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Barbara Lorraine Gillman

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**DEVELOPMENT AND CHARACTERIZATION OF PASTAS
CONTAINING UNDERUTILIZED CRAB MINCE**

By

Barbara Lorraine Gillman

B.S. University of Maine, **1998**

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

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(in Food Science and Human Nutrition)

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The University of Maine

August, 2001

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DEVELOPMENT AND CHARACTERIZATION OF PASTAS CONTAINING UNDERUTILIZED CRAB MINCE

By Barbara Lorraine Gillman

Thesis Advisor: Dr. Denise Skonberg

An Abstract of the Thesis Presented
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Almost three and a half million pounds of Atlantic Rock and Jonah crab, having a value of almost \$1 million, are landed annually in Maine. These landings generate approximately 2.5 million pounds of post-processing waste each year. The by-product consists **mainly** of shell, viscera, and unpicked meat. Mechanical separation of crustacean by-product can result in an additional 15-20% food grade minced meat, which can be utilized to produce value-added products for human consumption. One such product that may be enhanced by crab mince is fresh pasta. Americans are eating more pasta than ever before and fresh pasta consumption is very popular. The development of a crab mince-containing seafood-flavored pasta would not **only** provide a gourmet flair and a more nutritionally balanced meal, but also utilize high quality crab meat that is typically discarded.

The objectives of this study were to: 1) determine the mechanical feasibility, yield, nutrient composition, and quality of crab mince; 2) evaluate the effects of sodium

lactate, lactic acid, rosemary, and diacetyl on the chemical and microbial quality of refrigerated crab mince; 3) determine if fresh pastas containing different concentrations of crab mince could be successfully extruded; and 4) evaluate the consumer acceptance of fresh pasta products containing crab mince.

The first study evaluated the separation of minced meat from the carapace and legs of Jonah crabs. Due to the hardness of the shell, crab meat could not be mechanically separated from the legs, however crab meat from the carapace was successfully separated, and resulted in an average 64% minced meat yield from the starting product. The crab mince consisted of 77.8% moisture, 5.8% ash, 1.3% fat, and 15.1% protein. The shelf-life of the crab mince, which was evaluated by pH, Total volatile base nitrogen (TVBN), Thiobarbituric acid reactive substances (TBARS), and microbial analyses, was less than four days. TVBN concentrations were above 70 mg N/ 100g and APC counts were between 10^8 - 10^9 CFU/g by day four. Since the crab mince contained only 1-2% fat, lipid oxidation was not a limiting factor for shelf-life.

The addition of sodium lactate, lactic acid, rosemary, and diacetyl was effective in improving shelf life of the mechanically separated crab mince. Lactic acid had the most significant effect on maintaining crab mince quality. Combinations of additives might prove most effective in enhancing quality of refrigerated crab mince during storage, since individual additives improved only some aspects of mince quality. Microbial counts of crab mince in the shelf-life study were between 10^5 - 10^7 CFU/ g throughout the study. Sanitizing all parts of the mechanical separation machine before processing the carapaces resulted in significantly lower microbial counts in the mince ($10^3 - 10^5$ CFU/g) even after three to five days of refrigerated storage.

Pasta containing 10 and 20% crab mince with additives was successfully extruded. As percent crab increased, pH, TVBN, and microbial counts also increased. During the first three weeks of refrigerated storage microbial counts (*APC*, yeasts, and molds) remained unchanged, however, due to temperature abuse microbial counts significantly increased to $10^5 - 10^6$ for *APC*, and between $10^2 - 10^4$ CFU/g for yeasts and molds, limiting the pasta shelf-life to a minimum three weeks. As weeks storage increased, there was a slight degradation in pasta physical quality as measured by cooking loss and firmness. As percent crab increased, there was a significant increase in cooking loss; a decrease in firmness was not significant. Storage time percent crab had no effect on cooking weight. As pasta samples were cooked, width and thickness of pasta significantly increased. As percent crab increased, “L” and “b” values of pasta significantly decreased. Additives had a significant effect on pasta color. After pasta was cooked, it was an average of 14% lighter, 244% more green, and 19% less yellow than raw pasta samples.

Results from consumer sensory analyses of pasta containing 10 or 20% crab mince with additives with and without red colorant indicated that panelists liked all samples slightly to moderately. There were no significant differences between samples for flavor, texture, aroma, or overall acceptability. Pasta containing red colorant and 10% crab mince with additives received a significantly lower color score (5.3) than the rest of the samples (average 6.7). Panelists’ comments indicated that the pasta had only a slight seafood flavor and a gritty texture due to residual shell particles in the pasta.

This preliminary study was conducted to determine whether mince from crab processing by-product could be successfully extracted and utilized in a seafood-flavored

fresh pasta. Pastas containing 10 and 20% crab mince were successfully developed and slightly liked by consumers during sensory analyses. However, further research on flavor development and reduction of grittiness is needed to ensure successful commercialization of this novel pasta product.

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TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
LIST OF TABLES.....	x
LIST OF FIGURES.....	xiii
Chapter	
1. INTRODUCTION.....	1
The Crab Industry.....	1
Total By-product Utilization.....	4
Current Uses of Crustacean Processing By-product.....	5
Mechanical Separation.....	6
Constraints to Utilization of Minced Crab Meat.....	8
Extending Shelf-life of Crab Mince with Additives.....	12
Sodium Lactate.....	13
Lactic Acid.....	17
Rosemary.....	21
Diacetyl.....	27
Consumer Seafood Trends.....	29
Consumer Pasta Trends.....	31
Pasta Quality.....	33
Microbial Quality.....	33
Physical Quality.....	38
Food Safety Concerns for Fresh Pasta Containing Crab Mince	48
Objectives.....	52

2. MATERIALS AND METHODS: MECHANICAL SEPARATION OF CRAB MINCE	54
Objectives.....	54
Experimental Design.....	54
Ingredients.....	54
Chemical Analyses.....	56
Moisture.....	56
Ash.....	56
Minerals.....	56
Fat.....	57
Crude Protein.....	58
pH.....	59
Total Volatile Base Nitrogen.....	59
Thiobarbituric Acid Reactive Substances.....	60
Microbial Analyses.....	61
Total Plate Counts.....	61
Yeast and Mold Counts.....	61
Coliforms.....	62
3. MATERIALS AND METHODS: EFFECTS OF ADDITIVES ON THE SHELF-LIFE OF CRABMINCE	63
Objectives.....	63
Experimental Design.....	63
Ingredients.....	64

Formulations.....	65
Chemical Analyses.....	66
Microbial Analyses.....	66
Statistical Analyses.....	66
4. MATERIALS AND METHODS: DEVELOPMENT AND QUALITY OF OF PASTA CONTAINING CRAB MINCE WITH ADDITIVES.....	67
Objectives.....	67
Experimental Design.....	67
Ingredients.....	68
Formulations.....	69
Chemical Analyses.....	71
Proximate Analyses.....	71
pH.....	71
Water Activity.....	72
Total Volatile Base Nitrogen.....	72
Microbial Analyses.....	72
Physical Analyses.....	73
Cooking Time.....	73
Cooking Weight, Cooking Loss, Width and Thickness.....	73
Instron.....	74
Colorimetric Analyses.....	75
Statistical Analyses.....	75

5. MATERIALS AND METHODS: SENSORY ANALYSES AND EVALUATION OF PASTA CONTAINING CRAB MINCE WITH ADDITIVES	76
Objectives.....	76
Experimental Design.....	76
Ingredients.....	77
Formulations.....	78
Chemical Analyses.....	79
Microbial Analyses.....	80
Physical Analyses.....	80
Sensory Analyses.....	80
Statistical Analyses.....	82
6. RESULTS: MECHANICAL SEPARATION OF CRAB MINCE.....	84
Ease of Separation.....	84
Chemical Analyses.....	84
Proximate Analyses.....	84
pH.....	85
TVBN.....	87
TBARS.....	88
Microbial Analyses.....	89
7. RESULTS: EFFECT OF ADDITIVES ON THE SHELF-LIFE OF CRAB MINCE.....	92
Chemical Analyses.....	92
Yield and Proximate Analyses.....	92
pH.....	92

TVBN	93
TBARS	96
Microbial Analyses	97
8. RESULTS: DEVELOPMENT AND QUALITY OF PASTA CONTAINING CRAB MINCE WITH ADDITIVES	101
Chemical and Microbial Analyses of Crab Mince	101
Chemical Analyses of Pasta Containing Crab Mince with Additives	102
Proximate Analyses	102
Water Activity	104
pH	105
TVBN	108
Microbial Analyses of Pasta Containing Crab Mince with Additives	109
APC	109
Yeasts	112
Molds	113
Physical Analyses of Pasta Containing Crab Mince with Additives	115
Cooking Loss	115
Cooking Weight	116
Firmness	117
Width and Thickness	119
Instrumental Color	124
9. RESULTS: SENSORY ANALYSES AND EVALUATION OF PASTA CONTAINING CRAB MINCE WITH ADDITIVES	132
Chemical and Microbial Analyses of Crab Mince with Additives	132

Chemical Analyses of Pasta Containing Crab Mince with Additives.....	134
Proximate Analyses.....	134
Water Activity.....	135
pH.....	136
TVBN.....	136
Microbial Analyses of Pasta Containing Crab Mince with Additives.....	137
Physical Analyses of Pasta Containing Crab Mince with Additives.....	138
cooking Loss.....	138
Cooking Weight.....	138
Firmness.....	139
Width and Thickness.....	139
Instrumental Color.....	141
Sensory Analyses of Pasta Containing Crab Mince with Additives.....	145
10.DISCUSSION.....	152
Mechanical Separation of Crab Mince.....	152
Shelf-life of Mechanically Separated Crab Mince with Additives.....	154
Proximate and Mineral Analyses.....	154
pH.....	155
TVBN.....	156
TBARS.....	158
Microbial Analyses.....	159
Development and Quality of Pasta Containing Crab Mince with Additives.....	164

Proximate Analyses.....	164
Water Activity, pH , and TVBN.....	165
Microbial Analyses.....	i67
Physical Analyses.....	171
cooking Loss.....	171
Cooking Weight.....	172
Firmness.....	173
Width and Thickness.....	174
Instrumental Color.....	175
Sensory Analyses.....	179
11. CONCLUSIONS.....	182
REFERENCES.....	184
BIOGRAPHY OF THE AUTHOR.....	192

LIST OF TABLES

Table 1.	Treatments and Codes for Mechanically Separated Crab Mince.....	54
Table 2.	Treatments and Codes for Crab Mince with Additives.....	63
Table 3.	Additives Incorporated into Crab Mince.....	65
Table 4.	Treatments and Codes for Pasta Containing Crab Mince.....	68
Table 5.	Additives Incorporated into Crab Mince.....	70
Table 6.	Formulations of Fresh Pasta Treatments.....	71
Table 7.	Treatments and Codes for Pasta Containing Crab Mince.....	76
Table 8.	Additives Incorporated into Crab Mince.....	78
Table 9.	Formulations of Fresh Pasta Treatments.....	79
Table 10.	Percent Moisture, Ash , Fat and Protein of Crab Mince.....	85
Table 11.	Selected Mineral Concentrations of Crab Mince.....	85
Table 12.	pH of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	86
Table 13.	TVBN Concentrations of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	88
Table 14.	TBARS Levels of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	88
Table 15.	APC of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	90
Table 16.	Yeast Counts of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	90
Table 17.	Mold Counts of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	91
Table 18.	Coliforms of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	91

Table 19. pH of Crab Mince with Additives during Refrigerated Storage.....	93
Table 20. TVBN Concentrations of Crab Mince with Additives during Refrigerated Storage.....	94
Table 21. TBARS Levels of Crab Mince with Additives during Refrigerated Storage.....	97
Table 22. APC of Crab Mince with Additives during Refrigerated Storage.....	99
Table 23. pH, TVBN, and APC of Crab Mince during Refrigerated Storage.....	101
Table 24. Percent Moisture, Ash, and Fat of Crab Mince and Pasta Samples.....	103
Table 25. Selected Mineral Concentrations of Crab Mince and Pastas C o n t m g Crab Mince.....	104
Table 26. Water Activity of Fresh Pasta during Refrigerated Storage.....	105
Table 27. pH of Fresh Pasta during Refrigerated Storage.....	107
Table 28. TVBN of Fresh Pasta during Refrigerated Storage.....	109
Table 29. APC of Fresh Pasta during Refrigerated Storage.....	111
Table 30. Yeast Counts of Fresh Pasta during Refrigerated Storage.....	113
Table 31. Mold Counts of Fresh Pasta during Refrigerated Storage.....	114
Table 32. Percent Cooking Loss of Fresh Pasta during Refrigerated Storage.....	116
Table 33. Percent Cooking Weight of Fresh Pasta during Refrigerated Storage.....	117
Table 34. Firmness of Fresh Pasta during Refrigerated Storage.....	118
Table 35. Width and Thickness of Raw and Cooked Pasta after One Week Refrigerated Storage.....	121
Table 36. Width and Thickness of Raw and Cooked Pasta after Three Weeks Refrigerated Storage	122

LIST OF FIGURES

Figure 1.	pH of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	86
Figure 2.	TVBN Concentrations of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	87
Figure 3.	Aerobic Plate Counts of Crab Mince Separated from Carapace and Legs during Refrigerated Storage.....	89
Figure 4.	TVBN Concentrations of Crab Mince without Lactic Acid	95
Figure 5.	TVBN Concentrations of Crab Mince with Lactic Acid	95
Figure 6.	APC of Crab Mince without Lactic Acid.....	100
Figure 7.	APC of Crab Mince with Lactic Acid	100
Figure 8.	APC of pasta.....	110
Figure 9.	Firmness of Pasta.....	118
Figure 10.	Average “2” Values of Raw and Cooked Pasta Containing 10 or 20% Crab Mince with Additives	130
Figure 11.	Average “a” Values of Raw and Cooked Pasta Containing 10 or 20% Crab Mince with Additives	130
Figure 12.	Average “b” Values of Raw and Cooked Pasta Containing 10 or 20% Crab Mince with Additives.....	131
Figure 13.	Participation by Age	145
Figure 14.	Participation by Gender	145
Figure 15.	Pasta Consumption Frequency	146
Figure 16.	Single Most Important Quality Characteristic for Pasta.....	146
Figure 17.	Colored Pasta Purchasing Frequency	147
Figure 18.	Do You Like Seafood-flavored Pastas?.....	148
Figure 19.	Would You Be Interested in Purchasing a Pasta Product that Contains Seafood?	148

INTRODUCTION

The Crab Industry

Crabs are decapod crustaceans representing more than 50 families with thousands of species worldwide. Most crabs are located in marine waters at all depths of the ocean although some species live in freshwater. Commercially important species found in U.S. waters include: 1) the blue crab (*Cullinectes sapidus*), 2) the Dungeness crab (*Cancer mugister*), 3) the king crab (several species in the family *Parulithodes*), 4) the stone crab (*Menippe mercenaria*), 5) the Jonah crab (*Cancer borealis*), 6) the red or golden crabs (*Geryon quinque-dens* or *G. fenneri*) and 7) the snow or tanner crabs (*Chionoecetes bairdi*, *C. opilio*, or *C. tunneri*) (Osterling, 2000). Along the coast of Maine, the commercially important species are Atlantic rock crab (*Cancer irroratus*), of which over 2.5 million pounds were harvested in 1999, deepsea red crab (*Geryon quinque-dens*), of which over 740,000 pounds were harvested in 1999, and Jonah crab (*Cancer borealis*), of which over 50,000 pounds were harvested in 1999 (National Marine Fisheries Service, 2001).

The Rock and Jonah crab are harvested from waters that are 40 meters and 40+ meters deep, respectively, and are primarily picked by hand and marketed by small companies and family businesses (Kryzynowek et al., 1982). Typically live crabs are brought from the ocean in the afternoon and stored in totes in refrigerated coolers (5°C) until processed the next morning. Before steaming, dead crabs are removed from the totes and only live crabs are steamed at 210°F for 17.5 minutes or until an internal body temperature of 185°F is reached. The crabs legs and claws (knuckles included) are removed from the crab carapace (body) and separated into individual totes. The crab parts are either covered in ice or placed in ice water and stored in refrigerated coolers (5°C) to

stop cooking and reduce the temperature so the pickers can remove the meat from the shell. Once the parts are cooled down, the pickers use wooden mallets to smash the shell and remove the meat with their lobster picks. The meat is usually packaged either **as** empress claws or **as a *mix***. The empress claws have a small amount of shell remaining on the tip of the claw so that when eaten the consumer simply holds onto the shell and pulls the meat off the cartilage that is located inside the shell. Empress claws are marketed mostly for appetizers. The *mix* is a combination of knuckle meat and leg meat. Both the empress claws and *mix* are generally packaged in one pound or eight ounce containers, held at refrigeration temperatures (5°C), and are currently sold for \$9.75 and \$10.00 per pound, respectively, either on site or in grocery stores (Peter Victor, CCP, Personal Communication). Small-scale processors, such as Cranberry Point Products, located in Gouldsboro, Maine, also cryogenically freeze crabs when they have an abundance of them so the crabs can be sold at any point during the year. However, if the crabs are going to be frozen, they are first debacked, the gills are scraped out with a knife, and the viscera is washed out of the body before being steamed. This process, which **is** commonly referred to as clustering, reduces the microbial counts in the meat by removing the gills and viscera, which are the major sources of bacterial contamination on the crabs beside the shell. Once the crabs are steamed and iced down, they are placed in a cryofreezer that has a temperature of -70°F for 12 minutes. The frozen crabs are then held in frozen storage (-20°F) until needed and at that point they are thawed and the meat is picked. Crabs, in general, can be sold live, frozen, or the meat can be picked and canned or pasteurized and sold fresh.

Crab meat is low in cholesterol and fat and is a great source of high quality protein. Ke et al. (1990) reported that crab body meat contains **82.9%** water, **16.0%** protein, **0.86%** fat, and **1.7%** ash. Similarly, other reports have shown Jonah crab meat to contain about **78%** moisture, **16.2%** protein, 1% to **2%** fat, and **1.5%** ash (Krzynowek et al., 1982). These values may change based on factors such as sex, animal size, season, and cooking method (Lee et al., 1993). Depending on the processing method, the crab by-product may also have higher mineral content. Lee et al. (1993) reported a similar moisture, protein, and fat content for mechanically separated (deboned) Blue crab meat as reported for picked crab meat; however the ash percentages and calcium content, which ranged from **12.65 mg/g** to **37.80 mg/g**, were much higher for deboned product than picked crab meat. Gates and Parker (1992) reported a lower ash content of **1.57%** for deboned blue crab leg meat and **2.14%** for deboned blue crab minced meat. However, the difference between the ash content that Lee et al. (1993) obtained and Gates and Parker (1992) obtained may be due to differences in the types of mechanical separators that were used (Lee et al., 1993).

An average yield of picked crab meat is **10-12%** (Ward, 1990; Peter Victor, CCP, Personal Communication) and the meat can be picked by hand at an average rate of **2-3** pounds per hour. Currently, workers remove most meat from the shell by hand. One of the exceptions is king crab leg meat, which is squeezed out of the shell by rollers before being packed in cans and thermally processed (Ward, 1990). Some mechanical methods of removing the meat from the shell have been explored; however, consistent high quality results have yet to be obtained. Some of these methods include vacuuming, shaking the meat out of the shell by vibration, removing the meat from the shell by centrifugal force,

squeezing the meat out of the shell with rollers, and crushing the shell with a hammer mill and allowing the meat to float to the top of a brine solution and extracting it (Ward, 1990).

Total By-product Utilization

Once meat is extracted from the crabs, the rest of the body, known as post-processing waste or by-product, which consists primarily of shells, viscera, and unpicked meat from the body cavity, is typically dumped in landfills (Shahidi and Synowiecki, 1991). Almost three and half million pounds of Atlantic Rock and Jonah crab, having a dockside value of almost \$1 million, are landed annually in Maine (National Marine Fisheries Service, 2000). These landings generate approximately 2.5 million pounds of post-processing waste each year. The major concerns regarding this waste production is that biodegradation of crustacean waste is very slow (Shahidi and Synowiecki, 1991). The shells of crustaceans are largely insoluble and very resistant to biodegradation (Healy et al., 1994) and the shell proteins putrefy and render the enzyme components inactive and unable to break down the waste (Shahidi and Synowiecki, 1991). This accumulation of waste presents environmental problems due to space limitation, odor, and the high moisture content available for microbial growth. With the advent of increasingly more stringent environmental regulations, some processors have begun paying composters to handle the waste. Cranberry Point Products, mentioned before, currently grinds all of its crab waste and gives it to a Christmas tree company for fertilizer but in the past they have had to pay for the waste to be removed (Peter Victor, CCP, Personal Communication). When only an average 10-12% of the crab is utilized and the other 88-90% is disposed of, the waste removal costs are higher than most small-scale seafood processors can manage.

Instead of paying for the remaining product to be discarded, there is now an emphasis in the global fisheries marketplace on total product recovery and utilization. New seafood product formulations that incorporate by-product can reduce waste formation, lower overhead costs, and help sustain and promote the aquaculture and seafood industries (Meyers, 1994).

Current Uses of Crustacean Processing By-product

Waste product from the processing of crustaceans contains substantial amounts of protein, mineral, and chitin, which can be utilized in various industries. Carotenoid pigments, such as astaxanthin, which can be removed from the shell by oil extraction, are utilized by the aquaculture industry and added to fish diets (i.e. salmonoid diets) as a flesh and integument color enhancer important for consumer acceptance (Meyers, 1994). Meal created from crustacean by-product is being used as a protein supplement in shrimp and fish diets because of its high quality and value as a fish attractant (Meyers, 1994).

Another use of crab processing by-product is as an ingredient in animal feeds. Protein is the most expensive ingredient in animal diets aside from pre-vitamin mixes, which are incorporated in very small amounts. Thus, the farming industry (i.e. poultry, beef, fish, pigs) has been utilizing amino acid hydrolysates from high protein waste products in addition with grain proteins to produce a less expensive, high quality feed.

Chitin and chitosan, produced by the deacetylation of chitin, are currently used for removal and recovery of amino acids, proteins, dyes, heavy metals, and pesticides (Shahidi and Synowiecki, 1991). Chitosan has been utilized for pharmaceutical use as a drug carrier, in enzyme and cell immobilization, and is also a component in eye bandages and contact lenses (Shahidi and Synowiecki, 1991). Chitosan has also been utilized by

the agricultural industry **as** an edible food coating for reducing moisture loss, inhibiting bacterial growth, and reducing oxygen levels on fruits and vegetables during storage (Li et al., 1997). Chitosan is also **an** ingredient in beauty products such as body creams, toothpaste, shampoo, and cosmetics. Biodegradable films for packaging, animal food, and sausage casings can also be produced from shell waste.

The agricultural industry also utilizes crustacean by-products to produce compost. The food industry utilizes the volatile flavor compounds from the shell waste to make bases, stocks, and soups to provide an authentic crab flavor (Meyers, 1994).

These uses of crustacean processing by-product certainly help to reduce the amount of accumulated waste, however, they are not being utilized to their full potential. Additionally, the majority of the by-product is utilized in non-food or animal feed items although there is a substantial amount of food grade protein in the by-product that if extracted could be utilized in the development of new seafood-based products for the consumer. This high value utilization could turn “trash into treasure” and increase profits for small-scale processors all along the coast of Maine and the rest of New England.

Mechanical Separation

Mechanical separation, otherwise known **as** deboning, is the separation of meat from bone or shell by a mechanical separator. In deboning, product is typically picked of meat manually or by machine and the discards are placed in a mechanical separator. The discards can be placed into the hopper of the machine manually or automatically in one type of mechanical separator. A metal cylinder, which rotates clockwise when the machine is on, is used to grind **and** disintegrate the product into smaller pieces. The cylinder has jagged pieces of metal along the outside to help facilitate the grinding. Meat

is ground finely enough to be pushed through the microslits, located along the entire length of the cylinder, to the inside of the cylinder where it exits the back **of** the machine. Bone or shell fragments, which remain on the outside **of** the cylinder, are transferred to the front of the machine and exit the machine through the end plate.

Mechanical separation is currently used to increase the yield of poultry and fish processing. Some common products that are already produced using mince from mechanical separation include chicken nuggets, chicken patties, fish sticks, and surimi products. Lee et al. (1997) successfully produced low-fat chicken sausages made with **72%** mechanically deboned chicken meat and **24%** whole boneless and skinless chicken thigh meat that was low fat and had an eight-day shelf life.

