In tray-type freeze driers, energy is typically transferred to the product through the shelf which the product rests on. This energy facilitates the conversion of ice water to vapor and increases the rate of sublimation (Pikal 1990; Franks 1990).

3.1. Freezing effects on proteins

The conformation of a protein is related to its functionality and is dependent on the chemical bonds which connect peptides together to form the primary, secondary, tertiary and quaternary structures. The chemical bonds are sensitive to environmental changes e.g. temperature, pressure, pH and ionic strength. Therefore, any change in the environment has the potential to alter the chemical bonds thus changing the conformation and as a result alter functionality (Walstra 2003a). Please note, conformational changes will be referred to as denaturation throughout this manuscript, however, denaturation is not intended to imply a loss of protein functionality. Denaturation is commonly used to impart functionality

(Grinberg and others 1993), however, excessive denaturation can lead to aggregation and loss of functionality (Walstra 2003a).

In FD, the freezing process can induce protein denaturation in several ways. First, lowering the temperature can induce what is known as cold denaturation. Privalov (1990) described this general protein unfolding mechanism as a temperature-dependent interaction of protein nonpolar groups with water, which increases with decreasing temperature. Upon cooling below the freezing point, much of the liquid water is converted to ice. The conversion of liquid water into ice may result in a substantial increase in solute concentration caused by segregation of the solute from the ice crystals into non-freezable water. This process is accentuated by a slow freezing rate, which better allows for redistribution of the solutes as water slowly crystallizes, whereas a more rapid freezing rate minimizes solute concentration because ice crystals form rapidly and can envelope solutes. Solute concentration normally results in increased ionic strength and pH which favor protein denaturation as well. Solute concentration can also lead to concentration dependent changes in the system, such as salt or sugar crystallization or protein aggregation, which may reduce functionality (Abdul-Fattah and others 2007).

Freezing rate also markedly affects the number and size of ice crystals which form during freezing. Rapid freezing promotes smaller, more numerous ice crystals, thus maximizing the ice surface area. Adherence of proteins to the more hydrophobic ice crystal surface (relative to water) can induce denaturation (Chang and others 1996). In general though, solute concentration effects are considered to be more perturbing than ice surface induced denaturation, so faster freezing is usually preferred (Abdul-Fattah and others 2007).

3.2. Dehydration effects on proteins

In freeze dried products, final moisture content typically ranges from 2-10% by weight. This level of dehydration results in the removal of ‘bound’ water which is intimately hydrogen bonded at the protein surface. Because protein structure is largely determined by its interaction with water, this dehydration can greatly destabilize the native structure. This in turn can lead to aggregation and loss of gelling functionality. According to the water substitution hypothesis (Carpenter and Crowe 1989), introduction of a surrogate hydrogen bonding agent may serve to stabilize the proteins as water is removed. They indicated that disaccharides are the agents of choice for this process.

3.3. Protein aggregation inhibition

Protein denaturing events during drying are thought to greatly contribute to aggregation and loss of functionality. Aggregation is considered to be predominantly hydrophobically driven. Denaturation or protein unfolding generally results in greater exposure of hydrophobic groups which in an aqueous environment leads to aggregation (Cleland and others 1993). During heating, heat-set gels undergo denaturation, followed by aggregation. To produce firm and elastic gels, the rate of aggregation should be slower than the denaturation rate (Belitz and others 2004). This allows time for the exposure of reactive groups before protein-protein interactions take place. Excessive protein-protein interactions which occur in aggregated proteins contribute to low quality gels by decreasing the interaction of proteins with water. By increasing net charge of proteins, greater electrostatic repulsion occurs and has been shown to inhibit aggregation leading to firmer gels with higher WHC (Picone and others 2011). Cleland and others (1993) noted how aggregation

proliferates near the pI of proteins and away from the pI in the presence of high ionic strength solvents due to charge neutralization. Using cod myosin, Brenner and others (2009) showed aggregate size decreased with increasing pH (6.0-8.0) after heating and cooling.

4. Lyoprotection

To inhibit freezing and dehydration induced protein denaturation, lyoprotectants are added to freeze-dried protein formulations. Many lyoprotectants are available; however, only disaccharides stabilize proteins to freezing and dehydration denaturation (Arakawa and others 2001). In aqueous solutions, the stabilization of proteins in the presence of sugars is the result of preferential hydration of the proteins which results in an inhibition of denaturation during perturbation (Lee and Timasheff 1981, Arakawa and Timasheff 1982). Arakawa and Timasheff (1982) attributed enhanced protein stabilization in the presence of lactose and glucose to an increase in the surface tension of water, exclusion volume of the sugars and the chemical nature of the protein surface. The increase in surface tension of the water coupled with exclusion from the protein surface appears to make protein unfolding require more energy and thus stabilize the protein. Semenova and others (2002) noted that this preferential hydration mechanism does not, however, explain many phenomena observed in protein-sugar solution experimental data such as an increase in protein solubility and inhibition of protein aggregation to name a few. These phenomena may be explained by the partial hydrophobicity of disaccharides, however**.** Vagenende and others (2009) proposed that glycerol may act as an amphiphilic interface between hydrophobic regions on a proteins

surface and the polar solvent thus inhibiting hydrophobically driven aggregation. The authors further postulated that this mechanism may be valid for other polyols as well.

For disaccharides, two mechanisms are generally accepted for their stabilizing ability of dehydrated proteins. The first involves an increase in the glass transition temperature upon lyoprotectant addition. All molecules present in an amorphous, glassy material are hindered in terms of molecular motion and therefore stabilized to denaturation (Levine and Slade 1992). The second mechanism involves the lyoprotectant serving as a replacement hydrogen bonding partner when 'bound water' is removed from the protein during dehydration (Carpenter and Crowe 1989). Generally, a lyoprotectant 'cocktail' is preferred where certain ingredients are added to raise the glass transition temperature while others are included to stabilize the protein to dehydration. Allison and others (2000) showed how the addition of a high molecular weight carbohydrate (dextran) for the purpose of raising the glass transition temperature, combined with sucrose or trehalose resulted in greater storage stability than either alone.

4.1. Trehalose

Trehalose is one of many polyols which stabilize proteins to virtually all stresses in aqueous solution and research indicates that trehalose may be the single best lyoprotectant available. Many forms of life are capable of surviving in a dehydrated state by producing disaccharides, especially sucrose and/or trehalose, which inhibit cellular and enzymatic damage (Crowe and others 1998). In terms of the glass transition temperature (T_g), trehalose does possess the highest compared to other disaccharides (Furuki and others 2009; Jain and Roy 2009). In addition, trehalose can form a unique dihydrate crystal. This crystal formation

may explain why trehalose glasses are less susceptible to depression of the T_g by small amounts of water (Crowe and others 1998; Kilburn and others 2006). However, research has shown that formation of a glass alone does not explain the stability of proteins. Trehalose and other polyols have also been shown to hydrogen bond directly with proteins after freeze drying causing improved protein stability (Allison and others 1999; Carpenter and Crowe 1989). According to Jain and Roy (2009), the exceptional stabilization of proteins by trehalose is due to the sum of many positive benefits which no other polyol can match.

5. References

Abdul-Fattah AM, Kalonia DS, Pikal MJ. 2007. The challenge of drying method selection for protein pharmaceuticals: Product Quality Implications. J Pharm Sci 96(8):1886-1916.

Allison SD, Chang B, Randolph TW, Carpenter JF. 1999. Hydrogen bonding between sugar and protein is responsible for inhibition of dehydration-induced protein unfolding. Arch Biochem Biophys 365(2):289-98.

Allison SD, Manning MC, Randolph TW, Middleton K, Davis A, Carpenter JF. 2000. Optimization of storage stability of lyophilized actin using combinations of disaccharides and dextran. J Pharm Sci 89(2):199-214.

Amato PM, Hamann DD, Ball HR, Foegeding EA. 1989. Influence of poultry species, muscle groups, and NaCl level on strength, deformability, and water retention in heat-set muscle gels. J Food Sci 54(5):1989.

Arawaka T, Prestrelski SJ, Kenney WC, Carpenter JF. 2001. Factors affecting short-term and long-term stabilities of proteins. Adv Drug Deliver Rev 46:307-26.

Arakawa T, Timasheff SN. 1982. Stabilization of protein structure by sugars. Biochemistry-US 21:6536-44.

Barbut S. 1997. Microstructure of white and dark turkey meat batters as affected by pH. Brit Poultry Sci 38:175-82.

Barbut S, Maurer AJ, Lindsay RC. 1988. Effects of reduced sodium chloride and added phosphates on physical and sensory properties of turkey frankfurters. J Food Sci 53(1):62-6.

Belitz HD, Grosch W, Schieberle P. 2004. Food Chemistry. 5th ed. Berlin: Springer-Verlag Berlin Heidelberg. p 62-3.

Brennan JG. 1989. Dehydration of foodstuffs. In: Hardman TM, editor. Water and Food Quality. New York: Elsevier Science Publishers LTD. p 33-70.

Brenner T, Johannsson R, Nicolai T. 2009. Characterization of fish myosin aggregates using static and dynamic light scattering. Food Hydrocolloid 23:296-305.

Carpenter JF and Crowe JH. 1989. An infrared spectroscopic study of the interactions of carbohydrates with dried proteins. Biochemistry-US 28:3916-22.

Carvajal PA, Lanier TC, MacDonald GA. 2005. Stabilization of proteins in surimi. In: Park JW, editor. Surimi and Surimi Seafood. 2nd ed. Boca Raton, FL: CRC Press. p 163-225.

Chang BS, Kendrick BS, Carpenter JF. 1996. Surface-induced denaturation of proteins during freezing and its inhibition by surfactants. J Pharm Sci 85(12):1325-30.

Chang H, Feng Y, Hultin HO. 2001a. Role of pH in gel formation of washed chicken muscle at low ionic strength. J Food Biochem 25:439-57.

Chang H, Hultin HO, Dagher SM. 2001b. Effect of $MgCl_2$/sodium pyrophosphate on chicken breast muscle myosin solubilization and gelation. J Food Biochem 25:459-74.

Chung Y, Ho M, Chyan F, Jiang S. 2000. Utilization of freeze-dried mackerel (*Scomber australasicus*) muscle proteins as a binder in restructured meat. Fisheries Sci 66:130-5.

Cleland JL, Powell MF, Shire SJ. 1993. The development of stable protein formulations: A close look at protein aggregation, deamidation and oxidation. Crit Rev Ther Drug 10(4):307-77.

Crowe JH, Carpenter JF, Crowe LM. 1998. The role of vitrification in anhydrobiosis. Annu Rev Physiol 60:73-103.

Daum-Thunberg DL, Foegeding EA, Ball HR. 1992. Rheological and water-holding properties of comminuted turkey breast and thigh: Effects of initial pH. J Food Sci 57(2):333-7.

Davenport MP, Kristinsson HG. 2011. Channel catfish (*Ictalurus punctatus*) muscle protein isolate performance processed under different acid and alkali pH values. J Food Sci 76(3):240-7.

Feng Y, Hultin HO. 2001. Effect of pH on the rheological and structural properties of gels of water-washed chicken-breast muscle at physiological ionic strength. J Agric Food Chem 49:3927-35.

Foegeding EA, Bowland EL, Hardin CC. 1995. Factors that determine the fracture properties and microstructure of globular protein gels. Food Hydrocolloid 9(4):237-49.

Furuki T, Oku K, Sakurai M. 2009. Thermodynamic, hydration and structural characteristics of alpha, alpha-trehalose. Front Biosci 14:3523-35.

Franks F. 1990. Freeze drying: From empiricism to predictability. Cryo-Lett 11(2):93-110.

Gordon A, Barbut S. 1992. Mechanisms of meat batter stabilization: A review. CRC Cr Rev Food Sci 32(4):299-332.

Grinberg VY, Burova TV, Grinberg NV, Mashkevich AY. 1993. On the effect of denaturation degree of food proteins on their functional properties. In: Schwenke KD, Mothes R, editors. Food Proteins: Structure and Functionality. Weinheim, Germany: VCH. p 40-7.

Haard NF. 1992. Biochemical reactions in fish muscle during frozen storage. In: Bligh EG, editor. Seafood Science and Technology. Oxford: Fishing New Books p 176-209.

Hayakawa T, Ito T, Wakamatsu J, Nishimura T, Hattori A. 2010. Myosin filament depolymerizes in a low ionic strength solution containing L-histidine. Meat Sci 84:742-6.

Hermansson AM, Harbitz O, Langton M. 1986. Formation of two types of gels from bovine myosin. J Sci Food Agr 37:69-84.

Hermannson AM, Langton M. 1988. Filamentous structures of bovine myosin in diluted suspensions and gels. J Sci Food Agr 42:355-69.

Huda N, Abdullah A, Babji SB. 2001. Functional properties of surimi powder from three Malaysian marine fish. Int J Food Sci Tech 36:401-6.

Ito Y, Tatsumi R, Wakamatsu J, Nishimura T, Hattori A. 2003. The solubilization of myofibrillar proteins of vertebrate skeletal muscle in water. Anim Sci J 74:417-25.

Jain NK, Roy I. 2009. Effect of trehalose on protein structure. Protein Sci 18:24-36.

Kijowski JM, Mast MG. 1988. Effect of sodium chloride and phosphates on the thermal properties of chicken meat proteins. J Food Sci 53(2):367-70.

Kilburn D, Townrow S, Muenier V, Richardson R, Alam A, Ubbink J. 2006. Organization and mobility of water in amorphous and crystalline trehalose. Nat Mater 5:632-5.

Kim YS, Park JW. 2008. Negative roles of salt in gelation properties of fish protein isolate. J Food Sci 73(8):585-8.

Knipe CL, Olson DG, Rust RE. 1985. Effects of selected inorganic phosphates, phosphate levels and reduced sodium chloride levels on protein solubility, stability and pH of meat emulsions. J Food Sci 50:1010-3.

Knipe L. 2004. Use of phosphates in meat products. Meat Industry Research Conference; October 2.

Krishnamurthy G, Chang H, Hultin HO, Feng Y, Srinivasan S, Kelleher SD. 1996. Solubility of chicken breast muscle proteins in solutions of low ionic strength. J Agric Food Chem 44:408-15.

Kristinsson HG, Hultin HO. 2003a. Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. J Agric Food Chem 51:7187-96.

Kristinsson HG, Hultin HO. 2003b. Effects of low and high pH treatment on the functional properties of cod muscle proteins. J Agric Food Chem 51:5103-10.

Langton M, Hermansson A. 1992. Finer-stranded and particulate gels of β–lactoglobulin and whey protein at varying pH. Food Hydrocolloid 5(6):523-39.

Lee JC, Timasheff SN. 1981. The stabilization of proteins by sucrose. J Biol Chem 256(14):7193-7201.

Lesiów T, Xiong YL. 2003. Chicken muscle homogenate gelation properties: Effect of pH and muscle fiber type. Meat Sci 64:399-403.

Levin H, Slade L. 1992. Glass transitions in food. In: Schwartzberg H, Hartel RW, editor. Physical Chemistry of Foods. New York: Marcel Dekker. p 83-221.

Leyva-Mayorga MA, Ramirez JA, Martin-Polo MO, Hernandez HG, Vazquez M. 2002. Use of freeze-dried surimi in low-fat meat emulsions. Cienc Tecnol Aliment 3(5):288-94.

Mackie IM. 1994. Fish protein. In: Hudson BJF, editor. New and Developing Sources of Food Proteins. London: Chapman & Hall. p 95.

Montejano JG, Morales OG, Diaz R. 1994. Propiedades reol reológicas de geles de surimi liofilizado de trucha (*Cyanoscion nothus*) y tilapia (*Orochromis nilotica*). Rev Españ Ciencia Tecnol Alim 34(2):165-177.

Musa KH, Aminah A, Wan-Aida WM. 2005. Functional properties of surimi related to drying methods. Malays Appl Biol 34(2):83-7.

Niki H, Igarashi S. 1982. Some factors in the production of active fish protein powder. B Jpn Soc Sci Fish 48(8):1133-7.

