AN ABSTRACT OF THE THESIS OF

Jin-Shan Shie for the degree of Master of Science in Food Science and Technology presented on December 5, 1997. Title: Development of Optimum Pasteurization Conditions for Surimi Seafood.

Abstract approved:

Jae W. Park

Texture, color and microbiological characteristics of surimi seafood were measured at various pasteurization conditions. Three different pasteurization temperatures (93, 85, and 75°C) were chosen at various pasteurization times (from 0 to 120 min). Both pasteurization temperature and time affected whiteness, shear strain, and aerobic plate count (APC) of surimi seafood. As the pasteurization temperature and time increased, the shear strain and whiteness of surimi seafood decreased. The higher and longer the pasteurization temperature and time, the lower the shear strain and whiteness. The time required to obtain a zero APC count at 93, 85, and 75°C pasteurization temperature was 5, 15, and 15 min, respectively. Shear stress, L^* and a^* values of surimi seafood were significantly changed during the first 5 min pasteurization, but not between 15 to 120 min at three pasteurization temperatures (93, 85 and 75°C).

A thermal death time (TDT) curve of *Enterococcus faecium* E-20 was obtained at the temperature range of 60 to 85°C using the thermal death time tube method. The z value of TDT curve was 16.3°C, and the D-values were in the range of 30.60 min at 60°C to 1.15 min at 85°C. F_{250} pasteurization values were 0.014, 0.01, 0.012, 0.014, and 0.019 min at 65, 70, 75, 80, and 85°C, respectively. At 60°C, the F_{250} pasteurization value (0.044 min) was much higher than other temperatures. Our results suggested that the US surimi seafood industries might have overcooked their products. In conclusion, 15 min at 75 or 85°C were the optimum pasteurization conditions for this study.

Development of Optimum Pasteurization Conditions for Surimi Seafood

by

Jin-Shan Shie

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented December 5, 1997
Commencement June 1998

Master of Science thesis of Jin-Shan Shie presented on December 5, 1997

APPROVED:

Major Professor, representing Food Science and Technology

Head or chair of Department of Food Science and Technology

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Jin-Shan Shie, Author

ACKNOWLEDGMENT

I want to thank Dr. Jae W. Park, my major professor, for his guidance, encouragement and support throughout the entire course of my study. I also want to thank Dr. Suad Ghazala for her technical advice and for providing *Enterococcus faecium* E-20 and Dr. Brian Himelbloom for his technical advice. My sincere gratitude goes to my thesis committee, Dr. Edward Koble, Dr. Mark Daeschel, and Dr. Kenneth B. Johnson, for their advice and instruction.

I want to express my appreciation to Nancy Chamberlain for editing my thesis. I would also like to thank all of the staff and students at OSU Seafood Laboratory for their help and friendship.

Finally, I would like to express my deep appreciation to my family who always supports me with their love. It is to them that this work is dedicated.

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DEVELOPMENT OF OPTIMUM PASTEURIZATION CONDITIONS FOR SURIMI SEAFOOD

Chapter 1 Introduction

Surimi has become Americanized with an annual production of 150,000 to 190,000 M/T since the early 1980s (Park, 1994a). This intermediate product is used as a major ingredient in surimi seafood; such as, surimi crab, shrimp, lobster, and scallop meat. The success of these surimi seafood products was partly due to their economic values in relation to the natural counterparts. These surimi seafood products do not fluctuate in availability of supply or quality as with higher-priced natural shellfish. Since the introduction of surimi seafood to the United States in 1978, the consumption reached 68,000 M/T in 1989. Its market has grown continuously at 3-5% every year and reached 72,500 M/T in 1994 (Park, 1994a). However, over the last two years the market has remained constant.

Since surimi seafood is ready to serve, it is very important to know the microbial content. Pasteurization is the most important step in securing the microbial quality of surimi seafood. No standard pasteurization value for surimi seafood products has been established within the US industry since its inception in 1980s. Park (1994b) surveyed the pasteurization practices within the U.S. surimi seafood industry. Surprisingly, there was a great deal of variation in terms of time and temperature of pasteurization within the industry. Under the United States Dept. of Commerce (USDC) guidelines for pasteurization of the voluntary PUFI (Packed Under Federal Inspection) or HACCP

(Hazard Analysis Critical Control Point) sealed surimi seafood, it is recommended that vacuum packed products must be heated at 85°C (internal temperature) for 20 min followed by fast chilling. The chilling method must bring the product temperature to 4°C within 30 min (Comar, 1987). This cooking and chilling regime only applies to a manufacturer who voluntarily participates in the PUFI or HACCP program. Even when the HACCP plan becomes mandatory in the US seafood industry as of December 18, 1997, no standard methods for surimi seafood pasteurization are likely to be reinforced.

Pasteurization in surimi seafood not only affects the microbial quality, but also affects the texture and color qualities of the finial products (Alvarez et al., 1995; Chan et al., 1995; Bertak and Karahadian, 1995; Hsu, 1990, Lee, 1986; Park, 1995; Verrez-Bagnis et al., 1993; Yang, 1997). Pasteurization with proper heat treatment would enhance the texture of surimi seafood in regards to sensory attributes. Longer cooking at higher temperatures, more negative attributes result with. The surimi seafood industry has experienced textural softness, brownish discoloration, and off-odor particularly when a significant portion of surimi was replaced by a relatively large content of starch and other additives and the finished products is pasteurized at higher temperature for a longer time (Park, 1994b).

It is very fortunate that the US surimi seafood industry has never experienced outbreaks leading to serious illness or death since its establishment. However, there is a great potential for possible outbreaks using nonstandardized pasteurization methods. Therefore, there is a great need for an efficient and/or optimum pasteurization procedure

for surimi seafood. The objectives of this study were to: 1) investigate the effects of various pasteurization conditions on the texture, color and microbiological characteristics of surimi seafood; 2) Determine a F_{250} pasteurization value for surimi seafood (using *Enterococcus faecium* E-20 as a target microorganism).

Chapter 2 Literature Review

Manufacturing of surimi seafood

Surimi is isolated myofibrillar proteins and is stabilized with cryoprotectants to prevent protein denaturation during frozen storage (Lanier, 1986; Lee, 1984). The production of surimi requires continuous processing steps (Fig. 2.1). Since the early 1980s, an annual production of frozen surimi has reached 150,000 to 190,000 M/T. This intermediate product is the major ingredient in manufacturing surimi-based products; such as, surimi crab, shrimp, lobster, and scallop meat. Surimi crabmeat (hereafter surimi seafood) is manufactured through a series of continuous processing steps such as comminution, extrusion, fiberization, cutting and packaging, and pasteurization (Park and Lanier, 1997)(Fig. 2.2). Basically, each processing step can affect the quality of products. In the processing of surimi seafood, several different raw materials are added. They are starch, protein additives, salt, food grade chemicals, flavorings, and colorings. These raw materials must be inspected carefully and stored in proper conditions to achieve good quality of products.

Microbiology of surimi and surimi seafood

Many substances or organisms hazardous to health can be ingested with fishery products. These include various parasites, toxic chemical pollutants and variety of toxins found in fish. However, microorganisms constitute the largest proportion of fish and shellfish-borne diseases (Eyles, 1986). The US Government Accounting Office has indicated that seafoods account for about 3-5% of all food-borne illnesses (Lampila,

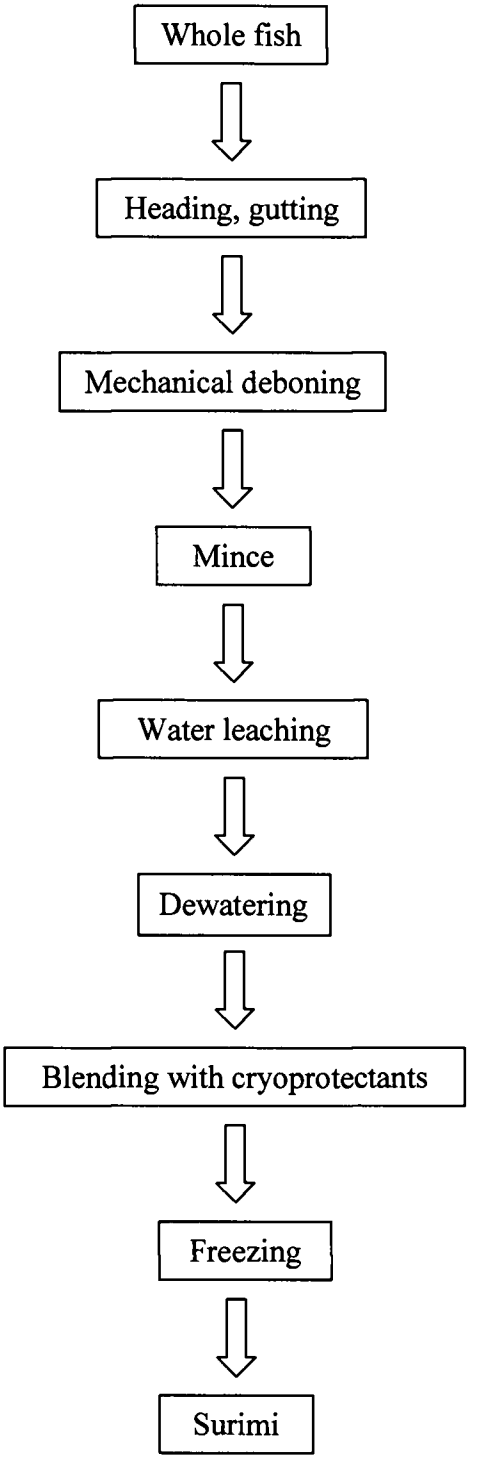
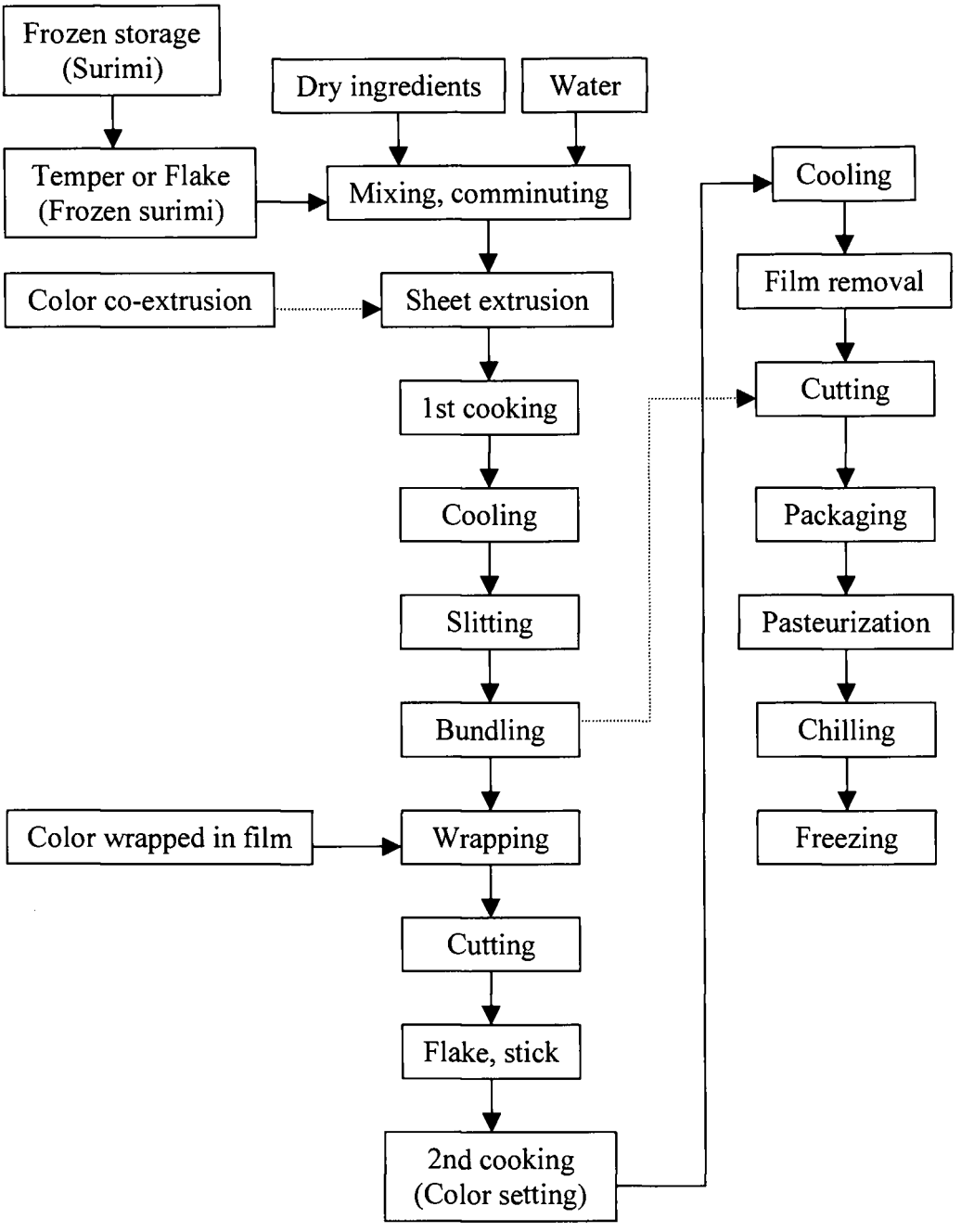


Fig. 2.1. Flow chart of surimi production



demonstrates a co-extrusion color application.

Fig. 2.2. Flow chart of surimi seafood production

1990). The majority of outbreaks are caused by either temperature abuse or post-processing contamination.

Based on the major source of the responsible agent, fish-borne and shellfish-borne diseases are divided into three categories (Bryan, 1980): 1. Agents naturally present in aquatic environment include *Clostridium botulinum*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, *Vibrio vulnificus*, *and Aeromonas hydrophila.* 2. Agents derived from pollution of aquatic environments include *Salmonella* species, and 3. Agents derived from workers, equipment or the environment of food handling, processing service establishments such as, *Staphylococcus aureus*, *Bacillus cereus*, *and Clostridium perfringens.*

The microbial quality of surimi seafood is affected by the quality of raw surimi and other ingredients, processing equipment, pasteurization procedures, cooling, packaging and storage conditions (Himelbloom, 1997; Himelbloom et al., 1991a). Each step in the processing of surimi seafood can impact its final microflora. Overall, the microbial types present in fish mince are similar to those present in the whole fish depending on where the fish were caught (Nickelson et al., 1980). Mechanical deboning of fish can increase the microbial count. Raccach and Baker (1978) reported that mechanical deboning of either frames or headed and gutted fish increased the bacterial count about tenfold. In surimi processing, washing fillets before mincing can remove some microorganisms (Himelbloom, 1997), but washing the mince hardly affects the microbial flora. Therefore, washing thoroughly before mincing is more desirable to

control microorganisms. The temperature of wash water has to be below 10°C to inhibit the growth of microorganisms. The adding of small molecular carbohydrates (sucrose, sorbitol) to mince as a cyroprotectant can help the growth of microorganisms, since many microorganisms grow best in carbohydrate-rich media. During processing, surimi and surimi seafoods can be contaminated by airborne microorganisms, as well as microorganisms that adhere to processing equipment. After pasteurization of the final products, if the cooling procedure is not fast enough to bring down the temperature, injured psychrotrophic microorganisms can grow back. Proper storage conditions are also very important to the microbial quality of surimi seafood products. If the surimi seafood products are stored under improper refrigerated conditions (>5°C), pathogenic microorganisms could be found. *Bacillus cereus* has been found in vacuum-packed and pasteurized flaked surimi seafood at 22°C (Hollingworth, et al., 1991).

Listeria monocytogenes is a food borne pathogen affecting pregnant women and their fetuses, the elderly, and immunosuppressed individuals (Dillon and Patel, 1992). Since this microorganism can cause serious illness, FDA has set a zero tolerance on any ready-to-eat foods. This food borne microorganism has been found in a variety of seafood products. Weagant et al. (1988) tested 57 samples of seafood products, such as raw shrimp, cooked and peeled shrimp, cooked crabmeat, raw lobster tails, scallops, squid, and surimi seafood. They found that 35 of tested samples were positive for *Listeria* species and 15 of 57 samples were positive for *L. monocytogenes*. *L. monocytogenes* is tolerant to a great number of adverse conditions. It can grow in 10% sodium chloride solutions, survive in frozen storage after 1 year. It also grows very well

at refrigerated temperatures (Ingham, 1991). But *L. monocytogenes* can be easily killed by heating the product to 77°C. It is probable that this microorganism can be introduced into surimi seafood through post-processing contamination.

Staphylococcus aureus may be found on raw seafood at the time of catching or may enter the raw products during primary handling and processing. It can grow at >10°C (Yoon and Matches, 1988) and it most commonly enters surimi seafood products during processing and preparation. Its presence in food usually indicates cross-contamination or mishandling. According to the FDA's informal guidance, the product may be actionable if tested positive for the toxin or if $1*10^4$/g (MPN) organisms are found (Ward and Price, 1992). *S. aureus* can grow well in protein-rich foods and is tolerant of high levels of salt, but it competes poorly with the normal spoilage flora. The contamination of *S. aureus* can be controlled with a high standard of personal hygiene among processing workers. Proper refrigeration can also prevent the growth of this microorganism, since it will not grow below 6°C or above 46°C (Eyles, 1986).