Minced meat of some crab by-products is also being used as extenders in seafood stuffings, soups, and chowders (Gates and Parker, 1992). However, the primary use of mechanically separated mince is in the production of aquaculture and agricultural feeds (Ward, 1990).

According to Thompson (1985), over **30** million pounds of mechanically deboned crab meat have been recovered annually in the United States. The mechanical separation of blue crab by-products has resulted in an additional **15-20%** food grade meat (Gates and Parker, 1992). When evaluating the quality and appearance of mechanically separated blue crab meat, Gates and Parker (1992) obtained a mince meat yield of **3.18%** white meat, **13.89%** mixed minced meat ("**10.71%** if slabs were separated"), **2.62%** minced leg meat, and **6.39%** minced claw meat for a total mince recovery of **22%** of the uncooked blue crab weight. Lee et al. (1993) were able to recover up to **50.3%** of mince from composite blue crab processing by-product when gills were removed. Using the same

recovery figures, a small-scale processor in Maine, who processes an average 1200 pounds of product per day, would receive an additional 180 – 240 pounds of food grade mince through mechanical separation (Peter Victor, CCP, Personal Communication). Interestingly, the total meat that would be picked by hand, which averages about 10% of the total product, would only be 120 pounds of meat (Keithly et al., 1988; Ward, 1990). Therefore, the processor could recover more meat for human consumption from mechanical separation than from picking. Certainly, the price for minced meat is not **as** high as for empress claws or **from a mix** of crab meat but the value of minced meat is 20 times the value of meal sold to animal and fish feed companies (Gates and Parker, 1992).

Constraints to Utilization of Minced Crab Meat

Some of the primary concerns for companies looking to utilize mechanical separation to increase meat yield are the difficulty of meat-shell separation, the cost of additional equipment and labor, the quality of the mince, and the further development of the mince into a value-added product. True, mechanical separation can potentially increase the meat yield of a crab processing operation, but at what additional cost and effort? For example, what additional processing steps would be added to production line if mechanical separation were included? If a high quality product were going to be utilized for human consumption, a minimum of four processing steps would have to be added to the production line. First, the crabs would need to be debacked to gain access to the inside of the crab, the **gills** would have to be scraped out, and the viscera **has** to be washed out before the crab is steamed. The next logical question is what parts of the crab can be deboned? Studies on the successful mechanical separation of blue crab have been noted but Jonah crab is a different species and the hardness of the shell may affect its

ability to be deboned. Not all material is suitable for mechanical separation. Perhaps, only some parts of the crab can be deboned while others cannot. The most meat yield will come from the body, which is typically left unpicked because it is too difficult to pick by hand (Peter Victor, CCP, Personal Communication). Furthermore, Jonah crab shells are extremely hard and very smooth. Will the machine be able to readily grind the product or will additional processing steps need to be completed prior to mechanical separation to make the product ready? How many workers will be needed to run the deboner efficiently? Finally, how many pounds of crab can be mechanically deboned per hour and what percentage meat yield can be recovered from Jonah crab by-products? The quantity of deboned product needs to be substantial and marketable to insure the success of a total recovery approach. The gross profit also needs to be more than the labor or equipment costs to make it feasible for a small-scale processor.

Another major concern for the processor is the quality of the crab mince. Fresh seafood is highly perishable due to its biological composition (Ashie et al., 1996) and is limited by enzymatic and microbiological degradation (Ashie et al., 1996; Marshall and Jindal, 1997). Marshall and Jindal (1997) reported that fish products have reached the maximum shelf-life when microbial counts reach 10^7 CFU/ g. The refrigerated shelf life of picked crab meat is typically seven to ten days (Ward, 1990). However, the shelf life of crab mince is reduced when mechanically deboned for several reasons. Mechanical separation increases microbial contamination from workers and equipment. When crabs are cooked, all viable microbes are killed and the meat inside is essentially sterile (Wentz et al., 1985; Ashie et al., 1996). However, contamination occurs throughout the processing line as the meat comes in contact with non-sterile items. Surveys have shown

that **90-93%** of crab processing facilities that operate under good sanitary conditions produce crab meat that contains 10^5 CFU/ g (Wentz et al., **1985**). Lee et al. (**1993**) stated that mechanical separation increased the mesophilic and psychrotrophic counts in composite blue crab by-product between 10^4 CFU/ g to 10^7 CFU/ g. Based on microbial counts they determined a refrigerated shelf life for deboned crab mince of two days. Raccach and Baker (**1978**) reported a 10-fold increase in microbial counts after the by-product was mechanically deboned. Headed and gutted cod, pollock, and whiting had microbial counts from 10^4 CFU/ g prior to being deboned and 10^5 CFU/g afterwards. Gates and Parker (**1992**) established that hourly clean up of the mechanical separation machine (Baader) improved the quality of the blue crab mince, yet microbial counts of blue crab after separation were 10^5 to 10^7 CFU/ g.

One of the processing concerns with mechanical separation in general is the addition of oxygen and metals scraped from the machine. Lipid oxidation is propagated by an attack of oxygen, metals, heat or light (Nawar, **1996**). This causes the product to have a rancid odor, off-taste, and reduces the shelf-life. Important metals involved in lipid oxidation are Cu^{2+} , Fe^{2+} , and Fe^{3+} (Ashie et al., **1996**). Fortunately, most crab meat has between one and two percent fat so lipid oxidation is not one of the key factors responsible for quality loss (Ke et al., **1990**). In fact, microbial contamination is the primary source of quality loss in mechanically deboned mince products. What **makes** mince product such an easy environment for microbial growth is the maceration of tissues causing an increased rate of chemical reactions providing a nutrient rich environment (Raccach and Baker, **1978**). A combination of high moisture content, near neutral pH, a nutrient dense environment, and high microbial load results in a very short

shelf life for refrigerated mince products. In fact, looking at previously described studies it is apparent that steps other than proper sanitation need to be taken by the processor to reduce or inhibit the growth of bacteria to ensure a longer shelf life of more than two days.

Lastly, further development of the minced product is something that needs to be addressed by the processor. When crab meat is mechanically deboned the product is a thick slurry, which is not aesthetically pleasing. Thus, the mince needs to be formed, gelled, or put into a “package” before consumers may be willing to buy it.

One example of packaging mince into a form that consumers like is the coextrusion of grain flour and deboned underutilized fish species to produce shelf stable foods or healthy snack products. Snack foods are typically high in calories and fat but low in vitamins, protein, and other nutrients (Suknark et al., **1998**).

Maga and Reddy (**1985**) evaluated the consumer acceptability of Indian rice flour based pakodas (snack food) that had minced carp incorporated at 25, 30, and 35%. Plain rice flour extrudates had a protein content of 8.3%, but with the addition of minced carp the protein contents in the extrudates were increased to 13.3, 14.3, and 15.4%, respectively. The pakodas, the fried extrudates or finished snack product, had an increase of protein content of 100%, 114%, and 128% corresponding with a 25%, 30%, and 35% minced carp addition.

Clayton and Miscourides (**1992**) also developed a snack product by coextruding Atlantic cod and rice flour. The fish mince from the cod had a protein content of 14% but when azotropically dewatered to fish flour the protein content was 87.4%. By using a high protein flour in conjugation with a high temperature short time (HTST) extruder, the

researchers produced a protein enriched (18% protein), low moisture and “highly expanded porous and crisp” snack product.

Suknark et al. (1998) determined the acceptability of a tapioca starch: catfish mince snack product for specific target market groups. A 60:40 (w/w) ratio of starch to minced fish was blended and the moisture content was adjusted to 40%. The data indicated of two ethnicities, American and Asian, the Asian consumers rated overall acceptance of fish snacks as “like moderately to like slightly” while American consumers rated overall acceptance of fish snacks as “neither like nor dislike” to “like slightly”.

These coextruded products have not appealed to U.S. consumers as well as they may have in Asian countries and further product development utilizing this high quality protein is needed (Suknark et al., 1998). Food product development is extremely expensive, labor intensive, and can be very risky. Despite marketing efforts, the products may not sell. Additionally, small scale-processors may not have the space, the equipment, or the money to set up a product development facility. Thus, the processor either pays to have by-product removed or if possible, gives it away.

Extending Shelf-life of Crab Mince with Additives

As stated before seafood, and, in particular, mechanically deboned seafood has a short shelf life due to microbial deterioration and lipid hydrolysis and oxidation. The use of additives, however, has been shown to extend the shelf life of many refrigerated seafood products. One of the major sources of deterioration in mechanically deboned seafood products is microbial (Raccach and Baker, 1978). The primary microbial spoilers of seafood products include gram-negative psychrotrophic bacteria including *Pseudomonads* and *Acinetobacter-Moraxella* (Jay, 2000a). Additives that have been

utilized in seafood **as** antimicrobial agents include acetic acid, citric acid, lactic acid, sodium lactate, potassium lactate, phosphates, nitrite/nitrates, and salt. Additives that have been utilized in seafood as antioxidant agents include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), propyl gallate (PG), ascorbic acid, α -tocopherol, ascorbate, and erythorbic acid. Antimicrobials and antioxidants that may be particularly effective at extending the shelf life of seafood are sodium lactate, lactic acid, rosemary, and diacetyl. They have shown antimicrobial efficacy in seafood, meat, or poultry products, have proven effective against gram negative bacteria either in meat or media systems, have known antioxidant efficacy in seafood, meat, or poultry products, and may also enhance the flavor of seafood products (Shelef et al., **1980**; Jay, **1982a**; Farag et al., **1989**; Shelef, **1994**; Ouattara et al., **1997**; Alakomi et al., **2000**; Del Campo et al., **2000**).

Sodium Lactate

Sodium lactate is used **as** a humectant and flavor enhancer **as well as** an antimicrobial agent in meat and poultry products (Shelef, **1994**). Sodium lactate is applied to cattle, sheep, and poultry carcasses **as** a 1% - 3% dipping or spraying solution to lower viable microbial counts during storage (Shelef, **1994**). It is a colorless, non-volatile compound that **has** an acrid taste and is effective against psychrotrophic gram-negative bacteria. Although sodium lactate's mode of action to inhibit microbial growth remains unclear, two possible mechanisms have been proposed. First, sodium lactate is a weak lipophilic acid and it can pass through the cell membrane in its undissociated form and dissociate within the cell to acidify the cell interior. Second, sodium lactate may have the ability to lower the water activity of an environment, making it less suitable for

bacteria to grow (Shelef, 1994). Sodium lactate is available as a 60% aqueous solution and its pH is near neutral. Levels of sodium lactate that are allowed as flavor enhancements are not to exceed 2% of the final weight of the product, however, higher percentages can be used for antimicrobial and antioxidative purposes (Kulshrestha and Rhee, 1996). A level of 2% is recommended, because studies have noted that higher percentages resulted in lower sensory scores. Sodium lactate is also a chelator of metals in foods and may be able to stabilize fatty acids and reduce lipid oxidation in a food system (Shelef, 1994). It can also increase the cooking yield of meat products (Williams et al., 1995; Williams and Phillips, 1998).

Papadopoulos et al. (1991a,b) injected beef top rounds with 0, 1, 2, 3, or 4% sodium lactate, then cooked and vacuum-packaged the rounds and stored them at 0°C for up to 84 days. The addition of 3% and 4% sodium lactate increased the shelf life of cooked, vacuum packaged beef top rounds up to 84 days. As days storage increased, APCs also increased and the increase ~~was~~ inversely related ($p < 0.001$) to the percent sodium lactate treatment Papadopoulos et al. (1991a). There was a general trend for pH to decrease over time. The authors noted a maximum of 3% sodium lactate absorption in the raw meat based on lactic acid measurements. Sodium lactate did not affect the water activity of the beef but it did result in higher cooking yields. The sensory results indicated that the addition of sodium lactate resulted in lower warmed over flavors in cooked roasts and in an enhanced fresh flavor Papadopoulos et al. (1991b). The addition of 1% sodium lactate improved palatability while the addition of 4% sodium lactate resulted in minor throat irritations for some of the panel members when testing the rounds that had been stored for less than 56 days.

Several studies have also been conducted on sodium lactate addition to comminuted or ground beef. Shelef et al. (1997) noted that the addition of 2% sodium lactate to ground beef held in refrigerator storage increased the shelflife by 2 – 4 days compared to the control. Similarly, Kulshrestha and Rhee (1996) looked at the effects of 3% sodium lactate with or without 0.05% sodium ascorbate in a reduced fat (10%) beef-carageenan patty. The patties were formed, cooked, aerobically or vacuum packaged, and stored at 4°C or –20°C for six days. The results indicated that APCs for all treatments increased throughout storage but that sodium lactate had the most antimicrobial effect in aerobically or vacuum packaged patties compared to no treatment, or the addition of 0.05% sodium ascorbate, 0.3% sodium tripolyphosphate, or 0.05% sodium ascorbate + 0.3% sodium tripolyphosphate. Aerobically or vacuum packaged beef patties with sodium lactate had lower TBA values compared to the control when stored at 4°C. Sodium lactate had no effect on pH and no protective effect on flavor. The addition of lactate did increase the cooking yields of the patties. Rhee et al. (1997) also noted that the addition of 3% sodium lactate reduced microbial growth compared to the control in ground beef top rounds aerobically stored for 18 days at 4°C. The pH of the beef samples treated with sodium lactate did not change over time. Beef samples that were pre-treated with 0.05% sodium ascorbate and then treated with sodium lactate had no increase in TBARS levels, however non-pre-treated samples treated with sodium lactate did have an increase in TBARS accumulation. The authors attributed the effect of sodium lactate on the TBARS due to its antimicrobial effect rather than an antioxidative effect. Sodium lactate reduced the bacteria available to accumulate or remove TBARS.

Williams and Phillips (1998) studied the effects of sodium lactate on the sensory and objective characteristics of fresh boneless and skinless chicken breasts. Chicken breasts were dipped in either tap water or a **2%** sodium lactate solution at various pHs, packaged, and held at $2 \pm 1^{\circ}\text{C}$ for **12** days. As storage time increased, microbial counts increased although they were lower in chicken treated with sodium lactate than the control at days 7 and **12** of storage. The pH of the sodium lactate solution was adjusted using **88%** lactic acid and the pHs tested were 4.00, 4.50, 5.00, 5.50, and **7.30** (no adjustment). The sodium lactate was most effective at retarding microbial growth at pH 5.00. However, antimicrobial effects were noticeable at pH 7.30 as well. At pH 4.5 and 5.0, there was discoloration and protein denaturation in the chicken breasts. Chicken breasts treated with the pH of 4.5 and 5.0 solutions were not tested for consumer acceptance. Fifteen percent of panelists reported acidic aftertastes in chicken that had been treated with sodium lactate that was adjusted to a pH of 5.00. Off flavors were detected in samples treated with sodium lactate through day three of sampling. After day 7, off-flavors were detected in all samples.

Williams et al. (1995) determined the shelf life and consumer acceptance of fresh catfish fillets treated with sodium lactate that were stored under simulated retail conditions. Fresh catfish fillets were treated with 0, 1, or **2%** sodium lactate adjusted to a pH of 5.50 by 1M hydrochloric acid and displayed in a retail case filled with ice for 10 hours per day and stored at 1.67°C overnight for a total of **8** days. Fillets with **2%** sodium lactate had significantly ($p < 0.05$) lower APCs compared to the control through eight days of storage. There was no significant difference between fillets treated with 1% or **2%** sodium lactate. Psychrotrophic counts were lower in treated fillets compared to the

control. Fillets treated with 2% sodium lactate had significantly lower psychrotrophic counts compared to fillets treated with 1% sodium lactate, however, all counts were similar by day four of storage. Fillets treated with 2% sodium lactate also had lower total coliforms than the control or fillets treated with 1% sodium lactate. There was no significant difference in pH between any of the treatments throughout the study. The TBA values increased throughout storage yet fillets treated with 2% sodium lactate had significantly lower TBA values than fillets treated with 1% sodium lactate or the control from day 2 to 8 of storage. Fillets treated with 1% and 2% sodium lactate had acceptable sensory scores throughout the storage study while the controls were only presented to the panelists up to day five due to the development of an intense off-odor. Based on their results, the authors stated that fillets treated with 2% sodium lactate had an extended shelf life of 4 to 7 days compared to the control.

Lactic Acid

Lactic acid is generally recognized as safe (GRAS) and is one of the compounds produced by lactic acid bacteria. Lactic acid bacteria are primarily used in the dairy industry in starter cultures in the fermentation process of cheese, butter, cultured buttermilk, cottage cheese, and cultured sour cream (Jay, 2000c). Lactic acid is also used in dairy, egg, beer, bread, and candy products, condiments, and infant foods to improve shelf stability, and taste (Shelef, 1994). Lactic acid has been examined extensively as an antimicrobial agent against food spoilers. It has been shown to be particularly efficient at inhibiting the growth of gram-negative bacteria while other antimicrobials have not.

Alakomi et al. (2000) examined the permeabilizing ability of lactic acid on gram-negative bacteria, *Escherichia coli* O157:H7, *Salmonella enterica* serovar Typhimurium,

and *Pseudomonas aeruginosa* with NPN, a hydrophobic probe whose fluorescence is greatly enhanced when in a glycerophospholipid environment. Uptake of a hydrophobic probe, such as NPN, indicates damage in the gram negative bacteria outer membrane (OM), which normally excludes hydrophobic substances. Typically gram negative bacteria can inhibit hydrophobic compounds such as detergents, lysozymes, and bacteriocins from entering the cell by protecting the OM with specific lipopolysaccharide (LPS). Thus, the cell remains biologically active. However, since lactic acid is a small water-soluble molecule, it can pass through the periplasm through the water-filled porin protein in the outer membrane. Once inside, it is hypothesized that the lactic acid permeabilizes the cell by causing the release of LPS. Once permeabilization has taken place, hydrophobic antimicrobials can enter the cell. Thus the lactic acid may make gram-negative bacteria more sensitive to other antimicrobials. Alakomi et al. (2000) examined the effectiveness of lactic acid in comparison to HCl and EDTA, a known permeabilizer. When in the presence of lactic acid, the uptake of NPN into the gram negative cells was significantly greater than uptake in just the presence of HCl at the same pH. The organisms took on more NPN in the presence of lactic acid than in the presence of EDTA. Lactic acid further sensitized all bacteria to the detergents SDS and Triton X-100, and to lysozyme. The bacteria were not as sensitive when in the presence of HCl except for the *P. aeruginosa*. Additionally, results from fatty acids analysis indicated that in the presence of lactic acid, bacteria released more LPS than in the presence of HCl or EDTA. The authors noted that the disruption of the outer membrane possibly occurs by both undissociated and dissociated forms of the acid. They also stated

that lactic acid combined with other antimicrobials may provide a hurdle technology to help reduce food spoilage and increase safety.

Marshall and Kim (1996) examined the microbial and sensory properties of refrigerated catfish fillets dipped or sprayed with acetic acid (AA), lactic acid (LA), or a combination of the two according to one of the following treatments: 1) 1 – 4% AA dipped for 15 seconds, 2) 1 – 4% LA dipped for 15 seconds, 3) 3% AA dipped for 5 – 60 seconds, 4) 3% LA dipped for 5 – 60 seconds, 5) 1% AA + 1% LA, dipped for 5 – 60 seconds, 6) 2% AA + 2% LA, dipped for 5 – 60 seconds, 7) 3% AA or combinations of AA and LA at 1% or 2% dipped for 30 seconds. The addition of acid lowered the initial pH of the fillets by 0.2 – 0.4 compared to the control, water-treated, or sodium lactate treated fillets. Fillets dipped for 30 – 60 seconds in 3 – 4% AA or a combination of 2% AA plus 2% LA showed inhibited microbial growth up to four days and extended refrigerated shelflife to 16 days. Fillets dipped in either 3 – 4% LA or 1% AA plus 1% LA for 5 – 60 seconds had an extended shelf of 12 days. When fillets were dipped for 15 seconds in 1- 4% AA or 1 – 4% LA there was little difference between the microbial generation times. There was also no extension of shelflife in fillets dipped in 4% AA for 15 seconds compared to 3% AA for 60 seconds. Results from sensory analyses indicated fillets treated with acetic acid had acidic odor and flesh discolorations. The authors recommended the concentration of AA or LA not to exceed 2% and a dipping time not to exceed 30 seconds. Nykanen et al. (1998) examined the antimicrobial effects of whey, whey derived lactic acid, and sodium lactate on rainbow trout from the same lot harvested from hatchery waters that were 4 to 7°C in the spring and 11 to 14 °C in the autumn. Whole gutted fish were dipped in 20g/ L of whey, whey-derived lactic acid, or

sodium lactate solutions for two minutes, then drained, vacuum-packaged, and stored at 0°C for 10 days. The pH values of the fillets were higher in the spring than in the autumn. Microbial counts in the spring and autumn were ≤ 1.2 CFU/cm² and 1.9 – **3.0** CFU/cm², respectively. In the spring, fillets treated with whey had the lowest microbial counts after 10 days storage. The difference between fillets treated with whey and the control were **0.8** log, 1.0 log, and 0.1 log for mesophilic, psychrotrophic, and anaerobic and facultative anaerobic bacteria, respectively. In the autumn, fillets treated with lactic acid had the lowest microbial growth. The difference between fillets treated with lactic acid and the control were 1.9 log, 1.9 log, and 1.6 log for mesophilic, psychrotrophic, and anaerobic and facultative anaerobic bacteria, respectively. Fillets treated with lactic acid had significantly lower mesophilic and psychrotrophic counts than fillets treated with whey after 7 days storage. The difference between fillets treated with whey and the control in the autumn were 1.1 log, 1.0 log, and 0.9 log for mesophilic, psychrotrophic, and anaerobic and facultative anaerobic bacteria, respectively. The authors found that dipping fish in sodium lactate solution did not retard microbial growth. Results from a duo-trio test indicated that consumers could tell a difference between fillets treated with sodium lactate or whey and the control, however, they could not detect a difference between fillets treated with lactic acid and the control.

In an effort to produce a beef product with high quality throughout storage Dorsa et al. (1997) examined the efficacy of an acetic acid, lactic acid or trisodium phosphate spray to reduce and inhibit the growth of *Escherichia coli* 0 157:H7, *Listeria innocua*, and *Clostridium sporogenes* in refrigerated beef carcasses infected with up to 10⁵ CFU/ g of each pathogen. **Six** spray washes were applied to the carcasses including tap water

(W), 1.5% lactic acid (LA), 3% lactic acid, 1.5% acetic acid (AA), or 3% acetic acid, or 12% trisodium phosphate (TP). Spraying the carcasses with all treatments resulted in an initial reduction of 5.6 CFU/ cm² to 1.3 – 2.0 CFU/ cm². The carcasses sprayed with water had microbial growth after two days of storage and reached an APC of 10⁷ CFU/cm² by day 7. Carcasses treated with acid sprays had inhibited APCs for the first days and suppressed growth throughout the experiment compared to the control. On the last day of storage APCs were 5.5 log CFU/ cm², which was significantly less than the control. Level of *E. coli* in the control carcasses reached 5.8 log CFU/ cm². The final populations of *E. coli* on beef carcasses treated with 1.5% and 3% LA were below 0.8 log CFU/ cm² and 0.1 log CFU/ cm², respectively. Spraying the carcasses with water resulted in an initial reduction of *L. innocua* by 2.0 log CFU/ cm², which was not significantly different from the control. After 21 days storage *L. innocua* levels were ≤ 0.2 log CFU/ cm² in carcasses treated with 1.5% LA and undetectable in carcasses treated with 3% LA. Spraying the carcasses with water resulted in an initial reduction of *C. sporogenes* by 1.6 log CFU/ cm². Carcasses treated with acids had either 1.6 log CFU/ cm² or undetectable levels of *C. sporogenes*.

Rosemary

Rosemary has been used for years in the food industry for its flavor and antioxidant effects. Fresh and dried rosemary are applied as flavor enhancers on vegetables, meat, poultry, and seafood products (Shelef et al., 1980). Fresh and dried rosemary and rosemary extracts have been noted for their antioxidant properties. Rosemary also has been shown to have antimicrobial effects (Shelef et al., 1980; Farag et al., 1989; Ouattara et al., 1997; Del Campo et al., 2000). It has been stated that the actual

spice is more inhibitory to microbes than the extract of spices (Jay, 2000c), however, due to the potent flavor of the spice the flavorless extracts have also been studied in media and meat systems. Rosemary's antioxidant activity is either due to chelation of metals, the donation of hydrogen atoms to radicals, or a combination of the two. Rosemary's antimicrobial activity is thought to come from polar phenol compounds, specifically diterpenoids, such as carnosic acid and carnosol (Del Campo et al., 2000). Most literature has focused on rosemary's antioxidant activity in food and media systems, however, there has been some research on its antimicrobial activity, primarily in media systems.

