

Abstract

RIEMANN, ALEXANDER ERICH. Gelation properties of comminuted meat paste from cold and warm blooded species as affected by rapid heating. (Under the direction of Dr. Tyre Lanier).

The hypothesis was tested that total thermal input, rather than merely heating rate, is the determining factor in heat-induced gel formation by muscle proteins. Comminuted pastes (2% NaCl and 78% total moisture content) made from Alaskan pollock surimi and turkey breast were heated at 0.5°C/min, 20°C/min or 80/98°C/min to 70°C and held up to 40 min prior to cooling. Fracture stress and strain, rigidity modulus (G'), cook loss and acid phosphatase activity were measured following each treatment. Results showed that rapid heating plus a brief holding time at the endpoint temperature produced similar meat gel textural properties as those cooked by a conventional slow ramp heating just to the same endpoint temperature. Thus the thermal input of the process, above the activation energy of protein denaturation leading to gelation, can be predictive of the resulting gel properties. Moreover, this indicates that the equivalent point method can be used to identify a range of process parameters which would yield cooked protein gels of desirable properties. Additionally, rapid heating, with advantages of process efficiency, space, and control, can likely be utilized by processors of certain muscle foods without compromising product quality.

Rapid heating for preparation of cylindrical (1.9 or 3 cm dia) surimi gels by ohmic and a new focused microwave applicator were compared to conventional heating of gels in a

water bath at 90 or 100°C. Both rapid heating methods (ohmic and microwave) produced superior gels from a surimi blend containing significant heat-activated protease activity as compared to water bath heating at either temperature. These were comparable to preparing gels from the same surimi by water bath with the addition of beef plasma as a protease inhibitor. Ceramic end caps on the teflon tubes used for microwave processing improved the uniformity of heating by microwave. Gels prepared from protease-free surimi in 3 cm casings by microwave heating did not achieve similar results to the same gels made by water processing, which likely resulted from the microwave unit being under powered and not properly focused.

**GELATION PROPERTIES OF COMMINUTED MEAT PASTE FROM COLD
AND WARM BLOODED SPECIES AS EFFECTED BY RAPID HEATING.**

by
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BIOGRAPHY

Alexander Erich Riemann was born on July 21, 1974 in Edison, New Jersey. He is the son of Mary and Mathew Riemann who came from Europe in 1972. He has a 6 year older brother, Christopher Riemann who is a retinal surgeon in Cincinnati, Ohio. Alex grew up in Edison and went to Edison High School where he graduated in June 1992. Alex along with his parents moved to NC in the summer of 1992. He began his college career at North Carolina State University in Zoology. He graduated with his BS in May 1997. In August 1998, he began the graduate program, but was not under the direction of Dr. Tyre Lanier until May 1999, where he began his thesis work at NC State's seafood laboratory.

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Introduction

Comminuted meat products are those which have been mechanically processed to very small particles, such that the blended meat system (which usually contains a variety of both meat and non-meat ingredients) is relatively homogenous throughout. The blended paste, in which the meat proteins have been partially solubilized by salt addition to enhance their even distribution, is heated to unfold the proteins, inducing their gelation. The myofibrillar fraction of muscle is known to be particularly active in this regard (Acton and Ziegler 1983). This results in formation of a solid matrix that entraps other constituents, particularly fat and water. Examples of comminuted meat products include frankfurters and similar cooked sausages, luncheon meats, surimi-based shellfish analogs, and many formed poultry, fish and red meat products such as nuggets, etc.

From this list of examples it is apparent that the meat used in these products may be derived from both warm- and cold- blooded species. Typically these meats are also derived either from lower value species (in the case of fishes) or from less choice cuts of meat (containing higher fat and/or connective tissues) or that recovered as a paste by mechanical deboning of previously hand-deboned carcasses. Comminuted products from land animals seem to have originated mainly in the European nations, whereas comminuted fish products were developed mainly in Asian island nations. Thus while the basic nature of the products is similar, when one compares comminuted products made from fishes vs those from land animals there are considerable differences in both processing and typical ingredients, often deriving from the culture of the originating countries.

Surimi-based Comminuted Products

Surimi is a wet frozen concentrate of primarily the myofibrillar proteins of fish, manufactured by water leaching of mechanically deboned fish flesh (Lanier 1986). Although the stroma (connective tissue) proteins are also concentrated by the surimi manufacturing process, these exist at low concentrations in fish relative to meat from land animals (Mackie 1993). This partially explains why the surimi process has never been successfully adapted to processing the meat of land animals, as too high a content of collagenous tissue in a meat blend can lead to poor gelation and formation of unsightly gelatin pockets in a cooked product (Nowsad and others 1999). The sarcoplasmic fraction of muscle, which is 20 –30 % of the total proteins present (Foegeding and others 1996), is largely lost during surimi manufacturing. For this reason the process is also considered too wasteful for application to land animals, since (in western nations, at least) their meat typically commands a lower profit margin than seafoods. Typically it is the mechanically deboned meats of poultry that have been targeted as candidates for a surimi process due to their low cost, but the deboning equipment used induces emulsification of the fat that makes water leaching very difficult (Ball and others 1987).

Once solubilized by salt addition and comminution, surimi readily gels upon heating to produce a chewy, elastic texture (Shimizu 1985; Lee 1986; Lanier 1986). Montejano and others (1984) compared the properties of gels prepared from a fish surimi with those of gels made from the lean meat of beef, pork and turkey. Under equal conditions of water, fat, and protein content the surimi evidenced stronger and more cohesive gels. Such superior gelling properties of surimi have previously been attributed

to its higher relative content of myofibrillar protein as compared to lean, unleached muscle (Nowsad and others 2000). However, it seems likely that the relatively lower degree of muscle cytoskeletal structure present in the leached meat of surimi may also contribute, by facilitating better protein distribution in the gel (Chang and others 2001).

Often mammalian or avian meats are mixed with surimi in Asian foods, but such mixtures have not been acceptable from an aesthetic (market) or regulatory standpoint in the U.S. The most popular western products manufactured from surimi are imitation crabmeat and other forms of shellfish analogs, termed 'surimi seafoods' (Vondruska 1985).

Comminuted Products from Mammalian and Avian Meats

Prior to the recent advent of low-fat versions of comminuted meat products, most were typically of higher fat content (about 30%). Such products have often been described as meat 'emulsions' (Yetim and others 2001). Hansen (1960) assumed that fat in a meat batter forms a discontinuous phase which is stabilized by a continuous phase of solubilized protein. Others describe comminuted meat gels as consisting of solid fat particles suspended in a protein sol, later entrapped by the gel that forms upon heating (Saliba and others 1987). Some high fat versions of surimi-based products, termed 'fish sausages', are consumed in Asian nations (Tanikawa 1985). Lower fat versions of traditional European or north American comminuted meat products substitute macromolecular hydrocolloids or gums for the fat component to yield a product that has similar textural properties (Grigelmo-Miguel and others 1998).

Traditionally the high fat 'sausage' products were made as a means of utilizing fatty or collagenous trimmings of the carcass, and these still form the bulk of most higher-fat content products. The demand for lower-fat products has led to new methods of defatting these. There is also increased use of mechanically deboned meat, usually derived from manually deboned carcasses, which ranges 5-20% in fat content typically and may contain up to 1% bone particles or residue (Grigelmo-Miguel and others 1998). Typical deboners used by poultry processors first grind the meat-containing bone, then force the meat through small apertures to strain out bone particles (Calhoun and others 1999). In a newer process used for mammalian deboning, termed "advanced meat recovery system" (AMR), bones (typically from mammalian sources) such as cervical vertebrae are presized into 10 – 15 cm lengths and then pressed in a piston-like device that forces mainly meat, with some marrow, from the bones (AMSA 1997). Lean meats from dairy animals are often used to improve gelation properties and reduce fat in sausage products (Fisher and others 2000).

Chemistry of Muscle Protein Gelation

Muscle proteins can be classified by their solubility characteristics into myofibrillar proteins (contractile element proteins; primarily salt soluble above physiological ionic strength), sarcoplasmic proteins (largely metabolic proteins; easily solubilized in water) and stromal proteins (connective tissue proteins; largely insoluble in water or salt water) (Ziegler and Acton 1984b). Grabowska and Sikorski (1976), as well as Schmidt (1982), found that sarcoplasmic and stromal fractions possess no gelation ability. However, Lan

and others (1995b) demonstrated that 7 and 10% solutions of sarcoplasmic protein at pH 6.0 and 5% NaCl can gel when heated.

Myofibrillar proteins in their near-native conformation generally exhibit good gel forming ability when properly solubilized by salt and heated (Ziegler and Acton 1984a). Myosin, which comprises 55-60% of myofibrillar proteins, is thought to be the primary myofibrillar protein responsible for gel formation of heated meat pastes (Visessanguan and others 2000). Myosin is a multidomain protein, composed of two large heavy chains and four light chains arranged into an asymmetrical molecule with two globular heads attached to a long alpha helical rod-like tail (Privalov and Medved 1982). The myosin molecule may be further segregated into several regions in the laboratory by proteolytic attack. Smyth and others (1996) showed that these regions each denature at different temperatures, indicating that it is unlikely that the entire myosin molecule would unfold instantaneously during heating at a particular temperature.

Fennema (1976) defined protein gels as a three dimensional network of protein fibers formed by intermolecular (protein-protein) interactions which hold or trap water. Paul and Palmer (1972) postulated that the flexibility and the number of connections between polymer molecules determine the elasticity and strength of protein gels, respectively. It is also known that different types of intermolecular bonds become more prevalent at different temperatures during heating, such that this might also affect gel properties.

Samejima and others (1981) suggested that heat-induced gelation of myosin initially involves the formation of aggregates by the globular myosin heads with involvement of disulfide bonds. As heating proceeds, the tail portions unfold and a

myosin network forms. Acton and Dick (1989) later reviewed research on the sequence of events during heat-induced gelation of myosin. Initially aggregation of the globular head regions of myosin occurs upon heating to 30 – 50 °C. Upon heating to 50-70 °C, a structural change occurs in the helical rod segment of myosin, leading to network formation through cross-linking of these segments. As the temperature is increased, hydrogen bondings decrease, though such bonds can reform to strengthen the resulting gel upon subsequent cooling. As heating leads to conformational change (unfolding) of the myosin, there is a greater tendency for inter-chain hydrophobic interactions, which are also strengthened with increasing temperature (Wicker and others 1989). Foegeding and others (1996) believed that the head portion of myosin undergoes irreversible aggregation involving hydrophobic interactions and oxidation of SH groups. This is followed by cross-linking between unfolding tail portions from similar reactions (Lee and Lanier, 1995).

Fish muscle pastes have the unique ability to form gels at temperatures well below 40 °C (i.e., below the denaturation temperature for myosin), a phenomenon known as ‘setting’ (Lanier and others 1982). Nondisulfide, covalent polymerization of myosin heavy chains is responsible for this setting phenomenon, attributed to glutamyl-lysine bonding by an endogenous transglutaminase (Kamath and others 1992). Setting is not exhibited by the muscle pastes of homeotherms; this may result either because of the greater stability of the myosin in land animals or a lesser endogenous transglutaminase activity (Lee and Lanier 1995).

Cooking of Comminuted Meat Products

Most of the western-style comminuted meat products, particularly those containing substantial amounts of fat such as hot dogs and many luncheon meats, are heated relatively slowly (usually over 1-2 hr) to the desired endpoint temperature (typically 70-75 °C) (Komarik and others 1974). Supposedly this slow heating rate results in better gel texture and optimum fat and water-binding properties. Hermansson (1978) elaborated on Ferry's (1948) two-step (denaturation > aggregation) model of protein gelation that, when protein aggregation is suppressed until after denaturation occurs, the resulting network can be expected to exhibit a finer texture and higher degree of elasticity than if random aggregation and denaturation occur simultaneously, or if aggregation precedes denaturation. Ziegler and Acton (1984b) interpreted this as an explanation of why slower heating of protein gels apparently resulted in stronger texture. He attributed the low temperature setting ability of fish pastes to the better 'ordering' of the gel structure when denaturation proceeded slowly.

Foegeding and others (1986b) observed that rapid heating (50 °C/hr) produced weaker myosin gels from beef than a slower heating rate of 12 °C/hr. They similarly surmised that slower heating rates would result in more extensive denaturation of the proteins prior to aggregation, thus allowing for the creation of more active sites for aggregation and therefore more extensive aggregation. Saliba and others (1987) determined that the strength of cooked frankfurter batters (gels) decreased linearly as heating rate was increased from 0.25 to 0.9°C/min. They likewise explained that slower heating rates could allow for more time for the proteins to unfold and interactions to occur thus enabling a stronger gel matrix to form. Camou and others (1989) reported a

decrease in the strength of gels made from pork salt soluble protein as the heating rate was increased from 17 – 85 °C/hr and similarly attributed this to more complete gel formation at the slower heating rates. Barbut and Mittal (1990) showed that the rigidity (G') of cooked beef meat batters (gels) decreased as the heating rate was increased from .31 to 1.62 °C/min, citing the explanation of Foegeding and others (1986a), that slower heating rates can allow more time for proteins to unfold and interact, thus enabling a stronger gel matrix to be formed. They also stated that the main factor driving the proteins to coagulate or form a gel is the rate of their association, where slower rates favor more ordered interactions and a stronger gel formation. Arntfield and Murray (1992) also showed that, for a 10% ovalbumin network in .15M NaCl, its gel rigidity decreased as heating rate was increased. They explained that the slower heating rates allow more denaturation to occur prior to aggregation. Yongsawatdigul and Park (1996) obtained very fast heating rates for pollock surimi pastes by use of ohmic heating and found that the strength of gels decreased as the heating rate was increased from 1 to 30 °C/min, with the effect of heating rate. Their explanation was that the slower heating regime is likely to prolong the myosin crosslinking reaction because transglutaminase is subjected to an optimum temperature range (25 – 30 °C) for a longer period, thus more “setting” can occur. Yoon and Park (2001) showed that slower heating rates induced stronger whey protein concentrate gels and likewise explained that, during slower heating, protein molecules in solution are allowed sufficient time to aggregate molecular strands into a more ordered structure, thus forming a stronger gel matrix.