Botulism is a fatal disease caused by *Clostridium botulinum*. *C. botulinum* is widely distributed in soil and aquatic environments. Type E of this microorganism has been isolated from aquatic sources and is most commonly implicated in outbreaks of botulism caused by fishery products (Eyles, 1986). Non-proteolytic types of *C. botulinum* in surimi seafood can be inhibited by 2.4% of NaCl and by heat processing (Eklund, 1987). Surimi seafood products are vacuum-packed, therefore, this environment is especially good for *C. botulinum* which grows under anaerobic conditions even at

refrigerated temperatures. Proper refrigeration and pasteurization are two major methods to prevent the growth of *C. botulinum*, since the toxin production is very slow at temperatures below 10°C and the spores of *C. botulinum* are very invulnerable to heat (Dolman, 1970 and Eyles, 1986).

Vibrio parahaemolyticus is the most common food-borne illness in Japan, and it is a potential cross-contaminant of surimi seafood (Blake et al., 1980). Diarrhea is the main clinical sign of infection, and the disease is usually self-limiting. This microorganism was once found in cooked salted Atlantic pollock surimi (Ingham and Potter, 1988). Infection of *V. parahaemolyticus* is most often caused by improper cooking of seafood products or contaminated after cooking, but infections in the US are not very common.

Pasteurization of seafood and surimi seafood products

Pasteurization is commonly used in seafood including surimi seafood products to extend the refrigerated shelf life. The relatively mild heating conditions result in color, texture, and flavor characteristics that are similar to fresh products, but with greatly extended shelf life (Rippen and Hackney, 1992).

Sous vide technology is another heating method used significantly with seafood products. This technology is very similar to pasteurization, but sous vide products are produced primarily for flavor development and suitability for central distribution (Rippen

and Hackney, 1992). Sous vide processing cooks food in a pouch filled with its own juices to maintain the aroma and flavor. All volatile compounds stay within the package and minimum nutrients are lost during cooking (Rhodehamel, 1992). The literal French meaning of sous vide is "under vacuum". It is a technologically advanced method of cooking whereby fresh food is vacuum sealed in impermeable plastic pouches, cooked at length in a low temperature in a circulating water bath, then chilled and held at refrigerated temperatures for up to three weeks (Baird, 1990). The product is subsequently reheated before consumption.

Most spoilage microorganisms and pathogens are heat sensitive and can be destroyed by low to moderate heat. However some microorganisms are heat-resistant and often found in seafood products as listed in Table 2.1 (Mulak et al., 1995). The heat resistance was calculated at 70°C, since this is the official reference temperature in France.

The US experience in the pasteurization of seafoods is based largely on the processing of blue crab meat (*Callinectes sapidus*) because of its high commercial value. No target microorganism has been identified for the pasteurization of crabmeat and the process is based on historical data that gave the desired shelf life. A z value of 8.9°C was picked arbitrarily in the absence of a specific target microorganism (Rippen and Hackney, 1992). Although no controlled study was conducted to equate various crabmeat pasteurization schedules with shelf life, empirical data accumulated from mid-

Atlantic commercial blue crab processing gave the guidelines listed in Table 2.2 (Rippen and Hackney, 1992).

Table 2.1. $D_{70°C}$ of several heat-resistant microorganisms isolated from seafood products (Mulak et al., 1995).

Microorganism	Heating medium	$D_{70°C}$ (min)	z (°C)	$F_{70°C}$ (min)
Pseudomonas paucimobilis	Phosphate buffer	1.160	7.7	ND[a]
	Fish fillet	1.640	5.8	14.760
	Fish terrine	3.320	9.1	29.880
Enterococcus faecium	Phosphate buffer	0.161	4.7	ND
	Fish fillet	0.087	4.3	0.780
	Fish terrine	0.380	4.4	3.800
Micrococcus varians	Phosphate buffer	0.020	4.2	ND
	Fish fillet	0.028	4.0	0.250
	Fish terrine	0.150	5.2	1.350
Staphylococcus aureus	Phosphate buffer	0.085	8.7	ND
	Fish fillet	0.017	5.2	0.170
	Fish terrine	0.069	6.3	0.690
Pseudomonas putida	Phosphate buffer	0.002	5.0	ND
	Fish fillet	0.002	4.8	0.018
	Fish terrine	0.001	4.8	0.009

a: ND: not determined

Table 2.2. Observed relationship of blue crab $F_{85}^{8.9}$ pasteurization values to refrigerated shelf life (Rippen and Hackney, 1992).

$F_{85}^{8.9}$ pasteurization value (min)	Shelf life (months)
10-15	1.5
15-20	2-4
20-25	4-6
25-30	6-9
30-40	9-18
>40	12-36

The heat resistance of some pathogenic microorganisms in crabmeat has been studied. Lynt et al. (1977) used TDT (thermal death time) tubes method to study the heat resistance of five strains of *C. botulinum* type E (Beluga strain, Alaska strain, Crab G21-5, Crab 25V-1, Crab 25V-2) in sterilized crabmeat. The original concentration of the microorganism was around 10^5-10^6/g. The D value at various temperatures is listed in Table 2.3. In five strains, Crab G21-5 had the highest z value of 15.2°F and Alaska strain had the lowest z value of 12.6°F. Cholera is an acute bacterial disease caused by infection of the small intestine with *Vibro cholerae*. The D value of *V. cholerae* in crabmeat has been reported by Shultz et al. (1984). The culture concentration was around 10^6 cell/g. As shown in Table 2.4, the D values range from 8.15 min at 49°C to 0.3 min at 71°C. Harrison and Huang (1990) studied the heat-resistant ability of *L. monocytogenes* in blue crabmeat. They inoculated the blue crabmeat with *L. monocytogenes* strain Scott to reach the concentration of 10^7 cells/g, and then distributing 7.5g of inoculated crabmeat into sausage casings. A water bath adjusted to 50 to 60°C, was used to generate the D and z values. They reported that the z values of 8.4 and

6.99°C were derived from the TSA (trypticase soy agar) and modified Vogel-Johnson agar data. The D values at different temperatures were also calculated (Table 2.5).

Table 2.3. Heat resistance of *Clostridium botulinum* type E in five strains of crabmeat (Lynt et al., 1977).

Temperature (°C)		74	77	80	82	85
Beluga	D[a]	12.9	4.1	1.7	0.7	0.3
	U[b]	16.4	6.2	2.4	1.0	0.4
	L[c]	9.5	1.9	0.9	0.4	0.2
Alaska	D	10.4	3.0	1.4	0.5	
	U	12.5	3.7	1.8	0.7	
	L	8.5	2.3	0.9	0.3	
G21-5	D	6.8	2.4	1.1	0.6	
	U	7.8	3.1	1.6	0.9	
	L	5.7	1.7	0.6	0.3	
25V-1	D				0.6	
	U				1.0	
	L				0.2	
25V-2	D				0.5	
	U				0.7	
	L				0.3	

a: D is the D value average in min
b: U is the upper value in min
c: L is the lower value in min

Table 2.4. D values of *Vibrio cholerae* in crabmeat (Shultz et al., 1984).

Temperature (°C)	D-value (min)	7 D value (min)
49	8.15	57.05
54	5.02	35.14
60	2.65	18.55
66	1.60	11.20
71	0.30	2.10

Table 2.5. D values of *Listeria monocytogenes* at temperature 50-60°C (Harrison and Huang, 1990).

Plating medium	D value (min)		
	50°C	55°C	60°C
Trypticase soy agar	40.43	12.00	2.61
Modified Vogel-Johnson agar	34.48	9.18	1.31

Other seafood products such as shrimp and smoked fish have also been studied for heat pasteurization values. Lerke and Farber (1971) reported that after pasteurization for 1 min in a 180°F water bath, no *Staphylococcus aureus* (original inoculated with 10^7-10^8 cells/g) and *Salmonella* (*Salmonella senftenberg* 775W, original inoculated with 10^8 cells/g) was recovered from 6 oz mylarpolyethylene pouch of shrimp (*Pandalus jordani*). Eklund et al. (1988) demonstrated the feasibility of pasteurization for vacuum-packaged hot-smoked chum salmon (*Oncorhynchus keta*). They reported that after pasteurization for 85, 65, and 55 min at 185, 192 and 198°F water baths, respectively, there was no

toxin produced by *C. botulinum* type E in vacuum-packaged chum salmon stored at 25°C for 21 days.

A two-step cooking method is commonly employed in manufacturing of surimi seafood products. In the first step, the surimi gels enable to hold all ingredients together by heat-induced gelation of myofibrillar proteins. The second step is used for pasteurization while texture is developed throughout the complete gelatinization of starch. Since the establishment of surimi seafood manufacturing in the US in the early 1980s, no standard pasteurization values for surimi seafood products have been established. Under the USDC guidelines for pasteurization of the voluntary PUFI (Packed Under Federal Inspection) or HACCP (Hazard Analysis Critical Control Point) sealed surimi seafood, it is recommended that vacuum packed products must be heated at 85°C (internal temperature) for 20 min followed by fast chilling. The chilling method must bring the product temperature to 4°C within 30 min (Comar, 1987). This guideline is only used for those manufacturers that voluntarily participate. Park (1994b) surveyed the pasteurization practices within the U.S. surimi seafood industry. Surprisingly, there was a great deal of variation in terms of time and temperature of pasteurization within the industry. It is very fortunate that the US surimi seafood industry has never experienced outbreaks leading to serious illness or death since its establishment. However, there is a great potential for possible outbreaks using nonstandardized pasteurization methods.

Color and texture quality of surimi seafood

Texture and color are two important qualities of surimi seafood products. Pasteurization temperature and time significantly affect the texture of surimi seafood. Determination of the optimum temperature-time relationship is critically important to obtain the desired texture (Lee, 1986). Texture quality of surimi seafood is greatly influenced by setting. Several studies reported that low-temperature settings before heating of surimi gels can increase the shear stress significantly (Alvarez et al., 1995, Chan et al., 1995, Park et al., 1994). Pasteurization also plays a key role in developing the texture of surimi seafood. Proper cooking can increase the gel rigidity of surimi gels (Lee and Kim, 1986; Verrez-Bagnis, et al., 1993), but prolonged cooking may deteriorate its texture quality. Alvarez et al. (1995) reported that prolonged heating of sardine surimi at 90°C (saturated steam oven) decreased the gel strength. Bertak and Karahadian (1995) reported that heating might cause free water to rebind with starches in surimi seafood, thus decreasing expressible moisture, which contributed to decreases in firmness and chewiness of the product.

For surimi seafood products, the higher the whiteness, the better the color quality. Several studies have shown that heating temperature and time affect the color of surimi gels. Hsu (1990) reported that the whiteness of pike eel *(Muraenesox cinereus)* sausages was significantly affected by frozen storage (-20°C) and its interactions with the leaching, grinding and heating processes; they did not report whether heat processing gave a positive or negative effect on the color of the fish sausages. Park (1994a) reported that in

Alaska pollock (*Theragra chalcogramma*) and Pacific whiting (*Merluccius productus*) surimi gels, the lightness values in two-stage heating (25°C for 3 hr followed by 90°C for 30 min) were higher than one stage heating (90°C for 30 min), but the relationship between heating temperature and time to the color of surimi gels was not studied.

Enterococcus faecium and *Enterococcus faecalis*

Enterococcus faecium is a thermoduric enterococcus microorganism that has been implicated as a potential spoilage type. This organism most likely survives in foods which are processed in mild pasteurization conditions and can withstand the presence of salt and nitrite at normal usage (Magnus et al., 1986; Simpson et al., 1994). The thermal resistance of some streptococci under varied experimental conditions has been well documented (Magnus et al., 1986, 1988). The heat-resistant ability of these streptococci varies at different heating mediums (Table 2.6). Simpson et al. (1994) studied the thermal resistance of *E. faecium* (ATCC 19432) influenced by the pH and salt concentration. They found that *E. faecium* was less heat-resistant in acidic conditions (pH 5 and 6) than neutral or basic conditions. Higher salt concentrations (0.5-10%) generally resulted in higher D values. The same phenomena was also found in *E. faecalis*. White (1963) reported that *E. faecalis* (L6, C, G) was more susceptible to heat at low and high pH than at pH approaching neutrality. Age of the culture also affects the heat-resistance of streptococci. White (1953) reported that heat resistance of three strains of *E. faecalis* (L5, L6, C and G) was increased by transferring the culture to fresh medium. Heat resistance of the culture fell during the lag phase of growth and reached a

minimum as rapid reproduction began. Other factors such as incubation temperature of the heated culture, salt concentration in the recovery medium of the culture also affect heat resistance of *E. faecalis* (Beuchat and Lechowich, 1968a, b).

Table 2.6. The heat resistance of *E. faecium* and *E. faecalis* at 70°C under various heating conditions (Magnus et al., 1986, 1988).

Microorganism	D_{70} value (min)	z value (°C)	Heating medium
E. faecium E-20	1.37	7.16	Sorenson's buffer
E. faecium P-1a	1.94	8.11	Sorenson's buffer
E. faecium 19434	1.46	9.28	Sorenson's buffer
E. faecium E-20	3.42	11.27	Ham broth
E. faecium P-1a	3.28	9.56	Ham broth
E. faecium 19434	2.79	12.82	Ham broth
E. faecalis DFS	0.193	8.08	Sorenson's buffer
E. faecalis P-2a	0.2	8.27	Sorenson's buffer
E. faecalis 19433	0.274	9.06	Sorenson's buffer
E. faecalis DFS	0.032	4.52	Ham broth
E. faecalis P-2a	0.556	8.69	Ham broth
E. faecalis 19433	0.023	4.12	Ham broth
E. faecium E-20	4.70	7.46	Ham packet
E. faecium P-1a	7.89	7.46	Ham packet
E. faecium E-20	1.11	12.4	Cooked meat broth

Chapter 3

Functional and Microbiological Characteristics of Surimi Seafood at Various Pasteurization Conditions

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To be submitted to Journal of Food Science,

Institute of Food Technologist, Chicago, Illinois

1997

Abstract

Shear stress, shear strain, aerobic plate count (APC), and color of surimi seafood gels using Alaska pollock surimi were measured at various pasteurization conditions. A two-step thermal treatment was used. Step 1; all samples were placed in a water bath (90°C) for 6 min to mimic commercial cooking of surimi seafood. Step 2; samples were placed in 93, 85, and 75°C water baths to conduct pasteurization and to complete cooking processes for other ingredients such as starches. The pasteurization time applied was between 0 and 120 min. The time required to obtain a zero APC count at 93, 85, and 75°C was 5, 15, and 15 min, respectively. The whiteness values of pasteurized surimi seafood were the highest at 75°C. The b^* value indicated a sign of browning reaction and was lower at 75 and 85°C than 93°C. Shear strain values decreased by 24.38, 23.52, and 16.63% after 120 min of pasteurization at 93, 85, and 75°C, respectively. Shear stress, L^*, and a^* values of surimi seafood significantly changed for the first 5 min of pasteurization, but did not change between 15 to 120 min at various pasteurization conditions.

Key words: pasteurization, surimi seafood, shear stress, shear strain, color, APC

Introduction

Surimi is stabilized fish myofibrillar proteins and is used as a major ingredient for surimi seafood products; such as, surimi crab, shrimp, lobster and scallop meat. The US consumption of surimi crabmeat (hereafter surimi seafood) has reached 68,000 MT in 1989 and it continues to grow about 3-5% every year (Park, 1994a). The surimi seafood market has reached a plateau the last two years. The earlier success of these surimi seafood products was due to their economic value in relation to its natural counterparts. They are also convenient to prepare and ready to eat. Since surimi seafood is ready to serve, the microbiological quality of these seafood products is very important. The pathogenic microorganisms commonly associated with fish and fishery products are *Salmonella*, *Listeria monocytogenes*, *E. coli*, and *Staphylococcus aureus* (Eyles, 1986). The FDA considers regulatory action when enterotoxigenic *E. coli* (ETEC) is present at 1×10^3/g in processed seafood which requires minimal or no processing by the consumer (Ward and Price, 1992). According to the FDA's informal guidance for *S. aureus*, the product may be actionable if tested positive for the toxin or if 1×10^4/g (MPN) organisms are found. The FDA has established a zero tolerance for *L. monocytogenes* in cooled and ready-to-eat foods and also has a zero tolerance for *Salmonella* in seafood.

Pasteurization is a significant process in the manufacturing of surimi seafood, because it completes the gelation of surimi proteins mixed with other functional ingredients and kills vegetative cells of microorganisms. Yoon and Matches (1988) reported that the APC of freshly processed surimi seafood reached the maximum of 10^9

cells/g after 20 days storage at 15°C and decomposition started when the APC reached 10^7cells/g. Hollingworth et al. (1991) reported that the initial microbial levels (APC and proteolytic count) of commercially prepared, vacuum-packed, pasteurized surimi seafood were approximately 10^2/g and *Bacillus cereus* (spore forming bacterium) was the only species found that was potentially pathogenic to humans.