Vareltzis et al. (1997) added rosemary extract, incorporated as a dip, to the fillets and mince of Horse mackerel (*Trachurus trachurus*), a fatty fish, and Mediterranean hake (*Merluccius mediterraneus*), a low fat fish, to determine its effect on lipid oxidation throughout frozen storage. Results showed that rosemary slowed oxidation throughout storage and that treated fillets and mince of both mackerel and hake had significantly lower levels of MDA and higher PUFA content compared to untreated samples. Rancidity scores correlated well with MDA and PUFA content and treated samples received lower rancidity scores than the untreated samples during sensory analysis. Vareltzis concluded that rosemary extract was effective in reducing lipid oxidation in both filleted and minced samples of fatty and lean fish throughout storage.

The antioxidant effects of α -tocopherol and rosemary extract in a sardine oil model system and frozen-crushed fish meat were examined by Wada and Fang (1992). Each of the following treatments was mixed with sardine oil, and frozen-crushed bonito meat and stored in dishes held at 30°C and 60°C for the sardine oil, and 30°C for the bonito meat, for 15 days: 1) control containing no antioxidants, 2) 0.5% of α -tocopherol

(toc) **3) 0.02%** rosemary extract (rm), **4)** combination of 0.5% of α -tocopherol and **0.02%** rosemary extract (toc + rm), and 5) 0.02% BHA. Peroxide values in the **oil** samples were between 0 – and 1500 meq/ kg throughout storage. Oil samples treated with rosemary or α -tocopherol had suppressed induction of lipid oxidation after five days compared to the control and noticeable lipid oxidation was seen at day 10. The combination of the two proved to be the most effective natural treatment and the resulting PV was comparable to **oil** treated with BHA. As temperature increased from **30°C** to **60°C**, the authors noted an increase in PV. Lipid oxidation was seen to occur at day one when samples were held at **60°C** compared to day 10 when held at **30°C**. Lipid oxidation in the bonito meat was measured by TBARS. The combination of α -tocopherol and rosemary extract was the most effective treatment compared to all others, including BHA. The control had the highest TBA values throughout storage. The efficacy of the antioxidants were as follows: toc + rm > toc > rm > BHA. The combination of α -tocopherol and rosemary extract was also very effective in inhibiting the oxidation of polyunsaturated fatty acids. The authors noted the synergism between α -tocopherol and rosemary extract stating that, as radicals in the system were formed α -tocopherol acted as a radical scavenger, becoming a tocopheroxy radical, and then regenerating to its reduced form following the donation of hydrogens by the rosemary.

Boyd et al. (1993) examined the efficacy of a dip made of *tert*-butylhydroquinone ascorbic acid (TBHQ-AS) and Herbolox-W (HERB), a commercially produced rosemary extract, alone and in combination, to inhibit oxidation in cooked, flaked gray trout fillets stored at **-20°C** for 90 days. Fillets treated with TBHQ-AS or TBHQ-AS + HERB had the lowest TBARS values throughout storage compared with the rest of the fillets. Fillets

treated with HERB had significantly ($p < 0.05$) lower TBARS than untreated fillets held at -20°C and -70°C . Treated fillets had higher free fatty acid (FFA) formation compared to untreated fillets held at -20°C and -70°C , although it was noted that FFA formation did not always correspond with oxidation patterns. Throughout storage (-20°C) the control fillets lost the most PUFA while fillets treated with TBHQ-AS and HERE3 had the highest **PUFA** retention. Results from sensory analyses of the fillets indicated that the control fillets had the most fresh fish flavor. The addition of TBHQ-AS to the fillets resulted in bitter or sour flavors and fillets that were treated with HERE3 were found to have the flavor closest to the control. The antioxidant effectiveness of rosemary has also been shown in vegetable and fish **oils** (Frankel and Huang, 1996), restructured chicken nuggets (Lai et al., 1991), and heat sterilized meat (Guntensperger et al., 1998).

Del Campo et al. (2000) studied the effects of Oxy'less, a rosemary extract that is commercially produced and sold **as** an antioxidant, on the inhibition of bacteria, yeasts and molds in media and in the presence of several food ingredients in media. Rosemary extract had no effect on gram-negative bacteria but inhibited gram-positive bacteria in media held at 30°C . Two types **of** mold that were tested, *P. roquefortii* and *B. cinerea*, were slowed by the rosemary although not inhibited. The two yeasts, *R. glutinis* and *C. laurentii* were also not inhibited by the extract. The authors indicated that the rosemary had a more pronounced inhibitory effect on bacteria at refrigerated (5°C and 10°C) versus non-refrigerated temperatures (25°C and 30°C). At 30°C and 10°C , rosemary extract concentrations of 0.5% and 0.13%, respectively, were required to inhibit *S. aureus*. The rosemary extract also had a more inhibitory effect at a lower pH. A concentration of

0.13% of the rosemary extract inhibited the growth of *L. plantarum* at pH 4.5 while no inhibition was observed when 1% extract was used at pH 7.

B. cereus and *S. aureus* were utilized to investigate the effects of food ingredients on the antimicrobial activity of the rosemary extract at 30°C (Del Campo et al., 2000). The authors noted that fat and proteins reduced the antimicrobial activity of the rosemary extract. Bovine serum albumin (BSA) (1mg/ ml) and sterilized dairy cream (50 µl/ ml or 100 µl/ ml) mixed in tryptic soy broth reduced the antimicrobial activity of the rosemary extract. When BSA was mixed with TSB, a concentration of 0.5% rosemary extract did not inhibit *S. aureus* and 0.25% extract was needed to kill *B. cereus*. When 50 µl/ ml sterilized dairy cream were placed in TSB, 0.5% rosemary extract was needed to kill *B. cereus* while 1% rosemary extract was needed to kill *B. cereus* when 100 µl/ ml of cream was added. When 50 µl/ ml and 100 µl/ ml of cream mixed with TSB, 1% rosemary extract was needed to kill *S. aureus*. When *S. aureus* and *B. cereus* were mixed with skimmed milk, rosemary extract up to 1% had no effect on the growth of *S. aureus* but it delayed the growth of *B. cereus* for 24 hours.

Shelef et al. (1980) examined the efficacy of finely ground rosemary, sage, and allspice in inhibiting the growth of 24 gram positive and 22 gram negative bacteria. At the beginning of the study, the rosemary itself had microbial counts of 1.1×10^5 CFU/ g. Gram positive bacteria were more sensitive to the rosemary than gram negative bacteria. A concentration of 0.3% ground rosemary inhibited 22 out of the 24 gram positive bacteria tested. Concentrations of 0.5% and 2% inhibited or delayed the growth of 3 and 10 of the 22 gram negative, respectively. As concentrations of the spice increased, the antimicrobial effects also increased. A combination of 0.15% rosemary and sage, which

are often used together as flavor enhancers in meat products, inhibited the growth of **21** gram positive bacteria.

Farag et al. (1989) examined the antimicrobial activity of six essential oils from sage, rosemary, caraway, cumin, clove, and thyme against three gram negative bacteria, four strains of gram positive bacteria, one acid fast bacterium, and one yeast. **Similar** to Shelef et al. (1980), they reported that the gram-positive bacteria were more sensitive to the spice oils than the gram negative and that sage, cumin, and rosemary **oil** had very little inhibitory effect against gram-negative bacteria. However, rosemary **oil** had a minimum inhibitory concentration (MIC) (mg/ ml) of **12.00** for *Pseudomonas fluorescens*, **4.50** for *Serratia marcescens* and, 4.50 for *Escherichia coli*, which were all gram-negative bacteria. Rosemary extract had a MIC of **1.25** for all gram-positive bacteria except *Sarcina* spp., which required **1.50**. The MIC for the acid-fast bacterium and yeast were **1.25** and **1.75**, respectively.

When Ouattara et al. (1997) tested the efficacy of several essential oils to inhibit growth of gram positive and gram-negative bacteria the inhibition was similar for both types, however, after **48** hours the gram-negative bacteria were less affected. In their study, rosemary extracts inhibited the growth of following bacteria at a **1/100** dilution of the essential oils: *B. thermosphacta*, *P. fluorescens*, *S. liquefaciens*, *C. piscicola*, *L. curvatus*, and *L. sake*. The inhibition of the last three bacteria was extended beyond **48** hours. One of the reasons given for the different sensitivity between gram-positive and gram-negative bacteria was the presence of a lipopolysaccharide wall that inhibits fatty acids from entering into gram-negative bacteria (Ouattara et al., 1997).

Diacetyl

Diacetyl is produced by lactic acid bacteria, primarily heterolactics, such as *L. lactis* subsp. *lactis* biovar *diacetylactis* (Jay, 2000c). It is used primarily in the dairy industry as a flavoring compound. It is a bright yellow-colored volatile liquid, which has a pungent buttery smell and imparts a butter flavor to foods. It is also found naturally in red and white wines, roasted coffee, brandy, fermented foods, and silage (Jay, 1982a). Diacetyl **has** been shown to be more effective against gram-negative bacteria, yeasts, and molds than gram-positive bacteria (Jay, 1982a).

Sun and Oliver (1994) evaluated the effects of **BHA**, diacetyl, and lactic acid in inhibiting *Vibrio vulnificus* in oysters. Naturally infected oysters were purchased from a local seafood wholesaler, starved for two days, and placed in seawater tanks in which 0.05% concentrations or lower of the additive had been incorporated. After 24 hours the oysters were removed, stored at 5°C, shucked, and analyzed for APC and *V. vulnificus*. Holding the oysters in tanks at 5°C usually resulted in a 10-fold decrease in *V. vulnificus*. The addition of lactic acid or BHA concentrations up to 0.05% resulted in no inhibition of growth in *V. vulnificus* and in some cases growth was increased. The addition of diacetyl at concentrations of 0.05 and 0.1% decreased *V. vulnificus* 10 – 100 fold compared to the control. There was generally only one log decrease in APC counts compared to the control regardless of treatment but this reduction was not significant.

Jay (1982a) evaluated the efficacy of diacetyl in inhibiting microbial growth of 9 lactic acid bacteria, 14 non-lactic gram positive bacteria, 13 nonpseudomonad gram-negative bacteria, 16 yeasts, and 6 molds in refrigerated ground beef inoculated with 400 ppm diacetyl and stored for 8 days, **as well as** on pour plates with various concentrations

of diacetyl and pH. Results from the ground beef samples indicated that 400-ppm diacetyl effectively inhibited the growth of all microorganisms throughout the 8-day storage period. The control samples had initial APCs of 6.05 log CFU/ g and 10.50 log CFU/ g at the end of 8 days. Samples treated with diacetyl had APCs of 6.50 log CFU/ g after 8 days storage. Gram-negative bacteria followed the same growth pattern as APCs in the control samples and went from an initial 5.90 log CFU/ g to 5.60 log CFU/ g after 8 days storage in beef treated with diacetyl. The pH of the control increased from 5.8 to 7.6 by day 8. The pH of beef treated with diacetyl increased by only 0.1 after 8 days of storage. The pH had a substantial effect on the efficacy of the different concentrations of diacetyl. At pH 5.0 microbial growth was substantially inhibited compared to at pH 8.0. The group most sensitive to diacetyl was the *Pseudomonas*, in which all 12 were inhibited at 300 ppm at all pH except 8.0, in which case only 9 were inhibited. Lactic acid bacteria were the most resistant to the diacetyl and only one of the bacteria was inhibited at 300 ppm diacetyl and pH 5.0. Gram-positive non-lactics were all inhibited at 300 ppm and a pH of 5.0. Six and two of these bacteria were inhibited at 300 ppm and pH 7.0 and 8.0, respectively. Four yeasts grew at specific pHs below 7 with a concentration of 300 ppm diacetyl, while they all grew at pH 8.0 with a concentration of 300 ppm diacetyl. At 300 ppm all molds were inhibited except at pH 8.0 where they all grew. Jay noted that combinations of diacetyl with organic acids such as lactic acid and acetic acid may require a lower concentration of diacetyl to produce similar results.

Jay (1982b) also published a similar report on the effectiveness of diacetyl in media as an antimicrobial for lactic acid bacteria, gram-positive non-lactic acid bacteria, gram-negative bacteria, and yeasts. Parameters tested included the effect of different

commercially produced diacetyls, the effect of pH, the effect of additives, the effect of diacetyl on nongrowing cells, the inhibitory lethality effects of diacetyl, and the effect of diacetyl on anaerobes. The results indicated that diacetyl produced from different companies had similar antimicrobial activities. The sensitivity of bacteria to diacetyl was according to the following order from most to least sensitive: gram-negative bacteria, yeasts, gram-positive non-lactic acid bacteria, and lactic acid bacteria. Gram-positive bacteria were not inhibited by diacetyl. Gram-positive non-lactic acid and gram-negative bacteria were inhibited by 300 $\mu\text{g}/\text{ml}$ diacetyl at pH 7.0. *All* yeasts were inhibited by 300 $\mu\text{g}/\text{ml}$ regardless of pH. Diacetyl was more effective on **PCA** substrate and much less effective in cooked-meat medium and brain heart infusion broth. For example, at 344 $\mu\text{g}/\text{ml}$ only a yeast was inhibited and at **860** $\mu\text{g}/\text{ml}$ diacetyl two yeasts plus six gram-negative bacteria were inhibited in cooked-meat medium, which had a pH of 7.2, respectively. Furthermore, anaerobes were also not inhibited at **860** $\mu\text{g}/\text{ml}$ diacetyl in cooked-meat medium. **As far as** additives were concerned, gluconic acid had no effect on the activity of diacetyl while glucose, acetate, and Tween **80** were all antagonistic. Results indicated that diacetyl was not inhibitory to nongrowing cells, however, concentrations of 258 or 344 $\mu\text{g}/\text{ml}$ were lethal to gram-negative bacteria and inhibitory and lethal to two of four yeasts tested, respectively.

Consumer Seafood Trends

Seafood consumption is on the rise in the U.S. (Anonymous, 1991). Total fish and shellfish per capita consumption has risen from 11.7 pounds in 1970 to 14.5 pounds in 1997 (Putnam and Allshouse, 1999). Additionally, fresh and frozen shellfish per capita consumption ~~has~~ increased 42%, from 2.4 pounds in 1970 to **3.8** pounds in 1997.

Interestingly, crabs were selected by 22% of New York and New Jersey consumers polled in a survey **as** the most frequently purchased seafood item (O'Dierno, 1995). When consumers were asked why they purchased seafood products, the number one reason was taste and the second was for health reasons. Some consumers also felt that fish and seafood had a gourmet appeal (O'Dierno, 1995). Consumers recognize that seafood is a good source of protein, minerals, and unsaturated fatty acids (Anonymous, 1991). However, seafood is still consumed much less than its other meat counterparts. Per capita consumption of chicken **has** risen from **33.8** pounds per year in 1970 to **64.8** pounds per year in 1997. Beef is also consumed in much higher amounts than seafood, although it **has** decreased from **131.7** pounds per capita in 1970 to **111.0** pounds per capita in 1997. One of the reasons associated with the lower consumption of seafood is **difficulty of** preparation. Consumers appear unsure of how to cook fish products properly to avoid under or overcooking while ensuring that the product is safe to eat. Additionally, seafood is much more odiferous than other meats and the aroma left after seafood preparation can be unappealing. Thus, consumers primarily eat fish outside of the home, which leads to a lower consumption of these products compared to other types of meat commonly prepared in the home. In 1989, consumers spent **\$28.3** billion on fishery products (Anonymous, 1991). An estimated \$19.1 billion was spent at food service establishments such **as** restaurants, carry-outs, and catering facilities, \$9 billion was spent at retail stores such **as** grocery stores for home consumption, and \$181.7 million was spent for industrial fish products (Anonymous, 1991).

In order to increase consumption of seafood products while catering to consumer demands for easily prepared foods, seafood processors need to find **a** niche market

(Rippen, **1991**). Some of the "hot" marketing concepts that could help increase consumption of seafood include the development of ethnic, gourmet, convenient, flavorful products that are easy to make. Already prepared dishes that require simple cooking including microwaving, cooking in the oven, or boiling may be the wave of the future. Additionally, incorporating seafood into products that are already consumed frequently may be one approach to increase seafood consumption in the U.S. For example, formulating minced crab meat into a "package" such as pasta, which is already consumed more than once per week (Pszczola, **2000**), might be a way of utilizing a high quality protein to produce a gourmet seafood product that increases consumption of seafood while riding on the coattails of pasta's popularity.

Consumer Pasta Trends

The National Pasta Association states that more Americans are eating pasta than ever before (NPA, 2000). The U.S. per capita consumption of flour and cereal products was **200** pounds in **1997**, an increase from **145** pounds in **1980** and **136** pounds in **1970** (Putnam and Gerrior, **1999**). In the last quarter century, wheat flour per capita consumption increased **28%**, from **110.9** pounds to **141.7** pounds per year (King et al., **2000**). Durum flour comprised **11%** of the total wheat flour per capita consumption and in the last quarter century consumption increased from six pounds per year to **12.9** pounds per year (King et al., **2000**). Statistics indicate that consumers eat pasta more than once per week (Pszczola, **2000**). Additionally, children eat more pasta than any other age group. In **1997**, pasta was a **\$5.5** billion market in the United States that was rapidly growing.

Pasta is recognized as a good source of complex carbohydrates that is low in fat. Pasta is sold either dried, fresh, cooked, or frozen (Rubin, **1996**). There are over **150** different shapes of pasta including fettuccine, spaghetti, ziti, **orzo**, ravioli, cannelloni, fusilli, and vermicelli. With consumers currently spending less than a half an hour in the kitchen to prepare a meal, they are demanding convenient, fast, ready-to-eat, and prepared foods (Anonymous, **1991**) and pasta companies are meeting those demands especially in the fresh pasta market. A survey conducted of refrigerated fresh pastas available from a local Bangor supermarket revealed over **17** varieties of pasta including the popular plain pasta choices such as angel hair, fettuccine, and linguine, **as well as** flavored pasta such **as** hot red pepper linguine, garlic and herb linguine, and cracked black pepper linguine. More gourmet choices are now available which include stuffed **or** filled pastas such as cheese and mushroom ravioli, ricotta and spinach tortellini, chicken and prosciutto tortellini, mozzarella and pepperoni tortellini, and roasted garlic and asiago ravioli. Fresh pastas are available in either nine ounce containers for three servings and family size containers, which have up to five servings **or 20** ounces. Most nine ounce packages are priced between **\$1.79 to \$2.99** in local supermarkets in Bangor, Maine. A variety of refrigerated, fresh pasta sauces including marinara sauce, alfredo sauce, and red pepper sauce are also conveniently located next to the pasta. Pasta companies have focused on increasing consumption by providing more nutritious and well-balanced complete pasta choices as well. In **1998**, all food manufacturers were required to fortify specific grain products with folic acid to reduce birth defects in newborns (Ternus, **1996**). In **1999** a patent was issued for a calcium-fortified pasta that contains at least **800-8,000** mg of calcium per pound of product. Whole-wheat pasta, which contains 5 grams of fiber

per serving, compared to regular pasta, which contains only 2 grams of fiber per serving is now available. Additionally, wheat-free pastas for consumers with gluten allergies and high-protein pastas, made of **soy** flour, wheat germ, and yeast or dairy products that contain 20 - 100% more protein than a regular serving of pasta, are also available (Rubin, 1996). Pasta choices are also becoming hotter and more flavorful **as** gourmet pastas with ethnic flair are hitting the markets. There are also flavored, colored pastas that are aesthetically pleasing, which come in all shades of the rainbow including green, red, black, and speckled. New pastas containing fish and shellfish are also gaining popularity as "health conscious" pasta dishes. Some of these dishes include Maryland Crab Shells and Seafood Pasta Stir-Fry (Pszczola, 2000).

Pasta Quality

Microbial Quality

Some of the primary concerns in pasta quality are firmness and cooking quality of the noodle. However, fiesh pasta typically **has** a pH between 5 - 7, a high water activity (-0.93), and a moisture content between 25 - 30%. Thus one of the primary concerns regarding fresh pasta is microbial quality (Trovatelli et al., 1988; Giannuzzi, 1998a; Giannuzzi, 1998b; and Lopez et al., 1998).

Trovatelli et al. (1988) assessed the microbial quality of home-made and commercially manufactured fresh, filled dumplings that were vacuum-packaged and stored in refrigerated display cases in Bologna, Italy and the surrounding district. The filled pastas contained either a mixture of minced meat, cheese or other dairy products, fresh vegetables, spices, and bread crumbs. The dumplings were evaluated for aerobic

plate count, total coliforms, fecal coliforms, *S. aureus*, *C. perfringens*, and *Salmonella*.

Home-made pasta dumplings had the highest aerobic plate counts in the range of $10^5 - 10^6$ CFU/ g. None of the samples had aerobic plate counts below 10^4 CFU/ g, and a high percentage of the samples had APCs higher than 10^6 CFU/ g. The manufactured pasta had microbial counts primarily between $10^4 - 10^6$ CFU/ g and none of the samples tested had counts over 10^6 CFU/ g. Sixty-two percent of the homemade pasta had coliform counts $< 10^2$ CFU/ g and 38% had coliform counts $> 10^2$ CFU/ g, while 79% of the manufactured pasta dumplings had counts below 10^2 CFU/ g. Twelve percent and seven percent of the dumplings had fecal coliform counts greater than 10^2 CFU/ g in homemade and manufactured dumplings, respectively. Most samples contained coagulase-positive staphylococci but only 14% and 8% of the manufactured and homemade pasta dumplings, respectively contained more than 10^4 CFU/ g per gram. *Salmonella* and *C. perfringens* were not detected in any of the pasta samples. The authors noted that seasonal differences affected only the *S. aureus* counts, such that both homemade and manufactured dumplings contained higher levels of *S. aureus* in the summer months than during the rest of the year. Aerobic plate counts were not affected by seasonal differences.

The International Commission for Microbiological Specification has set a standard for APC counts for fresh pasta dumplings (Trovatelli et al., 1988). APC counts in homemade and manufactured pasta are not to exceed 10^6 CFU/ g. *S. aureus* is not to exceed 10^4 CFU/ g and 10^3 CFU/ g, respectively in homemade and manufactured pastas. *C. perfringens* is not to exceed 10^3 CFU/ g in manufactured pasta. No limit was stated for homemade pastas. No limit was established for *Salmonella* spp.

Lopez et al. (1998) examined the microbiological quality of purchased fresh filled pasta containing either ricotta and vegetables (**RS**) or cheese and meat (**CM**). The raviolis were evaluated for microbial population, characterization of bacterial isolates, water activity, and pH. The pH of the **RS** and **CM** ravioli were 5.5 and **6.1**, respectively. The **RS** had a water activity of **0.946** and the pasta had a water activity of **0.948**. The **CM** had a water activity of **0.948** and the pasta had a water activity of **0.947**. The total mesophiles were **6.4** and **5.4** log CFU/ g for **RS** and **CM**, respectively. The authors noted that due to the different ingredients utilized to make the raviolis, the microbial populations were heterogeneous. Gram negative bacteria including *Serratia marcescens*, *Klebsiella pneumoniae*, *E. coli*, *Pseudomonas* spp., *Pseudomonas paucimobilis*, and *Enterobacter sakazakii* were all detected. However, gram negative bacteria made up only 5% of the identified bacterial isolates. *L. monocytogenes* was found in one of the ravioli samples. Yeasts species included *Candida pelliculosa*, *Candida krusei*, *Rhodotorula* spp., *Candida famata*, *Pichia membranaefaciens*, and *Candida* species. All spore forming aerobic populations were *Bacillus* species, although no pathogenic *Bacillus* species were detected. The total coliforms were **5.6** and **4.9** log CFU/ g for **RS** and **CM**, respectively. The fecal coliforms were **4.6** and **3.6** log CFU/ g for **RS** and **CM**, respectively.

