AN ABSTRACT OF THE DISSERTATION OF

Jacek Jaczynski for the degree of Doctor of Philosophy in Food Science and Technology presented on March 18, 2002.

Title: "Physicochemical and Microbiological Characteristics of Surimi Seafood under Thermal and E-beam Processing"

Abstract approved: _____

Jae W. Park

Effects of thermal and electron beam (e-beam) processing on microbial inactivation and physichochemical properties of surimi and surimi seafood were investigated. Thermal-death-time (TDT) concept was used to model *Staphylococcus aureus* inactivation by heat and e-beam. Gurney-Lurie charts were used to estimate temperature during thermal processing of surimi seafood. Dose mapping technique was used to estimate dose absorbed by surmi seafood during e-beam processing. The dose absorbed increased up to 2 cm and then it gradually decreased, reaching a minimum at 5 cm depth of surimi seafood. It was determined that one and two-sided e-beam can efficiently penetrate 3.3 and 8.2 cm of surimi seafood, respectively. The D-value for thermally inactivated *S. aureus* was 0.65, 1.53, 6.52, 49.83, and 971.54 s, at 95, 85, 75, 65, and 55°C, respectively. The D-value for *S. aureus* inactivated with e-beam was 0.34 kGy. The z-value for thermally inactivated *S. aureus* between 55-95°C was not linear. Thermal processing degraded texture and color of surimi seafood. E-beam enhanced firmness and decreased the b* value of surimi seafood. Myosin heavy chain (MHC) degraded proportionally to the e-beam dose. Actin was not affected by e-beam except treatment at 25 kGy. Hydrophobic interactions and disulfide bonds were formed in raw Alaska pollock surimi when treated with e-beam.

Physicochemical and Microbiological Characteristics of Surimi Seafood under Thermal and E-beam Processing

by

Jacek Jaczynski

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented March 18, 2002
Commencement June 2002

ACKNOWLEDGMENTS

I would like to thank to my major professor, Dr. Jae Park. Dr. Park has helped me a lot from the very beginning of my program at Oregon State University. I do not intend to exaggerate with words. All I want to say is sincere and straight from my heart: "THANK YOU". However, during my every-day lab struggles, more important was scientific support that I could rely on in "large quantities" from Dr. Park. His little notes always turned out to be of crucial importance and finally allowed me to puzzle my thoughts together and carry on with the projects.

Of course, I would not have come here without spiritual support from my brother and my parents. I am very thankful for that what they have done for me so far and I have to admit they are very stubborn - they are still willing to support me. Thank you folks.

I also want to send a big smile to my wife – Gosia, who was able to stand all my different habits when I studied for finals or I just wrote a manuscript. She was very benevolent and easy-going at the same time.

I would like to express my deep gratitude to my colleagues who helped me so much during the entire course of study. I appreciate their commitment and involvement.

I also feel indebted to all the students I have met on my trip through Graduate School. I hope they will be obstinate enough to wade through all the shallows and finally see a little candle in the tunnel – it's Graduation Ceremony, our final bus stop on the route called OSU.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

Page

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF TABLES

PHYSICOCHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF SURIMI SEAFOOD UNDER THERMAL AND E-BEAM PROCESSING

INTRODUCTION

INTRODUCTION

Food and food packaging technology must continually improve in order to provide better nourishment and overall food quality (Brody 1988). At the same time, in the wake of the September 11 incident, the food industry must take proper steps to assure a continuous risk-free food supply (Hollingsworth 2002, Applebaum 2002). Improvement of current pasteurization practices will play a key role in achieving that goal. There is a great need therefore for research and education regarding minimally processed chilled-distribution foods.

Most food products classified as "fresh-like" are in fact "minimally processed". The term "minimally processed" includes, for example oranges, which are sorted and waxed and fish, which are scaled, eviscerated, and chilled. There is a concern however that minimal processing and package engineering may actually increase the probability of pathogenic microbial infection or intoxication. This is reinforced by abuses of the food system, which falls into the following categories:

1) Failure to exercise quality control over the food components.
2) Failure to comprehend and implement effective in-plant HACCP programs.
3) Use of elevated temperatures in processing operations.
4) Too slow reduction of in-product temperatures.
5) Less-than-clean operations resulting in microbial contamination.

6) Packaging in less-than-clean rooms.

7) Anomalous pasteurization techniques.

8) Defective packages.

9) Prolonged time in distribution or any other element of the channel.

These areas of abuse, in combination with consumer demand for safer food products and increasing regulatory pressures on food safety, obviously necessitate more studies on this subject. Presently, multiple approaches are available. However, most of them are in developmental stage.

THERMAL PASTEURIZATION

Pasteurization or moderate temperature thermal processing is used to extend the refrigerated shelf life of pre-packaged foodstuffs. Pasteurization is defined as the elimination of viable pathogenic organisms along with a reduction of common spoilage organisms. Pasteurization is not a true sterilization because bacterial spores and other latent microbial forms are not necessarily inactivated. The relatively mild heating conditions however, result in color, texture, and flavor characteristics that are similar to fresh products, and greatly extend the shelf life compared to the fresh counterparts (Rippen and Hackney 1992).

Most spoilage microorganisms and pathogens are heat sensitive and can be destroyed by low to moderate heat. The D and z-values of pathogens and spoilage microorganisms can be calculated from data available in literature. The standard pasteurization procedures, when performed correctly, produce high-quality, safe products. However, sensory or shelf life problems may arise that can generally be solved by understanding the principles of the process:

1) F-value (min), a mathematically calculated number describing the total heating time of the process (accumulated heat exposure), which depends on

the initial microbial load, composition of the microbial population, composition of the product, storage temperature, and container integrity.

2) D-value (min), decimal reduction time or the time required to reduce a population of target bacteria by 1-log (90%). The D-value is specific to the microorganism, media, and temperature. It is affected by water activity, pH, and chemical composition.

3) z-value (°C or °F), the number of degrees (°C or °F) required for the D-value to traverse 1-log (90%). The z-value gives an indication of the relative impact of different temperatures on an organism. The z-value is essential in process calculations and can be used to calculate D-values at intermediate temperatures.

Presently, commercial applications for pasteurization and related processes are increasingly integrated into comprehensive HACCP-based programs, which accommodate new products, innovative packaging, and pathogen control requirements (Rippen and Hackney 1992).

Besides the conventional approach to thermal pasteurization (hot water), a new trend has recently appeared. The idea is to increase the efficiency of the process, which translates into either shorter pasteurization time (maintaining the same microbial inactivation) or higher microbial inactivation during the same pasteurization period. One such approach is continuous flow microwave pasteurization (CFMP), which applies microwave radiation for thermal pasteurization. This process can be applied to continuously flowing products. Microwave heating rates are faster than conventional thermal pasteurization, therefore, increasing the efficiency of the process (Harlfinger 1992). The microwave cavity directly and evenly heats the entire product. Therefore, there are no off-flavors (product burning). Sensory tests proved that products pasteurized by the microwave technique were preferred by panelists over the same product pasteurized by conventional means (Harlfinger 1992). In addition, turbulent flow is not required and product density is of less concern when microwave radiation is

applied. Furthermore, there is higher efficiency of energy utilization since the electrical energy is converted to microwaves that are directly passed onto the product. In conventional pasteurization, either steam or water is first heated and then the energy passes onto the product. This inevitably causes energy loss.

Another pasteurization system that is in commercial use employs steam as the heating medium. The steam pasteurization system (SPS) seems to be particularly effective for the elimination of *Eechrichia coli* and *Salmonella* on beef carcasses (Nutsch and others 1998). The SPS blankets the carcasses with pressurized steam to uniformly reduce bacteria over the entire surface. Then, the carcasses are rapidly chilled in the "superchiller" section of the SPS where the carcass temperature is reduced to 60°F with a cold-water spray. The SPS system can be included as a critical control point in the HACCP plan. The only concern is the possibility of a system breakdown. If steam pasteurization, as a critical control point is inoperable, the process must halt (Nutsch and others 1998).

Kozempel (1998) applied a similar approach by using saturated steam to decontaminate the surface of poultry meat with subsequent quick chilling under a partial vacuum. The system killed 99.99% (4-log) of bacteria within 26 milliseconds without cooking the meat. This was accomplished by first eliminating the air surrounding the meat. Initially, the product was briefly exposed to steam. Then, the product was subjected to equally quick surface cooling by re-evaporating the steam condensate (that had formed on the meat during heating) into a vacuum. Since both the heating and cooling steps are finished within milliseconds there is not enough time for the meat to cook. This opportunity arises because of the higher activation energy of meat protein denaturation compared to the lower activation energy of disrupting the most vulnerable bacterial enzymes. Since most contamination of intact meat is on the surface, SPS could virtually eliminate this danger to meat consumers, provided that the resulting products are correctly handled.

Another approach that employs steam as the heating medium is continuous steam fusion pasteurization (Stroup 1972). This system handles a wide range of products from low-viscosity fluids to pastes and slurries with or without particulates. The key to this process is a variable-speed agitator, which rotates on an axis parallel to the product flow through a vertical tube. A row of steam injectors along each side of the tube provides rapid heating while turbulence created by the agitator fuses steam to evenly heat the product as it flows through the tube. The product reaches cooking temperature in just a few seconds, and steam is efficiently combined with the product so that particulates are not overcooked. In addition, since all cooker surfaces are at the same temperature as the product, there is no burn-on.

SOUS-VIDE

A French method of food preparation called sous-vide (a French term meaning "under vacuum") has generated considerable interest in the food industry. *"Sous-vide"* is in a food category commonly referred to as new-generation refrigerated foods. The term sous-vide is also used to describe the final end product. This novel thermal process is milder than traditional pasteurization. The product is cooked in the package in its own juice, thus the flavor is locked in (Adams 1991). The product is subsequently reheated before consumption.

Rhodehamel (1992) explored the potential danger of sous-vide products from the FDA's standpoint. The primary microbial concerns are *Salmonella*, *Listeria monocytogenes* and toxins generated by *Clostridium botulinum*. Sous-vide products are generally formulated on the basis of desired organoleptic qualities and not on safety considerations (Rhodehamel 1992). The current preservation procedures for sous-vide, therefore, may not be sufficient to maintain the product at a microbially safe level. Consequently, Adams (1991) concluded that due to the

microbial safety concerns of sous-vide the only reasonable approach is a HACCP program fully implemented and accepted in the industry. An additional drawback of sous-vide products is the requirement of strict temperature control during distribution, which limits the shelf life and distribution range (Rhodehamel 1992). Consequently, the reason for slow acceptance of sous-vide products may in part be due to safety concerns.

Ghazala and others (1995) investigated the microbiological aspects and safety of sous vide foods. They tested *S. faecium* for heat resistance in cooked meat and nutrient broth. The z-value obtained at 60-85 °C was 12.4 °C; however when only 60-75 °C was considered, the z-value was 9.4 °C. It was therefore suggested that the use of z-value, due to its variation, should be carefully considered when applied to the establishment of a pasteurization process.

Briley (1992) concluded that the popularity of sous-vide is expected to increase due to Americans' interest in convenience foods. Other factors that will contribute to the increased popularity of sous-vide are the increase in individuals over 55 years old, the increasing numbers of workingwomen, and the changing structure of the US family. Consequently, the safety concerns associated with these products will become increasingly more important. Continued research in this area is, therefore, needed.

OHMIC (ELECTRICAL) HEATING

Thermal processing is a traditional pasteurization method in the food industry. Currently, most thermal sterilization techniques employ indirect heating, where an external medium applies heat to the food product. However, thermal convection and conduction limits the process by the inherent heat transfer coefficient (h) and thermal diffusivity (α), respectively. The temperatures required to kill microbial spores also induce a number of reactions, which reduce food

quality. For instance, the outside of the can needs to be held at the process temperature for several minutes before the particles at the center of the can also reach the process temperature. During this time, the rest of the food will have been significantly over-processed. The result is that canned food, although commercially sterile, is often of low quality.

An alternative technique is direct heating, in which heat is generated within the material itself. Ohmic heating, which uses the inherent electrical resistance of a material to generate heat, is a relatively new process in food engineering, and has already attracted broad attention. The resulting mathematical models, though, are often complex and highly nonlinear. A better understanding of the interaction between temperature and voltage fields for ohmically heated liquid-particle mixtures is therefore required. In addition, critical parameters need to be identified, and the effects of features, such as particle shape, size, orientation, and distribution need to be investigated. Therefore, for successful commercial applications, it is important to demonstrate that ohmic heating can produce safe, high quality food, and also to develop and validate an accurate model.

Ohmic heating for mixtures of solids and liquids is an additional challenge, because electrical conductivity is a non-linear function of temperature. Therefore, the current tends to channel through the material and can result in the solids heating faster than the liquid, which is the opposite of conventional heating methods, where liquids heat faster than solids. Even for a single particle in a liquid, the heating rate is a function of particle shape and particle orientation to the applied electric field. Therefore, it is difficult to ohmically heat particulate foods.

The advantage of ohmic heating, however, is more uniform heating of liquid or particles suspended in a liquid, than classical indirect heating methods. Therefore, it is possible to improve the ratio between the desired sterilization effect and the undesired reactions that impair the quality of processed food (Fryer 1993). To properly evaluate the reaction kinetics and to optimize the process, a description of residence time distribution and temperature distribution in the processed food

must be known. Typically, materials heated ohmically are heterogeneous and highly non-Newtonian liquids.

Halden and others (1990) determined changes in the electrical conductivity of food during ohmic heating. A number of effects, such as starch transition, melting of fats and cell structure changes, affected electrical conductivity. They demonstrated that in some cases the presence of an electric field induced enhanced diffusion of cell fluids into the food, which increased the rate of change of conductivity with temperature above that utilized by conventional heating. They hypothesized that electro-osmotic effects were responsible for this observation.

Yongsawatdigul and others (1997) ohmically heated Pacific whiting surimi paste to investigate degradation of myosin heavy chain (MHC) caused by endogenous proteinase over a range of 40-85°C and 0.5-35 min. The degradation rate increased with temperature and reached a maximum at 57°C. The rate of MHC degradation then decreased with higher temperature and reached a minimum at 75°C. They also determined the apparent degradation reaction order, activation energy and inactivation energy (E_a) at 1.4, and 142.3 and 83.1 kJ/mol, respectively.

Palaniappan and Sastry (1991a, 1991b) investigated the application of ohmic heating to meat and vegetables. The samples were subjected to constant voltage in a static ohmic device. To increase the conductivity of the tested samples, the samples were dipped in salt solutions. They determined that conductivity under these testing conditions increased linearly with temperature at the voltage gradient. However, when the field strength decreased, the conductivity-temperature curve gradually became non-linear, and under conventional heating conditions, a sharp transition was observed at about 70 °C. At this temperature, texture deterioration was significant. An explanation similar to Halden and others (1990) that electro-osmotic effects were responsible was also proposed.

Yongsawatdigul and Park (1996) showed the superior effects of fast heating rates obtained from ohmic heating of surimi gels, which resulted in increased shear

stress. They determined that the increased shear stress was due to increased cross-linking of myosin heavy chain (MHC). Slow heating rates, though, increased protein proteolysis as shown by the degradation of MHC and low shear stress.

Parrott (1992) examined the applicability of ohmic heating to aseptic and bag-in-box filling systems for high value added and ready-to-eat meal products. The benefits of ohmic heating were found to be:

1) Continuous production without slow surface heat-transfer.
2) Rapid, even treatment of liquid and particulates with minimal heat damage and residence-time differences.
3) Ideal process for shear-sensitive products because of low flow velocity.
4) Increased nutrient retention.
5) Fresher-tasting, higher-quality products than with alternate heat-preservation techniques.
6) Optimization of capital investment and product safety.
7) Reduced fouling as compared to conventional heating.
8) Ease of process control with instant shutdown.
9) Ambient-temperature storage and distribution when combined with an aseptic filling system.

Sastry and Palaniappan (1992) attempted to determine whether the lethal effect in ohmic heating results from temperature alone or in combination with electrical current. They compared the death kinetics of microbes under conventional and ohmic heating. Identical thermal conditions were used for both techniques to eliminate thermal effects. The results indicated that for the field strengths commonly associated with ohmic heating, no significant lethal effect due to the electrical current existed. However, mild electrical pre-treatment was found to result in reduced subsequent thermal inactivation requirements. At high field strengths, associated with electroporation techniques, though microorganisms can be destroyed by disruption of the cell membrane, although the effect has primarily been observed for vegetative cells.

Palaniappan and Sastry (1991b) developed and tested a device for ohmic heating applicable for fluids. The device was used for determination of the electrical conductivity of foods under conventional and ohmic heating conditions. They found that the electrical conductivity of juices increased with temperature and decreased with solids content. However, there was a significant difference between the temperature effect on the conductivity of orange juice and tomato juice. The effect of temperature was greater for orange juice, whereas the effect of solids content was greater for tomato juice. The relationship between these parameters was found to be linear, both under conventional and ohmic conditions. They also determined that there was an increase in conductivity with decreasing particle size.

Yongsawatdigul (1995) and others described and tested an ohmic heater applicable for the gelation of meat pastes. They found that ohmic heating was capable of faster heating rates than conventional water bath heating. Ohmic heating resulted in maximum functionality of surimi gel compared to water bath heating. This was possibly due to minimized degradation of myosin heavy chain and actin obtained with ohmic heating. Ohmic heating also resulted in a continuous gel network structure.

Cho and others (1996) tested the growth of *Lactobacillus acidophilus* under ohmic and conventional heating. They applied electrical current at 15 and 40 V to *Lactobacillus acidophilus* culture to heat the bacteria directly during fermentation. They found that the lag phase for bacterial growth was greatly affected by heating method, but the magnitude of these changes depended on the fermentation temperature. Ohmic heating did not significantly change the generation time and caused a slight, but statistically significant decrease in maximum growth. Therefore, it was concluded that electric current enhances the early stages, but it inhibits the late stages of microbial growth. Ohmic heating as compared with conventional heating, also resulted in a higher final pH and lower bacteriocin activity.

RADIO FREQUENCY (RF)

A radio frequency generator is used in RF heating systems to create an alternating electric field between two electrodes. A food product is conveyed between electrodes where the alternating energy causes polar molecules in the material to continuously reorient to face opposite poles, which resembles the bar magnets in an alternating magnetic field. The friction resulting from the molecular movement causes the material to rapidly heat throughout the entire mass. The amount of heat generated in the product is determined by the frequency, the square of the applied voltage, product dimensions, and the dielectric loss factor of the material. The dielectric loss factor is an essential parameter, which is a measure of the ease with which the material can be heated by radio frequency waves.

Kolbe and others (2001) investigated Debye resonances in food and packaging materials and their effects on selective capacitive dielectric heating. They quantified the dielectric properties and demonstrated the rapid heating of muscle foods. Such heating was maximized by continual adjustment of impedance match to track rapidly-changing temperature-dependent properties.

Ponne and others (1994) showed that whether RF, steam, or hot water was applied for blanching vegetables, there was no difference in quality change. However, the rapid, precise RF heating process offers the advantages of reduced processing times, consistent quality, and simplified process control. In addition, RF heating occurs instantly throughout the thickness of the material providing fast and uniform heating. Conventional heating, in contrast, relies on conduction to transfer heat to the center of the material and, therefore, can be a much slower process.

The following are additional advantages of radio frequency heating:

1) Heating occurs selectively in those areas where heat is needed because water, un-polymerized synthetic resins, and uncured latices are much more responsive to RF energy than most other dielectric materials.

2) More water is automatically removed from wet areas because wetter areas absorb more RF power than dryer areas, which results in a more uniform moisture distribution.
3) The load may be supported by electrodes or conveyed under or between them. Self-supporting webs or strands do not need to touch the product, thus avoiding surface marking and contamination.
4) Power is consumed primarily by the workload. There are no losses from heating masses of cast iron or huge volumes of hot air. In addition, no long warm up or cooling times are required. Consequently, power is consumed only when the load is present and only in proportion to the load.
5) Power control is accurately metered and recorded. A meter constantly displays the amount of power being applied to heat the product.
6) The full range of power control from minimum to maximum is traversed in seconds. Adjustments take effect immediately, therefore thermal lag time is zero. Automatic changes in power level, due to physical properties or load size, are instantaneous.
7) When a dielectric material is polymerized, dried, or cured, its loss factor is lowered, reducing its response to RF energy. RF heating is, therefore, automatically limited to the level required to complete the process.
8) The RF heater is a very sensitive moisture meter. Its power output is a measure of the average moisture content of the load. Changes in power output therefore can be used to trigger alarms or provide feedback signals for process control.

IONIZING RADIATION

Vegetative bacteria as well as parasitic food-borne pathogens, which cause preventable illness and death, still remain a substantial public health problem

(Lagunas-Solar 1995). Food irradiation with low doses of gamma rays, X-rays, and electron beams can effectively control these vegetative bacteria and parasitic food-borne pathogens (Monk and others 1995). Following good practice guidelines and HACCP, irradiation doses as low as 1 to 3 kGy yield adequate safety margins for food-borne pathogens, such as *Campylobacter*, *Cryptosporidium*, *Escherichia coli*, *Listeria*, *Salmonella*, and *Toxoplasma* in raw meat, poultry, seafood, and produce (Monk and others 1995).

The food industry, however appears reluctant to fully embrace irradiation pasteurization despite the obvious and painful failure of alternative approaches to prevent food-borne infections. Much of this reluctance stems from the perception that consumers will reject the process and will refuse to buy irradiated food. Indeed, surveys have shown considerable consumer confusion and ignorance about food irradiation (Bruhn 1995). At the same time, however, in the wake of the September 11 incident, the food industry needs to take proper steps to assure a continuous risk-free food supply (Hollingsworth 2002, Applebaum 2002). Food irradiation may offer a solution.

The FDA has approved the following sources of ionizing radiation for the treatment of foods: gamma rays produced by the natural decay of radioactive isotopes of cobalt-60 or cesium-137, x-rays with a maximum energy of five million electron volts (MeV), and electrons with a maximum energy of 10 MeV. The electron volt [eV] is the amount of energy acquired by an electron when it is accelerated by one volt in a vacuum. X-rays are produced when high-energy electrons strike a thin metal film and are identical in action to gamma rays. Other forms of radiant energy include light, heat, microwave, and radio waves.

Fish and shellfish are significant sources of food-borne pathogens. Radiation has been demonstrated to control *Salmonella*, *Shigella*, *Staphylococcus aureus*, enteropathogenic *Escherichia coli*, *Vibrio cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and hepatitis A virus (Clavero and others 1994). Ionizing radiation at 1 kGy was adequate to eliminate *Vibrio vulnificus* in oysters (Ama and others

1994). In addition, when combined with a traditional depuration, or controlled purification treatment, radiation at 2 kGy significantly decreased the number of hepatitis A virus in clams and oysters.

There is a concern, however, that marine fish might be contaminated with *Clostridium botulinum* type E, which can grow at refrigeration temperatures as low as 38°F. *C. botulinum* is relatively resistant to radiation and would not be significantly affected by low doses. In addition, if contaminated and irradiated fish are sealed in oxygen impermeable packages, *C. botulinum* type E may grow because of decreased competition from other bacteria. The product would then become toxic without the usual signs of spoilage. This outcome, however, seems unlikely considering the rare occurrence of *C. botulinum* type E and the availability of oxygen-permeable packaging materials.

The technology and type of equipment required to irradiate food are identical to the equipment used to sterilize medical supplies and devices. Many commercial irradiation plants exist in the United States, and they have established an excellent safety record. Commercial plants that use cobalt-60 as the source of radiation, store it underwater when not in use. Cobalt pellets are encapsulated twice in stainless steel tubes arranged in racks. These racks are withdrawn from the water when materials are to be irradiated, and the food is passed through the radiation field. A maze constructed of thick concrete walls, numerous safety interlock systems, strict operating procedures, and proper training protect workers from radiation.

Giddings (1984) investigated three basic types of ionizing radiation (electron beams, gamma, and x-rays) in fish product processing with regard to pasteurization, sanitation, insect destruction, and sterilization of *Clostridium botulinum*. He indicated high efficiency and throughput of radiation processing for fresh-market fishery product.

Food-borne pathogens were greatly reduced or eliminated from foods by low doses of ionizing radiation. The shelf life of poultry, pork, and beef was also

significantly extended by radiation treatment. Thayer (1993) demonstrated that ionizing radiation can inactivate parasites, eliminate or greatly reduce the populations of microbial pathogens, and extend the shelf life while preserving the desired nutritional and sensory properties of refrigerated poultry and red meats. In addition, a synergistic effect between radiation and vacuum or modified atmosphere packaging was observed.

Food is usually packaged to prevent microbial contamination and spoilage. Ionizing radiation can also be applied to food-packaging materials. It is usually accomplished by: (1) radiation sterilization of the packaging materials for subsequent aseptic packaging, or (2) radiation processing of the prepackaged food. In aseptic packaging, a radiation-sterilized package is filled with a sterile product in a microbiologically controlled environment. Demands on the toxicological safety are less stringent for irradiation of prepackaged food, since the food and packaging material are irradiated simultaneously. For both applications, though the radiation stability of the packaging material is a key consideration (Killoran 1972). The radiation must not compromise the stability or physicochemical properties of the packaging material. The irradiated material must also protect the food from environmental contamination while maintaining its organoleptic properties. Single-layer plastics, however, cannot meet the requirements of either application. Multi-layered structures, on the other hand, produced by co-extrusion, lamination, or co-injection molding would likely satisfy the demands of radiation processing for prepackaged food (Allen and others 1988).

ULTRAVIOLET RADIATION (UV)

UV radiation has been found to inactivate not only the viable cells but also the aflatoxins (Rustom 1997). These highly toxic, mutagenic, teratogenic, and carcinogenic compounds contaminate a wide variety of important agricultural

products. Removal or inactivation of aflatoxin in food and feedstuffs is therefore a major global concern.

Crouse and others (1991) exposed beefsteaks to UV radiation. Aerobic and anaerobic counts significantly decreased in UV-treated steaks and no effect on shear force or myofibril fragmentation was observed. Maunder (1977) tested UV radiation to sterilize the surfaces of plastic containers, which are highly heat unstable. They found UV effective against spoilage bacteria that were present only on the surface of the tested containers. Gao and others (1997) tested UV irradiation for reducing *Salmonella* present on eggs and in egg handling facilities. They evaluated parameters such as test materials, UV intensity, time of UV exposure, and initial *Salmonella* contamination levels and showed that each of these elements greatly varied the capacity for UV sterilization.

PULSED ELECTRIC FIELD (PEF)

High voltage pulsed electric field (PEF) technology is a promising innovation in non-thermal processing. Application of intense PEF results in irreversible cell membrane breakdown of microorganisms in foods while causing little loss of food flavor and taste as compared to traditional heat pasteurization. Treatment by pulses of 1-5 msec duration above a threshold minimum of 15 - 20kV/cm average field strength is considered adequate to inactivate most vegetative cells. An operational goal for PEF pasteurization is to achieve a reduction of 6-10 logs of vegetative bacterial populations without reaching temperatures of common thermal pasteurization. Consequently, physichochemical properties of food and vitamins are retained in this process, which results in good food quality. In addition, the process kills both spoilage and pathogenic organisms, and can be used in conjunction with conventional thermal processing methods to provide a greater kill ratio and longer shelf life.

The effectiveness of PEF in injuring or killing microbes in foods seems to depend on the complexity and size of the target organism. Sensitivity to PEF treatment follows the approximate order: parasites> yeasts & molds> Gm(-) bacteria> Gm(+) bacteria. However, Gm(+) bacterial spores, yeast ascospores, parasitic oocysts and viruses are quite resistant to PEF. In addition, extended PEF treatment at elevated temperatures may damage a substantial population of certain bacterial spores, but it is unlikely that PEF alone will ever reach the 12-log reduction of *Clostridium botulinum* spores demanded for commercial sterilization of low-acid foods. Electrical factors affecting PEF treatment success include treatment temperature, field strength, duration of treatment, the number of pulses delivered, and pulse shape.

Zhang and others (1994) successfully inactivated *S. cerevisiae* in apple juice with short-duration, high-intensity PEF. By applying ten 2.5-μs pulses of 50kV/cm, 6-log inactivation of *S. cerevisiae* was achieved. The apple juice temperature was 29.6°C during the treatment, much lower than the 80°C required for heat pasteurization. Therefore, PEF has potential for inactivating microorganisms at temperatures below those that significantly affect the physicochemical properties, such as color and flavor. However, work is needed to prove that relevant food poisoning microorganisms are actually inactivated.

While traditional pasteurization will also reduce the activity of many enzymes to a large extent, PEF is unlikely to have a similar effect. It must be shown that the lack of inactivation of certain enzymes does not have undesirable consequences on the safety of the product. PEF is also likely to cause electrochemical changes, but these may be so small that product safety may not be affected. Again, evidence is needed to verify this assumption.

Wouters and others (1997) examined the effect of PEF on microorganisms. High voltage (PEF) treatment showed potential as a non-thermal food decontamination technique. For a treatment time of less than a second, microorganisms in foods were inactivated at ambient or refrigerated temperatures.

In addition, the fresh-like quality of the food was preserved. Therefore, it was concluded that during PEF treatment, for microbial inactivation, temperature increase was negligible. The bactericidal effect of PEF, instead was related to the electric field strength, treatment time, number of pulses, and pulse width. Additionally, microbial inactivation by PEF was determined to be a function of the type of microorganism, the microbial growth stage, inoculum size, pre-culture condition, ionic concentration, and conductivity of the suspension fluid.

Zhang and others (1995) tested high-strength, short-duration PEF for inactivation of *E. coli* in liquid foods. A 40 kV pulse generator was used to supply high voltage electric pulses to a treatment chamber with two parallel plate electrodes. The strength of PEF ranged from 35 to 70 kV/cm with 2-μs pulses, and the number of pulses per treatment varied from 1 to 80. *E. coli* were suspended in a simulated milk ultra-filtrate and treated with PEF in a batch mode. The suspension fluid was maintained at a constant temperature 7, 20, or 33°C and a 9-log reduction of *E. coli* was achieved. This study demonstrated that high-strength PEF treatment was adequate for the pasteurization of liquid foods.

OZONE (O_3)

The reduction and control of pathogens are essential for fresh food products like meats, fish, fruit, and vegetables. Bacteria, molds, viruses, and other single-cell microorganisms cause spoilage of fresh foods and decrease shelf life. Ozone has the capability of destroying bacterial formation. Ozone even enhances the taste of some food by oxidizing chemicals, such as pesticides and by neutralizing ammonia and ethylene off-gases produced by decay. The use of ozone does not leave toxic by-products or residues and is non-carcinogenic. Consequently, the FDA declared ozone as GRAS (Generally Recognized as Safe), when applied under

good manufacturing practices. In addition, ozone inevitably reverts back to its original form, oxygen.

All chemicals used in food and water treatment have a negative impact on human risk factors, as well as on the environment. Ozone is the most effective natural bactericide and viricide of all available disinfecting agents. The germicidal power of ozone is generally specific with respect to the individual species. Ozone's primary action on molds is to suppress growth and this effect can set in rapidly, particularly in the initial stage of mold surface growth. Ozone immediately attacks the easily accessible cells on the surface, but has a limited penetration depth. Ozonation applied for the storage of refrigerated meat, therefore destroyed surface microorganisms, particularly *Pseudomonas* responsible for spoilage.

Increasing the moisture content of the environment favorably influences the germicidal effect of ozone. This is caused by the swelling of microorganisms and thus, makes them more susceptible to destruction. Experiments conducted with beef showed that ozone is most efficient if the surface has a moisture content around 60%. Freshly caught fish can also be stored longer if washed in water containing ozone. In addition, if fish are stored in ice produced from water containing ozone, shelf life extension is obtained.

Restaino and others (1995) tested the antimicrobial effects of ozonated water in a recirculating concurrent reactor against four Gm(+) and four Gm(-) bacteria, two yeasts, and spores of *Aspergillus niger*. More than a 5-log reduction of *Salmonella typhimurium* and *E. coli* were obtained in ozonated water. In the presence of organic material, death rates were significantly reduced. However, death rates among the Gm(-) bacteria, *S. typhimurium*, *E. coli*, *Pseudomonas aeruginosa*, and *Yersinia enterocolitica* in ozonated water were not significantly different. Among Gm(+) bacteria, though, *L. monocytogenes* was significantly more sensitive than either *Staphylococcus aureus* or *Enterococcus faecalis*. More than 4.5-logs of *Candida albicans* and *Zygosaccharomyces bailii* were

instantaneously killed in ozonated water. However, less than 1-log of *Aspergillus niger* spores were killed after 5-min exposure.

Sheldon and Brown (1986) studied the effects of an ozone contact system on the quality of poultry carcasses and recirculating poultry chill water. They reported a 2-log reduction of the microbial burden on carcasses. In addition, ozonation greatly improved the microbial quality of chill water and had no adverse effect on carcass color, lipid oxidation, or flavors.

Yang and Chen (1979) determined the stability of ozone in water as affected by water temperature, initial ozone concentration, and length of holding time. The germicidal effects of ozone were affected by contact time, temperature, pH, and the presence of inorganic and organic materials in solution. Longer contact time, lower pH, and temperature resulted in a greater bactericidal effect. However, the bactericidal effects of ozone were reduced in solutions containing higher concentrations of organic materials.

TEMPERATURE PREDICTION DURING THERMAL PROCESSING OF SURIMI SEAFOOD

ABSTRACT

An interactive temperature prediction for surimi seafood was constructed and validated based on Gurney-Lurie temperature charts. The prediction model showed good agreement with experimental temperature profiles. The heat transfer coefficient (h) was determined by the lumped mass method and decreased with an increase of temperature difference (ΔT) between the sample surface and the water bath. The h had an effect on the convective heat transfer, resulting in a slower overall heat transfer at the beginning of heating. Thermal diffusivity (α) of surimi seafood was determined from experimental temperature profiles. The α increased slightly as the temperature difference (ΔT) between sample and water bath increased. The low α observed at small ΔT reduced conduction, resulting in a slower overall heat transfer.

INTRODUCTION

Surimi seafood is commonly pasteurized in hot water or steam (Park 2000). There is a two-step heat transfer in the pasteurization of surimi seafood; convection heat transfer from the hot water to the product's surface and then heat conduction within the product. Numerous temperature prediction models have been developed (McAdams 1954; Hsu 1963; Su and others 1999). Provided that thermal diffusivity, thermal conductivity, and the heat transfer coefficient are known,

Gurney-Lurie charts allow temperature prediction at any location and time in a brick-shaped product heated in an un-steady state heat transfer system, (Toledo 1991).

Temperature prediction at the cold spot is particularly important for microbial safety. The cold spot is defined as the region within a food sample that is the last to reach the temperature of the surroundings (water or steam)(Rippen and others 1993). If bacteria remain alive in the cold spot it will proliferate and contaminate the food. In pasteurization, when hot water is used to inactivate microorganisms, the "killing factor" is heat, a thermal energy (Kebede and others 1996). If the cold spot receives enough energy to inactivate the bacteria, then all other regions have received at least the same amount of energy. This form of pasteurization treatment is recognized as sufficient to maintain microbial safety (Rippen and others 1993).