Texture is the primary quality aspect of surimi seafood. Several studies have been conducted regarding surimi gels prepared at various thermal conditions such as setting, heating time, and temperature affecting the gel strength of surimi gels (Alvarez et al., 1995; Autio et al., 1989; Chan et al., 1995; Lee, 1986; Park et al., 1994). Color and flavor are important quality aspects of surimi seafood. Park (1995) investigated the effects of different setting-heating conditions on colors of surimi gels. Set gels showed a higher L^* value especially when the moisture content was low. Bertak and Karahadian (1995) demonstrated that the heating time and method affected the color and texture of surimi seafood. They found that baked samples were softer and less chewy than microwaved samples and heating tended to result in whiter samples, regardless of heating method or end-point temperature. Hsu (1990) also reported that frozen storage of pike eel surimi and its interactions with leaching, grinding and heating processes significantly affected the whiteness and gel strength of the fish sausages.

Pasteurization can be used to extend the refrigerated shelf-life of prepackaged seafood. The relatively mild heating conditions resulted in color, texture, and flavor characteristics similar to fresh products, but with greatly extended shelf-life (Rippen and

Hackney, 1992). Under the United States Dept. of Commerce (USDC) guidelines of pasteurization for the voluntary PUFI (Packed Under Federal Inspection) or HACCP (Hazard Analysis Critical Control Point) sealed surimi seafood, it is recommended that vacuum packed products must be heated at 85°C (internal temperature) for 20 min followed by fast chilling. The chilling method suggested must bring the product temperature to 4°C within 30 minutes (Comar, 1987). This cooking and chilling regime has only applied to manufacturers who voluntarily participate in the PUFI and HACCP program (Park, 1996). Eklund (1987) requested that the industry maintain a 2.4% water-phase salt level in surimi seafood to prevent the growth of *Clostridium botulinum.* It is expected to change when HACCP becomes a mandatory seafood inspection plan in the US after Dec 18, 1997. However, no optimum thermal conditions for the pasteurization of surimi seafood have been issued.

Park (1994b) surveyed the pasteurization practices within the US surimi seafood industry. As indicated in Table 3.1, surprisingly, there was a great deal of variation in terms of time and temperature of pasteurization within the industry. It is very fortunate that the US surimi seafood industry has never experienced outbreaks leading to serious illness or death since its establishment in the early 1980s. However, there is a great potential for possible outbreaks using nonstandardized pasteurization methods. Extreme pasteurization conditions may not only affect the surviving microorganisms of the finished products, but also affect their color, texture and shelf-life. However none study has been conducted on the relationships among the surviving microorganisms, texture, and color of surimi seafood at different pasteurization conditions. The objective of this

research was to investigate the effects of various pasteurization conditions on the texture, color and microbiological characteristics of surimi seafood.

Table 3.1. Pasteurization time and temperature used in the US surimi seafood industry.

Company	Frozen[a]	Frozen	Refrigerated
	External Temperature	Internal Temperature	
A	190°F (87.3°C)/30 min		Same as Frozen
B		85°C/15-30 min	Same as Frozen
C		185°F (85°C)/20 min	Same as Frozen
D	190°F (87.8°C)/22 min	To reach 170°F (77.6°C)	Same as Frozen
E	90°C/45 min	180°F (82.2°C)/10 min	Same as Frozen
F		70°C/20 min	Same as Frozen
G		160°F (71.1°C)/10 min	185°C (85°C)
H		To reach 82°C	Same as Frozen
I		85°C/17 min	Same as Frozen
J		80°C/5 min	85°C/5 min

a: Vacuum-packed products are either marketed as frozen or refrigerated.

Materials and Methods

Materials

A common commercial surimi seafood formulation (Table 3.2) was developed using KA grade Alaska pollock (*Theragra chalcogramma*) surimi (American Seafoods Co, Seattle, WA) and other food grade ingredients. The moisture content of surimi and surimi seafood was 74.31±0.59% and 68.26±1.46%, respectively (AOAC, 1995). Salt, sugar, soy oil, egg white, and wheat flour were purchased from a local market. PG 15 starch (waxy maize starch modified with cross-link and hydroxypropylation) and corn starch were obtained from Cerestar (Hammond, IN). Givaudan-Roure, Inc. (Brampton, Ontario, Canada) generously supplied natural and artificial crab flavors.

Table 3.2. Formula of surimi seafood

Ingredient	Weight %
Surimi	35.54
Water	35.54
Salt	1.65
Sugar	1.5
Wheat flour	4
Corn starch	7.25
PG 15 starch	0.5
TiO_2	0.02
70% (w/w) liquid sorbitol	5.68
Liquid egg white	4.55
Soy oil	2.27
Crab flavors	1.5

Sample preparation

Frozen surimi samples were tempered in cold running water for 1 hr before cutting into small chunks (3-5 cm^3). Surimi chunks were chopped in a Stephan UM5 universal food processor (Stephan Machinery Corp., Columbus, OH) at low speed for 1.5 min. Salt was added and the surimi paste was chopped for 0.5 min, then water and all other ingredients were added and chopped for 1 min. For the final 3 min, the surimi paste was chopped under vacuum (0.5 bar) at high speed. During the whole chopping procedure, the temperature of the surimi paste was maintained below 8°C using a NesLab constant-temperature bath (Portsmouth, NH) containing a solution (50:50) of ethylene glycol and water. A sausage stuffer (The Sausage Maker, Buffalo, NY) was used to stuff the surimi paste into the stainless steel tubes (i.d.=1.86 cm, length=17.5 cm) with screwable caps.

A two-step thermal treatment was used. Three tubes were placed in a plastic bag (Alpak, Portland, OR) and vacuum-sealed. Then the bag was put into another plastic bag and vacuum-sealed again to prevent leakage. Six bags were submerged in a 90°C bath containing about 18 L of water and held for 6 min for the gelation of surimi proteins to mimic the commercial method of continuous sheet cooking. According to Yongsawatdigul et al. (1995), surimi paste kept in the same size tube reached 73°C after 6 min holding at 90°C. It was assumed that this treatment would complete the gelation of myofibrillar proteins. All samples were held at room temperature for 8 min before additional heat treatment to simulate idle time in commercial operation between the first cooking and pasteurization. As a pasteurization step, various thermal treatments were

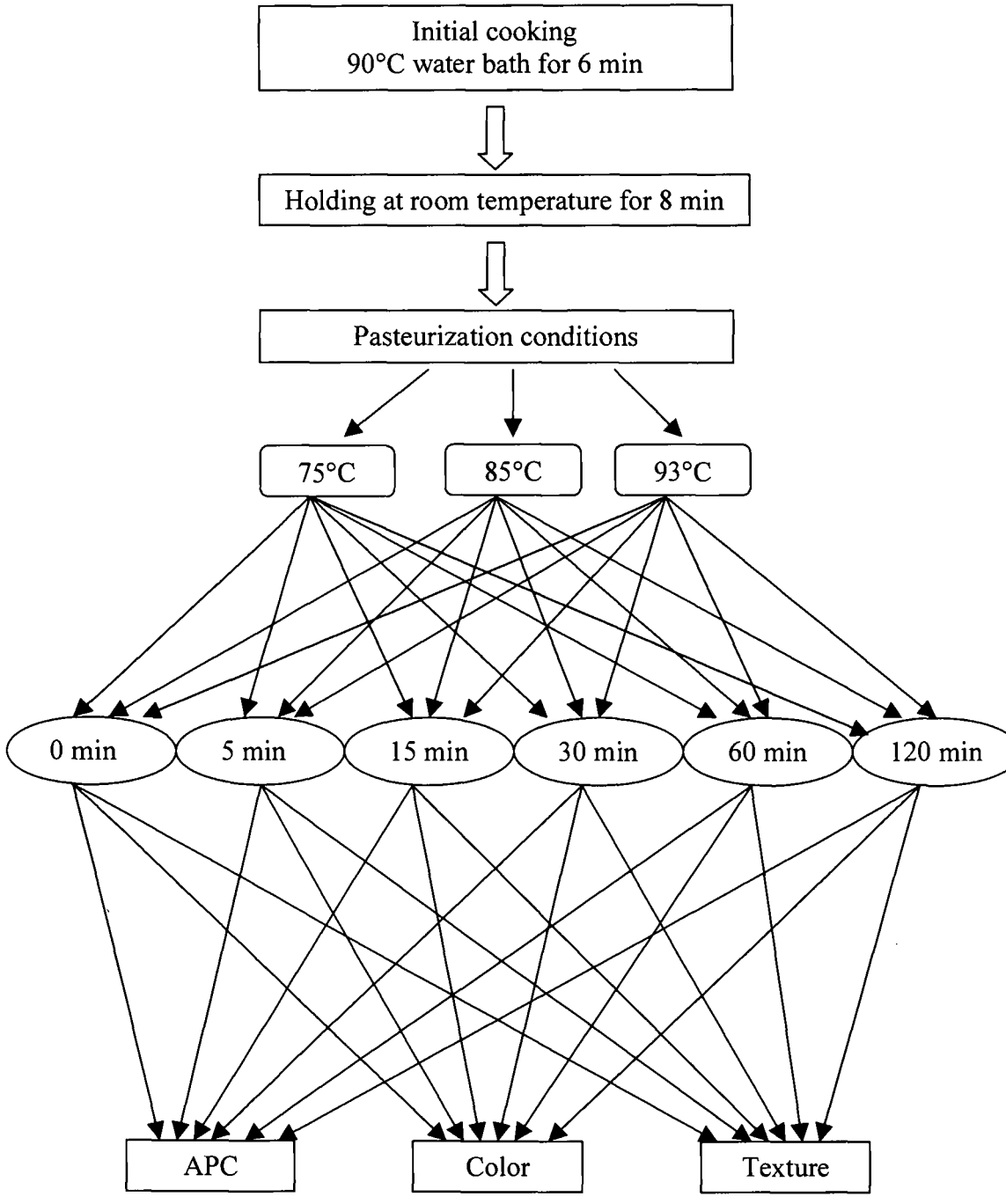


Fig 3.1. Experimental outline

used. Three different pasteurization temperatures: 93, 85, 75°C, and six different pasteurization times: 0, 5, 15, 30, 60, 120 min were chosen as outlined in Fig 3.1. For pasteurization, tubes were placed in a bath containing about 18 L of water (Blue M Electric Company, model: WB-1120A). In the selection of pasteurization temperature, 93°C was picked over 95°C because the temperatures of heating medium (water and steam) used in industry were 92-93°C (Park, 1994b). During pasteurization, the internal temperature of surimi paste was measured via a thermocouple attached to a data logger (model 21X, Campbell Scientific, Logon, UT).

Color analysis

Five samples from each pasteurization condition were removed for color analysis at room temperature (Park, 1995). A CIE color scale was used to measure the degree of lightness L^* (black [0] to light [100], a^* (red [60] to green [-60], b^* (yellow [60] to blue [-60]) using a Minotla Chroma Meter CR 300 (Minolta Camera Co. Ltd., Osaka, Japan). Whiteness of gels was calculated using L^*-3b^* (Park, 1994a).

Shear stress and shear strain by torsion method

Gel samples were removed from refrigerator (~5°C) and held at room temperature (~22°C) for 2 hr prior to torsion test. Gels were cut into 2.9 cm length and both ends were glued to plastic discs using Crazy glue (Borden, Inc., Columbus, OH). Ten gel samples of each pasteurization condition were milled into a dumbbell geometry (length = 0.29 cm, end diameter = 1.86 cm, and minimum diameter = 1.0cm). The torsion test was performed using a Hamann gelometer (Gel Consultant, Raleigh, NC). By twisting

samples, shear stress and shear strain at mechanical failure were measured (Hamann, 1983).

Aerobic plate count

A 10 g gel sample of each pasteurization condition was aseptically transferred to a blender jar and 90 ml of 0.1% sterile bactopeptone solution (Bacto-Peptone; Difco Laboratories, Detroit, MI) was added. The gel samples were blended at whip speed for 2 min using an Osterizer 10-speed blender. Aerobic plate counts (APC) were measured using a spread-plating method by spreading 0.1 ml samples onto plate count agar (PCA, Difco, Detroit, MI) supplemented with 0.5% (w/v) NaCl. Triplicate plates were measured at each pasteurization condition. All plates were incubated at 37°C for 48 hr.

Statistical analysis

Effect of different pasteurization conditions on whiteness, L^*, a^*, b^*, shear strain, and shear stress of gel samples were analyzed to determine a statistical difference using the analysis of variance (STATGRAPHICS, 1992). Least significant difference (LSD) at 5% was used to determine significant differences between mean values.

Results and Discussion

Effects of color

L^* value decreased significantly for the first 5 min of pasteurization ($p<0.05$) but remained unchanged during 15-120 min at three pasteurization temperatures (93, 85 and 75°C)(Fig. 3.2). Starch granules absorbed water and became swollen upon initial heating

so that gel colors changed from opaque to more translucent and the L^* values decreased (Charley, 1982). After 5 min pasteurization, there was no statistical difference of L^* value between different pasteurization temperatures, but L^* values at 75°C were higher than 85 and 93°C. This was probably because starch at 75°C had a lower degree of gelatinization than 85 and 93°C. Surimi seafood gels at 75°C were more opaque than 85 and 93°C and the L^* values at 75°C were higher. Yang (1997) used Alaska pollock (*Theragra chalcogramma*) surimi to investigate the effects of starch and thermal conditions on the color of surimi gels and reported that there was no significant difference of L^* value at different heating times.

As shown in Fig. 3.3, the a^* values of surimi seafood were very consistent at different pasteurization times at all of three different pasteurization temperatures (except zero pasteurization time). There were no significant differences ($p>0.05$) of a^* value in pasteurization times and temperatures. Park (1995) investigated the effects of moisture content and physical conditions on the color of pollock and whiting surimi gels, and confirmed that there was no significant difference of a^* value at different heating and setting conditions.

Both pasteurization temperature and time affected the b^* value of surimi seafood ($p<0.05$)(Fig 3.4). The higher the pasteurization temperature and the longer the pasteurization time, the higher b^* values were obtained. This was probably due to the Maillard (browning) reaction between sugars, fish proteins or amines, and water at higher temperatures. The higher temperature and longer time of the pasteurization resulted in

more active Maillard browning reaction (Whistler and Daniel, 1985). There was no difference between two temperatures (75 and 85°C) for their effect on the b^* value during the entire pasteurization time (120 min).

Both pasteurization temperature and time ($p<0.05$) affected the whiteness of surimi seafood. The whiteness of surimi seafood pasteurized at 93°C was lower than that of 85°C at 60 and 120 min, but no statistical difference was found (Fig 3.5). When the pasteurization temperature and time increased, the whiteness value of the surimi seafood decreased. After pasteurization for 120 min, the whiteness value decreased by 2.2%, 3.9% and 5.9% at 75, 85 and 93°C, respectively. This demonstrated that the whiteness of surimi seafood decreased more when the higher temperatures and longer pasteurization times were applied. The whiteness of pike eel (*Muraenesox cinereus*) surimi sausage was also significantly affected by heat processing (Hsu, 1990).

As the pasteurization temperature and time of surimi seafood increased, the L^* value decreased and the b^* value increased. This is why the whiteness of surimi seafood decreased according to the equation (Whiteness = L^*-3b^*)(Park, 1994a). For surimi seafood, the whiter meat is perceived as that with the highest color quality. High pasteurization temperatures and long pasteurization times can deteriorate the color quality of surimi seafood and are not recommended.