Giannuzzi (1998b) established a Hazard Analysis Critical Control Points (HACCP) plan for fresh pasta filled with a ricotta filling for commercial manufacturing and homemade processing. The author noted the need for a HACCP plan due to the likely contamination of filled pasta by *Bacillus cereus*, *Staphylococcus aureus*, and if raw eggs were used *Salmonella enteritidis*. The moisture content of the pasta was between **30-31%** and the water activity of the ricotta filling and filled raviolis was **0.97** and **0.96**,

respectively. The pH of the ricotta filling was between 5.4 - 5.8, the dough had a pH of 5.7, and the cooked product had a pH of 5.8. The total microbial count of the ricotta filling was 10^6 CFU/ g which came from the ricotta or spices. The dough had an initial microbial count of 10^4 CFU/ g and after being filled the ravioli had a microbial count of 10^8 CFU/ g. Once the raviolis were cooked according to manufacturer's instruction, the total microbial count was 10^2 CFU/ g, and no pathogens were detected. Although all vegetative forms of pathogens were killed during the cooking process, *B. cereus* and *Clostridium*, which are spore-forming pathogens may survive the cooking process. Thus, the authors determined that cooking and holding time, after the pasta is cooked but before it is eaten, are critical control points that need to be monitored closely.

After the initial evaluation of the fresh filled pasta manufacturing process, several methods to improve the microbial quality of the finished product were suggested. These included cleaning the mixer and kneader with a 0.02% active chlorine solution for 30 minutes after processing, and cleaning the dough rubbing and stretching machines with a high powered vacuum to remove all dried residue. Additionally, placing hand washing basins where operators worked and using separate cold storage to avoid cross contamination of raw materials and final product were also suggested. After these suggestions were utilized another assessment of the facility was completed with better results. The total microbial count of dough, ricotta filling, and ricotta-filled ravioli were 10^4 , 10^5 , and 10^5 CFU/ g, respectively.

Giannuzzi et al. (1998b) also held ricotta-filled raviolis at 0, 4, 8, and 10°C to determine the shelf-life of products held at different temperatures. The authors noted that maximum shelf-life of ricotta-filled ravioli in chilled storage was reached at microbial

counts of 10^6 CFU/ g. At microbial levels greater than 10^6 CFU/ g toxin production by pathogens can occur. According to their results, ricotta-filled ravioli had a shelflife of 14.0, 7.2, 6.7, and 1.2 hours when held at 0, 4, 8, and 10°C.

Similarly, Giannuzzi (1998a) developed a mathematical model of microbial growth in fresh filled pasta stored at different temperatures. In Argentina, filled pasta is a popular dish that is filled with either meat, cheese, vegetables, chicken, and/or spices. These pastas, however, are frequently subjected to temperature abuse and establishing a model to predict the shelf-life of these products would increase the safety of the product. The finished pasta and the ricotta filling that were evaluated in this study were held at 0, 4, 8, and 10°C and evaluated for psychrotrophic bacteria, *Enterobacteriaceae*, and mold and yeast counts. The ricotta filling and pasta filled with ricotta had a pH between 5.8 - 6.2, a moisture content between 30 - 31%, and water activity between 0.96 and 0.97. Psychrotrophic bacteria grew the fastest out of all microbes in both the ricotta and ricotta-filled ravioli regardless of temperature. Ricotta filling had psychrotrophic counts of 1.95 log (CFU/g) to 2.80 log (CFU/g) at 0°C and 10°C, respectively, with lag phases of 1.60 days and 0.11 days at the respective temperatures. *Enterobacteriaceae* counts ranged from 0.94 log (CFU/g) at 0°C with a lag time of 3.78 days to 1.58 log (CFU/g) at 10°C with a lag time of 0.86 days. The mold and yeast counts in the ricotta filling ranged from 0.18 log (CFU/g) at 0°C with a lag time of 1.43 days and 0.62 log (CFU/g) at 10°C with a lag time of 0.92 days. Psychrotrophic counts ranged from 1.63 to 2.55 log (CFU/g) with a lag time of 1.11 days and 0.18 days, for pasta held at 0°C and 10°C, respectively. The *Enterobacteriaceae* counts ranged from 0.65 log (CFU/g) at 0°C with a lag time of 1.71 days to 1.07 log (CFU/g) at 10°C with a lag time of 0.22 days. The mold and yeast counts

in the raviolis containing ricotta filling ranged from 0.59 log (CFU/g) at 0°C with a lag time of 1.29 days to 0.98 log (CFU/g) at 10°C with a lag time of 0.15 days. Ravioli with ricotta had higher initial counts and maximum population density for all microorganisms compared to just the ricotta filling. The maximum population density in the ravioli was highest for psychrotrophic organisms and ranged from 8.69 log (CFU/g) at 0°C to 9.00 log (CFU/g) at 10°C. The maximum population density for *Enterobacteriaceae* ranged from 7.53 log (CFU/g) at 0°C to 8.22 log (CFU/g) at 10°C. The maximum population density for molds and yeasts ranged from 6.40 log (CFU/g) at 0°C to 7.06 log (CFU/g) at 10°C.

Physical Quality

Durum semolina is produced when the endosperm of durum wheat kernels are separated from the rest of the seed in 150 to 500µm particles that are hydrated, mixed, pressed, and extruded sequentially (Feillet et al., 1996). During industrial processing, durum semolina, durum flour, and hard wheat flour are used to produce pasta. There are various types of durum wheat flour currently produced by flour mills for pasta making. Fancy durum flour has a minimum protein content of 11.5%, ash content of $0.85 \pm 0.05\%$ and the suggested application for this flour with the highest ash content is short goods such as elbows and shells. Extra fancy durum flour has an 11.5% minimum protein content, an ash level $0.75 \pm 0.05\%$ and is utilized in the production of sheeted goods such as ravioli. Number one semolina also has a minimum protein content of 11.5%, an ash content of $0.75 \pm 0.05\%$ and is used in the production of long goods such as lasagna, spaghetti, and fettuccine (ConAgra, 2000). During industrial processing of dried pasta, the flour and water are mixed in a mixing chamber and then extruded through a die. Both

of these steps are usually done under vacuum to prevent air bubble formation, which causes a chalky appearance and lower mechanical strength in the final product (Smewing, **1997**). The pasta is then dried at either a low temperature (**40 - 50°C**) or high temperature ($\geq 80^{\circ}\text{C}$) (Marconi et al., **1999**). The primary function of the drying step is to reduce the moisture content from 31% to 10- 12% (Smewing, **1997**). Drying is a critical stage in the production of dried pasta. If the pasta is dried too slowly, microbial growth may occur and spoil the product. If the pasta is dried too quickly, it may crack. The pasta is then packaged, stored at room temperature, and distributed to retail stores. If fresh pasta is extruded on a home or restaurant pasta machine, vacuum may not be a feature on the machine. Fresh pasta is not dried, although it is recommended that the pasta be laid on wooden racks at room temperature (20°C) up to **24** hours to allow some moisture removal so condensation is prevented during packaging (German, **1993**). Homemade or restaurant fresh pasta is typically bagged in plastic bags and refrigerated. Industrially made fresh pasta is typically packaged in plastic containers under modified atmosphere, stored at refrigerated temperatures (5°C), and distributed to retail stores.

When pasta is extruded, there is a continuous protein film on the outside of the pasta noodle surrounding the inner starch granules, and a protein matrix is aligned in parallel layers to the outside protein film (Smewing, **1997**). The protein responsible for the matrix is gluten, the major storage protein of wheat. Over 50% of gluten's amino acid composition is comprised of glutamic acid/glutamine and proline. Lysine, arginine, glutamic acid, and aspartic acid, which are responsible for gluten's low water solubility, make up 10% of the amino acid residues. Cysteine and cystine residues comprise **2-3%** of gluten's amino acid composition and these undergo sulfhydryl-disulfide interchange

reactions that help in the polymerization of gluten proteins. About 10% of the amino acid residues are glutamine and hydroxyl based, which are responsible for the protein's water binding capacity, and the hydrogen bonding of these amino acids provide the cohesion-adhesion properties. Thirty percent of the amino acid residues are hydrophobic, and these form protein aggregates which bind to other hydrophobic compounds such as lipids and non-polar substances (Damodaran, **1996**).

Damodaran (**1996**) states that gluten is made up of glutenins and gliadins, and that glutenins, specifically, determine the wheat quality of the dough. Good wheat quality, which produces a viscoelastic dough, is determined by the amount of polymerization between low molecular weight glutenins (< **90,000**) and high molecular weight glutenins (> 90,000). Poor wheat quality is attributed to low molecular weight glutenins polymerizing with themselves. Hydrophobic and sulfhydryl-disulfide interactions between the HMW glutenins and LMW glutenins create polymers and form a sheet that can entrap gas. As kneading and mixing occur during processing, these polymers form the protein matrix within the dough and a **maximum** resistance is reached. The longer it takes to reach the **maximum** strength or resistance of the dough, the better the wheat quality. If the dough is kneaded past the point of **maximum** resistance, the disulfide cross **links** can be broken and the polymers will be sheared into smaller polymers (Damodaran, **1996**).

When pasta is cooked, the gluten proteins absorb water and partially unfold. The higher the protein concentration the higher the water absorption, retention, and swelling (Damodaran, **1996**). When cooking pasta, it is critical that the proteins surrounding starch granules absorb water more rapidly than the starch granules to entrap the starch before it

can gelatinize and leach out. Starch gelatinization and protein coagulation occur at the same temperature and moisture *so* the two are in competition for the water (Smewing, **1997**). Poor wheat quality is characterized by the aggregation of the proteins in discrete masses with a large amount of starch loss *as* compared to a continuous matrix.

Pasta cooking quality is dependent on protein quality as well *as* quantity. During industrial processing, if the pasta is dried at a low temperature (**40 - 50°C**), protein quality and quantity are equally important in determining the pasta cooking quality. At a high-temperature drying stage ($\geq 80^{\circ}\text{C}$), the protein quantity is a more determinant factor of pasta cooking quality (Feillet et al., **1996**; Marconi et al., **1999**). When Marconi et al. (**1999**) made pasta *of* different spelt wheat cultivars the protein quality was low but the quantity was high, thus at high-temperature drying the large amount *of* protein was able to create a strong enough network capable of preventing starch granules from escaping during cooking.

Pasta quality is also attributed to optimal processing conditions (Cole et al., **1990**; Debbouz and Doetkott, **1996**; Waananen, **1996**; Icard-Verniere and Feillet, **1999**; Marconi et al., **1999**). These processing conditions include extrusion speed, temperature, pressure (Abecassis et al., **1994**), and the effect of mixing conditions (Icard-Verniere and Feillet, **1999**). Whether pasta is dried at a low or high temperature may also have an affect on its overall cooking quality of the pasta. For example, spelt cultivars of “inferior quality” compared with durum semolina produced high quality pasta when dried at a high temperature (Marconi et al., **1999**). Bergman et al. (**1994**) saw decreased cooking loss because of cowpea supplements being denatured during high-temperature drying resulting in a strong network entrapping the *soft* wheat starch granules.

Significant amounts of research have been conducted on the cooking quality of dried pasta, however, there **has** been little research on the physical characteristics of fresh pasta. **As** stated before, microbial quality is the primary concern of fresh pasta because it ensures the safety of the product, however, physical evaluations are also needed to determine the quality of the pasta for the consumer. The following summaries discuss the chemical and physical parameters **as** affected by the use of different flours other than semolina or the substitution **of** semolina by a high protein ingredient.

A 2-ounce serving size of pasta provides only 10% of the suggested daily protein intake and is limiting in two essential amino acids, lysine and threonine (Rayas-Duarte et al., **1996**). Since pasta is often times eaten as a meal without other sources of protein researchers have been investigating the quality of high-protein pastas as a means to increase the protein content in pasta while producing a product with acceptable physical qualities.

Rayas-Duarte et al. (**1996**) evaluated the effects of adding light and dark buckwheat, whole amaranth, and lupin flours at 0, 5, 15, 25, and **30%** incorporation into a semolina pasta. Some of the analyses conducted on the dried pastas included protein content, lysine content, color, firmness, cooking weight, and percent cooking loss. The control durum wheat flours contained ~ **14%** protein and only the addition of lupin flour significantly increased the protein content compared to the controls.

The color scores for the pastas with additional wheat flours ranged from **4.3** to **10.5**. Pastas containing lupin flour, regardless of different concentrations, maintained a raw score of **10**. Pastas containing light buckwheat and amaranth had significantly lower color scores than pastas made with extra fancy and fancy durum semolina. After the

pastas were cooked, the scores ranged from **3.8** to **11.0** and followed the same trend as the raw although the scores were lower.

Cooking weight of the pastas is the amount of water uptake during boiling or the amount of hydration (Rayas-Duarte et al., **1996**). The expected cooking weight of dried pasta made with semolina is about **3** times its original weight. Pasta containing light and dark buckwheat increased **3** times its original weight while pastas containing amaranth and lupin increased **2.9** times their original weight.

Percent cooking loss is the weight of total solids lost in the cooking waters (Rayas-Duarte et al., **1996**). Percent cooking loss should not exceed **7 -8%** in pasta made with semolina flour. The pastas made with durum semolina extra fancy and fancy had an average percent cooking loss of **6.4%**. Average percent cooking loss of pastas containing light buckwheat, dark buckwheat, amaranth, and lupin were **7.2**, **8.0**, **8.1**, and **7.8%**, respectively. The percent cooking loss of these pastas were within the range of acceptable quality. The authors noted that the ability to form a gluten matrix is unique to durum and semolina flours that have the protein gluten. The addition of other flours that do not possess gluten can dilute the gluten proteins, and the matrix is not formed as tightly. Without this tight gluten matrix holding the pasta together, leaching of solids, primarily starch components, into the cooking water can occur.

Firmness, which is determined in grams * centimeter for pasta products, is conducted on cooked pasta samples whereby a special plastic tooth shears spaghetti noodles. Average spaghetti firmness values were **5.3 g*cm** for the reference semolina, **4.9 g*cm** for the durum flour controls, **4.1 g*cm** for the light buckwheat, **3.5 g*cm** for the dark buckwheat, **3.3 g*cm** for the amaranth, and **5.6** for the lupin.

Since lysine is one of the limiting amino acids in pasta products, the authors also evaluated the level of lysine in these alternative pastas. A typical amount of lysine in conventional pasta is 2.1 g/ 100g of pasta. The lysine content in pastas containing buckwheat, amaranth, and lupin flours was 5.1, 5.2, and 5.7 g/ 100g while the lysine content of pasta made from durum semolina was 1.9g/ 100g.

With an increasing concentration in light buckwheat flour at 25 - 30%, dark buckwheat at 15% or higher, and lupin flour, there was increase in grittiness detected by the panel members during sensory evaluation. There was also a significant increase in mustiness and earthy flavors in pasta containing dark buckwheat and amaranth incorporated at 25% and 25 -30%, respectively.

Bergman et al. (1994) evaluated the development of a high-temperature soft wheat pasta that was supplemented with 10, 20, and 30% cowpea meal in terms of cooking quality, color, and sensory quality of the pasta. The authors noted that semolina, which is the most common wheat used to produce pasta, makes up only 5% of the world's wheat production and is more expensive than common wheat. Thus, the utilization of soft wheat with the addition of a high protein meal, such as cowpea, combined with high-temperature drying may produce a product similar to conventional pasta. All pasta samples were mixed for four minutes, extruded through a macaroni die, and dried for 0.5 hours at 25°C for case hardening, one hour at 40°C for predrying, two hours at 80°C for drying, and another two hours at 40°C for final drying. The pastas were cooled, placed in plastic bags, and stored in cardboard boxes at room temperature. The pastas were analyzed for proximate composition, color, optimum cooking time, cooking loss, and cooked weight. The moisture contents of the pastas ranged from 7.1 to 9.7%, and pasta

made with **100%** durum semolina had the highest moisture content. The protein content for pasta made from durum semolina was **16.0%** while the protein content of pasta made with **100% soft** wheat flour was **10.9%**. The addition of cowpea to the soft wheat flour significantly increased the protein content of the pastas. Color scores for durum pasta was **6** out of a possible **12** points, with a score of **12** being the best. Color scores for pasta made with **100% soft** wheat pasta was **3.5**. The authors attributed the very low durum pasta color score to the fact that the pasta was not extruded under vacuum which allows for the interaction of air bubbles, and the color was not evaluated until one year after it was extruded, thus, enzymes, such as lipoxygenases, could have degraded some of the carotenes. The addition of cowpea meal improved the color of the pasta compared to soft wheat pasta. The authors noted that the cowpea flour had a slight yellow color compared to the soft wheat flour. They also attributed some of the color improvements to the high temperature drying and Maillard browning reactions that occur between reducing sugars and lysine. As supplementation increased, pasta samples became darker, more yellow, more red, and less green. Pasta with **30%** cowpea had “L”, “a”, and “b” values of **49.0**, **3.1**, and **17.5**, respectively, and a color score of **5.0**. The range of cooking weight for pastas with cowpea supplementation was **26.2** to **29.4%**, while pasta made of durum semolina and **100% soft** wheat flour had cooking weights of **23.1%** and **29.5%**, respectively. There was a significant decrease in cooking weight observed at **30%** cowpea supplementation compared to the control but no significant changes at lower supplementations were observed. The cooking loss of pasta made with durum semolina and **100% soft** wheat flour pasta was **5.3** and **10.0%**, respectively. The addition of cowpea significantly reduced the cooking loss of the pasta compared to the pasta made

with **100%** soft wheat flour. Results from sensory analyses indicated that consumers could not detect a difference between pasta samples. The authors attributed this to the fact that over **30%** of the panel members ate cowpea on a regular basis *so* the difference may not have been **as** noticeable to them **as** someone from another part of the world where cowpea is not regularly consumed.

Marconi et al. (1999) evaluated the pasta-making quality of five European spelt wheat cultivars, including Ebners Rotkorn, Rouquin, Triventina, Balmegg, and Oberkulmer. Whole meal flour was obtained and each flour was mixed for 15 minutes with tap water, extruded at $40 \pm 5^{\circ}\text{C}$ and a pressure of 60 ± 10 atm. All spaghetti was dried in 7 hour drying cycles. These pastas were made purely from spelt wheat cultivars and no semolina was added in the formulation. The spaghetti was evaluated for proximate analyses, color, optimal cooking time, total organic matter, and cooking quality (as assessed by sensory analyses of the stickiness, bulkiness, and firmness). The mean protein content of the pastas containing spelt wheat was **15.7%** (db) All spelt wheat pastas had significantly higher protein content than durum wheat. The color analyses revealed that the pastas made from spelt wheat had lower “L” and “b” values, or less white and less yellow colors than durum wheat. According to sensory analyses, the pastas were given one score which ranged from 52 for Rouquin to **93** for Triventina. Interestingly, durum wheat received an overall score of **86**, which was less than for pasta made of Triventina.

Matsuo et al. (1972) evaluated the effect of different protein contents on the cooking quality of spaghetti. Two samples of No. **3** Canada Western Amber Durum (CWAD), one with a high protein content of **17.8%** and the other with a low protein

content of **9.8%**, were utilized to make spaghetti. Included with these different durum wheats were rapeseed flour (**49.2%**protein), fish protein concentrate (**79.6%**protein), soya flour (**52.0%**protein), powdered egg albumin (**68.4%**protein), as well as gluten, gliadin, and the soluble protein fraction of durum semolina. These ingredients were incorporated into spaghetti made of semolina in **1, 3, 5, and 10%** additions. The ingredients were mixed for three to five minutes and then extruded through a spaghetti die. Tenderness, compressibility, and protein content of the spaghetti were evaluated. The protein content in CWAD samples ranged from **8.5 to 16.6%**. The higher the supplementation the higher the protein content in the pasta. Adding rapeseed flour, fish protein concentrate, soya flour, and egg albumin all resulted in increased protein content of the pastas. The addition of durum glutenin and durum gliadin, which were both incorporated at **6%**, resulted in pastas with **15.3%** protein content. The addition of durum albumin (soluble protein fraction) resulted in a pasta containing **34%** protein.

Increasing the protein content of pasta with rapeseed flour or fish protein concentrate decreased the protein quality of the pastas (as measured by tenderness and compressibility). The tenderness of pasta containing fish protein concentrate was between **50 and 52 mm/ second * 10⁻³**. CWAD samples had tenderness values between **35 - 59 mm/ second * 10⁻³**. The compressibility, which measures the extent to which a sample can be compressed under a constant force, ranged between **70 to 100**. High compressibility scores were the result of soft samples with very little recovery or springiness while low compressibility scores were the result of high recovery or springiness. Samples with rapeseed flour and fish protein concentrate were soft with little elasticity. The addition of soya flour or protein soluble fraction had no effect on cooking

quality while egg albumin, glutenin, and gliadin (although to a lesser extent) resulted in good cooking quality, due to their firmness and low compressibility. The authors suggested the reason for the improvement in pasta containing egg albumin was due to the soluble egg albumin coagulating during heating, which improved firmness and elasticity of the product. When pasta is boiled and the gluten becomes wet it loses its extensibility and becomes firm and rubbery, however, the addition of extra gluten helped to maintain the firmness of the pasta noodle. The authors noted that the protein content should be at least 11% to have good pasta cooking quality, however, not all proteins are equally suitable. The only proteins that improved cooking quality in this study were egg albumin and wheat gluten.

Food Safety Concerns for Fresh Pasta Containing Crab Mince

There are several pathogens that could be present in a fresh pasta product containing crab mince that could pose food safety issues. The pathogen of primary concern is *Listeria monocytogenes*. *L. monocytogenes*, if present in seafood pasta, would most likely come from the crab mince. *L. monocytogenes* is a gram positive, asporogenic, facultative anaerobe that can survive and grow in temperatures ranging from 1°C to 45°C and can survive and grow in a pH range of 4.1 to 9.6 (Jay, 2000d). *L. monocytogenes* is distributed throughout the environment and can be found in a number of food products including fruits and vegetables, raw milk, soft cheese, animal or plant fresh food products, fresh/frozen meat and poultry, seafood, and ready-to-eat products. If ingested *L. monocytogenes* can cause beta-hemolysis of erythrocytes and destroys the phagocytic cells that engulf the microorganism (Jay, 2000d). Most individuals are actually exposed to levels up to 5 logs about 3.8 times per year but if the individuals are healthy they are

highly resistant to infection. Those individuals who are most susceptible to Listeriosis are the elderly, immunocompromised, or pregnant women. The symptoms are host dependent and the more severe symptoms seen are meningitis, sepsis, abortion, premature birth, and stillbirth in pregnant women (Jay, 2000d). *L. monocytogenes* is not thermally resistant and if products are properly cooked *L. monocytogenes* is destroyed (Jay, 2000d). In the United States *L. monocytogenes* is considered an adulterant in ready-to-eat products and there is a zero tolerance policy for *Listeria monocytogenes* in these products (Jay, 2000d).

Possible in-plant sources of contamination of lobster and snow crab by *Listeria* spp. have been examined (Chiasson et al., 1998). The authors indicated that the presence of total aerobes and staphylococci counts were significantly correlated with *Listeria* but fecal counts were not. *Listeria* spp. was found on food contact surfaces including drum meat separators, crab crushers, aprons, gloves, work tables, knives, and shucking machines, however, higher incidences of *Listeria* spp. were most often detected on surfaces that did not come in direct contact with food. These surfaces included foot stools, drains, employee boots and pants, floors, and truck boxes. The authors noted that even if good manufacturing processes are in place to reduce the risk of *Listeria* spp. in the final finished product, contamination can still occur and sanitary attention needs to be focused on non-food contact surfaces as well.

Once *Listeria* is in the final product, it can persist in a variety of environmental conditions. A challenge study looking at the growth of *L. monocytogenes* in value-added raw and cooked shrimp nuggets made from shrimp processing by-product was conducted (Lyver et al., 1998). A mixture of five *L. monocytogenes* subspecies were injected into raw and cooked nuggets for a total *L. monocytogenes* count of 10^3 CFU/ g. The nuggets

were packaged in Cryovac bags with either air, air with an oxygen absorbent, 100%CO₂, or 100%CO₂ with an oxygen absorbent. Packages were held at 4°C or 12°C for 28 days.