Niki H, Kato T, Deya E, Igarashi S. 1983. Water holding capacity, emulsifying capacity and storage ability of active fish protein powder. B Jpn Soc Sci Fish 49(1):91-6.

Niki H, Matsuda Y, Suzuki T. 1992. Dried forms of surimi. In: Lanier TC, Lee CM, editors. Surimi Technology. New York: Marcel Dekker Inc. p 209-42.

Northcutt JK, Lavelle CL, Foegeding EA. 1993. Gelation of turkey breast and thigh myofibrils: Changes during isolation of myofibrils. J Food Sci 58(5):983-6.

Offer G, Trinick J. 1983. On the mechanism of water-holding in meat: The swelling and shrinking of myofibrils. Meat Sci 8(4):245-81.

Okada M. 1992. History of surimi technology in Japan. In: Lanier TC, Lee CM, editors. Surimi Technology. New York: Marcel Dekker Inc. p 3-21.

Picone CSF, Takeuchi KP, Cunha RL. 2011. Heat-induced whey protein gels: Effects of pH and the addition of sodium caseinate. Food Biophysics 6:77-83.

Pikal MJ. 1990. Freeze-drying of proteins, Part 1: Process design. Biopharm 3(8):18-27.

Privalov PL. 1990. Cold denaturation of proteins. Crit Rev Biochem Mol 25(4):281-305.

Ramirez JA, Diaz-Sobac R, Morales OG, Vazquez M. 1999. Evaluation of freeze-dried surimi from tilapia and fat sleeper as emulsifiers. Cienc Tecnol Aliment 2(4):210-4.

Reynolds J, Park JW, Choi YJ. 2002. Physiochemical properties of pacific whiting surimi as affected by various freezing and storage condition. J Food Sci 67(6):2072-8.

Sato S, Tsuchiya T. 1992. Microstructure of surimi and surimi-based products. In: Lanier TC, Lee CM, editors. Surimi Technology. New York: Marcel Dekker Inc. p 501-18.

Semenova MG, Antipova AS, Belyakova LE. 2002. Food protein interactions in sugar solutions. Curr Opin Colloid In 7:438-44.

Shaviklo GR, Thorkelsson G, Arason S, Kristinsson HG, Sveinsdottir K. 2010. The influence of additives and drying methods on quality attributes of fish protein powder made from saithe (*Pollachius virens*). J Sci Food Agr 90(12):2133-43.

Shiau C, Pan BS. 1981. Effect of processing condition on properties of minced mackerel products. In: Tsen CC, Lii, C, editors. Recent Advances in Food Science and Technology. Vol. 2. Taipei, Taiwan, R.O.C.: Hua Shiang Yuan Publishing Co. p 198-210.

Stefansson G, Hultin HO. 1994. On the solubility of cod muscle proteins in water. J Agric Food Chem 42:2656-64.

Tadpitchayangkoon P, Yongsawatdigul J. 2009. Comparative study of washing treatments and alkali extraction on gelation characteristics of striped catfish (*Pangasius hypophthalmus*) muscle protein. J Food Sci 74(3):284-91.

Vagenende V, Yap MGS, Trout BL. 2009. Mechanisms of protein stabilization and prevention of protein aggregation by glycerol. Biochemistry-US 48:11084-96.

Varnam AH, Sutherland JP. 1995.d Meat and Meat Products: Technology, chemistry and microbiology. London: Chapman & Hall. p 404-5.

Venugopal V, Chawla SP, Nair PM. 1996. Spray dried protein powder from threadfin bream: preparation, properties and comparison with FPC Type-B. J Muscle Foods 7(1):55-71.

Walstra P. 2003a. Proteins. In: Physical Chemistry of Foods. New York: Marcel Dekker Inc. p 203-49.

Walstra P. 2003b. Soft Solids. In: Physical Chemistry of Foods. New York: Marcel Dekker Inc. p 683-771.

Wright BJ. 2007. Effect of ultrastructural disruption and protein dispersion on gel-forming in myofibrillar gels. [DPhil dissertation]. Raleigh, NC: NC State Univ. 289 p. Available from: NCSU Libraries.

Xiong YL, Brekke CJ. 1991. Protein extractability and thermally induced gelation properties of myofibrils isolated from pre- and postrigor chicken muscles. J Food Sci 56(1):210-5.

Xiong YL, Ho C, Shahidi F. 1999. Quality characteristics of muscle foods. In: Xiong YL, Ho C, Shahidi F, editors. Quality Attributes of Muscle Foods. New York: Kluwer Academic. p 8-9.

Yasui T, Fukazawa T, Takahashi K, Sakanishi M, Hashimoto Y. 1964. Phosphate effects on meat: Specific interaction of inorganic polyphosphates with myosin B. J Agric Food Chem 12(5):399-404.

Yoo B, Lee CM. 1993. Thermoprotective effect of sorbitol on proteins during dehydration. J Agric Food Chem 41:190-2.

MATERIALS AND METHODS

Rationale

In this study, freeze drying was selected as the drying method since it is considered the least damaging method of drying available for proteins. Treatments that would minimize damage from freeze-drying might thus apply to other drying processes, such as spray-drying, which are more economical, but also harsher (Niki and others 1992). To induce greater charge separation of proteins, and possibly thereby induce greater stability of proteins, the pH of breast meat (typically near 5.8) suspensions was adjusted upward to 7.2, 9.0 or 11.0. Also, treatments were made prior to drying wherein the pH of the meat suspensions, after adjustment to pH 11.0, was then 'shifted' back to 9.0 or 7.2; both as a comparison with non-pH shifted proteins, and also because such a pH shifting treatment has been shown to induce better functionality in meat proteins (Kristinsson and Hultin 2003b). The quantity of trehalose (10%) added as a lyoprotectant was selected so that the final gels prepared with freeze-dried powder would contain 8-10% trehalose. Surimi seafood products commonly have cryoprotectant concentrations in this range on a wet weight basis.

To analyze the effects of pH and trehalose during freeze-drying on the gel forming ability of the powders, gels were prepared at 12% protein which corresponds with the protein concentration of many commercial meat products like frankfurters. However, the gels were prepared with 6% protein from freeze-dried powder and 6% protein from raw chicken breast. This formulation was chosen since dried meat powders would most likely find use as a

functional ingredient in meat products, supplementing rather than totally replacing fresh meat ingredients.

Since trehalose affects protein gelation and rheological properties, all gels and solutions were prepared with equal trehalose content; 10.2% for gels and 5.1% for solutions. To remove the effect of pH during analysis, all gels and solutions were tested at pH 7.2 in addition to the pH at which the proteins were freeze-dried (Figure 1).

1. Experimental design

A randomized complete block design with 10 levels of pH and 2 levels of trehalose was used in this study (Figure 1). Each treatment was replicated twice where each replicate constituted one block (total of 2). A randomized complete block-split plot design better describes this experiment since 4 treatments are prepared at 2 pH values, however, this design only slightly improved the statistical analysis. Therefore, the RCBD was used since interpretation is simpler.

2. Freeze Dried Protein Powders

2.1. Solution preparation

Boneless skinless chicken breast meat was obtained from a local chicken processor (Pilgrim's Pride, Sanford NC), packaged in ~ 3.5 kg portions in freezer bags and frozen at -10 °C until use (<5 months). Frozen chicken was thawed at 4 °C for at least 24 h before the removal of visible fat and connective tissue. Thawed chicken was chopped with chilled, deionized (DI) water at a ratio of 2.5:1 chicken to water for 2 min at 20,000 rpm using an ice-

water jacketed, Stephan UMC-5 vertical-cutter/mixer (Stephan Machinery Corp., Columbus, OH) under vacuum in order to form a paste. The paste was then transferred to an ice-water jacketed vessel and diluted with 2 parts chilled, DI water before adding trehalose (Hayashibara International, Okayama Japan) and blending with a Brookfield Counter Rotating Mixer (Brookfield Engineering Laboratories, Stoughton MA). Treatments were prepared at pH 5.8, 7.2, 9.0 and 11.0 using chilled, 2 M NaOH added under constant blending with a Brookfield Counter Rotating Mixer. Two additional treatments were prepared by first raising the pH to 11.0, then lowering the pH to 9.0 or 7.2 using 2 M HCl to test the effects of shifting pH before freeze drying. Lastly, DI water was added to bring the final protein concentration to 5.25% w/w and trehalose content to 10% w/w. Each solution was immediately transferred to an 8.5 x 12 x 2.5 in aluminum tray and vacuum sealed in a plastic bag before freezing in a dry ice-ethanol bath for 25 min. Each treatment was then transferred to a -50 °C freezer for temporary storage before freeze drying.

2.2. Freeze drying

Four frozen trays were freeze-dried together in a VirTis Genesis 25XL freeze dryer (SP Scientific, Gardiner NY). The shelf temperature was held at -10 °C for 1 week followed by 20 °C for 1 week with a condenser temperature of -80 °C. Freeze dried cakes were crumbled and passed through a #20 mesh sieve. Material that did not pass through the sieve was discarded. This material, which appeared to be connective tissue, was present in similar quantity (~25 g) for all freeze-dried treatments. Powders were vacuum-sealed in plastic bags and stored at -50 °C until further analysis.

3. Proximate analysis of powders

3.1. Moisture Content

Moisture content of freeze-dried powders was determined by volumetric Karl Fischer titration using a Brinkmann Metrohm 701 Titrino (Metrohm Ltd, Herisau Switzerland) with one component, Hydranal composite-5 reagent (Sigma Aldrich, St. Louis MO). Approximately 200 mg of each powder was titrated to completion at room temperature. Each treatment was measured in triplicate.

3.2. Protein Content

Nitrogen (%) was determined using a PerkinElmer 2400 CHN elemental analyzer (PerkinElmer Inc., Waltham MA). Crude protein content was determined from percent nitrogen using a conversion factor of 6.25. Each treatment was measured in triplicate.

3.3. Trehalose Content

The percent trehalose present in the freeze-dried powders was determined by the following formula:

Trehalose (%) = 100 – protein (%) – moisture (%) – ash (%)

3.4. Ash Content

Ash content (%) was determined by dry ashing at 550 °C in a furnace for 24 hrs. Each treatment was measured in triplicate.

4. Preparation of freeze-dried protein solutions

Each treatment was tested at both pH 7.2 and at the pH just prior to drying (‘treatment pH’) in triplicate. Adjustment of the pH involved rehydrating each powder in ~86 mL

deionized water (4°C) on a stir plate under refrigeration. Once dispersed, NaCl and trehalose were added followed by addition of 1 M tribasic potassium phosphate (~pH 12.5) or 1 M monobasic potassium phosphate (~pH 4.5) to adjust the pH. Since the treatments required different amounts of phosphate solution to change the pH, differences in molarity of potassium phosphate between treatments were eliminated by the addition of 1 M potassium phosphate solution prepared at pH 5.8, 7.2, 9.0 or 11.0 using tribasic and monobasic potassium phosphate. Air was removed prior to viscosity measurement by holding each solution under vacuum at -20 in Hg for 30 min. The final composition was 3.0 % w/w protein, 2.4 % w/w NaCl, 5.1 % w/w trehalose and 28 mM potassium phosphate. All solutions were hydrated and tested within 3 hours.

5. Viscosity measurement

The viscosity profile of powders dispersed in deionized water at 3.0 % w/w protein was determined by shear rate sweep from 0.1 to 100 s^{-1} at 4 °C using a StressTech Rheometer (Rheological Instruments AB, Lund, Sweden) with a smooth cup and bob attachment. Samples were pre-sheared at 50 s^{-1} for 30 s and allowed to equilibrate for 20 s before measurement. Flow behavior was described by the power law model $\sigma = k\dot{\gamma}^n$ where σ = shear stress, k = consistency index, n = flow behavior index and $\dot{\gamma}$ = shear rate. Each solution was measured in triplicate.

6. Gelation

6.1. Paste preparation

Pastes were prepared from a mixture of frozen chicken breast meat and freeze-dried chicken powder to test the use of freeze-dried chicken powder as a functional protein ingredient. Frozen meat was thawed under refrigeration for at least 24 hr and trimmed of exterior fat and connective tissue. A paste was first prepared in bulk from thawed chicken breast meat (TCBM) chopped with DI water (4° C) at a ratio of 2.5:1 chicken meat to water and 3% w/w NaCl in an ice-water jacketed, Stephan UMC-5 vertical-cutter/mixer under vacuum. The TCBM paste was vacuum sealed in plastic bags, stored under refrigeration (4 °C) and used within 6 h. Using a portion of the TCBM paste for each treatment, the pH was adjusted as needed with 1 M tribasic potassium phosphate (~pH 12.5) to the final treatment pH. Then, freeze-dried chicken powder was added and mixed under vacuum before the addition of the remaining NaCl and trehalose. When necessary, additional tribasic potassium phosphate was added to adjust the final treatment pH. Lastly, using chilled 1 M potassium phosphate solutions previously prepared at pH 5.8, 7.2, 9.0 or 11.0 the molarity of potassium phosphate was adjusted to 300 mM. The final composition of each treatment was 6.0% w/w protein contributed from each of freeze-dried chicken and TCBM (12% protein total), 2.0% w/w NaCl, 10.2% w/w trehalose and 300 mM potassium phosphate at either the pH of the freeze-dried powder or pH 7.2 ± 0.2.

As controls, pastes were also prepared from TCBM at 12% w/w protein as described above with the same concentration of NaCl, trehalose and potassium phosphate at pH 5.8, 7.2,

9.0 and 11.0 ± 0.2. All pastes were vacuum-sealed in plastic bags, stored under refrigeration (4° C) and cooked within 6 h.

6.2. Gel preparation

Using a manual sausage stuffer, pastes were stuffed into cylindrical, stainless steel tubes (40 cm x 19 mm) lubricated with cooking spray and sealed on both ends. Each paste was cooked in a water bath using a 2-step heating regiment; 50 °C for 30 min to denature meat proteins followed by 75 °C for 20 min to promote protein aggregation and gel formation. After cooking, samples were placed in ice-water slurry for 15 min before storage at 4 °C for 24 hrs prior to analysis.

7. Fracture Testing

Fracture stress and strain were measured by torsion analysis as described by Kim et al (1986). Notched plastic discs (27 mm diameter by 1 mm height) (Accu-tool LLC, Apex, NC) were glued with cyanoacrylate glue (Loctite 401 Instant Adhesive, Henkel Corp., Westlake, OH) to the ends of 28.7 mm long cylindrical gel pieces. The gels were ground into a capstan shape with a center diameter of 10 mm using a modified milling machine. Each sample was twisted at 2.5 rpm until fracture using a Haake Viscotester VT550 (Haake, Paramus NJ). Fracture stress and strain were calculated by the method of Barrangou et al. (2005).

8. Total moisture content

Moisture content of chicken pastes and gels was determined by oven drying for 24 hrs at 102 +/-2 °C using ~2.0 g samples of each. Each treatment was measured in triplicate.

9. Water holding capacity

The water holding capacity of each gel was measured using the microcentrifuge-based method described by Kocher and Foegeding (1993). Using a cork borer, 3 cylinders (10 x 4.3 mm) were cut from each gel. Each cylinder was inserted into a Forensic Micro-spin filter (MidSci, St. Louis MO) placed inside a 2.0 mL centrifuge tube for fluid collection during centrifugation. Samples were centrifuged at 153 x g (2000 rpm) for 10 min in a horizontal Beckmann Microfuge® 11 horizontal-rotor microcentrifuge (Beckman Instruments, Inc., Palo Alto, CA). The weight of the tubes and sample was recorded before and after centrifugation to determine the amount of fluid loss. Moisture content of each gel was determined by oven drying for 24 hrs at 102 +/-2 °C using ~2 g samples. Each treatment was measured in triplicate. Percent held water (HW) was determined as:

$$\text{HW (\%)} = \frac{\text{total g of water in sample - g water released}}{\text{total g water in sample}} \times 100$$

10. Cook loss

The percent loss of water and soluble solids as a result of cooking the pastes was determined from the percent difference in moisture content of the pastes and gels as determined by oven drying for 24 hrs at 102 +/-2 °C using ~2 g samples of each. Percent cook loss (CL) was determined as:

$$\text{CL (\%)} = \frac{\text{\% moisture in paste - \% moisture in gel}}{\text{\% moisture in paste}} \times 100$$

11. Statistical analysis

Statistical analysis was conducted using the generalized linear model (GLM) in SAS 9.1 (SAS Institute, Inc., Cary, N.C.). One-way blocked analysis of variance (ANOVA) with Tukey's procedure was used to determine significant differences between means.