To maintain the highest quality of color and texture, the gradient of temperature across the sample is a primary concern. This implies a different, more complex approach than for microbial consideration. To predict color and textural changes that occur during pasteurization, it is necessary to monitor time-temperature across the sample. This imposes some difficulties since thermal changes are dynamic. Heat penetrates a certain distance but simultaneously changes over time (Carlsaw and Jaeger 1959).

A dynamic system, where temperature changes with distance and time, appears to be functional (Hsu 1963; Toledo 1999). A three dimensional temperature profile is developed in the form of a surface response, which is a function of thermal processing time and distance from the cold spot of the sample. The amount of heat the sample has been exposed to during the thermal processing period is equal to the integrated volume under the three-dimensional surface.

Literature information on modeling temperature changes during thermal processing of surimi seafood is extremely limited. AbuDagga and Kolbe (1997) modeled thermal conductivity and specific heat of Pacific whiting surimi as

affected by moisture content and temperature. To our knowledge, there have been no reports on predicting temperature changes for surimi seafood processed thermally in a water bath. Temperature prediction is critical for optimization of microbial safety and physical quality of surimi seafood.

Our overall objective was to measure the thermal properties of surimi seafood using a conventional water bath. In detail, this study was: 1) to determine the convective heat transfer coefficient and thermal diffusivity for surimi seafood, and 2) to predict the temperature of surimi seafood as affected by various processing conditions.

MATERIALS AND METHODS

Samples

Un-pasteurized commercial surimi seafood (crabsticks) were obtained from Sea Blends (Seattle, WA) and transported in ice slush to the OSU Seafood Lab. Samples were kept in ice until used. The approximate dimension of an individual stick was 13-15 cm long and 1.4 cm in diameter. Before thermal treatment, the sticks were tightly placed on plastic trays and vacuum packed in a plastic film commonly used for surimi seafood packaging.

Water bath

An eighteen-liter water bath with water circulation was employed to mimic a typical industrial pasteurization process. Prior to the experiment, uniform temperature distribution in all locations and an accurate temperature reading of the water bath was verified.

Temperature measurement

A datalogger (CR10X, Campbell Scientific, Logan, UT) was used to monitor the temperature changes in the samples. The datalogger was programmed in Edlog version 6.3 (Campbell Scientific, Inc.) and five thermocouples (type T) took temperature readings every 5 s. The thermocouples were connected to temperature reading probes (59 mm long) using a threaded plug-socket system. Before the experiment, the probes were placed into the samples by puncturing the package and inserting the probe inside. To maintain the vacuum inside the package soft rubber washers were applied around the punctured holes and kept in place by bolts screwed on the probe's thread. Careful attention was taken to put the probes in the "cold spot" area of the samples. The geometrical center of the package was assumed as the "cold spot". After the experiment, the package was opened and the position of the probe's tip was verified. The datalogger was interfaced with a PC to display the temperature history of the thermocouples. The data was recorded by Tcom version 1.2 (Campbell Scientific, Inc.).

Heat transfer coefficient

The lumped mass method was used to determine the transient heat transfer coefficient (Incropera and Dewitt 1990) for hot water, which was used as the heating system. This method assumes that the heated solid is spatially uniform at any instant during the transient process. Therefore, the temperature measured at any location of the solid should be equal to the temperature at the surface of the solid. An aluminum block (122 x 122 x 9 mm), since it has small Biot number ($Bi = hL/k < 0.1$, where L indicates thickness of block (m) and k denotes thermal conductivity (W/m-°C)), was used. A hole was drilled and the probe was inserted for temperature measurement. The temperature was taken with the data logger

every second. The heat transfer coefficient was calculated using the following equation

$$h(t) = -\frac{\rho L C_p}{2\Delta t} \frac{T(t+\Delta t) - T(t-\Delta t)}{T(t) - T_a(t)}$$

h – heat transfer coefficient, W/m^2-°C,

ρ – density, kg/m^3,

L – thickness of the block, m,

Cp – specific heat, J/kg-°C,

Ta – ambient temperature, °C,

t – time, s,

T – temperature at time t, °C,

Δt – time interval for data collection, s.

Thermal diffusivity

For determination of thermal diffusivity (α) eight surimi seafood sticks were arranged in a single layer and placed on a small tray (15 x 11.5 x 2.0 cm) made of 0.05 mm polypropylene (5 PP). A thermocouple (O.F. Ecklund, Cape Coral, FLA) was inserted in the geometrical center and the temperature profile was recorded using CR10X data logger. Gurney-Lurie charts were used to calculate α from the experimental temperature profile as described by Singh (1982). The heat transfer coefficient, determined as described above, was used for α calculations. Thermal conductivity (k) was assumed to change (quadratic polynominal) from 0.534 to 0.680 W/m-°C over the temperature range of 30 - 80°C, as described by AbuDagga and Kolbe (1997) for 78% moisture surimi paste.

Gurney-Lurie charts

The charts plot partial differential equations and arrange them to find a solution. The charts are plots of a dimensionless temperature ratio ($Y = T_1\text{-}T/T_1\text{-}T_0$) against the Fourier number ($X = \alpha t/ x_1^2$). The Gurney-Lurie chart plots the dimensionless position ($n = x/x_1$; $n = 0$ at the center) and the 1/Biot number ($m = k/hx_1$) as parameters. In our study, Gurney-Lurie charts were used assuming that the sample shape was an infinite slab.

T_1- water bath temperature, °C,

T - temperature at location x at time t, °C,

T_0 - initial temperature, °C.

α - thermal diffusivity, m^2/s,

t - time, s,

x - distance from center to location where temperature T was reached at time t, ,

x_1- distance from center to the surface, m,

k - thermal conductivity, W/m-°C,

h - heat transfer coefficient, W/m^2-°C.

Validation

A single layer package of surimi seafood sticks with an initial temperature of 5°C was heated at 90°C for 45 min. The thermocouple was inserted 3 mm away from the center. The same variables were used for the temperature profile simulation. Double layered surimi seafood sticks with an initial temperature of 7°C were heated at 90°C for 100 min. The thermocouple was inserted 7 mm from the center. The simulation was obtained by entering those values into the spreadsheet. A package consisting of a triple layer of surmi seafood sticks with an initial

temperature of 3°C was heated at 90°C for 170 min. The thermocouple was inserted 2 mm from the center. The simulation was obtained by inputting those values into the spreadsheet.

RESULTS AND DISCUSSION

The heat transfer coefficient (h) was demonstrated as a function of temperature difference between the sample surface and hot water (ΔT). The heat transfer coefficient exists at the interface between the heating medium (water) and the heated sample (Geankoplis 1993). It acts as a protective thin film, slowing convective heat penetration. The h was particularly an effective barrier when ΔT was large, especially (Fig. 1.1) at the beginning of heating. The h was also plotted as a function of time (data not shown). This confirmed that h was crucial for the first minute of heating. The heat transfer coefficient (h) was also an important factor that influenced convective heat migration from the heating medium to the sample surface. For this reason, it is referred to as film/surface resistance.

The convective heat transfer coefficient is a parameter that is very specific to the heating system (Su and others 1999, Toledo 1999). Therefore, for accurate temperature predictions, h has to be determined for the system in which the temperature prediction is desired. Also, comparison of h for different systems may be erroneous (Su and others 1999). Factors, such as system geometry, fluid properties, flow velocity, and temperature difference, will contribute to an error in predictions if h has not been determined for that particular system (Geankoplis 1993).

Su and others (1999) determined h for surimi paste. They compared three methods for h determination and concluded that the inverse method gave the most accurate results for their experimental set-up. In our studies, the lumped mass method was used due to the ease in determination of h. Our results were slightly

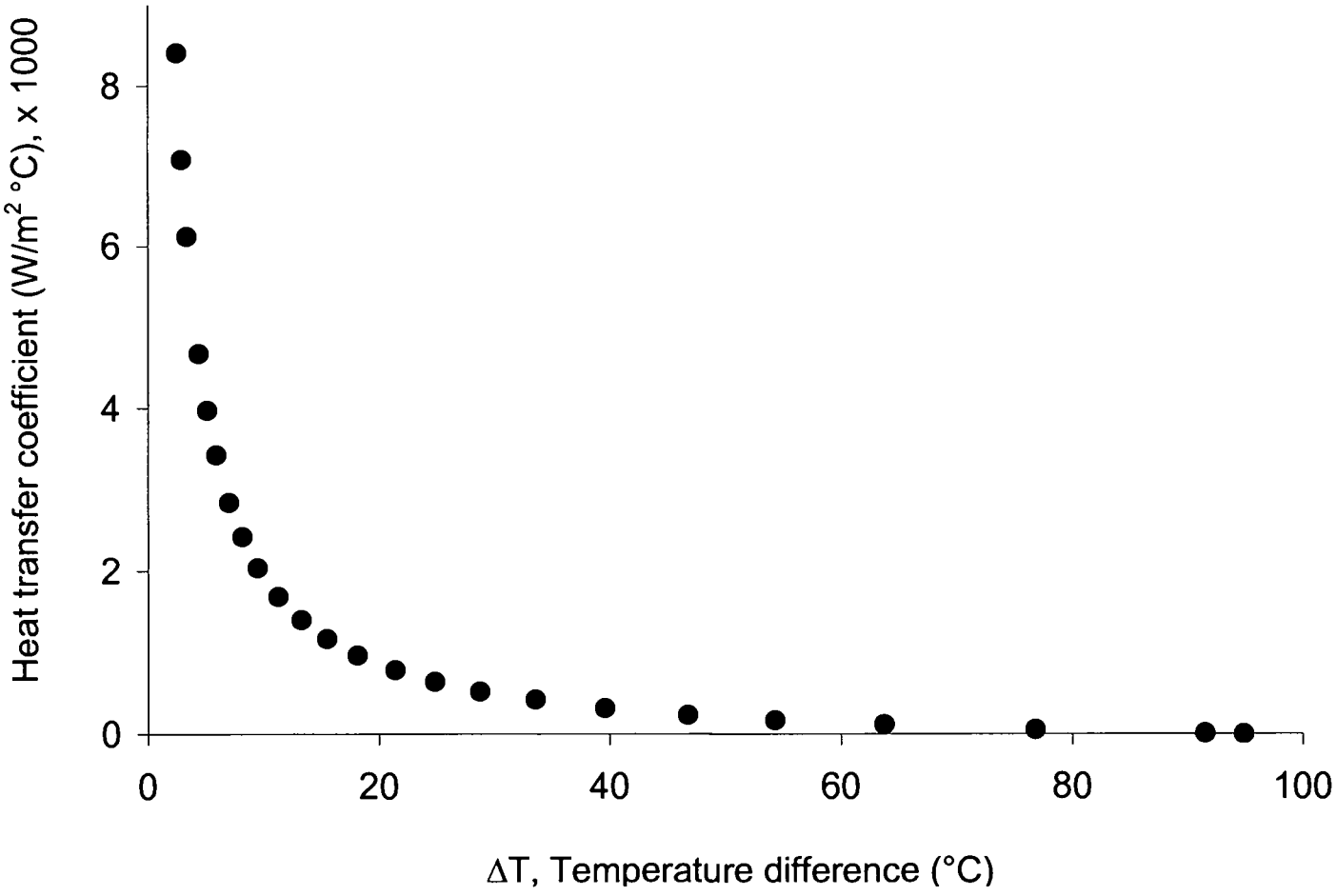

Figure 1.1: Heat transfer coefficient as a function of temperature difference

different, most likely due to the different method, sample, and heating medium. However, h estimated by the lumped mass method resulted in an accurate temperature prediction. Zhang and Cavalieri (1991) reported that h on the surface of green beans slowed heat transfer for the first 150 s of the heating period. When our data for h estimation was plotted against time (data not shown), a similar trend was observed.

Thermal diffusivity (α) as a function of temperature difference (ΔT), calculated using the heat transfer coefficient (h), is shown in Fig. 1.2. In our study, α was determined from the experimental temperature profile when the thermocouple was placed in the geometric center (Singh 1982). For the determination of α, the heat transfer coefficient (h) previously determined was applied. The larger the ΔT, the higher the α (Fig. 1.2). Therefore, heat penetration by conduction was very fast at the beginning. As the sample temperature approached the water temperature (low ΔT), α decreased rapidly, thus slowing heat conduction and contributing to a slower overall temperature increase. At the beginning of the heating period, h slowed the overall heat penetration. However, once the heat overcame the protective layer of h, high α contributed to very fast heat conduction. Conversely, as the heating was almost complete (low ΔT), h was high, contributing to the fast convective heat transfer. However, due to low α (slow conduction), the overall heat conduction was slower.

Huang and Mittal (1995) determined α for meatballs during boiling to be 1.6×10^{-7} m^2/s. These results are slightly higher than ours. However, they did not estimate α as affected by temperature. In addition, meatballs were shaped as spheres, which may have affected heat transfer differently. Muzilla and others (1990) determined that thermal diffusivity was affected most by heat capacity, thermal conductivity, and moisture content in pork/soy mixtures. They estimated α ranging from $1.14 - 2.01 \times 10^{-7}$ m^2/s. Unklesbay and others (1992) estimated thermal diffusivity for beef/corn flour blends, ranging from 0.831 to 1.200×10^{-7}

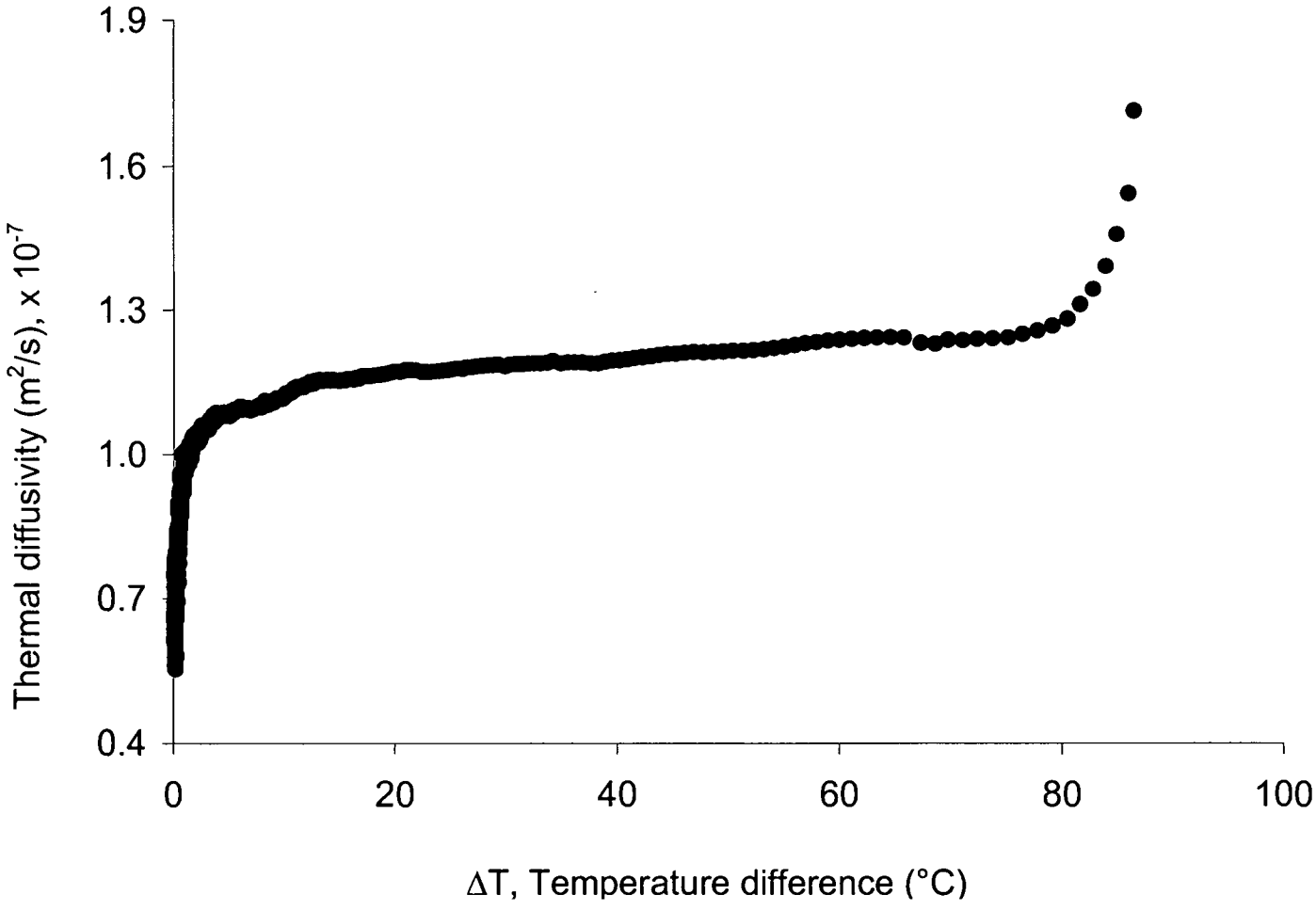

Figure 1.2: Thermal diffusivity of surimi crabsticks as a function of temperature difference

m^2/s during extrusion cooking. These values are very similar to ours, possibly due to the similar composition of the food product (proteins and starch) used.

Fig. 1.3 shows an example output from the temperature prediction model for surimi seafood that was constructed based on the Gurney-Lurie chart with h and α determined as described above. The model allows the input of four variables of a product: processing temperature, processing time, initial temperature, and product thickness. These variables may be changed at any time, resulting in respective changes. In this manner, thermal processing of surimi seafood can be optimized. It may be used to design a new thermal processing method for surimi seafood. It can also be used to verify existing thermal processing methods. However, it needs to be noted that a surface response model (Fig. 1.3) was based on the heat transfer coefficient (h) and thermal diffusivity (α) determined based on our experimental set-up. To apply this model in a different system, the specific h and α must be determined and incorporated into the spreadsheet.

Product thickness is typically determined as one half of the smallest dimension of the product (Geankoplis 1993). Therefore, Fig. 3 represents the temperature prediction for a 1.8 cm thick package. The surface response showed the temperature profile for the entire thermal processing time and also shows the temperature changes that occur at different locations of the package. In this manner, thermal changes that occur in the package can fully be demonstrated.

The surface response model also allows the user to select two locations within the package to determine the temperature profile (Fig. 1.3). Using the two locations [1 mm under the package surface (8 mm from the center) and at the center (0 mm from the center)], the user may determine how the center reaches the desired temperature (90°C) within the thermal processing time (30 min).

Fig. 1.4 shows comparisons of the simulation with the experimental temperature values. The simulated temperature profiles were obtained by inputting process time, processing temperature, sample size, and initial temperature into the spreadsheet (Fig. 1.3). The correlation coefficient (R^2) between the simulated and

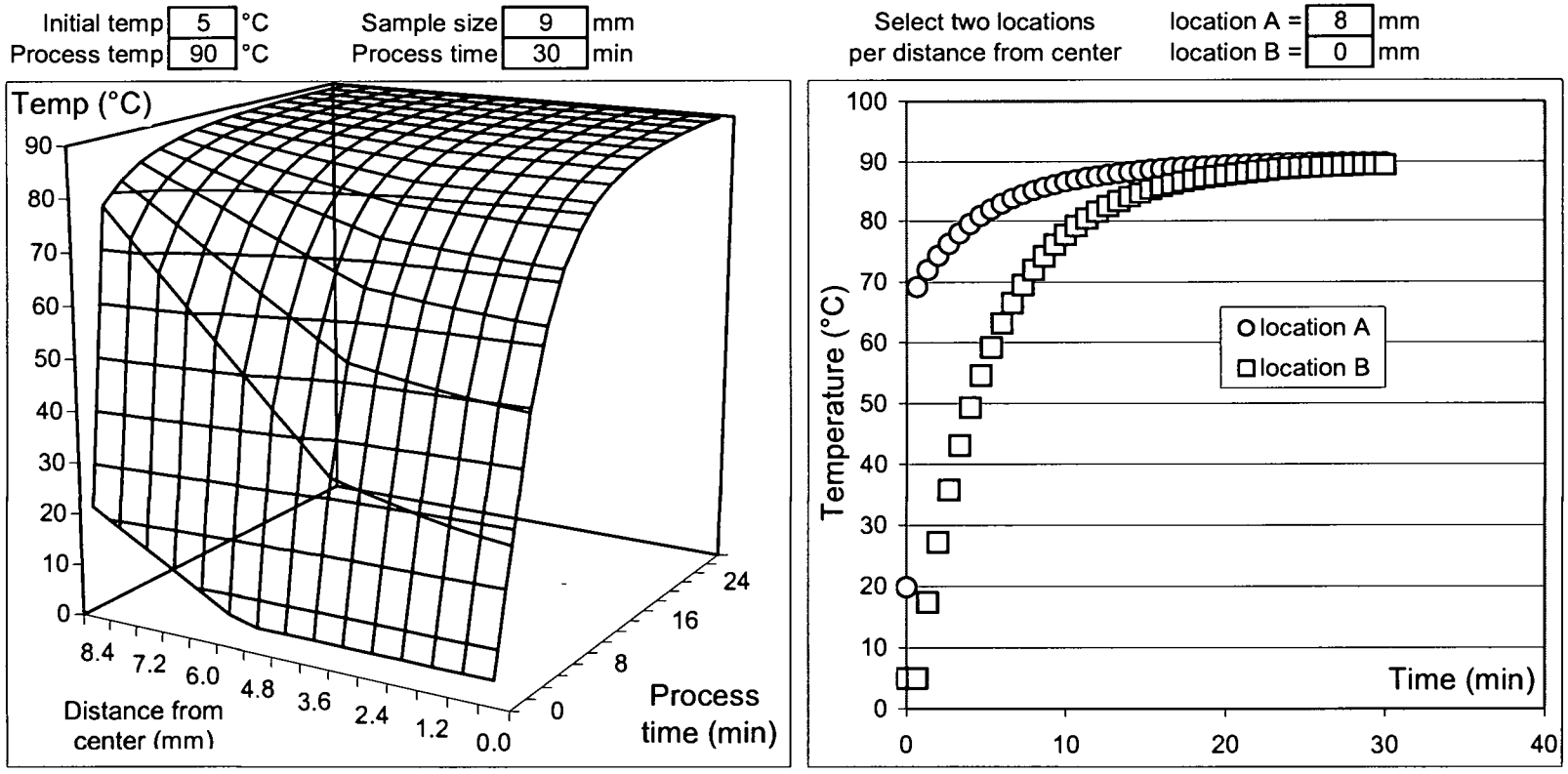

Figure 1.3: Temperature prediction model as affected by various processing parameters

experimental temperature curves was calculated. The R^2 was 0.999, 0.999, and 0.998 for single, double, and triple layer packages, respectively, indicating a good fit between the simulated and experimental values.

Erdogdu and others (1998) developed a mathematical model to predict temperature distribution during the cooking of shrimp. They assumed variable thermal conductivity, specific heat, and density. Our model also incorporates equations to account for the changes of thermal conductivity, specific heat, density, as well as thermal diffusivity, and the conductive heat transfer coefficient. Erdogdu and others (1998) concluded that their model accurately predicted the temperature distribution in shrimp.

Chang and others (1998) employed a finite element method to solve the unsteady state heat transfer equations for the heating of turkeys. They also used the surface heat transfer coefficient determined by transient temperature measurements for that heating system. Chang and others (1998) reported that simulated temperatures were within 1.33, 1.47, and 1.22°C of experimental values.

In our studies, the heat transfer coefficient was also determined for the heating system. Average differences between the experimental and simulated temperatures observed were much lower than in other studies: 0.36, 0.44, and 0.99°C for single, double, and triple layer packages, respectively. In studies with cooking meatballs, Huang and Mittal (1995) reported that the average root-mean-square of deviations between the observed and predicted temperature histories (2-90°C) ranged from 3.0 to 5.1°C.

CONCLUSIONS

Temperature simulation based on the Gurney-Lurie transient temperature charts for heat conduction in an infinite slab was developed for thermal processing of surimi seafood (crabsticks). The spreadsheet, using four variables (processing

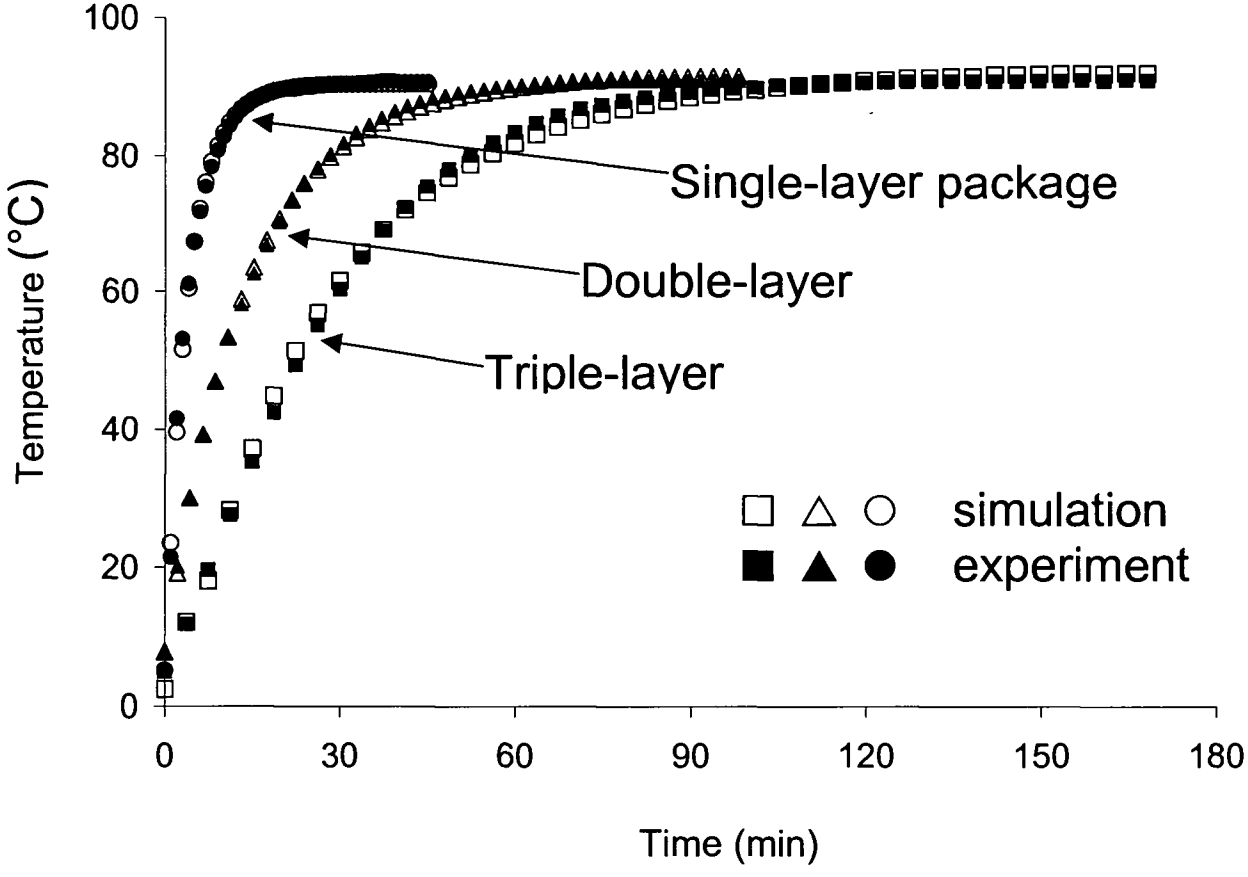

Figure 1.4: Comparison of simulation data with experimental values of surimi crabsticks

time, processing temperature, package size, and the initial temperature of the product),was developed to display the temperature profile as a surface response, as well as a temperature profile at any selected locations inside the package. The validation study demonstrated good agreement between the simulation and experimental temperature profile.

PREDICTIVE MODELS FOR MICROBIAL INACTIVATION AND TEXTURE DEGRADATION IN SURIMI SEAFOOD DURING THERMAL PROCESSING

ABSTRACT

Models for *S. aureus* inactivation and texture degradation during thermal processing of surimi seafood were developed based on the modified thermal-death-time (TDT) concept. The models allow input of various processing parameters, resulting in a surface response that determines microbial inactivation and texture degradation. D-values for microbial inactivation were determined between 55-95°C. The z-value, however, was not linear. Therefore, the polynomial equation was used to calculate the D-values at intermediate temperatures. Texture degradation between 55-95°C followed the power function. Therefore, power "n" was used as the degradation rate for modeling purposes. Polynomial equation was used to calculate "n" at intermediate temperatures.

INTRODUCTION

Pasteurization is a mild or moderate heat treatment with subsequent cooling. The purpose is to eliminate targeted bacterial pathogens and greatly reduce spoilage bacteria, in order to extend product shelf life (Park 2000). Properly designed pasteurization should result in a negligible microbial load of vegetative bacteria (FDA 1998). However, only pasteurization of smoked fish, crawfish tail meat, crabmeat and crabmeat analogs have been studied in detail (Rippen and Hackney 1992).

Sous-vide technology is gaining popularity because the products maintain their fresh-like flavor. This thermal process is milder than traditional pasteurization. Consequently, sous-vide products are generally formulated for desired organoleptic qualities rather than for safety. A drawback is a strict temperature control during distribution (Rhodehamel 1992). Hyytia-Tress and others (2000) found sous-vide processing inadequate for eliminating *Clostridium botulinum* spores. They concluded that the safety of sous-vide products is therefore questionable.

Surimi seafood formulations include ingredients in addition to raw surimi (Park and Lanier 1997). Therefore, the final surimi seafood product may be affected by the quality of these ingredients as well as manufacturing practices (Himelbloom 1997). Yoon and others (1988) reported that just one additional step in manufacturing, cutting to obtain flakes, resulted in a one log increase of the microbial load as compared to un-cut crab leg surimi seafood.

The United States Department of Commerce Guidelines for Pasteurization of the PUFI (Packed Under Federal Inspection) or HACCP for vacuum packed surimi seafood recommends 85°C (internal temperature) for 15 min followed by fast chilling. A water-phase salt level of 2.4% was also recommended to control *C. botulinum*. Microbiological hazards during surimi seafood processing may include pathogen survival, post-processing pathogen contamination, and growth of *C. botulinum*. Therefore, proper pasteurization, cooling, and storage have been suggested as critical control points for surimi seafood manufacture (Park 2000).

Park (1994, 2001) surveyed pasteurization practices in the US surimi seafood industry. He found great variation with regard to the time-temperature regimes. Some treatments may not be appropriate for maintaining microbial safety while others may be abusive with regard to physical properties. In addition, none of the present pasteurization methods have been scientifically validated with regard to microbial inactivation and texture degradation. Consequently, the FDA has recently recalled 16,965 kg of surimi seafood in Washington State (FDA 1998).

The first US incident of product recall occurred as early as 1988 for surimi seafood imported from Japan and distributed to three states (Park 2000).

The FDA established zero tolerance for *L. monocyotogenes* and *Salmonella* in ready-to-eat products. If enterotoxigenic *E. coli* is present at 1 x 10^3/g, the FDA will consider regulatory action. According to FDA's informal guidance, the product may be recalled if tested positive for toxins of *Staphylococcus aureus*, or if 1 x 10^4/g organisms are present (Ward and Price 1992).

S. aureus is associated with fishery products. It grows between 6 - 47.8°C (Eyles 1986, Smith and others 1983). Increased concentrations of *S. aureus* usually indicate cross-contamination and mishandling. *S. aureus* can survive salt concentrations as high as 10%, but competes poorly with other microorganisms. According to Mulak and others (1995), *S. aureus* is theromresistant, making it a good microbial target. Proper thermal processing and distribution under refrigerated conditions, as well as good hygiene of plant workers should prevent contamination. To our knowledge, there have been no studies of *S. aureus* inactivation/texture degradation in surimi seafood over wide temperature range.

Our objectives were to measure the inactivation of *S. aureus* and texture degradation of surimi seafood during thermal processing, and to develop predictive models.

MATERIALS AND METHODS

Microbial inactivation

Samples

Surimi seafood sticks were obtained from Sea Blends (Seattle, WA). The sticks were ground to a paste. The paste was inoculated with equal volumes of six

strains of *S. aureus*, resulting in a 5% inoculum size. The paste was incubated at 37°C for 72 h, resulting in 10^8-10^9 CFU/g. The paste was mixed during incubation to assure uniform distribution of bacteria. After incubation, 12 g aliquots were vacuum packed in plastic bags (18.5 x 10 cm). The paste was uniformly distributed, resulting in a thickness of 0.6 mm. The bags were subjected to various heat treatments, then, rapidly cooled to near 0°C and the survivors were enumerated.

Bacterial enumeration

S. aureus enumeration was performed on staphylococcus 110 agar (Difco Laboratories, Detroit, MI) by a serial 10-fold dilution using the spread plating method. The presence of *S. aureus* was confirmed by gram staining, catalase, and coagulase tests.

Thermal treatment

An eighteen-liter water bath was employed to mimic the industrial pasteurization process. Prior to the experiment, uniform temperature distribution of the water bath was verified. Five temperatures and various times were used: (1) 55°C for 19, 38, 57, 76, 95, and 114 min; (2) 65°C for 50, 100, 150, 200, 250, and 300 s; (3) 75°C for 9, 18, 27, 36, 45, and 54 s; (4) 85°C for 2, 4, 6, 8, 10, and 12 s; (5) 95°C for 1, 2, 3, 4, 5, and 6 s. The experiments were performed in duplicate.

D- and z-values

Survivors were plotted on a logarithmic scale as a function of heating time at each temperature, resulting in a survivor curve. D-value, the decimal reduction time necessary to reduce the population by 90 % (1 log), was calculated as a negative reciprocal of the slope of the survivor curve (Toledo 1991)(Equation 1).

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{D} * t \qquad \text{Eq. 1}$$

N – number of survivors at time t,

N_0 – initial microbial concentration,

D – D-value, decimal reduction time,

t – time.

The D-values were plotted on a log scale as a function of temperature, resulting in a thermal resistance curve. The z-value was calculated as a negative reciprocal of the slope of the thermal resistance curve (Toledo 1991)(Equation 2). However, since the D-value did not follow a linear relationship, the z-value was not used for model purposes. Polynomial regression resulted in a much better fit. Therefore, the polynomial equation was used in the model calculation to estimate the D-value at intermediate temperatures. Microsoft Excel was used for the calculations.

$$\log(D) = -\frac{1}{z} * T \qquad \text{Eq. 2}$$

D – D-value at temperature T,

z – z-value,

T – temperature.

Model development

Temperature of surimi seafood during thermal processing was simulated by a model described by Jaczynski and Park (2002) based on the Gurney-Lurie charts. The D-value and polynomial equation describing the temperature dependence of the D-value (instead of z-value) were used in the development of a microbial inactivation model. Microsoft Excel was used for the calculations.