L. monocytogenes in raw nuggets held at 4°C grew from 10³ CFU/g to 10⁷ CFU/g by 21 days of storage in all packaging atmospheres except 100%CO₂, which had a *L.*

monocytogenes count of only 10⁵ CFU/ g. *L. monocytogenes* in raw nuggets held at 12°C reached 10⁷ CFU/ g in 7 days in all packaging atmospheres except 100% CO₂ which took 21 days to reach 10⁷ CFU/ g. *L. monocytogenes* in cooked nuggets held at 4°C, which were initially 10³ CFU/ g reached 10⁷ CFU/ g after 21 days in packages with air and air plus an oxygen absorbent, however, *L. monocytogenes* only reached 10⁵ CFU/ g to 10⁶ CFU/g after 28 days storage in packaging with 100% CO₂ with or without oxygen absorbents. At 12°C, all nuggets had 10⁷ CFU/ g of *L. monocytogenes* in 7 - 14 days except for packages with 100% CO₂ which had counts of 10⁷ CFU/ g after 21 days of storage. The authors noted that none of the packaging atmospheres inhibited the growth of *L. monocytogenes*. The replacement of air with 100% CO₂ did increase the lag phase of *L. monocytogenes*. They also noted that spoilage microflora consisting primarily of lactic acid bacteria and *Bacillus* spp. had no effect on the growth of *L. monocytogenes*.

Rosemary, sodium lactate, and lactic acid have also been shown to reduce or inhibit the growth of *L. monocytogenes*. Ground rosemary was found to effectively inhibit *L. monocytogenes* at 0.7% - 1.0% of tryptic soy broth solution held at 24°C (Ting and Diebel, 1992). Pelroy et al. (1994) noted that sodium lactate inhibited the growth of *L. monocytogenes* in comminuted chicken and beef model systems, as well as cook-in-bag beef roasts. Three percent sodium lactate in combination with a three percent water-phase salt with or without 125 ppm NaNO₂ prevented the increase of *L. monocytogenes*

(10 CFU/g) injected in cold-process comminuted, raw salmon held at 5°C and 10°C prior to smoking. The addition of 2% sodium lactate prevented the growth of *L. monocytogenes* in salmon stored at 5°C but was less effective at 10°C. *L. monocytogenes* levels did not increase for 14 days of storage at 10°C for salmon treated with 2% sodium lactate and 3% water-phase salt but reached to within one to two logs of the control after 14 days. However, a combination of 2% sodium lactate, 3% NaCl, and 125 ppm NaNO₂ effectively inhibited *L. monocytogenes* growth at 10°C. Lactic acid has inhibited several strains of *L. monocytogenes* in tryptic soy agar broth (TSAB) (Sorrells et al., 1989). The maximum pH for no growth of *L. monocytogenes* in broth that contains lactic acid is between 4.4 – 4.6 regardless of temperature. The maximum pH needed varied due to strain differences. The minimum pH for growth occurred at a pH between 4.6 – 4.8, again depending on strain and temperature.

During pasta extrusion minimal heat is applied and the final product may contain *L. monocytogenes*. However, fresh pasta is not a ready-to-eat product, and the thermal death times of *L. monocytogenes* can be exceeded during the cooking process. Beuchat and Brackett (1989) evaluated the thermal inactivation of *L. monocytogenes* that was injected into meat, cheese, and egg ravioli at a level of 3×10^5 CFU/g. Ravioli samples were cooked on days 0 and 9 of refrigerated storage. Nine ravioli were placed in 200 mL of **boiling** water. The water returned **to boil** after 4.3 minutes and samples were taken out of the water at 3, 5, and 7 minutes after boiling had resumed. No *L. monocytogenes* were detected in the cooked ravioli. The D-values of *L. monocytogenes* strain Scott A injected into previously canned, pasteurized blue crab meat up 10^7 CFU/g were 40.43, 12.00, and 2.61 minutes at 50, 55, and 60°C, respectively (Harrison and Huang, 1990).

Other pathogenic organisms that could potentially be present in the pasta containing crab mince due to cross-contamination are *Salmonella enteritidis*, *Escherichia coli*, *Shigella dysenteriae*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Staphylococcus aureus*, and *Bacillus cereus* (Jay, 2000d,e,g,h,i). There is not a zero tolerance for these pathogens in the United States. Howard and Dewi (1995) reported that toxic substances can be produced when microbial levels are above 10^6 . Additionally, most of these pathogens require a high microbial concentration before making a person ill. If crab mince and raw product are manufactured in a clean environment, the final product is stored at refrigerated temperatures ($1 - 5^{\circ}\text{C}$), properly cooked, and the product is eaten promptly after being cooked, these pathogens should not reach high enough counts or produce a toxin that could make someone ill.

Objectives

The overall purpose of this research project was to develop and characterize fresh pastas containing minced crab meat. The project was split into the following four studies:

- Study # I - *Mechanical Separation of Crab Mince*

The specific objectives were to determine ease of separation, yield, nutrient composition, and quality of crab meat mechanically separated from the carapace and legs of Jonah crabs.

- Study # 2 – *Effect of Additives on the Shelf-life of Crab Mince*

The specific objectives were to evaluate the effects of rosemary, diacetyl, sodium lactate, and lactic acid on the chemical and microbial quality of refrigerated crab mince.

- Study #3 – *Development and Quality of Pasta Containing Crab Mince with Additives*

The objectives were to determine if fresh pastas containing different concentrations of crab mince with additives could be successfully extruded. The chemical, microbial, and physical quality of the pastas were also evaluated throughout a five week refrigerated storage period.

- Study #4 - *Sensory Analyses and Evaluation of Pasta Containing Crab Mince with Additives*

The objectives were to evaluate the consumer acceptance of fresh pasta products containing crab mince with additives and to evaluate the chemical, microbial, and physical quality of the pastas.

MATERIALS AND METHODS: MECHANICAL SEPARATION OF CRAB MINCE

Objectives

The objective of this study was to determine mechanical feasibility, yield, nutrient composition, and quality of crab meat mechanically separated from the carapace and legs of Jonah crabs.

Experimental Design

The experiment was set up to determine the mechanical feasibility, yield, nutrient composition, and quality of crab meat mechanically separated from the carapace and legs of Jonah crabs. There were a total of three batches. Two of the batches were crab meat separated from the carapace and the other batch was crab meat separated from the legs (Table 1).

Table 1. Treatments and Codes for Mechanically Separated Crab Mince

Code	Treatment
CA	Carapace A
L	Legs
CB	Carapace B

Ingredients

Approximately 20 pounds of steamed Jonah crab (*Cancer borealis*) legs with first joint removed, and carapaces with limbs, gills, and viscera removed were obtained from a small-scale crab processor (Cranberry Point Products; Gouldsboro, ME). The carapace and leg parts were separated into totes filled with ice just prior to mechanical separation. Four to five pound batches of crabs were weighed and the crabs were mechanically

separated with a Paoli One-Step Deboner Model #22-849 (Rockford, IL). The end plate setting on the meat bone separator was set at one-quarter open and the bar breaker setting was set at 0.120. A total of three batches of crabs were mechanically separated in the following order: Carapace A, which consisted of only cooked bodies (39°C) with the gills and viscera removed without any meat removed; Legs, which consisted of the last three joints of cooked legs (24°C) without any meat removed; and Carapace B, which consisted of cooked bodies (25.5°C) with the viscera and gills removed. The first two treatments were processed sequentially since more crab carapaces had to be steamed, delimbed, eviscerated, and de-gilled for the batch designated Carapace B. There was a **30** minute delay between the end of mechanical separation of the Legs and the beginning of mechanical separation for Carapace B. During that lag time, crab mince remaining in the machine dried in the microslits and prevented any Carapace B from coming out of the machine. Minced meat from Carapace B was manually scraped out of the separator's cylinder. The minced meat coming out of the machine for Carapace A and Legs treatments was collected in a stainless steel bowl and immediately weighed. *All* minced meat was placed in Whirl-Pak bags (Nasco; Fort Atkinson, WI) and spread evenly throughout the bag to create the greatest surface area. The bags were immediately placed in a cooler with ice *so* that sides of the bag came in contact with the ice. The crab mince was transported back to the University of Maine, Orono, **ME**, where it was held in refrigerated storage (5°C). On days 1, 4, 7, and 11 of refrigerated storage, crab mince was evaluated for chemical and microbial analyses. Approximately 50 g of crab mince from each of the three batches was placed in Whirl-Pak bags (Nasco; Fort Atkinson, WI) and held in frozen storage (-18°C) until proximate analyses were performed.

Chemical Analyses

Moisture

Five-gram samples of the treatments were placed in pre-weighed scintillation vials (Wheaton Scientific; Milville, NJ). The vials and samples were placed in a vacuum oven (National Appliance Co.; Portland, OR) set at 70°C for **24** hours. The vials and samples were re-weighed and percent moisture was determined by using the following calculation: $((\text{vial wt} + \text{sample wt}) - (\text{vial} + \text{dry sample wt})) / \text{sample wt} \times 100$. Samples were analyzed in duplicate.

Ash

The dried samples and vials from moisture analysis were reweighed and placed in a Thermolyne Model F-A1730 muffle oven (Dubque, IA) set at 550°C for **6** hours. The ashed samples and vials were re-weighed and percent ash was determined by using the following calculation: $((\text{vial wt} + \text{ash wt}) - (\text{vial wt})) / (\text{sample wt}) \times 100$. Samples were analyzed in duplicate.

Minerals

The ashed samples in scintillation vials were dissolved in 1 mL each of concentrated hydrochloric acid (J.T. Baker; Phillipsburg, NJ) and nitric acid (EM Science; Gibbstown, NJ). Ten mL of distilled water were added to the vials and the samples were mixed on a vortexer Model K-550-G (Vortex-Genie; Bohemia, NY). The samples were transferred to quantitative **flasks** and diluted to a volume of 100 mL with distilled water. Samples were analyzed for calcium, potassium, magnesium, and

phosphorus using an Inductively Coupled Argon Emission Spectrophotometer(ICP) by the Analytical Lab located in Deering Hall, University of Maine. Mineral concentrations were determined using the following calculation: (mineral ppm \times dilution factor)/**wt** of wet sample. Samples were analyzed in duplicate.

Fat

The fat content of the crab mince samples was analyzed according to the AOAC (1996a) method **922.06**. Flat bottom flasks were dried in a Fisher Isotemp Oven Model 350 (Fisher Scientific; Pittsburgh, PA) forced air oven at 100°C for 10 minutes. The flasks were placed in a dessicator to cool and the weight **of** the flask was recorded. Approximately four g of sample were placed in a screw top dilution bottle. Two ml of **95%** ethyl alcohol (EM Science; Gibbstown, NJ) and ten mL of 8.1N HCl (J.T. Baker; Phillipsburg, NJ) was added to the dilution bottle. The dilution bottles were placed in a 70-80°C bath for 40 minutes and the samples were stirred at frequent intervals. The dilution bottles were taken out of the water bath, ten mL of **95%** ethyl alcohol was added to the dilution bottle and the samples were allowed to cool. Twenty-five mL of **99%** ether (J.T. Baker; Phillipsburg, NJ) was added to the bottle, the bottle was capped, and shaken vigorously for 1 minute. Twenty-five mL of petroleum ether (J.T. Baker; Phillipsburg, NJ) was added to the bottle, the bottle was capped, and shaken vigorously for 1 minute. The fat and ether (upper liquid) from the dilution bottles was extracted by pipetting and transferred to pre-dried flat bottom flasks. The addition of **99%** ether and petroleum ether was repeated two more times using 15 mL of each ether. After the addition of ether, the fat and ether were transferred to the pre-dried flat bottom flasks for the last two extractions. The flat bottom flasks were placed on hot plates at setting **2** overnight to

allow the ether to evaporate. The **flasks** were dried in a Fisher isotemp oven Model 350 (Fisher Scientific; Pittsburgh, PA) forced air oven at 100°C for 10 minutes to drive off any moisture. The flasks were re-weighed and percent fat was determined by using the following calculation: $[(\text{flask} + \text{oil wt.}) - \text{flask wt.}] / \text{sample wt.} \times 100$. Samples were analyzed in duplicate.

Crude Protein

Approximately three grams of crab mince ~~was~~ dried in a vacuum oven (National Appliance Co.; Portland, OR) set at 70°C for **24** hours. The samples were ground in a Braun Model KSM2 **(4)** coffee grinder (Mexico) for 15 seconds. Approximately 0.5 g of dried, ground crab mince was placed in a Whatman # 1 filter paper (VWR; West Chester, PA) and folded over to contain the sample. Samples were analyzed for Kjeldahl nitrogen using a Foss Tecator Model **2400** Kjeltex Analyzer Unit (Foss Tecator Inc.; Hoganas, Sweden). Filter paper containing the dried samples were placed in digestion tubes and 15 mL concentrated sulfuric acid (EM Science; Gibbstown, NJ) and two cupric sulfate catalyst discs were added to the tubes. The digestion tubes were placed on Tectator Model **2020** Digestor (Tecator **Co.**; Hoganas, Sweden) set at **420°F**. Once samples were completely digested they were cooled, and **40 mL** of distilled water was added to the tubes to prevent crystallization. Distillation and titration with 0.1N sulfuric acid was performed by a Kjeltex Analyzer Unit (Foss Tecator Inc.; Hoganas, Sweden). Percent crude protein was determined by using the following calculation: $\%CP = [(\text{mL of titrant} - \text{blank values}) \times N \text{ sulfuric acid} \times 8.755] / \text{sample wt.}$, where N = the normality of the acid used. Samples were analyzed in duplicate.

pH

Approximately 15 g of each sample was placed in a Falcon test tube (VWR Brand; Boston, MA) and 15 mL of distilled water was added to each test tube. The samples were homogenized using a polytron (Kinematica; Switzerland) set at five for 30 seconds. The pH of the samples was determined with an Orion Model 320 PerpHecT LogR meter (Beverly, MA). Samples were analyzed in duplicate.

Total Volatile Base Nitrogen

Twenty-five g of crab mince samples were placed in a microblender (Waring; New Hartford, CT). Fifty mL of a 7.5% trichloroacetic acid solution (Sigma; St. Louis, MO) was added to the blender and the sample was mixed for 30 seconds until homogenized. The homogenized mince TCA mixtures were placed in centrifuge tubes (VWR; Boston, MA) and centrifuged at \approx 4000 rpm for 20 minutes. The supernatant was filtered through Whatman #1 filter paper into Falcon test tubes (VWR Brand; Boston, MA) and held in refrigerated storage (5°C) until analyses could be completed. Fifteen mL of each filtrate was placed in a micro-Kjeldahl distillation unit (Labconco Corporation; Kansas City, MO) set at level 7. Four mL of a 10% sodium hydroxide solution was added to the sample in the distillation unit. Total volatile base nitrogen, which volatilized from the crab mince sample during distillation was captured in 15 mL of a 4% boric acid solution (Sigma; St. Louis, MO) containing 8 drops of methyl red-methylene blue (Fisher Scientific Company; Fairlawn, NJ) indicator solution (two parts 0.2% alc methyl red solution with one part 0.2% alc methylene blue solution). The sample was titrated with 0.1N HCl solution (J.T. Baker; Phillipsburg, NJ). Total volatile base nitrogen was determined by using the following calculation: (mL HCl for sample \times mL HCl for blank)

x normality of HCl x **14.007** x (67.5 mL/15 mL) x (100 g/ 25 g). A single analysis was conducted on each replicate sample.

Thiobarbituric Acid Reactive Substances

Samples of crab mince were analyzed according to the method described by Tarladgis et al. (1960) and Rhee and Watts (1966). Four g of each sample were placed in 50 mL Falcon test tubes (VWR Brand; Boston, MA). Sixteen mL of cold buffer (50 nM PO₄, 0.1% EDTA, **0.1%PG**) was added to the test tubes and the samples were vortexed vigorously. The samples were homogenized on a Polytron (Kinematica; Switzerland) set at level 5 for **30** seconds. Four mL of **30%** trichloroacetic acid solution were added to the tubes and the samples were vortexed for **15** seconds. The slurry was filtered through a Whatman # 1 filter paper and collected in screw cap test tubes. Four mL of 20mM thiobarbituric acid solution was added to each test tube, the test tube was capped, and vortexed briefly. Test tubes were placed in a boiling water bath for 20 minutes. After **20** minutes, the test tubes were placed in an ice bath to stop the reaction. The cooled filtrates were transferred to disposable cuvettes and their absorbances were read at 530nm on a **DU-64** spectrophotometer(Beckman Instruments, Inc., CA). Each treatment was analyzed in duplicate.

Microbial Analyses

Total Plate Counts

Total plate counts were enumerated on plate count agar using standard AOAC (1996b) method 966.23. Twenty-five g samples were aseptically placed in Whirl-Pak stomacher bags (Nasco; Fort Atkinson, WI) and 225 mL of 0.1% bactopectone were added. Samples were stomached for two minutes using a Model **400** stomacher-lab blender (Tekmar Co.; Cincinnati, OH). Serial dilutions were made in 0.1 percent bactopectone. One mL aliquots were aseptically plated in sterile petri dishes (Fisher Scientific Co. LLC; Agwam, MA) and plate count agar was poured into the petri dishes. Plates were stored upside down and incubated at 23°C for **48** hours. Bacterial colonies were counted with the aid of a Quebec colony counter (American Optical Company; Buffalo, NY). Final results were expressed **as** colony forming units per gram of sample. Each treatment was analyzed in duplicate.

Yeast and Mold Counts

Yeasts and molds were enumerated on acidified potato dextrose agar using standard AOAC (1992a) method. Twenty-five g samples were aseptically placed in Whirl-Pak stomacher bags (Nasco; Fort Atkinson, WI) and 225 mL of 0.1% bactopectone was added. Samples were stomached for two minutes using a Model **400** stomacher-lab blender (Tekmar Co.; Cincinnati, OH). Serial dilutions were made in 0.1 percent bactopectone. One mL aliquots were aseptically plated in sterile petri dishes and acidified potato dextrose agar was poured into the petri dishes. Plates were stored right side up, wrapped in aluminum foil (Reynolds Wrap; Richmond, VA), and incubated at 23°C for

120 hours. Yeasts and molds were counted with the aid of a Quebec colony counter (American Optical Company; Buffalo, NY). Final results were expressed as colony forming units per gram.

Coliforms

Coliforms were detected using a modified AOAC (1992b) method. Twenty-five g samples were aseptically placed in Whirl-Pak stomacher bags (Nasco; Fort Atkinson, WI) and 225 mL of 0.1% bactopectone was added. Samples were stomached for two minutes using a Model 400 stomacher-labblender (Tekmar Co.; Cincinnati, OH). Triplicate sets of three serial dilutions were made in **3.6%** lauryl tryptose broth. Test tubes with LTB broth contained inverted Durham tubes. Gas production seen in those vials indicated the presence of gas producers. Test tubes with LTB broth were incubated at $35 \pm 1^\circ\text{C}$ for **48** hours. A loopful of sample from the LTB tubes with gas production was transferred into tubes containing **4%** Brilliant Green Broth (BGB). Test tubes with BGB broth also contained inverted Durham tubes. Gas production seen in those vials indicated the presence of coliform organisms. Test tubes with BGB broth were incubated at $35 \pm 1^\circ\text{C}$ for **48** hours. The most probable number of total coliforms was calculated based on the number of tubes with gas production.

MATERIALS AND METHODS: EFFECT OF ADDITIVES ON THE SHELF-LIFE OF CRAB MINCE

Objectives

The objective of this study was to evaluate the effects of rosemary, diacetyl, sodium lactate, and lactic acid on the chemical and microbial quality of refrigerated crab mince.

Experimental Design

The experiment ~~was~~ set up to test the effects of rosemary, diacetyl, lactic acid, and sodium lactate on the chemical and microbial characteristics of crab mince. Crab mince contained either no additives, 2 or 5 % dried rosemary with or without 2% lactic acid, 0.04% or **0.08%** diacetyl with or without **2%** lactic acid, or 2% sodium lactate. There were a total of eleven treatments (Table 2).

Table 2. Treatments and Codes for Crab Mince with Additives

Code	Treatment
2R	2% Rosemary
2R-L	2% Rosemary + 2% Lactic Acid
5R	5% Rosemary
5R-L	5% Rosemary + 2% Lactic Acid
4D	0.04% Diacetyl
4D-L	0.04% Diacetyl + 2% Lactic Acid
8D	0.08% Diacetyl
8D-L	0.08% Diacetyl + 2% Lactic Acid
SL	2% Sodium Lactate
C	Control
c - L	Control + Lactic Acid

Ingredients

Approximately 55 pounds of refrigerated, previously steamed Jonah crabs (*Cancer borealis*) with limbs, backs, gills, and viscera removed were obtained from a small-scale crab processor (Cranberry Point Products; Gouldsboro, ME). The crab bodies were stored in totes, covered with ice and held in refrigerated storage (5°C) overnight. The following day, five to six pound batches of crab bodies were taken out of refrigerated storage, weighed, and mechanically separated with a Paoli One-Step Model # 22-849 (Rockford, IL). The end plate setting on the meat bone separator was set at one-quarter open and the bar breaker setting was set at 0.120. The minced meat coming out of the machine was collected in a stainless steel bowl. The minced meat was placed in one gallon plastic bags (Ziploc; Racine WI) and spread evenly throughout the bag to create the greatest surface area. The bags were immediately placed in a cooler with ice so that all sides of the bag came in contact with the ice. The crab mince was transported back to the University of Maine, Orono, ME, where it was held in refrigerated storage (5°C) until further processing.

Lactic acid, diacetyl, and sodium lactate were obtained from Sigma (St. Louis, MO). Dried rosemary (McCormick; Hunt Valley, MD) was ground in a Braun Model KSM 2 (4) (Mexico) coffee grinder for 30 seconds. The ground, dried rosemary was placed in beakers, covered in plastic wrap (Saran®; Oakland, CA) and held at room temperature (20°C) until further processing.

Formulations

Crab mince was separated into 650 g portions and mixed with additives for 2 minutes in a Hamilton Beach Food Pro 2 food processor (Proctor-Silex, Inc.; Washington D.C) (Table 3). Ground, dried rosemary was added to the crab mince at 2% or 5% of the crab mince weight (w/w). Diacetyl was added to the crab mince at **0.04%(400 ppm)** or **0.08%(800 ppm)** of the crab mince weight (w/w). Lactic acid and sodium lactate were both added to the crab mince at **2%** of the crab mince weight (w/w). The crab mince-additive mixtures were placed in Whirl-Pak bags (Nasco; Fort Atkinson, WI) and held in refrigerated storage (5°C). Two replicates were prepared for each treatment. Approximately 50 g of crab mince without additives was placed in a Whirl-Pak bag (Nasco; Fort Atkinson, WI) and held in frozen storage (-18°C) until proximate analyses could be performed. On days 1, 3, 5, and 8 of refrigerated storage, samples were evaluated for chemical and microbial analyses.

Table 3. Additives Incorporated into Crab Mince

Treatment	Ingredients (g/ 100g)					Totals
	Crab Mince	Dried Rosemary	Diacetyl	Lactic Acid	Sodium Lactate	
2R	98.04	1.96	--	--	--	100
2R-L	96.15	1.92	--	1.92	--	100
5R	95.24	4.76	--	--	--	100
5R-L	93.46	4.67	--	1.87	--	100
4D	99.96	--	0.04	--	--	100
4D-L	98.00	--	0.04	1.96	--	100
8D	99.92	--	0.08	--	--	100
8D-L	97.96	0.08	--	1.96	--	100
SL	96.77	--	--	--	3.23	100
C	100.00	--	--	--	--	100
C-L	98.04	--	--	1.96	--	100

Chemical Analyses

Moisture, ash, minerals (including sodium), and fat were analyzed **as** described in "Evaluation of Mechanically Separated Crab Mince". The samples were analyzed in triplicate. pH, TVBN, and TBARS were analyzed as described in Study # 1. Each replicate sample was analyzed in duplicate.

Microbial Analyses

Total plate counts were analyzed **as** described in "Evaluation of Mechanically Separated Crab Mince". Each replicate sample was analyzed in duplicate.

Statistical Analyses

Statistical differences between treatments were evaluated by SYSTAT 9.0 (SPSS Inc.; 1999) using one-way analysis of variance (ANOVA) ($p < 0.05$). Differences between means were evaluated using Tukey's post hoc test (Neter et al., **1996**). A multi-way ANOVA was conducted by SYSTAT 9.0 (SPSS Inc.; 1999) to evaluate the effects of 1) additives (no additives, 2% or 5% dried rosemary, 0.04% or **0.08%** diacetyl), 2) the addition **of** lactic acid (0% or 2%), and 3) days storage (1, **3**, 5, or **8**).