RESULTS AND DISCUSSION

1. Proximate analysis of dried powders

Powders prepared with trehalose contained between 34.39% and 36.23% protein while FD powders prepared without trehalose contained between 83.51% and 89.03% protein (Table 1). Besides the innate variability in protein content among individual chicken breasts, variation within each treatment was contributed by the variable addition of NaOH and HCl during pH adjustment; protein content decreased as more NaOH / HCl was required during solution preparation. This addition of sodium / chloride also increased ash content.

Moisture content varied from 4.04% to 6.29% in trehalose-free FD powders while those prepared containing trehalose varied 2.40% to 3.25% (Table 1). Since the freeze drying regiment was the same for all treatments, the trehalose-containing treatments had lower final moisture content because their initial moisture content was lower.

2. Rehydrated powders

2.1. Appearance

Dispersions prepared from freeze-dried chicken breast meat were homogenous and smooth in appearance. Visible phase separation occurred only after standing for several hours, and only for dispersions prepared from treatments dried at pH 11.0. The remaining dispersions were stable even after 3 days holding at 4 °C. Dispersions prepared from treatments dried at pH 5.8, 7.2 and 9.0 appeared to form very weak gels after holding

overnight at 4 °C: these did not flow within the beaker when tilted slightly (<45°) but did flow when poured.

2.2. Apparent viscosity

All protein dispersions exhibited shear thinning behavior (flow behavior index $n < 1$) wherein the viscosity decreased with increasing shear rate (Table 2); R^2 values were > 0.99 for all treatments when curves were fitted to the power law model. Several factors such as protein conformation, colloidal interaction forces between proteins, amount of imbibed water and molecular weight influence the viscosity of protein solutions. As shear rate increases, viscosity can diminish through disruption of interaction forces, protein stretching and deformation, orientation with the direction of flow and/or reduction in the amount of imbibed water (Walstra 2003b). In meats, the rod or filament shaped myofibrillar proteins, actin and myosin, are the primary contributors to viscosity, and clearly these would orient within a flow field. Any remaining muscle fragments would have a higher hydrodynamic radius and thus affect viscosity effect more than unassociated myofibrillar proteins, especially if swollen at higher pH.

Both pH treatment (of which there were 10) and trehalose addition prior to drying significantly affected the flow behavior index (n) of protein dispersions but only pH treatment significantly influenced the consistency index (k) (Table 3). Dispersions were found to fall into three groups as judged by their apparent viscosity vs. shear rate profiles. Dispersions made from powders freeze-dried with and without trehalose at pH 5.8, 7.2 and 9.0, prepared both at the drying pH and at pH 7.2, exhibited a similar viscosity profile (Fig. 1-4). Their flow behavior index (n) ranged from 0.27 to 0.38 while k ranged from 2.33 to

5.17 (Table 2). Increasing the pH to 11.0 prior to freeze drying dramatically decreased the shear thinning behavior: these samples displayed a more Newtonian behavior (linear increase in viscosity with increasing shear rate) since *n* ranged from 0.82 to 0.92 (Table 2). Viscosity of these also decreased considerably as *k* ranged from 0.07 to 0.21 (Table 2). Raising the pH to 11.0, then decreasing it to 9.0 or 7.2 prior to freeze drying, affected shear-dependent behavior intermediate to the aforementioned two treatment groups with respect to values of *n* and *k*.

2.2.1 Mechanism of changes induced in viscosity of freeze-dried meat proteins

Prior to freeze-drying, the treatments used in this study were prepared by a procedure similar to that involved in a novel pH shifting process for surimi manufacture (Hultin and others 2005) , first described and patented by Hultin and Kelleher (1999). In this process, meat proteins are first solubilized by raising or lowering the pH of a dilute solution of homogenized meat and water to extreme values (pH < 3 or > 11). Then the proteins are removed from solution (dewatered) as aggregates after the pH is lowered to the isoelectric point (pH 5.3-5.5) (Nolsoe and Undeland 2009). In the alkaline version of pH shifting, as the pH is first raised the viscosity slightly increases with increasing pH, until reaching the range of pH ~9.5-10.5. An abrupt maximum in viscosity occurs within this range and a turbid appearance develops. Additional pH increase causes a marked decline in viscosity. Undeland and others (2003) measured the changes in viscosity of herring homogenates during pH shifting and found they were related to changes in protein solubility. The authors suggested that the viscosity reduction at pH >10.5 is associated with a large solubility increase, which may be caused by dissociation of myofibrillar proteins into monomers.

When muscle is first homogenized in water, the myofibrils are cleaved and fragmented into small myofibrillar protein particles. As ‘salt-soluble proteins’, the myofibrillar proteins do not dissociate from one another and these particles, still having the native structure of muscle, remain intact at physiological pH. However, as the pH is raised, charge separation progressively increases and the muscle fragments imbibe a great deal of water (Offer and Trinick 1983). Swelling of these muscle fragments thus leads to an increase viscosity, peaking in the region of pH 9.5-10.5. A similar swelling of meat fragments occurs without pH change when sufficient salt concentration is added to the aqueous milieu of the muscle fragments (Offer and Trinick 1983). In both cases, progressive charge separation eventually leads to solubilization of individual myofibrillar proteins. Thus, in the pH shifting process, at pH above 10.5 very little native muscle structure remains, and viscosity decreases as pH is further increased.

Clearly, pH shifting is able to effect disintegration of muscle structure, leading to solubilization of the constituent myofibrillar proteins and accompanying viscosity increase, which mimics the effects of chopping meat in salt and water but which apparently goes beyond the latter in its ability to totally deconstruct remaining muscle fragments and release free myofibrillar protein into solution (Wright 2007).

After having reached pH 11.0, the mixture exists primarily as a solution of free myofibrillar proteins, with little tendency to aggregation due to the high induced charge separation. It would seem that this could help stabilize proteins if dried in this state. Apparently the conformation of proteins solubilized at such high pH is different from that of native proteins solubilized at low pH in the presence of salt (Kristinsson and Hultin 2003a,

2003b); one consequence is that their gel-forming properties (upon heating) are apparently enhanced (Kim and Park 2008; Wright 2007).

If the pH is lowered after having attained pH 11.0, in the pH range 9.5-10.5 the proteins begin to reassociate. These loosely associated aggregates, with high charge separation of constituent proteins, apparently entrap considerable water and present a swelled mass of larger hydrodynamic radius such that viscosity exceeds that of the swollen muscle fragments when pH was initially raised to the range during pH shifting Undeland and others (2003). As pH is lowered further, these loose aggregates apparently contract, squeezing out water and decreasing in hydrodynamic radius such that viscosity again falls with decreasing pH. Near the isoelectric point, aggregation would become extensive, leading to precipitation from solution.

Undeland and others (2003) also showed a hysteretic effect in that, as pH was reduced from >10.5, the viscosity was greater than the same pH during the initial increase to pH 10.5. This indicates that the refolded proteins interacted with each other to a greater extent during the lowering of pH compared to when it was increased. These results provide evidence that the pH shifted proteins were altered in terms of their conformation and as a result their functionality differs compared to non-pH shifted proteins, and to the proteins that had not yet been taken to pH >10.5.

Our observed effects of pH treatment during drying on the viscosity of the rehydrated protein dispersions can be explained as follows. The relatively low viscosity (k) and high n of the drying pH 11.0 treatment were likely the result of damage sustained to the protein during drying. At pH 11 prior to drying, substantial protein dissociation caused by high electrostatic

repulsion would be expected. While high charge repulsion at pH 11.0 has been shown to impart some stability to the denaturing effects of subsequent heating of meat proteins (Brenner and others 2009) in this case it clearly did not help stabilize the proteins to the denaturing effects of drying. This is probably because proteins are forced into close association, despite high charge repulsion, by removal of water.

Kristinsson and Hultin (2003b) found considerable conformational unfolding occurring at pH 11, leading to a greater surface hydrophobicity. Thus proteins exposed to pH 11.0 would be more prone to aggregation by hydrophobic interactions. Even after having been lowered in pH, prior to drying, after exposure to pH 11, the proteins obviously sustained considerably more damage than proteins dried at the same pH without having been taken to pH 11. In contrast, Undeland and others (2003) had reported that lowering pH of wet meat proteins after exposure to pH >10.5 essentially restored functionality to the proteins. Clearly then, drying is quite damaging to meat proteins which have been essentially dispersed as free protein molecules and altered in surface hydrophobicity by exposure to such a high pH.

Meat proteins not exposed to this high pH exhibited higher viscosity, and greater shear-thinning behavior, after freeze drying. Over this pH range, drying pH had no effect on protein stability throughout drying and rehydration, as evidenced by viscosity properties of the rehydrated dispersions. Likewise, adjusting the final pH of rehydrated dispersions to 7.2 had little effect on viscosity properties of proteins dried over this pH range.

2.3. Gelation properties of mixed comminuted and freeze-dried meat protein pastes

The pH treatment associated with preparation of each of the gels tested (10 in all) significantly affected all measured gel properties while trehalose addition prior to drying significantly affected only fracture stress, fracture strain and held water of gels (Table 3).

2.3.1 Gels made containing powders not containing trehalose before drying

Gels made with FD powders and prepared at the drying pH without trehalose prior to drying, except for gels from the pH 5.8 treatment, had similar values for fracture stress and fracture strain as well as for held water and cook loss (Figures 5 and 6). For perspective, a leading brand of frankfurter typically exhibits fracture stress of about 25 kPa and strain of 1.5, so these are quite strong and deformable gels.

For gels prepared using powders dried at, and subsequently prepared at pH 5.8, the fracture stress, fracture strain and held water decreased while the cook loss increased relative to the other pH treatments. After slicing, the surface of the pH 5.8 gels had a grainy appearance and felt mushy to the touch. This was in stark contrast to the smoother surface and rubbery texture of the other gels. This suggests that this pH 5.8 treatment formed a more particulate-type gel. Gel structure is primarily controlled by pH and ionic strength wherein normally particulate gels form near the isoelectric point of the protein and/or at high ionic strength (Walstra 2003b). Particulate gels are characterized by an aggregated (‘fish net’ with knots, rather than finely woven or fine stranded) protein network which typically exhibits lower water holding capacity, fracture stress and fracture strain.

Fine stranded gels are characterized by a more evenly distributed protein network which exhibits higher water holding capacity, fracture stress and fracture strain (Langton and

Hermansson 1992). Those gels of Figures 5 and 6 not prepared from the pH 5.8 treatment exhibited similarly high water holding capacity (low cook loss, high held water) and fracture stress/strain. For gels prepared at pH 7.2 and 9.0, there was no significant difference between fracture stress and strain values for gels prepared with and without a pH shift (to pH 11 and back) treatment before freeze drying. However, water holding properties were diminished somewhat for those pH shifted treatments. Overall, the effect of this pH shifting (to pH 11 and down again) before freeze drying on gel properties was minimal.

To remove the effect of differing final gel pH on properties of gels made containing the freeze-dried powders, all treatments not dried at pH 7.2 (without added trehalose) were adjusted to pH 7.2 during the paste preparation. All treatments then exhibited similar values for fracture stress, fracture strain, held water and cook loss (Figures 7 and 8), with the exception of the gel prepared from powder dried at pH 11.0. When prepared at the drying pH, the highest fracture stress and strain values were found for the pH 7.2 treatment (Figure 5 and 6). Once those gels not dried at pH 7.2 were prepared at pH 7.2, all (except those containing powder dried at pH 11) increased in fracture stress and strain as well as in water holding capacity. Even the pH 5.8 drying treatment, which when gelled at pH 5.8 produced a particulate gel with poor properties, improved in gel properties when prepared at 7.2 to become similar to gels prepared from higher pH drying treatments.

Interestingly, the pH 11.0 treatment of Figures 7 and 8 dramatically decreased in fracture stress and strain as well as held water when its pH was changed to 7.2 during paste preparation. At this lower final pH, the gel made from this drying pH now exhibited characteristics of a particulate-type gel (Langton and Hermansson 1992). Kristinsson and

Hultin (2003b) noted that surface hydrophobicity of meat proteins increases when exposed to extreme pH. Protein denaturation during freeze drying also leads to greater exposure of hydrophobic residues at the protein surface (Arakawa and others 2001). Increased surface hydrophobicity may promote the formation of protein aggregates and aggregation can be further increased with increasing protein concentration (Walstra 2003c). We also observed above that viscosity of dispersions of the powder freeze-dried at pH 11, without added trehalose, and shifted downward to pH 9.0 were lower than that of dispersions taken only up to pH 9.0; this may also indicate a stronger tendency of proteins to associate once they have experienced a pH 11.0 treatment (Figures 1 and 3). The higher protein concentration in the paste coupled with the likely greater surface hydrophobicity induced at pH 11 probably led to aggregation of the proteins during pH reduction of the paste and/or during subsequent thermal treatment.

2.3.2 Gels made from powders containing trehalose before drying

Gels made containing meat powders freeze-dried with added trehalose showed increased fracture stress, fracture strain and held water compared to powders freeze-dried without trehalose (Figures 1-4; Table 4). Again, the pH 5.8 treatment produced a much weaker gel compared to the higher pH treatments. Sun and Holley (2011) found that at NaCl concentrations of 2-3% by weight, chicken meat gels have the best gel forming ability near pH 6.0. In the present study, all gels contained 2% NaCl but the ionic strength was significantly increased by the presence of 300 mM potassium phosphate. At high ionic strength, significant screening of charged groups on the proteins reduces electrostatic

repulsion between proteins; this allows them to aggregate more rapidly when heated leading to the formation of particulate gels (Walstra 2003b).

The pH 7.2 treatment gel again exhibited the highest fracture stress and strain for gels prepared at the drying pH. Overall however, little difference in fracture properties among the pH treatments was found.

The trend for held water measurements (Figure 10) remained similar to that exhibited by the gels made containing FD powders produced without added trehalose (Figure 6). Gels made from the higher pH treatments had higher values of held water. At constant ionic strength, higher pH induces increased water holding capacity in gels due to resulting increased electrostatic repulsion between protein strands, which allows for a greater volume of entrapped water in the expanded gel structure (Feng and Hultin 2001).

When gels made with FD powders from the different drying pH treatments were all prepared at pH 7.2, the difference caused by the drying pH was even smaller (Figures 11 and 12). No significant difference was found for fracture stress, fracture strain and cook loss between these gels and held water measurements were very similar as well.

These results support much published research which indicates that trehalose protects proteins from denaturation and aggregation induced by a variety of stresses, including temperature change and dehydration (Semenova and others 2002; Jain and Roy 2009). In a protein solution or dispersion, the stabilization mechanism for sugars like trehalose is a result of their exclusion from the protein surface, coupled with an ability to increase the surface tension of water (Arakawa and Timasheff 1982). This leads to stabilization of a more folded conformation (Jain and Roy 2009).

2.3.3 Mechanistic considerations in stabilizing gelation properties of meat proteins to drying

The gelation process of myofibrillar proteins is thought to be controlled by the rate of thermally induced unfolding and aggregation. Higher quality gels (higher fracture stress/strain, higher water holding ability, smooth and fine stranded texture) are thought to be produced when protein unfolding occurs prior to aggregation (Walstra 2003b). Thermally induced unfolding of proteins exposes hydrophobic groups previously buried within the interior of the protein. These hydrophobic groups then interact and contribute to the formation of smaller aggregates and higher quality stranded-type gels, when ionic repulsion of proteins is sufficient to prevent their clumping, but not too excessive to block protein association and gelation. By contrast, more rapid aggregation likely promotes larger protein aggregates and lower quality, more particulate gels (Foegeding and others 1991). By inhibiting denaturation during freeze drying and stabilizing the conformation after rehydration, trehalose addition thus likely delays the onset of thermally induced protein unfolding and leads to more ordered aggregation and thus to higher quality gels.