Validation study

Ground surimi seafood sticks were placed on plastic trays (15 and 30 mm deep) and inoculated with six strains of *S. aureus*. Samples were incubated at 37°C to reach a concentration of 10^9 CFU/g. The samples were then vacuum-packed and subjected to heat treatment (75, 85, and 95°C) for various time periods. Immediately following the treatment, the packages were chilled in ice slush and the survivors were enumerated. The results from the model calculations were compared to the experimental values.

Texture Degradation

Samples

Un-pasteurized surimi seafood sticks were un-bundled to a thin sheet. The thickness of the sheet was below 1 mm. The sheets were vacuum packed in plastic bags. The bags were subjected to various heat treatments. They were then rapidly chilled to near 0°C. The sheets were re-bundled to form sticks and measured for texture.

Thermal treatment

The same eighteen-liter water bath as described in microbial inactivation was used. Five temperatures and various times were used: (1) 55°C for 6, 12, 18, 24, 30, 36, 42, 48, 54, and 60 min; (2) 65°C for 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 min; (3) 75°C for 4, 8, 12, 16, 20, 24, 28, 32, 36, and 40 min; (4) 85°C for 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 min; (5) 95°C for 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 min. The experiments were performed in duplicate.

Texture measurement

A Warner-Bratzler shear device attached to the Sintech 1/G (MTS, Cary, NC) was set up to measure shear force (g). Whole-stick samples (1.9 cm diameter) were equilibrated to room temperature before testing with the shear device. At least six measurements per sample were taken.

Indices of degradation rate

Texture degradation did not follow a first order reaction. Instead, texture degraded at the tested temperatures according to the power function. Therefore, power "n" was estimated using the Microsoft Excel solver function and residual sum of squares technique (Ramsey and Schaffer 1997). Polynomial regression resulted in a best fit to describe the temperature dependence of "n" and was further used to estimate "n" at intermediate temperatures.

Model development

Temperature of surimi seafood during thermal processing was simulated by a model described by Jaczynski and Park (2002) based on the Gurney-Lurie charts. The power "n" and polynomial equation describing the temperature dependence of "n" (instead of D- and z-values) were used to develop the texture degradation model. Microsoft Excel was used for the calculations.

Validation study

Commercial surimi seafood sticks placed on 15 mm plastic trays and vacuum packed in a plastic film were subjected to heat treatment (75, 85, and 95°C) for various time periods. Immediately following the treatment, the packages were chilled in ice slush, equilibrated to room temperature, and measured for texture. The results from the model calculations were compared to the experimental values.

RESULTS AND DISCUSSION

Microbial inactivation

Microbial inactivation rates

Survivor curves are shown in Fig. 2.1 (95, 85, and 75°C) and Fig. 2.2 (65 and 55°C). The correlation coefficients (R^2) for the linear regression of survivors (on logarithmic scale) vs. time were 0.99, 0.99, 0.98, 0.99, and 0.99 at 55, 65, 75, 85, and 95°C, respectively. Therefore, survivor curves were assumed to be linear.

The D-values were calculated based on the negative reciprocal of the slope of the survivor curves (Table 2.1). The lowest D-value was 0.65 s at 95°C, indicating that *S. aureus* was readily inactivated at this temperature. However, at 55°C it took over 16 min to decrease the concentration of *S. aureus* by 90 % (1 log).

Table 2.1: Thermal D-values for *S. aureus* in surimi seafood

Temperature (°C)	D-value (s)
95	0.65
85	1.53
75	6.52
65	49.46
55	971.54

Mulak and others (1995) determined D_{70} = 5.1, 1.0, and 4.1 s for *S. aureus* in phosphate buffer, fish fillets, and fish terrine, respectively. The low D-value in fish fillets as compared to D-value in phosphate buffer may be attributed to the lower pH of the fillets (pH = 6.2-6.5, Lanier 2000) than the phosphate buffer (pH = 7.2) and also to the residual salt content in the fillets. Our studies, though, did not include survival tests at 70°C. Instead, based on the polynomial relationship found in our studies between the log D-value and temperature (55-95°C temperature range, R^2 = 0.9999), D_{70} was estimated to be 15.6 s. This value is higher than the D_{70} reported by Mulak and others (1995). Slower inactivation in surimi seafood as compared to phosphate buffer (Mulak and others 1995) might be due to the protective effect of the fish protein, starch, sugars, lipids, and sorbitol which are typical ingredients in a surimi seafood formulation. Also, the three dimensional gel structure of surimi seafood might have contributed to the protection of microbial

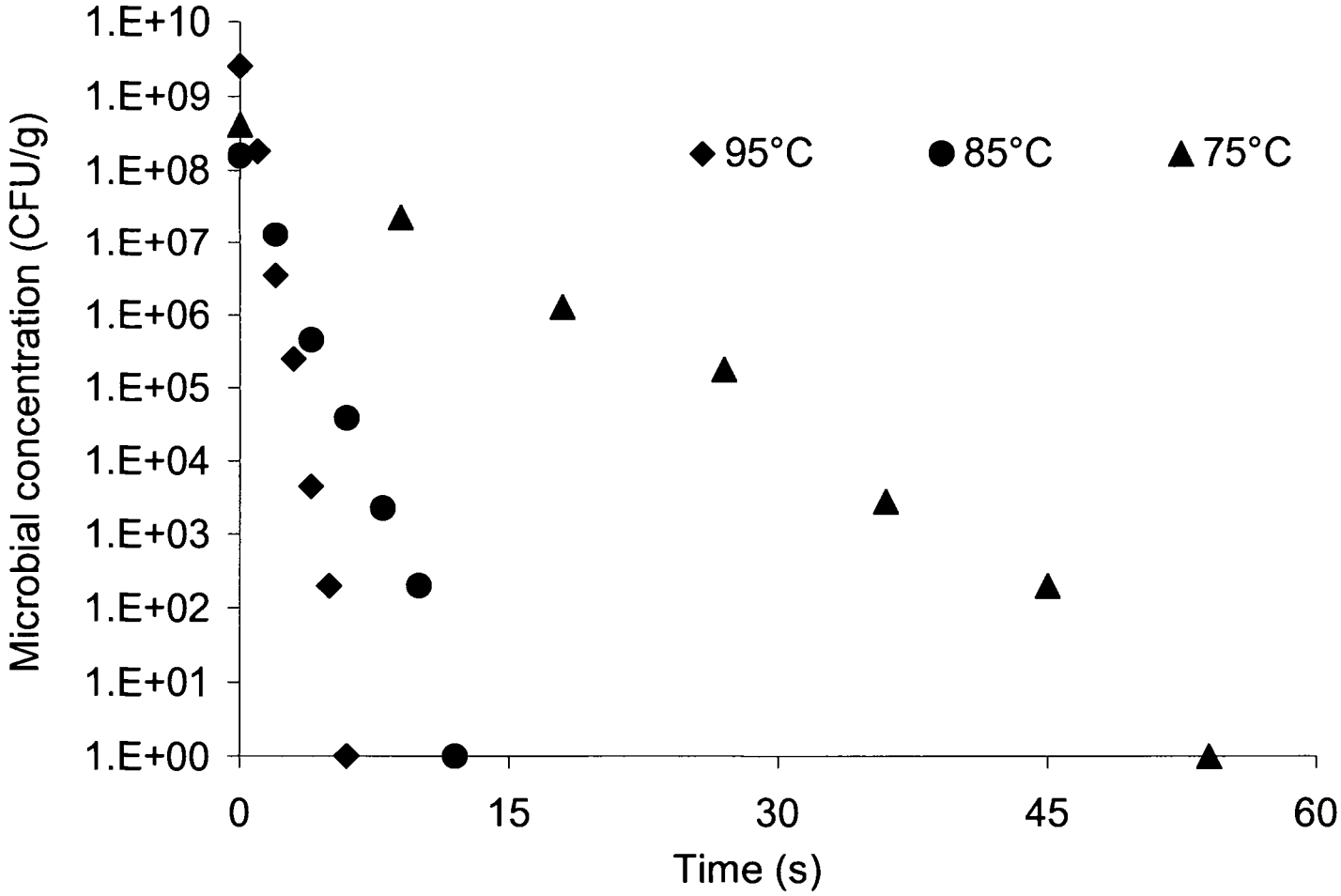

Figure 2.1: Survivor curves for *S. aureus* in surimi seafood at 95, 85, and 75°C

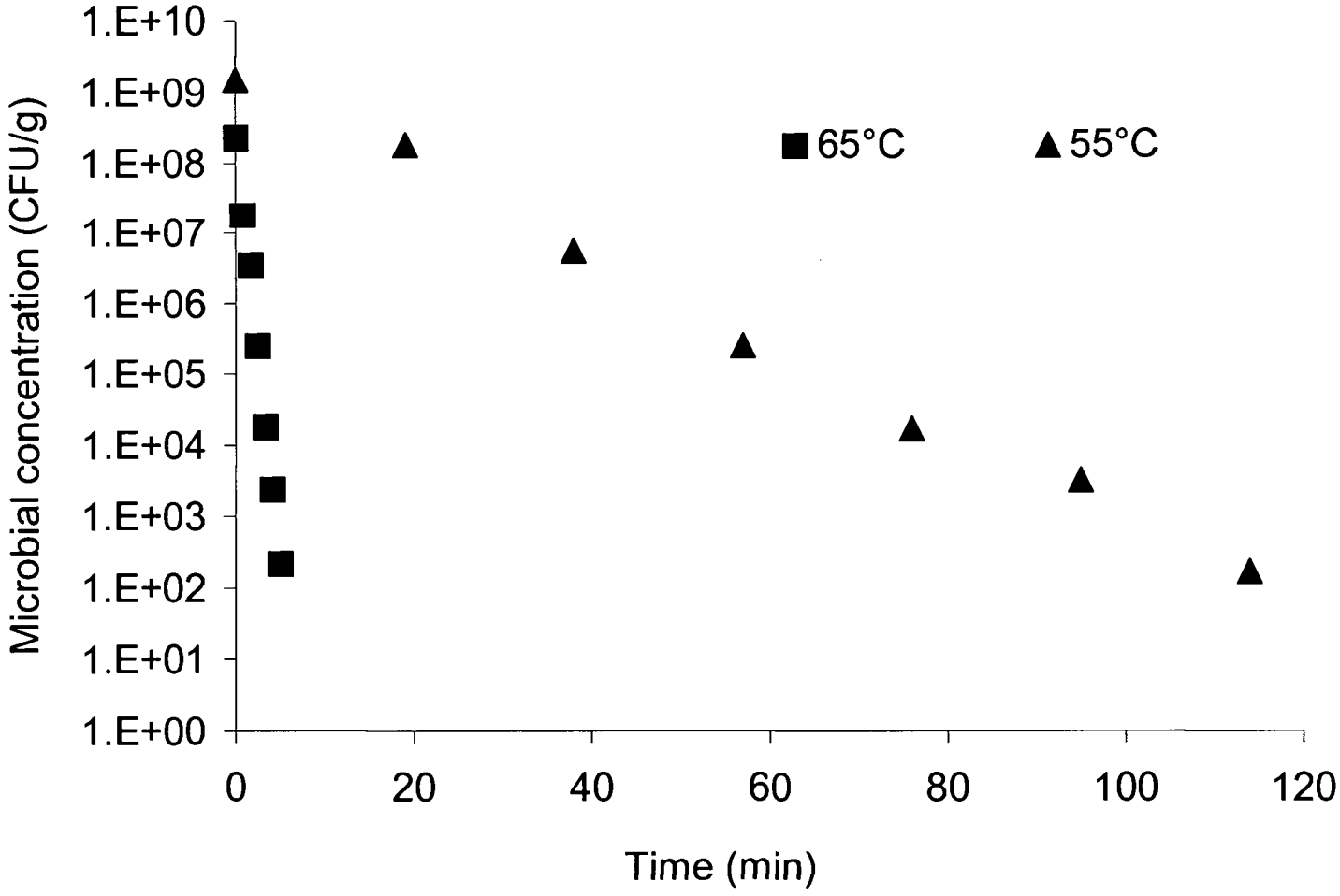

Figure 2.2: Survivor curves for *S. aureus* in surimi seafood at 65 and 55°C

cells from heat. In addition, six strains of *S. aureus* were used in our studies. Most likely, the most resistant strain exhibited the best survival. Therefore, a higher D-value was observed in our studies than that observed by Mulak and others (1995). Thayer and Boyd (1992) also observed higher resistance of a mixture of four strains of *S. aureus* than a single strain in mechanically de-boned chicken meat.

According to the International Commission on Microbiological Specifications for Foods (ICMSF 1996) D_{60} is 6.0 min for *S. aureus* in meat macerate. We did not perform tests at 60°C. However, based on D_{55} and D_{65} determined as 16.2 and 0.8 min, respectively, the estimated D_{60} was 3.15 min. It seems that meat macerate might have compounds that better protect microorganisms from heat than surimi seafood. In addition, 2 % salt is commonly added to surimi seafood formulations. Salt might have increased the stress to which microorganisms were subjected to during heating, resulting in faster inactivation. The ICMSF (1996) also indicated $D_{60} = 3 - 40$ min ($a_w = 0.92 - 0.80$, respectively) for *S. aureus* in pasta (semolina-egg dough). The D_{60} at $a_w = 0.92$ was similar to the D-value obtained in our studies, suggesting that inactivation of *S. aureus* in high moisture pasta and in surimi seafood may be similar. However, a_w of surimi seafood in our studies was not measured.

The z-value is normally calculated as a negative reciprocal of the slope of the D-value (on logarithmic scale) vs. temperature. The fundamental assumption of the z-value calculations is based on the linearity between the D-value (on logarithmic scale) and temperature (Toledo 1991, Geankoplis 1993). The equations based on this semilogarithmic plot are referred to as the thermal-death-time (TDT) model equations and the plot is referred to as thermal resistance curve. The z-value in the TDT model describes the thermal dependence of the microbial inactivation rate (D-value). This relationship allows the calculation of D-values at intermediate temperatures within the experimental temperature range. The thermal resistance curve for *S. aureus* in surimi seafood is shown in Fig. 2.3. Linear regression for the temperature range between 55–95°C yielded R^2=0.95 (Eq. 3).

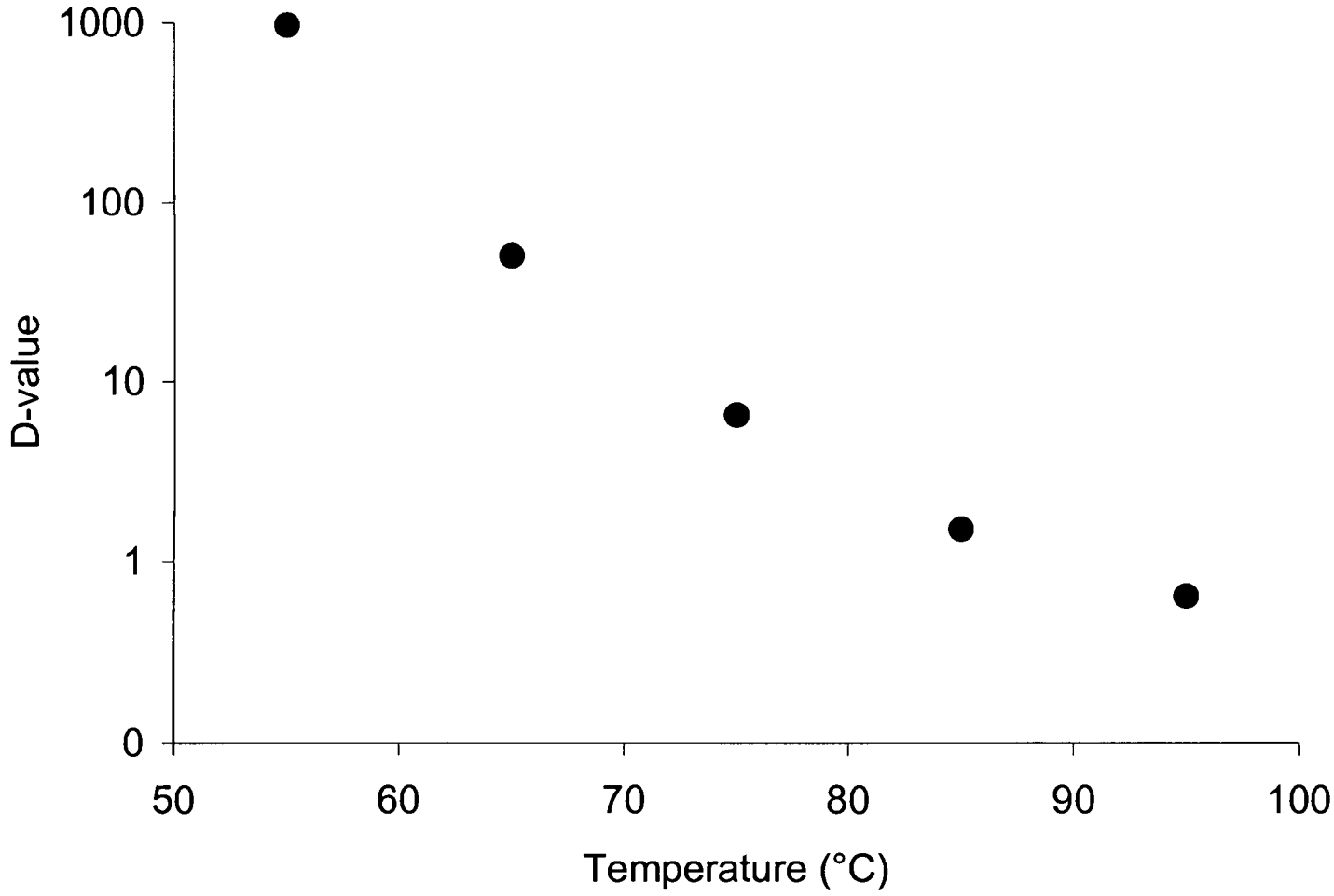

Figure 2.3: Thermal resistance curve of *S. aureus* thermally inactivated (55 – 95°C) in surimi seafood

$$\log(D-value) = -0.0786 * T(°C) + 6.9948 \qquad \text{Eq.3}$$

Eq. 3 was used to simulate D_{55}, D_{65}, D_{75}, D_{85}, and D_{95} resulting in 469.68, 76.88, 12.58, 2.06, and 0.34 s., respectively. Comparison with experimental D-values (Table 2.1) revealed approximately a two-fold difference between the experimental and simulated D-values. Therefore, it was assumed that the thermal resistance curve was not linear and the z-value was neglected.

Linear regression for D-values between 55–75°C resulted in R^2=0.99. The z-value for that temperature range equaled 9.2°C. ICMSF (1996) reported the z-value for *S. aureus* in milk is equal to 9.5°C. This value is similar to the z-value found in our studies between 55–75°C. Mulak and others (1995) reported z-value = 8.7, 5.2, and 6.3°C for *S. aureus* in phosphate buffer, fish fillets, and fish terrine, respectively. However, they calculated the z-value for a very narrow temperature range (50 – 58°C). Therefore, comparison between our values and theirs may be erroneous. Our results suggested that the thermal resistance curve for inactivation of *S. aureus* in surimi seafood did not show good linearity between 55 – 95°C.

The D-values (on logarithmic scale) were also plotted as the reciprocal of the absolute temperatures (similar to Arrhenius approach). However, the R^2 for the linear regression was equal to 0.97. The equation is shown below (Eq. 4).

$$\log(D-value) = 9499.1 * \frac{1}{T(°K)} - 26.321 \qquad \text{Eq. 4}$$

Eq. 4 was used to predict D_{55}, D_{65}, D_{75}, D_{85}, and D_{95} resulting in 534.85, 73.49, 11.32, 1.94, and 0.36 s., respectively. Comparison with the experimental D-values (Table 2.1) showed a better fit than Eq. 3, even though it was also unsatisfactory. Thayer and Boyd (1995) also used the reciprocal of the absolute temperatures to determine the D-value of microbial inactivation. They found a similar correlation

with $R^2 = 0.953$. However, in order to obtain the D-value from such equation it is necessary to back-convert log(D-value) to D-value.

The cubic polynomial equation was fitted to the thermal resistance curve (Eq. 5). This resulted in a satisfactory fit, yielding $R^2 = 0.9999$.

$$\log(D-value) = -0.0000126 * T^3 + 0.0044 * T^2 - 0.52 * T + 20.069 \qquad \text{Eq. 5}$$

This equation was also used to simulate D_{55}, D_{65}, D_{75}, D_{85}, and D_{95} resulting in 0.71, 1.59, 6.13, 48.60, and 940.52 s., respectively. These estimates were close to the experimental D-values (Table 2.1). Therefore, Eq. 5 was used for model calculations to estimate the D-values at various temperatures within the experimental temperature range (55-95°C).

Surface response model for inactivation of *S. aureus*

The temperature prediction model (Jaczynski and Park 2002) was used to estimate temperatures at any time and location of the surimi seafood sample. The cubic polynomial equation (Eq. 5) was used to calculate the D-values at those intermediate temperatures, which were between 55 – 95°C. Smith and others (1983) reported that *S. aureus* can be grown at up to 48°C. Therefore, it was assumed that inactivation of *S. aureus* below 55°C was negligible.

The inactivation model allows inputting five variables: product size, initial product temperature, processing time, processing temperature, and initial microbial concentration (Fig. 2.4). Based on the first four input variables (product size, initial product temperature, processing time, and processing temperature), the temperature prediction model estimates temperatures, which are subsequently used by Eq.5 to determine the D-values. The estimated D-values, in combination with process time and microbial concentration (input variables), are used to calculate the microbial

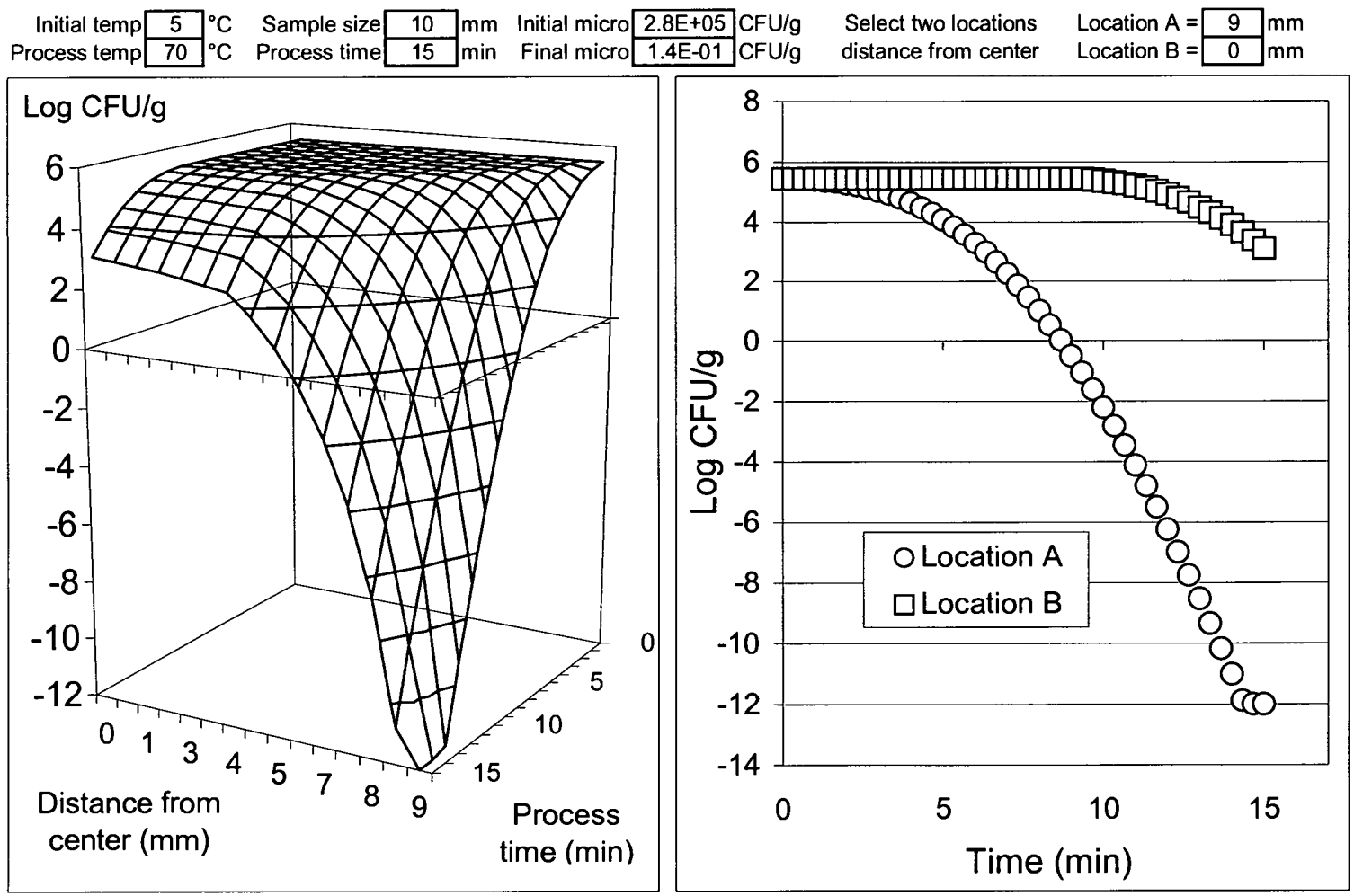

Figure 2.4: Microbial inactivation model as affected by various processing parameters

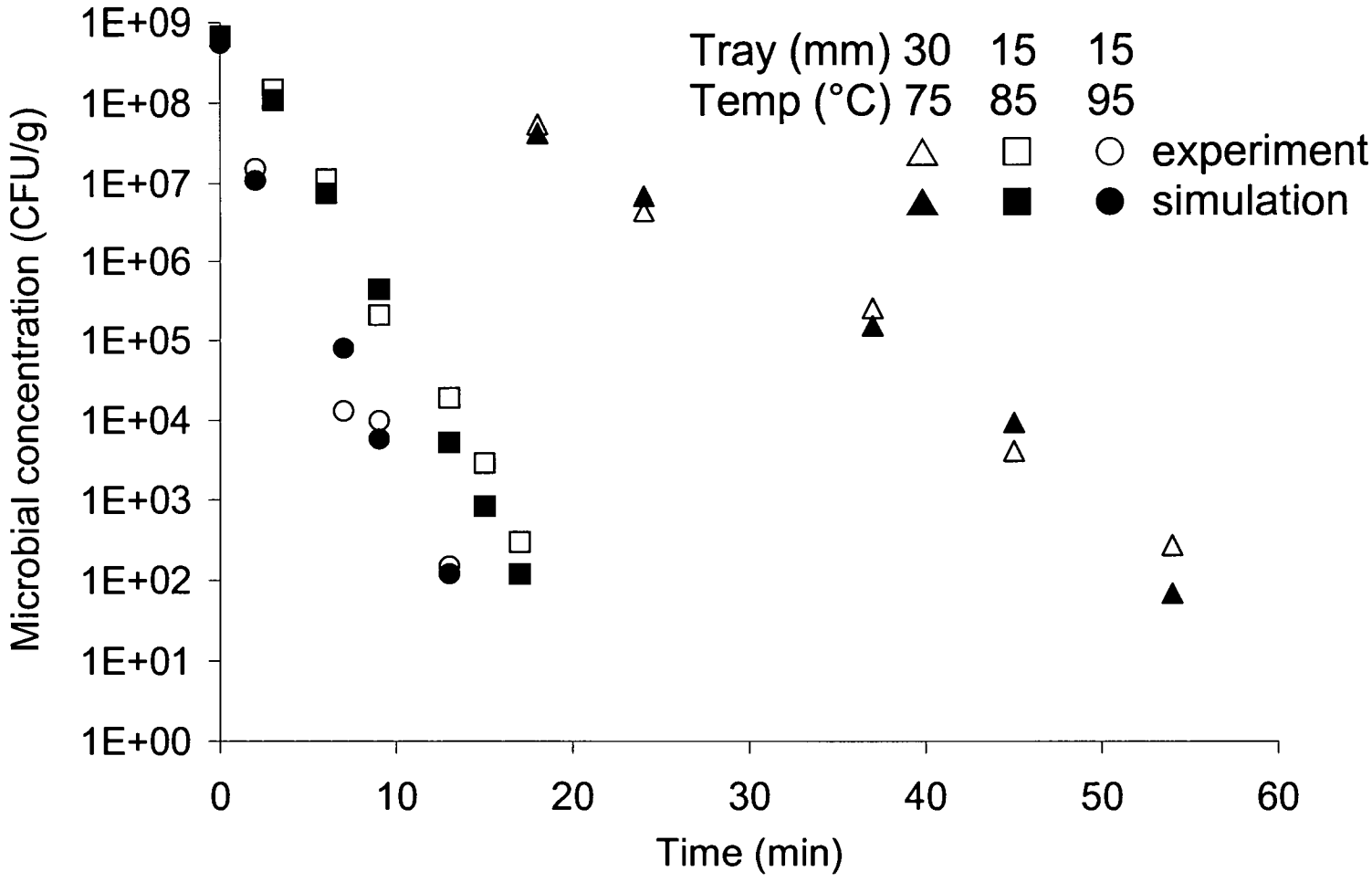

Figure 2.5: Comparison of simulated microbial inactivation with experimental values

concentration at any location of the product at any time of the process. The model also calculates the average microbial concentration after the surimi seafood package (2 cm thick, 5°C initial temperature, initial micro population of *S. aureus* of 2.8 x 10^5 CFU/g) was heated in a water bath at 70°C for 15 min. Fig. 2.4 demonstrates the change in microbial load. Model simulations were compared with experimental data (Fig. 2.5). The simulated values were within a 1-log concentration difference from experimental values. It must be noted that the model was based on the laboratory data for inactivation of *S. aureus* in surimi seafood in a water bath (55-95°C). Commercial settings could give different simulation results.

Texture degradation

Texture degradation rates

Texture degradation, unlike microbial inactivation, did not show a linear relationship, but followed the power function at every temperature tested (Fig. 2.6 and 2.7). Therefore, instead of the D-value, which assumes linearity of microbial iactivation (on log scale) to time, power "n" was estimated and used as the degradation rate (Table 2.2).