MATERIALS AND METHODS: DEVELOPMENT AND QUALITY OF PASTA CONTAINING CRAB MINCE WITH ADDITIVES

Objectives

The overall objectives of this study were to determine if fresh pastas containing different concentrations of crab mince with additives could be successfully extruded and to evaluate the chemical, microbial, and physical quality of the pastas throughout a five week refrigerated storage period.

Experimental Design

The experiment was set up to test the effects of type of additive utilized in the crab mince and percent crab mince incorporated into the fresh pasta on the chemical, microbial, and physical characteristics of the pasta. Crab mince contained either: 1) no additives, 2) 5% fresh, ground rosemary and 2% lactic acid, or 3) 0.08% diacetyl and 2% lactic acid. Pasta formulations contained either 0, 10, or 20% crab mince. There were a total of seven pasta treatments (Table 4).

Table 4. Treatments and Codes for Pasta Containing Crab Mince

Code	Treatment
CON	Control Pasta
10CR	Pasta containing 10% Crab Mince
10CR-R-L	Pasta containing 10% Crab Mince with 5% Fresh Rosemary and 2% Lactic Acid
10CR-D-L	Pasta containing 10% Crab Mince with 0.08% Diacetyl and 2% Lactic Acid
20CR	Pasta containing 20% Crab Mince
20CR-R-L	Pasta containing 20% Crab Mince with 5% Fresh Rosemary and 2% Lactic Acid
20CR-D-L	Pasta containing 20% Crab Mince with 0.08% Diacetyl and 2% Lactic Acid

Ingredients

Approximately 60 pounds of refrigerated, previously steamed **Jonah** crabs (*Cancer borealis*) with limbs, backs, gills, and viscera removed were obtained from a small-scale crab processor (Cranberry Point Products; Gouldsboro, **ME**). The crab bodies were stored in totes, covered with ice and held in refrigerated storage (5°C) overnight. The following day, each part of the Paoli One-Step Deboner Model #22-849 (Rockford, IL) was sprayed with Sanitizer 1610 (Candy & Co./ Peck's Products; Chicago, IL) and allowed to dry for about an hour. Five to six pound batches of crab bodies were taken out of refrigerated storage, weighed, and mechanically separated. The end plate setting on the meat bone separator was set at one-quarter open and the bar breaker setting was set at 0.120. The minced meat was collected in a stainless steel bowl and immediately weighed and recorded. The time for each batch of crabs to be mechanically separated was also

recorded. The minced meat was placed in one-gallon plastic bags (Ziploc; Racine, WI) and spread evenly throughout the bag to create the greatest surface area. The bags were immediately placed in a cooler with ice so that all sides of the bag came in contact with the ice. The crab mince was transported back to the University of Maine, Orono, **ME**, where it was held in refrigerated storage (5°C) until further processing.

No. 1 durum semolina was donated from North Dakota Milling (Grand Forks, **ND**). Lactic acid and diacetyl were purchased from Sigma (St. Louis, MO). Fresh rosemary (New England Herb Co.; Sunapee, **NH**) was obtained from Shaw's Supermarket and held in refrigerated storage (5°C) overnight. The rosemary leaves were separated from the stems and only leaves were ground in a Braun Model KSM 2 (**4**) (Mexico) coffee grinder for **30** seconds. The ground, fresh rosemary was placed in beakers, covered in plastic wrap (Saran®; Oakland, CA) and held in refrigerated storage (5°C) overnight.

Formulations

Selected additives for each treatment were added to the crab mince prior to incorporation with the flour and water (Table 5). Crab mince and additives were mixed in a Kitchenaid Model #KSM90 food processor (Kitchenaid; St. Joseph, MI) for **4** minutes. Ground, fresh rosemary and diacetyl were added to the crab mince at 5% and **0.08%** of the crab mince weight (w/w), respectively. Lactic acid was added to the crab mince at 2% of the crab mince weight (w/w). The crab mince-additive mixtures were placed in Whirl-Pak bags (Nasco; Fort Atkinson, WI) and held in refrigerated storage (5°C) overnight. **An** additional three batches of crab mince were mixed with additives for evaluation of the initial chemical and microbial characteristics **as well as** proximate analysis **of** the crab -

mince. Batches with approximately 240g crab mince for chemical and microbial analysis was held in refrigerated storage (5°C) until analyses were performed. Crab mince for proximate analysis was held in frozen storage (-18°C) until analyses were performed.

Table 5. Additives Incorporated into Crab Mince

Treatment*	Ingredients (g/ 100g)				Totals
	Crab Mince	Fresh Rosemary	Diacetyl	Lactic Acid	
Crab Mince	100.00	--	--	--	100
Crab mince with 5% rosemary + 2% lactic acid	93.46	4.67	--	1.87	100
Crab mince with 0.08% diacetyl + 2% lactic acid	97.96	--	0.08	1.96	100

Flour, room temperature tap water, and the crab mince mixtures were placed in a Bottene Inver **3** Pasta Maker (Bottene; Morano, Italy) and mixed for 6 minutes (Table 6). All pasta formulations contained 25% moisture based on the flour, crab mince, and water weight. Ten or twenty percent crab mince was added to the pasta and this percentage was based on the total flour and crab weight. The pasta was extruded using a fettuccine die and the noodles were cut manually with scissors designated for cutting pasta only. The pasta was placed on a No. 11 wooden, foldable drying rack (Walmart, Bangor, **ME**) for 15 minutes and allowed to air dry at room temperature \approx 20°C. Three replicate batches of pasta were made for each treatment. Each replicate batch was approximately 1.1 to 1.2 kg of pasta *so* that a total of 3.3 to **3.6** kg of pasta was extruded for each pasta treatment. Each replicate batch of pasta was packaged into five 12 ounce, clear, plastic containers with lids (Dart Container Corporation; Mason, MI) and held in refrigerated storage (5°C) for four weeks of storage. On day 21 of refrigerated storage, unforeseen temperature

abuse occurred and the temperature reached 14.5°C. Remaining samples were immediately placed in refrigerated storage and held at 5°C for the last week of the shelf-life study. Samples were evaluated for chemical, microbial, and physical analyses at week 1, 2, 3, 4, and 5.

Table 6. Formulations of Fresh Pasta Treatments

Treatment*	Flour	Water	Crab Mince	Fresh Rosemary	Diacetyl	Lactic Acid	Totals
10-CR	73.42	18.36	8.22	--	--	--	100
10CR-R-L	72.99	18.24	8.17	0.43	--	0.16	100
10CR-D-L	73.30	18.32	8.21	--	0.007	0.16	100
20CR	70.42	11.97	17.60	--	--	--	100
20CR-R-L	69.57	11.83	17.39	0.87	--	0.35	100
20CR-D-L	70.17	11.93	17.54	--	0.01	0.35	100
	75.02	24.98	--	--	--	--	100

* Percentages of
based on weight of crab mince

Chemical Analyses

Proximate Analyses

Moisture, ash, minerals (including sodium), and fat of crab mince, control pasta, pasta containing 10% crab mince, and pasta containing 20% crab mince were analyzed as described in "Evaluation of Mechanically Separated Crab Mince". Each sample was analyzed in triplicate.

pH

Approximately 10 g of crab mince and pastas containing crab mince were placed in Falcon test tubes (VWR Brand; Boston, MA) and 10 mL of distilled water was added to each test tube. The samples were homogenized using a polytron (Kinematica;

Switzerland) set at level 5 for 1 minute. The pH of the samples was determined with an Orion Model 320 PerpHecT LogR meter (Beverly, MA). A single analysis was conducted on each replicate sample.

Water activity

Water activity was only determined for pasta samples. Approximately 5 g of each sample was ground in a Braun KSM 2 (4) coffee grinder (Mexico) for 15 seconds. Samples were analyzed for water activity using an Aqua Lac CX-2 water activity meter (Decagon Devices Inc.; Pullman, WA). A single analysis was conducted on each replicate sample.

Total Volatile Base Nitrogen

Twenty-five g samples of crab mince and pastas containing mince were analyzed as described in "Evaluation of Mechanically Separated Crab Mince". Pasta samples were blended in the microblender (Waring; New Hartford, CT) for one minute as opposed to 30 seconds to effectively grind the sample. A single analysis was conducted on each replicate sample.

Microbial Analyses

Total plate counts and yeasts and molds of crab mince and pastas containing crab mince were analyzed as described in "Evaluation of Mechanically Separated Crab Mince". Each replicate sample was analyzed in duplicate.

Physical Analyses

Cooking Time

A twenty-five g sample of pasta was broken into 5 cm long pieces, added to a beaker containing 300 mL of boiling distilled water, and stirred frequently with a Scoopula spatula (Fisher Scientific, Pittsburgh, PA). A single piece of pasta was taken out at 30-second intervals and chewed to evaluate the texture. When “al dente” firmness was reached, the time was recorded as cooking time. All samples analyzed were cooked for the designated cook time of four minutes to ensure that the pasta was fully cooked.

Cooking Weight, Cooking Loss, Width and Thickness

Samples of pastas containing crab mince were analyzed for cooking weight and cooking loss using modified AACC (1995) method 16-50. Twenty-five g samples of raw pasta were weighed, broken into five cm long pieces, and the width and thickness of nine pasta pieces were measured for raw pasta width and thickness using a Mitutoyo Pigimatic caliper Model # CD-6"BS (Mitutoyo Corporation; Japan). The pasta pieces were added to a beaker with 300 mL of boiling distilled water and stirred at frequent intervals. Samples were boiled until the designated cook time of 4 minutes. Cooked samples were placed in Buchner funnels and rinsed with fifty mL of distilled water. The pasta was allowed to drain for 30 seconds. The cooking and rinse waters were collected in pre-weighed 500 mL Mason jars (Ball; Munice, IN), and dried in a Fisher isotemp oven Model 350 (Fisher Scientific; Pittsburgh, PA) set at 115°C for 24 hours. The Mason jars were placed in a dessicator to cool, then re-weighed to 0.01 g. The percent cooking loss was determined using the following calculation: $((\text{jar wt.} + \text{cooking loss wt.}) - \text{jar wt.}) \times 4$. Each of the replicate cooking loss samples was analyzed in duplicate. The pasta was immediately

transferred into 300 mL of room temperature distilled water and held for one minute to stop the pasta from cooking longer than the designated time. The sample was placed in a Buchner funnel and allowed to drain for 30 seconds. The cooked pasta samples were reweighed, and the percent cooking weight (percent of water uptake) was determined using the following calculation: $[(\text{cooked sample wt.} - \text{raw sample wt.}) / \text{raw sample wt.}] \times 100$. Cooking weight and loss for each replicate pasta batch were analyzed in duplicate. The width and thickness of nine pieces of cooked pasta were measured using a Mitutoyo Pigimatic caliper Model # CD-6"BS (Mitutoyo Corporation; Japan).

Instron

Firmness was analyzed using modified AACC (1995) method 16-50. An Instron Machine Model 4466 with a Series IX Automated Materials Testing System (Instron, Inc.; Canton, MA) equipped with a modified plastic tooth (thickest part of blade = 2.379 mm, thinnest part of blade = 0.397mm, angle of taper = 71.8°), 5-kg weigh beam, computer, and printer was used to analyze the firmness of the cooked pasta. An average thickness of the cooked pasta, which was 1.25mm, was entered into the computer. For each measurement, four strands of cooked pasta were placed adjacent to one another on the base plate of the Instron platform at right angles to the plastic tooth. The crosshead speed was set to 10.0 mm/minute and the firmness was measured as grams x cm. This procedure was repeated twice more with fresh sample. The firmness of the cooked pastas was determined using the following calculations: $((\text{load at max} \times 1000) / 9.81) \times \text{total displacement}$. Each of the replicate pasta batches was analyzed in triplicate.

Colorimetric Analyses

Instrumental colorimetric analyses were conducted on both raw and cooked pasta samples. A Hunter L, a, b Model II color difference meter (Hunter Associates Laboratory; Reston, VA) equipped with a six cm optical aperture was used to determine “L”, “a”, “b” values. Samples were placed in transparent plastic dishes in the following manner: each row of pasta was laid adjacent to another with no overlaps and then another row of pasta was placed directly on top of the first row at a 90° angle to the underlying row. “L”, “a”, “b” values were read and recorded and the samples were turned 1/3 of a turn and read twice more. Each of the replicate pasta batches was analyzed in triplicate.

Statistical Analyses

Statistical differences between treatments of crab mince and pasta containing crab mince were evaluated by SYSTAT 9.0 (SPSS Inc.; 1999) using one-way analysis of variance (ANOVA) ($p < 0.05$). Differences between means were evaluated using Tukey’s post hoc test (Neter et al., 1996). A multi-way ANOVA was conducted by SYSTAT 9.0 (SPSS Inc.; 1999) to evaluate the 1) effects of percent crab (0%, 10%, or 20%), 2) additives (5% rosemary plus 2% lactic acid or 0.08% diacetyl plus 2% lactic acid), and 3) weeks storage (one, two, three, four, or five weeks), on the chemical, microbial, and physical quality of the pastas containing crab mince.

MATERIALS AND METHODS: SENSORY ANALYSES AND EVALUATION OF PASTA CONTAINING CRAB MINCE WITH ADDITIVES

Objectives

The overall objectives of this study were to evaluate the consumer acceptance of a fresh pasta product containing crab mince and to evaluate the chemical, microbial, and physical quality of the pastas.

Experimental Design

The experiments were set up to test the effects of type of additive utilized in the crab mince, percent crab mince incorporated into the fresh pasta, and the addition of red colorant on the chemical, microbial, physical, and sensory characteristics of the pasta. Crab mince contained either no additives or 0.08% diacetyl and 2% lactic acid. Pasta formulations contained either 10 or 20% crab mince and 0 or 0.5% red colorant. There were a total of six treatments (Table 7).

Table 7. Treatments and Codes for Pasta Containing Crab Mince

Code	Treatment
10CR	Pasta containing 10% Crab Mince
20CR	Pasta containing 20% Crab Mince
10CR-D-L	Pasta containing 10% Crab Mince with 0.08% Diacetyl and 2% Lactic Acid
20CR-D-L	Pasta containing 20% Crab Mince with 0.08% Diacetyl and 2% Lactic Acid
10CR-D-L-R	Pasta containing 0.05% Colorant and 10% Crab Mince with 0.8% Diacetyl and 2% Lactic Acid
20CR-D-L-R	Pasta containing 0.05% Colorant and 20% Crab Mince with 0.8% Diacetyl and 2% Lactic Acid

Ingredients

Approximately 80 pounds of refrigerated, previously steamed Jonah crabs (*Cancer borealis*) with limbs, backs, gills, and viscera removed were obtained from a small-scale crab processor (Cranberry Point Products; Gouldsboro, **ME**). The crab bodies were stored in totes, covered with ice and held in refrigerated storage (5°C) overnight. The following day, each part of the Paoli One-Step Deboner Model # 22-849 (Rockford, IL) was sprayed with Sanitizer 1610 (Candy & Co./ Peck's Products; Chicago, IL) and allowed to dry for about an hour. Five to six pound batches of crab bodies were taken out of refrigerated storage, weighed, and mechanically separated. The end plate setting on the meat bone separator was set at one-quarter open and the bar breaker setting was set at 0.120. The minced meat was collected in a stainless steel bowl and immediately weighed and recorded. The time for each batch of crabs to be mechanically separated was also recorded. The minced meat was placed in one-gallon plastic bags (Ziploc; Racine, WI) and spread evenly throughout the bag to create the greatest surface area. The bags were immediately placed in a cooler with ice so that all sides of the bag came in contact with the ice. The crab mince was transported back to the University of Maine, Orono, **ME**, where it was held in refrigerated storage (5°C) until further processing.

Durum semolina was obtained from ConAgra (**Omaha**, NE). Food grade lactic acid and diacetyl were obtained from Sigma (St. Louis, MO). Natural Tomato Red Color for Pasta, Formula No. 4528 was donated by Colormaker (Anaheim, CA).

Formulations

Selected additives for each treatment were added to the crab mince prior to incorporation with the flour and water (Table 8). Crab mince and additives were mixed in a Kitchen Aid Ultra Power Model # KSM 900 food processor (Kitchenaid; St. Joseph, MI) at level 2 for 4 minutes. Diacetyl was added to the crab mince at 0.08% of the crab mince weight (w/w). Lactic acid was added to the crab mince at 2% of the crab mince weight (v/w). The crab mince-additive mixtures were placed in Whirl-Pak bags (Nasco; Fort Atkinson, WI) and held in refrigerated storage (5°C) overnight. An additional batch of crab mince was mixed with additives for evaluation of the initial chemical and microbial characteristics as well as proximate analysis. Crab mince for chemical and microbial analysis was held in refrigerated storage (5°C) until analyses were performed. Crab mince for proximate analysis was held in frozen storage (-18°C) until analyses were performed.

Table 8. Additives Incorporated into Crab Mince

Treatment	Ingredients (g/ 100g)			
	Crab Mince	Diacetyl	Lactic Acid	Totals
Crab mince	100.00	--	--	100
Crab mince with 0.08% diacetyl + 2% lactic acid	97.96	0.08	1.96	100

Flour and crab mince mixtures were placed in a Bottene Pasta Maker Inver 3 (Bottene; Italy). Room temperature water was weighed and red colorant was added to it at 0.005% of the combined flour, crab mince, and water weight of the dough (Table 9). The water and color mixture was placed in the pasta maker and ingredients were mixed for 6 minutes. All pasta formulations contained 25% moisture based on the total flour,

crab mince, and water weight. Ten or twenty percent crab mince was added to the pasta and this percentage was based on flour and crab weight. One batch was extruded per treatment.

Table 9. Formulations of Fresh Pasta Treatments

Treatment	Ingredients (g/100g)						Totals
	Flour	Water	Crab Mince	Diacetyl	Lactic Acid	Colorant	
1CR	72.55	19.34	8.10	--	--	--	100
20CR	71.43	10.71	17.86	--	--	--	100
1OCR-D-L	72.42	19.31	8.09	0.006	0.16	--	100
20CR-D-L	71.16	10.67	17.79	0.014	0.36	--	100
1OCR-D-L-R	72.07	19.22	8.05	0.006	0.16	0.50	100
20CR-D-L-R	70.81	10.62	17.70	0.014	0.35	0.50	100

based on total weight of crab mince. Percentage of colorant is based on total weight of flour, crab mince, and water.

The pasta was extruded using a fettuccine die and the noodles were cut manually to the length of commercially produced dried pasta with scissors designated for cutting pasta only. The pasta was placed on a No. 11 drying rack (Walmart; Bangor, ME) for 15 minutes and allowed to air dry at room temperature (20°C). Approximately 4000 g of pasta was extruded for each batch. Pasta batches were packaged into Whirl-Pak bags (Nasco; Fort Atkinson, WI) and held in refrigerated storage (5°C) until they were evaluated for chemical, microbial, physical, and sensory analyses.

Chemical Analyses

Moisture, ~~ash~~, minerals (including sodium), and fat of crab mince, pasta containing 10% crab mince without additives, and pasta containing 20% crab mince without additives were analyzed as described in "Evaluation of Mechanically Separated

Crab Mince". Each of three samples were analyzed in triplicate. TVBN, pH, and water activity of pastas containing crab mince were analyzed as described in "Shelf-life of Pasta Containing Crab Mince with Additives". Each pasta batch was analyzed in triplicate.

Microbial Analyses

Total plate counts of crab mince and pastas containing crab mince were analyzed as described in "Evaluation of Mechanically Separated Crab Mince". Yeast and mold counts of pastas containing crab mince were analyzed as described in "Evaluation of Mechanically Separated Crab Mince". Each crab mince treatment and pasta batch was analyzed in triplicate.

Physical Analyses

Cooking time, cooking weight, cooking loss, width and thickness, Instron, and colorimetric analyses were analyzed as described in "Shelf-life of Pasta Containing Crab Mince with Additives". Designated cooking time for pasta in this study was two and half minutes. Each pasta batch was analyzed in triplicate.

Sensory Analyses

The objective of the sensory analysis was to examine the consumer acceptance of different fish pasta formulations containing minced crabmeat. There were a total of six treatments: 1) fish pasta containing 10% crab mince, 2) fish pasta containing 20% crab mince, 3) fresh pasta containing 10% crab mince pre-mixed with 0.08% diacetyl and 2% lactic acid, 4) fresh pasta containing 20% crab mince pre-mixed with 0.08% diacetyl and

2% lactic acid, 5) fresh pasta containing 0.05% natural tomato red color and 10% crab mince pre-mixed with 0.08% diacetyl and 2% lactic acid, and 6) fresh pasta containing 0.05% natural tomato red color and 20% crab mince pre-mixed with 0.08% diacetyl and 2% lactic acid. Fresh pasta treatments were boiled the day before the sensory test in 4 quarts of boiling water per one pound of pasta for two and half minutes. The pasta was drained, and cold water was run over the pasta for 30 seconds to reduce the heat and stop the pasta from cooking further. The pasta was shaken and drained for 15 seconds. One tablespoon of light olive oil (Shaw's, Italy) was mixed per one pound batch of pasta. The pasta was separated into 28 g (1 ounce) portion sizes and placed in coded plastic Ziploc sandwich bags (SC Johnson, Inc.; Racine, WI). Pasta samples were held in refrigerated storage (5°C) overnight. The following day each panelist's samples were taken out of the refrigerator just prior to sensory evaluation. The Ziploc bags were opened so that steam could escape during the microwave process. The samples were placed in a microwave (Hotpoint, Louisville, KY) equipped with a rotating plate and microwaved on high for 55 seconds. After heating, each sample was placed in a 2-ounce opaque cup (Solo; Urbana, IL) labeled with randomized 3 digit codes, and the noodles were separated manually and fluffed.

The sensory panelists consisted primarily of members of the University of Maine community between the ages of 19 – 62. Panelists signed an informed consent form in order to participate in the sensory analyses. The protocol for the sensory test was approved by the Human Subjects Protection Committee of the University of Maine College of Natural Sciences, Forestry, and Agriculture.

Panelists were seated in booths with fluorescent lighting located in the Consumer Testing Center sensory suite, Holmes Hall, University of Maine. Samples were presented on trays to panelists in randomly selected order by computer. Specific instructions were given by a computerized ballot to guide the panelists through the taste test. Panelists were asked to provide information about age, gender, pasta preferences, and purchasing frequencies. Panelists were also provided a cup of water and asked to take a sip of the water to cleanse their palate before each sample. Pasta samples were evaluated for color, aroma, flavor, texture, and overall acceptability using a 9-point hedonic scale, with 1 = dislike extremely and 9 = like extremely (Peryam and Pigram, 1957). Results of sensory evaluation were collected by the SIMS 2000 program for Windows. *All* data regarding age, gender, pasta preferences, and purchasing frequencies were statistically evaluated by the SIMS 2000 program for Windows, with SAS. *All* data regarding scores for color, aroma, flavor, texture, and overall acceptability of the pastas were statistically evaluated using a one-way ANOVA performed by SYSTAT 9.0 (SPSS Inc.; 1999).

Statistical Analyses

Statistical differences between treatments were evaluated by SYSTAT 9.0 (SPSS Inc.; 1999) using one-way analysis of variance (ANOVA) ($p < 0.05$). Differences between means were evaluated using Tukey's post hoc test (Neter et al., 1996). A multi-way ANOVA was conducted by SYSTAT 9.0 (SPSS Inc.; 1999) to evaluate the 1) effects of percent crab (10% or 20%) and 2) additives (no additives or 0.08% diacetyl plus 2% lactic acid), on the chemical, microbial, and physical quality of the pasta containing crab mince. A second multi-way ANOVA was conducted by SYSTAT 9.0 (SPSS Inc.; 1999)

to evaluate the 1) effects **of** percent crab (10% **or** 20%) and **2)** the addition **of** colorant (0% **or** 0.05%) on the chemical, microbial, and physical **quality of** the pasta containing crab mince.

RESULTS: MECHANICAL SEPARATION OF CRAB MINCE

Ease of Separation

The first batch of crab parts consisted of only cooked bodies with the gills and viscera removed. The crab bodies had to be broken into two pieces so that the pieces were small enough to be mechanically deboned. The second batch of crab parts consisted of the last three joints of the cooked legs. While deboning the legs, smoke started coming out of the hopper of the separator and the side of the separator was too hot to touch by hand. Additionally, even though the legs were in small enough pieces to be deboned, the product did not come out at a smooth rate. The last batch of crabs was a replicate of batch of the first. It consisted of cooked bodies with the viscera and gills removed. The machine was not used for about a half an hour prior to this because we were waiting for additional sample. When we attempted to debone the bodies, no minced meat came out of the machine and the mince had to be manually scraped out of the cylinder. Two processors were needed to continually feed the crab parts into the separator, collect and package the mince, and retrieve more crab parts out of the refrigerator as needed.