Notably, trehalose addition prior to drying of meat proteins essentially eliminated effects of drying pH on gel fracture stress and fracture strain (Figure 11). This was even found for the gels prepared from the drying pH 11.0 treatment made from paste that was reduced to pH 7.2 before cooking: without added trehalose prior to drying, this pH treatment led to formation of a low quality gel. These results indicate that trehalose was quite effective at inhibiting freeze drying induced protein denaturation at high pH (11.0) to the extent that its

presence or absence was the determining factor for the type of gel that formed when the pH of the paste was lowered from 11.0 to 7.2.

2.3.4 Gels made from only comminuted chicken meat (not dried)

To determine if adding freeze-dried chicken breast meat powder to raw breast meat affected its gel properties, 'control' gels were made, not containing any FD powders, but instead being prepared only from comminuted chicken breast meat at the same composition and solution conditions as those gels which did contain freeze-dried powders.

In contrast to the gels made containing FD powder, these control gels increased in fracture stress/strain and in held water values as gel pH was increased from 5.8 to 11 (Figures 13 and 14). As was seen in the pH 5.8 treatment gel containing freeze-dried powder, here the pH 5.8 treatment was also a weaker, particulate-type gel (Table 4). This is in contrast to what is customarily seen in the comminuted meats industry: such products as chicken frankfurters and bologna are commonly made at similar NaCl and protein contents using meat having a pH near 5.8 (Barbut 1997). Such meat gel products are known to customarily exhibit strong, deformable properties with a smooth texture, typical of a fine stranded gel structure. As mentioned earlier, it seems likely therefore that the high level of added phosphates necessitated by this experimental design are likely responsible for inducing protein aggregation in these gels, leading to a particulate, weaker gel structure than otherwise would be expected. At higher pH, fine stranded gels are apparently obtained due to the higher charge repulsion induced between protein strands in the gel.

Gels at pH 7.2 and 9.0 exhibited essentially identical gel properties (Figures 13 and 14). Interestingly, compared to the pH 7.2 and 9.0 gels prepared containing FD powder, with or without added trehalose prior to drying, these gels were lower in fracture stress, strain and held water (Table 4). Values for fracture stress, fracture strain and held water of the pH 11.0 treatment gels made only from comminuted meat (Figures 13 and 14) fell between those exhibited by gels containing FD powder dried with and without added trehalose (Table 4).

This superiority of properties of gels made with added FD powders, as compared to without, is quite surprising. Published studies comparing any type of dried meat proteins to non-dried meat proteins consistently show lower gel forming ability of the dried proteins, regardless of drying method (Shaviklo and others 2010; Kijowski and Richardson 1996; Niki and Igarashi 1982; Niki and others 1992; Reynolds and others 2002).

However, in the present study the dried powders were prepared not from comminuted meat, but rather from meat which had been homogenized while in more dilute dispersion. Wright (2007), as well as Sato and Tsuchiya (1992) and Chang and others (2001a) gave evidence that when meat proteins are better dispersed prior to gelation, gel properties such as fracture stress, fracture strain, and held water increase. In typical comminuted meat pastes, intact myofibril fragments are normally found, indicating that considerable protein may still be entrapped in fragments of native muscle structure which was not fully broken down by conventional manufacturing processes (Sato and Tsuchiya 1992). Therefore, the improvement noted in gel properties when FD powders were added to comminuted meat seems likely due to the greater liberation of myofibrillar proteins from the myofibrils caused by aspects of the FD meat powder preparation.

In summary, comminuted chicken breast meat gels produced at pH 7.2 and 9.0 had reduced gel properties compared to those produced containing FD powders at the same pH, especially when the freeze-dried powder was prepared with trehalose. Only when the comminuted gels were prepared at pH 11.0 were the gel properties similar. Therefore, these results research suggest that improved dispersion and/or dissociation of the freeze-dried proteins improved their gelling properties.

Considering the viscosity data of Figures 1-4, the freeze-drying process seemed to damage those proteins exposed to a pH 11 shift, regardless of whether the pH was at any point lowered after that. However, in Figures 5-12, gels could be made containing powders from these drying pH treatments which were actually stronger, more deformable, and had better water holding properties than gels made only from undried meat. Apparently their greater dispersibility, from whatever changes were induced by pH change and/or freeze-drying, rendered them capable of strong gel-forming properties despite damage that negatively influenced viscosity properties. This certainly seems to bolster the hypothesis of Wright (2007) that better dispersion of proteins can be a strong contributing factor to improving gel-forming ability.

CONCLUSION

The results of this study indicate that freeze-dried chicken breast meat proteins, prepared and used under proper conditions, can contribute to the formation of gels which are strong, deformable and exhibit high water holding properties. Trehalose addition prior to freeze drying significantly improved fracture stress, fracture strain and held water in all gels made containing freeze-dried protein powder. Interestingly however, trehalose addition prior to drying had little apparent effect in stabilizing the viscosifying properties of the meat proteins. Viscosity and gel properties depend upon different characteristics of the proteins. Viscosity is the manifestation of the hydrodynamic radius of suspended molecules, or groups of molecule (even possibly of muscle fragments). Unfolding/denaturation of proteins typically leads to protein aggregation; limited aggregation could favor protein aggregates of high hydrodynamic radius and viscosity. Gelation depends upon heat-induced aggregation of proteins into a three dimensional network structure, expanded sufficiently to imbibe most of the water in the system and thus exist as a freestanding gel rather than aggregated precipitates. It is difficult to conjecture as to exactly how much conformational change occurred in individual proteins as a result of a particular drying treatment, or as to exactly how this affected subsequent aggregation properties.

Almost certainly, drying at or after shifting downward in pH from pH 11 did result in denaturation and aggregation during drying/rehydration that damaged viscosifying properties and, in the case of the 11-7.2 pH shifted treatment, caused the gels to become particulate in

nature. Thus, effecting greater charge-induced separation of proteins by raising pH seems to be ineffective as a means of stabilizing them to the denaturing/aggregating effects of drying.

At pH 7.2 and 9.0, gels prepared with freeze-dried chicken breast meat protein had superior gel properties compared to comminuted chicken breast meat gels. Improved dispersion of the freeze-dried proteins compared to comminuted ones was proposed as the mechanism which caused the improvement in gel properties. This new observation, coupled with past work by others (Wright, 2007; Sato and Tsuchiya 1992; Chang and others 2001a) certainly bolsters the hypothesis that gel-forming ability of meat-derived proteins is improved by completely and homogenously disintegrating native muscle structure to aid dispersion of individual proteins, prior to heating.

Freeze drying is known to be the most gentle, yet also a quite expensive and time-consuming, drying method. Clearly it is challenging to attempt to dry muscle proteins, which have denaturation temperatures and overall stability far below that of vegetable and dairy proteins, and still retain functionality. The results of this study do however indicate that production of functional meat protein powders may possibly one day be accomplished with a more cost effective method of drying by employing lyoprotectant additives like trehalose, coupled with maximizing dispersibility of the proteins by some acceptable means to optimize their functionality.

REFERENCES

Amato PM, Hamann DD, Ball HR, Foegeding EA. 1989. Influence of poultry species, muscle groups, and NaCl level on strength, deformability, and water retention in heat-set muscle gels. J Food Sci 54(5):1989.

Arawaka T, Prestrelski SJ, Kenney WC, Carpenter JF. 2001. Factors affecting short-term and long-term stabilities of proteins. Adv Drug Deliver Rev 46:307-26.

Arakawa T, Timasheff SN. 1982. Stabilization of protein structure by sugars. Biochemistry-US 21:6536-44.

Barbut S. 1997. Microstructure of white and dark turkey meat batters as affected by pH. Brit Poultry Sci 38:175-82.

Barbut S, Maurer AJ, Lindsay RC. 1988. Effects of reduced sodium chloride and added phosphates on physical and sensory properties of turkey frankfurters. J Food Sci 53(1):62-6.

Brenner T, Nicolai T, Johannsson R. 2009. Rheology of thermo-reversible fish protein isolate gels. Food Res Int 42:915-24.

Chang H, Feng Y, Hultin HO. 2001a. Role of pH in gel formation of washed chicken muscle at low ionic strength. J Food Biochem 25:439-57.

Feng Y, Hultin HO. 2001. Effect of pH on the rheological and structural properties of gels of water-washed chicken-breast muscle at physiological ionic strength. J Agric Food Chem 49:3927-35.

Foegeding EA, Brekke CJ, Xiong YL. 1991. Gelation of myofibrillar protein. In: Parris N, Barford R, editors. Interactions of Food Proteins. Washington D.C.: American Chemical Society. p 257-67.

Hultin HO, Kristinsson HG, Lanier TC, Park JW. 2005. Process for recovery of functional proteins by pH shifts. In: Park JW, editor. Surimi and Surimi Seafood. 2nd ed. Boca Raton, FL: CRC Press. p 107-39.

Hultin HO, Kelleher SD. 1999. Process for isolating a protein composition from a muscle source and protein composition. Patent US6005073.

Jain NK, Roy I. 2009. Effect of trehalose on protein structure. Protein Sci 18:24-36.

Kijowski J, Richardson RI. 1996. The effect of cryoprotectants during freezing or freeze drying upon properties of washed mechanically recovered broiler meat. Int J Food Sci Tech 31:45-54.

Kim YS, Park JW. 2008. Negative roles of salt in gelation properties of fish protein isolate. J Food Sci 73(8):585-8.

Kristinsson HG, Hultin HO. 2003a. Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. J Agric Food Chem 51:7187-96.

Kristinsson HG, Hultin HO. 2003b. Effects of low and high pH treatment on the functional properties of cod muscle proteins. J Agric Food Chem 51:5103-10.

Langton M, Hermansson A. 1992. Finer-stranded and particulate gels of β–lactoglobulin and whey protein at varying pH. Food Hydrocolloid 5(6):523-39.

Niki H, Igarashi S. 1982. Some factors in the production of active fish protein powder. B Jpn Soc Sci Fish 48(8):1133-7.

Niki H, Matsuda Y, Suzuki T. 1992. Dried forms of surimi. In: Lanier TC, Lee CM, editors. Surimi Technology. New York: Marcel Dekker Inc. p 209-42.

Nolsøe H, Undeland I. 2009. The acid and alkaline solubilization process for the isolation of muscle proteins: State of the art. Food Bioprocess Technol 2:1-27.

Offer G, Trinick J. 1983. On the mechanism of water-holding in meat: The swelling and shrinking of myofibrils. Meat Sci 8(4):245-81.

Reynolds J, Park JW, Choi YJ. 2002. Physiochemical properties of pacific whiting surimi as affected by various freezing and storage condition. J Food Sci 67(6):2072-8.

Sato S, Tsuchiya T. 1992. Microstructure of surimi and surimi-based products. In: Lanier TC, Lee CM, editors. Surimi Technology. New York: Marcel Dekker Inc. p 501-18.

Semenova MG, Antipova AS, Belyakova LE. 2002. Food protein interactions in sugar solutions. Curr Opin Colloid In 7:438-44.

Shaviklo GR, Thorkelsson G, Arason S, Kristinsson HG, Sveinsdottir K. 2010. The influence of additives and drying methods on quality attributes of fish protein powder made from saithe (*Pollachius virens*). J Sci Food Agr 90(12):2133-43.

Sun XD, Holley RA. 2010. Factors influencing gel formation by myofibrillar proteins in muscle foods. Compre Rev Food Sci F 10:33-51.

Undeland I, Kelleher SD, Hultin HO, McClements J, Thongraung C. 2003. Consistency and solubility changes in Herring (*Clupea harengus*) light muscle homogenates as a function of pH. J Agric Food Chem 51:3992-8.

Walstra P. 2003a. Changes in dispersity. In: Physical Chemistry of Foods. New York: Marcel Dekker Inc. p 476-546.

Walstra P. 2003b. Polymers. In: Physical Chemistry of Foods. New York: Marcel Dekker Inc. p 137-201.

Walstra P. 2003c. Proteins. In: Physical Chemistry of Foods. New York: Marcel Dekker Inc. p 203-49.

Walstra P. 2003d. Soft Solids. In: Physical Chemistry of Foods. New York: Marcel Dekker Inc. p 683-771.

Wright BJ. 2007. Effect of ultrastructural disruption and protein dispersion on gel-forming in myofibrillar gels. [DPhil dissertation]. Raleigh, NC: NC State Univ. 289 p. Available from: NCSU Libraries.

Table 1 – Proximate analysis of freeze-dried chicken breast meat powders[A]

Treatment					
pH	Trehalose (%)	Protein (%)	Moisture (%)	Ash (%)	Trehalose (%)
5.8	0	89.0 ± 2.2^{a}	5.0 ± 1.5^{a}	4.73 ± 0.84^{e}	
5.8	10	36.2 ± 0.8^{c}	2.5 ± 0.4^{a}	2.00 ± 0.03^{hi}	59.3 ± 1.2^{a}
7.2	0	88.6 ± 1.4^{ab}	6.3 ± 0.5^{a}	5.53 ± 0.18^{d}	
7.2	10	36.2 ± 0.8^{c}	2.9 ± 1.0^{a}	2.23 ± 0.08^{hi}	58.6 ± 0.3^{a}
9	0	86.3 ± 1.2^{ab}	4.7 ± 1.2^{a}	6.51 ± 0.23^{c}	
9	10	34.5 ± 0.7^{c}	2.4 ± 0.3^{a}	2.50 ± 0.02^{h}	60.6 ± 1.0^{a}
11	0	85.8 ± 0.5^{b}	5.6 ± 1.0^{a}	9.17 ± 0.07^{b}	
11	10	35.6 ± 0.5^{c}	3.2 ± 1.6^{a}	3.56 ± 0.05^{g}	57.7 ± 1.0^{a}
11-9	0	83.5 ± 0.6^{b}	4.0 ± 0.2^{a}	9.33 ± 0.12^{b}	
11-9	10	34.4 ± 0.6^{c}	3.2 ± 1.6^{a}	3.78 ± 0.01^{fg}	58.6 ± 2.3^{a}
11-7.2	0	84.0 ± 0.1^{b}	4.7 ± 1.1^{a}	10.10 ± 0.01^{a}	
11-7.2	10	35.6 ± 0.0^{c}	3.0 ± 1.4^{a}	4.00 ± 0.05^{ef}	57.4 ± 1.5^{a}

[A] Values are mean of two replicates ± standard deviation. Means in the same column followed by the same letter are not significantly different ($P > 0.05$)

Table 2 – Effect of pH and trehalose on flow behavior index (*n*) and consistency index (*k*) of freeze-dried chicken breast meat solutions[A]

Treatment			
pH	**Trehalose (%)**	***n***	***k***
5.8	0	0.33 ± 0.05^{d}	2.89 ± 1.83^{abcde}
5.8	10	0.35 ± 0.03^{d}	3.13 ± 0.90^{abcd}
5.8→7.2	0	0.27 ± 0.06^{d}	5.00 ± 1.35^{a}
5.8→7.2	10	0.38 ± 0.01^{d}	3.35 ± 0.24^{abc}
7.2	0	0.31 ± 0.01^{d}	4.24 ± 0.03^{a}
7.2	10	0.34 ± 0.09^{d}	4.03 ± 1.27^{a}
9.0	0	0.32 ± 0.03^{d}	3.79 ± 1.63^{ab}
9.0	10	0.34 ± 0.00^{d}	2.33 ± 0.02^{abcde}
9.0→7.2	0	0.29 ± 0.06^{d}	5.17 ± 1.12^{a}
9.0→7.2	10	0.32 ± 0.03^{d}	4.39 ± 0.33^{a}
11.0	0	0.84 ± 0.01^{ab}	0.19 ± 0.05^{de}
11.0	10	0.92 ± 0.02^{a}	0.07 ± 0.00^{e}
11.0→7.2	0	0.82 ± 0.02^{ab}	0.21 ± 0.01^{de}
11.0→7.2	10	0.92 ± 0.01^{a}	0.08 ± 0.01^{e}
11-9.0	0	0.73 ± 0.02^{bc}	0.34 ± 0.13^{de}
11-9.0	10	0.67 ± 0.03^{c}	0.51 ± 0.15^{cde}
11-9.0→7.2	0	0.66 ± 0.00^{c}	0.51 ± 0.16^{cde}
11-9.0→7.2	10	0.63 ± 0.06^{c}	0.70 ± 0.39^{cde}
11-7.2	0	0.63 ± 0.02^{c}	0.51 ± 0.09^{cde}
11-7.2	10	0.60 ± 0.00^{c}	0.91 ± 0.01^{cde}