Fig. 3.2. L* value of surimi seafood at various pasteurization conditions

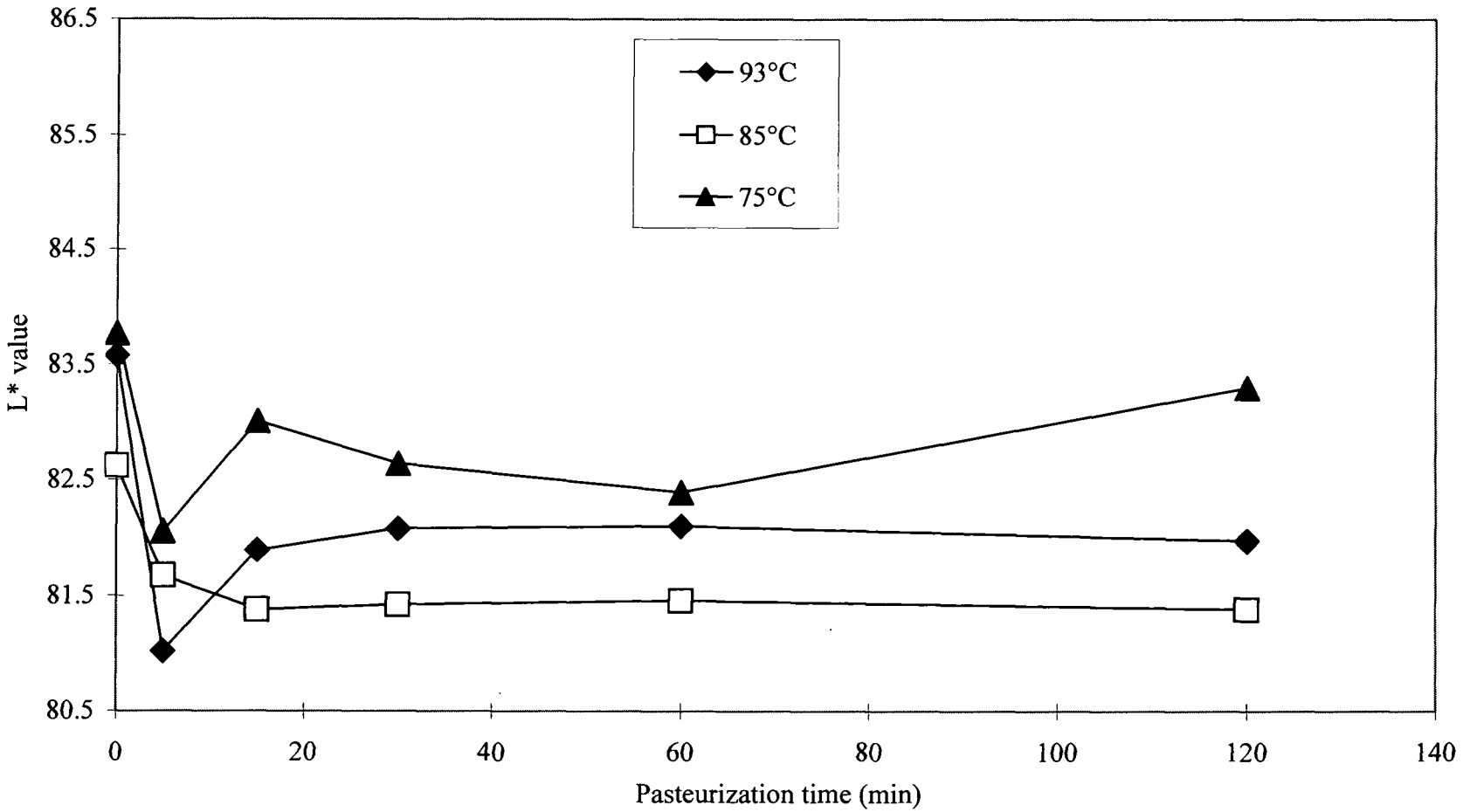


Fig. 3.3. a* value of surimi seafood at various pasteurization conditions

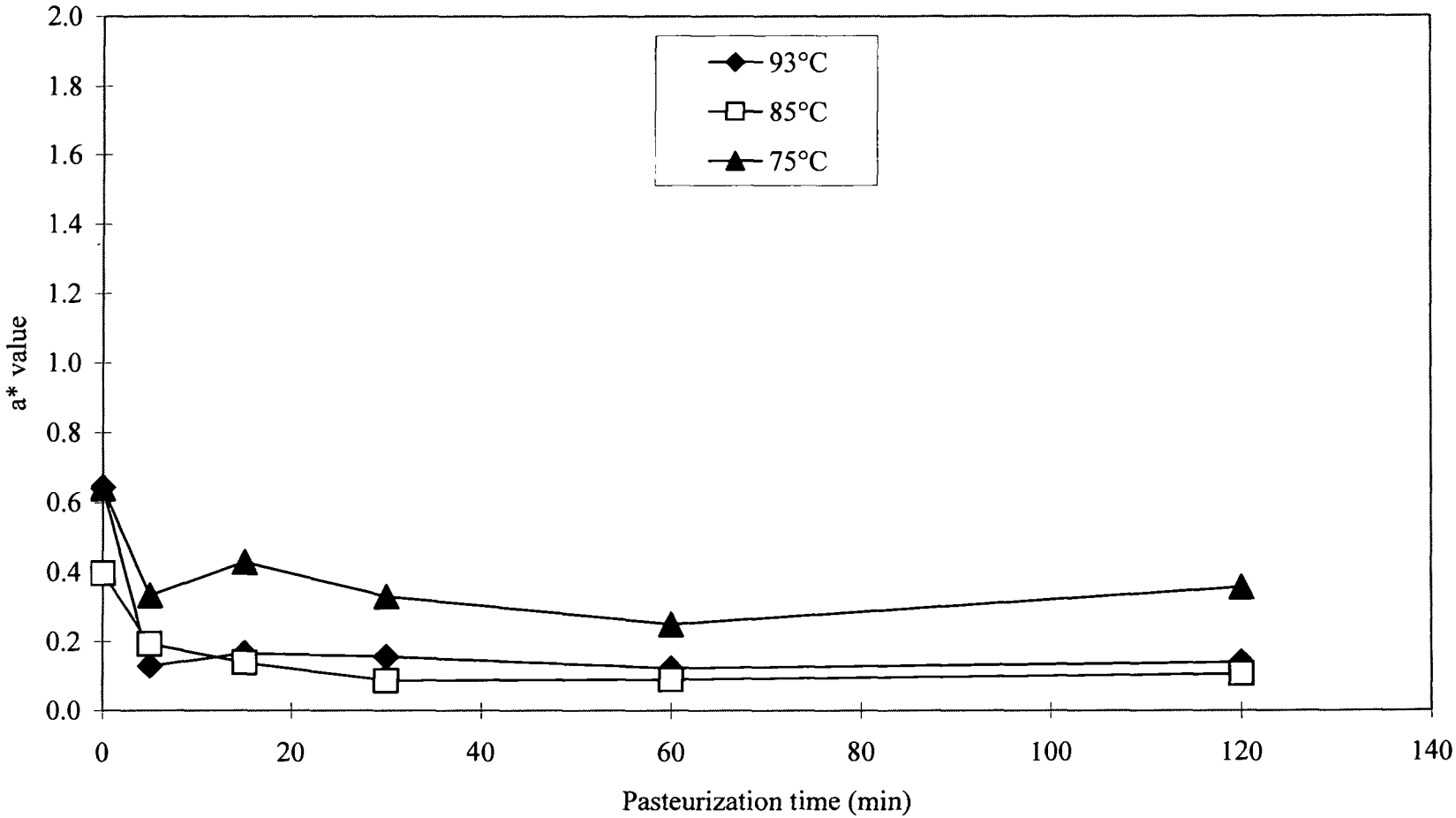


Fig. 3.4. b* value of surimi seafood at various pasteurization conditions

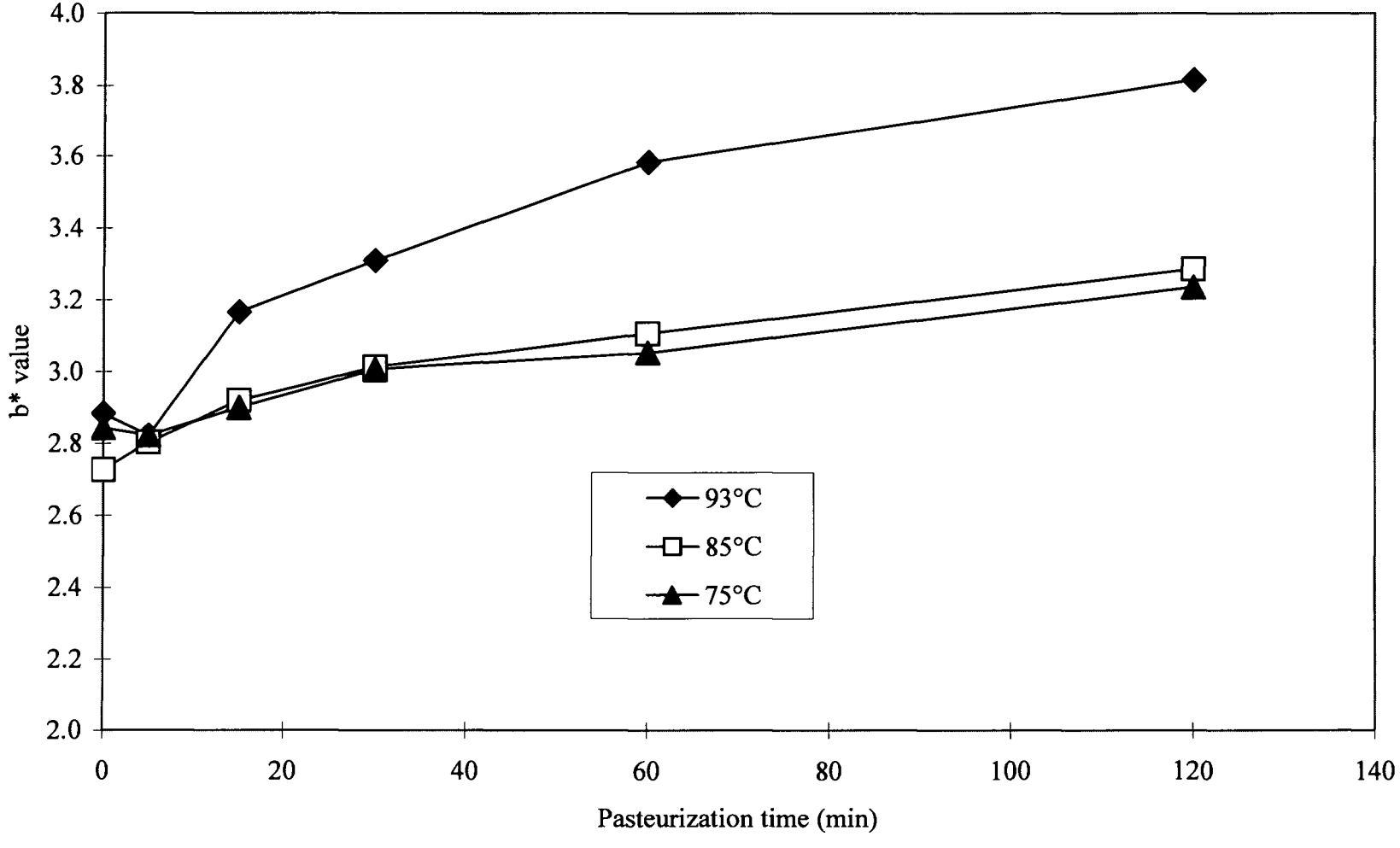
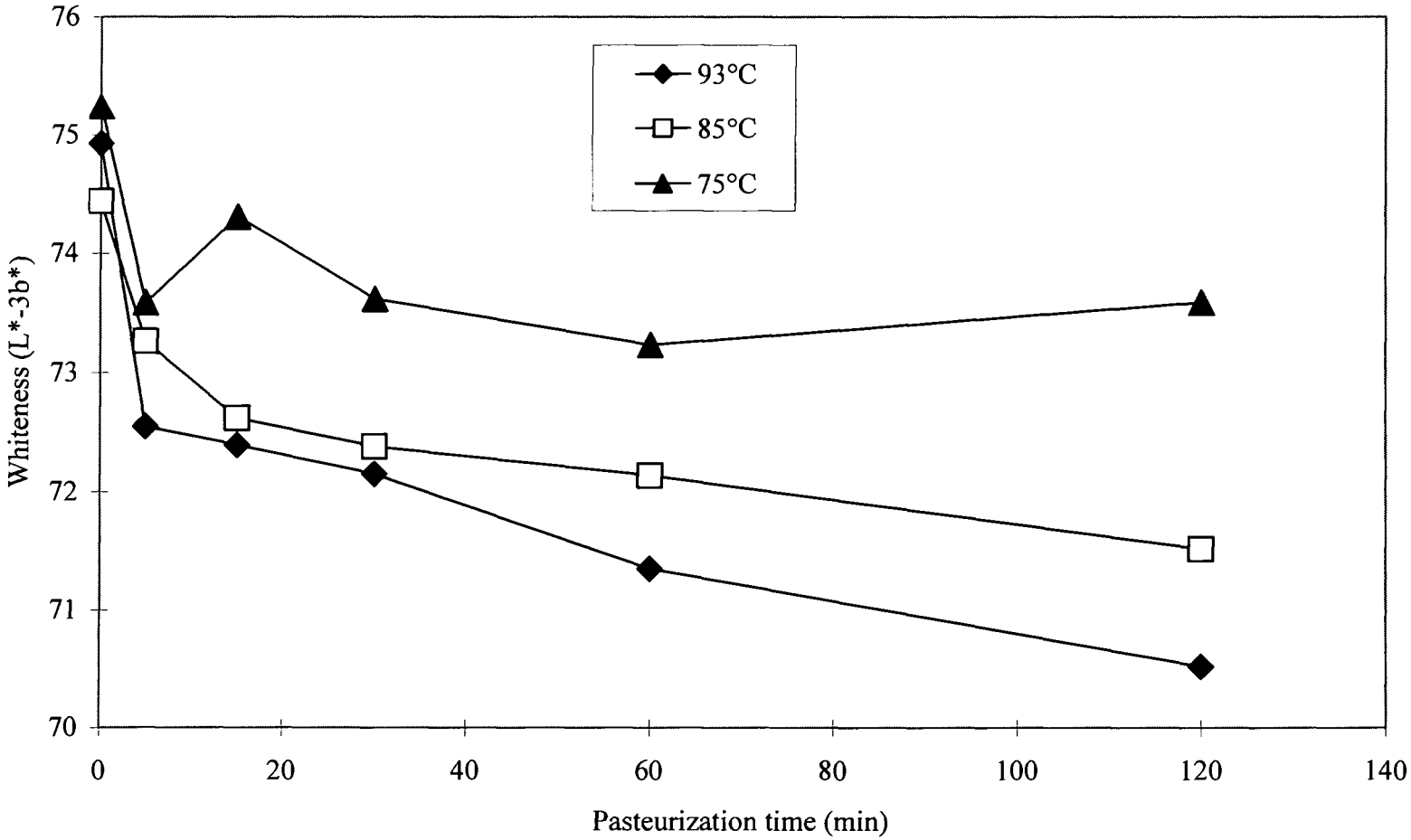


Fig. 3.5. Whiteness of surimi seafood at various pasteurization conditions



Effect of textual properties

Shear stress and shear strain of gels indicate strength and cohesiveness, respectively. After 5 min of pasteurization at 93, 85, and 75°C, the shear stress of surimi seafood increased by 44, 21, and 28%, respectively, and then remained constant for 120 min (Fig 3.6). Different starches have different gelatinization temperatures (Whistler and Daniel, 1985), but most starches can be gelatinized below 75°C. Since initial cooking at 90°C for 6 min can bring the internal temperature to 73°C (Yongsawatdigul et al., 1995), most starches could have completed gelatinization while surimi proteins were forming a strong gel. A rapid increase of shear stress after 5 min pasteurization at all temperatures (Fig 3.6) confirmed that initial cooking was not enough to develop surimi seafood texture. As reported by Wu et al. (1985), salt and sucrose in our samples might have shifted the starch gelatinization temperature to a higher temperature. Yamashita and Yoneda (1989) also reported that in a surimi-sucrose-sorbitol-salt-starch system, the gelatinization temperature of starch can be shifted to a higher temperature by 8-15°C. At a 75°C internal temperature of surimi seafood, the starch gelatinization might have started, but not completed in the presence of salt and sugar. As the pasteurization of surimi seafood continued, the starch imbibed more water from surroundings of the protein matrix resulting in increased shear stress. As a result, the shear stress values increased after 5 min of pasteurization at three different temperatures. This result confirms that the gel-reinforcing effect of starch in the heat-induced surimi gel was due to starch granules swollen in protein gel matrix causing texture to become firmer (Kim and Lee, 1987; Yang, 1997). Shear stress at 75°C was lower than at 85 and 93°C (Fig. 3.6) indicating that 75°C was not high enough for the starch to complete gelatinization in this

particular formula. For 93°C, after 30 min pasteurization, shear stress reached the highest point, and then gradually decreased. This indicated that extreme pasteurization conditions could cause low shear stress of surimi seafood resulting in lower texture quality.