In all the works cited previously, a negative effect of increased heating rate was observed on protein gel strength or rigidity. The explanation of such observations

was also consistent amongst these authors who believed that, based on the essentially two step mechanism of protein gelation (denaturation > aggregation & gelation) proposed by Ferry (1948), slower heating rates allowed more time for protein denaturation and/or 'alignment' prior to aggregation. This more 'ordered' alignment, which was presumed to occur during slow heating of protein sols, was the explanation for the greater strength and rigidity of the cooked gels.

Another common feature of these works is that, in every case, effects of heating rate were compared amongst gels which were heated to the same endpoint temperature. Thus the time of heating, and especially the time of heating above the temperature at which proteins would begin to unfold and aggregate (dependent on the species and conditions of the experiments) were different amongst treatments heated at different rates. Foegeding and others (1986a) had actually also posited another explanation for the heating rate effects. They indicated that faster heating rates may have produced weaker gels because the pastes under faster heating spent less time above the denaturation temperature of the proteins than when they were heated more slowly. There was no further elaboration by these authors on this possibility, nor did they attempt to experimentally test this alternate theory. Certainly, however, it represents an entirely different explanation of their observations; that time of protein aggregation, rather than time of denaturation prior to aggregation as so many have believed, was responsible for weaker gels obtained at higher heating rates.

Interestingly, the implications of the two opposing hypotheses are quite different. If the cause of weaker gels at higher heating rates is a too-rapid transition through the denaturation step, then it is unlikely that rapid heating under any conditions could lead to

gels of equivalent strength and properties. However, if (conversely) the explanation is that rapid heating to the same endpoint temperature merely does not allow sufficient time for aggregation to occur following denaturation, then it should be possible to extend this process time at the endpoint temperature (isothermally) sufficiently such that gels which are rapidly heated may attain equivalent strength and properties to those heated more slowly just to the same endpoint temperature. The experimental work of this thesis is the first published attempt to test this second, alternate hypothesis.

Water Holding Ability of Protein Gels

Water holding capacity describes the ability of a matrix of macromolecules present at relative low concentrations to physically entrap large amounts of water in a manner that inhibits exudation (Fennema 1996). Syneresis, defined as water leakage from a gel during refrigerated holding, can occur as the number of crosslinks or junctions in a gel gradually increase to compact the network and expel water (Ziegler and Acton 1984b). Cook loss is a reflection of a loss in water holding capacity. There are few explanations as to why cook loss occurs. One explanation for cook loss occurring during cooking of comminuted products of land animals may be the heat-induced shrinking of connective tissue (collagen). Such shrinking could possibly mechanically expel water from the gel. Patana –Anake and Foegeding (1985) showed that loss of water is significant during cooking of frankfurters from 50 to 70 °C. Yang and Froning (1992) found that a higher cook yield was seen in washed chicken mince as compared to unwashed mince. The protein amounts and pH were not equivalent, however, and the

higher cook yield was attributed to an increased concentration of myofibrillar protein in the washed mince.

Water loss under applied pressure is another common measure of water holding capacity of gels. Camou and others (1989) tested water loss by compressing samples to 20% of their original height, then centrifuging to separate the water expelled from the surfaces of the gel.

The mechanism of water holding ability in a gel has been widely disputed. Ziegler and Acton (1984b) concluded that water is immobilized in a gel through capillary action. Chang and others (2001) further elaborated on this by explaining that a better gel for water entrapment might occur as the proteins are better distributed by freeing them from their myofibril structure during the comminution process. Better dispersed proteins can supposedly form more numerous and strong crosslinks which can create small pockets that can entrap water via capillary action. This may be the reason why surimi has a good water holding capacity.

Another possible reason that a finely textured gel, comprised of evenly distributed proteins and cross-links, increases water holding capacity is that such a gel causes increased structuring of water (Ling 1992). Water holding capacity of meat gels may also be related to the numbers of polar groups available for binding water (Zayas 1997).

The pH of meat can dramatically affect water holding capacity. Lan and others (1995a) found that water loss, due to cooking, decreased as pH increased in comminuted meat gels. Water loss was greatest at the isoelectric point of the meat. As the pH is increased, the electrostatic repulsion between the muscle proteins increases, resulting in space between the proteins that can be occupied with water. Lan and others (1995a) also

concluded that, in addition to electrostatic repulsion, increased protein-solvent interactions may have occurred with increased pH.

Heating Rate Effects on Surimi

It is interesting that very rapid cooking, at rates in excess of 100 °C/min, is already commonly employed in the manufacture of surimi seafood products. This occurs in the initial setting of the meat paste to form a flexible gelled sheet, and is accomplished by steam heating of a very thin (2mm) sheet of paste. Slower heating rates are used to cook various types of kamaboko products in Japan, mainly resulting from the greater diameter of these products (several cm) and the use of conductive heating.

Yongsawatdigul and Park (1996) and Yoon and Park (2001) both found that rapid ohmic heating of surimi and whey gels respectively, with immediate cooling upon reaching an endpoint temperature, produced gels that were not as strong as gels cooked more slowly to the same endpoint temperature.

Surimi from most fish species contains some level of heat-activated protease. This may arise from various sources, such as leaching of gut enzymes during holding of intact fish, from parasitic cysts, or merely endogenous to the muscle of particular species (Lanier 1986). Such proteases typically are most active during heating to 50-60 °C (Park and others 1997) being largely inactivated once the product exceeds 70 °C (Yongsawatdigul and others 1995).

Obviously then if such protease activity is present in surimi, heating rate (assuming an endpoint temperature well in excess of 70 °C; typically 90 °C) could dramatically affect the texture of the resulting gel (Lanier 1981). Slower heating would

allow more time for the proteases to cleave myofibrillar proteins in the active temperature range, leading to drastically weakened gels in a matter of minutes (Yongsawatdigul and others 1995). Rapid heating eliminates this problem; (Yongsawatdigul and Park 1999) showed that myofibrillar proteins of Pacific whiting surimi, which contained no protease inhibitors, exhibited higher shear fracture stress and strain when heated at a higher heating rate of 30 °C/min.

Surimi from many species can also exhibit significant activity of endogenous transglutaminase (TGase). Typically this crosslinking enzyme is active at temperatures in the range of 0-40 °C, depending upon species, and usually activated by salt addition during comminution of the surimi paste (Lee and Lanier 1995 , Yongsawatdigul and Park 1996). Whereas rapid heating to >50 °C would rapidly inactivate this crosslinking activity, slow heating below these temperatures could result in significant gel strengthening due to formation of covalent glutamyl-lysine crosslinks (Lee and Lanier 1995; Yongsawatdigul and Park 1996). It is important to note that even distribution and proper arrangement of each type of cross-link are of equal or greater importance in determining their contribution to gel mechanical properties than just the number of crosslinkages that occur (Lee and Lanier 1995; Chang and others 2001).

Heating Methods for Comminuted Meat Products

Comminuted meats prepared from land animals are typically cooked by a slow heating regime in smoke houses that use dry indirect heating with added humidity. Surimi-based products are typically cooked by flame broiling or steam, sometimes at high heating rates (i.e., thin sheet-cooked products such as imitation crab). Several

technologies now exist which can rapidly heat (>5 °C/min) products of larger diameter. Yongsawatigul and others (1995) used ohmic heating to rapidly cook surimi pastes so as to minimize heat-activated proteolytic activity and thereby maximize gel strength. Ohmic heating employs an alternating electrical current passed through an electrically conducting food product (Biss and others 1989). Heat is internally generated because of the electrical resistance of the food. Heating rate is directly proportional to the square of the electric field strength and electrical conductivity of the food. The electric field strength can be adjusted by altering the applied voltage or the gap between the two electrodes that supply the alternating current to the food sample (Ruan and others 2001). Since heat is simultaneously generated in liquid and solid phases of a product, there is a uniform temperature increase (Parrot 1992). Ohmic processing of low acid ready to eat meals is commercially practiced, as has processing of whole strawberries and other fruits for yogurt (Ruan and others 2001). Advantages of ohmic heating include that the product heats uniformly, that higher temperature in particulates than liquid can be achieved, which is impossible for conventional heating and that high energy efficiency is achieved because 90% of the electrical energy is converted to heat (Ruan and other 2001). In continuous processing, problems can result if a single electrode pair is used to heat food material through a large change in temperature; substantial changes in liquid conductivity and thus heating rate may result along the length of the electrode, thus preventing the ability to control the apparatus through a feedback mechanism. Another problem included the dissolution of the electrode into the food over time. However new metals, which could be used for electrodes are available that prevent this (Ruan and others 2001).

Microwave heating of salt-containing meat gels can also be quite rapid. Greene and Babbit (1990) achieved a heating rate by microwave sufficient to greatly reduce the textural degradation normally seen in slower cooking of Arrowtooth flounder surimi pastes. Polar molecules in a sample to be heated by microwaves align with the constantly shifting electric field (millions of times per second depending on the microwave frequency, typically 915 Mhz or 2.4 Ghz; Metaxas 1991). The resulting constant rotation of the polar molecules generates heat through friction (Reiger and Schubert 2001).

However, the design of traditional microwave applicators ('ovens') creates a non-uniform heating pattern. Decareau (1985) and Datta and Hu (1992) concluded that, since heating rates by microwave are very rapid, conductive heat transfer cannot uniformly take place to even out hot spots created in the product. Microwave cavities are made of metal, as a result microwaves are reflected from the walls of the cavity. A large percentage of the reflected waves will be directed back towards incoming waves, the magnitude of which is the same. As the two collide, standing waves result. At the nodes between the standing waves the electric field can oscillate with twice the normal amplitude, affecting the distribution of energy at the surface of the material to be heated and causing uneven heating of the product (hot and cold spots), because of their unequal distribution (Rosenthal 1992). A metal like fan, called a mode stirrer, as well as rotating the food within the microwave is used to scatter microwaves to reduce hot and cold spots (Rosenthal 1992). Recently a new microwave applicator has been developed which focuses the applied microwave field (Joines and Drozd 1999) to achieve more uniform heating of product passed through the applicator at a constant rate.

Radio frequency (RF) heating can also be used for rapid heating of comminuted meat products having larger diameter. Whereas microwaves affect both ionic and polar molecules, RF uses longer wavelengths (1 to 300 MHz, with the most typical frequencies being 13.56, 27.12 and 40.68 MHz; Jones 1989) which heat mainly the highly polar molecules such as water (Sandeep 1999). Power generation is also different between the methods as microwaves are typically generated by a magnetron controlled by an applied voltage, whereas an RF generator is based on a triode thermionic valve and the material being processed becomes an essential electrical component in the circuit (Anonymous 1994). RF has been used in a process to heat cure raw meat products, such as meat, meat offal and fish (Jensen and others 1990).

Processing of foods by microwaves

Faraday's Law states that a time-varying magnetic field produces an electric field (Iskander 2000). In a microwave magnetron a magnetic field forces electrons to spiral around a cathode, which in turn induces alternating currents in anode cavities. This is the start of wave propagation (Chan and Reader 2000). The dielectric properties of foods determine the amount of energy that a material can absorb, and thereby influence the degree of heating achieved (Decareau 1985). These properties consist of the dielectric constant, which is the ability to store electromagnetic energy; and the dielectric loss, which is the ability to dissipate the electromagnetic energy and convert it to heat (Rosenthal 1992). These properties provide an indication of the electrical insulating ability of the material (Singh and Heldman 1993). Foods are very poor insulators, thus

they generally a large fraction of the energy when placed in a microwave field, resulting in instantaneous heating (Singh and Heldman 1993).

Compositional factors of food can determine the amount of heating that can occur. The moisture of food is one such factor. As moisture content increases, dielectric constant increases, enhancing heating of the food. Highly porous foods with a significant amount of air increase heating rates because the material will act as a good insulator, due to the low thermal conductivity of air (Singh and Heldman 1993). Addition of salt increases the rate of heating because the increased concentration of ions, as a result of the salt addition, promotes heating in microwaves. Oil can have an effect on heating as well. Although oil has a much lower dielectric loss factor than water, oil has half of water's specific heat, which is defined as the quantity of heat that is gained or lost by a unit weight of product to accomplish a desired change in temperature, without a change in state (Singh and Heldman 1993). Since oil will require less heat to increase in temperature, as a result of the specific heat, oil exhibits a higher rate of heating (Singh and Heldman 1993). Frozen foods have a decreased dielectric constant relative to liquid foods. Thus, when portions of a frozen food thaw, the resulting increase in dielectric constant may cause runaway heating in those spots, which is an exponential increase in temperature with respect to time, that occurs rapidly when the amount of microwave energy absorbed increases due to the increasing dielectric constant.

Dipole rotation and ionic polarization are two ways heat is generated in a microwave field. During dipole rotation, polar molecules within a food sample can generate heat by either friction of rotation or vibration. If the electric field is normal to the plane of incidence, torque forces to the polar molecules occur causing them to spin.

The energy absorbed via the friction of the rotating molecules is converted to heat. By rotating the electric field millions of times per second, the polar molecules rotate quickly and heat is generated. If the electric field is traveling in the plane of incidence, the polar molecules vibrate by stretching and compressing, causing heat generation. When an electric field is applied to food containing ions, these move at an accelerated pace due to their charge, causing collisions with one another which converts kinetic energy to thermal energy (Rosenthal 1992).

The depth or thickness of food products is important to the heating process since heat is produced as the microwaves penetrate. Penetration can be infinite, such as occurs in loss-free glasses and ceramics, or zero in reflective materials such as metals (Decareau 1992). Thus metallic surfaces are used to guide microwaves from the magnetron to the heating applicator. In traditional microwave applicators ('ovens'), a metallic mode stirrer is used to scatter microwaves around the cavity (Rosenthal 1992).

Reflection and refracting of the scattered microwaves within the applicator cavity can cause uneven heating of foods. Certain layers of multilayered foods may heat more than others. At certain interfaces between layers a portion of the microwaves will be reflected. If a reflection at an interface is large, reflected waves within the region between the two interfaces could form standing waves which contribute to uneven heating (Iskander 2000).

The geometry of the microwave applicator or oven, or even of the food package, can be altered to focus the energy field and achieve more even heating of foods (Iskander, 2000; Joines and Drozd 1999). Packaging for microwave-ready dinners is shaped in such a way so as to promote even heating without overheating foods at the edge of the package

(Decareau 1992). Joines and Drozd (1999) demonstrated that uniform heating can be achieved with microwave heating by focusing the microwave field exclusively to the area where the sample to be heated is. This focusing is done by geometrically changing the heating cavity from the standard square shape to a cylindrical shape.