[A] Values are mean of two replicates ± standard deviation. Means in the same column followed by the same letter are not significantly different ($P > 0.05$)

Table 3 – F and *P*-values for gel properties and powder law model variables[A]

		Fracture stress		**Fracture strain**		**Held water**		**Cook loss**		***n***		***k***	
Source	DF	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*	F	*P*
pH	9	28.23	**<0.001**	68.86	**<0.001**	54.14	**<0.001**	9.91	**<0.001**	155.56	**<0.001**	26.41	**<0.001**
Trehalose	1	98.79	**<0.001**	64.11	**<0.001**	55.96	**<0.001**	0.02	0.8992	4.88	**0.0396**	2.14	0.1595
pH*Trehalose	9	4.78	**0.0020**	3.65	**0.0084**	4.28	**0.0037**	1.38	0.2633	2.24	0.0666	0.99	0.4778
Block	1	2.67	0.1189	0.01	0.9083	0.94	0.3435	0.07	0.8010	0.01	0.9185	3.03	0.0977

[A] Values in bold are statistically significant ($P<0.05$)

Table 4 – Gelation properties of control (C)[A] and mixed freeze-dried and raw chicken breast meat gels prepared at the treatment pH and pH 7.2[B]

Treatment		Fracture stress (kPa)		Fracture strain		Held water (%)		Cook loss (%)	
pH	Trehalose (%)	Treatment pH	pH 7.2	Treatment pH	pH 7.2	Treatment pH	pH 7.2	Treatment pH	pH 7.2
5.8	0	13.5^{IJ}	44.2^{ABCDE}	0.8^{H}	2.1^{ABC}	62.8^{HI}	79.4^{CDEF}	2.4^{ABC}	0.9^{D}
5.8	10	16.9^{IJ}	56.3^{A}	0.8^{H}	2.4^{A}	67.2^{GH}	80.9^{BCDE}	2.9^{AB}	0.8^{D}
5.8C		11.6^{J}		0.8^{H}		59.2^{I}		3.1^{A}	
7.2	0	38.9^{BCDEF}		2.1^{ABC}		77.9^{CDEF}		0.6^{D}	
7.2	10	53.3^{AB}		2.3^{A}		80.2^{BCDEF}		1.3^{CD}	
7.2C		22.7^{GHIJ}		1.6^{EFG}		75.2^{F}		0.9^{D}	
9	0	37.7^{BCDEFG}	51.6^{ABC}	2.1^{ABCD}	2.2^{ABC}	80.2^{BCDEF}	80.2^{BCDEF}	0.9^{CD}	1.0^{CD}
9	10	42.6^{ABCDE}	53.5^{AB}	2.1^{ABC}	2.3^{AB}	80.6^{BCDEF}	80.7^{BCDEF}	0.3^{D}	0.4^{D}
9C		24.7^{FGHI}		1.7^{DEFG}		78.2^{CDEF}		0.6^{D}	
11	0	34.2^{DEFGH}	19.8^{HIJ}	1.6^{FG}	1.4^{G}	83.4^{ABC}	35.6^{G}	0.7^{D}	0.8^{D}
11	10	44.2^{ABCDE}	51.8^{ABC}	2.0^{ABCDE}	2.1^{ABC}	87.2^{A}	79.3^{CDEF}	0.7^{D}	0.5^{D}
11C		38.9^{BCDEF}		1.9^{CDEF}		85.2^{AB}		1.5^{BCD}	
11-9	0	28.6^{EFGHI}	39.8^{BCDEF}	1.9^{BCDEF}	1.9^{BCDEF}	76.0^{DEF}	76.9^{DEF}	0.5^{D}	0.6^{D}
11-9	10	45.9^{ABCD}	55.9^{A}	2.2^{ABC}	2.2^{ABC}	81.5^{BCD}	80.2^{BCDEF}	0.3^{D}	0.8^{D}
11-7.2	0	36.2^{CDEFG}		1.9^{CDEF}		75.3^{EF}		0.6^{D}	
11-7.2	10	49.2^{ABCD}		2.2^{ABC}		77.4^{DEF}		1.2^{CD}	

[A] Control gels prepared from comminuted chicken breast meat (no FD powder)
[B] Values are mean of two replicates. Means in the same column followed by the same letter are not significantly different ($P > 0.05$)

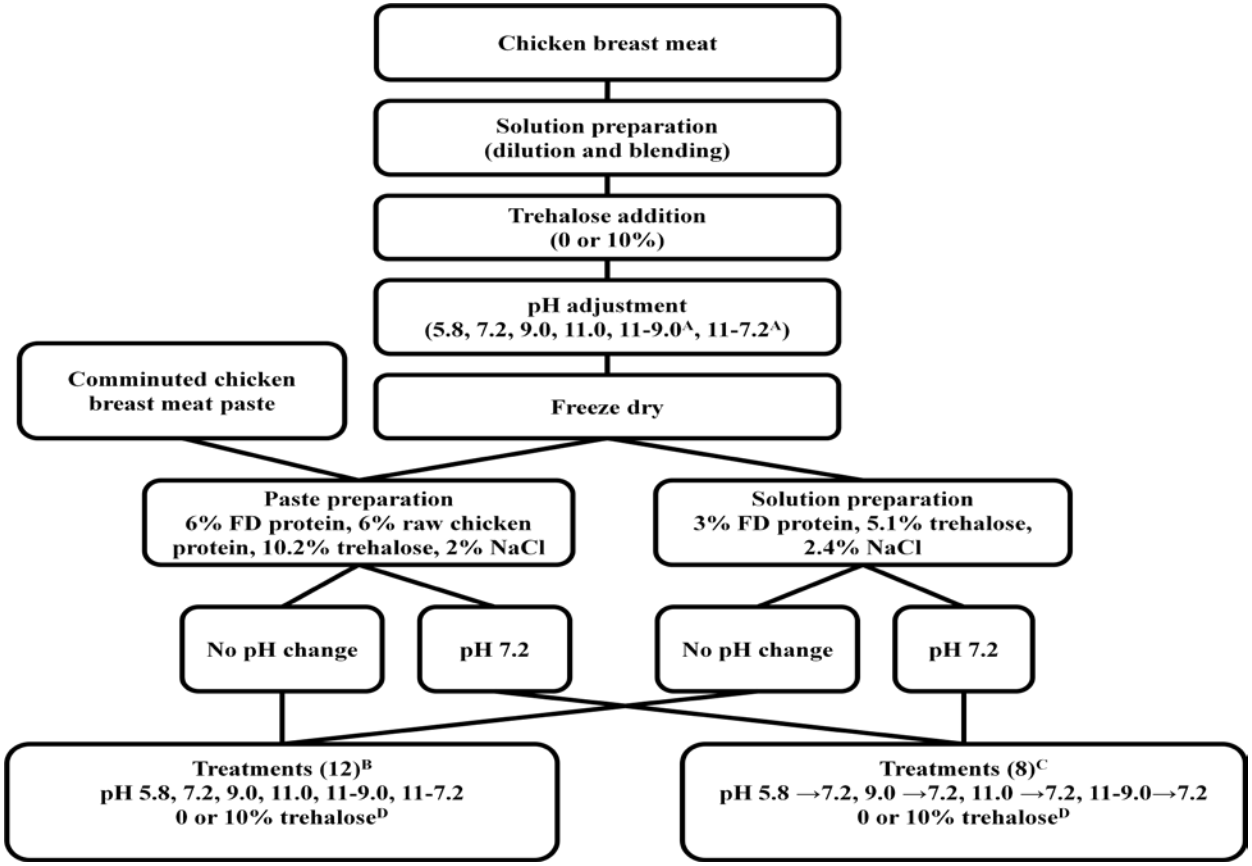

Figure 1 – Process flow diagram for preparation and analysis of freeze-dried chicken breast meat protein powders

[A] The solution pH was raised to 11.0, then lowered to 9.0 or 7.2

[B] Treatments were prepared at the same pH as when freeze-dried

[C] Treatments were adjusted to pH 7.2

[D] Trehalose content differed during freeze drying but was adjusted to 10.2% in the pastes and 5.1% in the solutions

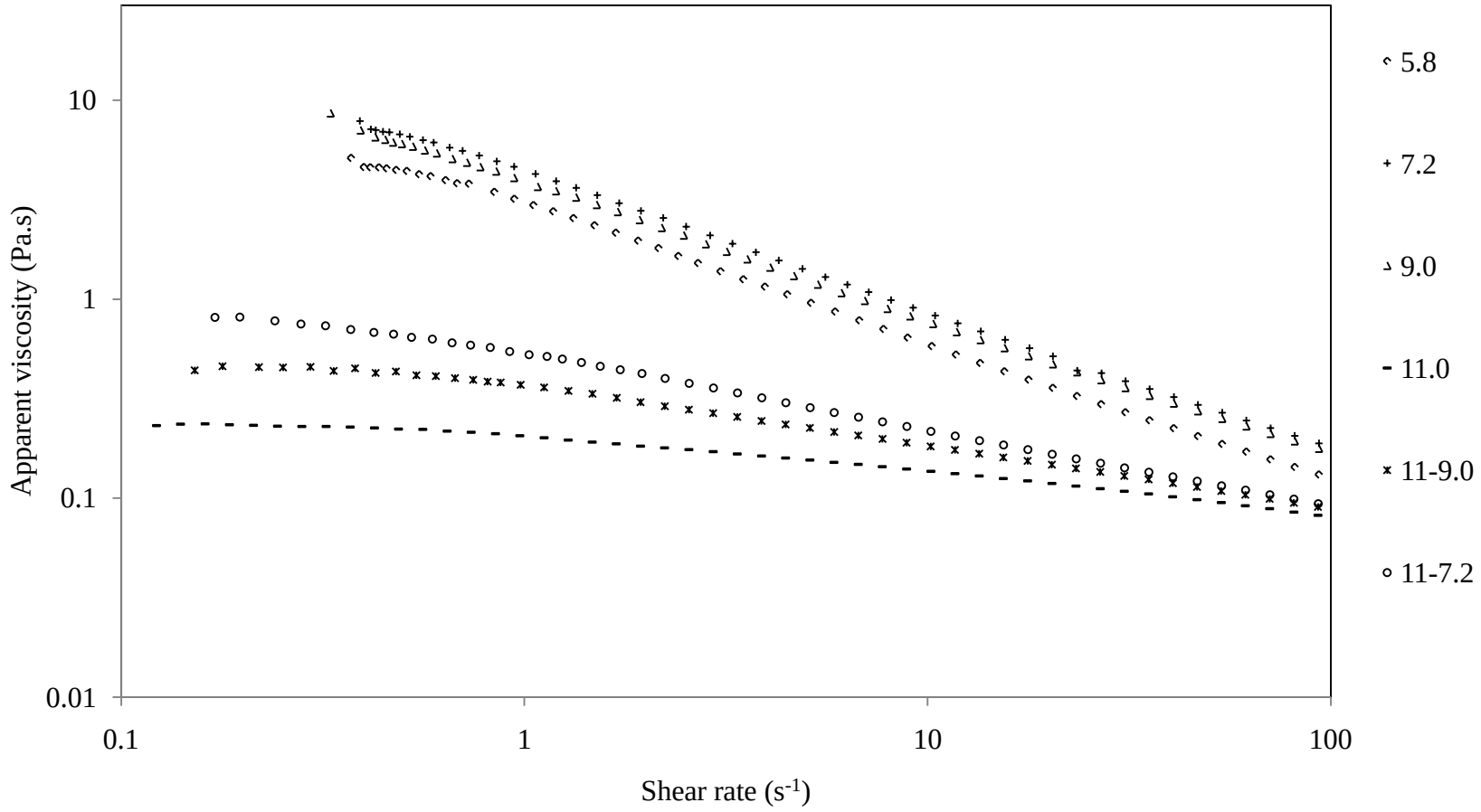

Figure 2 – Apparent viscosity (Pa.s) of 3% (w/w) protein dispersions (measured at the drying pH) from chicken breast meat freeze-dried without added trehalose

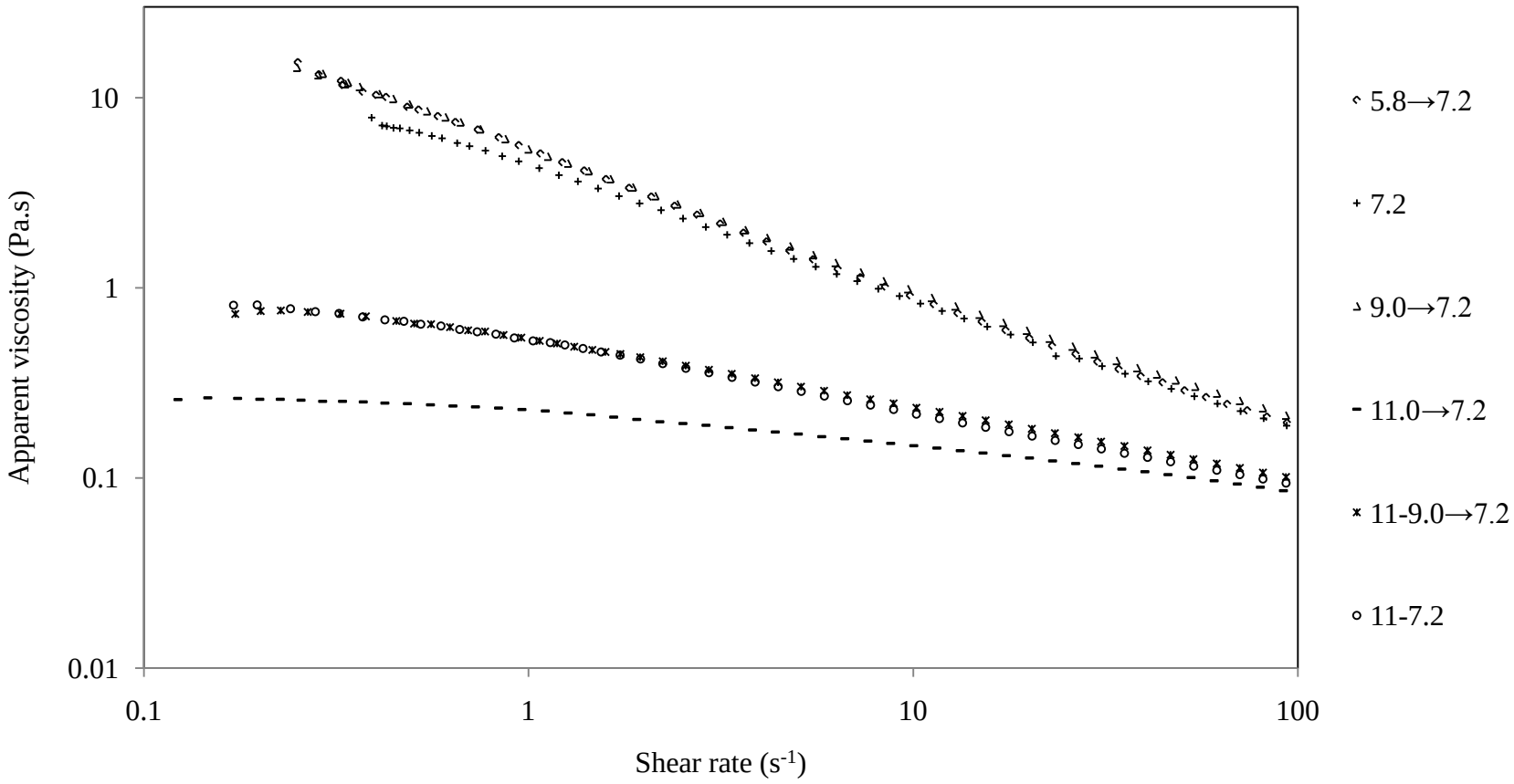

Figure 3 – Apparent viscosity (Pa.s) of 3% (w/w) protein dispersions (all measured at pH 7.2) from chicken breast meat freeze- dried without added trehalose