Table 2.2: Thermal degradation rate (power "n") of surimi seafood texture

Temperature (°C)	Power "n"
95	-0.207
85	-0.163
75	-0.129
65	-0.123
55	-0.115

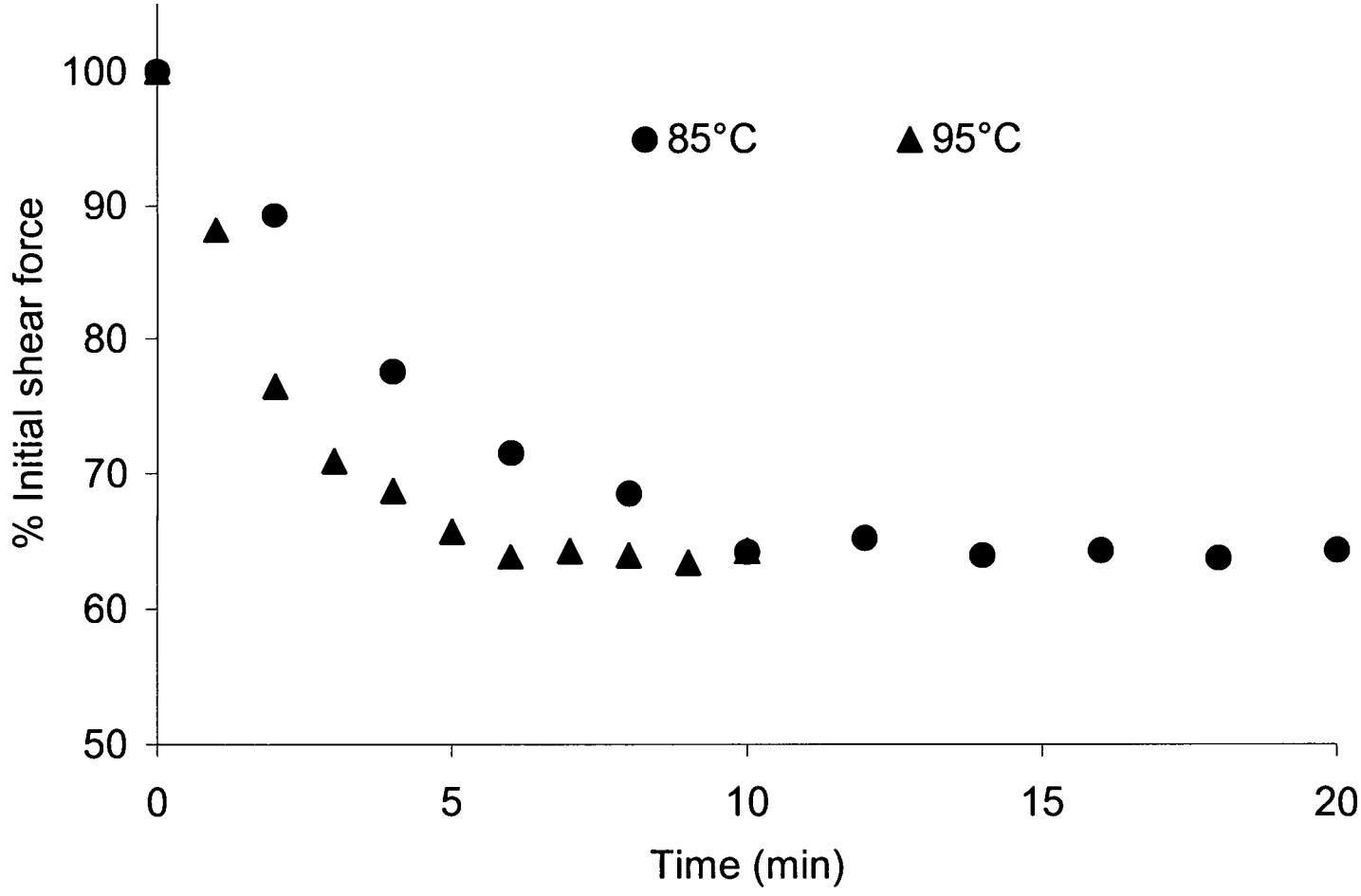

Figure 2.6: Thermal degradation of surimi seafood texture at 95 and 85°C

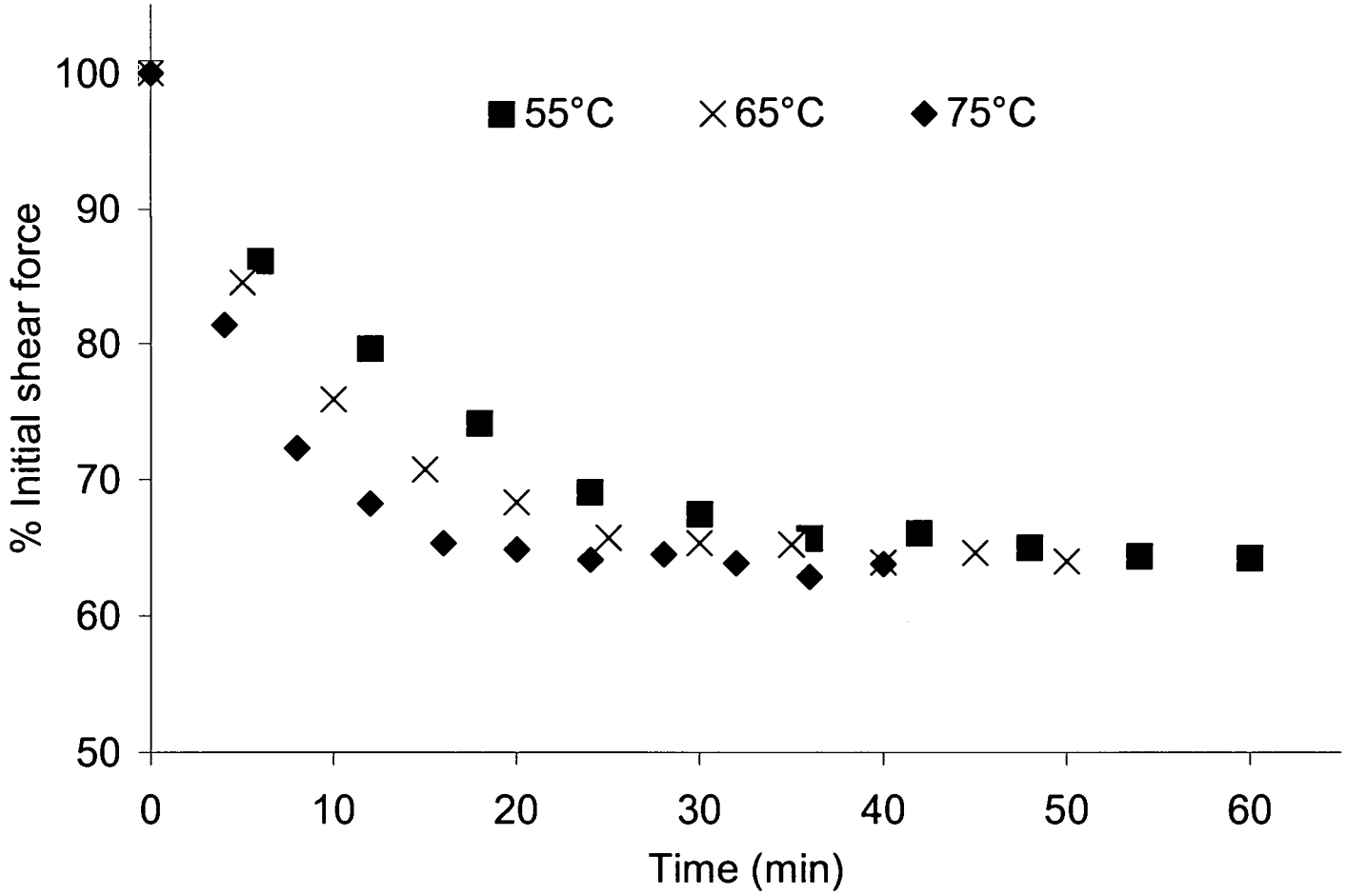

Figure 2.7: Thermal degradation of surimi seafood texture at 75, 65, and 55°C

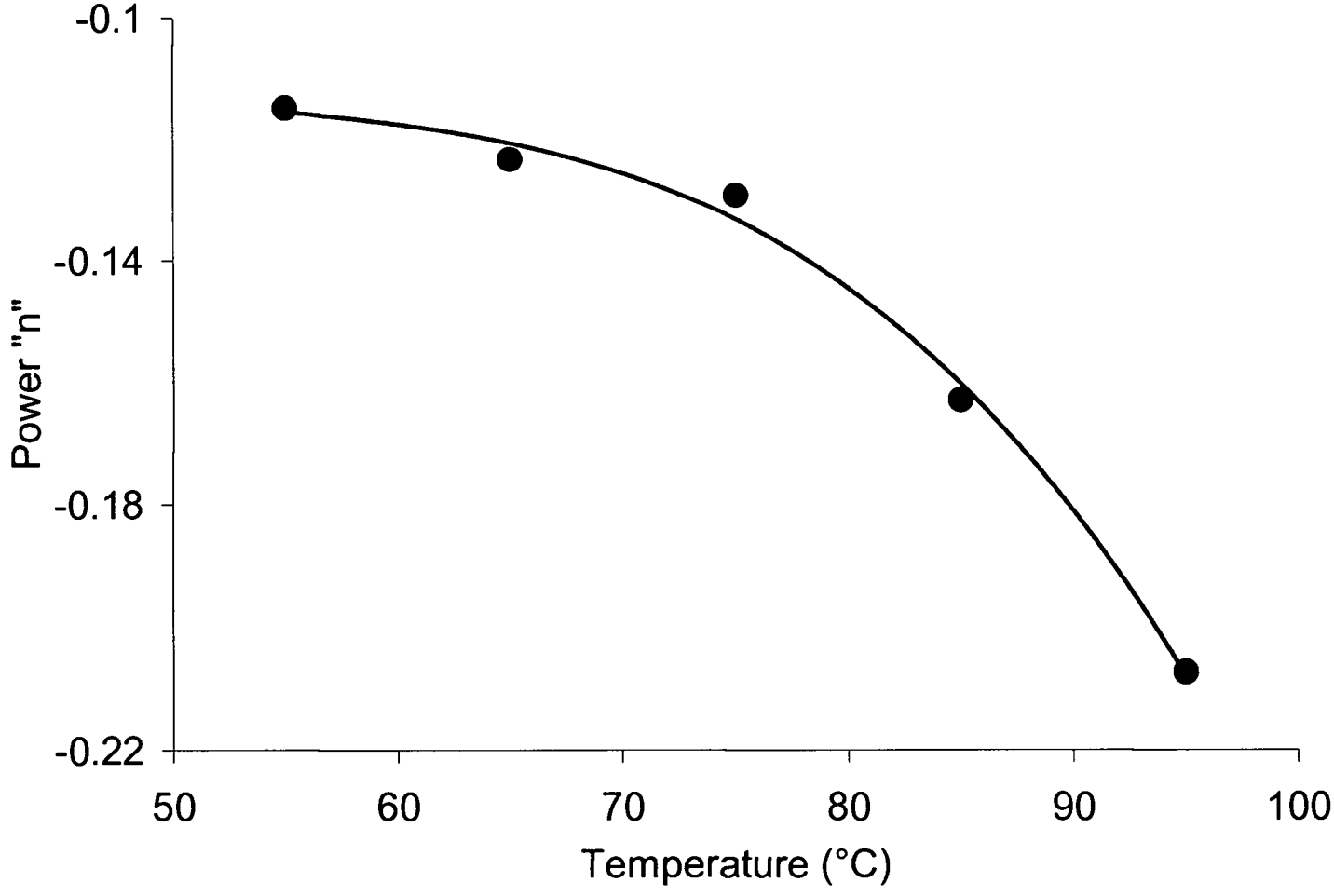

Figure 2.8: Thermal dependence of texture degradation rate (power "n")

In order to calculate power "n" at intermediate temperatures, a polynomial equation (Eq. 6) describing the thermal dependence of power "n" was used (Fig. 2.8).

$$Power"n" = -0.00000134 * T^3 + 0.000209 * T^2 - 0.01 * T + 0.07 \qquad \text{Eq. 6}$$

Shear force has been related to gel firmness (Kim and Park 2000, Montejano and others 1985). Shie and Park (1999) reported that excessive heating (93°C for 30 min) caused decrease in shear stress of surimi gels. Bertak and Karahadian (1995) also tested texture of surimi crabstick under various thermal treatments. It is difficult to compare our data and these literature reports that did not account for heat transfer. However, in general a decreasing trend of texture firmness was confirmed.

Surface response model for texture degradation

The temperature prediction model (Jaczynski and Park 2002) was used to estimate temperatures at any time and location of the surimi seafood sample. The polynomial equation (Eq. 6) was used to calculate power "n" at those intermediate temperatures. The texture degradation model allows the input of four variables: product size, initial product temperature, processing time, and processing temperature (Fig. 2.9). Based on the input variables, the temperature prediction model estimates temperatures, which are subsequently used by Eq.6 to determine power "n" values. The estimated power "n" values with process time, are used to calculate texture degradation at any location of the product and at any time of the process. The model also calculates the average texture degradation at the end of the process. Fig. 2.9 demonstrates the textural degradation when the surimi

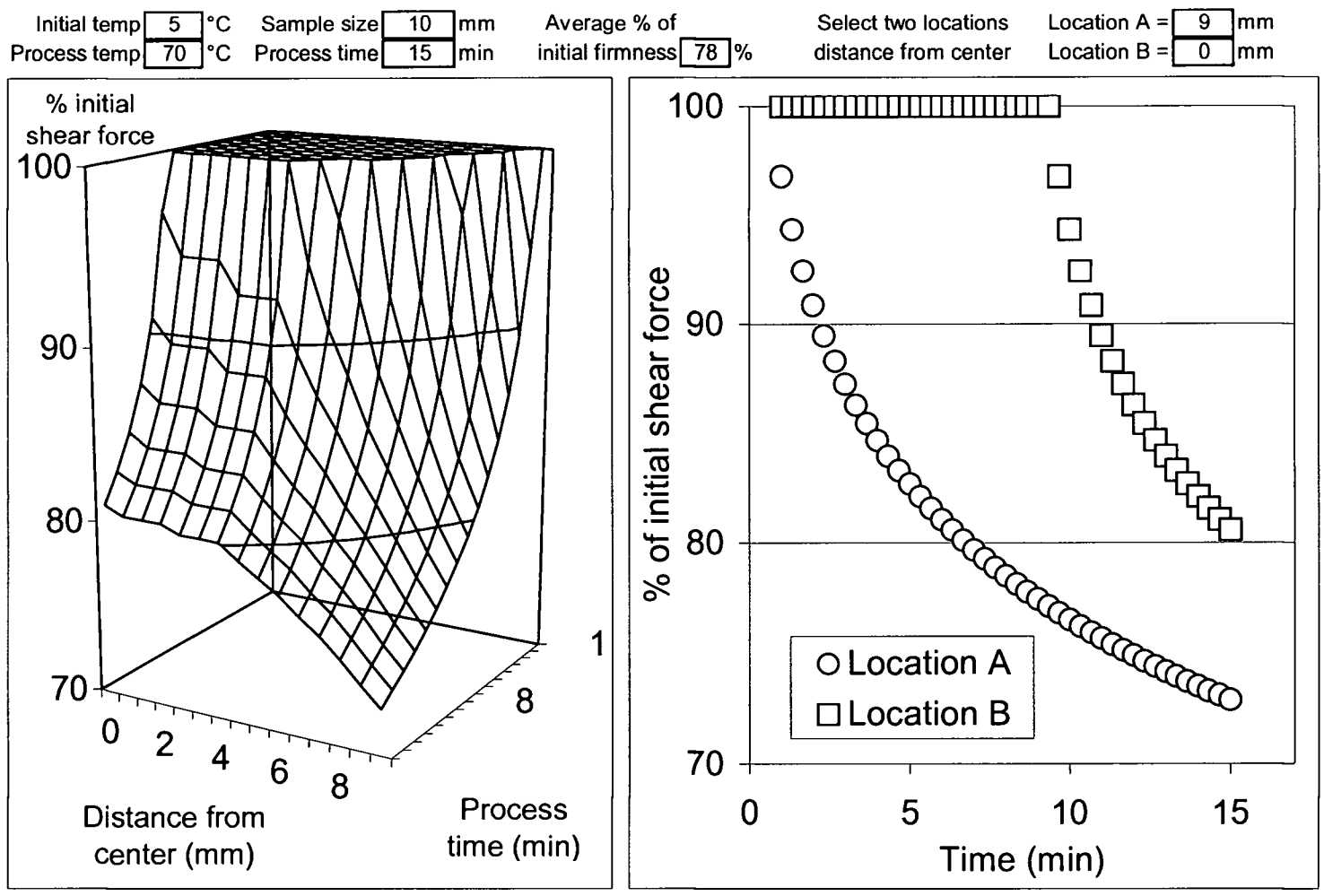

Figure 2.9: Thermal texture degradation model as affected by various processing parameters

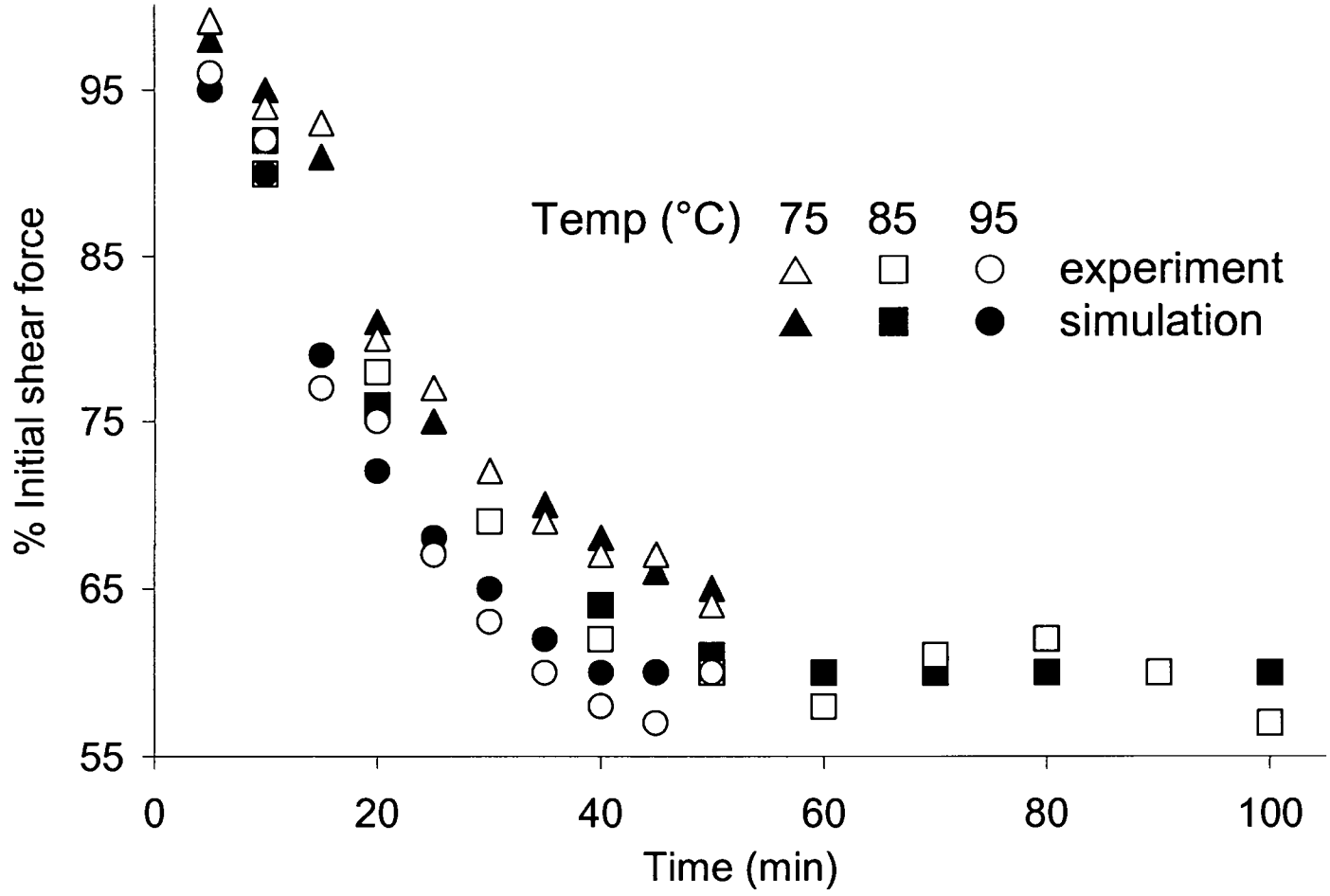

Figure 2.10: Comparison of simulated thermal texture degradation with experimental values

seafood package (2 cm thick, 5°C initial temperature) was heated in water bath at 70°C for 15 min.

Model simulations were compared with experimental data (Fig. 2.10), showing a good fit. The correlation coefficients (R^2) between the simulation and experiments were 0.99, 0.97, and 0.98 at 75, 85, and 95°C, respectively. It must be noted that the model was based on the laboratory data for texture degradation of commercial surimi seafood in a water bath. Commercial settings could give different simulation results.

PHYSICHOCHEMICAL PROPERTIES OF SURIMI SEAFOOD AS AFFECTED BY ELECTRON BEAM AND THERMAL PROCESSING

ABSTRACT

Physicochemical changes of surimi seafood under E-beam and thermal processing were investigated. Thermal processing caused color browning and texture softening. E-beam (up to 4 kGy) deteriorated neither color nor texture when the sample temperatures were between 5 and 23°C. However, firmer texture was measured when samples were treated by E-beam while frozen. None of the treatments changed rancidity of surimi seafood within the parameters tested. Thermal processing, as compared to E-beam, generated the same head-space volatiles, however, to a higher extent. E-beam was determined to be an excellent pasteurization alternative for surimi seafood in terms of retention of fresh-like physicochemical attributes.

INTRODUCTION

The surimi seafood industry currently uses either hot water or steam as a thermal processing medium for surimi seafood (i.e., crabmeat analog)(Park 2000). Traditionally, thermal processing entails detrimental physicochemical changes (Bertak and Karahadian 1995). It is feasible though to find a point at which the minimum loss of physicochemical properties and the maximum effects of microbial inactivation are simultaneously provided. However, the loss of physicochemical properties is common for thermally processed surimi seafood (Shie and Park 1999).

Starch and myofibryllar fish proteins have to gel in order to develop proper texture. It takes up to 20 min at 90°C for most of the starches and proteins to gel (Park 2000). To assure microbial safety, the United States Department of Commerce guidelines for pasteurization of the PUFI (Packed Under Federal Inspection) or HACCP for vacuum-packed surimi seafood recommended 85°C (internal temperature) for 20 min. It takes about 60 min to reach 85°C (internally) for a package of two layers of surimi crabsticks in hot water (90°C) (Jaczynski and Park 2001). Therefore, to comply with the PUFI, total heat required for the package would be approximately 80 min. It is obvious that the microbial safety must not be compromised for physicochemical quality. However, comparing only 20 min necessary for texture development with 80 min necessary for microbial safety, 60 min of over-cooking, with regard to texture quality, is unacceptable.

There are two crucial facts commonly found in the surimi seafood industry that further contribute to the pasteurization dilemma. First, there is no standardized pasteurization method available to the industry. Consequently, different manufacturers use different time-temeprature regimes (Park 1994) and, to our knowledge, none of them have been scientifically validated in terms of physicochemical changes (Park 1994, 2001). A survey conducted by Park (1994, 2001) showed that thermal processing varied significantly and products might have been overcooked, resulting in unfavorable physicochemical changes (Shie and Park 1999). Secondly, when the price of surimi more than doubled in 1991, the surimi seafood industry was forced to minimize surimi content in surimi seafood. Since that time, more additives, such as starches and proteins, have been used as surimi replacements, which affected the heating requirements (Park 2000, Shie and Park 1999). However, thermal processing methods remained unchanged. This resulted in lower quality of color and texture since processing conditions were not optimized (Yang and Park 1998).

Electron beam processing, in contrast, utilizes high-energy electrons as a pasteurizing/sterilizing agent. The source for electron beam is regular electricity

and, unlike gamma irradiation, E-beam does not use radioactive materials (Luchsinger and others 1996). If a problem occurs, the system can be inactivated by simply unplugging the electricity.

Electrons are accelerated to near the speed of light by means of a linear accelerator. Then, electrons are scanned through the product. This results in the scission of bacterial DNA. The damage to DNA renders the microorganisms incapable of reproduction. Therefore, the bacteria within the package are inactivated and the product is pasteurized/sterilized. Since E-beam enables the use of high dose rates (unlike cobalt 60), the exposure time of the food sample to the beam may be very short. In addition, E-beam does not alter the temperature of processed food during treatment. Therefore, E-beam is likely to minimize the degradation of food physicochemical properties (color, texture and flavor) (Giddings 1984). The application of E-beam in surimi seafood has not been investigated.

Our objective was to investigate the physicochemical properties of surimi seafood caused by E-beam and to compare them with those of thermal processing.

MATERIALS AND METHODS

Sample preparation

Un-pasteurized surimi seafood sticks (length appr. 10 cm, diameters appr. 2 cm) were obtained from a commercial factory (Bumble Bee Seafoods, Motley, MN). Sticks were tightly placed on plastic trays. Two different trays were used. A 15 mm deep tray held 6 sticks (one layer of sticks) and a 30 mm deep tray held 12 sticks (2 layers, 6 sticks in each layer). The samples were packed in plastic film under either aerobic (non-vacuum pack) or anaerobic (vacuum pack) conditions.

Then, the samples were subjected to various e-beam and thermal treatments. The experimental outline is shown in Fig 3.1.

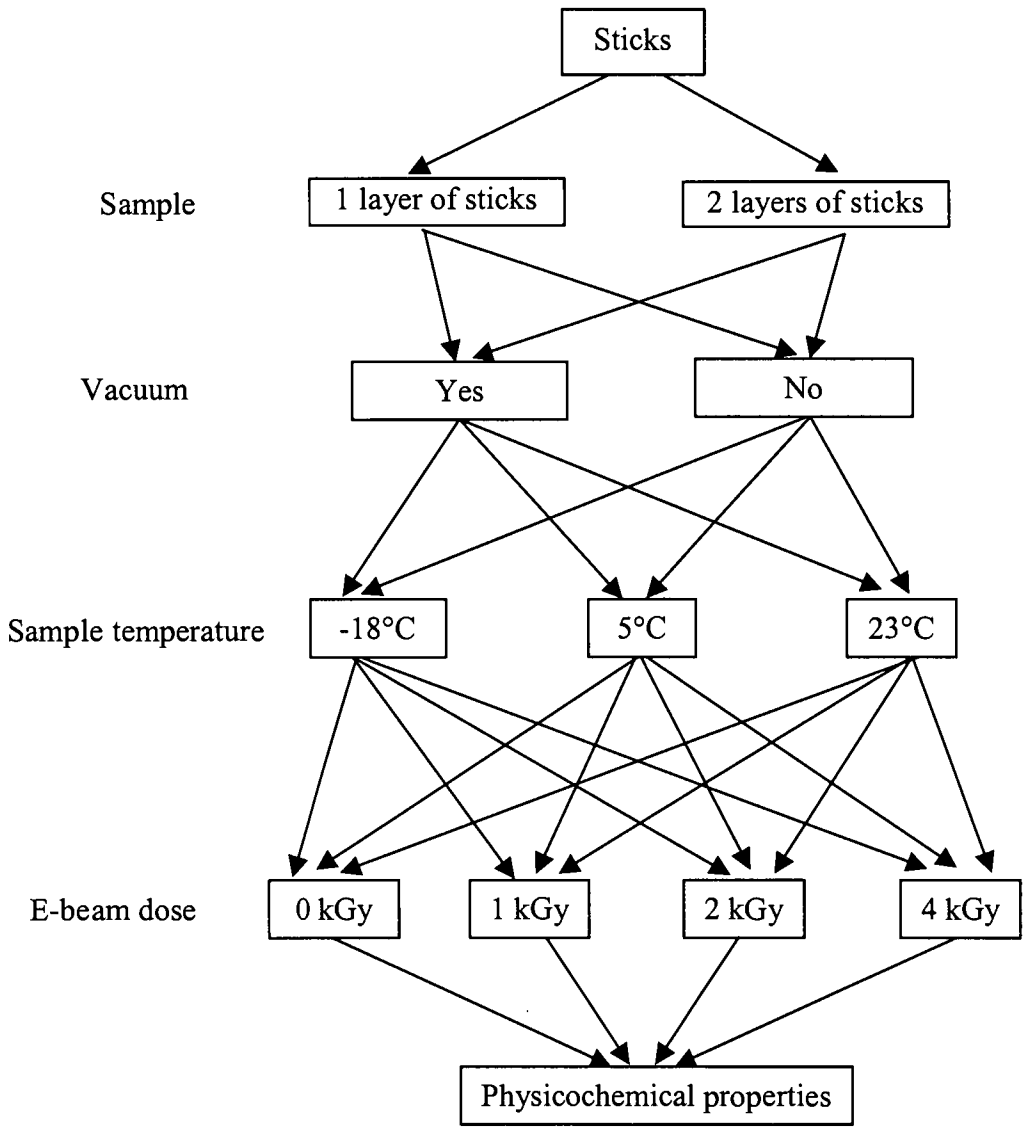

Figure 3.1: Outline of E-beam experiment

Thermal treatment

An eighteen-liter water bath with water circulation was used to mimic industrial pasteurization. Surimi seafood sticks were placed on 15 mm deep trays (1 layer of 6 sticks) and vacuum packed in a plastic film. The samples were heated at 90°C for 30 and 60 min, respectively. Samples were chilled in ice water immediately following the treatment. The same analyses were performed for E-beam treated samples.

E-beam treatment

Surimi seafood sticks were placed on two different trays as described above. Half of the samples for each size were anaerobically packed (vacuum), whereas the other half were aerobically packed (non-vacuum). Before treatment, the temperature of the samples was equilibrated to either –18 or 5 or 23°C. Samples were exposed to four dose levels (0, 1, 2, and 4 kGy).

Color

Tristimulus color values L*, a*, and b* were measured using a Minolta chroma meter CR-300 (Minolta Camera Co. Ltd., Osaka, Japan) (Park 1995). The external (red colored) layer of the stick was peeled off and the remaining stick was ground to a paste. The paste was transferred onto a Petri dish for color measurement. To eliminate the effect of compactness, the same amount of paste was applied for each measurement.

Texture

A Warner-Bratzler shear device attached to the Sintech 1/G (MTS, Cary, NC) was set up to measure shear force (g). Whole sticks were equilibrated to room temperature before being subjected to the shear device for texture measurement.

2-thiobarbituric acid assay

Oxidative rancidity was measured by 2-thiobarbituric acid (TBA) assay of malonaldehyde (MDA)(Yu and Sinnhuber 1957). A few drops of antioxidant (Tenox 6, Eastman Chemical Division, Kingsport, TN) and 3 mL of TBA were added to 0.2 g of homogenized sample. Then, 17 mL of TCA (trichloroacetic acid)-HCl reagent was added. The solution was flushed with nitrogen and closed. Another solution with the same volume of reagents, but without sample was prepared as a blank. The tubes were boiled for 30 min, and then cooled. Colored solution (15 mL) was centrifuged at 1500 rpm for 15 min. A clear, colored supernatant was transferred to a cuvette and the absorbance was measured at 535 nm. The results were reported as mg MDA/kg of sample.

Head-space analysis

Head-space was measured by gas chromatography with an electron ionization detector (G1800A GCD Hewlett-Packard, Palo Alto, CA). Sample paste (15 g) and NaCl (4 g) were placed in a 50 mL Erlenmeyer flask. Distilled and deionized water was added to bring final volume to 40 mL. The flask was covered with a septum, resulting in a head-space, where solid phase micro extraction (SPME) fiber (24 gauge, 100 μm, polydimethylsiloxane) (Supelco, Bellefonte, PA)

was inserted to adsorb the volatiles. The sample was vigorously stirred at 60°C for 30 min. Then the fiber was removed and injected into a GC injection port (splitless, 250°C) for desorption (5 min). Helium (grade 5)(Airgas, Grand Rapids, MI) was used as a carrier gas at 1.0 mL/min flow. With the oven set at 45°C (initial temperature), the sample was heated to 240°C at 5°C/min. The detector temperature was fixed at 280°C. The length and diameter of the column (Ec-Wax, # 907094)(Alltech, Deerfield, IL) were 30.0 m and 0.25 mm, respectively.

Statistical analysis

All analyses were performed at least in duplicate. To determine statistically significant differences between means of various treatments, a paired t-test based on the pooled standard deviation was used (Ramsey and Schafer 1997).

RESULTS AND DISCUSSION

Color

The L* value was not affected ($p>0.05$) by E-beam (Fig. 3.2). However, L* increased significantly for heat-treated samples ($p<0.05$), resulting in an L* value over 88 (90°C for 60 min). Since our samples were pre-heated as a continuous, thin sheet for less than 1 min to develop the initial texture, before being bundled as a stick, the increased L* value upon additional thermal process in this study was probably due to starch gelatinization (Charley 1982).

The a* value did not change ($p>0.05$) for E-beam treated samples (Fig. 3.3). The a* value of thermally-treated samples increased significantly from 2.5

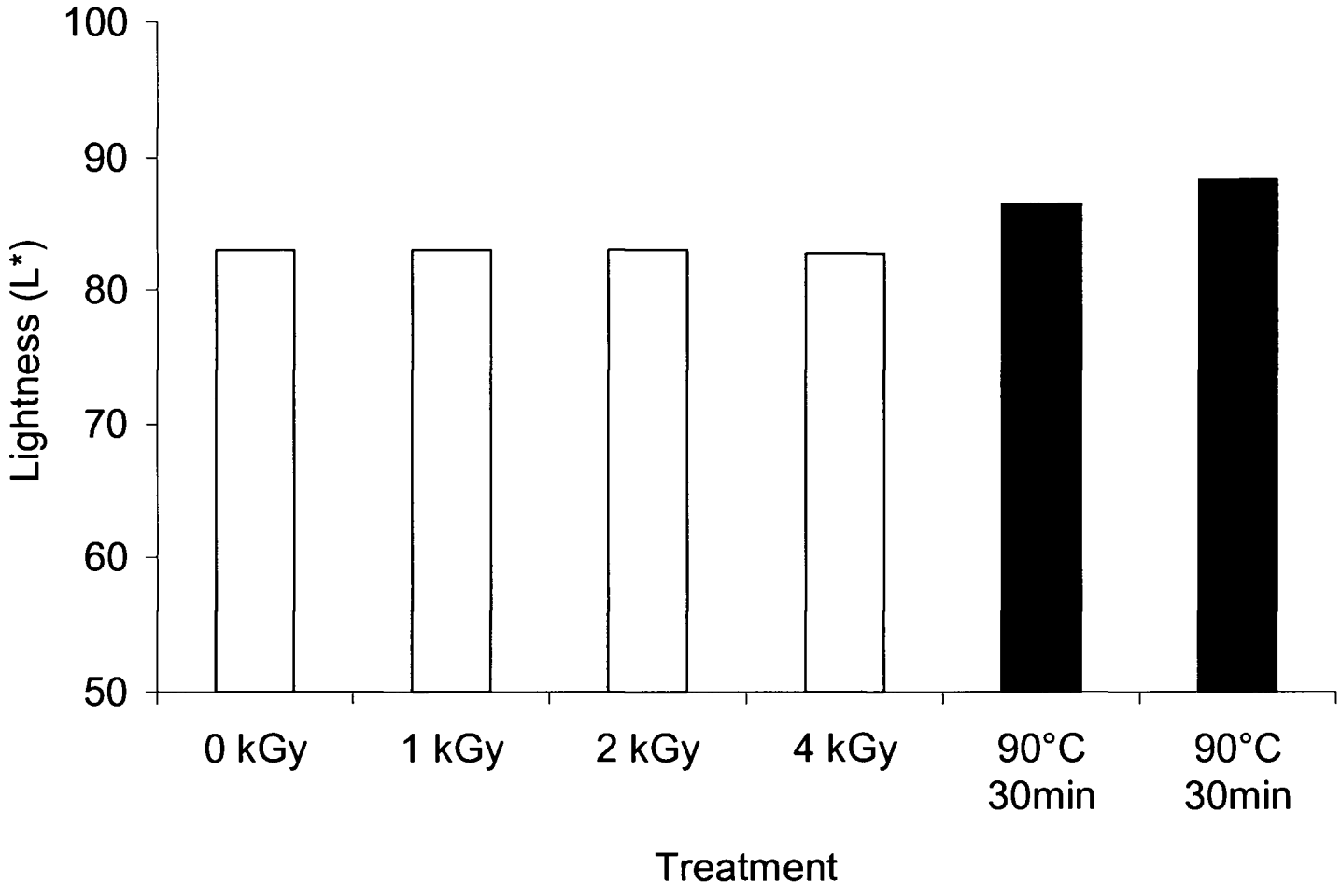

Figure 3.2: Lightness (L*) as affected by E-beam and heat

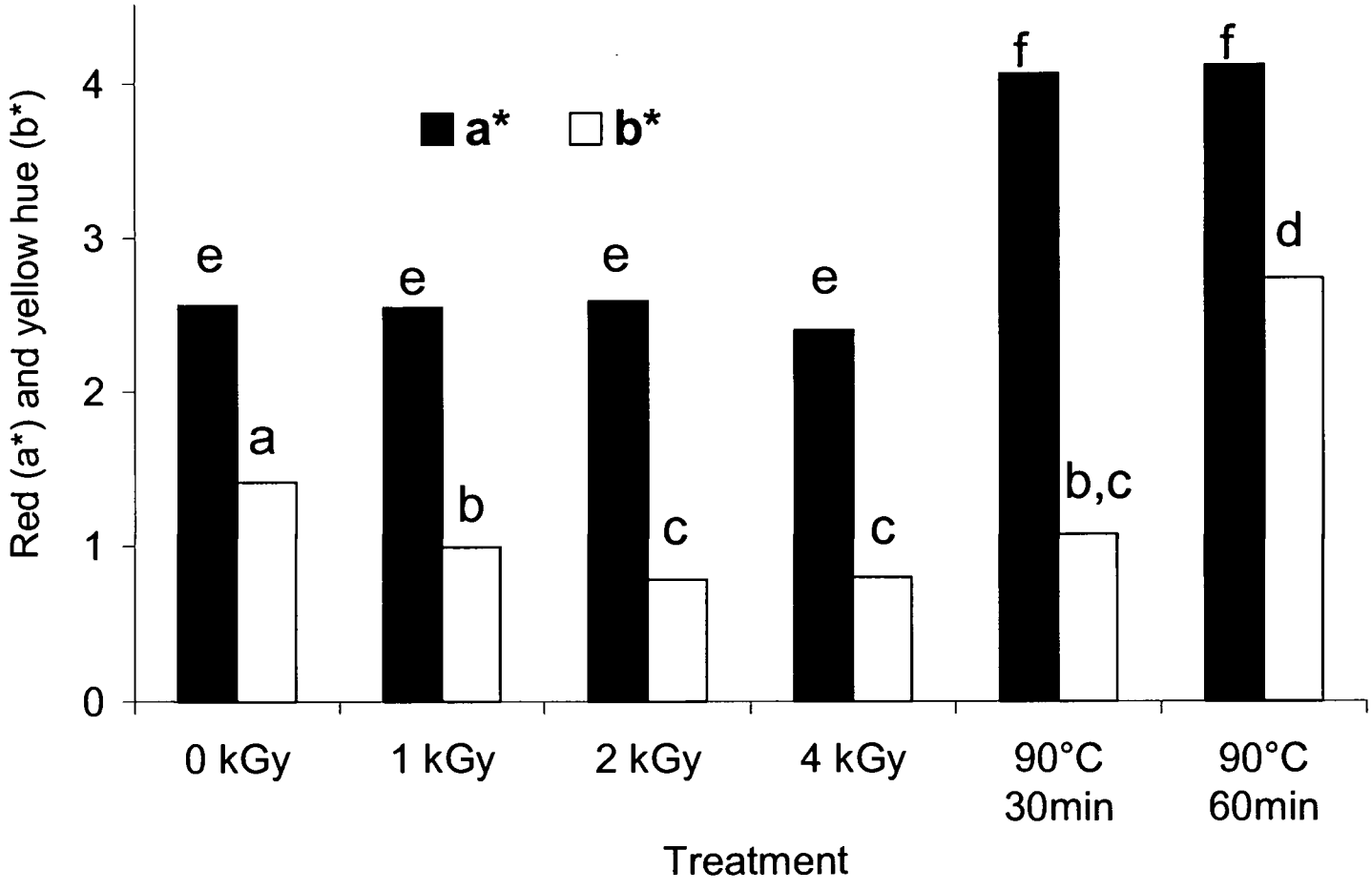

Figure 3.3: Red (a*) and yellow (b*) as affected by E-beam and heat

(control) to 4.0 and 4.1 for samples treated with heat at 90°C for 30 and 60 min, respectively ($p<0.05$)(Fig. 3.3).