Heat Transfer Fundamentals

The thermal conductivity of a food material is the rate at which heat will be conducted through a unit thickness of a material if a unit gradient temperature exists across that thickness (Singh and Heldman 1993). Conductive heat transfer requires no physical movement of the material and is the common mode of heat transfer in heating/cooling of opaque solid foods (Singh and Heldman 1993). Since the conductivity of water (which composes the majority of most comminuted meat products) is low, conductive heat transfer into the center of the product can be slow, especially if the product thickness is large.

Although microwave heating of larger diameter comminuted meat products can be more uniform, a gradient in temperature within the product during microwave heating may also be observed. In this case, however, the product will be hottest in its center, due to the outer surfaces of the product being in contact with a colder container or air.

Testing Methods Commonly Used to Measure Rheological Attributes of Comminuted Meat Products

Fracture methods are used to measure rheological properties of meat-based gels that relate to its sensory texture, such as its strength and deformability (Hamann 1983).

Park and others (1987) describes this test where samples are cut into short cylinders and then milled into dumbbell shaped specimens. These are twisted at a constant rate of rotation to fracture and the resulting torque and angular displacements at fracture are used to calculate true shear stress (gel strength) and strain values (gel deformability). Gels with intermediate strengths (5kPa – 70kPa) and strain of 1-3 can be tested effectively by torsion. Weaker gel samples may be lost during preparation for torsional testing.

Compression testing can also be used to obtain fundamental measures of fracture stress and strain (Steffe 1996). This type of testing is applicable to gels do not exceed a fracture strain of about 1.6. Cylindrical samples must be compressed axially between lubricated, smooth plates at a constant rate to the point of fracture for fundamental measurements to be made (Steffe 1996). Compression testing is also involved in the more empirical 'texturometer' approach to gel testing, which may not actually involve fracture of the specimen (Bourne 1978). A two-compression cycle to a set deformation limit is used, and from force and deformation curves may be calculated terms such as hardness, springiness, cohesiveness, gumminess and chewiness (Patana-Anake and Foegeding 1985).

Penetration (punch) testing is a more empirical fracture method developed originally for evaluating the gel-forming ability and gel properties of surimi-based gels. In the typical application for surimi, cylindrical gels 3 cm in diameter are cut into 2.54cm lengths. These are penetrated axially by a 5mm ball shaped probe at a constant rate of 1mm/sec until the sample fractures. Gel strength is measured as the fracture force (g), and gel deformability is determined by the depth of penetration at fracture (mm) (Lanier and others 1991).

Small strain rheology is used for the measurement of the viscoelastic behavior of food in a non destructive form of testing. Viscoelastic foods are foods that are both viscous and elastic. A viscous liquid does not store energy of deformation and therefore does not recover its shape following deformation. Conversely, a perfectly elastic solid responds to a stress with deformation, completely recovering from this deformation using energy it has stored. Viscoelastic measurements are useful for non-destructively monitoring the kinetics of gelation, and association of particular events in the gelation process to particular times or temperatures in the process. This type of testing uses oscillatory measurements where the rate (strain) is fixed and stress is measured, or the stress is controlled, where the stress amplitude is fixed and the deformation is measured. The sample (3mm thick) is placed on a stationary plate and an oscillating plate (either parallel or cone) is placed on the sample. Values such as G' (gel rigidity), as well Phase angle (A measure of gel fluidity) and G'' (loss modulus) can be continuously and non-destructively measured during heating and cooling.

Application of the Equivalent Point Method to Thermal Gelation.

The equivalent point method was initially devised, and has been widely applied, as a tool to compare and communicate equivalent heat treatments, as typically affecting bacterial reduction and/or enzyme inactivation in foods (Maesmans and others 1994). Swartzel (1982) characterized heating processes by a unique equivalent time and temperature which would result in the same reduction of a product constituent (bacteria, enzymic activity, etc.) as the variable time – temperature profile typically used for the lethal effect of pathogenic and spoilage organisms. This assumes that any thermal

treatment can be characterized by a unique equivalent point (time and temperature), independent from the kinetic behavior of the product constituents (Maesmans and others 1994). A specific activation energy (E_a) is used for each product attribute (bacterial load, enzymic activity, etc.), that is likely to be affected by the heating and cooling regime. An E_a is estimated in the occasion when the actual E_a is not known. By use of several equations and time temperature data from time temperature curves recorded during heating, holding (if any), and cooling (if any) versus lethality of the constituent (bacterial load, enzyme activity, etc) being studied, a straight line plot of $\ln(\text{time})$ vs $1/\text{temperature}$ results, which represents all possible combinations of time and temperature that cause the same concentration change in that quality attribute (Maesmans and others 1994). This step is repeated several times, each time with a different activation energy similar to the first activation energy chosen so that a range above and below of the original E_a is formed. All these lines will be found to intersect at a single point, which is the equivalent time and temperature that characterizes the heating process. The EPM concept makes it possible to determine the impact of a thermal treatment on different attributes without referring to the original temperature data, as compared to the traditional general method, which is limited to single constituent analysis (Kyereme and others 1999). Every attribute (lethality, enzymatic activity etc.) to be tested has an activation energy associated with it, the equivalent point will represent all attributes because it is comprised of the intersection of all activation energies within the system being heated. Some uses have included kinetic data generation and analysis of certain physical phenomena such as thermal lethality (Kyereme and others 1999).

Microbial reduction is often a main objective of heat processing, and thus microbial destruction is the measurement used most often to assess effectiveness of heating. A related measurement to this which is used to estimate the effectiveness of heating meat products is acid phosphatase (ACP) enzyme activity. ACP measurement has thus been used to estimate effectiveness of cooking to reduce microbial loads to safe levels in processed meat products (Davis and Townsend 1994; Lyon and others 2001). For example, an ACP of 4.88 U/kg was found to be equivalent to a 4.48 log of *Listeria monocytogenes* in chicken frankfurters (ABC Research Corp, 1999).

In principle it should also be possible to apply the EPM approach to predicting the effects of any thermal process on the gelation of muscle proteins, since gelation has an activation energy associated with it. Gelation may have similarities to enzyme reduction if an activation energy is required to be met before gelation could proceed. The difference is that thermal reduction requires the E_a for destruction, while gelation requires an E_a for initiation of the gel. Since initiation of gelation of any protein requires an activation energy (Bon and others 1999), then the EPM method could be used to determine the effect different heating regimes have on gelation. A question to be answered is if this general approach could be used to predict gel texture effects of any particular heating regime?

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**Rapid Heating Effects on Muscle Protein from Warm and Cold-blooded
Animals**

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ABSTRACT

The hypothesis was tested that total thermal input, rather than merely heating rate, is the determining factor in heat-induced gel formation by muscle proteins. Comminuted pastes (2% NaCl and 78% total moisture content) made from Alaskan pollock surimi and turkey breast were heated at 0.5°C/min, 20°C/min or 80/98°C/min to 70°C and held up to 40 min. Fracture stress and strain, rigidity modulus (G'), cook loss and acid phosphatase activity was measured. Results showed that rapid heating plus a brief holding time at the endpoint temperature produced similar textural properties to the slower, more conventionally heated, gels cooled immediately upon reaching the same endpoint temperature. These results thus confirm that thermal input above the activation energy of reactions dependent upon protein denaturation, such as gelation and enzyme/microbial inactivation, can be predictive of the resulting gel properties, and moreover can be used via the equivalent point method to identify other processes having similar effects. Moreover, rapid heating, with advantages of process efficiency, space, and control, can likely be utilized by processors of certain muscle foods without compromising product quality.

INTRODUCTION

Most comminuted meat products, particularly those containing substantial amounts of fat such as many frankfurters and luncheon meats, are heated relatively slowly (usually over 1-2 hr) to the desired endpoint temperature (typically 70-72C) (Zayas 1997). Such a slow heating rate supposedly results in better gel texture and optimum fat and water-binding properties (Komarik and others 1974). Several workers have noted that proteins form less strong or rigid gels as the heating rate used for their formation is increased (Foegeding and others 1986; Camou and others 1989; Barbut and Mittal, 1990; Arntfield and Murray, 1992; Yongsawatdigul and Park 1996, Yoon and Park 2001). The explanation commonly offered has been that more rapid heating allows insufficient time for proteins to denature and properly align prior to aggregation and gelation, resulting in gels that are more coarse and weak (Hermansson, 1978). This explanation is based upon the two-step theory of protein gelation advanced by Ferry (1948) which, simply stated, asserted that a protein dispersion first denatures upon heating to expose active sites which can then align and aggregate to form a gel.

Each of these studies, however, compared gels which were cooked to a common endpoint temperature and immediately cooled. Thus none of these workers considered the integrated effects of time, temperature and reaction rates on protein gelation as they compared effects of process treatments which differed in total thermal input (time/temperature integration) and time of heating above the denaturation temperatures of the gelling proteins.

It is well known that a high temperature/short time heating process can produce a similar level of microbial or enzymatic inactivation as a lower temperature/longer time process (Crespo and Ockerman 1977). Similarly, it is possible that a rapid heating rate plus a holding period prior to cooling could produce a gel having similar properties as one prepared at a slower heating rate, to the same endpoint temperature, with immediate cooling.

Foegeding and others (1986) also had considered that faster heating rates would allow less time at temperatures above the denaturation temperature of the proteins for aggregation to occur. There was no further elaboration by these authors on this possibility, nor did they attempt to experimentally test this alternate theory. Certainly, however, it represents an entirely different explanation of their observations: that time of protein aggregation, rather than time of denaturation prior to aggregation (as they and so many others have asserted), is responsible for weaker gels being obtained at higher heating rates.

The practical implications of the two opposing hypotheses are quite different. If the cause of weaker gels at higher heating rates is a too-rapid transition through the denaturation step, then it is unlikely that any manipulation of a rapid heating process could lead to gels of equivalent strength and properties. Alternately, rapid heating to the same endpoint temperature may not allow sufficient time for aggregation to occur following protein denaturation. If this were true, then extending the process time at the endpoint temperature (isothermally) may allow gels which have been rapidly heated to attain equivalent strength and properties as those which have been heated more slowly, to

the same endpoint temperature and rapidly cooled. The present experimental work is a test of this new hypothesis.

MATERIALS AND METHODS

Meat Materials

Alaskan pollock surimi was commercially processed and obtained frozen from Trident Fisheries (Seattle WA). Besides leached fish muscle the surimi contained 4% sugar, 4% sorbitol and 0.3% STP. Pretesting of the surimi (Hamann and others 1990) assured little evidence of heat-activated protease in this lot of surimi. Fresh turkey breast meat was obtained from Carolina Turkeys Inc. (Mt. Olive, NC) and ground in a meat grinder through a 2.0 cm diameter plate followed by regrinding through a 1.3 cm diameter plate, then hand mixed to achieve uniformity before vacuum packaging and freezing.

Gel Paste Preparation

Paste formulations for both turkey and surimi contained 2% NaCl and ice sufficient to achieve 78% total moisture content. To prepare pastes, frozen meat was tempered at room temperature for 1 hr and precut to flake consistency in a Stephan UM-5 vacuum cutter-mixer (Stephan Machinery Corp., Columbus OH) at 900 RPM for 1 min (no vacuum). Salt and ice were then added and the mixture was chopped at 2000 RPM under vacuum to an endpoint of 4°C. Pastes were vacuum packaged to remove as much air as possible. A corner of the bag was cut before placing in a manually operated sausage stuffer for extrusion of the paste into teflon (for microwave) or stainless steel (for

waterbath) tubes 1.9 cm i.d. and 17.8 cm long. Tubes were sealed at both ends prior to heating.

Heat Processing of Gels

These tubes were subsequently heated from 10°C to a 70°C internal endpoint, either rapidly by microwave at 20 or 98°C/min to test rapid heating effects, or slowly (0.5°C/min) by immersion in a programmable (heating rate) waterbath (Neslab Inc, Newington NH) to simulate conventional smokehouse process heating rates (Wilson 1960). Temperatures were measured at the center (both vertically and horizontally) of the gel. Upon reaching 70°C all samples were held for 0, 10, 20, 30 or 40 min prior to rapid cooling by immersion in ice water. Microwave heating was carried out using a recently patented (Joines and Drozd 1999) microwave applicator which focuses the applied microwave field to produce uniform heating of product (Industrial Microwave Systems, Morrisville NC). Samples were held static in the cylindrical microwave applicator (length 16 cm, radius 12.5 cm) and heated at power settings of 70 or 260 watts to obtain the 20°C and 98°C/min heating rates, respectively. After reaching a final temperature of 70°C, this temperature was maintained in gels during the subsequent holding period by utilizing feedback software on the controller for the microwave generator, with input from a data logger with an attached fiber optic temperature probe (model UM 4, Fiso Technology, Quebec Canada) inserted in the gel. Preliminary tests were performed to confirm uniform heating by this applicator; heated specimens were immediately sliced either longitudinally or every 1.3 cm cross-sectionally and observed by an infra red camera (Model Agema 570, Flir Systems Inc., Portland, Oregon).

Alternatively, a small amount of paste was loaded directly into a cone and plate apparatus of a controlled stress rheometer and heated at either 20°C/min (the most rapid heating rate possible for this apparatus) or 0.5 °C/min to an endpoint temperature of 70°C, then held for 0, 20 or 40 min prior to cooling at 5°C/min to 25°C.

Rheological Properties of Pastes/Gels

Rheological changes (storage modulus, G' , and phase angle) of pastes/gels were non-destructively and continuously measured as pastes were heated, held and cooled in the C 40 – 4 cone attachment of the constant stress, small strain rheometer (Stresstech, Rheologica instruments AB, Lund, Sweden). An oscillation of 0.1 Hz with a resistance stress of 100 Pa was used for testing.