Shear strain is the most important and sensitive parameter to describe functional quality of myofibrillar proteins in surimi gels (Lanier, 1986). At 93°C for 120 min, shear strain decreased by 24.36%, however, at 75°C, shear strain decreased by only 16.66% (Fig 3.7). Shear strain values of surimi seafood decreased more at higher pasteurization temperatures and longer pasteurization times. Shear strain at three different temperatures rapidly decreased after 5 min of pasteurization. Verrez-Bagnis et al. (1993) reported that when starch granules complete gelatinization, a part of starchy materials could leach out of the starch granules and lead to the formation of intergranular connections. As the starch went through the gelatinization, the protein-starch matrix became tighter and formed a closer network. This is most likely why the shear strain decreased. Bertak and Karahadian (1995) studied the effects of different heating methods on commercially produced leg style surimi seafood and reported that extra heating reduced firmness and chewiness. The extra heating may have destroyed the gel network matrix causing low shear strain

In general, surimi seafood with higher shear stress and shear strain is accepted as a premium product. Although heating is essential to develop the strength of gels (shear

Fig. 3.6. Shear stress of surimi seafood at various pasteurization conditions

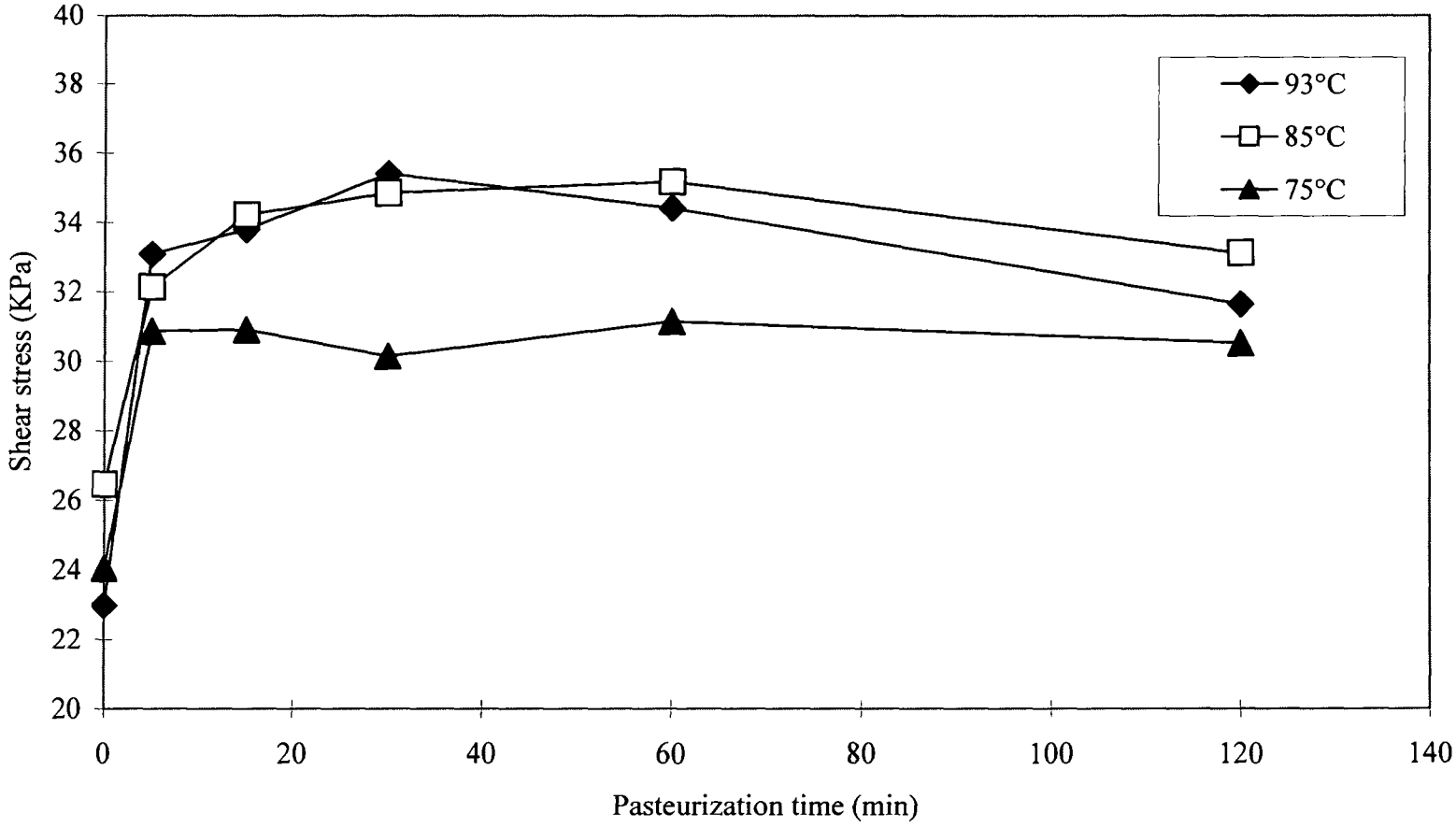
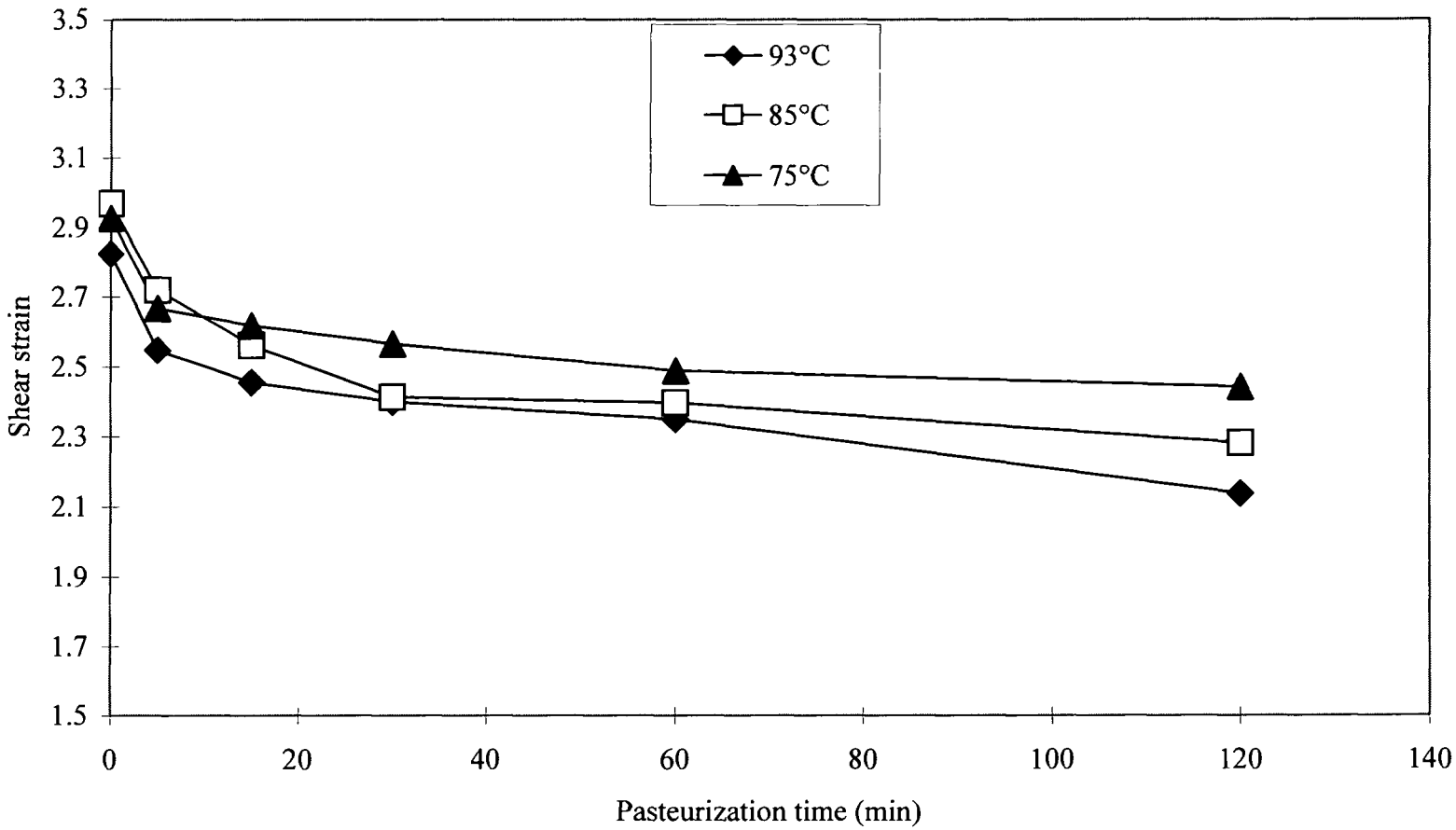


Fig. 3.7. Shear strain of surimi seafood at various pasteurization conditions



stress), extreme heating not only decreased the shear stress but also decreased the shear strain of the surimi seafood. To maintain the high quality of surimi seafood, the modification of the current pasteurization methods is suggested for a feasible solution using higher temperatures and shorter times.

Aerobic plate counts (APC)

APC of raw surimi used for this study was ~2×10^6 CFU/g. This number is higher than those reported by Himelbloom et al. (1991a) and Ingham and Potter (1987). Himelbloom et al. (1991a) reported the APC for high-grade surimi (Alaska pollock) was 5.5×10^4 CFU/g and 2.0×10^6 CFU/g for low-grade surimi (Alaska pollock) from two shore-based surimi plants in Alaska. Ingham and Potter (1987) reported the APC of Atlantic pollock surimi was 3.1×10^4 CFU/g. This indicates that factors such as season, source, grade, and processing procedures can make a difference in the microbial quality of surimi. After mixing surimi with other ingredients, the APC of surimi seafood paste varied from 5.9×10^5 to 7.7×10^6 CFU/g. Almost all (99%) of APC was destroyed by the initial cooking (Fig 3.8). The internal temperature of tubes were maintained above 60°C after holding at room temperature for 8 min (Fig. 3.9 and appendix 1). After reheating samples at 93°C water bath for 5 min, the internal temperature reached ~90°C. Therefore, it was not a surprise that the APC was zero after 5 min of pasteurization at 93°C. After 15 min of pasteurization at 75 and 85°C, the APC became zero. Although there was no measurement between 5 and 15 min of pasteurization time, the time required to obtain zero APC at 85°C pasteurization temperature should be less than time required at 75°C.

Fig. 3.8. APC of surimi seafood at various pasteurization conditions

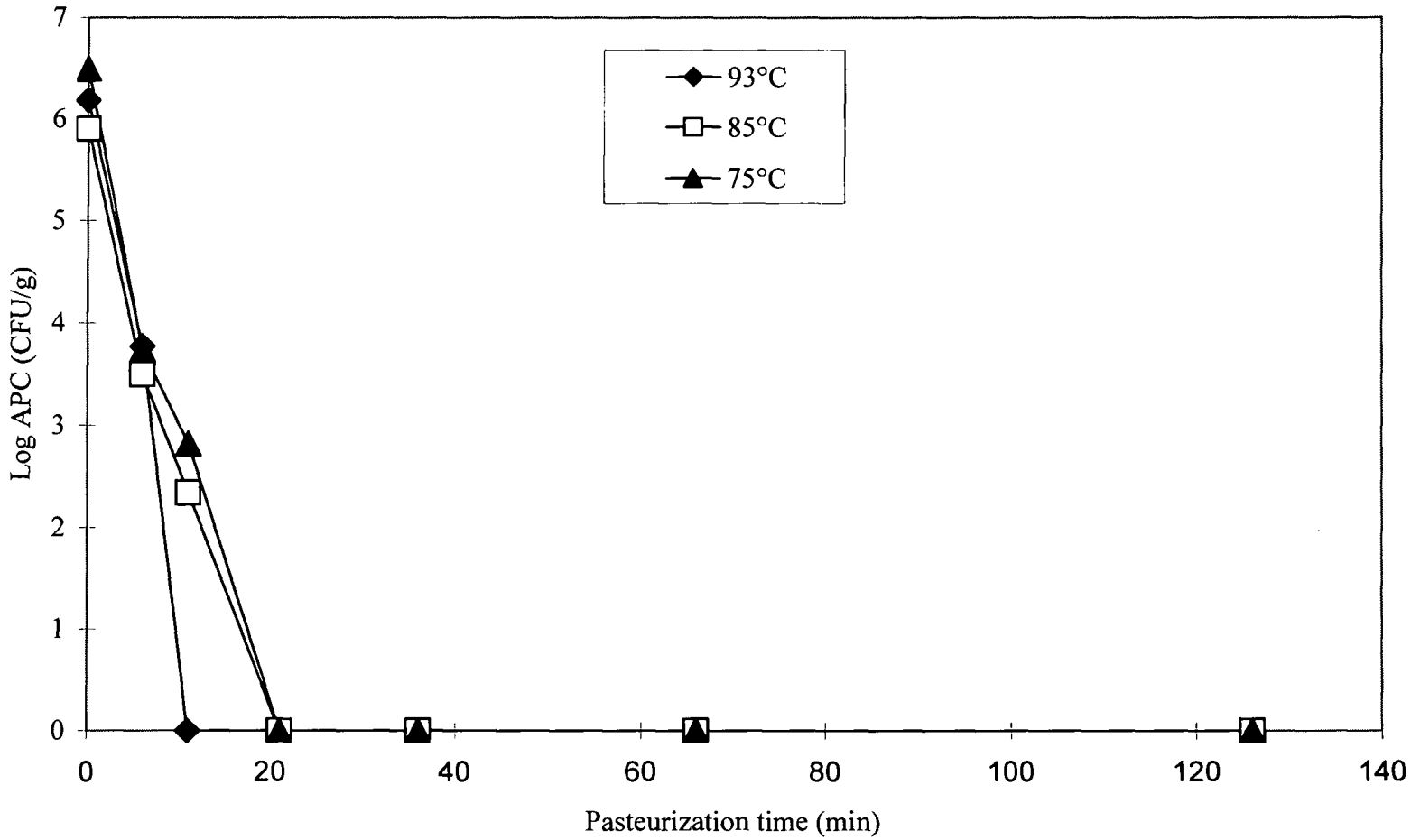
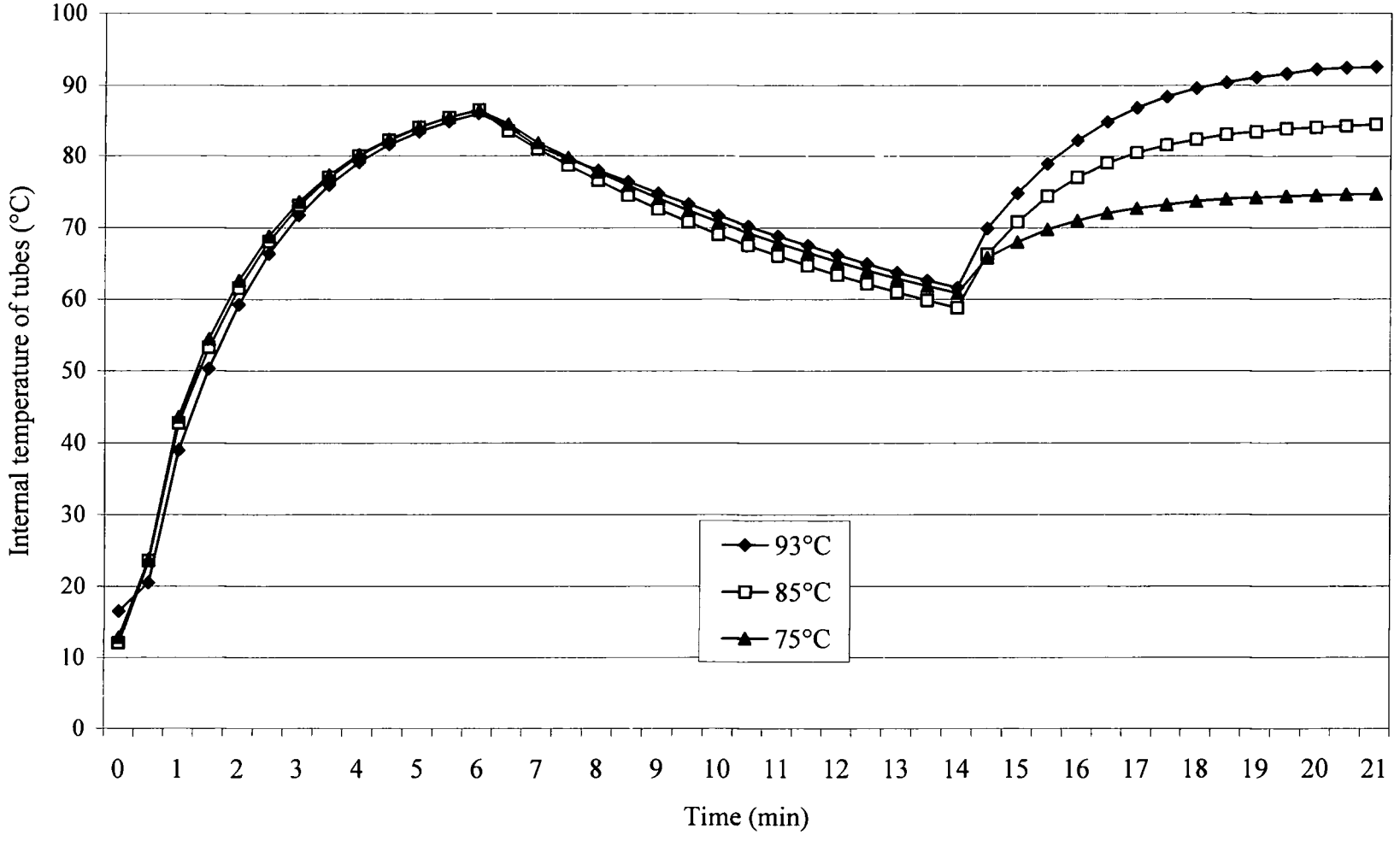


Fig 3.9. Temperature profiles of surimi seafood at various pasteurization conditions



The time required to destroy pathogenic microorganisms in food products is mainly dependent on the initial microbial load, the composition of the microbial population, and the target F-value (Rippen and Hackney, 1992). The internal temperature reached 75 and 85°C after ~6 min at 75 and 85°C water bath. Keeping surimi seafood at the internal temperature of 75°C for an additional 5 min was enough to obtain zero APC for this study. Regardless of the initial microbial load and composition of the microbial population as compared with our results using industry methods shown in Table 3.1, the surimi seafood in the market appeared to be overcooked. This may be one reason why there is often mushy and soft textured surimi seafood in the market especially when a large portion of surimi is replaced by starch and water.

Conclusion

Pasteurization temperatures and times significantly affected the shear strain, whiteness, and APC of surimi seafood. The texture and color qualities of surimi seafood significantly deteriorated at higher pasteurization temperatures and longer pasteurization times. At all three pasteurization temperatures (75, 85, 93°C), 15 min was enough to obtain the zero APC. At 15 min pasteurization time, the two major characteristics of surimi seafood (texture and color) were better at 75 and 85°C than 93°C. This indicates that 15 min at 75 or 85°C were the optimum pasteurization conditions for this study.