A gradual, yet statistically significant decrease of b* was observed for E-beam samples ($p<0.05$)(Fig. 3.3), resulting in meat whitening. Ozone, which may be generated during E-beam (Venugopal and others 1999), might have bleached the yellow hue, resulting in a reduced b* value. Heat-treated samples (90°C for 60 min) increased b*, from 1.4 (control) to 2.7. Similar conclusions of color browning were reported by Shie and Park (1999).

Factors, such as sample temperature during irradiation, oxygen availability, and sample thickness, did not influence color values significantly ($p>0.05$)(data not shown). Heat-processed samples, however, were visually distinguishable. They appeared more yellowish than both the control and E-beam-treated samples. Samples subjected to thermal treatment appeared to experience non-enzymatic browning. Similar conclusions concerning heat-induced browning were previously reported by Park (1995) as well as Shie and Park (1999). In general, whiter surimi seafood denotes better color quality.

Texture

The shear force for E-beam samples did not change ($p>0.05$) for the various dose levels (Fig. 3.4). The heat-treated samples showed a significant loss of shear force (firmness)($p<0.05$). Both samples, heated for 30 and 60 min, lost firmness to a similar degree, suggesting that even a 30 min treatment can result in significant texture loss. Heat, as the energy in thermal processing, might have excited molecules in the gel structure to an extent that caused their ejection from the gel lattice, resulting in gel destruction. Bertak and Karahadian (1995) studied the effects of heating on surimi seafood and concluded that heating reduced firmness. Shie and Park (1999) also reported loss of gel strength upon excessive heating.

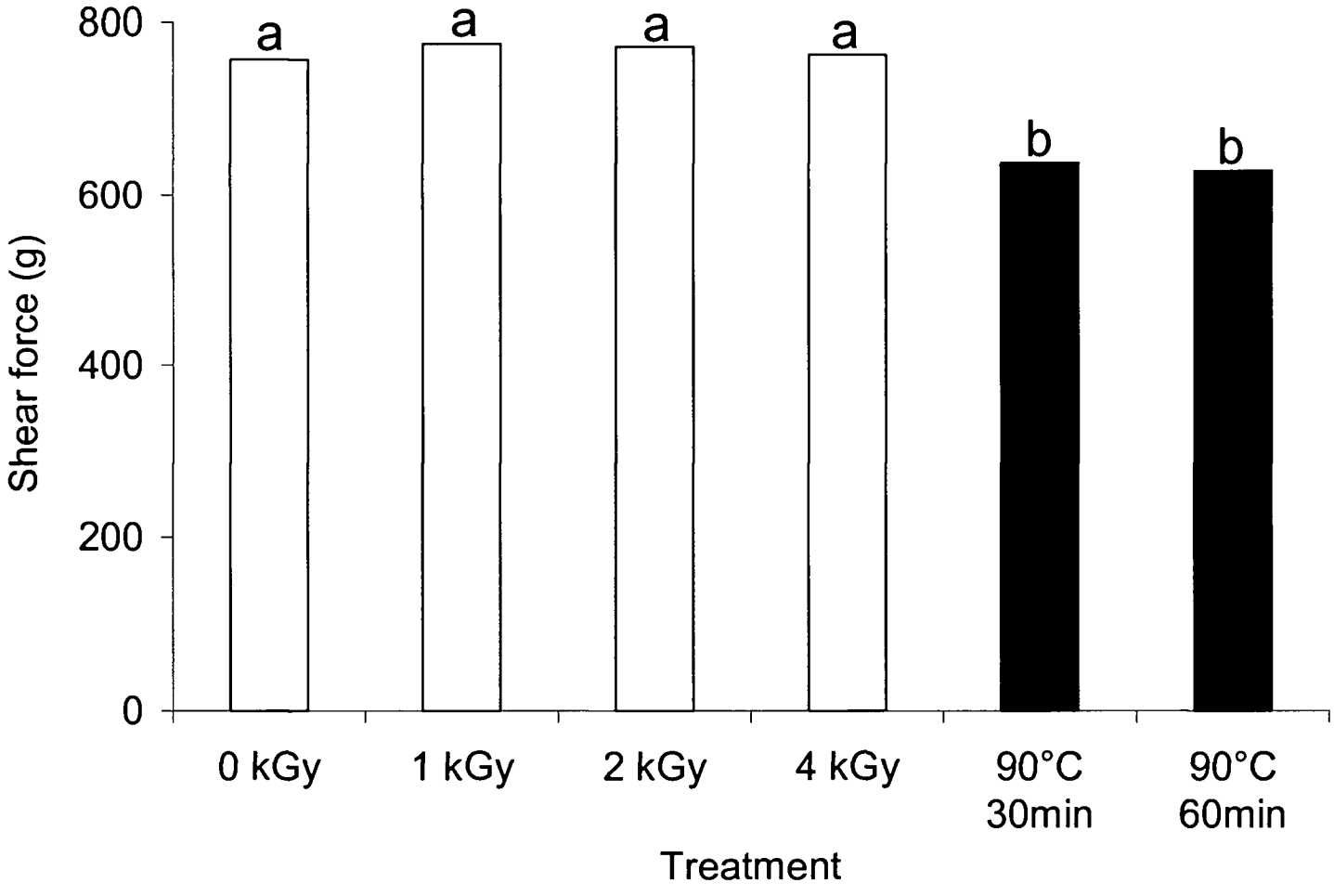

Figure 3.4: Shear force as affected by E-beam and heat

Statistical analysis revealed that E-beam treatment improved firmness when frozen samples were processed ($p<0.05$)(Fig. 3.5). This might have been caused by the formation of additional weak bonds by passing electrons through frozen samples. WHO (1994) reported that radiation induces protein denaturation and aggregation. It is likely that E-beam has a different mechanism for gel formation than heat. Therefore, E-beam might have improved firmness by introducing new bonds. The exact mechanism, however, is not clear. Further investigation is needed. In addition, it was determined that oxygen availability and sample thickness did not significantly affect shear force ($p>0.05$)(data not shown).

TBA

There was no significant change in lipid oxidation, except for samples exposed to 2 kGy (Fig. 3.6). The TBA value for 2 kGy samples was twice as high as that for the control. It may suggest that rancid odor may have developed faster in samples treated at 2 kGy than at any other dose. A possible explanation might be that electrons carrying 2 kGy of energy did not completely pass through the sample, resulting in their entrapment within the sample. That energy might have met the activation energy required to induce oxidation. Therefore, higher oxidation was measured at 2 kGy. Electrons at 4 kGy, however, possibly passed freely through the samples, leaving no trace in the sample. Electrons at 1 kGy might have been deposited within the sample similarly to electrons at 2 kGy. However, the ionizing energy might have been insufficient to overcome the activation energy for lipid oxidation. Therefore, the TBA remained unchanged. Further study for the effect of E-beam dosage on various lipid concentrations is needed.

Sample temperature during irradiation, oxygen availability, and sample thickness did not significantly affect TBA ($p>0.05$)(data not shown).

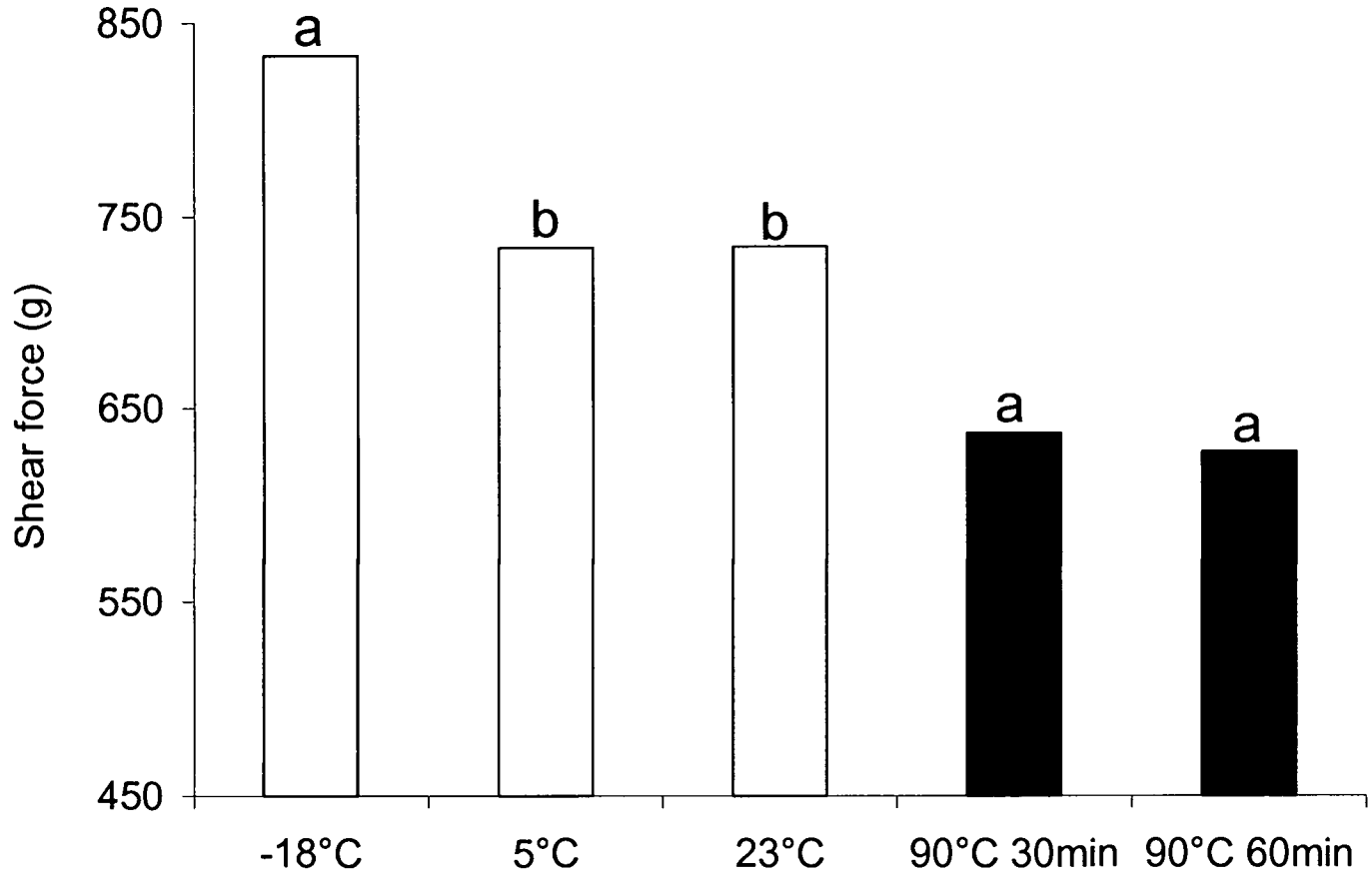

Figure 3.5: Effect of sample temperature during E-beam treatment on shear force

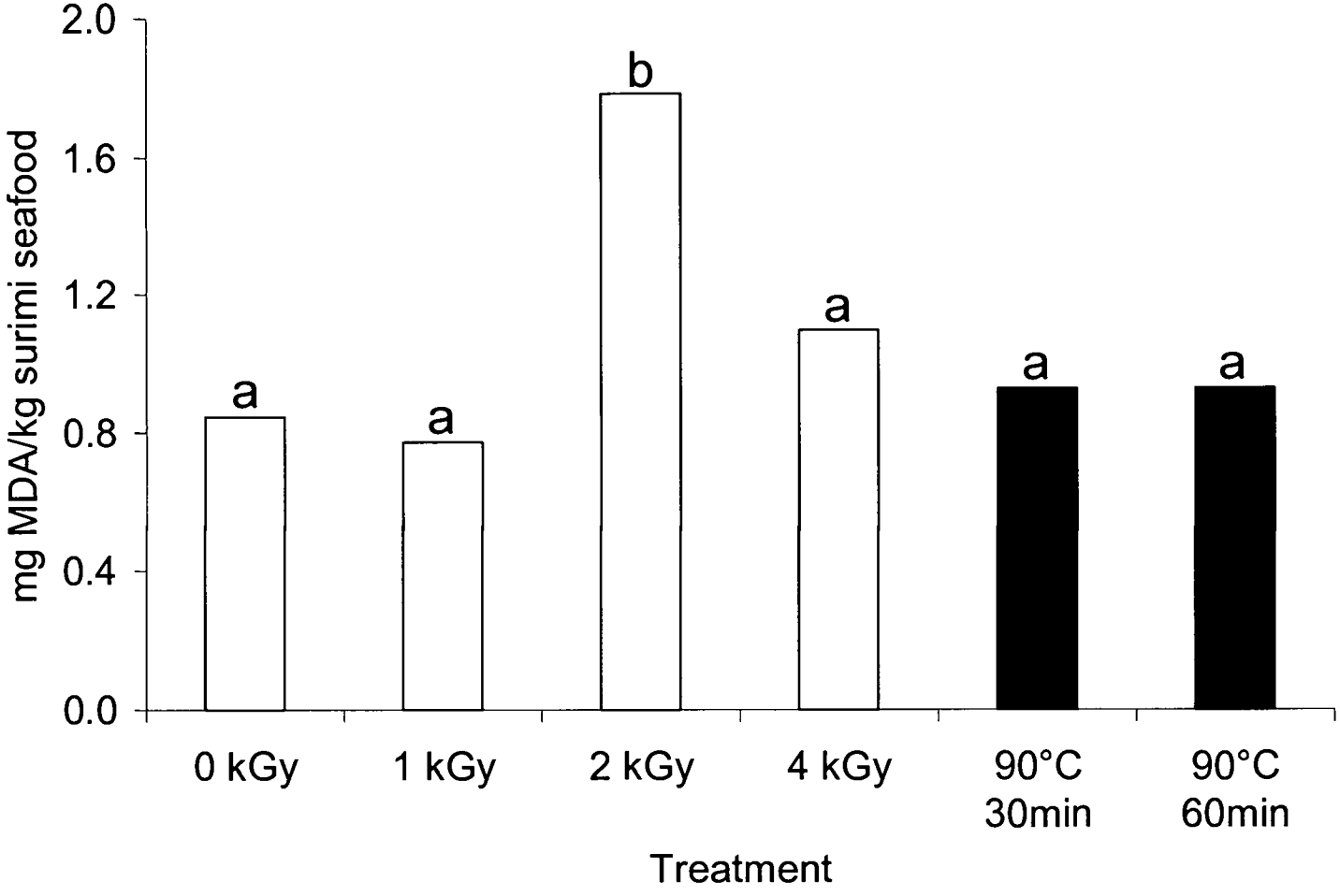

Figure 3.6: TBA values as affected by E-beam and heat

Head-space analysis

The composition of head-space and its changes upon E-beam and thermal treatments were determined (Fig. 3.7 and 3.8). The identified volatiles that may be responsible for the flavor of un-treated surimi seafood were ethyl esters of fatty acids, such as decanoic, octanoic, and dodecanoic (Fig. 3.7). furancarboxyaldehyde was also identified. However, head-space composition of heat-treated samples did not vary qualitatively from the control or the E-beam-treated samples.

WHO (1994) concluded that treatment up to 10 kGy did not cause the formation of new compounds. Chen and others (1996) tested the effects of gamma radiation (up to 2 kGy) on the flavor and aroma of crabmeat. They concluded that crab odor and flavor were similar for both treated and control samples, while off-flavors developed more rapidly in the control during 14-day storage. Poole and others (1990) also reported similar findings for scallops treated with low dose radiation (up to 3 kGy).

The level of ethyl ester of decanoic acids, which was the most abundant, decreased as the dose of E-beam increased (Fig. 3.7). Most likely, the decanoic acid was transformed into compounds that increased their respective percentage in the head-space (Fig. 3.7 and 3.8). Those compounds were ethyl ester of dodecanoic acid and furancarboxyaldehyde. The amount of ethyl ester of ocatanoic acid remained virtually unaltered (Fig. 3.7). Compounds such as furanmethanol and 4-H-pyran increased slightly (Fig. 3.8). Quantities of volatiles in the heat-treated samples changed more than those in the E-beam treated samples. It may be concluded that heat and E-beam caused similar changes in head-space, however, the results for heat-treated samples were more erratic. This may suggest that heat was more destructive to the olfactory attributes than E-beam within the tested parameters.

Lopez-Gonzalez and others (2000) compared the effect of E-beam and gamma radiation on beef patties. They found that patties irradiated by gamma rays

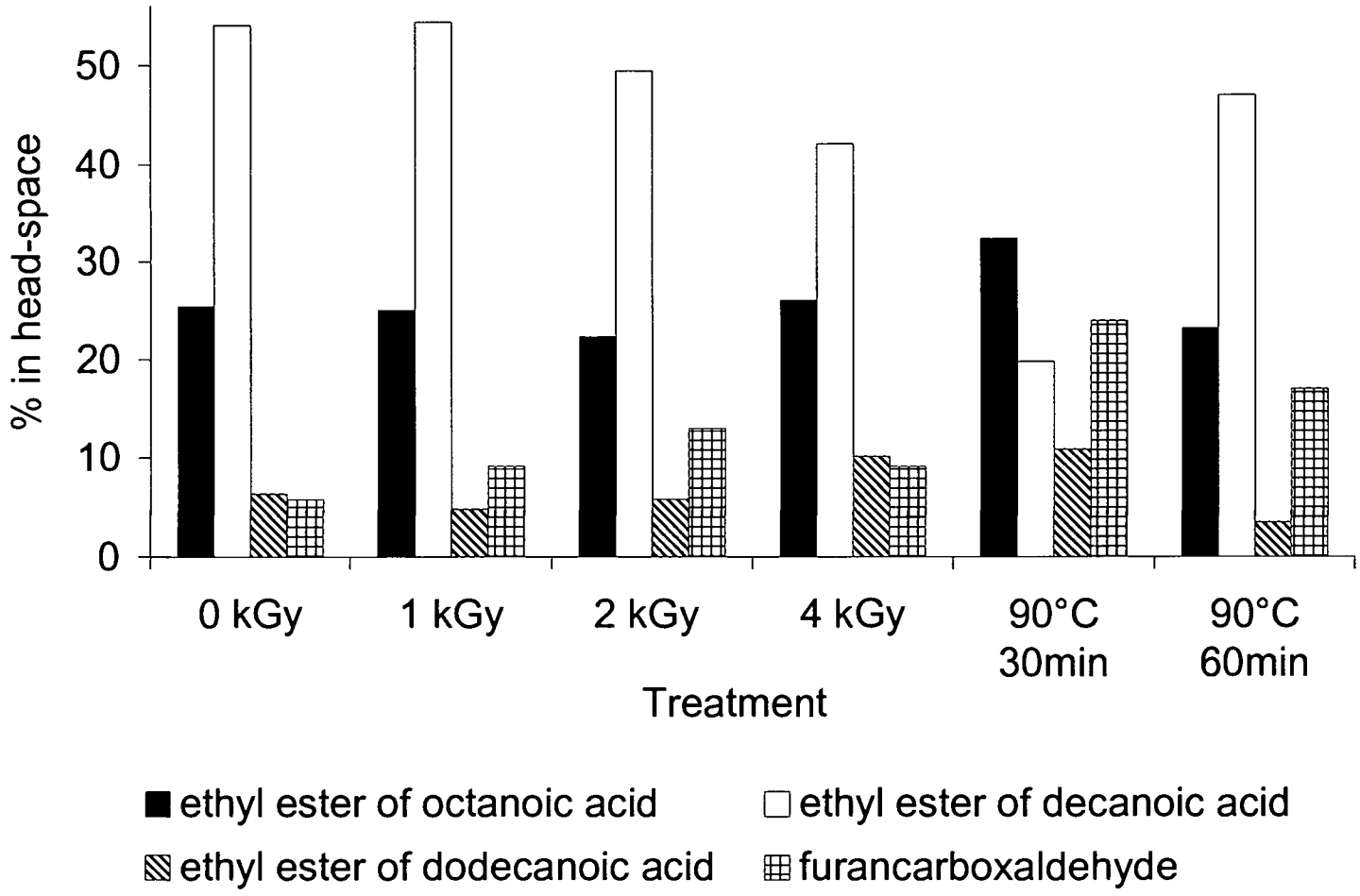

Figure 3.7: Major volatiles in head-space upon E-beam and heat

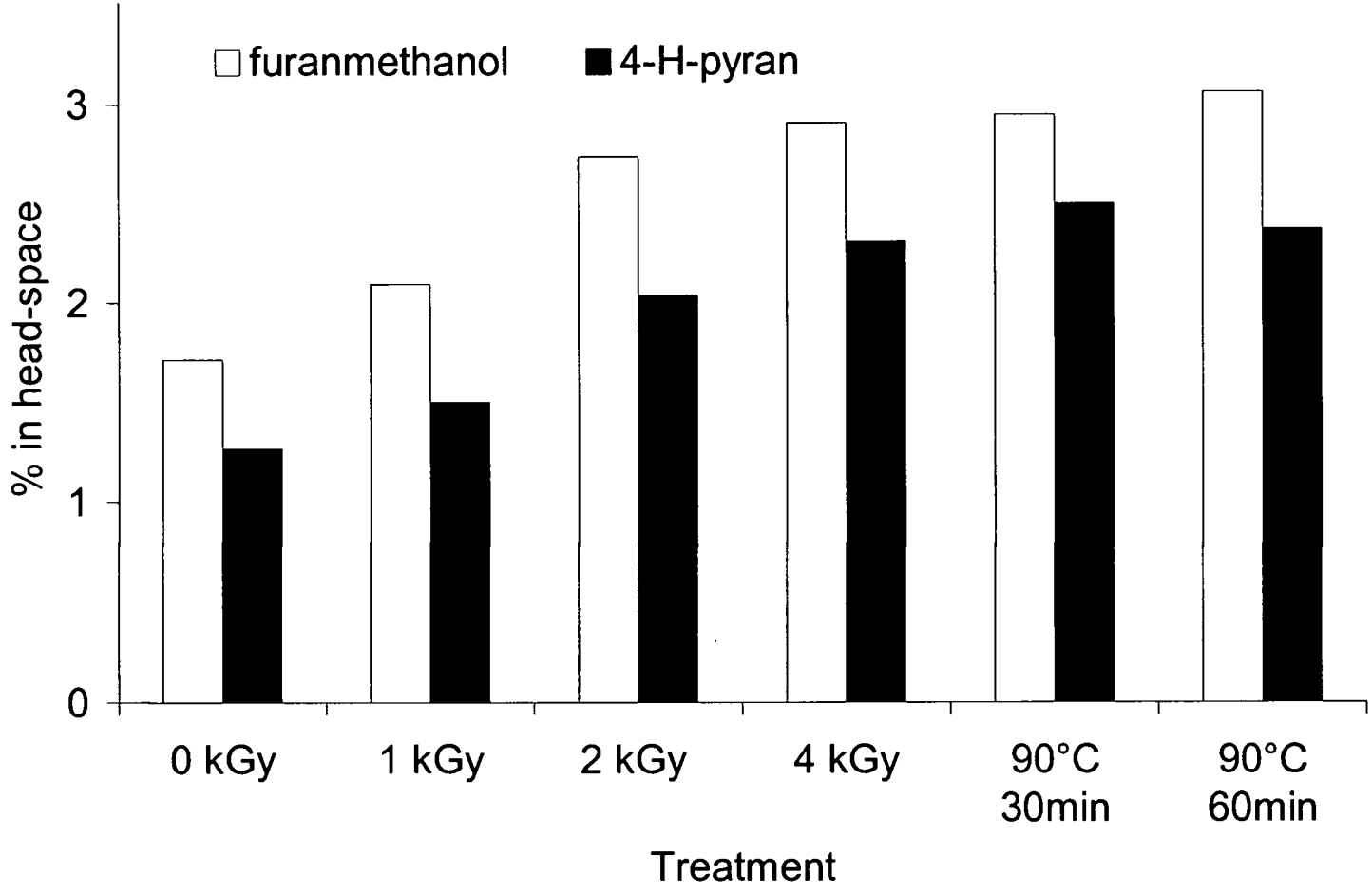

Figure 3.8: Minor volatiles in head-space upon E-beam and heat

had more intense cardboardy and sour flavors, and salty and sour tastes than patties irradiated by E-beam.

The oxygen availability during treatment may also accelerate lipid oxidation, which negatively contributes to flavor (Diehl 1995). During gamma processing, more O_2 from the atmosphere can diffuse into the food than is depleted by radiolysis, resulting in more unfavorable changes. In contrast to gamma processing, during E-beam treatment (high dosage rates) O_2 is depleted faster than it is replaced by diffusion of atmospheric O_2 (Venugopal and others 1999). Furthermore, at high dose rates (E-beam), free radicals form in such high concentrations that recombination of radicals rather than reaction with food components is favored (Hayashi 1991). Therefore, E-beam processing is likely to be less abusive than thermal treatment and gamma irradiation.

CONCLUSIONS

E-beam treatment up to 4 kGy did not significantly change color, texture, rancidity, and volatiles. When frozen samples were treated by E-beam, firmness increased. E-beam did not cause the formation of new compounds in the head-space. In contrast, thermal processing significantly deteriorated physical quality. Thermal processing at 90°C for 30 and 60 min caused color browning and firmness loss.

MICROBIAL INACTIVATION AND ELECTRON BEAM PENETRATION IN SURIMI SEAFOOD DURING E-BEAM PROCESSING

ABSTRACT

Electron penetration and microbial inactivation by E-beam in surimi seafood were investigated. Dose map revealed that one and two-sided E-beam could efficiently penetrate 33 and 82 mm thick surimi seafood, respectively. Modeling of microbial inactivation by E-beam demonstrated that two-sided E-beam may control *S. aureus* if the surimi seafood package is thinner than 82 mm. E-beam processing resulted in 0.34 kGy as the $D_{E\text{-}beam}$-value for *S. aureus*. A 12-log reduction of *S. aureus* was obtained with 4 kGy. Microbial inactivation was slower when frozen samples were subjected to E-beam.

INTRODUCTION

Fishery products require good pasteurization practices to maintain microbial safety (Mulak and others 1995, Harrison and Huang 1990). Psychrophilic microflora, inherent to seafood, grows well under refrigeration conditions. Therefore, seafood is particularly susceptible to spoilage.

The seafood industry traditionally uses either hot water or steam as a pasteurizing medium (Park 2000). However, various pasteurization regimes are used (Park 1994, 2001). Consequently, products may be overcooked, resulting in undesirable physical properties (Shie and Park 1999). It is feasible though to find a point at which minimum loss of physical properties and maximum bactericidal

effects occur simultaneously (Jaczynski and Park 2002a). However, some deterioration of physical properties is still inevitable (Bertak and Karahadian 1995, Jaczynski and Park 2002a, 2002b).

The FDA established zero tolerance for *L. monocyotogenes* and *Salmonella* in ready-to-eat products. If enterotoxigenic *E. coli* is present at 1 x 10^3/g, FDA will consider regulatory action. The product may be recalled if tested positive for staphylococcal toxins, or if 1 x 10^4/g organisms are present (Ward and Price 1992).

In the past, FDA has recalled surimi seafood. Recently, a seafood processor in Washington State had to recall 16,965 kg crabmeat (FDA 1998). According to the Center for Disease Control (CDC), foodborne-disease outbreaks implicating seafood are 5, 19, 4 and 12 times as frequent compared to beef, pork, chicken, and turkey, respectively. The CDC also reports that foodborne diseases in the USA claim 81 million illnesses, 616,377 hospitalizations, and 9000 deaths, annually.

Additionally, in the wake of the September 11 incident, the food industry also needs to take proper steps to assure a continuous risk-free food supply (Hollingsworth 2002, Applebaum 2002).

Sous-vide is a thermal process that is milder than traditional pasteurization and has gained popularity due to physical quality. Sous-vide products however are formulated for organoleptic qualities not for safety (Rhodehamel 1992). Sous-vide therefore raises safety questions, particularly *C. botulinum* (Hyytia-Tress and others 2000).

Electron beam, in contrast, utilizes high-energy electrons for pasteurization/sterilization. Electrons are accelerated to the speed of light by a linear accelerator. Then, electrons are passed to the product, thoroughly inactivating bacteria. The electron source is electricity and, unlike gamma radiation, E-beam does not use radioisotopes (Luchsinger and others 1996). Since E-beam uses high dose rates (unlike gamma radiation), the exposure time of food is short and food temperature is not affected. Therefore, E-beam is likely to minimize the degradation of food quality (Giddings 1984).

E-beam, unlike gamma rays, has limited penetration depth (Hayashi 1991), which may affect microbial inactivation. The overall effects though of gamma rays and electron beams are comparable (Urbain 1986, Hayashi 1991).

Tarte and others (1996) successfully applied E-beam to kill five strains of *Listeria* in pork. Garcia-Zepeda and others (1997) reported that *C. sporogenes* spores were un-detectable and reduced (0.5-log) in beef processed by E-beam and cooking, respectively. Shamsuzzaman and others (1995) showed that *L. monocytogenes* was un-detectable in samples treated with combined *sous vide* and E-beam, although microorganisms survived the *sous vide* treatment. Joint Expert Committes on Food Irradiation representing FAO/IAEA/WHO concluded that irradiation of any food up to 10 kGy caused no toxicological hazard and introduced no nutritional or microbiological problems (WHO 1981). E-beam application in surimi seafood, however has not been reported.

Our objective was to determine the antimicrobial properties of E-beam, as affected by electron penetration, in surmi seafood in order to develop a surface response model for microbial inactivation.

MATERIALS AND METHODS

Electron penetration

Sample preparation

Frozen Alaska pollock surimi was tempered and cut into small chunks. Surimi chunks were chopped in a silent cutter (Model UM5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min. Salt at 2 % was added and the surimi paste was chopped for 0.5 min at low speed. Final moisture was adjusted to 78% by adding ice, followed by chopping at low speed for another 1 min. Vacuum

(0.5 bar) and chopping at high speed was applied for 3 min. During chopping, the temperature was kept below 5°C. After chopping, the paste was placed in boxes (waxed paper)(4 x 4 x 20cm) and the air gaps were carefully removed. The boxes were vacuum packed in plastic and cooked in a water bath at 90°C for 45min. Immediately following cooking, the surimi gels were cooled in ice slush. The boxes were open and surimi gels of final dimensions were made. Two sizes of surimi gels were used: (1) 3 x 3 x 7cm and (2) 3 x 3 x 9cm.

E-beam treatment

Surimi gels were exposed to two dose levels (3 and 20 kGy) using one-sided E-beam fixed at 10 MeV. Sample temperature was equilibrated to room temperature before E-beam treatment. The experiments were performed in duplicate and the experimental outline is shown in Fig. 4.1.

Dose maping

Dosimeters were distributed every 1 cm from the top surface to the bottom of the surimi gels. Exposed dosimeters were read by a spectrophotometer at 605 nm and the dose absorbed at their respective locations locations was calculated. Absorbed doses were plotted against distance between dosimeters and the surimi gel surface, creating a dose map. The dose map allowed determination of R_{opt} (depth of surimi gel at which the absorbed dose equaled the dose at the surface of surimi gel), R_{50e} (depth of surimi gel at which the absorbed dose has decreased 50% of the absorbed dose at the surface of surimi gel), R_{max} (depth of surimi gel at which the absorbed dose reached its maximum value), and R_{50max} (depth of surimi gel at which the absorbed dose decreased 50% its maximum value). A polynomial

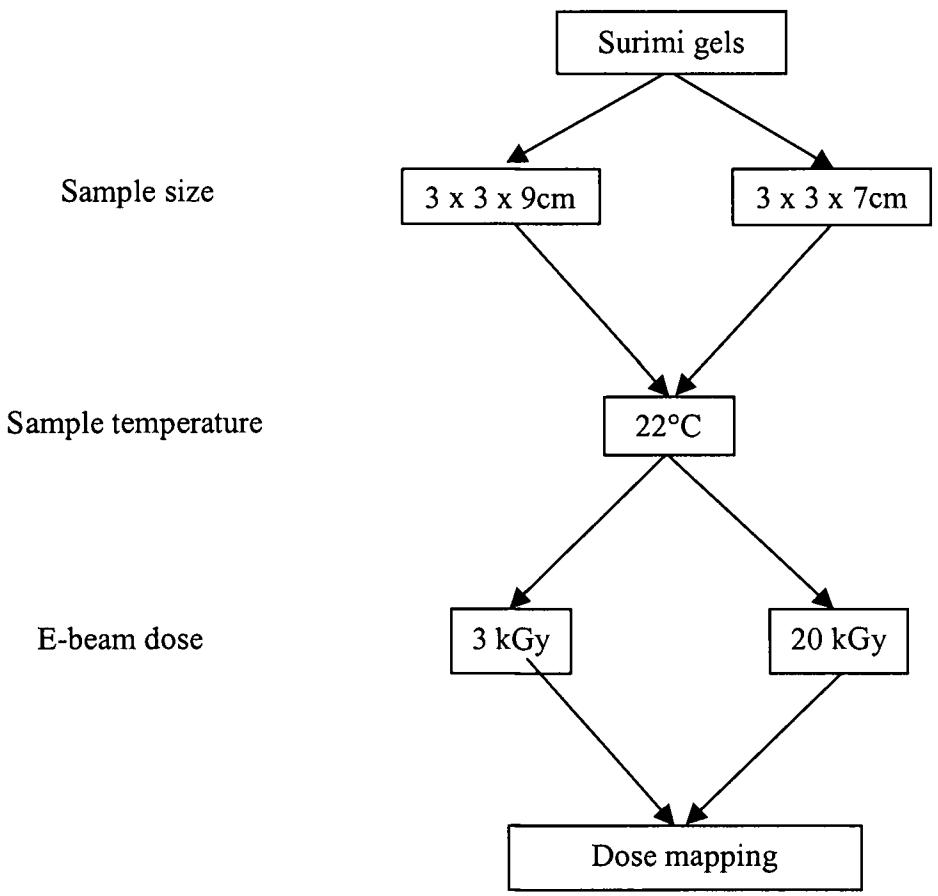

Figure 4.1: Outline of electron penetration experiment

regression ($R^2 = 0.9999$) equation was fitted to the experimental data. Microsoft Excel was used for the calculations.