Gelled cylindrical samples, prepared by heating in tubes in a waterbath or microwave applicator, were removed after cooling, held overnight in refrigeration, and subsequently cut into specimens 2.54 cm long, each end of which was glued to plastic disks (Gel Consultants Inc., Raleigh NC) using an instant adhesive. Dumbbell shaped samples were milled from each specimen to 1cm minimum diameter on a milling machine (Gel Consultants Inc.) and tempered to room temperature (without moisture loss) prior to torsion testing. For testing, gel specimens were vertically mounted and twisted to the point of fracture at 2.5 rpm on a Hamann Torsion Gelometer (Gel Consultants Inc.). Stress (KPa) and strain (dimensionless) at fracture were recorded for each sample, corresponding to the strength and deformability of the gels, respectively (Hamann and others 1990).

Cook Loss

Loss of moisture during cooking of gels by microwave or waterbath was calculated by subtracting the post-cooked weight of samples from the pre-cooked weight, expressed as a percentage of pre-cooked sample weight. Specimens were blotted before weighing, after cooking and cooling.

Acid Phosphatase Activity

An indication of the potential thermal kill effect of the various heat processes used was obtained by measuring acid phosphatase (ACP) activity following heating and cooling. Meats normally contain significant levels of acid phosphatase, which decrease in activity upon heating in a similar manner to heat inactivation of microbial populations, thus allowing the measurement of internal endpoint temperature using ACP (Davis and Townsend, 1994). A 5g portion of meat sample was mixed with 10 grams of deionized water and fully homogenized with a hand held homogenizer in a disposable tube for 1 min. This solution was then filtered through 1-micron filter paper into a cuvette. 75 microliters were pipetted and dispersed into 2 mL of Thermazyme ACP fluorophos substrate (100-test reagent set, Advanced Instruments Inc., Norwood, MA) in a cuvette, previously warmed in a heating block (Advanced Instruments Inc.) to 38°C. The mixture was then vortexed and the fluorescence measured in a fluorometer (Advanced Instruments Inc.). ACP activity was calculated automatically by the fluorometer.

Statistical Analysis of Data

The resulting data correspond to a complete, balanced 2-way ANOVA design with two replicates. Statistical analysis was performed using SAS v. 8.01 (PROC GLM).

RESULTS AND DISCUSSION

Fracture Testing of Gels

Rapid heating (20 or 98°C/min) to 70°C, followed by holding at this temperature for 10-20 min, yielded gels which exhibited similar fracture stress for surimi ($p > .1372$) and turkey ($p > .2245$) (Fig. 1), and fracture strain for turkey ($p > .5601$) (Fig 2), as those produced by heating slowly at 0.5°C/min to the same endpoint temperature with no subsequent holding period. Slow heating to 70°C, with no subsequent holding prior to cooling, is representative of the heat processes used for commercial processing of meat products (Wilson 1960). Fracture strains for surimi gels heated rapidly and held at 70°C 10-20 min. were actually higher than for gels heated slowly to 70°C and immediately cooled (Fig. 2).

At zero holding time at 70°C (the point in heating at which pastes just attained this endpoint temperature), gels heated slowly at 0.5°C/min were clearly stronger. This is consistent with previous reports that faster heating rates produce weaker gels, when heated only to the same endpoint temperature and immediately cooled (Foegeding and others 1986; Camou and others 1989; Yongsawatdigul and Park 1996).

Cook Loss

During holding at 70°C, turkey gels heated to this temperature at the faster heating rates lost water at a steady rate such that, by 20 min of holding, similar cook loss had occurred as in gels cooked at 0.5°C/min to 70°C and immediately cooled ($p > .4590$) (Fig. 3). Faster heating rates to 70°C with holding time longer than 30 minutes produced excessive cook losses. No cook loss was detected in surimi gel samples (data not shown). The greater loss of water in the turkey breast meat gels may be due to its relatively greater content of connective tissue which contracts upon heating (Zayas 1997), lower content of myofibrillar protein capable of binding water (Lee 1984; Lanier 1986; Yang and Froning 1992), and/or to the lesser degree of muscle structure disintegration as compared to surimi. Nowsad and others (2000) found that chicken meat washed several times (in imitation of the surimi manufacturing process) produced a higher cook yield than unwashed meats. Likely this is a result of greater muscle structure disintegration, resulting in better protein distribution and bonding within the heat-induced gel (Chang and others 2001).

Small Strain Rheology

Rapid heating (20°C/min) to 70°C, followed by holding at this temperature for 10-40 min, yielded gels from turkey which exhibited higher rigidity (G') as compared to those produced by heating slowly at 0.5°C/min to the same endpoint temperature and immediately cooled (Fig. 4a,b). Gels measured both at the endpoint temperature and after cooling adhered to this trend. Even the G' of rapidly heated gels measured just upon reaching the endpoint temperature of 70°C exceeded that of the slow heated samples.

This result differs from others who reported that slower heating rates result in gels with higher G' (Barbut and Mittal 1990; Arntfield and Murray 1992 and Yoon and Park 2001). Again, however, these workers compared all gels just at the time of reaching the same endpoint temperature.

G' of turkey gels heated rapidly to 70°C increased at a greater rate during subsequent holding at that temperature than did gels heated slowly to 70°C (Fig. 4a,b). Evidently gelation of rapidly heated pastes did not initiate (G' markedly increase) until prior to the 70°C holding phase. The decrease in G' noted at $50\text{-}60^{\circ}\text{C}$ during slow heating of turkey pastes (Fig. 4a) has been noted previously for this and other meats (Montejano and others 1984; Xiong and others 1994). Egeland and others (1986) and Zayas (1997) attributed this to the denaturation of light meromyosin, leading to a temporary increase of filamental “fluidity”.

Surimi pastes heated rapidly ($20^{\circ}\text{C}/\text{min}$) just to the endpoint temperature (70°C) exhibited lower rigidity (G') while still hot than gels heated more slowly ($0.5^{\circ}\text{C}/\text{min}$) (Fig. 5a,b). This was contrary to the results noted for turkey pastes but in agreement with other reports (Barbut and Mittal 1990; Arntfield and Murray 1992 and Yongsawatdigul and Park 1996). Further holding at 70°C did not result in the rapidly heated surimi gels ever attaining a similar gel rigidity as those heated slowly just to 70°C (no holding), when measured at 70°C . Note that surimi gels heated slowly to 70°C , unlike turkey gels heated identically, continued to increase in rigidity during subsequent holding at 70°C .

Comparing data for the cooled surimi gels cooked to 70°C and held for up to 40 min, the rapidly heated gels did attain and even exceed the G' of gels heated slowly just to the 70°C endpoint and immediately cooled (Fig. 5a,b). However, further holding at

70°C of the slowly heated gels (Fig. 5a) did result in substantial increase in G' of cooled gels, to values higher than those attained by the rapidly heated gels held at 70°C and cooled (Fig. 5b).

The differences between turkey and surimi pastes in effects of heating rate can be attributed to the 'setting' effect of pollock surimi: this is its ability to form gels at low temperature (0-40°C) due to non-disulfide crosslinking induced by an endogenous transglutaminase (Yongsawatdigul and Park 1996; Ashie and Lanier 2000). The rise in G' near 35°C which occurred only during slow heating of surimi pastes (Fig. 5a) may be evidence of this crosslinking activity. Setting prior to heating at higher temperatures is known to induce stronger gels than rapid heating to a high temperature, which rapidly inactivates the transglutaminase (Yongsawatdigul and Park 1996; Ashie and Lanier 2000).

In all treatments for both turkey and surimi gels (Figs. 4, 5), G' increased during cooling of the gels, which can be attributed to reforming of hydrogen bonds (Damodaran 1996).

Acid Phosphatase (ACP) Activity

Heating just to the 70°C endpoint temperature (zero holding time, Fig. 6) was sufficient to reduce the ACP activity of turkey breast meat paste to below 6 U/Kg, similar to results obtained by Davis and Townsend (1994). Rapid heating to this point predictably reduced ACP activity less than slower heating, since the total thermal input was greater for the slower heating rates. However, it is apparent that a similar reduction

in ACP activity ($p > .0739$) occurred for all heating treatments within 10 min of holding at 70 °C, which was similar to results obtained by Lyon and others (2001).

Just as thermal gelation is an event dependent upon protein denaturation and aggregation, the rates of which are determined by treatment time and temperature, so likewise is the inactivation of enzymes and microbial destruction. Activity of endogenous ACP has been used to estimate effectiveness of heating in the reduction of microbial loads to safe levels in processed meat products (Davis and Townsend 1994; Lyon and others 2001). For example, an ACP activity of 4.88 U/kg was found to be equivalent to a 4.48 log reduction of *Listeria monocytogenes* in chicken frankfurters when heated to 75C. Chicken frankfurters yielded ACP values of 119.39 and 8.19 for endpoint temperatures of 4.5 and 71.1C (ABC Research Corp 1999). Lyon and others (2001) measured an ACP activity of 1.29 U/kg at 71.1C for ground beef patties, which is the critical temperature for destruction of E.coli O157:H7. The food safety and inspection service has an end point temperature requirement of 71.1C for uncured poultry products. Davis and Townsend (1994) found that frozen turkey breast heated to 71.1C exhibited an ACP activity of 5.7 U/kg.

Implications for Processing of Gelled Meat Products

Rapid heating, with the addition of a brief holding time at the endpoint temperature, produced meat gels of similar textural and water-holding properties as slower heating to the same endpoint temperature with no holding at this temperature prior to cooling. Total process time for rapid heating (20 or 98°C/min) to achieve an equivalent gel was only 11 - 23 min (heat to 70°C + hold 10-20 min, then cool) as

compared with 89 min (heat to 70°C, then cool) at a slower heating rate (0.5°C/min) (Figs. 1-3), the latter process being representative of processes typically used in commercial smokehouses. Our results suggest that, while slow unfolding of proteins relative to their aggregation rate may occur to some extent during slower heating of meat pastes, ultimately it is the total thermal input (above the activation energy required for denaturation) that dictates development of the desired gel structure. Evidently the gel structure builds in strength during heating beyond the gel point, possibly through formation of disulfide bonds (Lee and Lanier 1995) and other bonds that result from further denaturation and/or rearrangement of proteins.

Microwave applicators such as that used in this study could be used to more rapidly heat meat paste products of large diameter than could feasibly be accomplished in conventional smokehouses. Besides dramatically reducing process time, such equipment would also allow instantaneous and precise on/off temperature control and a lower space requirement. Concerns that remain to be addressed are whether high fat-containing products, or those which employ added starches to retain elevated water contents for the purpose of fat reduction, would perform similarly in both slow and rapid heat processes. While it has long been known that a very slow heating rate can result in stronger surimi gels, due to activation of endogenous transglutaminase activity (Ashie and Lanier 2000), in practice it has proven to be more efficient to use rapid heating for processing most surimi-based foods. The present work suggests that processors of poultry products, and likely red meat products as well, could benefit also from the efficiencies inherent in rapid heating, without detriment to product quality.

Application to Process Optimization.

The equivalent point method (EPM) was initially devised, and has been widely applied, as a tool to compare and communicate equivalent heat treatments, as typically affecting bacterial reduction and/or enzyme inactivation in foods (Maesmans and others 1994). Swartzel (1982) characterized heating processes by a unique equivalent time and temperature which would result in the same reduction of a product constituent (bacteria, enzymic activity, etc.) as the variable time – temperature profile typically used for the lethal effect of pathogenic and spoilage organisms. Thus any thermal treatment can be characterized by a unique equivalent point (time and temperature), independent from the kinetic behavior of the product constituents (Maesmans and others 1994). Since both thermal kill and enzyme reduction are similar to gel formation in that all are manifestations of protein denaturation, the equivalent point method could possibly be used to predict the effect of various thermal treatments on protein gelation.

To test this assumption, each of the heating, holding and cooling regimes used in the present study were converted to equivalent points (time, temperature; Table 1) using calculations described in Swartzel (1982) and plotted (Fig. 7). If we connect the points representing the assumed acceptable slow-cooking process (0.5°C/min to 70°C with rapid cooling, upper left point on Fig. 7) to those points representing the rapid heating processes that yielded similar gel properties in terms of fracture stress and strain, cook loss, and ACP activity (80 or 98°C/min to 70°C, with holding for 20-30 min) for turkey breast meat gels, a triangular region is delineated (plotted lines, Fig. 7). This region represents a group of process parameters (all processes having equivalent points in this region) wherein acceptable gel attributes could be attained. Processes lying below the

lower energy boundary would be insufficient to achieve proper gel properties and/or ACP activity reduction. Processes lying above the upper boundary would produce unacceptable cook losses. It is conceivable that the two plotted boundary lines might be extrapolated outward to higher temperatures, encompassing a region of higher endpoint temperatures and heating rates than those tested in the present study wherein acceptable gel properties might also be obtained. Further work is of course required to confirm that such an extrapolation is valid.

This approach must also be extended to work with product formulations more closely resembling actual commercial products; i.e., with reduced meat contents and containing added fillers such as starches/water (low fat products) or finely divided fats (standard products). Such composite products could well perform differently from the simple meat gels evaluated in this study.

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Table 1: Heating regimes and corresponding equivalent time and temperatures.