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Chapter 4

Thermal Death Time of *Enterococcus faecium* E-20 in Surimi Seafood

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To be submitted to Journal of Food Science,

Institute of Food Technologist, Chicago, Illinois

1997

Abstract

Enterococcus faecium E-20, one of the most heat-resistant vegetative bacteria, was inoculated in raw surimi seafood paste. The inoculated surimi paste was heated at various temperatures from 60 to 85°C. Thermal resistance characteristics including survival curves, thermal death time curve, thermal resistance curve, D-values and z-value were determined. The pasteurization value F_{250} was also calculated. The D-values were in the range of 30.60 min at 60°C to 1.15 min at 85°C. The z-value of *E. faecium* E-20in surimi seafood was 16.3°C. The F_{250} value was much higher at 60°C than other temperatures.

Key words: *Enterococcus faecium* E-20, thermal resistance, D-value, z-value, F value, surimi seafood.

Introduction

Surimi crabmeat (hereafter surimi seafood) is ready to eat and most commonly used in seafood salads without further thermal treatment. Therefore, the microbial content of these seafood products is very important. The FDA will consider regulatory action when entrotoxigenic *E. coli* (ETEC) is present at 1×10^3/g in processed seafood which requires minimal or no processing by consumer (Ward and Price, 1992). According to the FDA's informal guidance for *Staphylococcus aureus*, the product may be recalled if tested positive for the toxin or if 1×10^4/g (MPN) organisms are found (Ward and Price, 1992). The FDA has also established a zero tolerance for *Listeria monocytogenes* and *Samonella* in ready-to-eat foods and seafood (Ward and Price, 1992). Pasteurization plays an important role in the manufacturing of surimi seafoods, because the gelation of surimi proteins is completed along with other functional ingredients and vegetative microorganisms are destroyed. The production of surimi seafood usually involves a two-step thermal treatment. The first step of thermal treatment is to make the gelation of surimi proteins. The second step is to pasteurize the finished products while gelatinization of starch components and gelation of other functional ingredients are completed. The former takes 1-2 min in a thin sheet ribbon shape, while the latter takes 20-80 min (Park, 1994). Pasteurization using mild heating conditions results in color, texture, and flavor characteristics which are similar to fresh products, but with greatly extended shelf life (Rippen and Hackney, 1992).

Pasteurization provides many advantages in surimi seafood, but the risks cannot be completely disregarded due to the growth of pathogenic microorganisms dangerous for the consumer. Park (1994) surveyed the pasteurization practices within the US surimi seafood industry. As shown in Table 4.1, surprisingly, there was a great deal of variation in terms of time and temperature of pasteurization within the industry. Over the last 15 years, it is very fortunate that the US surimi seafood industry has never experienced outbreaks leading to serious illness or death since its establishment. However, there is a great potential for possible outbreaks using unstandardized current pasteurization methods.

Shie and Park (1997) reported that pasteurization at a high temperature for a long time could deteriorate the texture and color qualities of surimi seafood. They also reported that after 10 min pasteurization at 93°C (6 min initial cooking at 90°C), the internal temperature of surimi seafood reached ~90°C and the aerobic plate count of surimi seafood was zero. For the voluntary United States Dept. of Commerce's (USDC) seafood inspection plans such as PUFI (Packed Under Federal Inspection) or HACCP (Hazard Analysis Critical Control Point), it is recommended that vacuum packed products must be heated at 85°C (internal temperature) for 20 min. No F values for surimi seafood products have been suggested (Comar, 1987). In comparing the study reported by Shie and Park (1997), it may be that the US industry overcooked surimi seafood to result in soft texture and yellowish color particularly when a large portion of surimi was replaced by starch, protein additives, and water. Although surimi seafood has become Americanized since the early 1980s, no research has been conducted to

determine a suggested F value for microbiologically safe and functionally good quality of surimi seafood.

Enterococcus faecium E-20 is one of the most heat-resistant vegetative bacteria. It has been implicated as a potential spoilage type (Brown et al., 1960, Ingram and Barnes, 1955) and most likely survives in mild pasteurization conditions. This specific microorganism was chosen as the target microorganism for this study because of its heat-resistance and non-pathogenic characteristics. The objectives of this study were (1) to determine the D-, z- and F_{250} values of *E. faecium* E-20 in surimi seafood in the temperature range of 60 to 85°C, and (2) to develop a suggested guideline for the optimum pasteurization procedures using the obtained z- and F_{250} values for the US surimi seafood industry.

Table 4.1. Pasteurization time and temperature used in the US surimi seafood industry

Company	Frozen	Frozen	Refrigerated
	External Temperature[a]	Internal Temperature[a]	
A	190°F (87.3°C)/30 min		Same as Frozen
B		85°C/15-30 min	Same as Frozen
C		185°F (85°C)/20 min	Same as Frozen
D	190°F (87.8°C)/22 min	To reach 170°F (77.6°C)	Same as Frozen
E	90°C/45 min	180°F (82.2°C)/10 min	Same as Frozen
F		70°C/20 min	Same as Frozen
G		160°F (71.1°C)/10 min	185°C (85°C)
H		To reach 82°C	Same as Frozen
I		85°C/17 min	Same as Frozen
J		80°C/5 min	85°C/5 min

a: Some companies used external temperature as a pasteurization target, while others used internal temperature of products.

Materials and Methods

Materials

KA grade Alaska pollock (*Theragra chalcogramma*) surimi (American Seafoods Co, Seattle, WA) was cut into about 1 kg blocks, vacuum packed, and stored at -25°C. The moisture content of surimi and surimi seafood was 74.31±0.59% and 68.26±1.46%, respectively (AOAC, 1995). In addition to surimi, other ingredients such as salt, sugar, wheat flour, liquid egg white, and soy bean oil (all from a local market) were used according to Table 4.2. Corn starch and PG 15 starch (waxy maize modified with cross-link and hydroxypropylation, Cerestar, Hammond, IN), and natural and artificial crab extract (Givaudan-Roure Inc, Brampton, Ontario, Canada) were also used.

Table 4.2. Formula of surimi seafood

Ingredient	Weight %
Surimi	35.54
Water	35.54
Salt	1.65
Sugar	1.5
Wheat flour	4
Corn starch	7.25
PG 15 modified starch	0.5
TiO_2	0.02
70% (w/w) liquid sorbitol	5.68
Soy bean oil	2.27
Crab extract	1.5
Liquid egg white	4.55

Enterococcus faecium E-20 culture

E. faecium E-20 culture was obtained from Dr. Sue Ghazala, Department of Biochemistry, Memorial University of Newfoundland, Canada. The lyophilized culture was revived in KF *Streptococcal* broth (KFSB; BBL, Cockeysville, MD) for 24 hr at a 37°C incubator. After revived, the culture was plated on Tryptic Soy Agar (TSA, Difco Laboratories, Detroit, MI) for 24 hr at 37°C and used as a stock culture. A stock culture was maintained at 4°C and subcultured on TSA every month to maintain its viability. When the culture was needed, one loop of pure culture was inoculated from TSA and revived in 40 ml KFSB for 16 hr to reach stationary phase.

Sample preparation

Frozen surimi sample was tempered in running water for 1 hr and then cut into 3-5 cm^3 chunks. Before chopping, the chopping bowl and cap were sterilized using 75% ethanol. Surimi chunks were comminuted at low speed for 1.5 min using a Stephan UM 5 universal food processor (Stephan Machinery Corp., Columbus, OH). Salt was added and the surimi paste was chopped for 0.5 min. All other ingredients and *E. faecium* E-20 culture were added and chopped for another 1 min. The concentration of *E. faecium* E-20 was controlled to around 10^7-10^8 CFU/g surimi paste. For the final 3 min, chopping continued at high speed under vacuum (~0.4 bar). During the entire chopping process, the temperature of surimi pastes was maintained below 8°C using a NesLab constant-temperature circulator (Portsmouth, NH) containing a solution (50:50) of ethylene glycol and water.

Prepared surimi seafood paste was injected into sterilized thermal death time (TDT) glass tubes (10×75 mm) using a 50 ml sterilized syringe. Each tube contained ~2 g of sample covered with sterilized aluminum foil. All prepared tubes were put into an ice-water bath to maintain a temperature around 0°C until thermal treatment (Lynt et al., 1977). During the injection, the remaining surimi seafood paste was kept at a 4°C refrigerator and the entire injection procedure was no longer than 1 hour. A total of 60 TDT tubes were prepared each time and subjected to one of the following temperatures: 60, 65, 70, 75, 80, 85°C.

Thermal treatment of *Enterococcus faecium* E-20

A preliminary study was conducted to determine the time required to obtain a zero count of *E. faecium* E-20 at 60, 65, 70, 75, 80, and 85°C. For each temperature, sixty TDT tubes were placed into a water bath. The time required to obtain zero count of *E. faecium* E-20 was divided into 10 equal time intervals, so the range of time intervals covered from one that wouldn't permit culture to survive (the last interval) and others would. At the same time, provided a gradation between the two extremes, in which some tubes contained surviving culture and others did not (the last interval). At each time interval, 6 tubes were removed out of the water bath and chilled in an ice-water bath immediately to bring down the temperature. During the heating and cooling, the internal temperature of tubes was monitored using a data logger (model 21 X, Campbell Scientific, Logon, UT). A detailed experimental outline is shown in Fig. 4.1. The entire experiment was repeated three times.

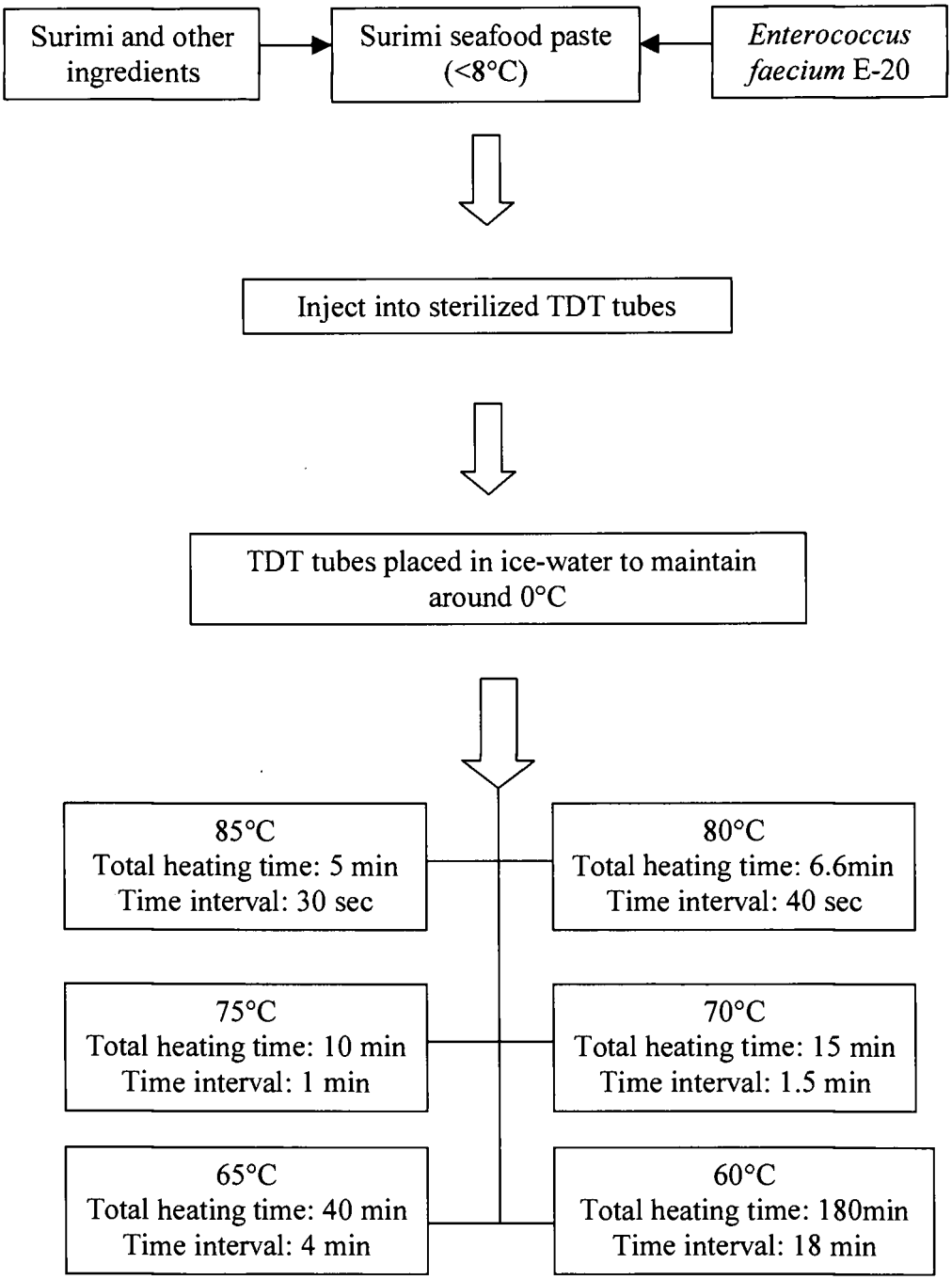


Fig. 4.1. Experimental outline

Enumeration of *Enterococcus faecium* E-20

Ten grams of sample were taken from each different thermal treatment and mixed with 90 ml of 0.1% peptone (Difco, Detroit, MI). The mixture was blended at whip speed for 2 min in a sterilized jar using an Osterizer 10-speed blender. After serial ten-fold dilutions, appropriate diluted samples (0.1 ml) were spread plated on KF *Streptococcal* agar (BBL, Cockeysville, MD). All plates were incubated at 37°C for 72 hr (Ghazala et al., 1995).

D-value, F-value and z value

F-value which is defined as a mathematically calculated number describing the total heating value of the process was obtained using the equal-time-interval method (Patashnik, 1953). This method was accomplished by tabulating the lethality value for each equal-time-interval temperature reading and making a summation thereof. During the heating of each treatment temperature, the internal temperature of the TDT tubes were monitored at different time intervals. The higher the temperature, the shorter the time interval. For example, at 60°C the internal temperature of the TDT tubes were monitored at 1 min intervals, and at 85°C, the internal temperature of TDT tubes were monitored at 5 sec time intervals. The lethal ratio (F/t) at a given temperature is the ratio of the time in minutes which was calculated using equation 4.1.

$$F/t = \log^{-1}[(T-250)/z] \qquad (4.1)$$

where T is the reading temperature (°F) at each time interval and z is the z value. After calculating the lethal ratio at each time interval, the lethal ratio was added together and then multiplied by the equal-time-interval to give a total process F value. According to equation 4.1, 121°C (250°F) was used as the reference temperature to calculate the lethal ratio, therefore the F_{250} value was calculated to represent the time in minutes required to kill a given number of microorganisms at 250°F.

The decimal reduction time (D-value), the time in minutes at a given temperature required to reduce a microbial population by 90%, was calculated using equation 4.2 (Bell and DeLacy, 1984).

$$D = t / (\log N_0 - \log N_1) \tag{4.2}$$

where D is the D-value, t is the heating time in minutes, N_0 is the microbial population at time 0 and N_1 is the microbial population remaining after t min heating.

The z-value which is defined as the degrees in Fahrenheit or Centigrade required for a thermal death time curve to traverse one log cycle was obtained using equation 4.3 (Rippen et al., 1993).

$$z = (T_2 - T_1) / (\log D_1 - \log D_2) \tag{4.3}$$

where z is the z value, T_1 is the temperature at time 1, T_2 is the temperature at time 2, D_1 is the D-value at temperature 1 and D_2 is the D-value at temperature 2. Microsoft Excel 97 was used for calculations and statistical analysis for regression.