Chemical Analyses

Proximate Analyses

The results from proximate analysis revealed that the crab mince obtained from the carapace was made up of an average 72.9% water, 9.9% ash, 1.4% fat, **and** 15.5% protein (Table 10). Crab mince obtained from the legs consisted of 71.8% water, 7.4% ash, 1.2% fat, and 14.4% protein.

Table 10. Percent Moisture, Ash, Fat and Protein of Crab Mince*

Treatment Code**	Moisture (%)	Ash (Yo)	Fat (%)	Protein (Yo)
CA	71.6	10.2	1.4	15.9
CB	74.2	9.6	--**	15.1
L	71.8	7.4	1.2	14.4

* Each value is the average of three analyses. Ash, fat, and protein values are given on a wet weight basis.

** No sample

*** CA= Carapace A; CB = Carapace B; L = Legs

The results from mineral analysis revealed that the crab mince obtained from the carapace was made up of an average of 25,303, 2,902, 1631, and 2810 mg/ kg of calcium, potassium, magnesium, and phosphorus, respectively (Table 11). Crab mince obtained from the legs contained 31,482, 2,987, 2,013, and 3,047 mg/ kg of calcium, potassium, magnesium, and phosphorus, respectively.

Table 11. Selected Mineral Concentrations of Crab Mince*

Treatment Code**	Mineral Concentration (mg/ kg)			
	Calcium	Potassium	Magnesium	Phosphorus
CA	30945 \pm 1648	2957 \pm 29	1858 \pm 58	2948 \pm 40
CB	19662 \pm 1011	2846 \pm 33	1403 \pm 25	2672 \pm 25
L	31482 \pm 5110	2987 \pm 364	2013 \pm 327	3047 \pm 395

* Each value is the average of three analyses \pm standard deviation. Values are given on a wet weight basis.

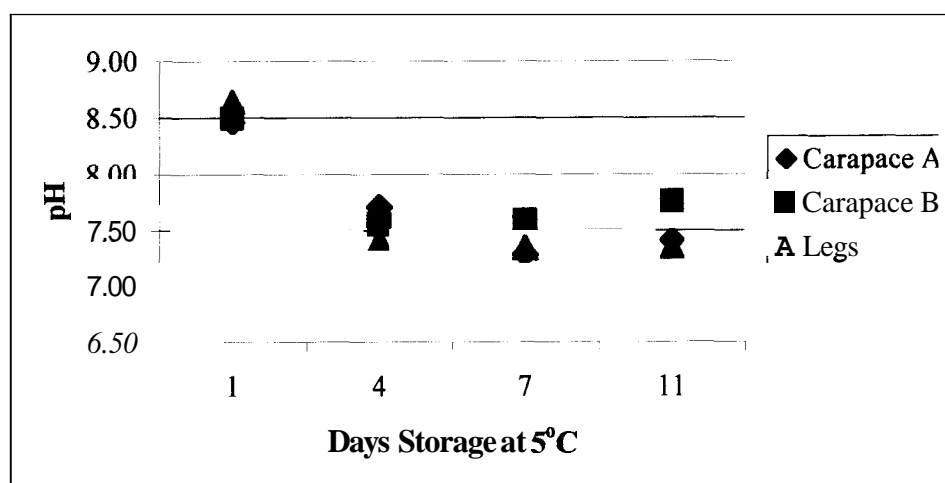
** CA= Carapace A; CB = Carapace B; L = Legs

pH

Throughout storage, there was a sharp decline in pH from day one to day four, followed by a plateau in pH from day 4 to day 7, and then a slight increase (Figure 1).

There was no notable effect of mince source (legs versus carapace) on pH. During the 11-day shelf-life study, the pH values of the crab mince obtained from the carapace declined from an average of 8.48 at day one to 7.44 at day seven and then increased slightly to 7.59 by day eleven (Table 12). The pH values of the crab mince obtained from the legs declined from 8.64 at day zero to 7.40 at day four and stabilized for the rest of the study.

Figure 1. pH of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*



* pH values are the average of duplicate analyses.

Table 12. pH of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*

Treatment Code**	Day 1	Day 4	Day 7	Day 11
CA	8.46 ± 0.01	7.70 ± 0.00	7.29 ± 0.13	7.41 ± 0.05
CB	8.49 ± 0.00	7.55 ± 0.07	7.58 ± 0.04	7.76 ± 0.11
L	8.64 ± 0.01	7.40 ± 0.00	7.34 ± 0.15	7.35 ± 0.21

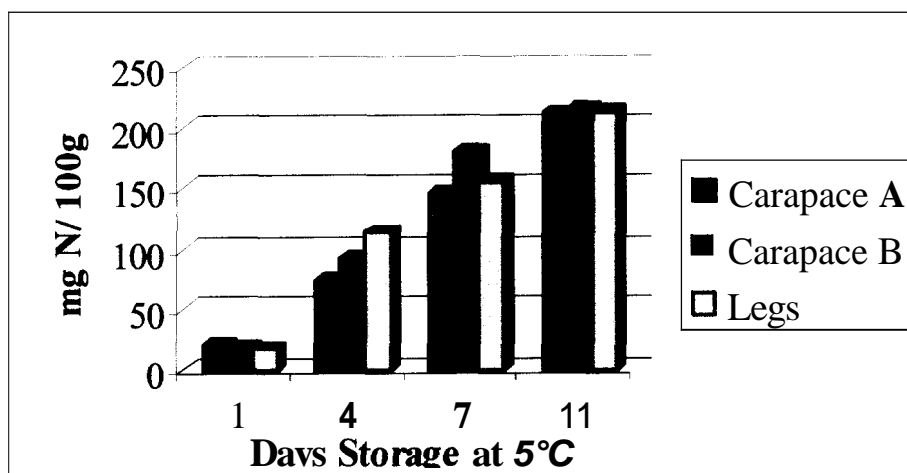
* Each value is the average of duplicate analyses ± standard deviation.

** CA= Carapace A; CB = Carapace B; L = Legs

TVBN

TVBN concentrations in crab mince increased approximately 10-fold over the course of the 11-day storage study (Figure 2). There was no notable effect of mince source (legs versus carapace) on TVBN concentrations. The TVBN concentration in crab mince obtained from the carapace increased from an average 19 mg N/ 100 g at day one to 211.8 **mg** N/ 100 g at day eleven (Table 13). The TVBN concentration in crab mince obtained from the legs increased from 16.9 mg N/ 100 g at day one to 210.9 mg N/ 100 g at day eleven.

Figure 2. TVBN Concentrations (mg N/ 100g) of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*



* TVBN concentrations are the average of duplicate analyses.

Table 13. TVBN (mg N/ 100g) Concentrations of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*

Treatment Code**	Day 1	Day 4	Day 7	Day 11
CA	19.4 ± 0.5	72.6 ± 4.3	143.9 ± 6.8	210.4 ± 42.4
CB	19.2 ± 0.0	90.7 ± 7.1	178.7 ± 2.5	213.3 ± 11.4
L	16.92 ± 1.1	112.92 ± 1.4	154.5 ± 2.5	210.9 ± 2.59

* Each value is the average of duplicate analyses ± standard deviation.

** CA= Carapace A; CB = Carapace B; L = Legs

TBARS

There were no consistent trends in TBARS concentrations observed for any of the crab mince samples during the course of the refrigerated storage study (Table 14).

TBARS concentrations were negligible throughout the 11 days of refrigerated storage and ranged from 0.06 µg/ g to 1.29 µg/ g of mince.

Table 14. TBARS Levels (µg/ g) of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*

Treatment Code**	Day 1	Day 4	Day 7	Day 11
CA	0.31 ± 0.01	0.06 ± 0.02	0.68 ± 0.10	0.39 ± 0.09
CB	0.34 ± 0.06	0.06 ± 0.01	0.80 ± 0.00	1.29 ± 0.59
L	0.19 ± 0.01	0.08 ± 0.02	0.61 ± 0.19	0.46 ± 0.02

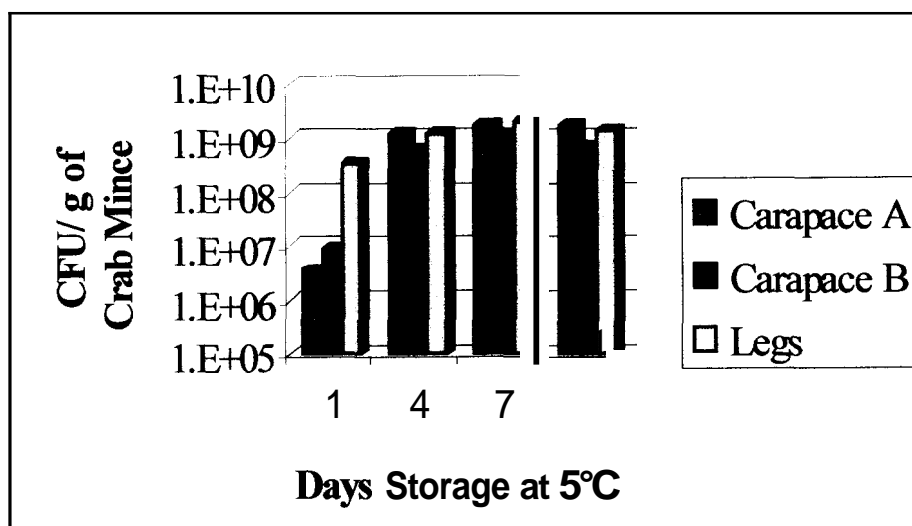
* Each value is the average of duplicate analyses ± standard deviation.

** CA= Carapace A; CB = Carapace B; L = Legs

Microbial Analyses

Throughout the 11 days of refrigerated storage, there was a sharp increase in aerobic plate counts from day one to day 4 and then a plateau in aerobic plate counts throughout of the rest of the study (Figure 3).

Figure 3. Aerobic Plate Counts (log CFU/g) of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*



* Aerobic plate counts are the average of duplicate analyses.

On day one, microbial counts reflected an increase in the order in which the batches were processed. Aerobic plate counts for the crab mince samples ranged from 3.35×10^6 – 1.74×10^9 (Table 15).

Table 15. APC (log CFU/ g) of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*

Treatment Code**	Day 1	Day 4	Day 7	Day 11
CA	4E+06 \pm 2E+06	1E+09 \pm 9E+07	2E+09 \pm 2E+08	2E+09 \pm 3E+08
CB	9E+06 \pm 9E+06	6E+08 \pm 2E+08	1E+09 \pm 4E+08	7E+08 \pm 2E+08
L	3E+06 \pm 2E+06	1E+09 \pm 2E+08	2E+09 \pm 3E+08	1E+09 \pm 2E+08

* Each value is the average of duplicate analyses \pm standard deviation.

** CA= Carapace A; CB = Carapace B; L = Legs

There was a general increase in aerobic plate counts of 2 – 3 logs of per gram in the crab mince samples throughout the 11-day shelflife study. In contrast, numbers for yeast and molds in the crab minces were very low, with average counts of less than 100CFU/ g throughout the 11-day shelf life study (Tables 16 and 17). No trends in coliform counts were observed for any of the crab mince samples (Table 18). Coliform counts ranged from 0 to 1100MPN/ g in crab mince samples.

Table 16. Yeast Counts (CFU/ g) of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*

Treatment Code**	Day 1	Day 4	Day 7	Day 11
CA	< 100	< 100	< 100	< 100
CB	< 100	< 100	< 100	< 100
L	< 100	< 100	< 100	< 100

* Each value is the average of duplicate analyses.

** CA= Carapace A; CB = Carapace B; L = Legs

Table 17. Mold Counts (CFU/ g) of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*

Treatment Code**	Day 1	Day 4	Day 7	Day 11
CA	< 100	< 100	< 100	< 100
CB	< 100	< 100	< 100	< 100
L	< 100	< 100	< 100	< 100

* Each value is the average of duplicate analyses.

** CA= Carapace A; CB = Carapace B; L = Legs

Table 18. Coliforms (MPN/ g) of Crab Mince Separated from Carapace and Legs during Refrigerated Storage*

Treatment Code***	Day 1	Day 4	Day 7	Day 11
CA	93	n.d.**	n.d.	n.d.
CB	1100	75	9	n.d.
L	4	n.d.	n.d.	4

* Each value is the average of duplicate analyses.

** n.d. = no cells detected at 3 cells per gram

*** CA= Carapace A; CB = Carapace B; L = Legs

RESULTS: EFFECT OF ADDITIVES ON THE SHELF-LIFE OF CRAB MINCE

Chemical Analyses

Yield and Proximate Analyses

Mechanical separation of crab mince resulted in 44 – 69% meat recovery from the starting by-product. Proximate analyses of crab mince indicated a 78.3% moisture content, 5.1% ash content, and 0.9% fat content. Protein content of the crab mince was not determined because of equipment failure. The mechanically separated crab mince contained calcium, potassium, magnesium, phosphorus, and sodium levels of $13,546 \pm 739$, $2,381 \pm 118$, $1,026 \pm 56$, $2,251 \pm 105$, and $3,184 \pm 83$ mg/kg, respectively.

pH

As storage time increased, pH significantly ($p = 0.000$) decreased from an average of 8.3 on day one to 7.5 on day eight based on multi-way ANOVA (Table 19). The addition of 2% lactic acid resulted in significantly ($p = 0.000$) lower pH values in the mince throughout the eight day storage period based on multi-way ANOVA. A combination of the addition of lactic acid and days storage had a significant ($p < 0.01$) effect on the pH concentrations of the crab mince. As days storage increased, the pH difference between samples treated with lactic acid and those that were not were less apparent. The type of additive utilized had a significant effect ($p = 0.000$) on the pH of the crab mince samples based on multi-way ANOVA. Crab mince samples with rosemary had significantly ($p = 0.000$) lower pH than mince samples with diacetyl. Crab mince samples with diacetyl had significantly ($p = 0.000$) higher pH than the control. There was no significant pH difference between crab mince samples with 2% or 5% rosemary.

There was also no significant pH difference between crab mince samples with 0.04% or 0.08% diacetyl. Sodium lactate had no effect on pH of crab mince samples.

Table 19. pH of Crab Mince with Additives during Refrigerated (5°C) Storage*

Treatment Code**	Day 1	Day 3	Day 5	Day 8
2R	8.52 ± 0.03"	7.99 ± 0.15 ^{abc}	8.27 ± 0.04 ^{bcd}	7.46 ± 0.39
2R-L	7.81 ± 0.28"	7.38 ± 0.35"	7.50 ± 0.23"	7.24 ± 0.32
5R	8.34 ± 0.07 ^{bc}	7.90 ± 0.28 ^{abc}	8.17 ± 0.08 ^{abcde}	7.52 ± 0.12
5R-L	7.88 ± 0.05 ^{ab}	7.53 ± 0.06"	7.65 ± 0.08 ^{ab}	7.08 ± 0.04
4D	8.77 ± 0.05 ^c	8.71 ± 0.11 ^{bc}	8.60 ± 0.23 ^{de}	7.87 ± 0.22
4D-L	8.08 ± 0.06 ^{ab}	7.99 ± 0.10 ^{abc}	7.84 ± 0.05 ^{abc}	7.47 ± 0.21
8D	8.72 ± 0.03"	8.70 ± 0.02 ^{abc}	8.60 ± 0.05 ^{de}	7.77 ± 0.04
8D-L	8.14 ± 0.03 ^{ab}	7.96 ± 0.07 ^{abc}	7.89 ± 0.05 ^{abcd}	7.66 ± 0.07
SL	8.77 ± 0.04"	8.76 ± 0.08 ^c	8.64 ± 0.03"	7.81 ± 0.16
C	8.77 ± 0.05"	8.38 ± 0.48 ^{abc}	8.48 ± 0.34 ^{cde}	7.29 ± 0.20
c - L	7.91 ± 0.06 ^{ab}	7.72 ± 0.17 ^{ab}	7.78 ± 0.12 ^{abc}	7.17 ± 0.02
p value	0.000	0.002	0.000	0.066

Each value is the average of two replications ± standard deviation, analyzed in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA and followed by Tukey's post hoc test.

** 2R = 2% Rosemary; 2R-L = 2% Rosemary + 2% Lactic Acid; 5R = 5% Rosemary; 5R-L = 5% Rosemary + 2% Lactic Acid; 4D = 0.04% Diacetyl; 8D = 0.08% Diacetyl; SL = 2% Sodium Lactate; C = Control; C-L = Control + 2% Lactic Acid

TVBN

TVBN concentration significantly ($p = 0.000$) increased from an average 18 mg N/ 100 g on day one to 37 mg N/ 100g on day eight as storage time increased based on multi-way ANOVA (Table 20). The addition of lactic acid to crab mince samples resulted in significantly ($p = 0.000$) lower TVBN concentrations compared to those without lactic acid based on multi-way ANOVA (Figures 4 and 5). As days storage increased samples

treated with lactic acid had significantly ($p < 0.05$) lower concentrations of TVBN compared to those not treated with lactic acid based on two-way ANOVA. Diacetyl and sodium lactate resulted in significantly ($p < 0.01$) lower TVBN concentrations compared to rosemary and control treatments based on multi-way ANOVA.

Table 20. TVBN Concentrations (mg N/ 100g) of Crab Mince with Additives during Refrigerated (5 °C) Storage*

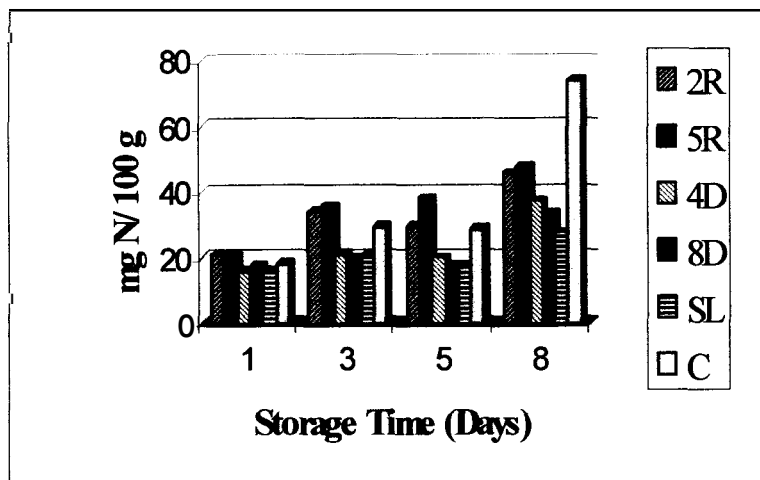
Treatment Code**	Day 1	Day 3	Day 5	Day 8
2R	20.7 ± 0.7 ^b	33.8 ± 2.1 ^{ab}	29.4 ± 3.7"	45.6 ± 8.9
2R-L	17.6 ± 0.0 ^{ab}	21.3 ± 2.0 ^{ab}	19.4 ± 1.4"	31.9 ± 12.3
5R	20.4 ± 0.4 ^b	35.9 ± 4.8 ^b	38.2 ± 6.6"	47.8 ± 0.2
5R-L	18.1 ± 0.0 ^b	28.6 ± 0.2 ^{ab}	27.6 ± 0.5"	32.3 ± 3.6
4D	16.0 ± 0.5"	21.0 ± 3.0 ^{ab}	20.0 ± 3.4"	37.4 ± 16.2
4D-L	15.3 ± 0.2"	21.3 ± 2.0 ^{ab}	15.6 ± 4.3"	26.6 ± 9.1
8D	17.1 ± 0.0 ^{ab}	20.0 ± 0.2 ^{ab}	17.5 ± 2.3"	33.4 ± 1.3
8D-L	17.4 ± 1.4 ^{ab}	17.0 ± 2.7"	16.3 ± 0.2"	18.4 ± 3.6
SL	16.1 ± 0.4"	20.5 ± 2.3 ^{ab}	17.5 ± 1.3"	27.5***
C	17.9 ± 0.4 ^{ab}	29.4 ± 13.0 ^{ab}	28.5 ± 16.0"	74.0 ± 45.
c-L	17.9 ± 2.9 ^{ab}	19.0 ± 0.9 ^{ab}	17.6 ± 2.1"	27.1 ± 1.3
* p value	0.005	0.016	0.033	0.213

Each value is the average of two replications ± standard deviation, analyzed in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA and followed by Tukey's post hoc test.

** 2R = 2% Rosemary; 2R-L = 2% Rosemary + 2% Lactic Acid; 5R = 5% Rosemary; 5R-L = 5% Rosemary + 2% Lactic Acid; 4D = 0.04% Diacetyl; 8D = 0.08% Diacetyl; SL = 2% Sodium Lactate; C = Control; C-L = Control + 2% Lactic Acid

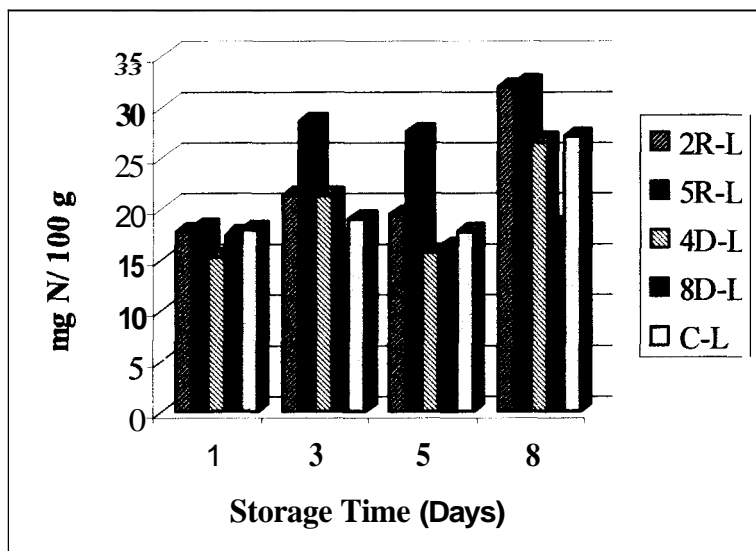
*** Single sample analyzed.

Figure 4. TVBN (mg N/ 100g) Concentrations of Crab Mince without Lactic Acid*



* T W N concentrations are the average of two replications, each analyzed in duplicate. 2R = 2% Rosemary; 5R = 5% Rosemary; 4D = 0.04% Diacetyl; 8D = 0.08% Diacetyl; SL = 2% Sodium Lactate; C = Control

Figure 5. TVBN (mg N/ 100g) Concentrations of Crab Mince with Lactic Acid



* T W N concentrations are the average of two replications, each analyzed in duplicate. 2R-L = 2% Rosemary + 2% Lactic Acid; 5R-L = 5% Rosemary + 2% Lactic Acid; 4D-L = 0.04% Diacetyl + 2% Lactic Acid; 8D-L = 0.08% Diacetyl + 2% Lactic Acid; C-L = Control + 2% Lactic Acid

TBARS

TBARS concentrations in the crab mince samples significantly ($p = 0.000$) increased from an average $0.79 \mu\text{g/g}$ mince on day one **of** refrigerated storage to $1.71 \mu\text{g/g}$ mince on day eight **of** refrigerated storage based **on** multi-way ANOVA (Table 21). Samples treated with lactic acid had significantly ($p < 0.01$) lower TBARS concentrations than those not treated with lactic acid. Based on two-way ANOVA, as days storage increased, samples treated with lactic acid had significantly ($p < 0.01$) lower concentration **of** TBARS compared to those not treated with lactic acid. The addition **of** diacetyl and sodium lactate to crab mince samples resulted in significantly ($p < 0.01$) lower TBARS concentrations compared to the control based **on** multi-way ANOVA.