Trial Numbers	Heating Regime	Equivalent Temperature (°C)	Equivalent Time (Minutes)
1	0.5 °C/min heat; no hold; 5 °C/min cooling	58.26	68.68
2	20 °C/min heat; no hold; 5 °C/min cooling	58.26	7.81
3	98 °C/min heat; no hold; 5 °C/min cooling	58.15	6.61
4	0.5 °C/min heat; 10 minute hold; 5 °C/min cooling	61.4	73.11
5	20 °C/min heat; 10 minute hold; 5 °C/min cooling	67.56	16.2
6	98 °C/min heat; 10 minute hold; 5 °C/min cooling	67.97	15.25
7	0.5 °C/min heat; 20 minute hold; 5 °C/min cooling	63.22	80.32
8	20 °C/min heat; 20 minute hold; 5 °C/min cooling	68.62	26.02
9	98 °C/min heat; 20 minute hold; 5 °C/min cooling	68.85	25.17
10	0.5 °C/min heat; 30 minute hold; 5 °C/min cooling	64.41	88.66
11	20 °C/min heat; 30 minute hold; 5 °C/min cooling	69.19	35.35
12	98 °C/min heat; 30 minute hold; 5 °C/min cooling	69.2	35.14
13	0.5 °C/min heat; 40 minute hold; 5 °C/min cooling	65.24	97.5
14	20 °C/min heat; 40 minute hold; 5 °C/min cooling	69.26	45.95
15	98 °C/min heat; 40 minute hold; 5 °C/min cooling	69.38	45.13

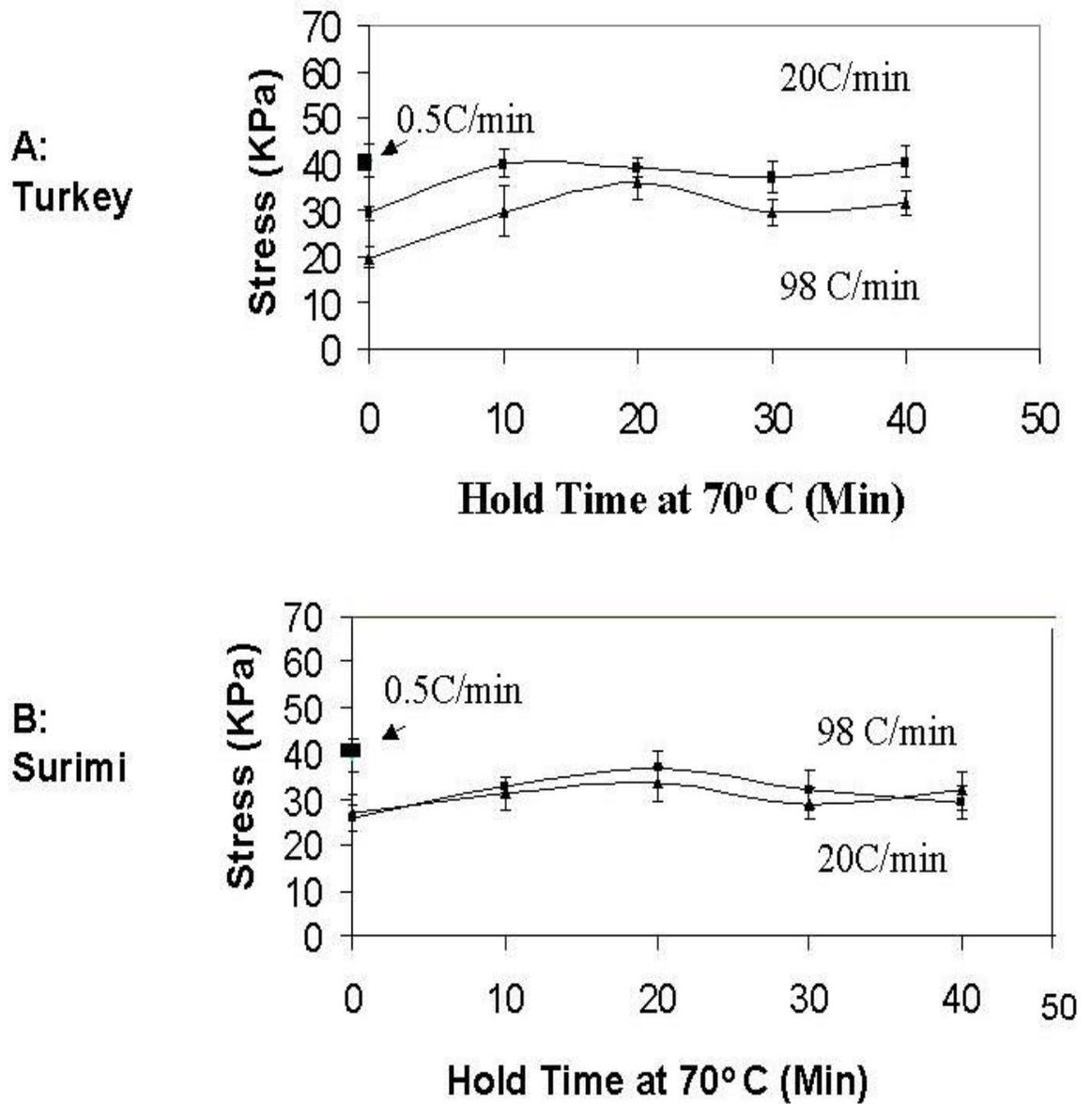


Figure 1: Fracture stress of (A) turkey and (B) surimi gels (after cooling to 25°C) during holding at 70°C, following heating to this temperature by either water bath (0.5°C/min) or microwave (20 or 98°C/min).

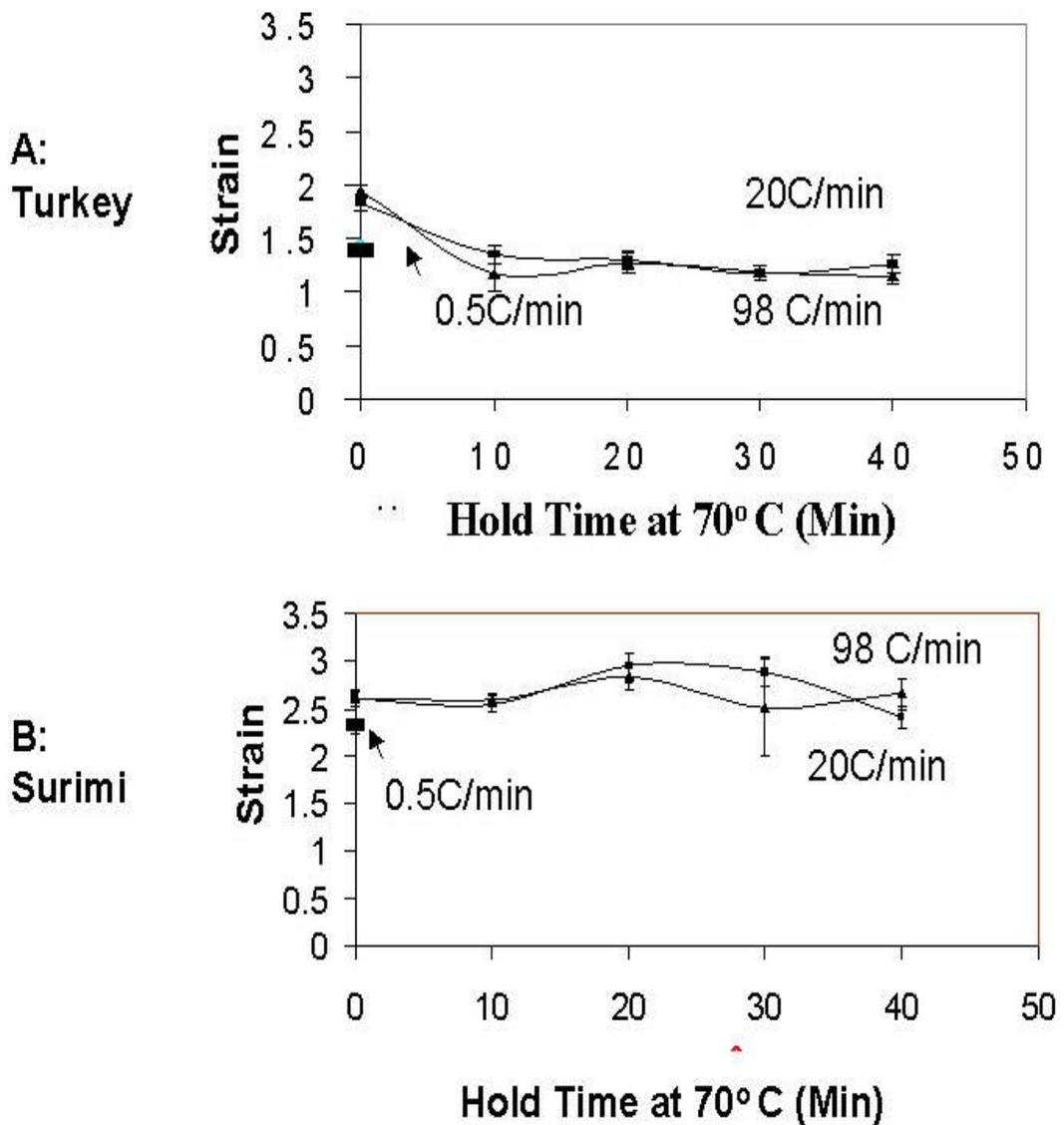


Figure 2: Fracture strain of (A) turkey and (B) surimi gels (after cooling to 25°C) during holding at 70°C, following heating to this temperature by either water bath (0.5°C/min) or microwave (20 or 98°C/min).

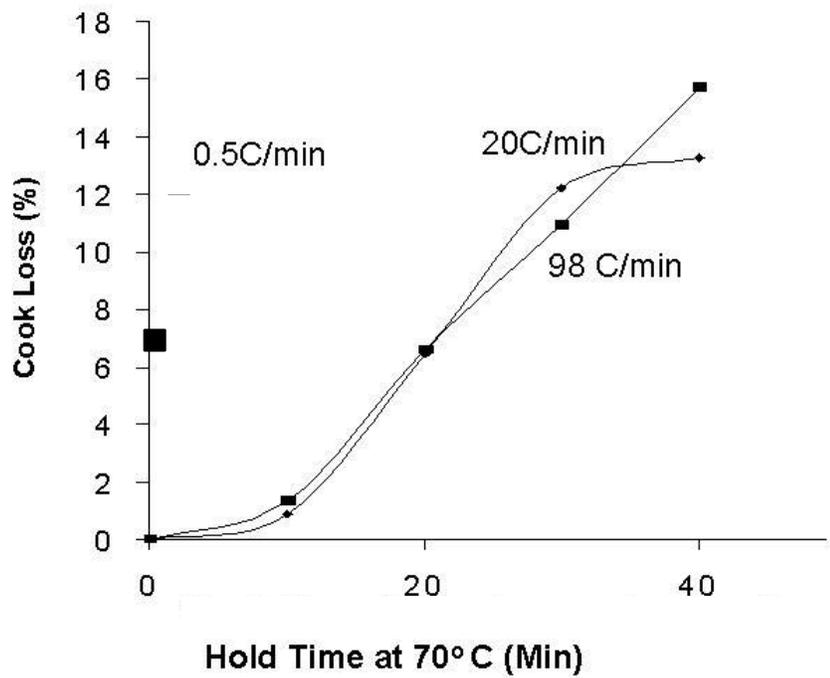


Figure 3: Water loss (after cooling to 25°C) of turkey gels during holding at 70°C, following heating to this temperature by either water bath (0.5°C/min) or microwave (20 or 98 °C/min).

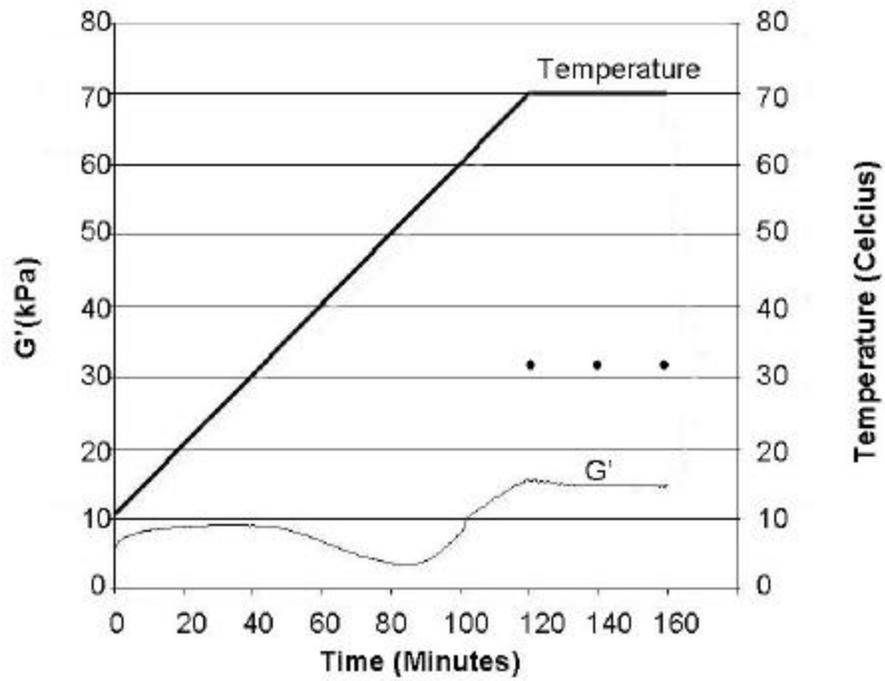


Figure 4A: Modulus of rigidity (G') of turkey pastes heated in a small strain rheometer at $0.5\text{ }^{\circ}\text{C}/\text{min}$ to 70°C and held 40 min before cooling at $5^{\circ}\text{C}/\text{min}$. Solid points are G' values at that point in heating or holding, after cooling to 25°C .

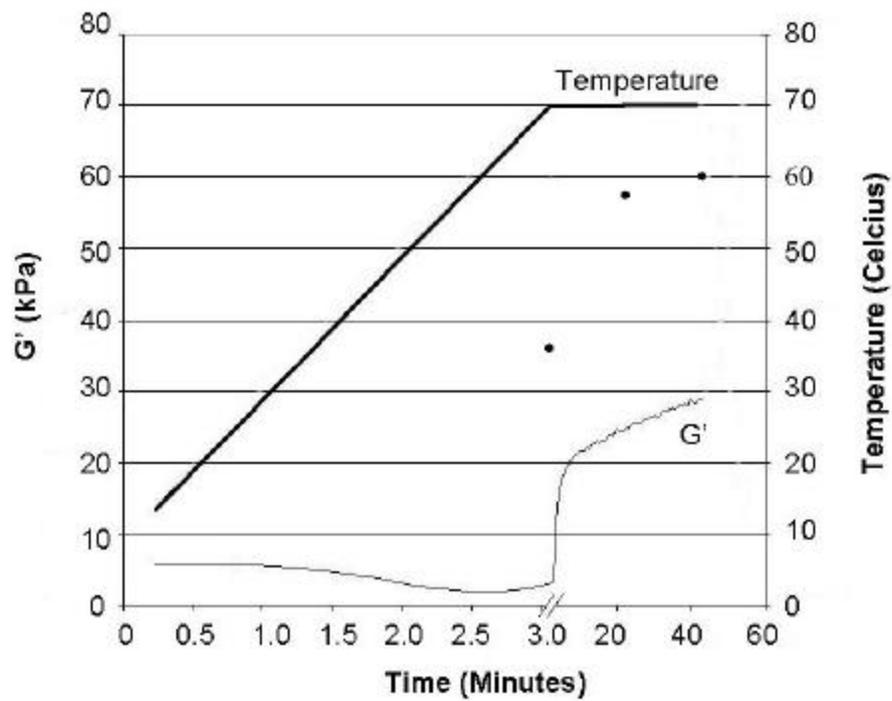


Figure 4B: Modulus of rigidity (G') of turkey pastes heated in a small strain rheometer at $20^{\circ}\text{C}/\text{min}$ to 70°C and held 40 min before cooling at $5^{\circ}\text{C}/\text{min}$. Solid points are G' values at that point in heating or holding, after cooling to 25°C .