Results and Discussion

Survival curves and D values

Survival curves of *E. faecium* E-20 (Fig. 4.2) and thermal death time curve (Fig. 4.3) in surimi seafood from 60 to 85°C were determined. These survival curves were similar in shape to those obtained in other studies. Ghazala et al. (1995) studied the thermal kinetics of *E. faecium* E-20 in cooked meat broth, while Magnus et al. (1986) studied the thermal resistance of *E. faecium* E-20 in pasteurized ham. The survival curves of 60 and 65°C showed the characteristic of heat-resistant microorganisms such as initial lag phases and shoulders. The initial lag phase may indicate bacterial clumping or chain formation, resulting in a temporary increase in heat stability, since only when the viability of each cell in the clump has to be destroyed before reductions in the viable cell count are observed (Hansen and Reiman, 1963). The shape of survival curves provides information about heat resistance of the population of the studied microorganism. Death rates at 70 to 85°C were much higher than those in 60 and 65°C (Fig. 4.2). The r^2, slopes, and intercepts of survival curves are shown in Table 4.3. The slopes at high temperatures were much higher than low temperatures indicating that increased temperature resulted in fast injury and death of cell. All survival curves characteristically

Figure 4.2. Survival curves of *Enterococcus faecium* E-20

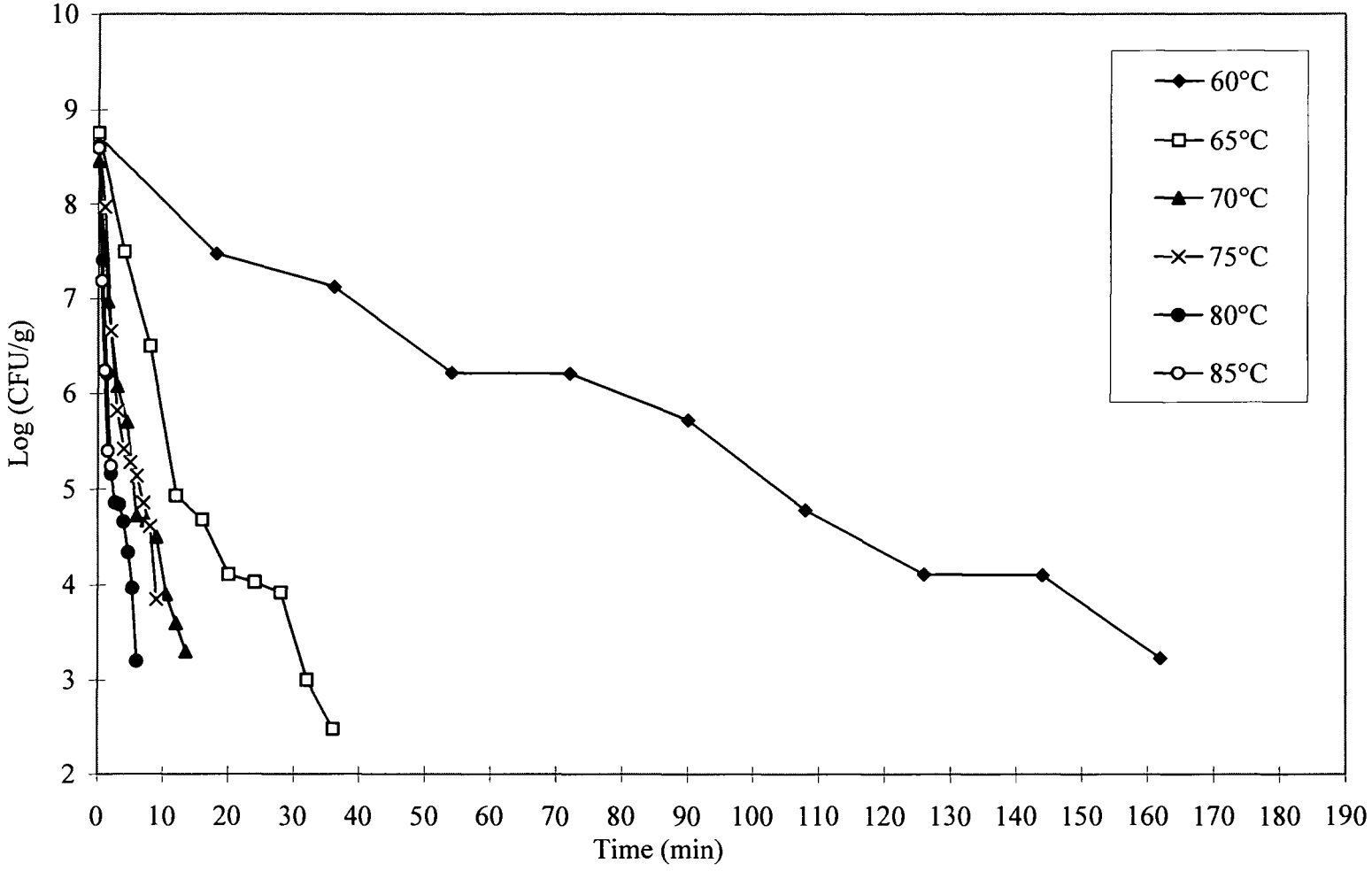
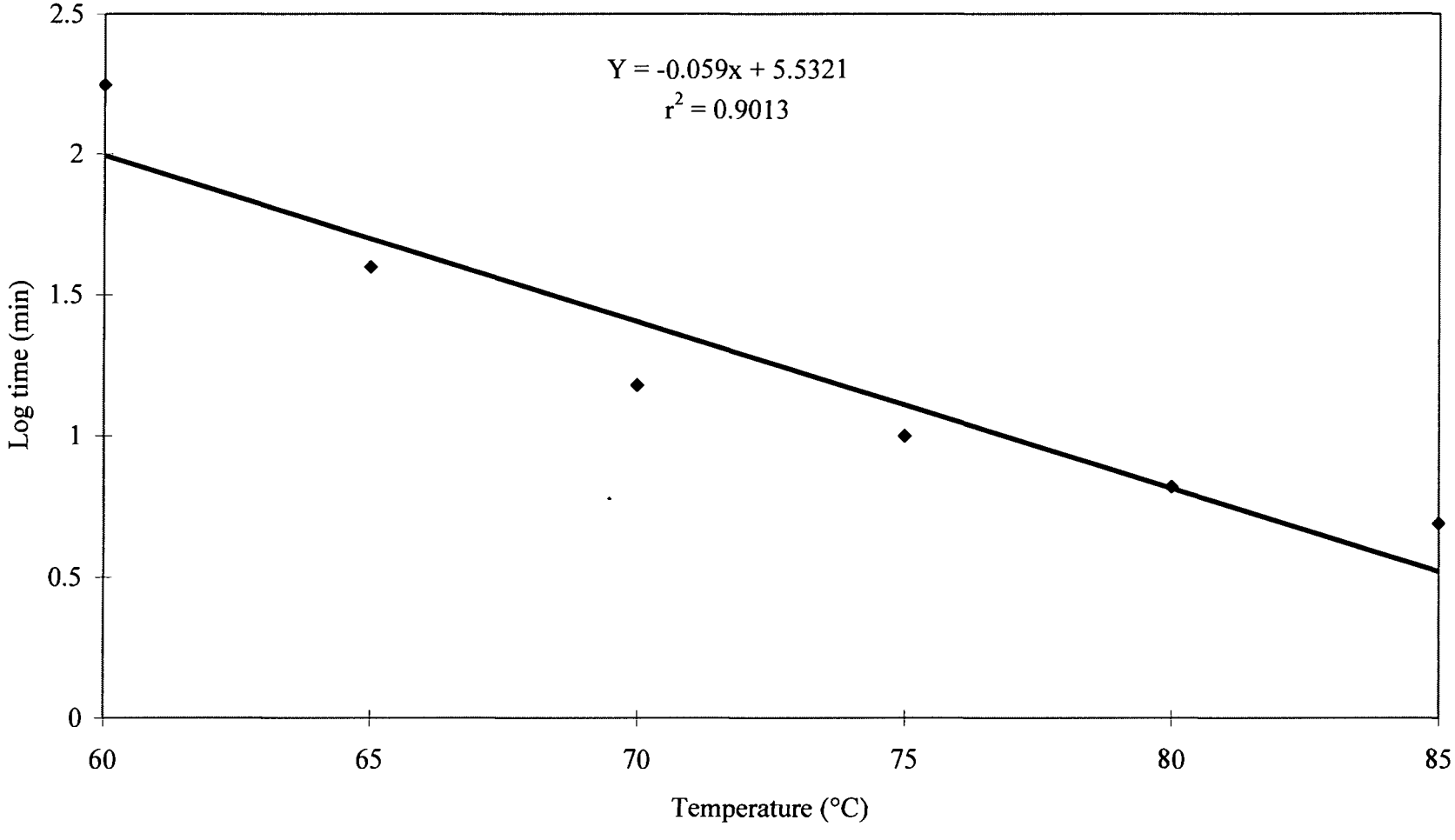


Figure 4.3. Thermal death time curve of *Enterococcus faecium* E-20



demonstrated a first-order destruction rate with the high r^2 value (>0.84)(Table 4.3). Thermal treatment using high temperatures were less accurate in this study since the r^2 values were lower for 80 and 85°C. This is probably because of the difficulty in handling samples accurately at short time intervals for high temperature treatments. For this experiment, we used a water bath as the heat source. Once the water bath was open, due to the heat loss from the water bath, it required some time to equilibrate to the desired heating temperature. In high temperature treatments, the water bath could not maintain the desired temperatures before the next sampling intervals because the time intervals were short (30 sec at 85°C, 40 sec at 80°C).

Table 4.3. R^2, slopes and intercepts of survival curves of *E. faecium* E-20 in surimi seafood.

Temperature (°C)	Y-intercept (log survivors)	Slope (min)	r^2
60	8.30	-0.0313	0.97
65	7.83	-0.1579	0.91
70	7.50	-0.3416	0.92
75	7.94	-0.4700	0.90
80	7.61	-0.7600	0.88
85	7.50	-0.8500	0.84

Table 4.4. D-values of *E. faecium* E-20 in surimi seafood

Temperature (°C)	D-value (min)
85	1.15
80	1.69
75	2.57
70	2.95
65	14.13
60	30.90

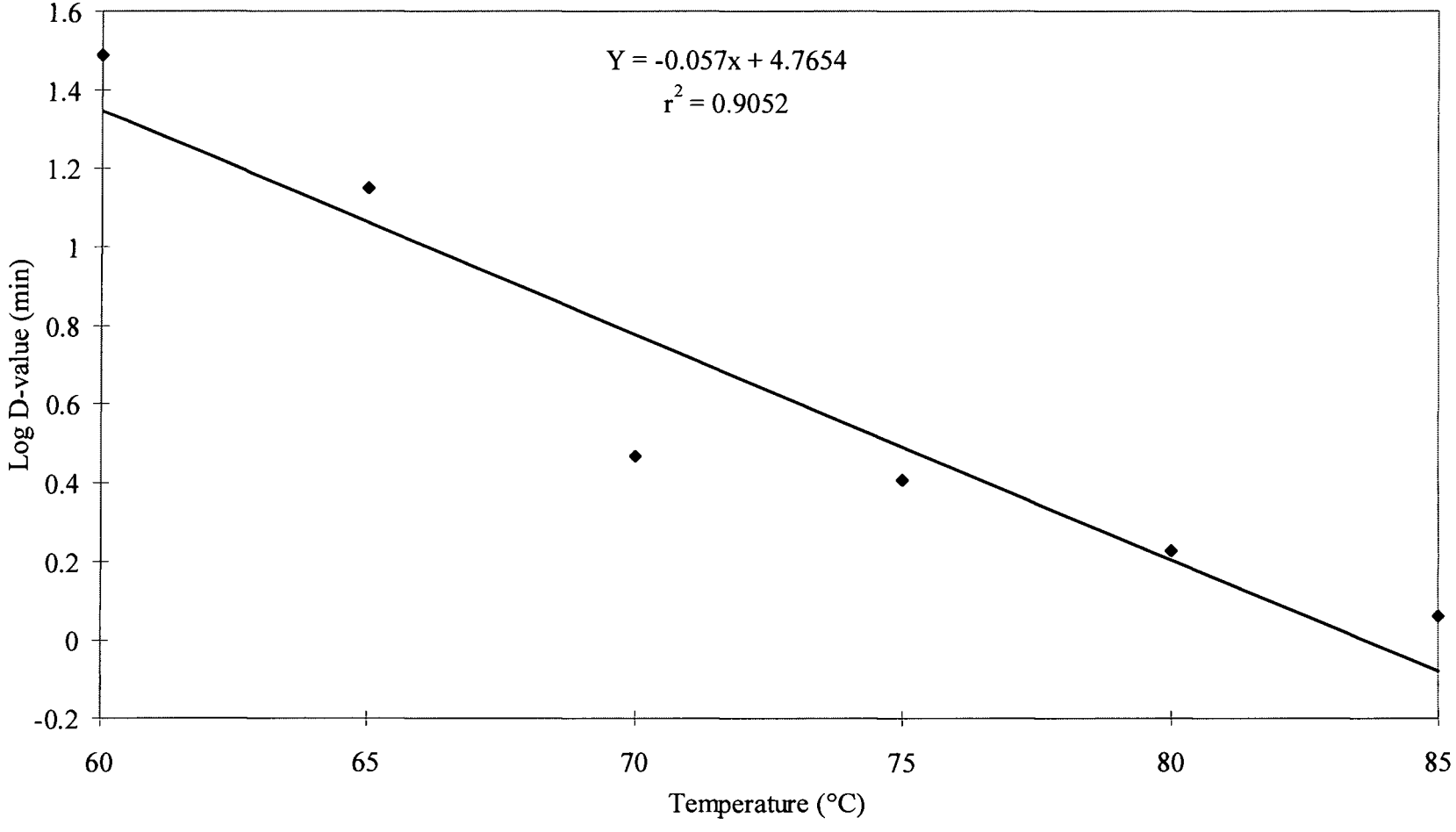
D-values of surimi seafood decreased when processing temperature increased form 60 to 85°C. Ghazala et al. (1995) reported that the D-values of *E. faecium* E-20 in cooked meat broth at a temperature range from 60 to 85°C were as follows: D_{60}= 22.53 min, D_{65} = 4.47 min, D_{70} = 1.11 min, D_{75} = 0.61 min, D_{80} = 0.29 min, D_{85} = 0.20 min. Magnus et al. (1988) studied the thermal resistance of *E. faecium* E-20 in pasteurized ham and reported D-values at 64, 66, 68, 70, 72°C were 26.2, 13.97, 8.82, 4.70, and 2.05 min, respectively. Comparing our results with those two studies, our D-values (Table 4.4) were higher than those reported by Ghazala et al. (1995), but appeared to be lower than those reported by Magnus et al. (1988). This difference could be explained by factors influencing heat resistance of microorganisms. These are: initial cell population, age of culture, complexity of suspending medium, pH of heating medium, and the recovery medium of the heat-treated microorganism (Beuchat and Lechowich, 1968a, b; Iyengar et al. 1957). It is well documented that the composition of the heating menstruum has a definite effect upon the degree of observed heat resistance (Magnus et al., 1986, 1988). The ability of the microorganism to survive at the heat stress increases

when the composition of the menstruum becomes more complex. Although the mechanisms of this protection are still unclear, it has been suggested that this is due to the protective effect of extracellular protein or the ability of solutes to reduce the water activity in the immediate environment of the cells protein (Verrips and Van Rhee, 1983). Therefore the heat resistance of the microorganism increases. Food constituents such as salt and fat are known to protect the microorganism from heat stress. The pH value of the heating menstruum affects the heat resistance of the microorganism. Bell and DeLacy (1984) studied the heat injury and recovery of *E. faecium* in chub-packed luncheon meat, and found that the presence of NaCl in the heating medium provided some protection from lethal heat damage for cells of *E. faecium*. Simpson et al. (1994) studied the thermal resistance of *E. faecium* as influenced by pH and salt and concluded that the D-values were influenced by thermal processing temperature, salt concentration, and pH of the test medium. The higher salt concentrations resulted in the higher D-values. Acidic conditions gave lower D-values than neutral or basic conditions. Surimi seafood has high protein content, neutral pH and the formula used had 10 different ingredients (high complexity). This may explain why the D-values obtained in this study were higher than for data in previous studies.

Thermal resistance curve, z-value and F value

Thermal resistance curve of *E. faecium* E-20 was developed (Fig. 4.4). The z-value calculated from the thermal resistance curve was 16.3°C. This z-value is higher than the z-value 12.4°C for cooked meat broth (Ghazala et al., 1995), and 7.46°C for ham (Magnus et al., 1988). Again, this is probably because of the different environmental

Figure 4.4. Z-value curve of *Enterococcus faecium* E-20 in surimi seafood



influences during the thermal processing and the handling procedures of the microorganism may have been factros. Several researches have demonstrated that the z-value measured in different menstruum is not constant (Bell and Delacy, 1984; Magnus et al., 1988; Simpson et al., 1994; Tsang and Ingledew, 1982). They suggested that the use of z-value in determining the time-temperature of pasteurization process should be considered cautiously.

The time required to obtain zero count of *E. faecium* E-20 at 60°C was much longer than at other temperatures (Table 4.5). As the heating temperature increased from 60 to 65°C, the time required to obtain zero count of *E. faecium* E-20 decreased from 180 to 40 min. This result indicates that *E. faecium* E-20 is more heat-resistant at low temperatures so that higher temperatures could shorten the heating time. It also supports our previous study. High temperature and short pasteurization time conditions were better for surimi seafood since long pasteurization time at high temperatures deteriorated texture and color quality (Shie and Park, 1997). The temperature profiles of TDT tubes at different heating temperatures were shown in appendix 2. Using the z value (16.3°C) of *E. faecium* E-20 obtained in this study, the F_{250} value used in commercial pasteurization practices in the US surimi seafood industry was calculated (Table 4.6). The F_{250} values ranged from 0.009 to 0.270 min. Comparing the F_{250} value at 60°C with other study temperatures (65 to 85°C), the F_{250} value (0.044 min) at 60°C is almost four times higher than other study temperatures (0.010-0.019 min). Again, this result supports that *E. faecium* E-20 is more heat-resistant at low pasteurization temperatures. A 0.044 min of F_{250} value was enough to obtain a zero count of *E. faecium* E-20 at temperature

range of 60 to 85°C. The F_{250} value of the USDC suggested method for the pasteurization of its voluntary PUFI or HACCP sealed surimi seafood (heat to reach 85°C internal temperature and hold for 20 min) is 0.12 min, this value is much higher than our result (0.044 min). Considering the microbial safety and comparing with our results, most of the surimi seafood manufacturers appeared to overcook their products.

Table 4.5. Time required to obtain zero count of *E. faecium* E-20 and F_{250} values at various heating temperatures.

Temperature (°C)	85	80	75	70	65	60
Time (min)	5	6.6	10	15	40	180
F_{250} (min)	0.019	0.014	0.012	0.010	0.014	0.044

Table 4.6. F_{250} values estimated from commercial pasteurization methods for frozen products used in the US surimi seafood industry.