Microbial inactivation

Sample preparation

Surimi seafood crabsticks (hereafter surimi seafood)(length appr. 10 cm, diameters appr. 2 cm) were obtained from a commercial factory (Bumble Bee Seafoods, Motley, MN). Sticks were ground into a paste. The paste was placed on 15 mm deep plastic trays. The paste was inoculated (5%) with a cocktail of six strains of *Staphylococcus aureus* consisting of equal volume of each strain and incubated for 72 hours at 37°C, resulting in a final concentration of 10^9 CFU/g. Following incubation, samples were packed in plastic film, subjected to various E-beam treatments, and the survivors were enumerated.

E-beam treatment

Ground surimi seafood was placed on 15 mm deep trays. Half of the samples were anaerobically packed (vacuum), the other half were aerobically packed (non-vacuum). Before treatment, sample temperature was equilibrated to –18, 5, and 23°C. Samples were exposed to four dose levels (0, 1, 2, and 4 kGy) of one-sided E-beam fixed at 10 MeV. The experiments were performed in duplicate and the experimental outline is shown in Fig. 4.2.

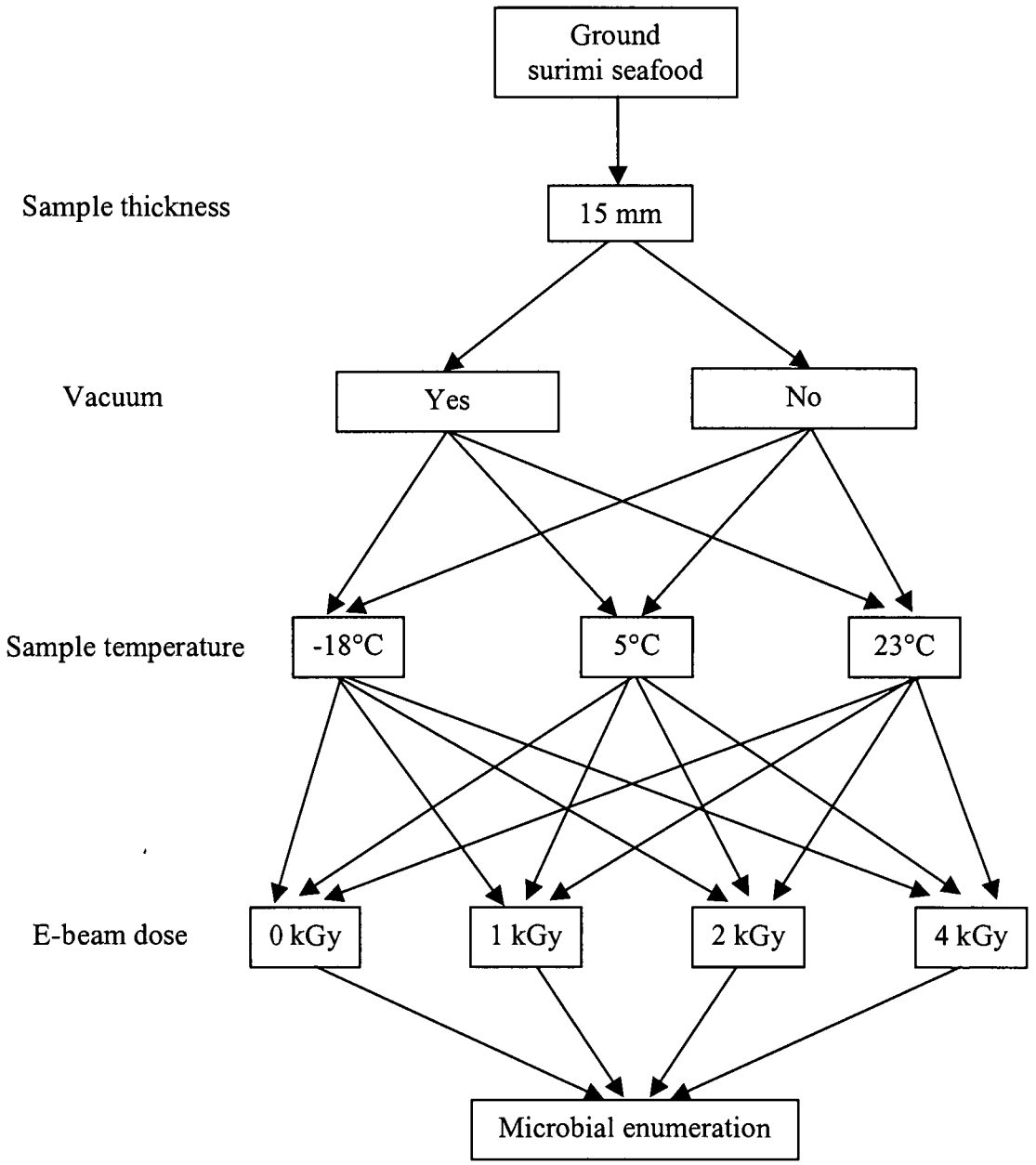

Figure 4.2: Outline of microbial inactivation experiment

Microbial enumeration

Enumeration of *S. aureus* survivors was performed on staphylococcus 110 agar (Difco Laboratories, Detroit, MI) by a serial 10-fold dilution using the spread plating. Following E-beam treatment, before the survivors were enumerated, the samples were thoroughly mixed to obtain uniform distribution of survivors. The presence of *S. aureus* was confirmed by gram staining, catalase, and coagulase tests.

Predictive Model of Microbial Inactivation by E-beam

$D_{E\text{-}beam}$-value

Survivors were plotted on a logarithmic scale as a function of dose, resulting in a survivor curve (Thayer and Boyd 1995). D-value (hereafter called $D_{E\text{-}beam}$-value in order to distinguish from D-value for thermal processes), the dose in kGy necessary to reduce the population by 90 % (1 log)(Thayer and Boyd 1995), was calculated as a negative reciprocal of the slope of the survivor curve (Toledo 1991, Thayer and Boyd 1995)(Equation 1).

$$\log\left(\frac{N}{N_0}\right) = -\frac{1}{D} * t \qquad \text{Eq. 1}$$

N – number of survivors at E-beam dose,

N_0 – initial microbial concentration,

D – $D_{E\text{-}beam}$-value, decimal reduction dose,

t – E-beam dose.

Model development

Dose absorbed, as a function of surimi seafood thickness was simulated by the polynomial equation obtained from the dose map. The dose absorbed was related to the $D_{E\text{-}beam}$-value obtained in the microbial inactivation experiment, resulting in a total log reduction of *S. aureus* population across the surimi seafood sample. The number of *S. aureus* (CFU/g) that survived E-beam treatment was calculated based on the total log reduction and the initial number of *S. aureus* (CFU/g). A simulation of microbial inactivation with one and two-sided E-beam was also performed. Microsoft Excel was used for the calculations.

Statistical analysis

All analyses were performed at least in duplicate. To determine statistically significant differences between means of various treatments, a paired t-test based on the pooled standard deviation was used (Ramsey and Schafer 1997).

RESULTS AND DISCUSSION

Electron Penetration

Fig. 4.3 shows the dose map for surimi seafood. The absorbed dose increased up to 2 cm deep from the product surface. Then, absorption gradually decreased, reaching a

minimum value at approximately 5 cm from the product surface. The surimi gels used in our experiments contained 78% moisture and specific density of

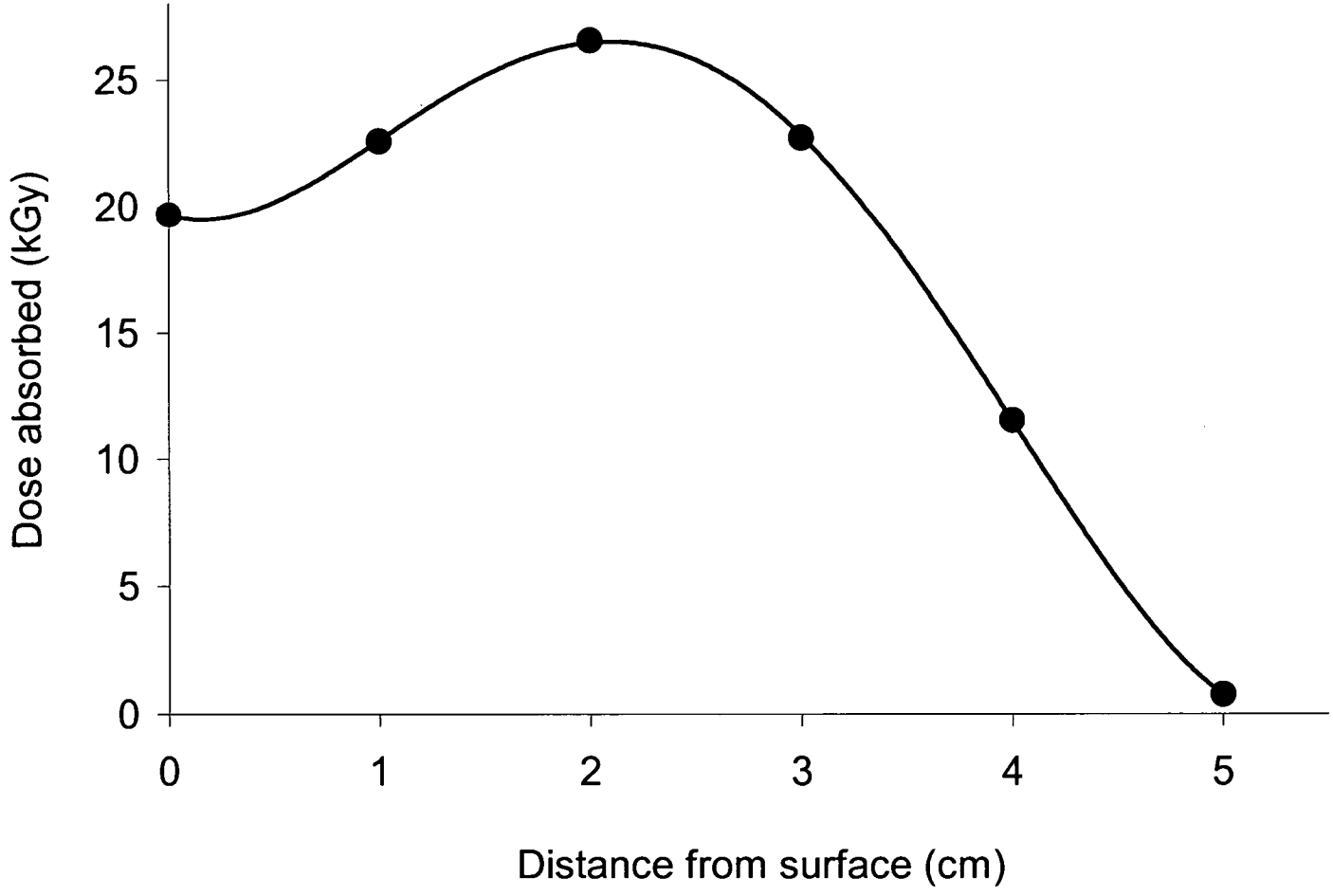

Figure 4.3: Dose map for surimi seafood

surimi seafood equals 1.067 g/cm^3 at 30°C (AbuDagga and Kolbe 1997). Venugopal and others (1999) reported that in aqueous solutions 10 kGy of E-beam fixed at 10 MeV resulted in a 12.5 kGy being absorbed 2 cm from the surface. They also observed no electron penetration below 5 cm. These results are similar to our findings. Diehl (1995) also reported that the dose absorbed increased under the surface and then decreased. This phenomenon has been attributed to the formation of secondary electrons that (Venugopal and others 1999).

The dose absorbed followed a polynomial function (Eq. 2) regardless of the dose applied and product thickness. In our experiments, 3 and 20 kGy of E-beam were applied to 5 and 7 cm thick surimi seafood samples. In all cases, the dose absorbed followed the same polynomial pattern (Eq. 2). Also, in each case the dose absorbed reached a minimum value at roughly 5 cm from the product surface.

$$DoseAbsorbed = 1.76 * X^4 - 17.73 * X^3 + 43.14 * X^2 - 12.32 * X + 99.96 \qquad \text{Eq.2}$$

X – distance from the product surface (cm).

The dose map allowed calculations of R_{opt}, R_{max}, R_{50e}, and R_{50max} equal to 33, 21, 41, and 39 mm, respectively. Based on the R_{50e}, two-sided E-beam could penetrate surimi seafood (thickness ≤ 82 mm), resulting in the dose absorbed across the entire thickness as being equal to or higher than the dose applied. Venugopal and others (1999) also suggested that E-beam could be successfully applied to processing of food with a density of 1 g/cm^3 up to 8-10 cm thick.

Microbial Inactivation

E-beam treatment at 1 kGy resulted in a 2.9-log reduction of *S. aureus*. Treatment at 2 kGy gave an additional 3.2-log reduction, resulting in a total

reduction of 6.1-log. Sterilization was observed at 4 kGy (Fig. 4.4) and the $D_{E\text{-}beam}$-value for *S. aureus* obtained with E-beam was equal to 0.33 kGy.

Venugopal and others (1999) reported a D-value of 0.29 kGy for *S. aureus* in shrimp. Thibault and Charbonneau (1991) determined that the D-value for *S. aureus* in Atlantic cod was dependent on the season when the fish were caught and ranged from 0.1-1.12 kGy. Licciardello and others (1989) obtained a D-value equal to 0.89 kGy for *S. aureus* treated with gamma radiation in clam and mussel. Lewis and others (1971) determined that gram-negative organisms are less resistant to radiation than gram-positive. They also reported the D-value for gram-negative bacteria ranged from 0.04-0.09 kGy.

Ehlerman (1993) showed that the effects of radiation are linear with dose, up to 15 kGy. Therefore, assuming linearity of the D-value, application of 4 kGy in our tests may have resulted in a 12-log reduction, as verified by no colonies in *S. aureus* enumeration at 4 kGy (Fig. 4.4).

Sample temperature during E-beam treatment had a significant effect ($p<0.05$) on microbial inactivation (Fig. 4.5). In frozen samples treated at 1 kGy, microbial reduction was about 1-log lower than in un-frozen surimi seafood. At a higher dose, the temperature effect was even more pronounced. At 2 kGy inactivation was best for samples at room temperature, followed by chilled, and frozen, respectively (Fig. 4.5). Brandao and others (1991) examined the effect of sample temperature during radiation on the inactivation of *Listeria monocytogenes* in shellfish. They concluded that the highest resistance was at –20°C. In addition, Thayer and Boyd (1995) investigated the temperature effect on inactivation of *L. monocytogenes* by gamma radiation. They reported an abrupt increase in microbial resistance starting at –5°C, followed by a linear increase of resistance with decreasing sample temperature.

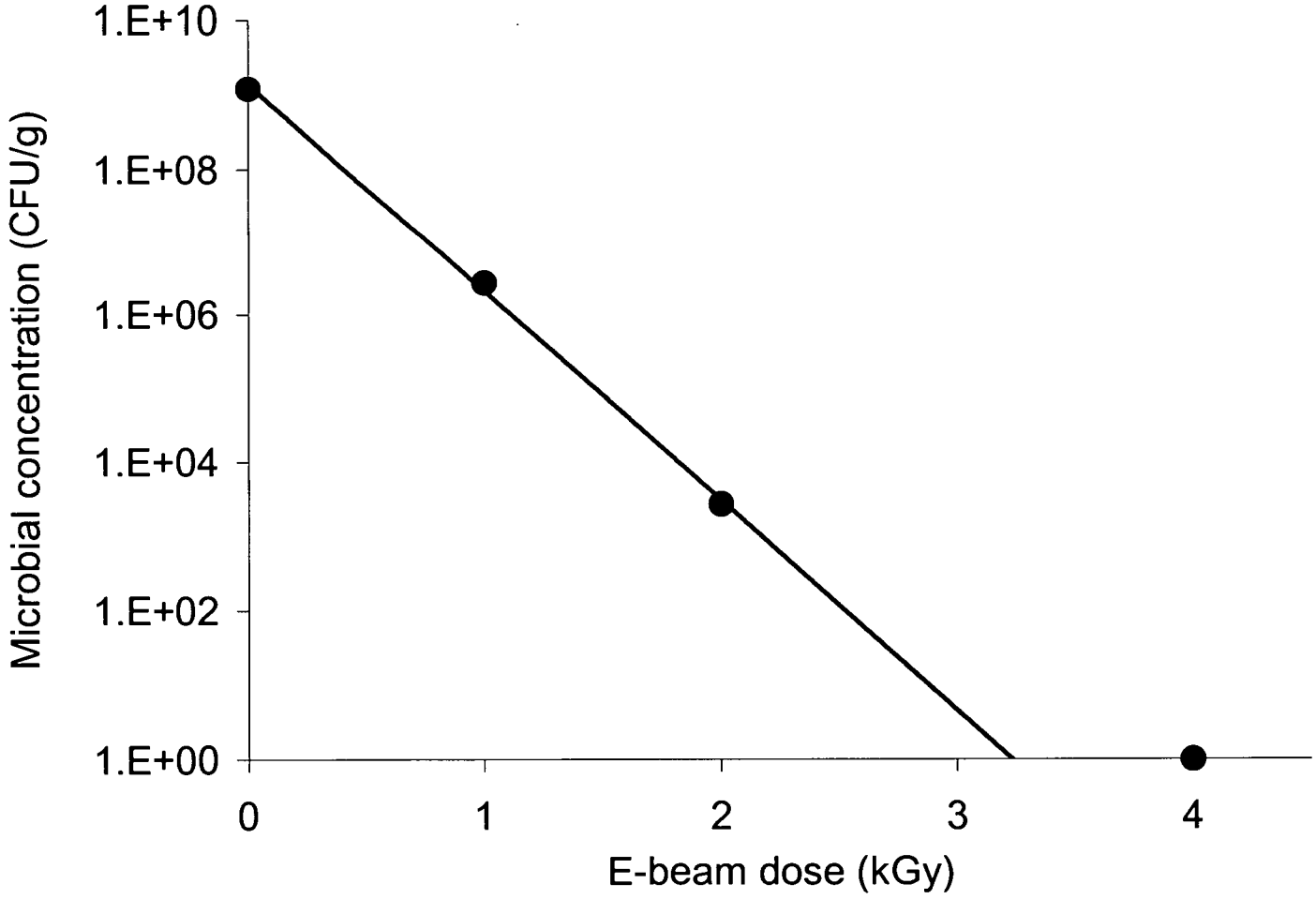

Figure 4.4: Survival of *S. aureus* treated with E-beam

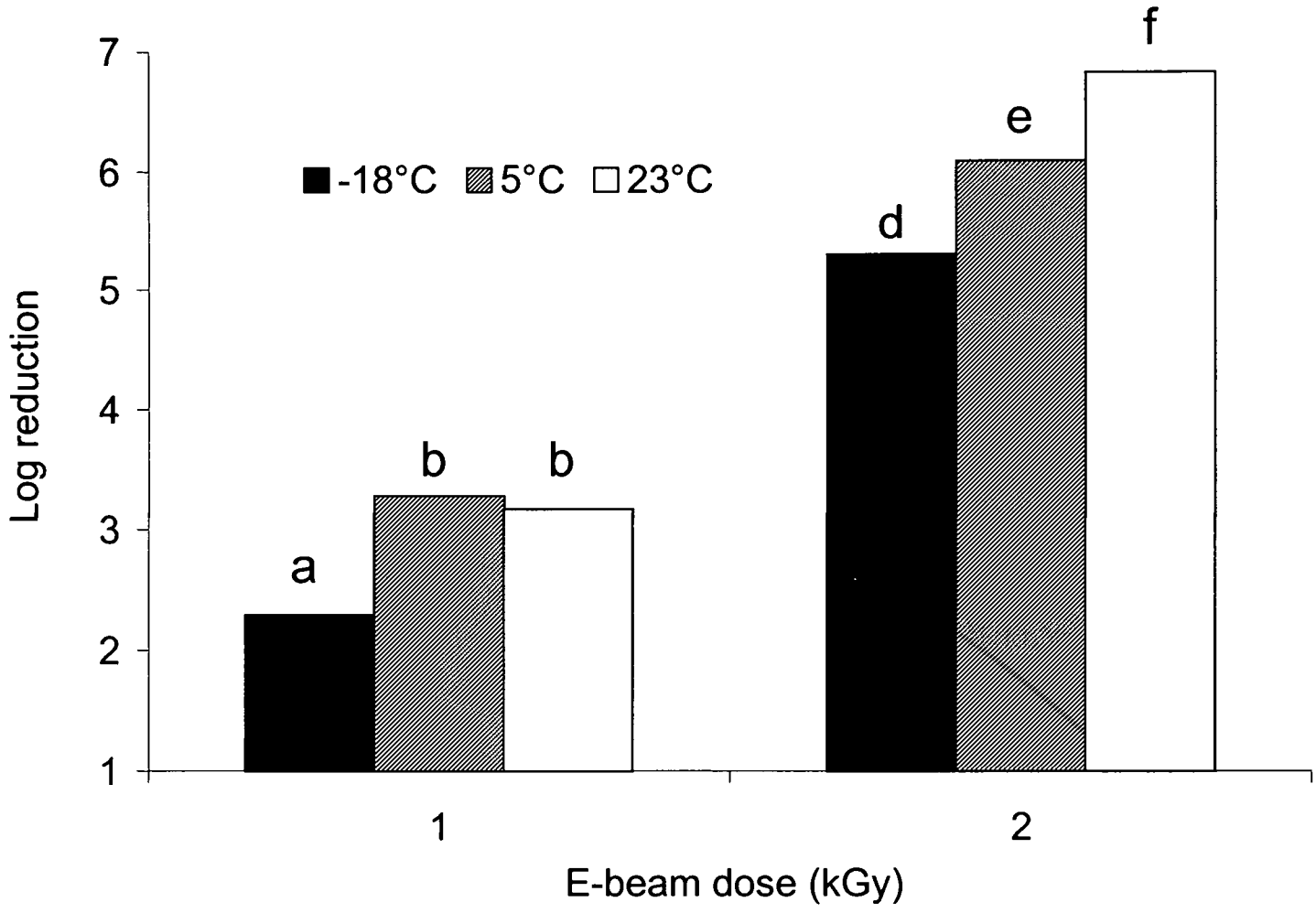

Figure 4.5: Temperature effect on inactivation of *S. aureus* by E-beam

E-beam generates free radicals, which kill bacteria. The reactivity of free radicals depends on their diffusibility, which is dependent on the availability of free water in the product. Movement of free radicals increases with temperature (Venugopal and others 1999). Availability of free water in frozen samples was lower than in un-frozen samples, contributing to slower movement of free radicals. Therefore, *S. aureus* exhibited the best survival in frozen surimi seafood.

Oxygen availability (vacuum packing) did not affect microbial inactivation ($p>0.05$)(data not shown).

Predictive Model of Microbial Inactivation by E-beam

The dose map allowed for the simulation of dose absorbed across a surimi seafood sample. By applying the $D_{E\text{-}beam}$-value to the simulated dose absorbed, total log reduction was estimated. Final concentration of survivors (CFU/g) was obtained by applying the total log reduction to the initial microbial concentration (CFU/g).

Fig. 4.6 shows a simulation of the dose absorbed (for one and two-sided E-beam) across surimi seafood based on a sample thickness of 82 mm and a target (applied) dose of 3 kGy. The plot for one-sided E-beam processing (Fig. 4.6, left) clearly shows that one-sided processing can efficiently penetrate only 33 mm (R_{opt}). Below 33 mm, the dose is lower than the target dose (applied). Therefore, the energy deposited below 33 mm would not assure the desired antimicrobial effect. However, the two-sided E-beam processing (Fig. 4.6, right) can efficiently penetrate up to 82 mm thick samples, maintaining the dose absorbed across entire sample at least at the same level as the target dose (applied). Therefore, two-sided processing represents a much better utilization of the applied energy to inactivate bacteria. However, simulations (two-sided E-beam) for surimi seafood thicker than 82 mm yielded lower dose absorbed than the target dose

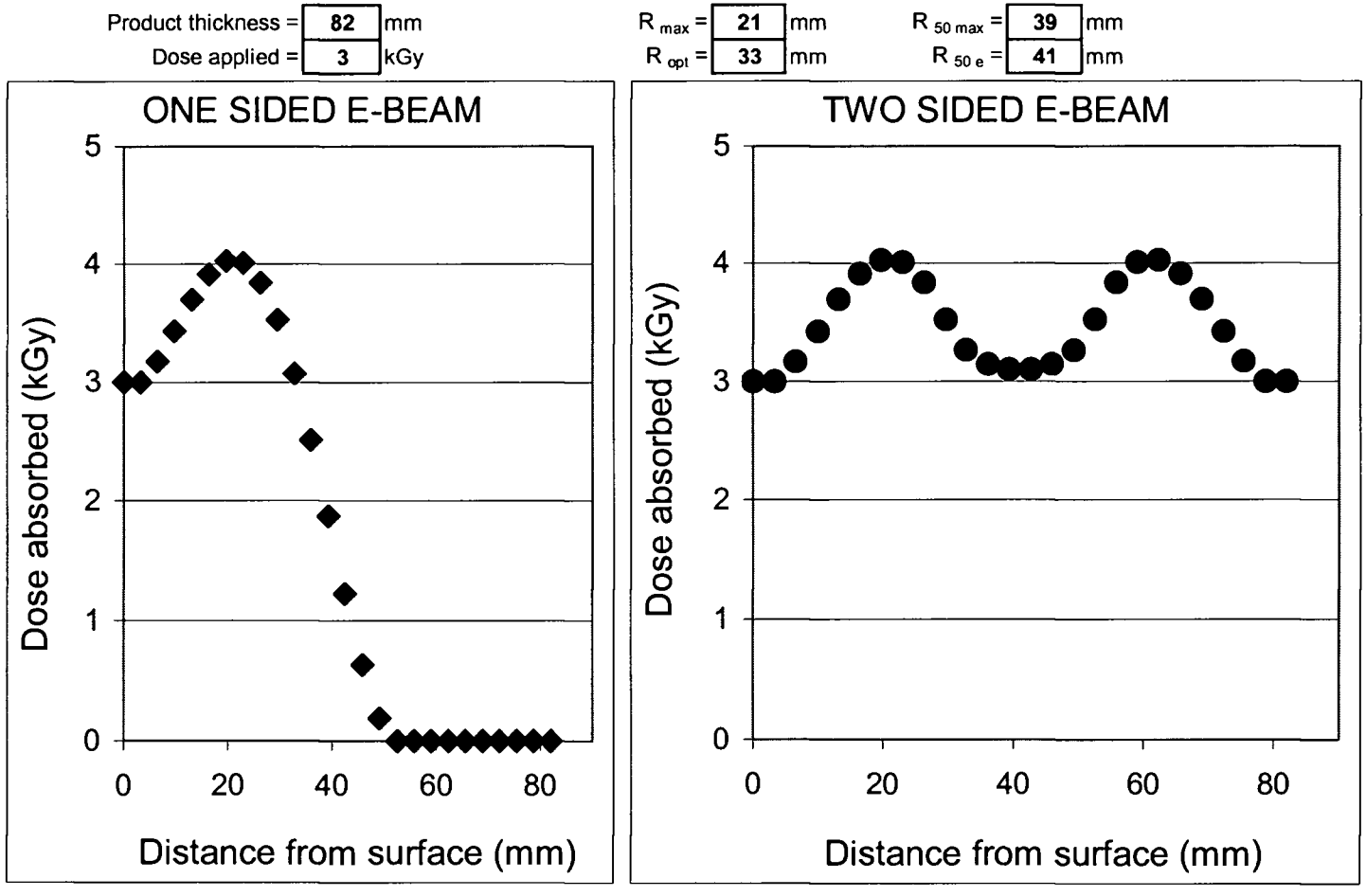

Figure 4.6: Predictive model for dose absorbed of one and two-sided E-beam as affected by various processing parameters

(applied) at a 41 mm depth (R_{50e}). Therefore, two-sided E-beam is not recommended if surimi seafood thickness exceeds 82 mm.

Fig. 4.7 shows the antimicrobial effect of E-beam. The simulation of microbial inactivation is based on a sample thickness of 82 mm, a target (applied) dose of 3 kGy, and a $D_{E\text{-}beam}$-value for *S. aureus* equal to 0.34 kGy. Since the antimicrobial effect of E-beam depends on electron penetration, the two-sided E-beam processing yields better results. Consequently, one and two-sided E-beam processing is not recommended for surimi seafood thicker than 33 and 82 mm, respectively. Simulation of microbial inactivation for samples thicker than 82 mm results in under-processing starting at 41 mm depth. For surimi seafood processing by E-beam, development of the "cold spot" for the depth 41 mm may be analogous to microwave or radio frequency. However, it is obvious that E-beam processing does not involve heat. The analogy refers only to under-processing, which results in elevated microbial survival in certain parts of the product.

ACKNOWLEDGMENTS

This research was partially funded by the NOAA Office of Sea Grant and Extramural Programs, U.S. Department of Commerce, under grant number NA76RG0476 (project number R/SF-27-PD), and by appropriations made by the Oregon State legislature. The views expressed herein do not necessarily reflect the views of any of those organizations. The U.S. government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. Authors would also like to express sincere appreciation to IBA (San Diego, Cal) and National Fisheries Institute (NFI) for their generous corporation and financial support toward our e-beam studies.

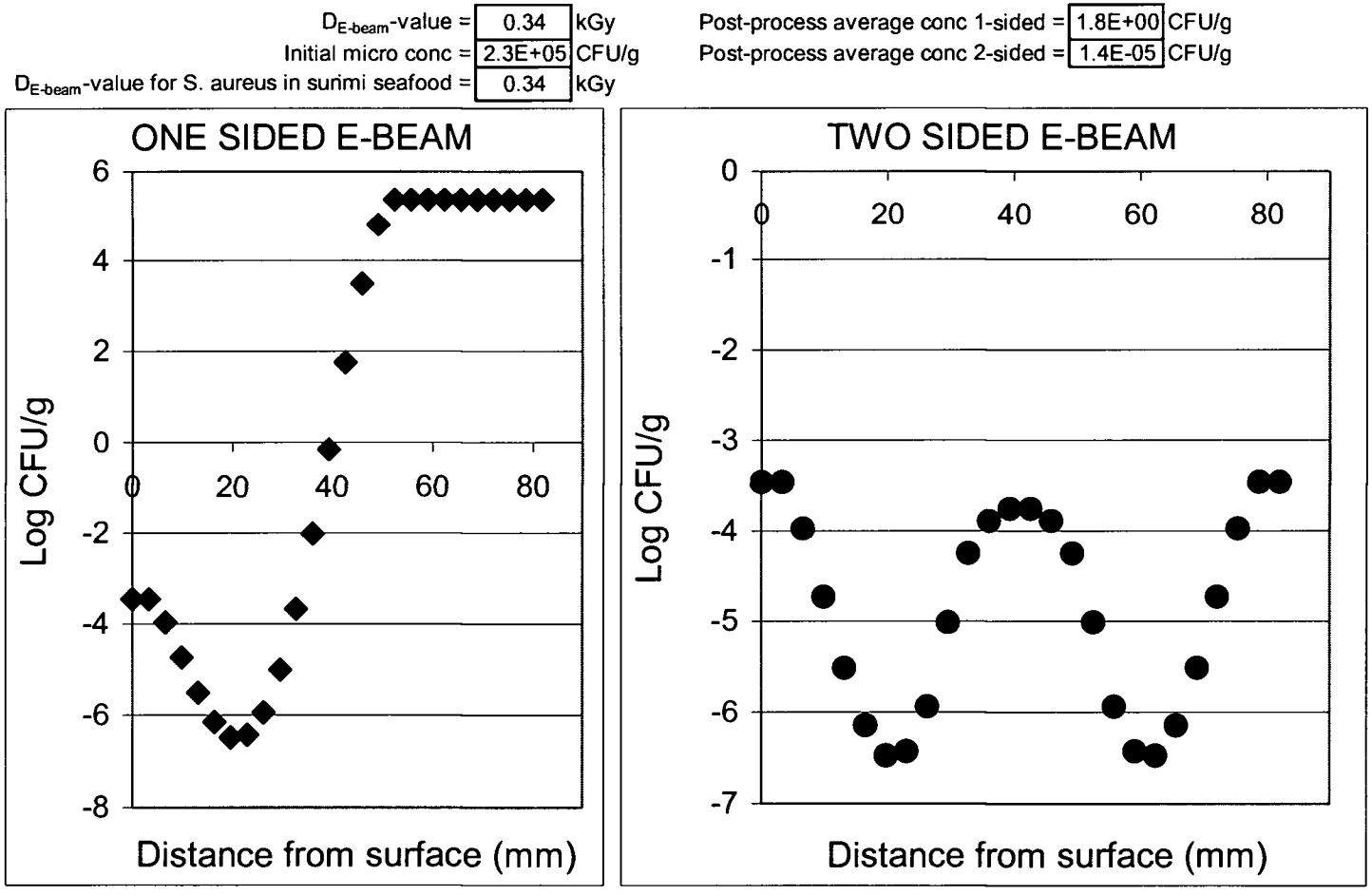

Figure 4.7: Predictive model for microbial inactivation by one and two-sided E-beam as affected by various processing parameters

GELATION PROPERTIES OF FISH PROTEINS AS AFFECTED BY ELECTRON BEAM

ABSTRACT

Alaska pollock surimi and surimi gels were treated with various e-beam doses to elucidate textural changes. Shear stress of surimi gels increased as dosage increased up to 6-8 kGy and then decreased at higher doses (up to 10 and 25 kGy). SDS-PAGE showed gradual degradation of myosin heavy chain (MHC) as dose increased. Degradation rate was slower when frozen samples were treated. The integrity of actin (AC) was not affected by e-beam. Sulfhydryl (SH) groups and surface hydrophobicity of raw surimi decreased as dose increased, indicating the formation of disulfide (SS) bonds and hydrophobic interactions. The SH and hydrophobicity of surimi gels increased as dose increased up to 6 kGy, suggesting hydrophobic interactions were more important than SS bonds in enhancing firmness of gels.