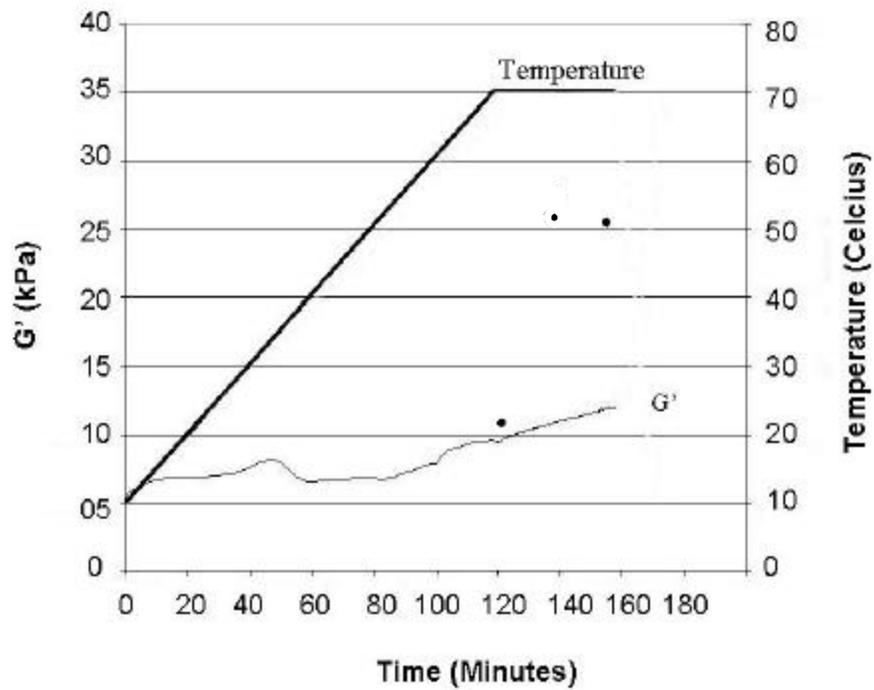


Figure 5A: Modulus of rigidity (G') of surimi pastes heated in a small strain rheometer at $0.5\text{ }^{\circ}\text{C}/\text{min}$ to 70°C and held 40 min before cooling at $5^{\circ}\text{C}/\text{min}$. Solid points are G' values at that point in heating or holding, after cooling to 25°C .

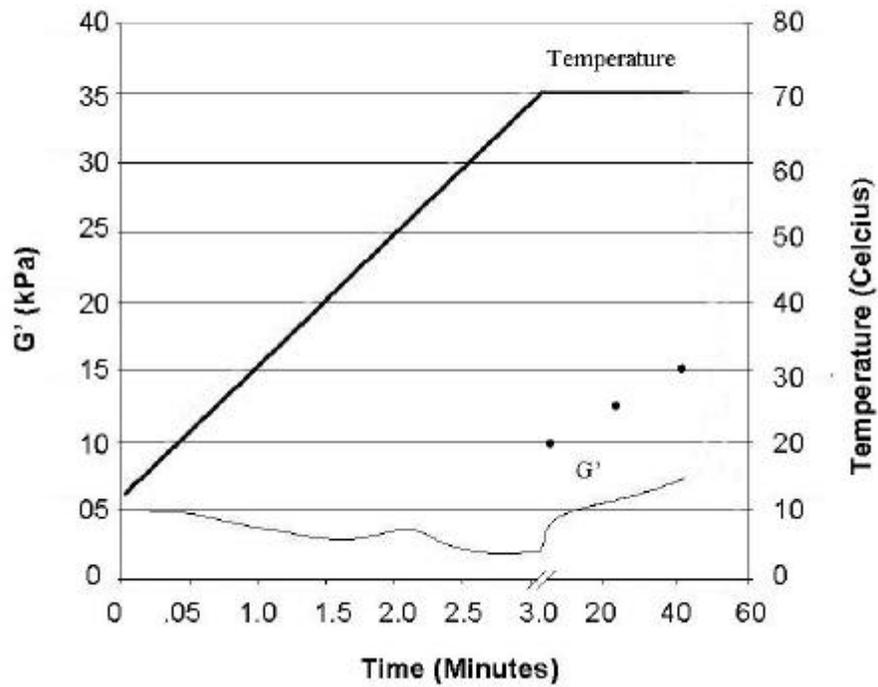


Figure 5B: Modulus of rigidity (G') of surimi pastes heated in a small strain rheometer at $20^{\circ}\text{C}/\text{min}$ to 70°C and held 40 min before cooling at $5^{\circ}\text{C}/\text{min}$. Solid points are G' values at that point in heating or holding, after cooling to 25°C .

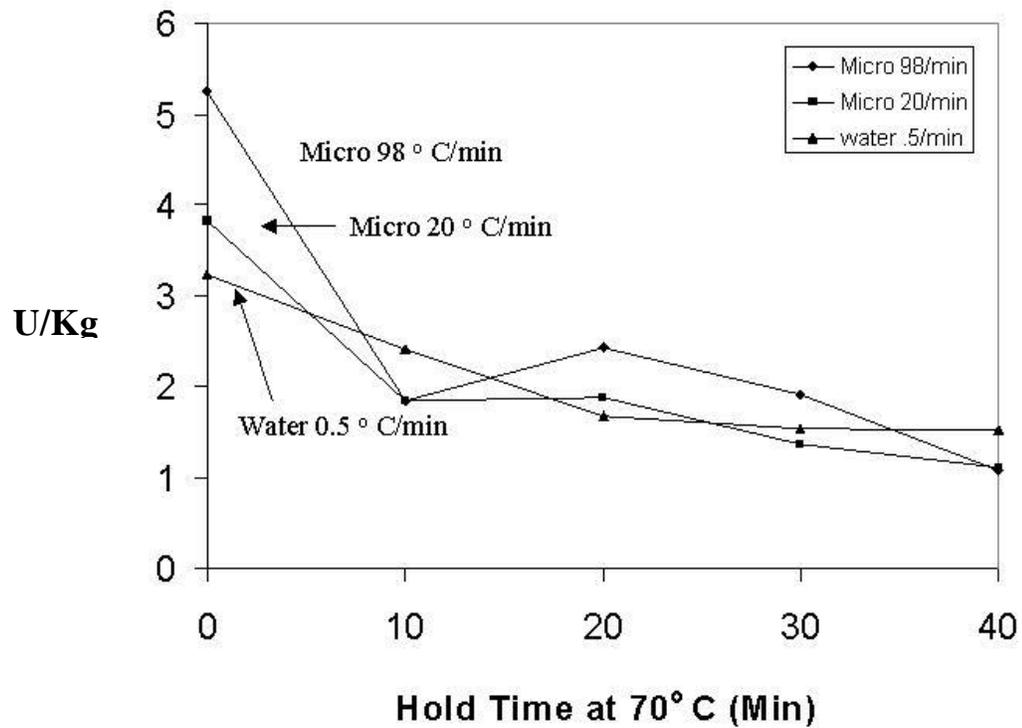


Figure 6: Acid phosphatase activity of turkey gels during holding at 70°C, following heating to this temperature by water bath (0.5°C/min) or microwave (20 or 98°C/min).

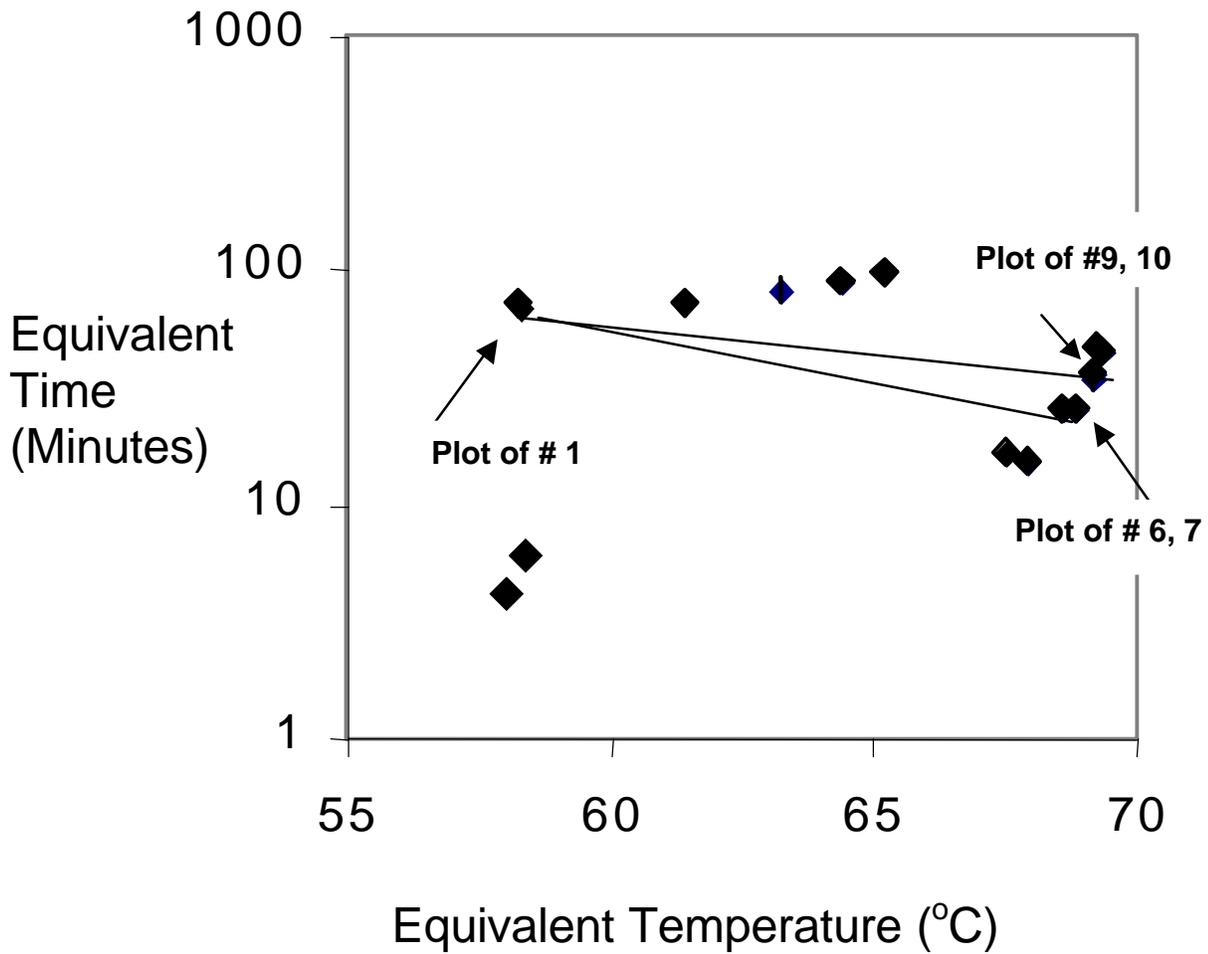


Figure 7: Semi-log plot of equivalent time (min) vs. equivalent temperature (°C) calculated for the various processing regimes. Lines denote lower and upper energy boundaries for obtaining nearly equivalent gel properties and ACP activity reduction for turkey breast meat.

APPENDIX

**Rapid Heating by a Focused Microwave Applicator
for Testing of Surimi Gel-Forming Ability**

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ABSTRACT

Rapid heating for preparation of cylindrical (1.9 or 3 cm dia) surimi gels by ohmic and a new focused microwave applicator were compared to conventional heating of gels in a water bath at 90 or 100°C. Both rapid heating methods (ohmic and microwave) produced superior gels from a surimi blend containing significant heat-activated protease activity as compared to water bath heating at either temperature. These were comparable to preparing gels from the same surimi by water bath with the addition of beef plasma as a protease inhibitor. Ceramic end caps on the teflon tubes used for microwave processing improved the uniformity of heating by microwave. Gels prepared from protease-free surimi in 3 cm casings by microwave heating did not achieve similar results to the same gels made by water processing, which likely resulted from the microwave unit being under powered and not properly focused.

INTRODUCTION

The gel-forming ability of surimi is the main determinant of its quality and value. Surimi from many fish species, but especially Pacific whiting and arrowtooth flounder, often possesses elevated levels of heat-activated protease activity that can weaken the gel structure during cooking in the temperature range of 50-70°C (Yongsawatdigul and others 1995). Such protease activity can occur in surimi due to gut contamination and/or parasitic cysts, depending on species and. For example, the protease responsible for textural degradation in Pacific whiting was identified as cathepsin L with an optimum activity at 55 °C (Seymour and others 1994). The optimum proteolytic activity for heat stable proteases in fish muscle is 55-60°C in almost all cases.

In the testing of surimi for gel-forming ability, surimi paste is exposed to this critical 50-70°C temp range for a significant period of time when cylindrical samples (required for rheological testing; Lanier 1981) are cooked by the traditional manner in a water bath (paste in tubes or casings of 1.9 – 3.0 cm internal diameter is heated in a 90°C waterbath for 15 - 60 min (Hamann and others 1990; Lanier 1991). This contrasts with the commercial heat processing of surimi-based crab analog products in a thin sheet by steam and/or radiant heating, which allows for a faster heat transfer and heating rate such that proteases are largely inactivated before significant textural damage can result.

Thus the established test methods used for estimating gel-forming ability, which employ water bath heating, are a poor predictor of actual performance of the surimi in such sheet-cooked surimi seafood products. As a result, the value of surimi intended for this product application is underestimated and surimi producers are unfairly penalized economically.