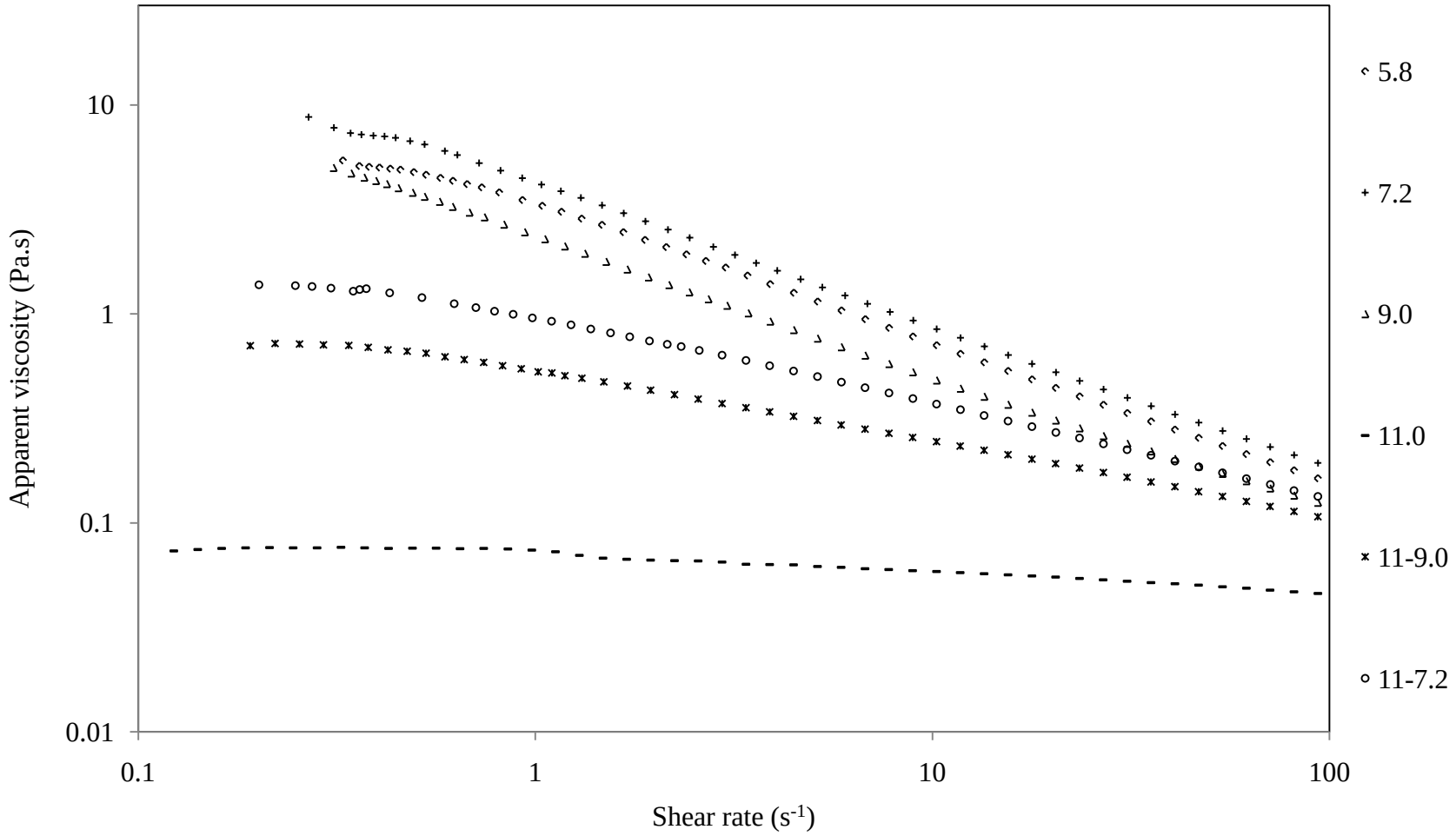

Figure 4 – Apparent viscosity (Pa.s) of 3% (w/w) protein dispersions (measured at the drying pH) from chicken breast meat freeze-dried with trehalose

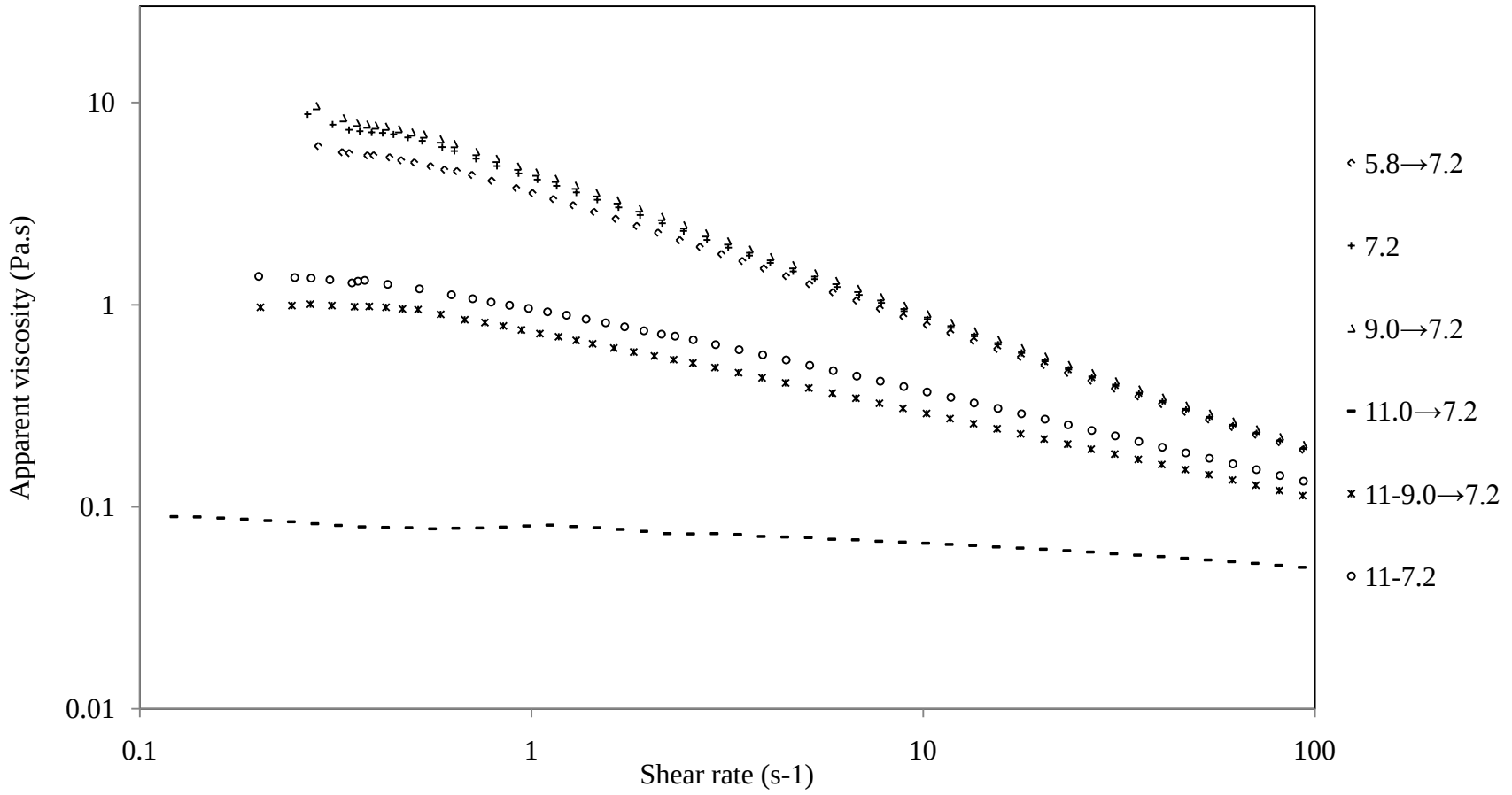

Figure 5 - Apparent viscosity (Pa.s) of 3% (w/w) protein dispersions (all measured at pH 7.2) from chicken breast meat freeze-dried with added trehalose

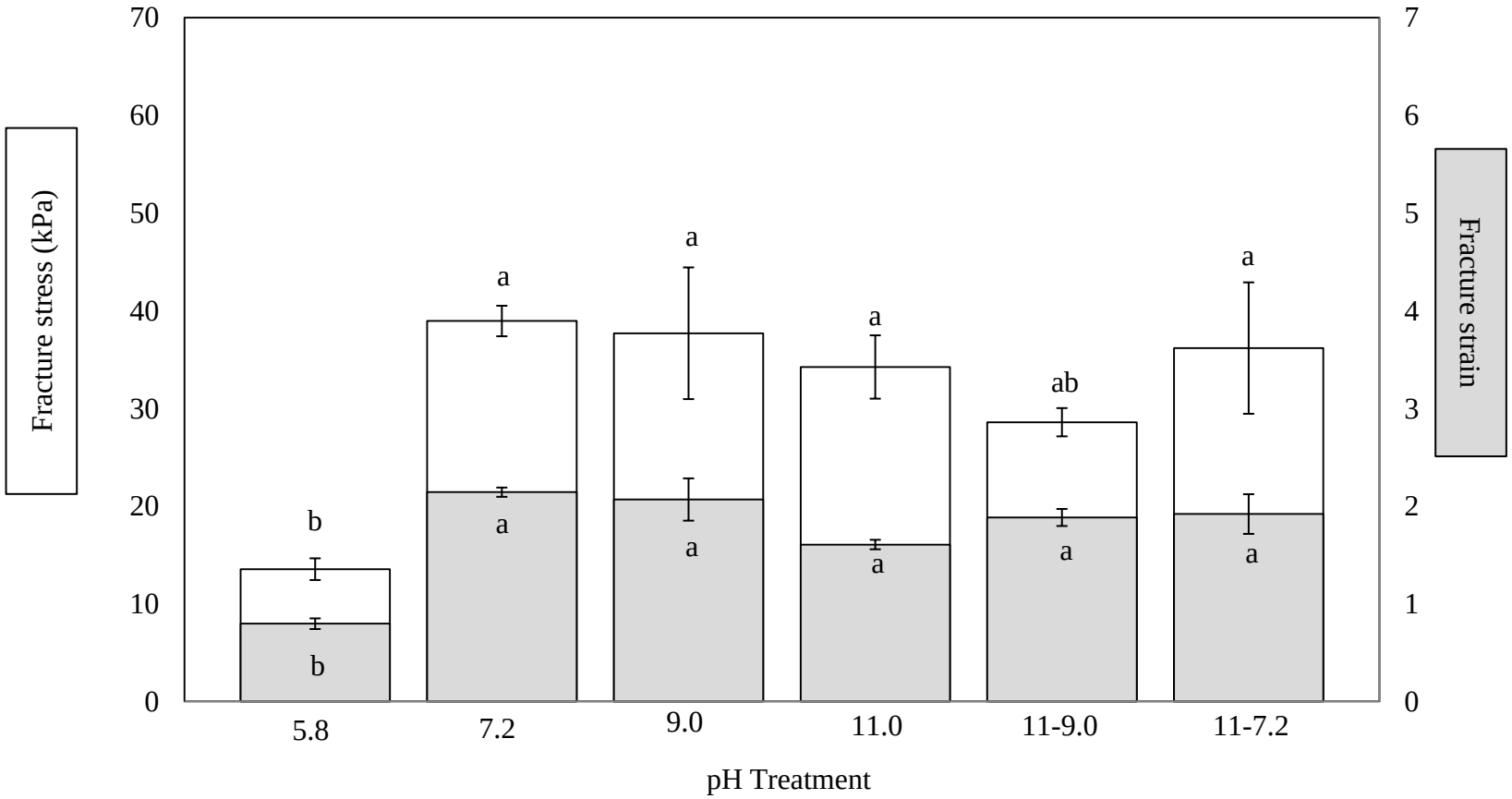

Figure 6 – Fracture stress (kPa) and fracture strain values of gels made (at the drying pH) containing chicken breast meat freeze-dried without added trehalose

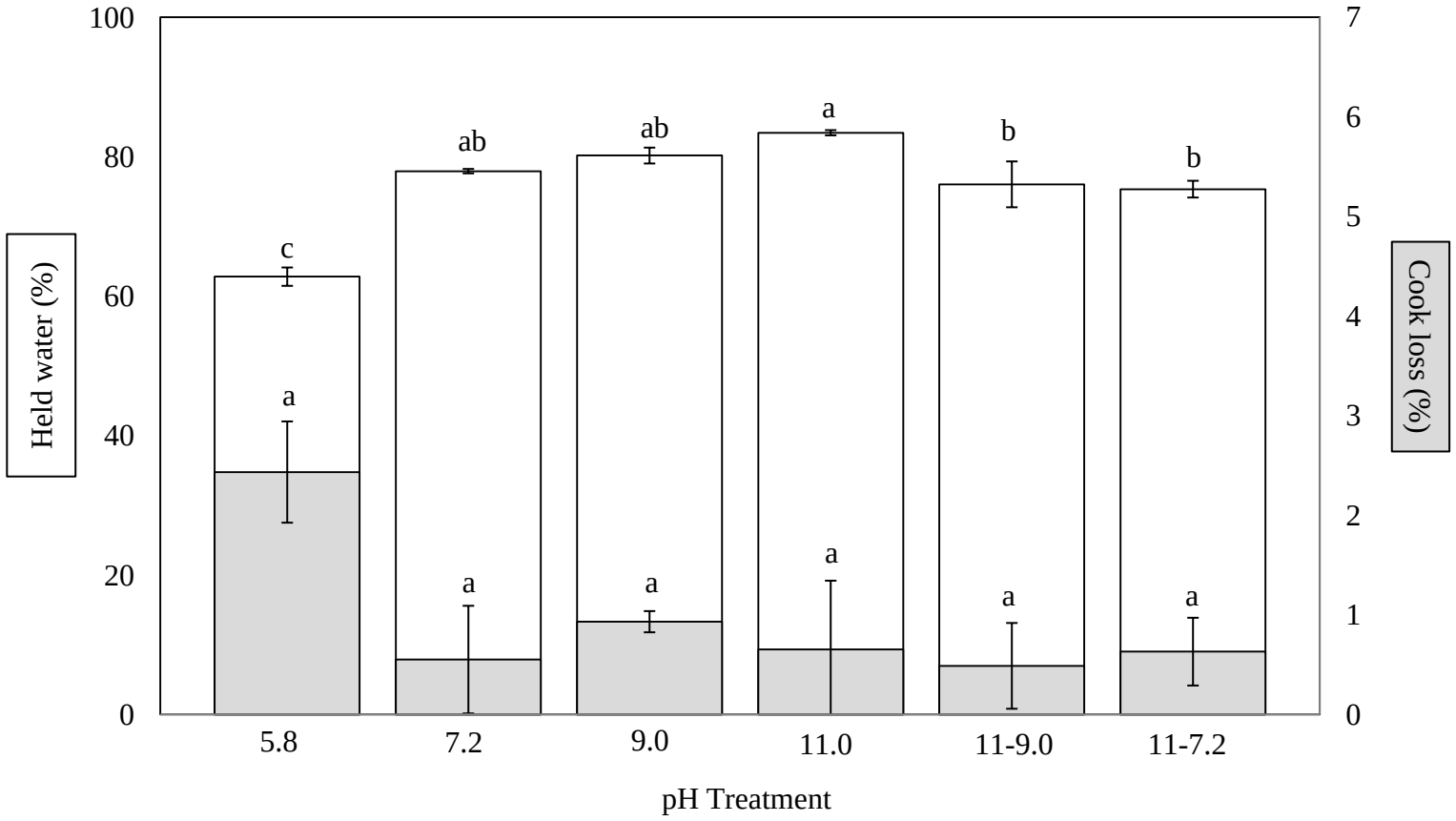

Figure 7 – Held water (%) and cook loss (%) of gels made (at the drying pH) containing chicken breast meat freeze-dried without added trehalose