INTRODUCTION

An important technological property of proteins is gel-forming ability (Lanier 2000). Gelation is the result of protein denaturation, leading to inter- and intramolecular covalent and non-covalent interactions, including disulfide bonds and hydrophobic interactions (Lee and Lanier 1995). It has been pointed out that the denaturation process is a prerequisite for the gelation of meat paste. It also has been widely accepted that myofibrillar proteins, particularly actomyosin and

myosin, play an important role in the thermal gelation of meat paste (Ziegler and Acton 1984).

The gel network formed during thermal processing is responsible for texture development. According to Ferry (1948), denaturation and aggregation are critical steps in heat-induced gelation. If more time is allowed for aggregation at proper temperature, with respect to denaturation, heat-denatured proteins line up in an orderly manner to form a better gel network. Thermal gelation may be explained as a two-stage process: native protein → denatured protein → associated protein (Ziegler and Acton 1984). Therefore, in order to understand texture development as a result of protein gelation, factors affecting protein-protein interactions, such as disulfide bonds and hydrophobic interactions of myosin and actomyosin, have to be investigated.

The surimi seafood industry takes advantage of the heat-induced gelation of fish proteins. During the crabstick manufacturing process, surimi paste is continuously extruded as a thin sheet (1-2 mm) and cooked for 50-100 sec on a belt/drum/ohmic cooker in order to develop the initial texture (Park 2000). Final texture is developed during the thermal pasteurization step (Park 2000).

Park (1994, 2001) surveyed pasteurization practices in the US surimi seafood industry. He found great variation with regard to time-temperature regimes. Some treatments may not be appropriate for assuring microbial safety while others may be abusive for physicochemical properties. Shie and Park (1999) additionally reported that excessive thermal processing caused degradation of color and texture of surimi seafood products. In general, thermal processing results in detrimental physicochemical changes (Bertak and Karahadian 1995).

Like other processed food industries, the seafood industry actively seeks non-thermal pasteurization techniques that would assure maximum retention of physicochemical properties while simultaneously assuring the microbial safety. Electron beam has proven effective in both the meat and fruit industries. A Joint FAO/IAEA/WHO (Food and Agriculture Organization / International Atomic

Energy Agency / World Health Organization) Expert Committee on irradiated foods has recommended unconditional acceptance of foods irradiated at ≤ 10 kGy (Thakur and Singh 1995). E-beam, unlike gamma radiation, does not rely on radioisotopes (Co^{60} or Cs^{137}) to generate ionizing energy. Instead, it uses regular electricity. Therefore, e-beam is more consumer friendly than gamma radiation in its application for food processing.

Recently, we reported that e-beam could effectively inactivate *Staphylococcus aureus* in surimi seafood (Jaczynski and Park 2002a). E-beam also showed superior retention of color and texture as compared to thermal pasteurization.

Venugopal and others (1999) suggested that during radiation treatment, a large portion of energy absorbed by proteins induces denaturation, however, much less is absorbed than with heating. Scission of peptide and disulfide bonds as a result of irradiation may then lead to protein aggregation (WHO 1994). Diehl (1995) reported that globular proteins irradiated in dilute solutions could undergo aggregation. Kumta and Gore (1970) also reported a similar observation for fish proteins. In addition, Taub and others (1979) suggested that, depending upon the dose, irradiation of meat proteins may induce such reactions as deamination, decarboxylation, reduction of SS linkages, oxidation of SH groups, decomposition of amino-acid side-group, and increase or decrease of peptide linkages. Whiting and Richards (1971) reported the increased shear resistance of chicken muscle by gamma irradiation. However, reports on the effects of e-beam on the texture properties of surimi seafood as a result of protein gelation are not available.

Our objective was to determine the fracture texture properties of surimi gels treated with various e-beam doses. Additional attempts were also made to understand protein-protein interactions in un-cooked fish proteins and surimi gels.

MATERIALS AND METHODS

Sample preparation

Surimi gels

Frozen Alaska pollock surimi was tempered and cut into small chunks. Surimi chunks were chopped in a universal food processor (Model UM5, Stephan Machinery Corp., Columbus, OH) at low speed for 1 min. Salt (2 %) was added and the surimi paste was chopped at low speed for 0.5 min. Final moisture was adjusted to 78% by ice, followed by chopping at low speed for 1 min. Vacuum (0.5 bar) and chopping at high speed was applied for 3 min. Temperature was kept below 5°C. The paste was then stuffed into gel molds, which resulted in hourglass-shaped surimi gels (length = 2.9 cm, end diameter = 1.9 cm, and minimum diameter = 1.0 cm) and cooked at 90°C for 15 min,. Samples were vacuum packed, frozen at –30°C and shipped to the e-beam facility (IBA, San Diego, CA).

Fish proteins

Alaska pollock surimi blocks were thawed to allow cutting into blocks 2.5 x 10 x 10 cm (height x width x depth). The blocks were vacuum packed, frozen at –30°C, and shipped to the e-beam facility (IBA, San Diego, CA).

E-beam treatment

Half of the surimi gels and Alaska pollock surimi blocks were thawed and treated after equilibrating to room temperature (23°C). The other half was treated

while still frozen at –18°C. Surimi gels and surimi blocks were treated at 0, 1, 2, 4, 6, 8, 10, and 25 kGy by one-sided e-beam at 10 MeV. Following treatment, all samples were frozen at –18°C and shipped to the OSU Seafood Laboratory (Astoria, OR) for analyses. The experimental outline is shown in Fig. 1.

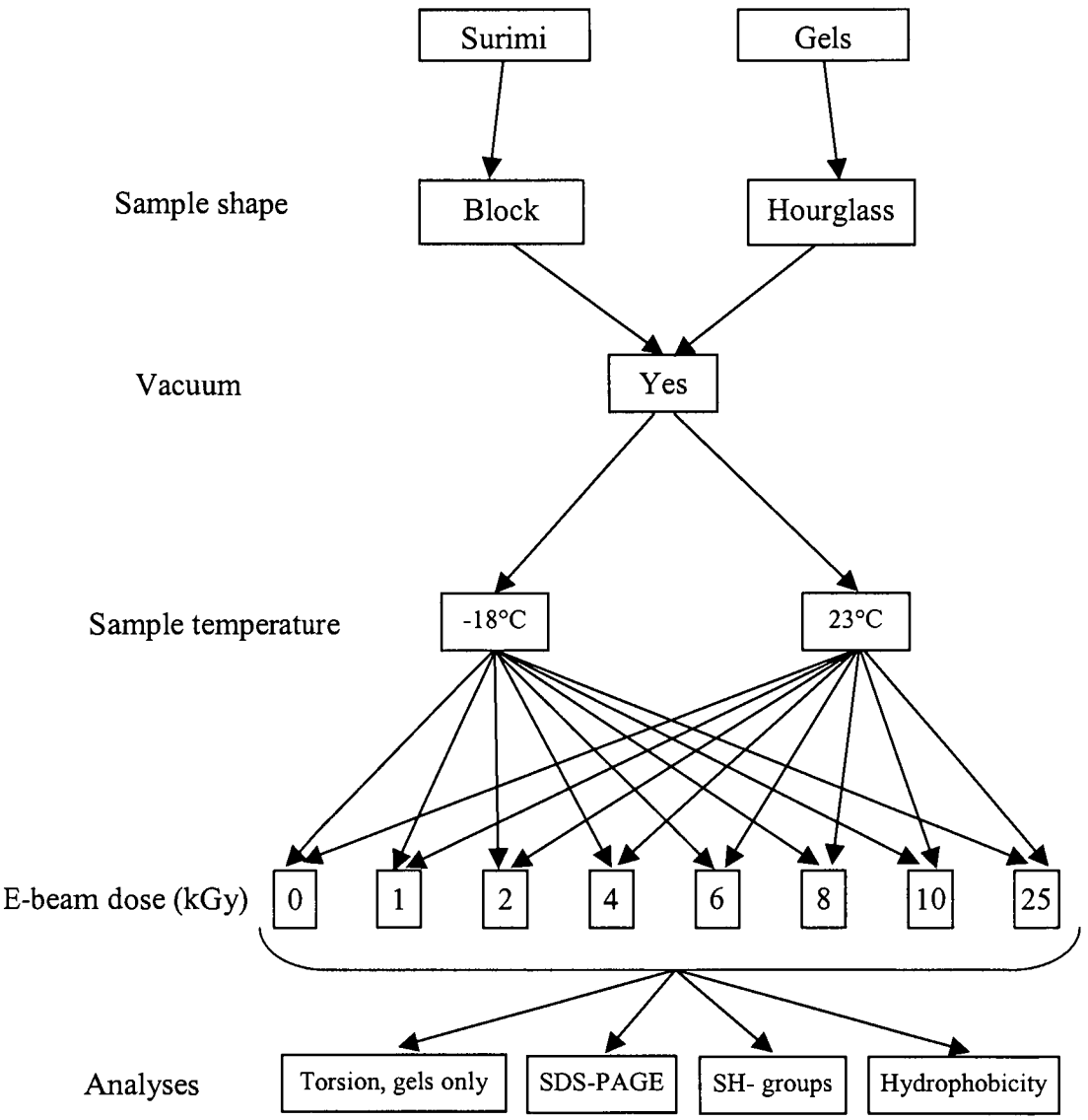

Figure 5.1: Experimental outline

Fracture stress and strain by torsion

Surimi gels were kept at room temperature for 2 hr prior to measurement. Hourglass-shaped gels were glued to plastic discs and subjected to torsional shear using a Hamman Gelometer (Gel Consultant, Raleigh, NC) set at 2.5 rpm. Shear stress and shear strain at mechanical fracture was measured to determine gel strength and gel cohesiveness, respectively (Hamann 1983).

SDS-PAGE electrophoresis

Protein preparation

Surimi (3 g) or surimi gel samples (3 g) was solubilized in 27 mL of 5 % sodium dodecyl sulfate (SDS) solution according to Morrissey and others (1993). Collected supernatant from protein solubilization was analyzed for protein concentration by the Lowry assay (Lowry and others 1951). Samples were diluted by 50-fold so that residual SDS did not interfere with the Lowry assay.

Based on the Lowry assay, protein concentration was adjusted to 2 mg/mL and then mixed with 5x sample buffer (1 M Tris-HCl (pH 6.8), 50 % glycerol, 10 % SDS, 14.4 mM β-mercaptoethanol, 1 % bromophenol blue, and ddH_2O), followed by heating at 90°C for 5 min (Bollag and others 1994). Aliquots of 12.5 μL (25 μg) of proteins per well, prepared as described above, were used for SDS-PAGE.

SDS-PAGE

Continuous (3 % separating gel) and discontinuous (12 % separating and 5 % stacking gel) SDS-PAGE under denaturing conditions at 200 mA of constant current were performed according to the method of Bollag and others (1994). Surimi and surimi gels treated with e-beam were analyzed for protein degradation/polymerization. The electrophoretic patterns of proteins were stained with Coomassie brilliant blue R-250 (Bio-Rad, Richmond, CA), followed by destaining with solution containing 25 % ethanol and 10 % acetic acid. GelBond PAG film (FMC Bioproducts, Rockland, ME) was used to support the 3 % gel.

Sulfhydryl groups and hydrophobicity measurements

Protein preparation

Solubilization of surimi (8 g) and surimi gel samples (8 g) was carried out in 22 mL of 20 mM Tris-HCl (pH 7.0) containing 0.6 M KCl using a PowerGen 700 GLH homogenizer (Fisher Scientific, Pittsburgh, PA) set at highest speed for 1 min. The homogenates were centrifuged in an Aristocrat 7020 centrifuge (Phillips-Drucker, Astoria, OR) at 2300 rpm for 15 min to remove insoluble proteins. During the solubilization and centrifugation the temperature was below 5°C.

Clear supernatant (20 mL) was collected and protein concentrations were determined using the Bradford dye-binding method. Bio-Rad dye reagent concentrate (5 mL reagent diluted with ddH_2O 1:4)(Bio-Rad, Richmond, CA) was mixed with 100 μL of supernatant (diluted as needed) and absorbance at 595 nm was measured with a Beckman DU640 spectrophotometer (Beckman Instruments, Richmond, WA). Based on the Bradford assay, protein concentration was adjusted to 1 mg/mL in all solubilized surimi and surimi gel samples. This concentration

was used for determination of reactive and total SH groups as well as for determination of surface hydrophobicity.

Total and reactive (surface) SH groups

Reactive (surface) and total SH groups were determined by the Ellman's method (1959). For determination of reactive SH groups, an aliquot (50 μL) of Ellman's reagent (10 mM 5,5'-Dithio-bis-(2-nitrobenzoic acid)) was added to 2.75 mL of protein solution. The mixture was set at 5°C for 1 hr (Sompongse and others 1996). The amount of reactive SH was measured at 412 nm with a molar extinction coefficient of 13600 $M^{-1}cm^{-1}$ using a Beckman DU640 spectrophotometer (Beckman Instruments, Richmond, WA).

For determination of total SH groups, 0.25 mL of protein solution was mixed with 2.5 mL of 0.1 M phosphate buffer (pH 8.0) containing 8 M urea. An aliquot (50 μL) of Ellman's reagent was then added to the mixture and set in a water bath at 40°C for 15 min. The amount of total SH was measured at 412 nm with a molar extinction coefficient of 13600 $M^{-1}cm^{-1}$ using a Beckman DU640 spectrophotometer (Beckman Instruments, Richmond, WA).

Surface hydrophobicity

Protein surface hydrophobicity was measured with 1-anilino-8-naphthal-enesulfonic acid (ANS)(Alizadeh-Pasdar and Li-Chan 2000). Protein solution (1 mg/mL) was serially diluted to 0.1, 0.3, and 0.5 mg/mL in 20 mM Tris-HCl (pH 7.0) containing 0.6 M KCl. Aliquots (20 μl) of 8 mM ANS were added to 4 mL diluted protein samples (Careche and Li-Chan 1997). After vortexing, the mixtures were sealed from light and incubated at 20°C for 15 min. The relative fluorescence

intensity was measured with a Perkin Elmer luminescence spectrometer LS50B (Perkin Elmer, Norwalk, CT) using a 1cm cell, excitation and emission wavelengths at 375 and 485 nm, respectively, excitation and emission slit widths of 5 nm each (Monahan and others 1995). Measurements were taken at room temperature (20°C). The surface hydrophobicity of solubilized surimi and surimi gel samples was calculated as the slope of the relative fluorescence intensity vs. protein concentration (Monahan and others 1995).

RESULTS AND DISCUSSION

Fracture shear stress and strain

A Joint FAO/IAEA/WHO Expert Committee on irradiated foods expressed unconditional acceptance of foods irradiated at ≤ 10 kGy (Thakur and Singh 1995). Shear stress of surimi gels subjected to the torsion test increased proportionally to e-beam dose up to 6-8 kGy, and then gradually decreased (Fig. 5.2). The texture of raw Alaska pollock surimi was not measured. Shear stress and strain indicate the strength and cohesive nature of surimi gels, respectively (Park and others 1994). Shear strain did not show a significant change (data not shown). Therefore, the results suggest that e-beam treatment (up to 6-8 kGy) improved gel firmness, however, cohesiveness remained unchanged. The firmness enhancment was even more pronounced when frozen samples where subjected to e-beam (Fig. 5.2).

When irradiated red hake mince was subsequently prepared for surimi gels, both compressive and penetration force decreased depending upon the dose (Dymsza and others 1990). These results were opposite to ours. In our studies, however the gels were treated with e-beam after the paste was cooked. This critical difference may be accounted for the discrepancy between our results and theirs. Fish proteins in mince

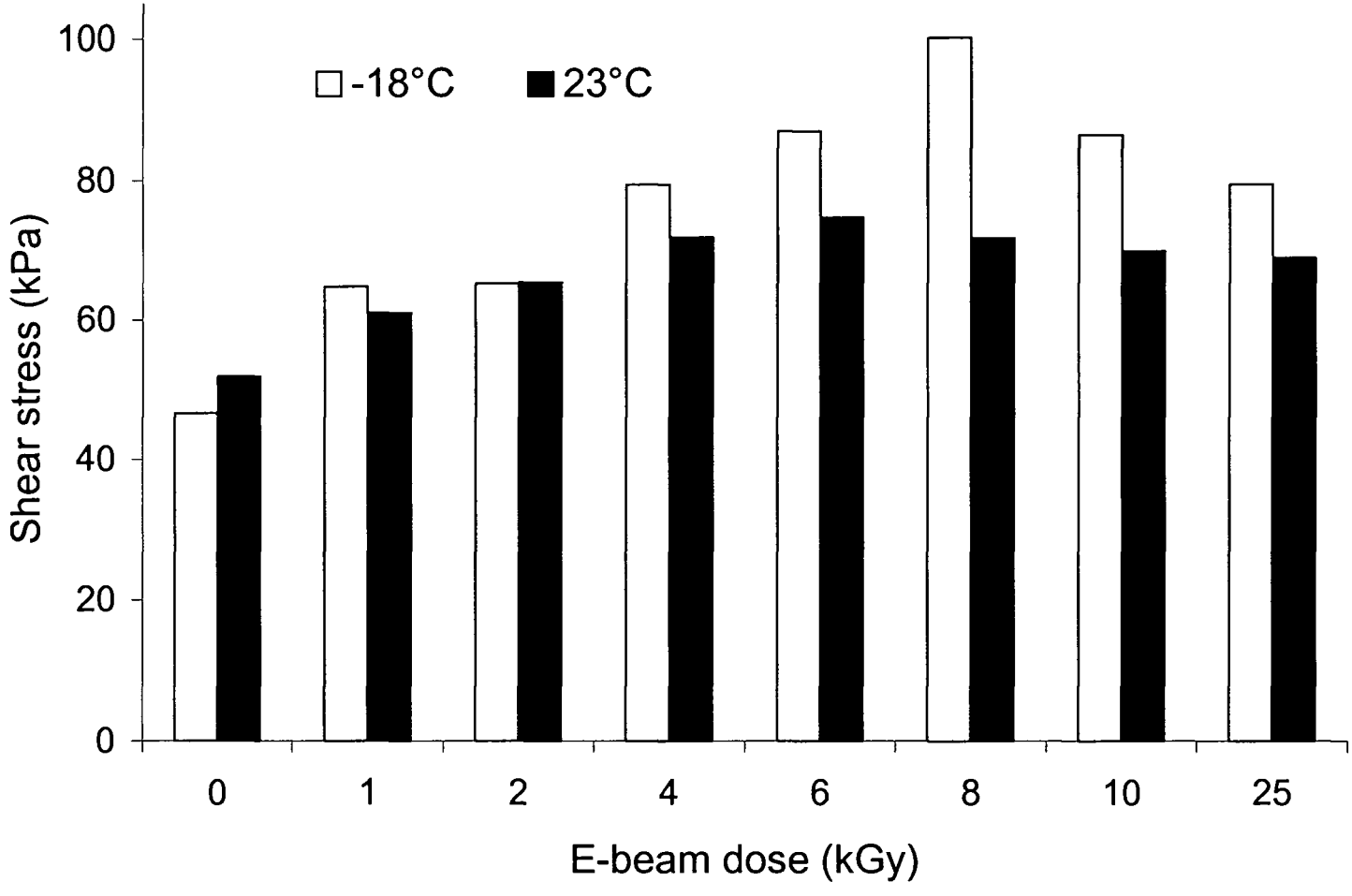

Figure 5.2: Effect of surimi gel temperature during e-beam treatment on shear stress

might have been partially denatured by radiation, therefore, decreasing the gel-forming ability upon subsequent heating.

Dagbjartsson and Solberg (1973) applied gamma radiation to precooked lobster and observed increased firmness. Whiting and Richards (1971) reported an increase in shear resistance (Kramer shear press) of chicken muscle treated with gamma radiation. These studies showed a similar trend of increased firmness, which concurs with our data.

Similar textural changes were also reported on other muscle foods irradiated with ultra violet light. Taguchi and others (1989) observed increased strength of sardine, beef, and pork surimi gels when subjected to UV radiation. Jiang and others (1998) and Ishizaki and others (1993) reported similar results for mackerel surimi actomyosin, and for sardine and pork pastes.

SDS-PAGE

The SDS-PAGE in 12 % polyacrylamide gel (Fig. 5.3) showed gradual degradation of myosin heavy chain (MHC) that is proportional to the increase of e-beam dose. Gradual disappearance of MHC resulted in a subsequent increase of smaller molecular weight proteins (200 to 50 kDa) that appeared in each lane below MHC. The complete disappearance of the MHC band was observed at 25 kGy for raw surimi and surimi gels irradiated while at 23°C. However, samples treated with 25 kGy while at –18°C showed a very thin MHC band, suggesting slower degradation at the lower temperature. Actin (AC) and other fractions of myofibrillar proteins were not less affected by e-beam than MHC. The electrophoresis was conducted under denaturing conditions of SDS and β-mercaptoethanol (β-ME)(Bollag and others 1994).

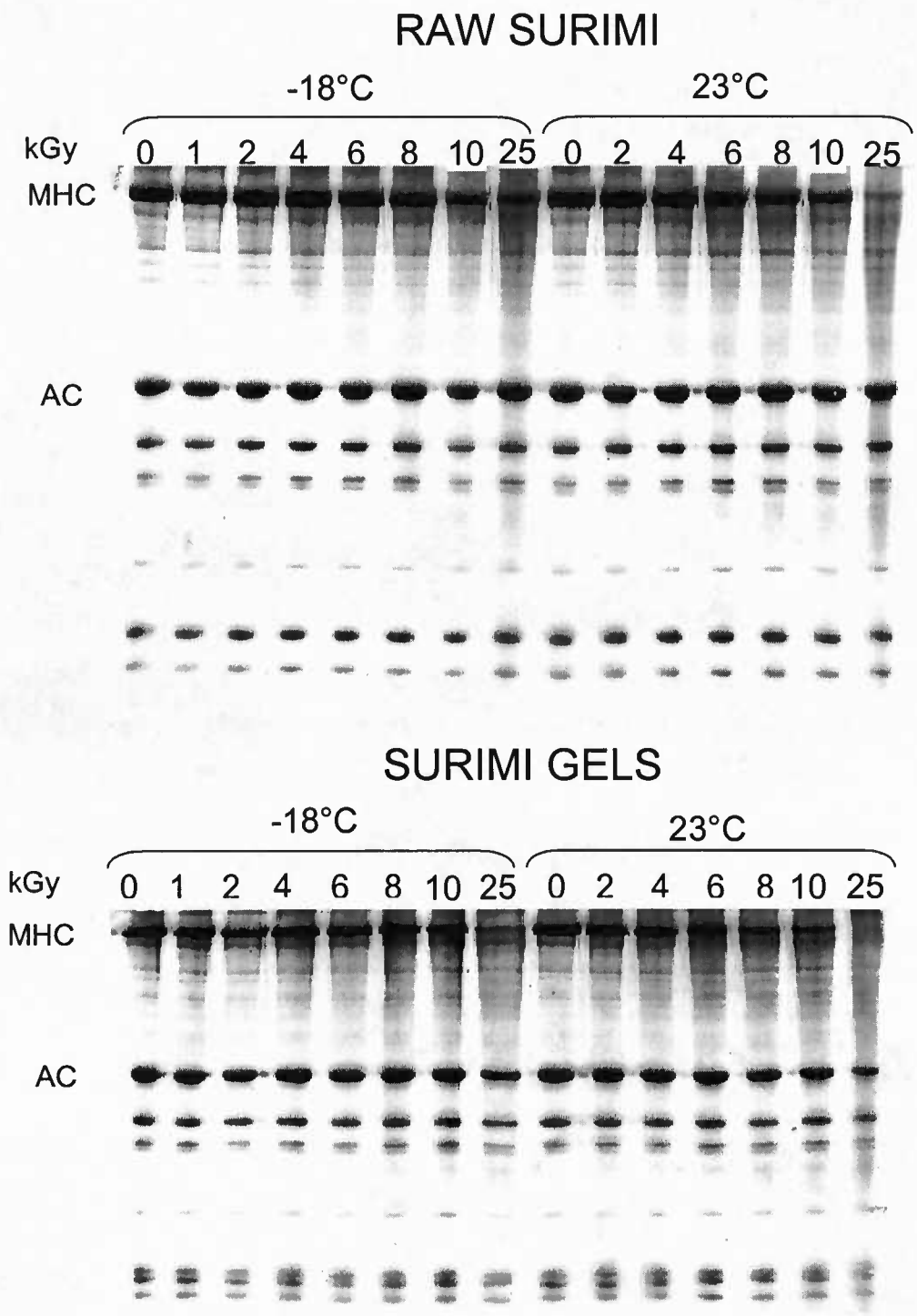

Figure 5.3: SDS-PAGE of surimi (top) and surimi gels (bottom) in 12 % gel. Sample temperature -18°C (left) and 23°C (right). MHC–myosin heavy chain, AC–actin.

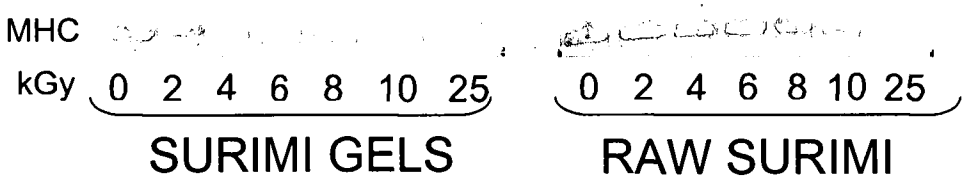

Figure 5.4: SDS-PAGE of surimi (right) and surimi gels (left) in 3 % gel. Sample temperature 23°C during e-beam treatment. MHC–myosin heavy chain.

If e-beam had induced cross-linking by disulfide bonds or hydrophobic interactions they would have not been detected due to cleavage of those bonds by β-ME and SDS, respectively. However, cross-linking involving means other than disulfide bonds or hydrophobic interactions would have been detected. Therefore, to verify the presence of high molecular weight polymers formed by non-disulfide covalent bond, SDS-PAGE in 3 % gel was carried out (Fig. 5.4)(Bollag and others 1994). However, the results showed no presence of high molecular weight polymers. If

MHC had polymerized, the polymers would have been visible. Therefore, it is suggested that e-beam degraded MHC and did not induce polymerization by means other than disulfide bonds or hydrophobic interactions.

Taub and others (1979) conducted SDS-PAGE using gamma irradiated (at various doses) and non-irradiated myosin samples. They reported a gradual disappearance of the band associated with the main peptide chain (210 kDa) as the dose increased. At the same time, there was no increase in bands corresponding to low-molecular weight fragments. They also observed a slower rate of myosin degradation when frozen samples were exposed to radiation. These observations were similar to ours, suggesting that e-beam and gamma radiation have a similar effect on myofibrillar proteins.

Jiang and Tsai (1998) investigated the cross-linking of mackerel actomyosin as affected by UV radiation using discontinuous (7.5 % separating and 3.75 % stacking gel) SDS-PAGE under denaturing conditions (10 % SDS and 5 % β-ME). Similar to our observations, they reported the disappearance of the MHC band. Ishizaki and others (1994) reported similar effects of UV radiation on the disappearance of MHC in flying fish. These findings also suggest that e-beam and UV radiation may have similar effects on myofibrillar proteins.

SH groups and hydrophobicity of raw surimi

Reactive (surface) and total SH groups in Alaska pollock surimi decreased as e-beam dose increased (Fig. 5.5). A similar phenomenon has been reported for thermally treated myofibrillar protein solutions (Itoh and others 1979, 1980). Therefore, it is suggested that e-beam, similar to heat, may oxidize SH, leading to the formation of SS bonds. Ishizaki and others (1993) observed the same trend in fish and pork proteins irradiated with UV, suggesting that UV radiation, similarly to e-beam and heat, induced oxidation of SH groups.

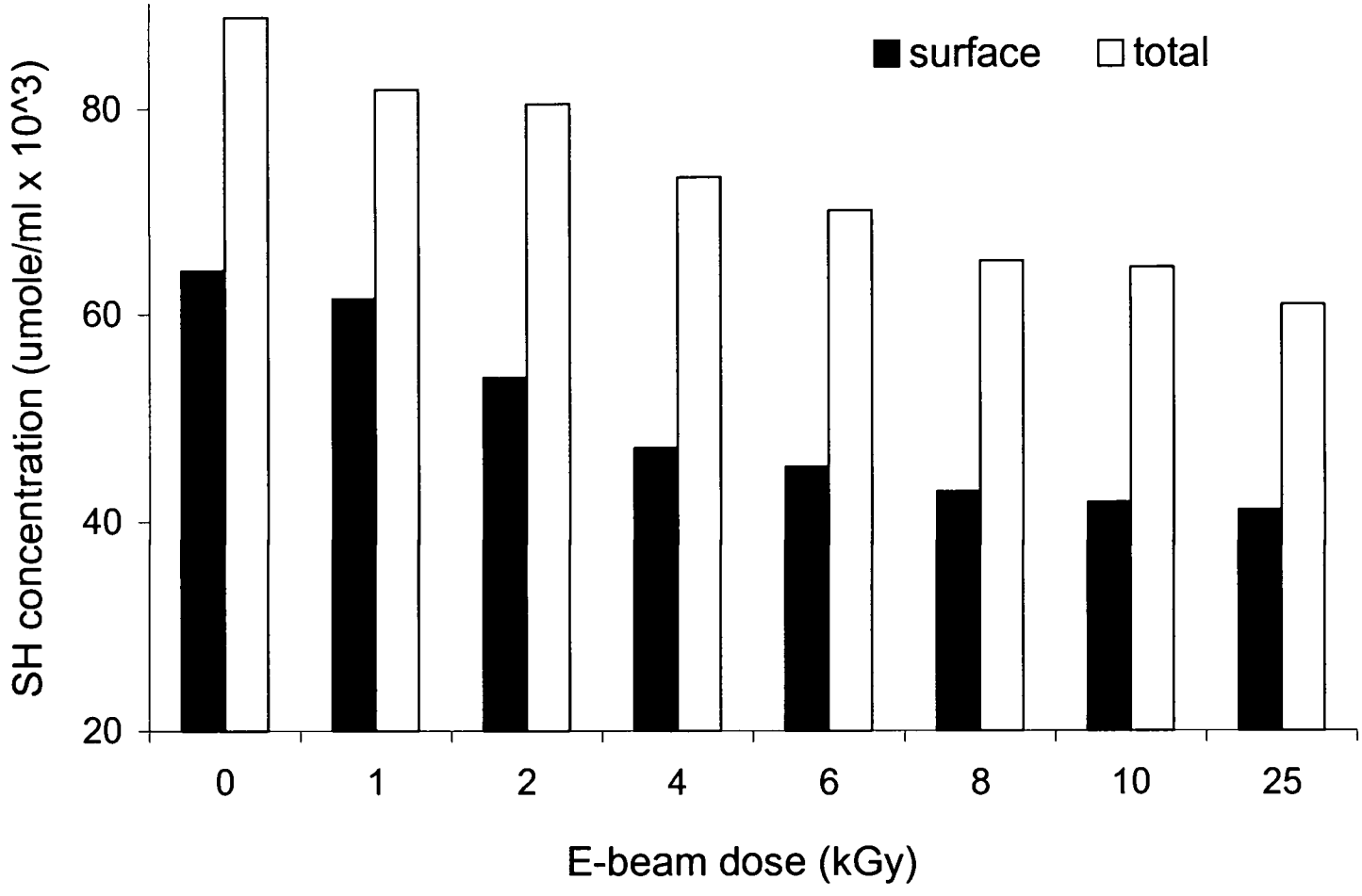

Figure 5.5: Total and reactive SH groups of Alaska pollock surimi as affected by e-beam

Surface hydrophobicity was measured using an ANS probe, which binds to the hydrophobic regions of proteins. Surface hydrophobicity of Alaska polock surimi decreased as the e-beam dose increased (Fig. 5.6). This suggests that the hydrophobic regions on the protein molecules interacted with each other via hydrophobic interactions.

Radiation, although to a lesser extent than heat, has been reported to induce protein denaturation (Venugopal and others 1999). Therefore, denaturation induced by e-beam led to unfolding of the fish proteins, exposing hydrophobic regions buried in the interior of protein structure. This increased negative entropy of the protein system corresponds to positive free energy that was thermodynamically un-favorable. Therefore, formation of hydrophobic interactions was favored as a way of reducing the free energy and thus stabilizing the protein system. Ishizaki and others (1993, 1994) reported similar observations for fish and pork muscles, and flying fish myosin, respectively, treated with UV radiation.