A more rapid heating method for the required cylindrical test samples, especially in the center of the cylinders where the rheological test is actually performed, would allow better prediction of on-line performance in sheet cooking of surimi seafoods. Such a rapid heating rate might be achieved by either microwave or ohmic heating, since neither depends on heat transfer from the surface to the interior of the surimi paste cylinder. Yongsawatdigul and others (1995) demonstrated that ohmic heating, generated by passing an electrical current through a material due to its electrical resistance, effectively increased the strength of gels made from Pacific whiting surimi of high protease content, as compared to gels cooked conventionally to the same endpoint temperature in a waterbath. One problem that exists with ohmic heating is when a single electrode pair is used to heat food material through a large change in temperature. Substantial changes in liquid conductivity and thus heating rate of the sample may result along the length of the electrode, thus preventing the ability to control the apparatus by a feedback mechanism (Ruan and others 2001).

A microwave applicator has been recently developed with a focusing apparatus which can produce uniform heating of materials in a cylindrical shape (Industrial Microwave Systems Inc., Morrisville NC). Prior to this development a disadvantage of processing food by microwave heating has been the non-uniform application of microwave energy by conventional oven designs, leading to hot and cold spots within the product during heating. Together with a feedback mechanism, the new focused applicator can be controlled and run away heating thus prevented.

The objective of this work was to explore rapid heating of cylindrical test gels of surimi by this new microwave applicator as a means of eliminating the undesirable

effects of heat stable proteases on gel rheology. This would lead to more accurate prediction of the gelling performance of surimi in commercial production of sheet-cooked and other rapidly heated surimi-based food products.

MATERIALS AND METHODS

Preparation of Surimi Pastes

Alaskan pollock and Pacific whiting surimi were commercially processed and obtained frozen from Trident Fisheries (Seattle WA). The surimi was cut, while still frozen, into 1kg blocks, vacuum packaged, and returned to the freezer. Surimi pastes were formulated as a mixture of 70% Alaskan pollock and 30% Pacific whiting surimi, prepared with or without 1% dried beef plasma (Proliant 600N, Ames IA) added as a protease inhibitor (Hamann and others 1990). Formulations also contained 2% NaCl and ice sufficient to achieve a 78% total moisture content.

To prepare pastes, frozen meat was tempered at room temperature for 1 hr and pre-cut to flake consistency in a Stephan UM-5 vacuum cutter-mixer (Stephan Machinery Corp., Columbus OH) at 900 RPM for 1 min (no vacuum). Salt and ice were then added and the mixture was chopped at 2000 RPM under vacuum to an endpoint of 4 °C. Pastes were transferred to a plastic bag and vacuum packaged to remove as much air from the sample as possible. A corner of this bag was cut before placing in a manually operated sausage stuffer and extruding the paste into teflon (for microwave) or stainless steel (for waterbath) tubes 17.5 long x 1.9cm dia.

In addition, tests were conducted wherein ceramic caps (2.54 cm length) were placed on the ends of teflon tubes stuffed with pollock surimi paste. This allowed

uniform heating of the paste near the ends of the tube, which did not occur due to the inadequate focusing of microwave energy. The improper microwave focusing occurred when the cavity was not completely filled during the entering or exiting of the sample, resulting in improperly heated sample ends. The ceramic used to form the end caps had similar dielectric properties to surimi, causing microwaves to be focused due to the cavity being fully filled before the entering and after exiting of the sample.

Plastic casings, 3 x 17.5 cm long, were filled with pollock (no whiting) surimi using the same procedures. For these trials, surimi pastes contained 3% NaCl with no moisture adjustment (moisture content of pollock surimi was 74–75%), characteristic of typical industrial testing protocols. Casing ends were twisted and secured with plastic tie strips.

Heating Methods

Rigid tubes (1.9 cm dia) containing surimi paste were heated either in the microwave cylindrical reactor (Fig. 1) to a 90°C endpoint (72 sec come-up at a heating rate of 66.6°C), in an ohmic heating device (J. Park, Oregon State University) at 5KHz, 180 volts for 80–90 sec to an endpoint temperature of 90°C, or in a water bath at 90 or 100°C for 15 min. For microwave heating, tubes were placed in a rotational hopper, which placed them individually on a belt that conveyed them through the microwave applicator at a controlled speed of 15.2 cm/min (exposure time of 77 seconds per 17.5 cm length of surimi paste). The power setting used was 170 watts (400 watts max). The 3 cm dia samples in casings were heated by microwave at a belt speed of 10 cm/min and power setting of 300, 360 or 400 watts, in an applicator utilizing same microwave wave

generator but specifically focused for the 3 cm casings to a 90°C endpoint temperature (135 sec come up time at a heating rate of 35 °C/min), or in a water bath for 20 min at 90 °C/min. Unless otherwise specified, tubes were immediately cooled in ice water following the heating regimes previously specified. Gels were removed from the tubes and cut into specimens 2.54 cm long, each end of which was glued to plastic disks using an instant adhesive. Dumbbell shaped samples were milled from each specimen to 1cm minimum diameter on a milling machine (Gel Consultants Inc., Raleigh NC) and allowed to attain room temperature prior to torsion testing, where the gel specimens were vertically mounted and twisted to fracture at 2.5 rpm on a Hamann Torsion Gelometer (Gel Consultants Inc.). Stress (kPa) and strain (dimensionless) at fracture were recorded for each sample, corresponding to the strength and deformability of the gels. Samples prepared in 3 cm casings were cut into 2.54 cm lengths and puncture tested using a 5 mm ball probe attached to a texture analyzer (Model TAXT2Li, 2 Kg load cell capacity, Texture Technologies Corp, Scarsdale, NY). Gel strength (grams at fracture) and deformability (penetration of probe in mm at fracture) were recorded for each sample.

RESULTS AND DISCUSSION

Effects of Plasma Addition and/or Rapid Heating

Pollock/whiting gels cooked by waterbath containing added beef plasma were stronger (higher fracture stress) but not more deformable (equal fracture strain) as compared to samples not containing plasma (Fig. 2a,b). Gels not containing plasma were stronger when cooked at 100°C than at 90°C. Weaker gels can be attributed to greater

protease activity in the absence of beef plasma and use of a slower heating rate (at 90°C). With addition of plasma, 90°C water bath processing actually produced stronger gels than did 100 °C water bath processing .

Rapid heating by microwave produced stronger gels than slower heating in a water bath (at 90 or 100°C), regardless of whether plasma protease inhibitor was added. Addition of plasma did additionally increase the strength of microwave heated gels as expected, since plasma is known to have a gel-enhancing effect apart from that of protease inhibition via its synergistic gelation with meat proteins (Kang and Lanier 2000). Rapid heating by microwave apparently inactivated proteases with similar effect on gel texture as by adding plasma to pastes and processing by water bath at 90°C (Fig.2 a,b). Rapid heating by microwave had essentially the same gel-enhancing effects on this surimi as did ohmic heating (Fig. 3a,b) (Yongsawatdigul and others 1995).

Effects of Ceramic End Caps

Gels prepared without ceramic end caps in the microwave applicator had lower mean fracture stress values with higher mean standard deviations as compared with those prepared by water bath (Fig. 4a). At the same power setting of 300, samples with end caps still exhibited lower fracture stress values but with lower mean standard deviations. However, with an increased power setting (360 watts), fracture stress values and mean standard deviations were similar to that of the waterbath treated. Higher fracture strain and mean standard deviations were exhibited by gels heated by microwave without ceramic end caps as compared to water bath heated samples (Fig. 4b). At the same power setting of 300 watts, microwave heated samples with end caps had similar fracture

stains and mean standard deviations as water heated samples, but increased in fracture strain when a higher power setting of 360 watts was used. Gels prepared in the larger diameter casings by microwave heating exhibited lower gel strengths, but similar mean standard deviations, as compared to water bath heated samples (Figure 5a,b). Gel deformability and mean standard deviations were similar between the two treatments.

Despite a redesign of the microwave applicator for 3cm casings, it was visually apparent that these were not uniformly cooked when viewed in cross-section (sufficiently cooked areas are more opaque). Incomplete heating of the ends of the casing samples was also apparent, as expected due to the absence of ceramic end caps. Because heat-activated protease activity in these pollock pastes was predetermined to be negligible, these two factors likely contributed to the lower gel strengths of gels prepared in casings by microwave heating. A redesigned microwave unit for 3 cm casings having a power output greater than 400 watts and a more carefully focused applicator is currently being developed which will likely resolve these problems. Additionally the incorporation of a brief holding time upon reaching the endpoint temperature after rapid heating could contribute to more uniform heating and thorough protein gelation (Riemann thesis 2002).

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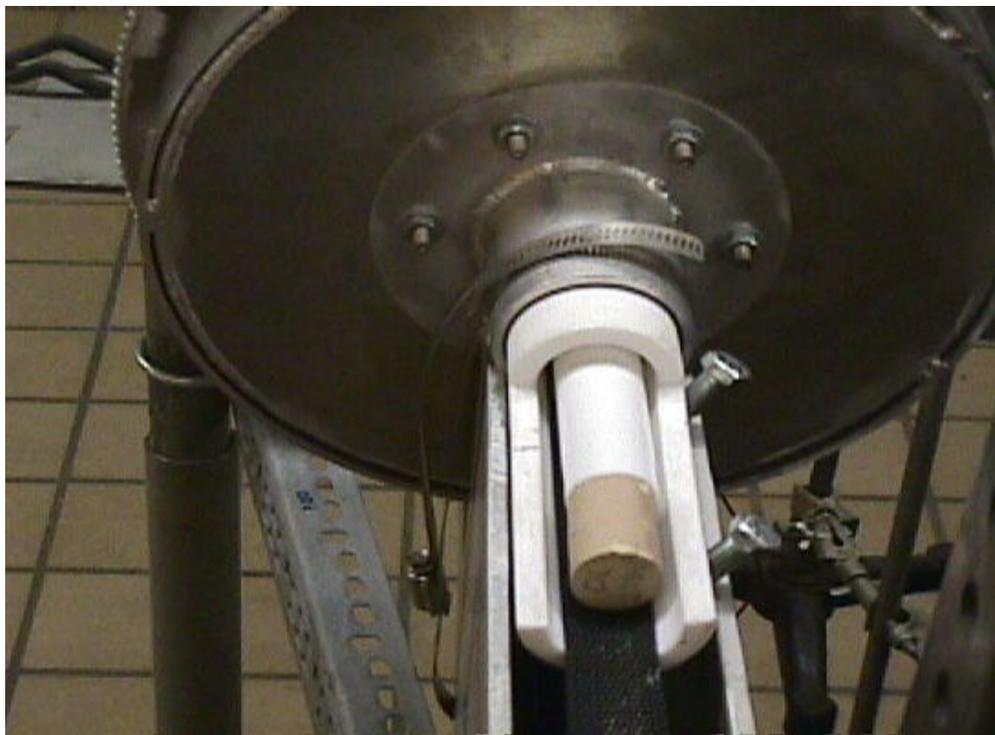
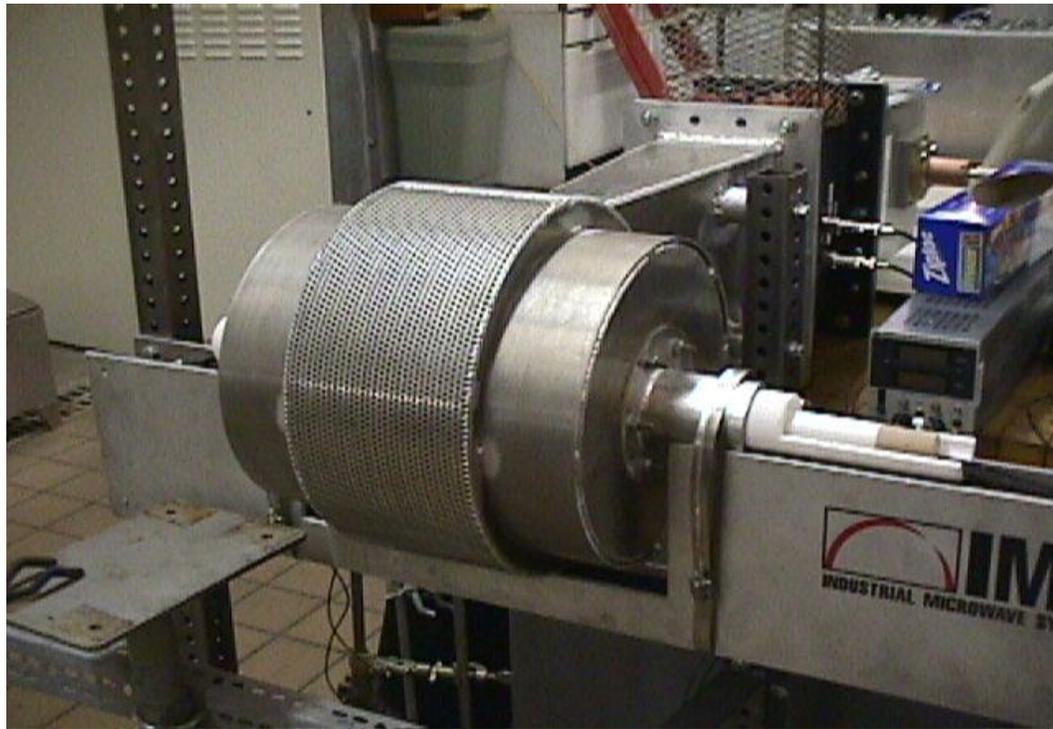


Figure 1: Different views of sample in teflon tube entering microwave cavity via a conveyor belt.

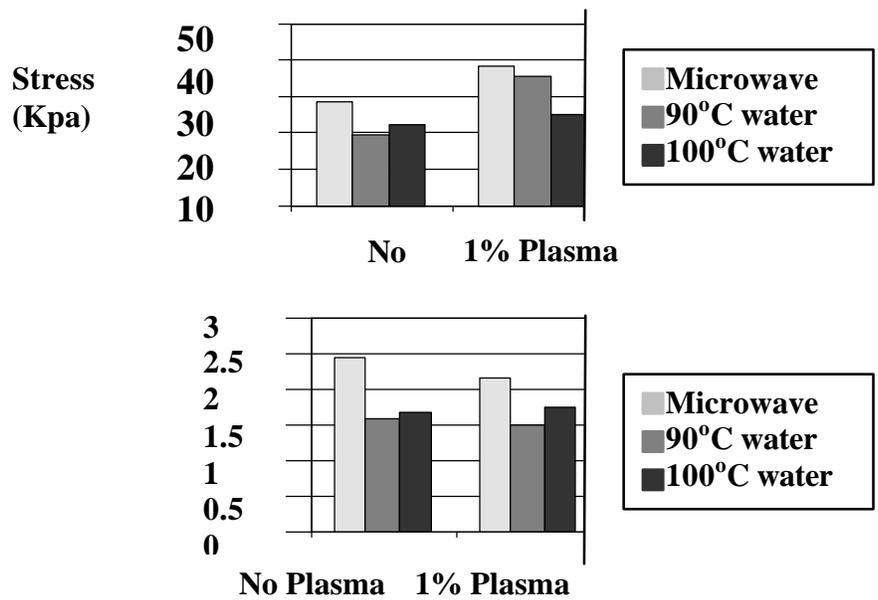


Figure 2 a & b: Stress and Strain values of 90 °C and 100 °C waterbath vs microwave with or without added 1% beef plasma.