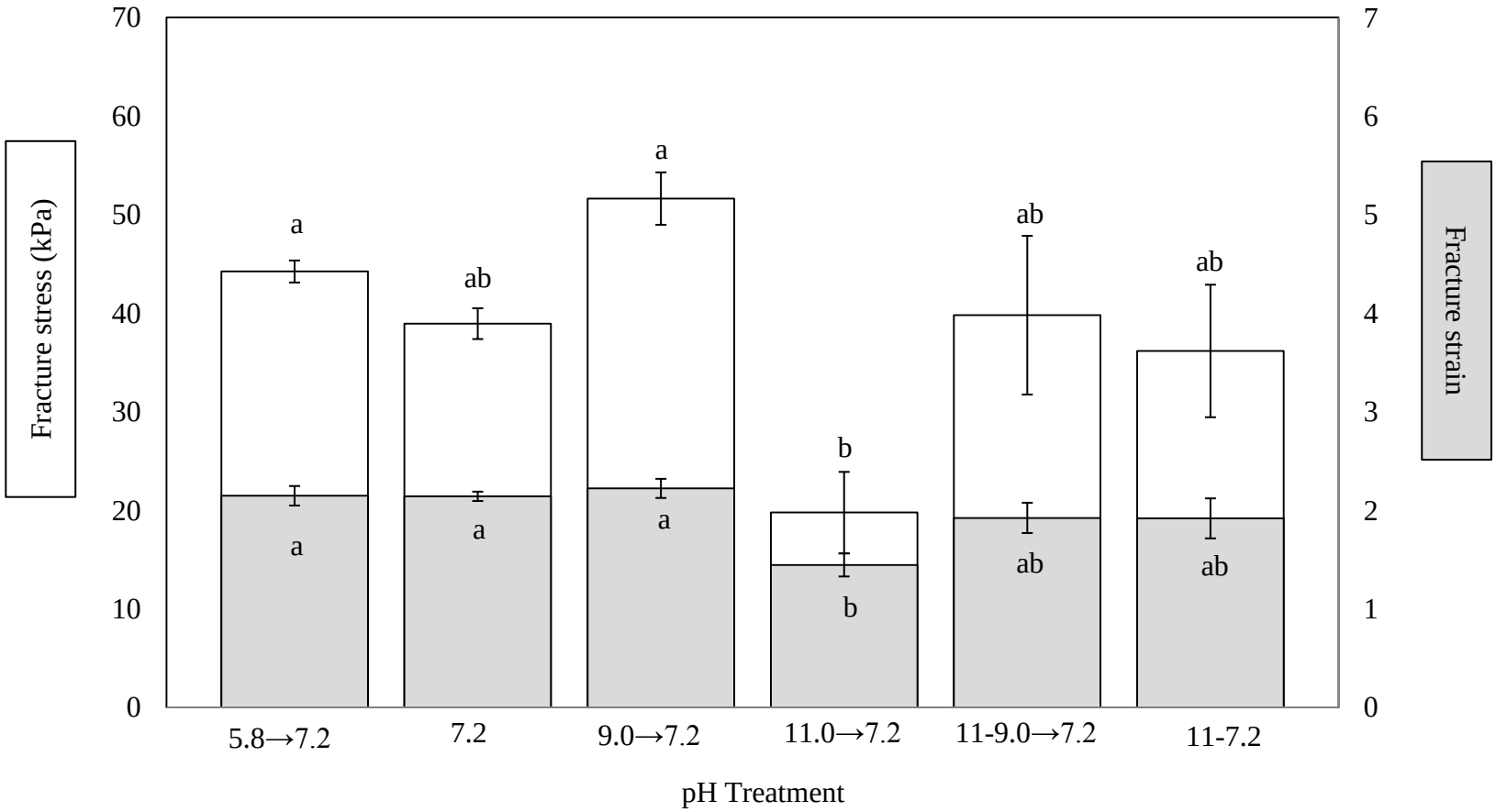

Figure 8 – Fracture stress (kPa) and fracture strain values of gels made (all at pH 7.2) containing chicken breast meat freeze-dried without added trehalose

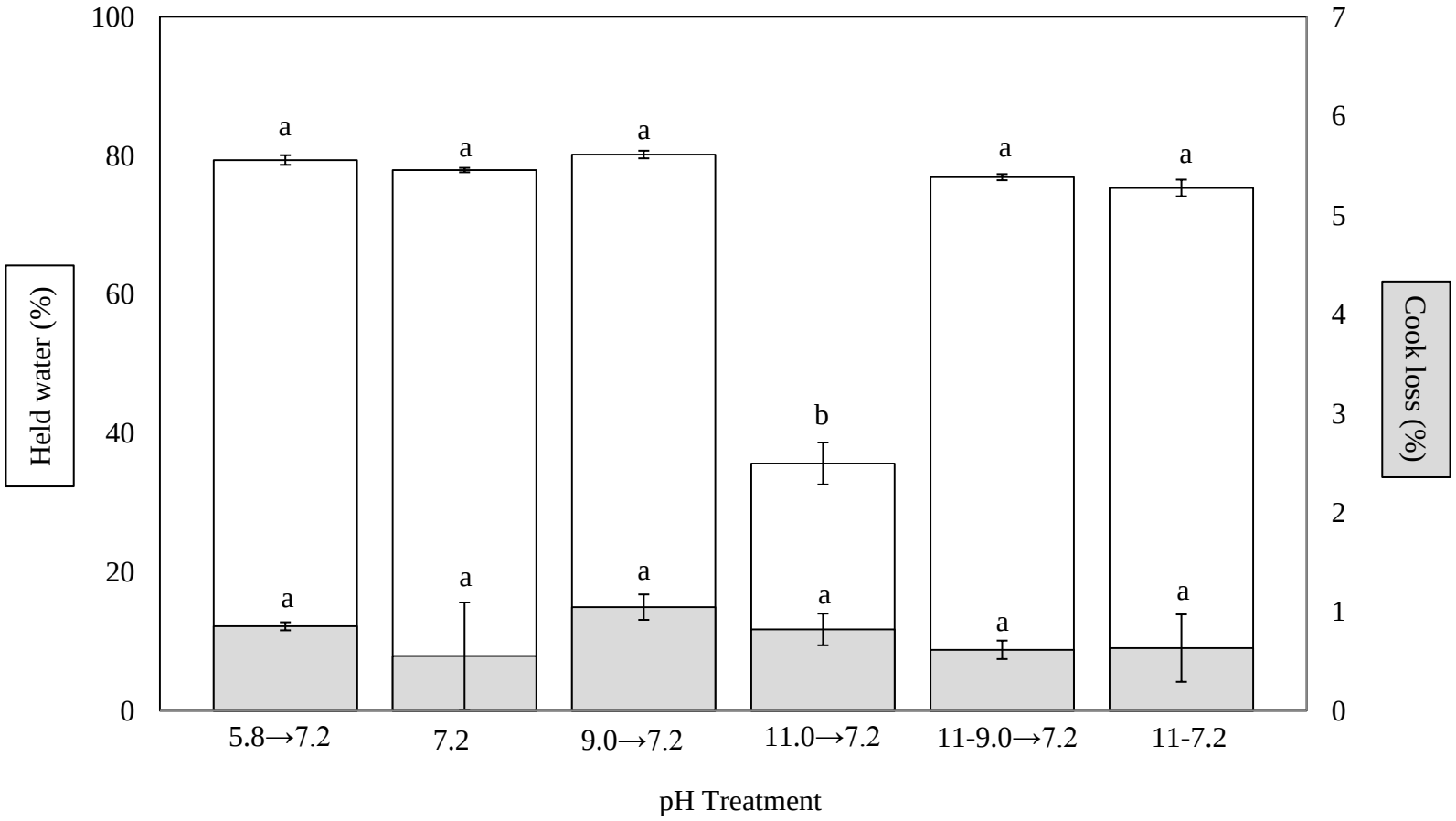

Figure 9 – Held water (%) and cook loss (%) of gels made (all at pH 7.2) containing chicken breast meat freeze-dried without added trehalose

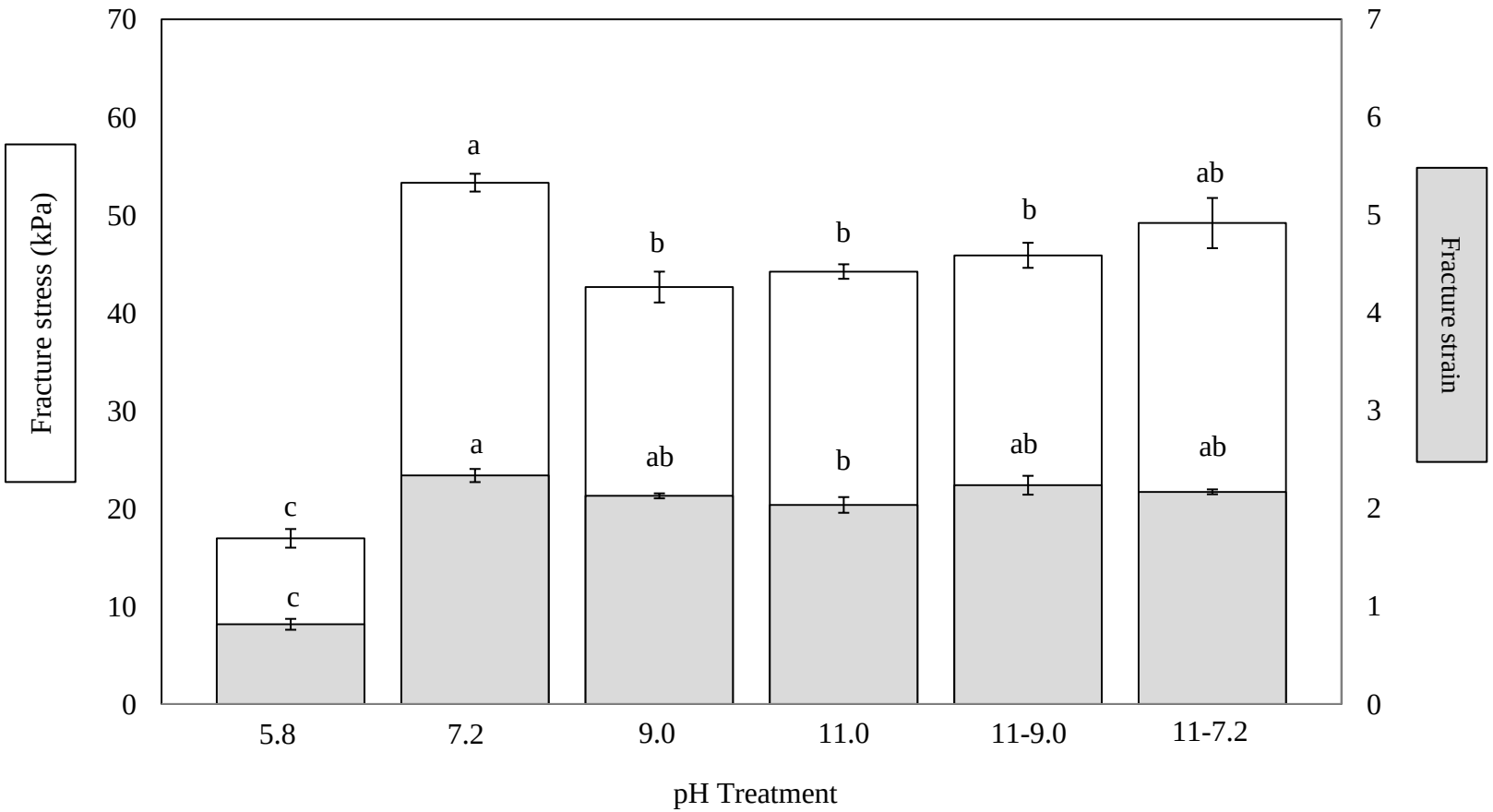

Figure 10 – Fracture stress (kPa) and fracture strain values of gels made (at the drying pH) containing chicken breast meat freeze-dried with added trehalose

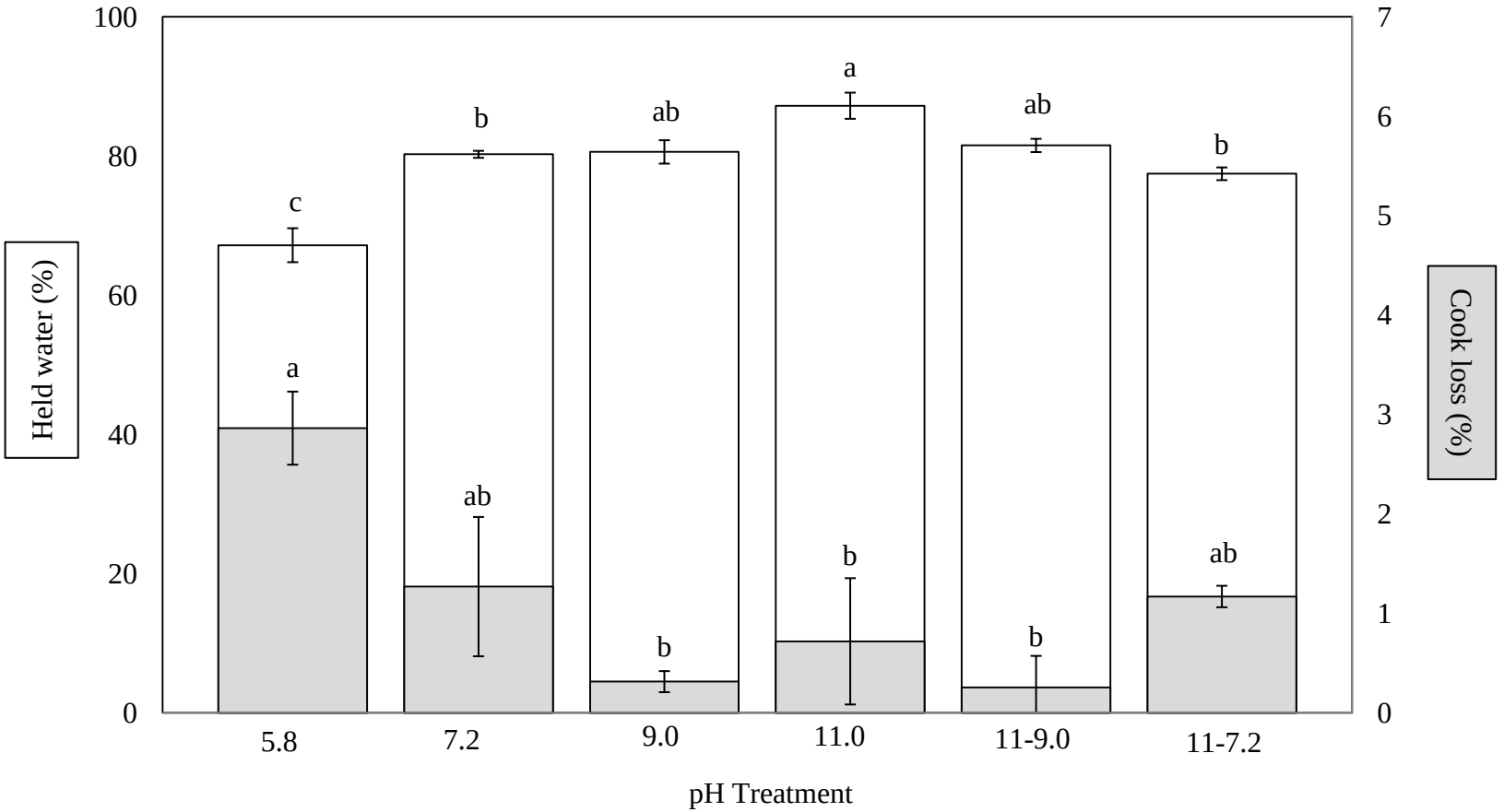

Figure 11 – Held water (%) and cook loss (%) of gels made (at the drying pH) containing chicken breast meat freeze-dried with added trehalose