SH groups and hydrophobicity of surimi gels

The surimi gels, unlike raw surimi, were cooked prior to e-beam treatment. Therefore, the proteins were already arranged in a heat-induced gel matrix. Reactive (surface) and total SH groups in surimi gels increased with the dose increase (up to 6 kGy) and then a gradual decrease was measured (Fig. 5.7). The reactive SH content increase (up to 6 kGy), was most likely due to protein unfolding and scission of the SS bonds that were formed during heating prior to e-beam processing. Unfolding induced exposure of SH groups buried in the protein interior. Ishizaki and others (1993) concluded that increase in reactive (surface) SH of sardine and pork muscle proteins treated with UV radiation was possibly due to protein unfolding. Urea (8M), however, was used to completely unfold the proteins

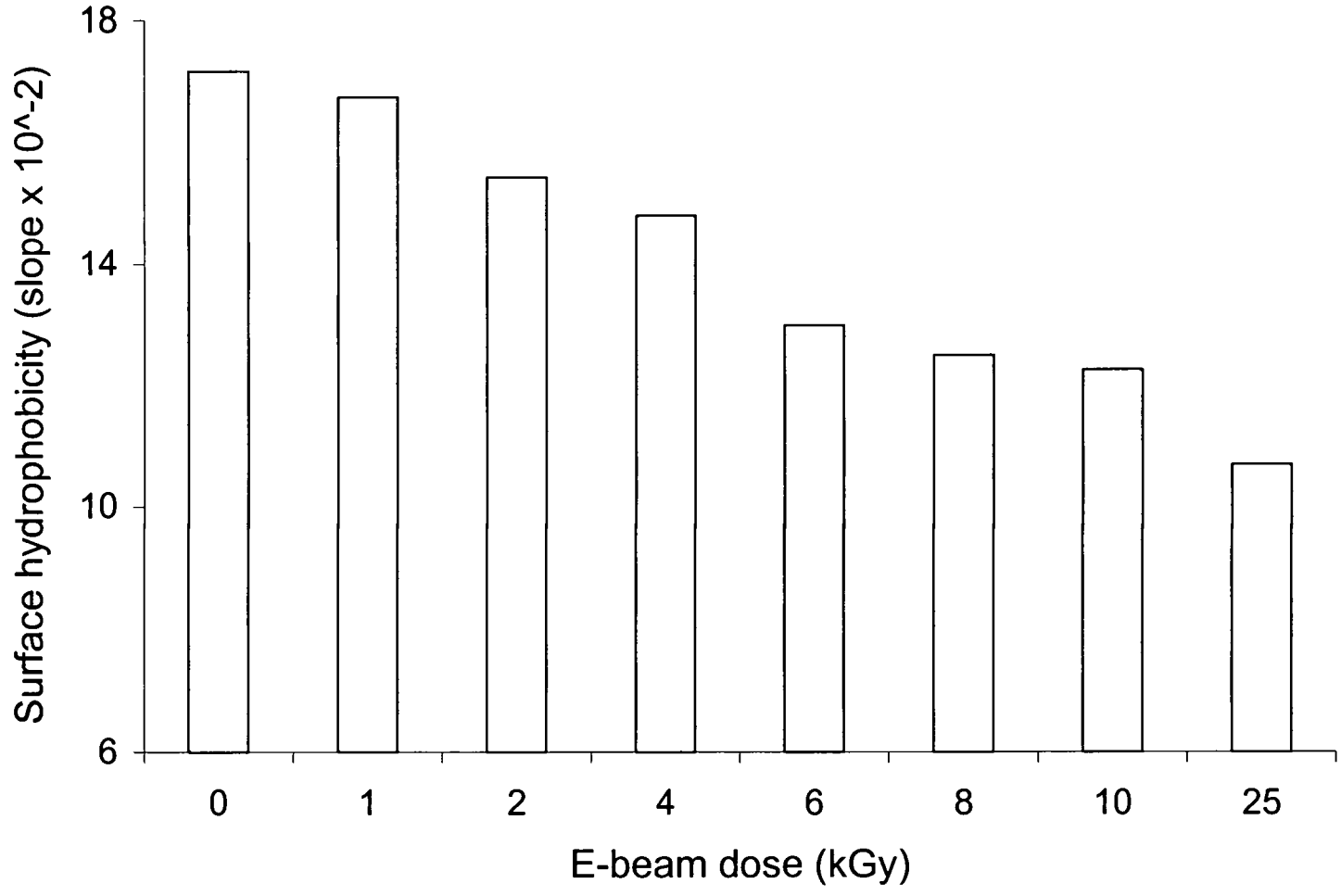

Figure 5.6: Surface hydrophobicity of Alaska pollock surimi as affected by e-beam

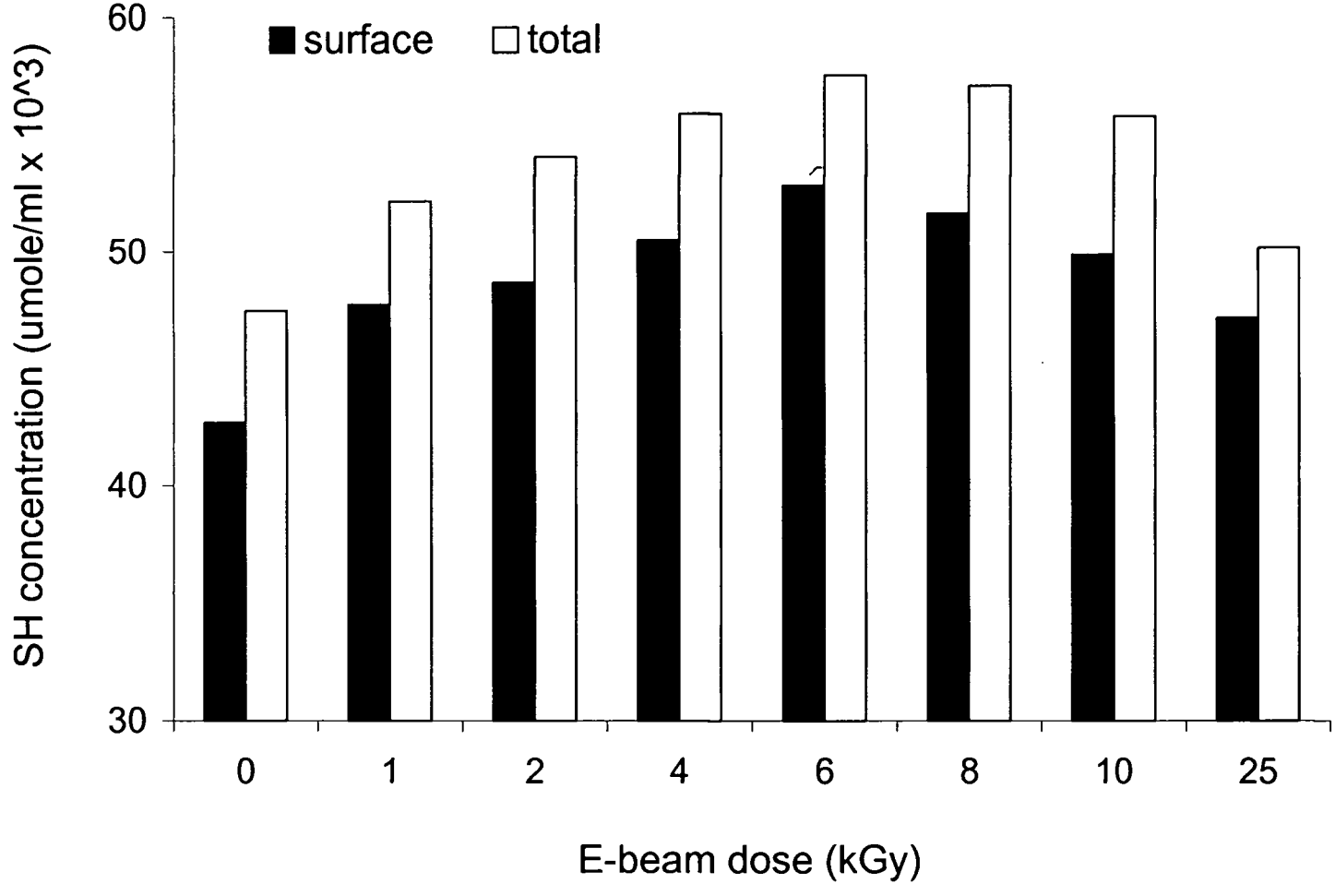

Figure 5.7: Total and reactive SH groups of surimi gels as affected by e-beam

during analysis of total SH. Therefore, protein unfolding may be excluded as a contributing factor to increased total SH. Additionally, Taub and others (1979) reported that reduction of disulfide bonds in proteins is as frequent with radiation of as with other treatments.

Surface hydrophobicity of surimi gels also increased up to 6 kGy (Fig. 5.8), and then decreased. Even though proteins were already heat-gelled prior to e-beam treatment, e-beam appeared to induce further unfolding, revealing hydrophobic regions buried in the protein interior. These regions interacted with each other contributing to firmer surimi gel texture (Fig. 5.2). At the same time a rigid, heat induced-gel network was already present. Most likely that rigid structure did not allow all hydrophobic regions to participate in the interactions, which contributed to higher surface hydrophobicity (Fig. 5.8). Ishizaki and others (1993, 1994) also concluded that hydrophobic interactions may have been major contributor to gel networking in myofibrillar proteins exposed to UV radiation.

CONCLUSIONS

E-beam up to 6-8 kGy enhanced firmness of surimi gels. E-beam induced degradation of MHC in raw surimi and surimi gels. The degradation was proportional to the dose increase and was slower when frozen samples were subjected to e-beam. However, the integrity of actin was not affected by e-beam treatment. E-beam induced the formation of disulfide bonds and hydrophobic interactions in raw Alaska pollock surimi. However, in surimi gels, scission of disulfide bonds rather than their formation was observed. Gel firmness, therefore was most likely enhanced by hydrophobic interactions.

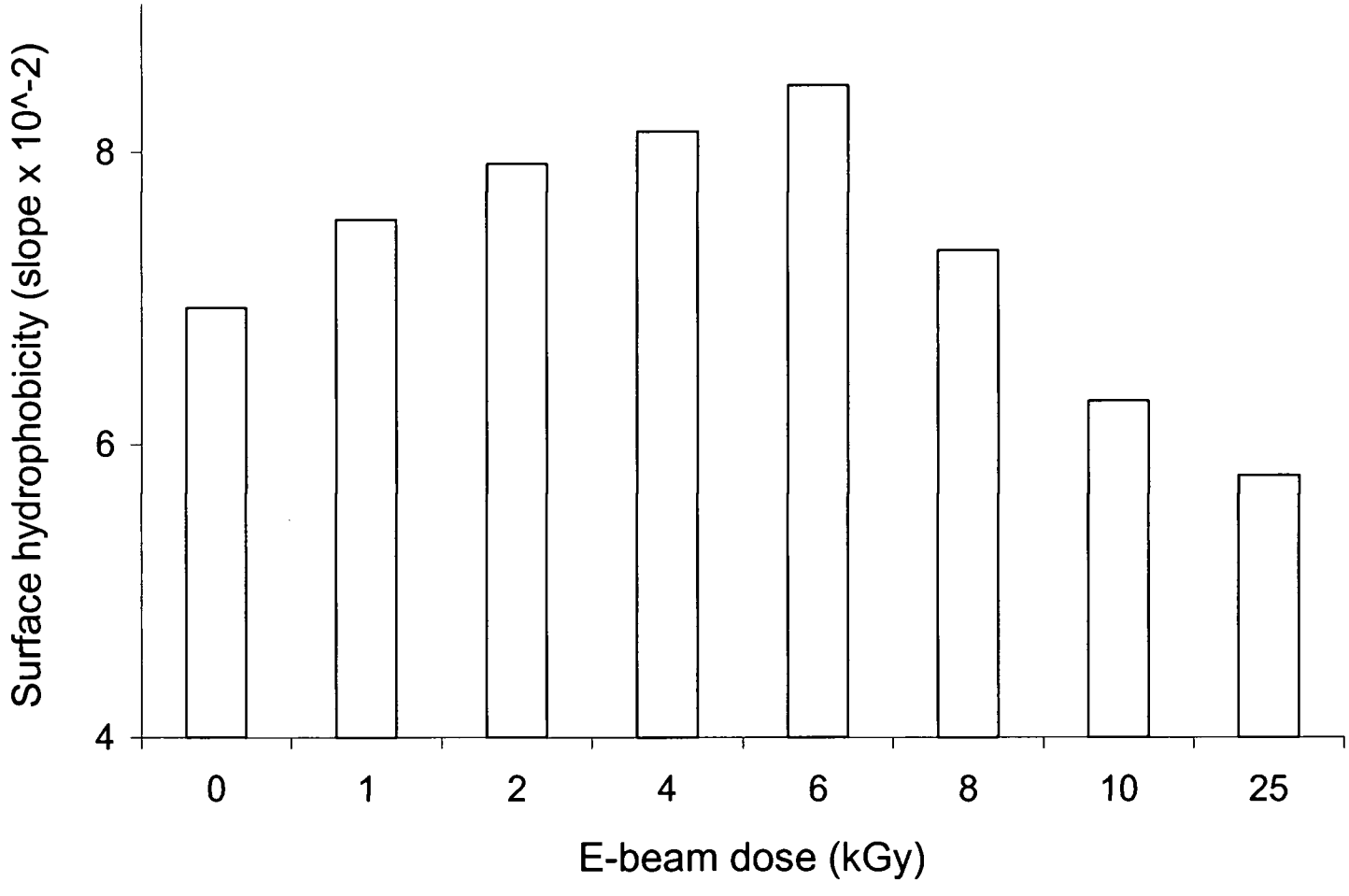

Figure 5.8: Surface hydrophobicity of surimi gels as affected by e-beam

ACKNOWLEDGMENTS

This research was partially funded by the NOAA Office of Sea Grant and Extramural Programs, U.S. Department of Commerce, under grant number NA76RG0476 (project number R/SF-27-PD), and by appropriations made by the Oregon State legislature. The views expressed herein do not necessarily reflect the views of any of those organizations. The U.S. government is authorized to produce and distribute reprints for governmental purposes notwithstanding any copyright notation that may appear hereon. Authors would also like to express sincere appreciation to IBA (San Diego, Cal) and National Fisheries Institute (NFI) for their generous corporation and financial support toward our e-beam studies.

BIBLIOGRAPHY

AbuDagga Y, Kolbe E. 1997. Thermophysical properties of surimi paste at cooking temperatures. J Food Eng 32(3):325-337.

Adams CE. 1991. Applying HACCP to sous vide products. Food Technol 45 (4):148-151.

Alizadeh-Pasdar N, Li-Chan ECY. 2000. Comparison of protein surface hydrophobicity measured at various pH values using three different fluorescent probes. J Agric Food Chem 48:328-334.

Allen DW, Leathard DA, Smith C. 1988. The effects of gamma irradiation of food contact plastics on the extent of migration of hindered phenol antioxidants into fatty food simulants. Chem Indust 12:399-406.

Ama AA, Hamdy MK, Toledo RT. 1994. Effects of heating, pH and thermoradiation on inactivation of *Vibrio vulnificus*. Food Microbiol 11:215-227.

Applebaum RS. 2002. Food industry response to terrorist attack. Food Technol 55(11):100.

Bertak JA, Karahadian C. 1995. Surimi-based imitation crab characteristics affected by heating method and end point temperature. J Food Sci 60(2):292-296.

Bollag DM, Rozycki MD, Edelstein SJ. 1994. Protein methods. 2nd ed. New York: A John Wiley and Sons. 415 p.

Brandao AH, Charlonneau R, Thibault C. 1995. Effect of ionizing radiation on *L. monocytogenes* in contaminated shrimp. Sciences des Aliments 15:261-272.

Briley ME. 1992. Sous-vide processed foods: are they safe for the elderly? Journal of Nutrition for the Elderly 11(3):45-52.

Brody AL. 1998. Minimally processed foods demand maximum research and education. Food Technol 52(5):62-66.

Bruhn CM. 1995. Consumer attitudes and market response to irradiated food. J Food Prot 58:175-181.

Byun MW, Kwon OJ, Yook HS, Kim KS. 1998. Gamma irradiation and ozone treatment for inactivation of *Escherichia coli* O157:H7 in culture media. J Food Prot 61(6):728-730.

Careche M, Li-Chan ECY. 1997. Structural changes in cod myosin after modification with formaldehyde or frozen storage. J Food Sci 62(4):717-722.

Carslaw HS, Jaeger JC. 1959. Conduction of heat in solids. 2nd ed. London: Oxford University Press. 510 p.

Chang HC, Carpenter JA, Toledo RT. 1998. Modeling heat transfer during oven roasting of unstuffed turkeys. J Food Sci 63(2):257-261.

Charley H. 1982. Food Science. 2nd ed. New York: John Wiley and Sons. 564 p.

Chen YP, Andrews LS, Grodner RM. 1996. Sensory and microbial quality of irradiated crabmeat products. J Food Sci 61(6):1239-1242.

Chiheb A, Debray E, Le-Jean G, Piar G. 1994. Linear model for predicting transient temperature during sterilization of a food product. J Food Sci 59(2):441-446.

Cho HY, Yousef AE, Sastry SK. 1996. Growth kinetics of *Lactobacillus acidophilus* under ohmic heating. Biotechnology and Bioengineering 49(3):334-340.

Clavero MRS, Monk JD, Beuchat LR, Doyle MP, Brakett RE. 1994. Inactivation of *Escherichia coli* O157:H7, *Salmonellae*, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. Appl Environ Microbiol 60:2069-2075.

Cockey R, Tarro M. 1974. Survival studies with spores of *Clostridium botulinum* type E in pasteurized meat of the blue crab *Calinectes sapidus*. Applied Micro 27:629-633.

Crouse JD, Koohmaraie M, Dickson JS. 1991. Storage and bacterial contamination effects on myofibrillar proteins and shear force of beef. J Food Sci 56(4):903-905.

Dagbjartsson B, Solberg M. 1973. Textural changes in precooked lobster (*Homarus americanus*) meat resulting from radurization followed by refrigerated storage. J Food Sci 28:165-167.

Diehl JF. 1995. Safety of Irradiated Foods. New York: Marcel Dekker. 454 p.

Dymsza HA, Lee CM, Saibu LO, Haun J, Silverman GJ, Josephson ES. 1990. Gamma radiation effects on shelf life and gel forming properties of washed red hake (*Urophycis chuss*) fish mince. J Food Sci 55(6):1745-1748.

Ehlerman DAE. 1993. Food irradiation: a challenge to authorities. Trends Food Sci Technol 4:184-189.

Ellman GL. 1959. Tissue sulfhydryl groups. Arch Biochem Biophys 82:70-77.

Erdogdu F, Balaban MO, Chau KV. 1998. Modeling of heat conduction in elliptical cross section. II. Adaptation to thermal processing of shrimp. J Food Eng 38(2):241-258.

Eyles MJ. 1986. Microbiological hazards associated with fishery products. Food Res 46:8-16.

Ferry JD. 1948. Protein gels. Adv Protein Chem 4:1-78.

Food and Drug Administration. 1998a. Fish and fishery products hazards and control guide. Center for Food Safety and Applied Nutrition. Washington: National Technical Information Service. 218 p.

Food and Drug Administration. 1998b. The FDA enforcement report. Center for Food Safety and Applied Nutrition. Washington: Food and Drug Administration.

Fryer PJ. 1993. Ohmic processing of solid-liquid mixtures: heat generation and convection effects. J Food Eng 18:101-125.

Gao F, Stewart LE, Joseph SW, Carr LE. 1997. Effectiveness of ultraviolet irradiation in reducing the numbers of *Salmonella* on eggs and egg belt conveyor materials. Applied Engineering in Agriculture 13(3):355-359.

Garcia-Zepeda CM, Kastner CL, Wolf JR, Boyer JE, Kropf DH, Hunt MC, Setser CS. 1997. Extrusion and low-dose irradiation effects on destruction of *C. sporogenes* spores in a beef-based product. J Food Prot 60(7):777-785.

Geankoplis CJ. 1993. Transport processes and unit operation. 3rd ed. Englewood Cliffs: A Simon & Schsuter Company. 921 p.

Ghazala S, Coxworthy D, Alkanani T. 1995. Thermal kinetics of *Streptococcus faecium* in nutrient broth/sous-vide products under pasteurization conditions. Journal of Food Processing and Preservation 19(4):243-257.

Giddings GG. 1984. Radiation processing of fishery products. Food Technol 38(4):61-65, 94-97.

Halden K, De Alwis AAP, Fryer PJ. 1990. Changes in the electrical conductivity of foods during ohmic heating. Int J Food Sci and Technol 25(1): 9-25.

Hamann DD. 1983. Structural failure in solid foods. In: Bagley EB, Peleg M, editors. Physical properties of foods. Westport: AVI Publishing Co.

Hayashi T. 1991. Food Irradiation. London: Elsevier Applied Science. 385 p.

Harlfinger L. 1992. Microwave sterilization. Food Technol 46(12):57-61.
Killoran JJ. 1972. Chemical and physical changes in food packaging material exposed to ionizing radiation. Radiat Res Rev 3:369-376.

Harrison MA, Huang Y. 1990. Thermal death times for *Listeria monocytogenes* (Scott A) in crabmeat. J Food Protect 53:878-880.

Helrich K. 1990. Official methods of analysis of the Association of Official Analytical Chemists. 15th ed. Arlington: Association of Official Analytical Chemists. 1298 p.

Himelbloom B. 1997. Surimi microbiology. In: Park JW, editor. Surimi and surimi seafood. Astoria: OSU Surimi Technology School, Astoria, OR.

Hollingsworth P. 2002. Close watch on America's food supply. Food Technol 55(11):20.

Hsu ST. 1963. Engineering heat transfer. New York: Van Nostrand Reinhold. 613 p.

http://www.cdc.gov/epo/mmwr as seen on November 14th, 2001.

Huang E, Mittal GS. 1995. Meatball cooking - modeling and simulation. J Food Eng 24(1):87-100.

Hyytia-Tress E, Skytta E, Mokkila M, Kinnunen A, Lindstrom M, Lahteenmaki L, Ahvenainen R, Korkeala H. 2000. Safety evaluation of *sous vide*-processed products with respect to nonproteolytic *C. botulinum* by use of challenge studies and predictive microbiological models. Appl Environ Microbiol 66(1):223-229.

Incropera FP, Dewitt DP. 1990. Fundamentals of heat and mass transfer. 3rd ed. New York: John Wiley & Sons. 919 p.

International Commission on Microbiological Specifications for Foods. 1996. Microorganisms in foods. Microbiological specifications of food pathogens. Book 5. London: Blackie Academic and Professional Publ.

Ishizaki S, Ogasawara M, Tanaka M, Taguchi T. 1994. Ultraviolet denaturation of flying fish myosin and its fragments. Fisheries Science 60(5):603-606.

Ishizaki S, Hamada M, Iso N, Taguchi T. 1993. Effect of ultraviolet radiation on rheological properties of thermal gels from sardine and pork meat pastes. Nippon Suisan Gakkaishi 59(7):1219-1224.

Itoh Y, Yoshinaka R, Ikeda S. 1979. Behavior of the sulfhydryl groups of carp actomyosin by heating. Nippon Suisan Gakkaishi 45:1019-1022.

Itoh Y, Yoshinaka R, Ikeda S. 1979. Formation of polymeric molecules of protein resulting from intermolecular SS bonds formed during the gel formation of carp actomyosin by heting. Nippon Suisan Gakkaishi 46:621-624.

Jaczynski J, Park JW. 2002a. *Staphylococcus aureus* inactivation/texture degradation in surimi seafood during thermal processing. Forthcoming.

Jaczynski J, Park JW. 2002b. Physicochemical properties of surimi seafood as affected by electron beam and thermal processing. Forthcoming.

Jaczynski J, Park JW. 2002c. Temperature prediction during thermal processing of surimi seafood. Unpublished data. OSU Seafood Laboratory, Astoria, OR.

Jaczynski J, Park JW. 2000d. Microbial inactivation and electron penetration during e-beam processing of surimi seafood. Unpublished data. OSU Seafood Laboratory, Astoria, OR.

Jaczynski J, Park JW. 2001. Unpublished data. OSU Seafood Laboratory, Astoria, OR.

Jiang ST, Leu SZ, Tsai GJ. 1998. Cross-linking of Mackerel surimi actomyosin by microbial transglutamizase and ultraviolet radiation. J Agric Food Chem 46:5278-5282.

Kebede E, Mannheim C.H. and Miltz, J. 1996. Heat penetration and quality preservation during thermal treatment in plastic trays and metal cans. J Food Eng 30(1/2):109-115.

Kim BY, Park JW. 2000. Rheology and texture properties of surimi gels. In: Park JW, editor. Surimi and surimi seafood. New York: Marcel Dekker, Inc. p 267-324.

Kolbe E, Park JW, Wells JH, Flugstad BA, Zhao Y. 2001. Capacitive dielectric heating system. U.S. Patent 6,303,166. Issued 10-16-01.

Kumta US, Gore MS. 1970. Chemical and physical changes in irradiated and frozen bombay duck. J Food Sci 35:456-460.

Lagunas-Solar MC. 1995. Radiation processing of foods: an overview of scientific principles and current status. J Food Prot 58:186-192.

Lanier TC. 2000. Surimi gelation chemistry. In: Park JW, editor. Surimi and surimi seafood. New York: Marcel Dekker, Inc. p 237-265.

Laycock A, Regier LW. 1970. Preservation of fish by irradiation. Vienna: International Atomic Energy Agency. p 13-25.

Lee H, Lanier TC. 1995. The role of covalent crosslinking in the texturizing of the muscle proteins sols. J Muscle Foods 6:125-138.

Lewis NF, Alur MD, Kumta US. 1971. Radiation sensitivity of fish microflora. Int J Exp Biol 9:45-49.

Licciardello JJ, D'Etremont DL, Lindstom RL. 1989. Radio-resistance of some bacterial pathogens in soft shell clams (*Mya arenaria*) and mussels (*Mytilus edulis*). J Food Prot 52:407-411.

Lopez-Gonzalez V, Murano PS, Brennan RE, Murano EA. 2000. Sensory evaluation of ground beef patties irradiated by gamma rays versus electron beam under various packaging conditions. J Food Qual 23(2):195-204.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. 1951. Protein measurements with Folin phenol reagent. J Biol Chem 193:256-275.

Luchsinger SE, Kropf DH, Garcia Zepeda CM, Hunt MC, Marsden JL, Rubio Canas EJ, Kastner CL, Kuecker WG, Mata T. 1996. Color and oxidative rancidity of gamma and electron beam-irradiated boneless pork chops. J Food Sci 61(5):1000-1005.

Mallett JC, Beghian LE, Metcalf TG, Kaylor JD. 1991. Potential of irradiation technology for improved shellfish sanitation. J Food Safety 11:231-245.

Maunder DT. 1977. Possible use of ultraviolet sterilization of containers for aseptic packaging. Food Technol 31(4):36-37.

McAdams WH. 1954. Heat transmission. 3rd ed. New York: McGraw-Hill Book Co. 532 p.

Monahan FJ, German JB, Kinsella JE. 1995. Effect of pH and temperature on protein unfolding and thiol/disulfide interchange reactions during heat-induced gelation of whey proteins. J Agric Food Chem 43:46-52.

Monk JD, Beuchat LR, Doyle MP 1995. Irradiation inactivation of food-borne microorganisms. J Food Prot 58:197-208.

Montejano JG, Hamann DD, Lanier TC. 1985. Comparison of two instrumental methods with sensory texture of protein gels. J Texture Stud 16(4):403-424.

Morrissey MT, Wu JW, Lin D, An H. 1993. Protease inhibitor effect on torsion measurements and autolysis of Pacific whiting surimi. J Food Sci 58:1050-1054.

Mulak V, Tailliez R, Eb P, Becel P. 1995. Heat resistance of bacteria isolated from preparations based on seafood products. J Food Protect 58:49-53.

Muzilla M, Unklesbay M, Unklesbay N, Unklesbay K, Helsel Z. 1990. Effect of moisture content on density, heat capacity and conductivity of restructured pork/soy hull mixtures. J Food Sci 55(6):1491-1493.

Nutsch AL, Phebus RK, Riemann MJ, Kotrola JS, Wilson RC, Boyer JE, Brown TL. 1998. Steam pasteurization of commercially slaughtered beef carcasses: evaluation of bacterial populations at five anatomical locations. J Food Prot 61(5):571-577.

Palaniappan S, Sastry SK. 1991a. Electrical conductivities of selected solid foods during ohmic heating. J Food Process Eng 14(3):221-236.

Palaniappan S, Sastry SK. 1991b. Electrical conductivity of selected juices: influences of temperature, solids content, applied voltage, and particle size. J Food Process Eng 14(4):247-260.

Park JW. 2001. Survey: Pasteurization methods in the U.S. industry. Unpublished. OSU Seafood Laboratory, Astoria, OR.

Park JW. 2000. Surimi and surimi seafood. New York: Marcel Dekker, Inc. 500 p.

Park JW, Lanier TC. 2000. Surimi and surimi seafood manufacturing. In: Martin R, Flicks G, editor. Fresh water and marine products handbook. Chapman and Hall. p 417-443.

Park JW. 1995. Surimi gel colors as affected by moisture content and physical conditions. J Food Sci 60(1):15-18.

Park JW. 1994. Pasteurization procedures in the US surimi seafood industry: survey. Unpublished data. OSU Seafood Laboratory, Astoria, OR.

Park JW, Yongsawatdigul J, Lin TM. 1994. Rheological behavior and potential cross-linking of Pacific whiting (*Merluccius productus*) surimi gel. J Food Sci 59(4):773-776.

Parrott DL. 1992. Use of ohmic heating for aseptic processing of food particulates. Food Technol 46(12):68-72.

Ponne CT, Baysal T, Yuksel D. 1994. Blanching leafy vegetables with electromagnetic energy. J Food Sci 59(5):1037-1041.

Poole SE, Wilson P, Mitchell GE, Wills PA. 1990. Storage life of chilled scallops treated with low dose irradiation. J Food Prot 53(9):763-766.

Radomyski T, Murano EA, Olson DG, Murano PS. 1994. Elimination of pathogens of significance in food by low-dose irradiation: a review. J Food Prot 57:73-86.

Ramsey FL, Schafer DW. 1997. The statistical sleuth: a course in methods and data analysis. New York: Wadsworth Publishing Company. p.742.

Restaino L, Frampton EW, Hemphill JB, Palnikar P. 1995. Efficacy of ozonated water against various food-related microorganisms. Applied Environ Microbiol 61(9):3471-3475.

Rhodehamel EJ. 1992. FDA's concerns with *sous-vide* processing. Food Technol 46(12):73-76.

Rippen TE, Hackney CR. 1992. Pasteurization of seafood: potential for shelf-life extension and pathogen control. Food Technol 46(12):88-94.

Rippen TE, Hackney CR, Ward DR, Martin RE, Croonenberghs R. 1993. Seafood pasteurization and minimal processing manual. Virginia Cooperative Extension, Hampton, VA.

Rustom IYS. 1997. Aflatoxin in food and feed: occurrence, legislation and inactivation by physical methods. Food Chem 59(1):57-67.

Sastry SK, Palaniappan S. 1992. Ohmic heating of liquid-particle mixtures. Food Technol 46(12):64-67.

Shamsuzzaman K, Lucht L, Chuaqui-Offermanns N. 1995. Effects of combined electron-beam irradiation and *sous-vide* treatments on microbiological and other qualities of chicken breast meat. J Food Prot 58(5):497-501.

Sheldon BW, Brown AL. 1986. Efficacy of ozone as a disinfectant for poultry carcasses and chill water. J Food Sci 51(2):305-309.

Shie JS, Park JW. 1999. Functional and microbiological characteristics of surimi seafood at various pasteurization conditions. J Food Sci 64(2):287-290.

Singh RP. 1982. Thermal diffusivity in food processing. Food Technol 36(2):87-91.

Smith JL, Buchanan RL, Palumbo SA. 1983. Effect of food environment on staphylococcal enterotoxin synthesis: A review. J Food Protect 46:545-555.

Sompongse W, Itoh Y, Obatake A. 1996. Role of SH in polymerization of myosin heavy chain during ice storage of carp actomyosin. Fisheries Science 62:110-113.

Stroup WH, Parker RW, Dickerson RW. 1972. Steam infusion heater for ultra high temperature pasteurization. J Dairy Sci 55(4):536-539.

Su A, Kolbe E, Park JW. 1999. A model of heat transfer coefficients over steam-cooked surimi paste. Aquat Food Product Technol 8(3):39-53.

Taguchi T, Ishizaki S, Tanaka M, Nagashima Y, Amano K. 1989. Effect of ultraviolet radiation on thermal gelation of muscle pastes. J Food Sci 54(6):1438-1440.

Tarte RR, Murano EA, Olson DG. 1996. Survival and injury of *L. monocytogenes, L. innocua* and *L. ivanovii* in ground pork following electron beam irradiation. J Food Prot 59(6):596-600.

Taub IA, Robbins FM, Simic MG, Walker JE, Wierbicki E. 1979. Effect of irradiation on meat proteins. Food Technol 5:184-193.

Thakur BR, Singh RK. 1995. Combination processes in food irradiation. Trends in Food Sci Technol 6:7-11.

Thayer DW, Boyd G. 1995. Radiation sensitivity of *L. monocytogenes* on beef as affected by temperature. J Food Sci 60(2):237-240.

Thayer DW. 1993. Extending shelf life of poultry and red meat by irradiation processing. J Food Prot 56(10):831-833.

Thibault C, Charbonneau R. 1991. Shelf-life extension of fillets of Atlantic cod (*Gadus morhua*) by treatment with ionizing rays. Sciences des Aliments 11:1-16.

Toledo RT. 1991. Fundamentals of food engineering. 2nd ed. Gaithersburg: Aspen Publishers, Inc. 602 p.

Unklesbay N, Unklesbay K, Hsieh F, Sandik K. 1992. Thermophysical properties of extruded beef/corn flour blends. J Food Sci 57(6):1282-1284.

Urbain WM. 1986. Food irradiation. Orlando: Academic Press. 351 p.

Venugopal V, Doke SN, Thomas P. 1999. Radiation processing to improve the quality of fishery products. Critical Reviews in Food Science and Nutrition 39(5):391-440.

Verrez-Bagnis V, Bouchet B, Gallant DJ. 1993. Relationship between the starch granule structure and the textural properties of heat-induced surimi gels. Food Structure 12:309-322.

Ward DR, Pierson MD, Minnick MS. 1984. Determination of equivalent process for the pasteurization of crabmeat in cans and flexible pouches. J Food Sci 49:1003-1017.

Ward DR, Price JR. 1992. Food microbiology: exact use of inexact science. In: NFI Green Book. Washington: National Fisheries Institute (3):34-42.

World Health Organization. 1994. Safety and nutritional adequecy of irradiated food. Geneva: World Health Organization.

World Health Organization. 1981. Wholesomeness of irradiated food. Geneva: WHO. Technical Report Series 651.

Wouters PC, Smelt JPM. 1997. Inactivation of microorganisms with pulsed electric fields: potential for food preservation Food Biotechnology 11(3):193-229.

Yang H, Park JW. 1998. Effects of starch properties and thermal-processing conditions on surimi-starch gels. Lebensmittel Wissenschaft & Technologie 31:344-353.

Yang PPW, Chen TC. 1979. Stability of ozone and its germicidal properties on poultry meat microorganisms in liquid phase. J Food Sci 44(2):501-504.

Yongsawatdigul J, Park JW, Kolbe E. 1997. Degradation kinetics of myosin heavy chain of Pacific whiting surimi. J Food Sci 62(4):724-728.

Yongsawatdigul J, Park JW. 1996. Linear heating rate affects gelation of Alaska Pollack and Pacific Whiting surimi. J Food Sci 61(1):149-153.

Yongsawatdigul J, Park JW, Kolbe E, Abu-Dagga Y, Morrissey MT. 1995. Ohmic heating maximizes gel functionality of Pacific Whiting surimi. J Food Sci 60(4):10-14.

Yoon IH, Matches JR, Rasco B. 1988. Microbiological and chemical changes of surimi based imitation crab during storage. J Food Sci 53(5):1343-1346.

Yu TC, Sinnhuber RO. 1957. 2-thiobarbituric acid method for the measurement of rancidity in fishery products. Food Technol 11:104-108.

Zhang Q, Cavalieri RP. 1991. Thermal model for steam blanching of green beans and determination of surface heat transfer coefficient. Transaction of ASAE 34(1):182.

Zhang Q, Pullman WA, Monsalve-Gonzalez A, Qin BL, Barbosa-Canovas GV, Swanson BG. 1994. Inactivation of *Saccharomyces cerevisiae* in apple juice by square-wave and exponential-decay pulsed electric field. J Food Process Eng 17(4):469-478.

Zhang QH, Qin BL, Barbosa-Canovas GV, Swanson BG. 1995. Inactivation of *Escherichia coli* for food pasteurization by high-strength pulsed electric fields. Journal of Food Processing and Preservation 19(2):103-118.

Ziegler GR, Acton JC. 1984. Mechanism of gel formation by proteins of muscle tissue. Food Technol 38:77-82.