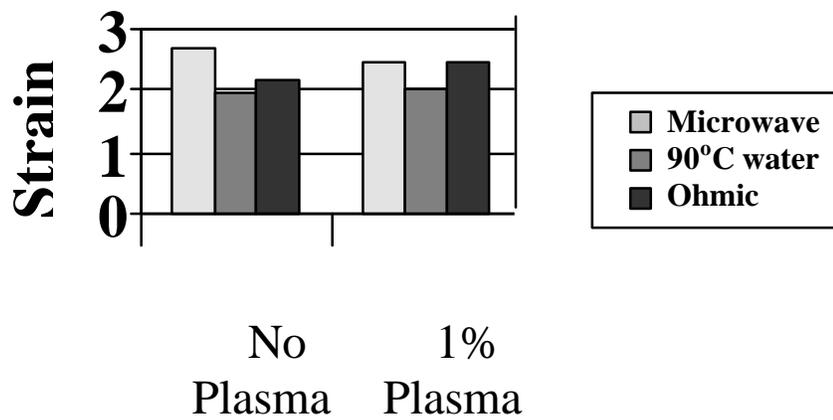
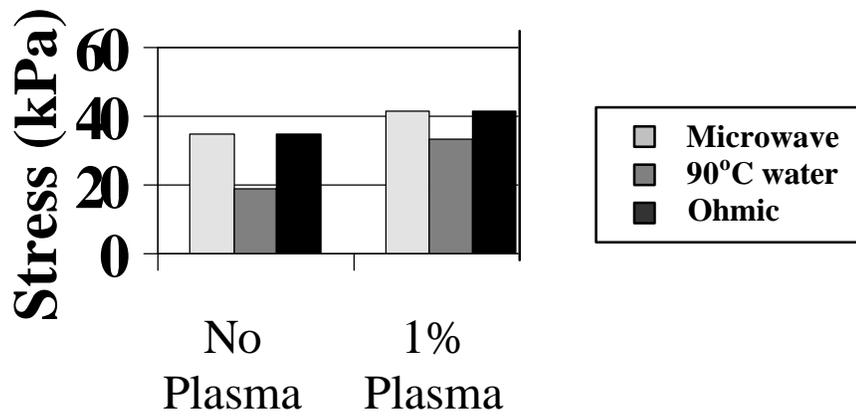


Figure 3 a & b: Stress and strain values of waterbath vs ohmic and microwave, with or without 1% added beef plasma

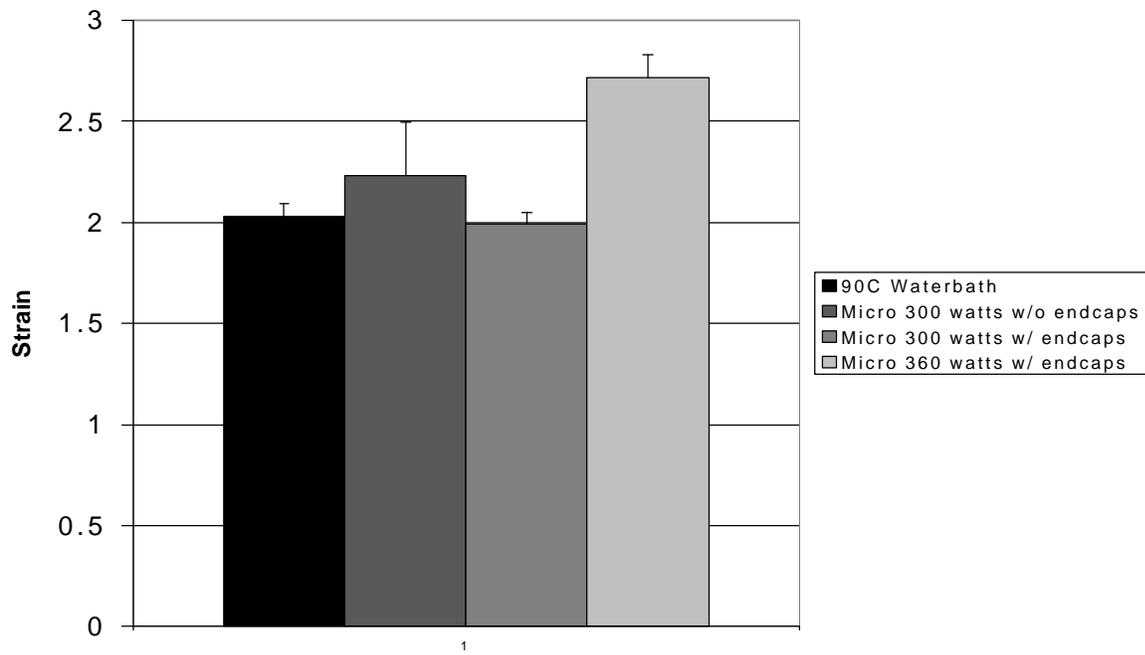
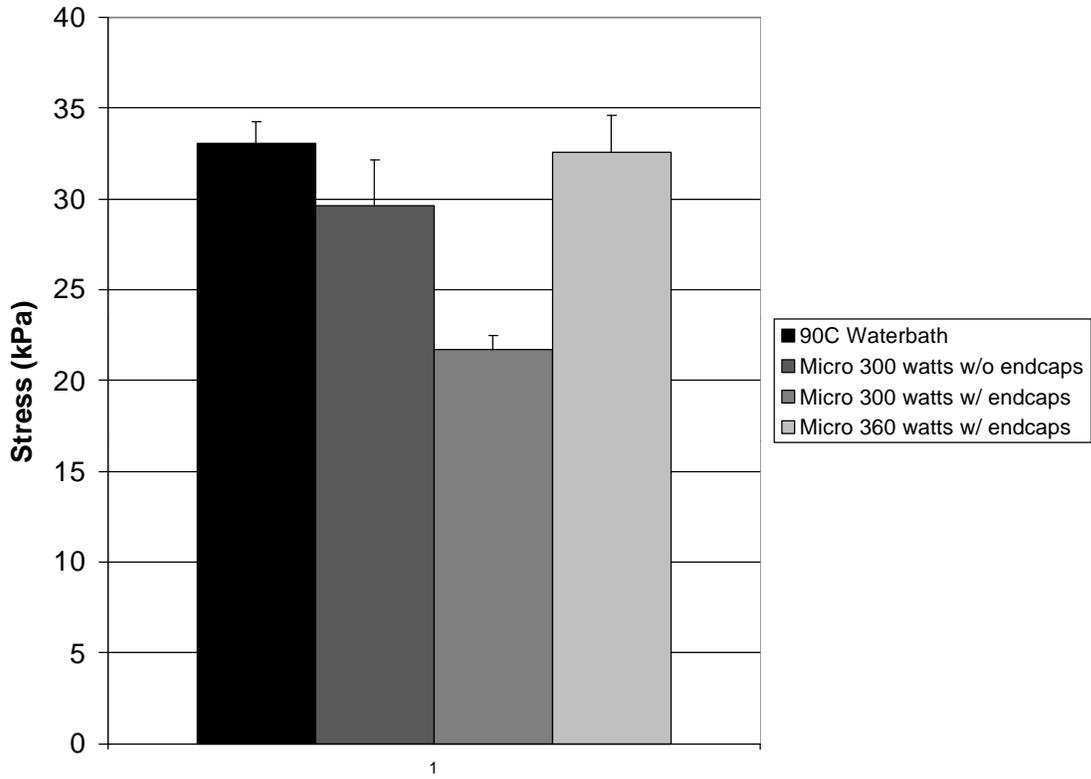


Figure 4 a & b: Stress, Strain and Standard Deviation values for Surimi heated in waterbath vs Microwave, with or without ceramic end caps to 90 °C.

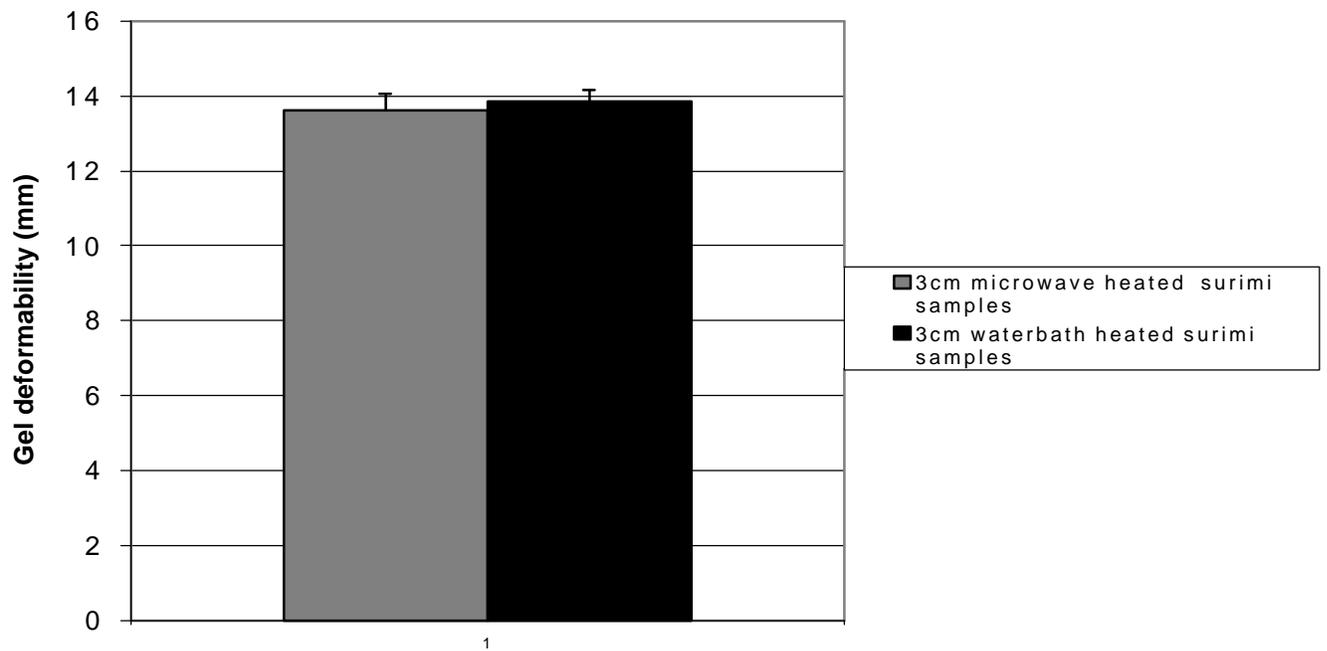
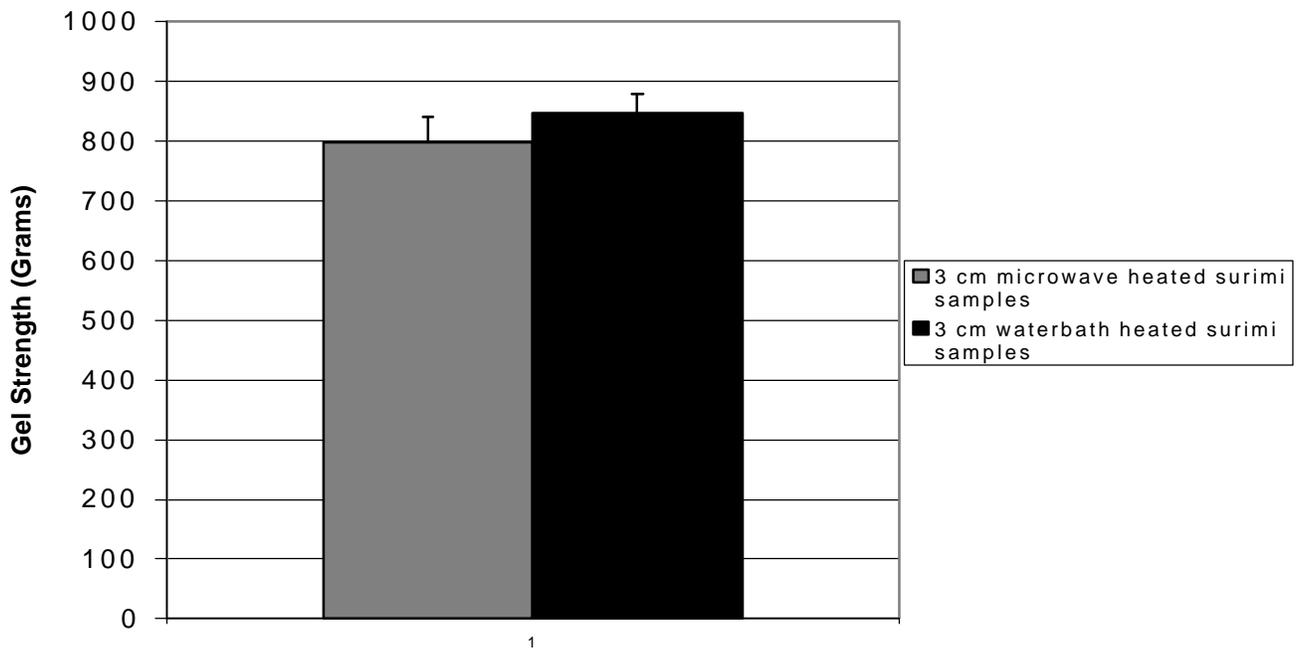


Figure 5 a&b: Gel strength (grams) and deformability (mm) of 3cm caseings surimi samples heated in waterbath vs microwave.