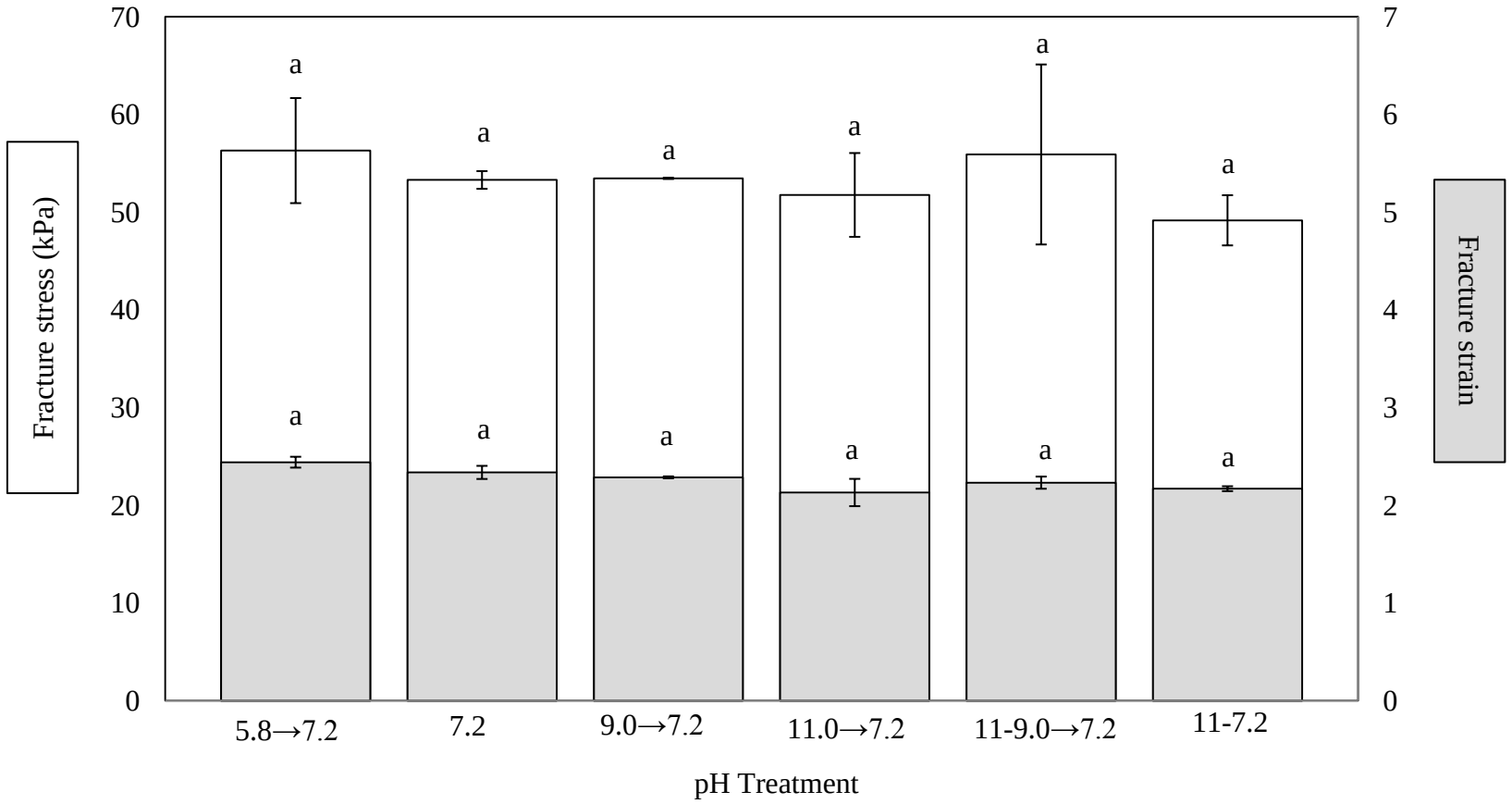

Figure 12 – Fracture stress (kPa) and fracture strain values of gels made (all at pH 7.2) containing chicken breast meat freeze-dried with added trehalose

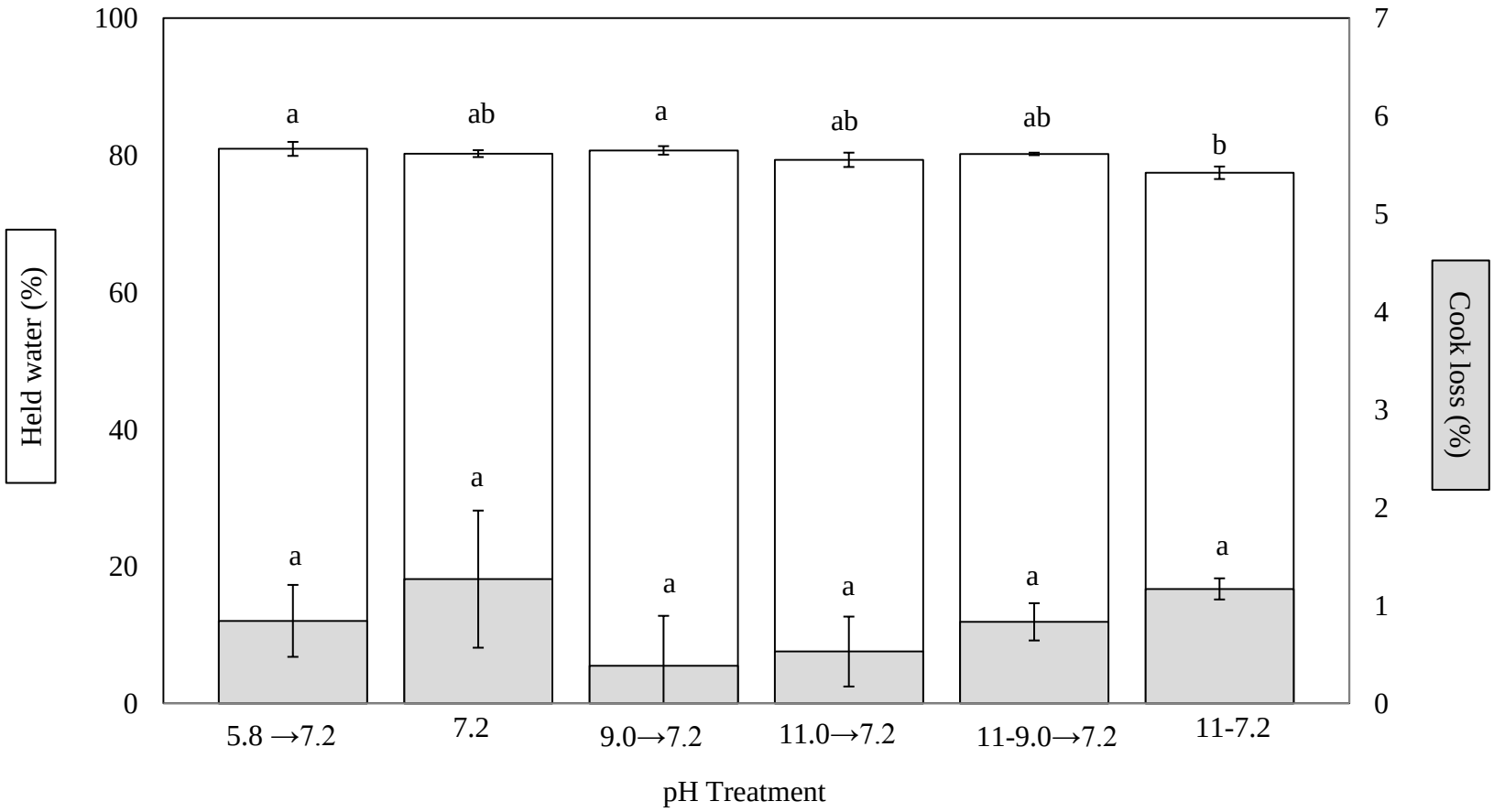

Figure 13 – Held water (%) and cook loss (%) of gels made (all at pH 7.2) containing chicken breast meat freeze-dried with added trehalose

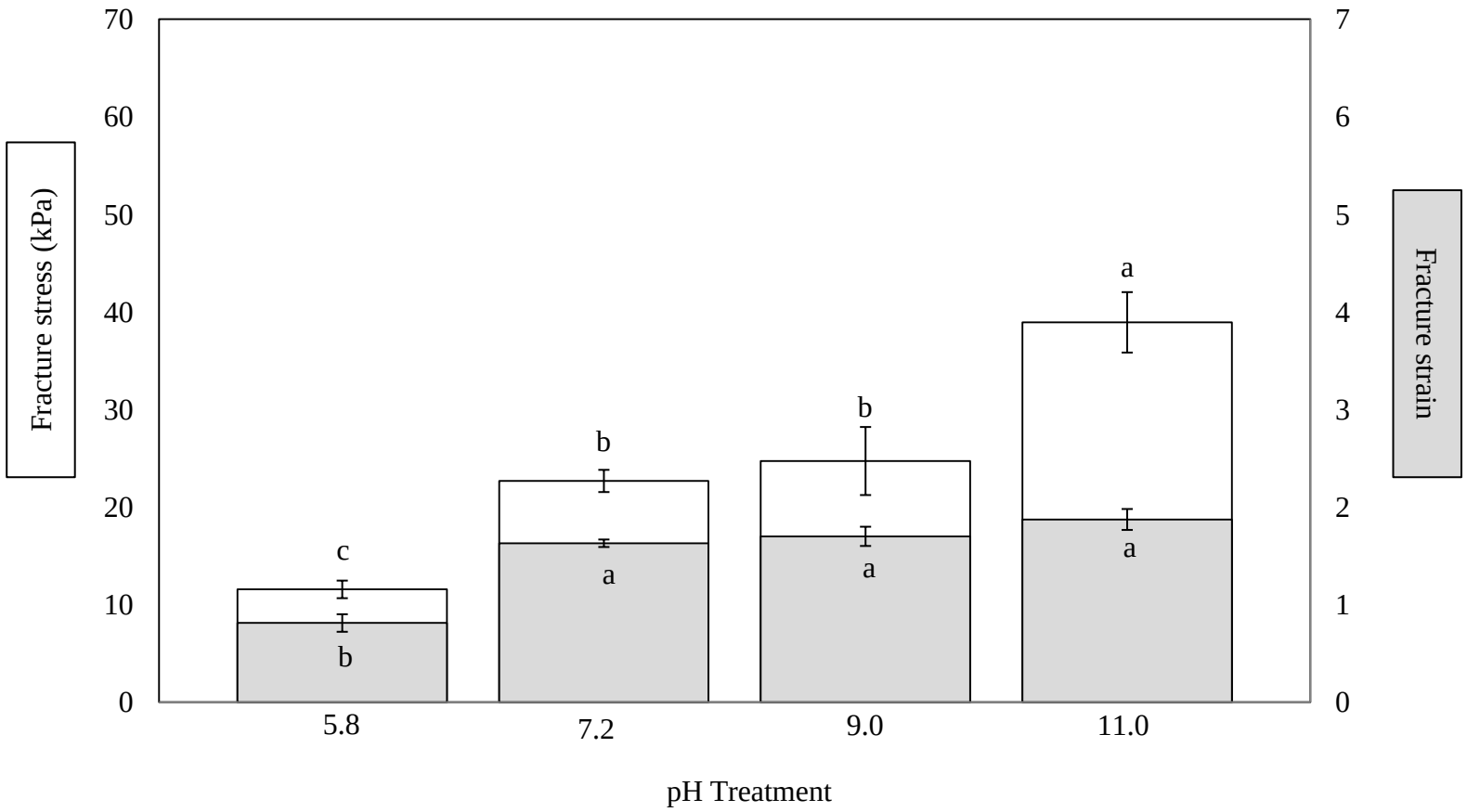

Figure 14 – Fracture stress (kPa) and fracture strain values of gels made only from comminuted chicken breast meat

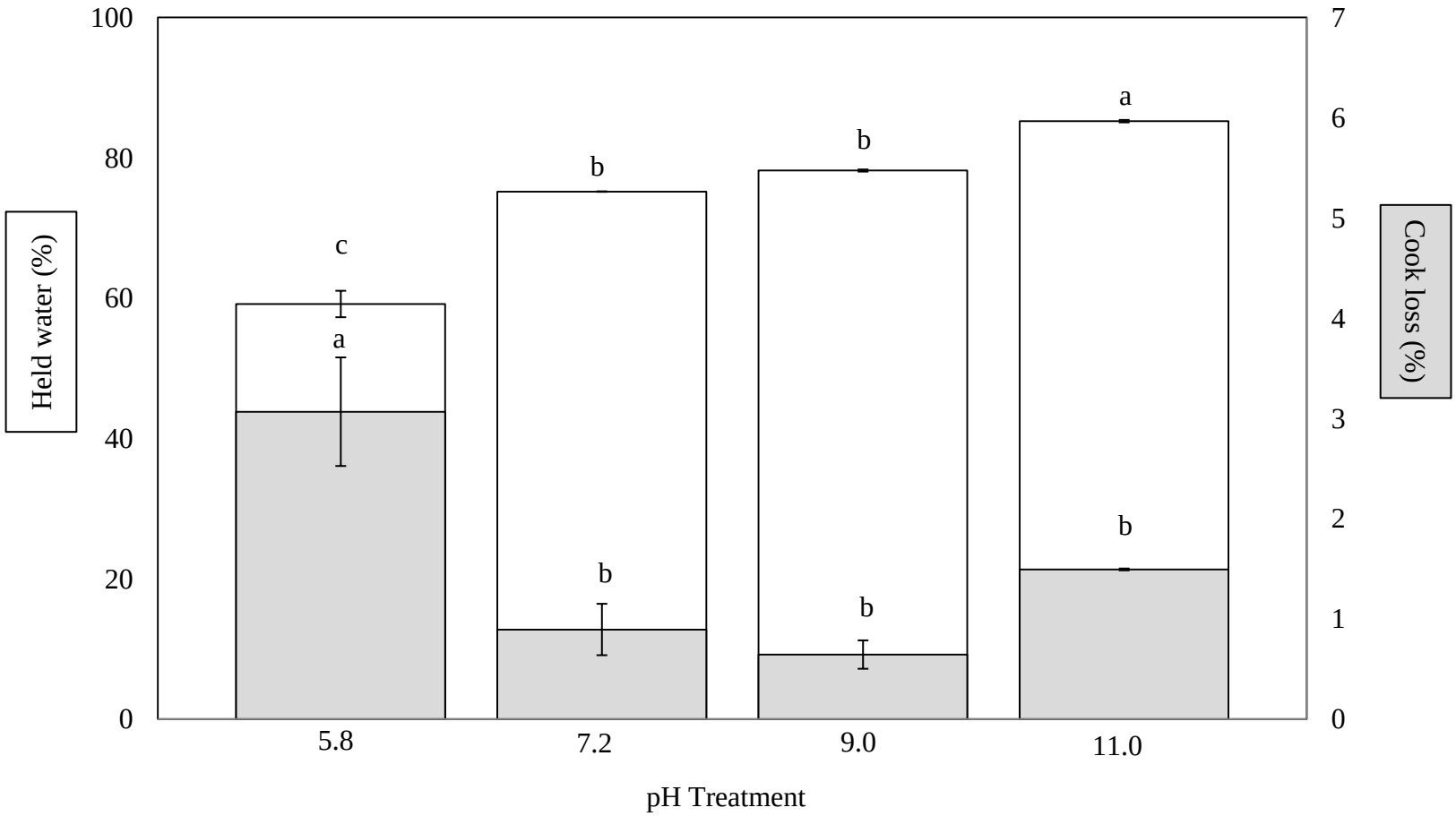

Figure 15 – Held water (%) and cook loss (%) of gels made only from comminuted chicken breast meat