Co.	Pasteurization procedure	Pasteurization procedure	F_{250} value (min)
	External Temperature	Internal Temperature	
A	190°F (87.3°C)/30 min		0.270
B		85°C/15-30 min	0.093-0.186
C		185°F (85°C)/20 min	0.124
D	190°F (87.8°C)/22 min	To reach 170°F (77.6°C)	0.197
E	90°C/45 min	180°F (82.2°C)/10 min	0.041
F		70°C/20 min	0.015
G		160°F (71.1°C)/10 min	0.009
H		To reach 82°C	-------
I		85°C/17 min	0.105
J		80°C/5 min	0.015

1. Pasteurization procedures were obtained from an industry survey (Park, 1994).
2. The F_{250} value was calculated using the external temperature, when the internal temperature was not used.
3. The F_{250} values were calculated without the effect of cooling lag during the pasteurization procedures. The products temperature was assumed to be 0°C instantaneously after pasteurization.

Conclusion

D- and z-values of *E. faecium* E-20 were affected by the menstruum of the microorganism. In surimi seafood, the bacterium can be killed more efficiently by raising the heating temperature than holding longer at a relatively low temperature. This result suggested that high temperature and short time pasteurization conditions are better since high temperature and long time pasteurization deteriorates the texture and color quality of surimi seafood. A 0.044 min of F_{250} value was enough to obtain a zero count of *E. faecium* E-20 at temperature range of 60 to 85°C. Considering our results on microbiological safety, most of the US surimi seafood manufacturers appeared to overcook their products. The data obtained in this study could be used to predict the pasteurization F values at various time/temperature combinations in surimi seafood.

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Chapter 5 Summary

Pasteurization temperature and time affected not only the microbiological quality but also the texture and color characteristics of surimi seafood. The longer pasteurization time at higher temperature, the lower the whiteness of the surimi seafood was obtained. As the pasteurization temperature and time increased, the shear strain of surimi seafood decreased. At all three pasteurization temperatures (75, 85, 93°C) the shear stress of surimi seafood dramatically increased when surimi seafood was subjected to pasteurization for 5 min, and then remained constant throughout the 120 min of pasteurization. At all three pasteurization temperature (75, 85, and 93°C), 15 min was enough to obtain a zero count of APC. From this study, 15 min was thought to be enough to obtain zero APC at all three pasteurization temperatures.

Using *Enterococcus faecium* E-20 as a target microorganism, F_{250} values >0.019 min was enough to obtain zero count of this specific microorganism at the temperature range from 65 to 85°C. At 60°C, the target microorganism was more heat-resistant than at other studied temperatures. The F_{250} value at 60°C was 0.044 min; it was two times higher than at any other studied temperatures. Comparing this F_{250} value with the current pasteurization conditions used by the U.S. surimi seafood industry, the industry methods have a tendency to overcook the surimi seafood products. Most of the surimi seafood manufacturers apparently use a higher pasteurization value than 0.02 min (F_{250}). This may be one reason why the surimi seafood manufacturers have often suffered from

textural softness, brownish discoloration, and off-odor particularly when a large portion of surimi was replaced by starch, protein additives and water.

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Appendices

Appendix 1

Temperature profiles of surimi seafood cooked at the initial cooking at 90°C and followed by various pasteurization conditions

Time(min)	93°C	85°C	75°C
0.0	16.5	12.1	12.9
0.5	20.5	23.6	23.7
1.0	39.0	42.7	43.6
1.5	50.3	53.3	54.5
2.0	59.3	61.5	62.6
2.5	66.3	68.1	68.8
3.0	71.7	73.1	73.6
3.5	75.9	77.0	77.3
4.0	79.1	80.0	80.2
4.5	81.6	82.3	82.4
5.0	83.5	84.1	84.1
5.5	84.9	85.5	85.5
6.0	86.1	86.6	86.5
6.5	84.2	83.6	84.6
7.0	81.4	81.0	81.9
7.5	79.6	78.7	79.8
8.0	78.0	76.6	77.8
8.5	76.4	74.5	75.0
9.0	74.8	72.6	74.1
9.5	73.3	70.8	72.4
10.0	71.7	69.1	70.8
10.5	70.2	67.5	69.3
11.0	68.8	66.1	67.8
11.5	67.5	64.7	66.5
12.0	66.2	63.4	65.2
12.5	65.0	62.1	64.1
13.0	63.8	61.0	62.9
13.5	62.7	59.9	61.9
14.0	61.6	58.8	60.9
14.5	69.9	66.3	65.5
15.0	74.8	70.8	68.0
15.5	78.9	74.4	69.8
16.0	82.3	77.0	71.0
16.5	84.9	79.0	72.0
17.0	86.9	80.5	72.7
17.5	88.4	81.6	73.2
18.0	89.6	82.4	73.7
18.5	90.4	83.1	74.0
19.0	91.1	83.5	74.2
19.5	91.6	83.9	74.4
20.0	91.9	84.1	74.5
20.5	92.2	84.3	74.6
21.0	92.4	84.5	74.7
21.5	92.5	84.7	74.8

Appendix 2

Temperature profiles of thermal death time tubes

Temp 60°C

Time (min)	Temp (°C)	Time (min)	Temp (°C)	Time (min)	Temp (°C)	Time (min)	Temp (°C)
0.0	0.5	43.0	60.0	86.0	60.0	129.0	59.9
1.0	51.8	44.0	60.0	87.0	60.0	130.0	59.9
2.0	58.6	45.0	60.0	88.0	60.0	131.0	59.9
3.0	59.5	46.0	60.0	89.0	60.0	132.0	59.9
4.0	59.8	47.0	60.0	90.0	60.0	133.0	59.9
5.0	60.0	48.0	60.0	91.0	60.0	134.0	59.9
6.0	60.1	49.0	59.8	92.0	60.0	135.0	59.9
7.0	60.1	50.0	59.9	93.0	60.0	136.0	59.9
8.0	60.1	51.0	59.9	94.0	60.0	137.0	59.9
9.0	60.1	52.0	59.9	95.0	60.0	138.0	59.9
10.0	60.1	53.0	59.9	96.0	60.0	139.0	59.9
11.0	60.1	54.0	60.0	97.0	59.8	140.0	60.0
12.0	60.1	55.0	60.0	98.0	59.7	141.0	59.9
13.0	60.1	56.0	60.0	99.0	59.8	142.0	59.9
14.0	60.0	57.0	60.0	100.0	59.9	143.0	59.9
15.0	60.0	58.0	60.0	101.0	59.9	144.0	60.0
16.0	60.0	59.0	60.0	102.0	59.9	145.0	60.0
17.0	60.0	60.0	60.0	103.0	60.0	146.0	60.0
18.0	60.0	61.0	60.0	104.0	60.0	147.0	60.0
19.0	60.0	62.0	60.0	105.0	60.0	148.0	60.1
20.0	60.0	63.0	60.0	106.0	60.0	149.0	60.1
21.0	60.0	64.0	59.9	107.0	60.0	150.0	60.1
22.0	59.8	65.0	59.9	108.0	60.0	151.0	60.1
23.0	59.8	66.0	59.9	109.0	60.0	152.0	60.1
24.0	59.9	67.0	60.0	110.0	60.0	153.0	60.1
25.0	59.9	68.0	60.0	111.0	60.0	154.0	60.0
26.0	59.9	69.0	60.0	112.0	60.0	155.0	60.0
27.0	59.9	70.0	60.0	113.0	60.0	156.0	60.0
28.0	60.0	71.0	60.0	114.0	60.0	157.0	60.0
29.0	60.0	72.0	59.6	115.0	60.0	158.0	60.0
30.0	60.0	73.0	59.7	116.0	60.0	159.0	60.0
31.0	60.0	74.0	59.8	117.0	60.0	160.0	60.0
32.0	59.9	75.0	59.9	118.0	60.0	161.0	60.0
33.0	59.9	76.0	59.9	119.0	59.8	162.0	59.9
34.0	59.9	77.0	60.0	120.0	59.8	163.0	59.8
35.0	60.0	78.0	60.0	121.0	59.8	164.0	59.9
36.0	59.9	79.0	60.0	122.0	59.8	165.0	59.9
37.0	60.0	80.0	60.0	123.0	59.9	166.0	60.0
38.0	60.0	81.0	60.0	124.0	59.8	167.0	60.0
39.0	59.9	82.0	60.0	125.0	59.9	168.0	60.0
40.0	60.0	83.0	60.0	126.0	59.8	169.0	60.0
41.0	60.0	84.0	60.0	127.0	59.9	170.0	60.0
42.0	60.0	85.0	60.0	128.0	59.9	171.0	60.0

Time (min)	Temp (°C)
172.0	60.0
173.0	60.0
174.0	60.0
175.0	60.0
176.0	60.0
177.0	60.0
178.0	60.0
179.0	60.0
180.0	60.0
181.0	58.4
182.0	11.5
183.0	2.0
184.0	0.8
185.0	0.1

Temp 65°C

Time (min)	Temp (°C)	Time (min)	Temp (°C)	Time (min)	Temp (°C)
0.0	0.2	21.5	64.9	43.0	1.2
0.5	48.1	22.0	64.9	43.5	0.9
1.0	59.5	22.5	64.9	44.0	0.7
1.5	62.7	23.0	64.9	44.5	0.5
2.0	63.8	23.5	64.9	45.0	0.3
2.5	64.3	24.0	64.9		
3.0	64.7	24.5	64.9		
3.5	64.9	25.0	64.9		
4.0	65.0	25.5	64.9		
4.5	65.1	26.0	64.9		
5.0	65.2	26.5	94.9		
5.5	65.2	27.0	64.9		
6.0	65.2	27.5	64.9		
6.5	65.2	28.0	64.9		
7.0	65.2	28.5	64.9		
7.5	65.2	29.0	64.9		
8.0	65.2	29.5	64.9		
8.5	65.1	30.0	64.9		
9.0	65.1	30.5	64.9		
9.5	65.1	31.0	65.0		
10.0	65.1	31.5	64.9		
10.5	65.0	32.0	64.9		
11.0	65.0	32.5	64.9		
11.5	65.0	33.0	64.9		
12.0	64.9	33.5	64.7		
12.5	64.9	34.0	64.4		
13.0	64.9	34.5	64.3		
13.5	64.9	35.0	64.4		
14.0	64.9	35.5	64.5		
14.5	64.9	36.0	64.6		
15.0	64.9	36.5	64.7		
15.5	64.9	37.0	64.8		
16.0	64.9	37.5	64.8		
16.5	64.9	38.0	64.9		
17.0	64.9	38.5	64.9		
17.5	64.9	39.0	65.0		
18.0	64.9	39.5	65.0		
18.5	64.9	40.0	65.0		
19.0	64.9	40.5	38.1		
19.5	64.9	41.0	17.5		
20.0	64.9	41.5	7.4		
20.5	64.9	42.0	3.1		
21.0	64.9	42.5	1.6		

Temp 70°C

Time (min)	Temp (°C)	Time (min)	Temp (°C)
0.0	0.3	8.3	69.8
0.3	15.2	8.5	69.7
0.5	30.3	8.8	69.8
0.8	45.3	9.0	69.8
1.0	56.0	9.3	69.9
1.3	62.0	9.5	69.9
1.5	65.2	9.8	69.8
1.8	67.0	10.0	69.8
2.0	67.8	10.3	69.8
2.3	68.3	10.5	69.8
2.5	68.6	10.8	69.9
2.8	68.8	11.0	69.9
3.0	69.0	11.3	69.8
3.3	69.2	11.5	69.7
3.5	69.3	11.8	69.8
3.8	69.2	12.0	69.8
4.0	69.3	12.3	69.8
4.3	69.4	12.5	69.9
4.5	69.5	12.8	69.8
4.8	69.5	13.0	69.7
5.0	69.6	13.3	69.8
5.3	69.5	13.5	69.8
5.5	69.5	13.8	69.9
5.8	69.6	14.0	69.9
4.0	69.3	14.3	69.8
4.3	69.4	14.5	69.7
4.5	69.5	14.8	69.8
4.8	69.5	15.0	69.8
5.0	69.6	15.3	57.0
5.3	69.5	15.5	31.2
5.5	69.5	15.8	20.2
5.8	69.6	16.0	14.2
6.0	69.6	16.3	10.2
6.3	69.7	16.5	7.5
6.5	69.7	16.8	5.6
6.8	69.7	17.0	4.3
7.0	69.7		
7.3	69.7		
7.5	69.8		
7.8	69.8		
8.0	69.8		
8.3	69.8		

Temp 75°C

Time (min)	Temp (°C)	Time (min)	Temp (°C)
0.0	0.2	10.4	58.7
0.2	0.7	10.7	41.7
0.4	25.1	10.9	29.8
0.7	41.1	11.2	21.1
0.9	51.4	11.4	14.3
1.2	58.5	11.7	9.3
1.4	63.9	11.9	6.1
1.7	67.6	12.2	4.1
1.9	70.0		
2.2	71.4		
2.4	72.3		
2.7	72.9		
2.9	73.3		
3.2	73.5		
3.4	73.7		
3.7	73.8		
3.9	73.9		
4.2	73.3		
4.4	73.5		
4.7	73.7		
4.9	73.8		
5.2	73.9		
5.4	74.0		
5.7	74.0		
5.9	74.0		
6.2	74.1		
6.4	74.2		
6.7	74.2		
6.9	74.3		
7.2	74.3		
7.4	74.4		
7.7	74.4		
7.9	74.4		
8.2	74.4		
8.4	74.5		
8.7	74.5		
8.9	74.5		
9.2	74.5		
9.4	74.5		
9.7	74.5		
9.9	74.5		
10.2	74.6		

Temp 80°C

Time (min)	Temp (°C)	Time (min)	Temp (°C)	Time (min)	Temp (°C)
0.0	0.1	3.5	78.4	7.1	35.0
0.1	17.3	3.6	78.4	7.1	39.6
0.2	37.7	3.7	78.4	7.2	25.6
0.2	46.3	3.7	78.4	7.3	22.2
0.3	52.7	3.8	78.5	7.4	18.9
0.4	57.8	3.9	78.5	7.5	16.2
0.5	61.8	4.0	78.6	7.6	13.8
0.6	65.0	4.1	78.6	7.6	11.9
0.7	67.6	4.2	78.7	7.7	9.3
0.7	69.7	4.3	78.7	7.8	8.2
0.8	71.4	4.4	78.6	7.9	7.2
0.9	72.7	4.5	78.6	8.0	6.4
1.0	73.7	4.6	78.6	8.1	5.8
1.1	74.6	4.6	78.6	8.1	5.3
1.2	75.3	4.7	78.7	8.2	4.8
1.2	75.9	4.8	78.7		
1.3	76.4	4.9	78.7		
1.4	76.8	5.0	78.7		
1.5	77.1	5.1	78.7		
1.6	77.2	5.1	78.7		
1.7	77.4	5.2	78.7		
1.7	77.5	5.3	78.7		
1.8	77.6	5.4	78.8		
1.9	77.9	5.5	78.8		
2.0	78.0	5.6	78.9		
2.1	78.0	5.6	78.8		
2.2	78.0	5.7	78.8		
2.2	78.0	5.8	78.8		
2.3	78.0	5.9	78.8		
2.4	78.1	6.0	78.8		
2.5	78.1	6.1	78.8		
2.6	78.2	6.1	78.8		
2.7	78.2	6.2	78.8		
2.7	78.2	6.3	78.8		
2.8	78.3	6.4	78.7		
2.9	78.3	6.5	78.7		
3.0	78.2	6.6	78.8		
3.1	78.2	6.6	78.8		
3.2	78.3	6.7	72.9		
3.2	78.3	6.8	62.7		
3.3	78.4	6.9	52.7		
3.4	78.4	7.0	42.1		

Temp 85°C

Time (min)	Temp (°C)	Time (min)	Temp (°C)
0.0	0.6	3.5	83.0
0.1	1.1	3.6	83.0
0.2	2.5	3.7	83.1
0.2	21.3	3.7	83.1
0.3	34.0	3.8	83.1
0.4	43.1	3.9	83.2
0.5	1.0	4.0	83.2
0.6	57.5	4.1	83.2
0.7	62.8	4.2	83.1
0.7	67.1	4.3	83.2
0.8	71.2	4.4	83.2
0.9	73.8	4.5	83.2
1.0	75.6	4.6	83.3
1.1	77.1	4.6	83.3
1.2	78.3	4.7	83.3
1.2	79.2	4.8	83.3
1.3	80.0	4.9	83.4
1.4	80.7	5.0	83.4
1.5	81.3	5.1	81.3
1.6	81.6	5.1	68.1
1.7	81.9	5.2	55.2
1.7	82.1	5.3	42.7
1.8	82.3	5.4	34.4
1.9	82.6	5.5	29.3
2.0	82.7	5.6	20.6
2.1	82.8	5.6	16.0
2.2	82.8	5.7	12.5
2.2	82.8	5.8	9.9
2.3	82.9	5.9	7.9
2.4	82.9	6.0	6.5
2.5	82.9	6.1	5.3
2.6	83.0		
2.7	83.0		
2.7	83.0		
2.8	83.0		
2.9	83.0		
3.0	83.0		
3.1	83.0		
3.2	83.0		
3.2	83.1		
3.3	83.0		
3.4	83.0		