Table 21. TBARS Levels ($\mu\text{g/g}$) of Crab Mince with Additives during Refrigerated (5°C) Storage

Treatment Code	Day 1	Day 3	Day 8
2R	$0.95 \pm 0.12^{\text{ab}}$	0.96 ± 0.59	1.43 ± 0.12
2R-L	$0.69 \pm 0.22''$	0.60 ± 0.01	0.94 ± 0.24
5R	$1.51 \pm 0.10^{\text{b}}$	1.09 ± 0.18	2.98 ± 0.14
5R-L	$1.47 \pm 0.08^{\text{b}}$	0.93 ± 0.01	2.54 ± 0.56
4D	$0.59 \pm 0.04''$	0.90 ± 0.27	1.07 ± 0.68
4D-L	$0.38 \pm 0.04''$	0.68 ± 0.24	0.82 ± 0.43
8D	$0.56 \pm 0.20''$	0.96 ± 0.59	2.64 ± 0.40
8D-L	$0.50 \pm 0.17''$	0.51 ± 0.19	0.63 ± 0.20
SL	$0.70 \pm 0.26''$	0.90 ± 0.17	0.84 ± 0.24
C	$0.77 \pm 0.13''$	1.19 ± 0.30	4.29 ± 3.26
c - L	$0.53 \pm 0.09''$	1.63 ± 0.49	0.68 ± 0.01
p value	0.000	0.2 16	0.058

Each value is the average of two replications \pm standard deviation, analyzed in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA and followed by Tukey's post hoc test.

**2R = 2% Rosemary; 2R-L = 2% Rosemary + 2% Lactic Acid; 5R = 5% Rosemary; 5R-L = 5% Rosemary + 2% Lactic Acid; 4D = 0.04% Diacetyl; 8D = 0.08% Diacetyl; SL = 2% Sodium Lactate; C = Control; C-L = Control + 2% Lactic Acid.

Microbial Analyses

Microbial counts significantly ($p < 0.01$) increased from an average 9.6×10^6 CFU/ g on day one of refrigerated storage to 1.0×10^9 CFU/ g on day eight of refrigerated storage based on multi-way ANOVA (Table 22). The addition of lactic acid to crab mince samples reduced microbial counts by approximately half a log although it was not significant based on multi-way ANOVA ($p = 0.053$) (Figure 6 and 7). The

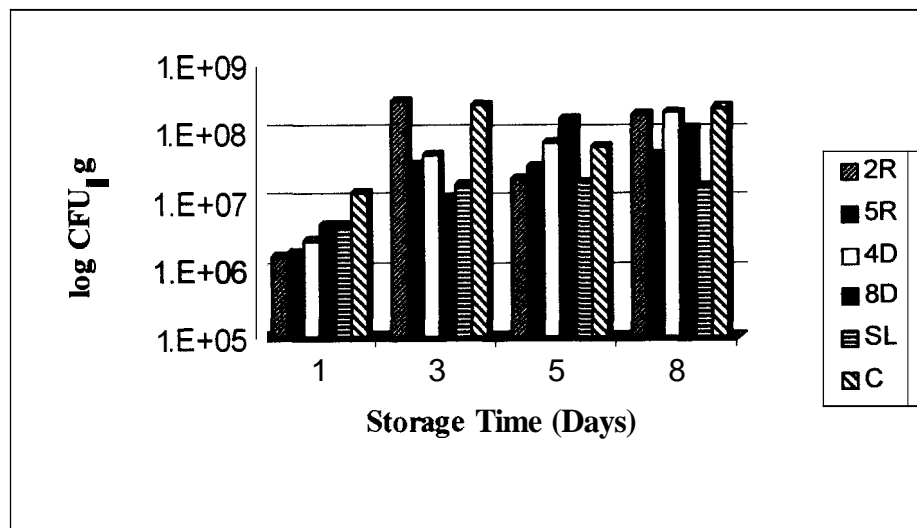
addition of sodium lactate, 5% rosemary, and **0.08%** diacetyl to crab mince samples resulted in significantly ($p < 0.05$) lower microbial counts compared to the control based on multi-way ANOVA.

Table 22. APC (log CFU/ g) of Crab Mince with Additives during Refrigerated (5°C) Storage

Treatment Code	Day 1	Day 3	Day 5	Day 8
2R	2E+06 ± 2E+05	3E+08	2E+07 ± 1E+07	2E+08 ± 2E+08
2R-L	7E+05 ± 4E+05	5E+07 ± 2E+07	8E+06 ± 3E+06	7E+07 ± 2E+07
5R	2E+06 ± 2E+06	3E+07 ± 2E+07	3E+07 ± 2E+07	5E+07 ± 3E+07
5R-L	6E+05 ± 1E+05	2E+07 ± 2E+07	4E+06 ± 4E+06	8E+06 ± 1E+06
4D	3E+06 ± 3E+06	5E+07 ± 1E+07	7E+07 ± 6E+07	2E+08 ± 2E+08
4D-L	5E+07 ± 7E+07	1E+08 ± 9E+07	10E+07 ± 1E+08	2E+08 ± 5E+07
8D	5E+06 ± 1E+06	1E+07 ± 7E+06	2E+08 ± 1E+08	1E+08 ± 6E+07
8D-L	2E+06 ± 2E+06	4E+07 ± 3E+07	7E+07 ± 10E+07	3E+07 ± 4E+07
SL	4E+06 ± 4E+06	2E+07 ± 2E+05	2E+07 ± 2E+06	2E+07 ± 8E+06
C	1E+07 ± 1E+07	2E+08 ± 2E+08	6E+07 ± 7E+07	2E+08 ± 2E+08
c-L	2E+07 ± 1E+07	1E+08 ± 5E+07	8E+07 ± 4E+06	1E+08 ± 9E+07
p value	0.479	0.089	0.481	0.440

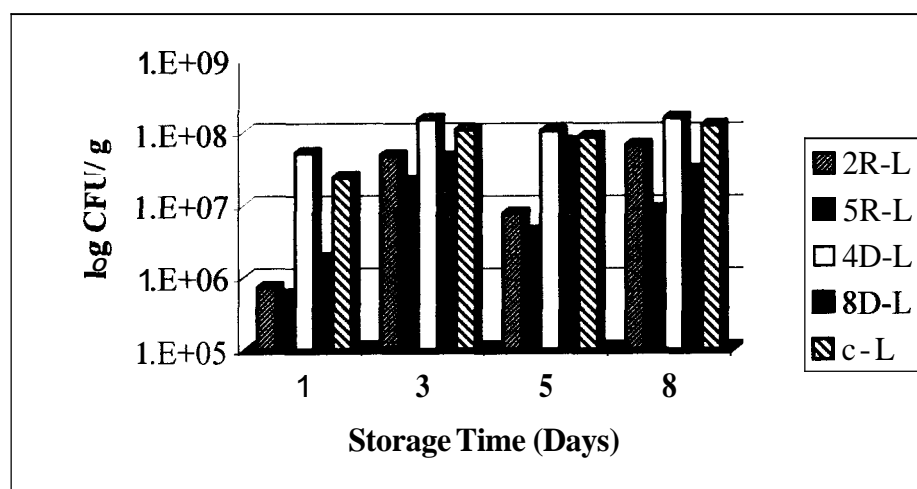
*Each value is the average of two replications ± standard deviation, analyzed in duplicate. Means in the same column not sharing a superscript are significantly (p < 0.05) different based on one-way ANOVA and followed by Tukey's post hoc test.

Figure 6. APC (log CFU/g) Levels in Crab Mince without Lactic Acid



* APC levels are the average of two replications, each analyzed in duplicate.
 2R = 2% Rosemary; 5R = 5% Rosemary; 4D = 0.04% Diacetyl;
 8D = 0.08% Diacetyl; SL = 2% Sodium Lactate; C = Control;

Figure 7. APC (log CFU/g) Levels in Crab Mince with Lactic Acid



* APC levels are the average of two replications, each analyzed in duplicate.
 2R-L = 2% Rosemary + 2% Lactic Acid; 5R-L = 5% Rosemary + 2% Lactic Acid;
 4D-L = 0.04% Diacetyl + 2% Lactic Acid; 8D-L = 0.08% Diacetyl + 2% Lactic Acid;
 c-L = Control + 2% Lactic Acid

RESULTS: DEVELOPMENT AND QUALITY OF PASTA CONTAINING CRAB MINCE WITH ADDITIVES

Chemical and Microbial Analyses of Crab Mince

TVBN concentrations, pH, and, **APC** of crab mince are located on Table 23.

Additives had a significant effect ($p < 0.000$) on the pH of the mince, based on one-way ANOVA. The addition of two percent lactic acid with either 0.08% diacetyl or five percent rosemary resulted in significantly lower pH ($p = 0.00$) and microbial counts ($p < 0.02$) in the mince compared to crab mince without additives. **APC** values were between 2×10^4 to 1×10^5 CFU/ g with the treated crab mince, which was a half a log lower than the control treatment. There were no significant differences in TVBN concentration among the three treatments based on average values.

**Table 23. pH, TVBN, and APC of Crab Mince during
Refrigerated (5°C) Storage***

Treatment Code**	pH	TVBN (mg N/ 100g)	APC (log CFU/g)
CR	8.72 ± 0.01^c	20.5 ± 2.7	$8E+05 \pm 2E+05^c$
CR-R-L	7.35 ± 0.03^b	17.0 ± 0.3	$1E+05 \pm 9E+04^a$
CR-D-L	$6.73 \pm 0.03''$	18.9 ± 0.4	$2E+04 \pm 3E+03^b$
p value	0.00	0.100	0.022

Each value is the average of three analyses \pm standard deviation. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CR = Crab mince; CR-R-L = Crab mince with 5% rosemary plus 2% lactic acid;
CR-D-L = Crab mince with 0.08% diacetyl plus 2% lactic acid

Mechanical separation of crab mince resulted in an average 70% yield from the starting by-product. Moisture, fat, and ash contents of the crab mince were 79.8%, 1.5%, 4.5%, respectively (Table 24). Protein content of the crab mince was not determined because of equipment failure. The mechanically separated crab mince had calcium, potassium, magnesium, phosphorus, and sodium concentrations of 5,094, 2,338, 887, 2,139, and 1,891 mg/ kg, respectively (Table 25).

Chemical Analyses of Pasta Containing Crab Mince with Additives

Proximate Analyses

The addition of crab mince increased the moisture and ash content of the pasta, however, fat content was not noticeably affected (Table 24). As percentage crab mince content increase, *so* did the moisture content from 22% for the control to 26.4% for the 20% crab treatment. Percent ash of the 20% crab treatment was twice **as** high (1.4%) as that in the control pasta. Fat content of all three pasta treatments were consistently low (– 2.3%). Protein content of the crab mince was not determined because of equipment failure.

Table 24. Percent Moisture, Ash, and Fat of Crab Mince and Pasta Samples*

Treatment Code	Moisture (%)	Ash (%)	Fat (%)
CR	79.8	4.5	1.5
CON	22.0	0.7	2.3
10CR	23.7	1.0	2.3
20CR	26.4	1.4	2.4

* Each value is the average of three analyses. Values are given on a wet weight basis.

** CR = Crab mince; CON = Pasta control; 10CR = Pasta containing 10% crab mince 20CR = Pasta containing 20% crab mince

The control pasta had calcium, potassium, magnesium, phosphorus, and sodium contents of 226, 2,060, 392, 1,355, and 67 mg/ kg, respectively (Table 25). As percent crab increased, calcium and sodium levels significantly ($p = 0.000$) increased in the pasta based on one-way ANOVA. The addition of crab mince to the pastas resulted in increases of phosphorus and magnesium; although only pasta containing 20% crab mince was significantly different from the control for magnesium based on one-way ANOVA. The addition of crab mince did not significantly increase potassium levels in the pasta.

Table 25. Selected Mineral Concentrations of Crab Mince and Pastas Containing Crab Mince*

Treatment Code**	Mineral Concentration (mg/ kg)				
	Calcium	Potassium	Magnesium	Phosphorus	Sodium
CR	5013 \pm 229 ^d	2312 \pm 206	947 \pm 57 ^c	2205 \pm 135 ^b	1920 \pm 88 ^d
CON	226 \pm 11 ^a	2060 \pm 110	392 \pm 21 ["]	1355 \pm 74 ["]	67 \pm 2 ["]
10CR	1186 \pm 123 ^b	2182 \pm 216	457 \pm 54 ^{ab}	1510 \pm 158 ["]	238 \pm 24 ^b
20CR	2248 \pm 75 ^c	20412117	513 \pm 31 ^b	1580 \pm 114 ^a	441 \pm 42 ^c
p value	0.000	0.319	0.000	0.001	0.000

* Each value is the average of three analyses \pm standard deviation. Values are given on a wet weight basis.

** CR = Crab mince; CON = Pasta control; 10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince

Water Activity

The water activity of all seven pasta treatments ranged between 0.92 and 0.95 (Table 26). Additives used in the crab mince, weeks storage, and a combination of percent crab and additives had a significant ($p < 0.01$) effect on water activity based on multi-way ANOVA. Water activity increased from week one to week two, decreased from week two to week four and there was a slight increase from week four to week five. The addition of additives lowered the water activity of the pastas. The average water activity values of pasta containing crab mince, pasta containing crab mince with 5% rosemary plus 2% lactic acid, and pasta containing crab mince with 0.08% diacetyl plus 2% lactic acid were 0.936, 0.933, and 0.929, respectively. Both pastas containing 10 and 20% crab mince had water activity values of 0.933, however, pastas containing 10% crab mince with additives had significantly higher ($p < 0.01$) average water activity (0.933) compared to pastas containing 20% crab mince with additives (0.929).

Table 26. Water Activity of Fresh Pasta during Refrigerated (5°C) Storage*

Treatment Code**	Week 1	Week 2	Week 3	Week 4	Week 5
CON	0.93 ± 0.00	0.95 ± 0.00	0.93 ± 0.01 ^{ab}	0.93 ± 0.00	0.94 ± 0.01
10CR	0.93 ± 0.01	0.94 ± 0.01	0.94 ± 0.01 ^{ab}	0.93 ± 0.00	0.94 ± 0.00
10CR-R-L	0.93 ± 0.01	0.94 ± 0.00	0.94 ± 0.01 ^{ab}	0.93 ± 0.00	0.94 ± 0.00
10CR-D-L	0.93 ± 0.01	0.94 ± 0.00	0.93 ± 0.00 ^{ab}	0.93 ± 0.01	0.93 ± 0.00
20CR	0.93 ± 0.01	0.95 ± 0.01	0.94 ± 0.00 ^b	0.93 ± 0.01	0.94 ± 0.01
20CR-R-L	0.92 ± 0.01	0.94 ± 0.00	0.94 ± 0.00 ^{ab}	0.93 ± 0.01	0.94 ± 0.00
20CR-D-L	0.92 ± 0.02	0.94 ± 0.00	0.92 ± 0.01 ^a	0.92 ± 0.03	0.92 ± 0.02
p value	0.672	0.064	0.032	0.593	0.109

* Each value is the average of three replications ± standard deviation analyzed in duplicate.

Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

pH

As weeks storage increased, there was a gradual increase in pH from week 1 to week 4 and then a significant ($p = 0.000$) increase from an average 6.73 at week 4 to 6.90 at week 5 based on one-way and multi-way ANOVA (Table 27). The addition of crab mince without additives to pasta resulted in significantly ($p = 0.000$) higher pH levels compared to the control based on multi-way ANOVA. There was also a significantly ($p = 0.000$) higher pH in pasta containing 20% crab mince compared to pasta containing 10% crab mince based on multi-way ANOVA. At week 1, pH of the control, pasta containing 10% crab mince, and pasta containing 20% crab mince, were

6.02, 6.94, and **7.32**, respectively. The addition **of** additives to crab mince resulted in significantly ($p = 0.000$) lower pH values in the pasta compared to pasta containing crab mince without additives based on multi-way **ANOVA**. There was no significant difference between pasta containing crab mince with 0.08% diacetyl plus two percent lactic acid or five percent rosemary plus two percent lactic acid. **A** combination **of** percent crab and additives had a significant ($p < 0.05$) effect on pH based on multi-way **ANOVA**. With an increase in percent crab, the differences in pH values between pasta containing mince with or without additives were more apparent.

Table 27. pH of Fresh Pasta during Refrigerated (5°C) Storage*

Treatment Code**	Week 1	Week 2	Week 3	Week 4	Week 5
CON	6.02 ± 0.03"	6.10 ± 0.04"	6.08 ± 0.04"	6.08 ± 0.05"	6.19 ± 0.03"
10CR	6.94 ± 0.15 ^{cd}	7.07 ± 0.11 ^b	7.05 ± 0.12 ^{cd}	7.00 ± 0.14'	7.19 ± 0.08'
10CR-R-L	6.41 ± 0.08 ^{ab}	6.54 ± 0.11 ^{ab}	6.55 ± 0.12 ^b	6.53 ± 0.12 ^b	6.72 ± 0.15 ^b
10CR-D-L	6.48 ± 0.18 ^b	6.54 ± 0.23 ^{ab}	6.52 ± 0.22 ^b	6.49 ± 0.21 ^{ab}	6.69 ± 0.23 ^b
20CR	7.32 ± 0.12 ^d	7.66 ± 0.12'	7.47 ± 0.15 ^d	7.48 ± 0.15 ^d	7.63 ± 0.11 ^d
20CR-R-L	6.70 ± 0.17 ^{bc}	6.83 ± 0.15 ^b	6.75 ± 0.16 ^{bc}	6.74 ± 0.14 ^{bc}	6.90 ± 0.12 ^{bc}
20CR-D-L	6.71 ± 0.18 ^{bc}	6.81 ± 0.26 ^b	6.74 ± 0.19 ^{bc}	6.76 ± 0.20 ^{bc}	6.96 ± 0.16 ^{bc}
p value	0.000	0.000	0.000	0.000	0.000

Each value is the average of three replications ± standard deviation analyzed in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid

TVBN

Over the course of the 5-week storage study, time had a significant ($p < 0.05$) effect on TVBN concentrations based on multi-way ANOVA (Table **28**). The range of TVBN concentrations for the pasta samples were between 3.5 mg N/ 100g and **9.4** mg N/ **100** g. TVBN concentrations decreased from week one to week three and then increased from week three to week five. The addition of crab mince without additives resulted in significantly ($p = 0.000$) higher TVBN concentrations compared to the control pasta based on multi-way ANOVA. There was also a significantly ($p = 0.000$) higher TVBN concentration in pasta containing **20%** crab mince compared to pasta containing 10% crab mince based on multi-way ANOVA. As weeks storage increased, the difference in TVBN concentrations between pasta with 10% and **20%** crab mince increased based on two-way ANOVA. There was no significant difference between pasta containing crab mince with or without additives.

Table 28. TVBN (mg N/ 100g) of Fresh Pasta during Refrigerated (5°C) Storage*

Treatment Code**	Week 1	Week 3	Week 5
CON	4.6 ± 0.0^a	3.8 ± 0.8["]	3.5 ± 0.6["]
10CR	6.2 ± 0.4^b	4.8 ± 0.5^a	5.9 ± 0.2^b
10CR-R-L	6.5 ± 0.6^b	5.3 ± 0.2^a	5.7 ± 0.2^b
10CR-D-L	7.1 ± 0.6^{bc}	5.1 ± 0.7^a	5.9 ± 0.5^b
20CR	8.2 ± 0.9^c	8.3 ± 0.6^b	9.4 ± 0.5^c
20CR-R-L	8.6 ± 0.3^c	8.5 ± 0.8^b	8.6 ± 0.7^c
20CR-D-L	7.7^{bc}	8.8 ± 0.9^b	7.8 ± 1.1^c
p value	0.000	0.000	0.000

Each value is the average of three replications ± standard deviation analyzed in duplicate.

Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

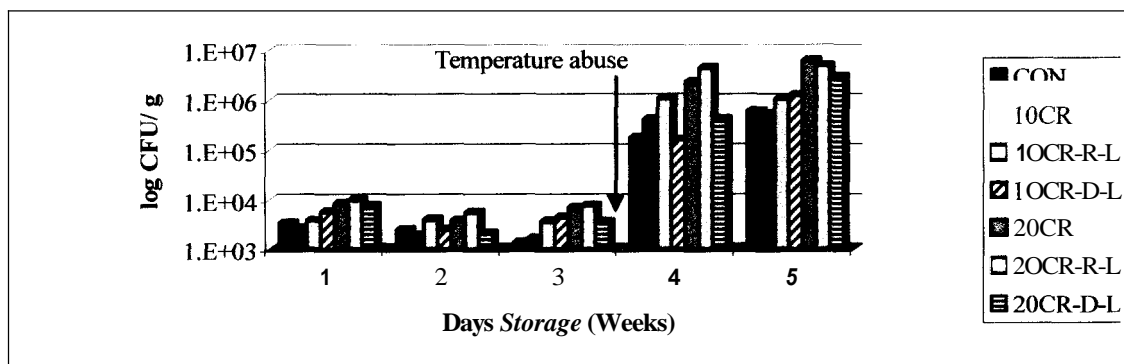
** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid

Microbial Analyses of Pasta Containing Crab Mince with Additives

APC

Over the course of the study, microbial counts remained unchanged from week one to week three of refrigerated storage. From week three to week five, there was a significant ($p = 0.000$) increase in microbial counts based on multi-way ANOVA (Figure 8).

Figure 8. APC (log CFU/ g) of Pasta*



*Aerobic plate counts are the average of **three** replications, each analyzed in duplicate.

Aerobic plate counts for pasta with and without crab mince ranged from 1.3×10^3 CFU/ g to 6.0×10^6 CFU/ g (Table 29). The addition of 20% crab mince without additives resulted in significantly ($p = 0.000$) higher aerobic plate counts compared to pasta without crab mince based on multi-way ANOVA. During weeks one through three of storage, there was no significant difference in microbial counts of pasta with 10% or 20% crab mince based on multi-way ANOVA. During weeks four and five of storage there were significantly ($p < 0.01$) higher microbial counts in pasta with 20% crab mince than in pasta with 10% crab mince based on multi-way ANOVA. The difference in microbial counts between pasta with 10% and 20% crab mince was more apparent at week five. There were no significant differences between pasta containing 10% crab mince without additives and the control. The addition of **0.08%** diacetyl plus two percent lactic acid to crab mince resulted in lower microbial counts in the pasta compared to pasta containing crab mince without additives although the reduction was not significant. The addition of five percent rosemary plus two percent lactic acid resulted in higher microbial counts compared to pasta containing crab mince without additives although the increase was not significant.

Table 29. APC (log CFU/ g) of Fresh Pasta during Refrigerated (5°C) Storage*

Treatment Code**	Week 1	Week 2	Week 3	Week 4	Week 5
CON	3E+03 ± 3E+03 ^a	2E+03 ± 1E+03	1E+03 ± 4E+02	2E+05 ± 2E+05	6E+05 ± 10E+05
10CR	3E+03 ± 2E+02 ^a	2E+03 ± 7E+02	2E+03 ± 4E+02	4E+05 ± 3E+05	5E+05 ± 7E+05
10CR-R-L	4E+03 ± 6E+02 ^a	4E+03 ± 2E+03	4E+03 ± 4E+03	1E+06 ± 2E+06	1E+06 ± 1E+06
10CR-D-L	6E+03 ± 4E+03 ^a	2E+03 ± 1E+03	4E+03 ± 1E+03	2E+05 ± 2E+05	1E+06 ± 1E+06
20CR	8E+03 ± 3E+03 ^a	4E+03 ± 8E+02	7E+03 ± 5E+03	2E+06 ± 3E+06	6E+06 ± 2E+06
20CR-R-L	9E+03 ± 2E+03 ^a	5E+03 ± 3E+03	7E+03 ± 4E+03	4E+06 ± 4E+06	5E+06 ± 4E+06
20CR-D-L	7E+03 ± 3E+03 ^a	2E+03 ± 1E+03	3E+03 ± 1E+03	4E+05 ± 6E+05	3E+06 ± 5E+06
p value	0.035	0.067	0.133	0.142	0.103

* Each value is the average of three replications ± standard deviation analyzed in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

Yeasts

Over the course of the 5-week study, microbial counts significantly increased ($p = 0.000$). Yeast counts in pasta with and without crab mince ranged from 0 to 8.5×10^3 CFU/g (Table 30). From week one to week four of refrigerated storage, yeast growth was minimal, however, between weeks four and five there was a significant ($p = 0.000$) increase in yeast counts on the pasta samples. The control pasta had the lowest yeast counts throughout storage followed by pasta containing 10% crab mince without additives although the differences were not significant. There were no significant differences between pasta containing crab mince with or without additives until week five. The addition of five percent rosemary plus two percent lactic acid or 0.08% diacetyl plus two percent lactic acid to crab mince significantly ($p = 0.000$) reduced the growth of yeast in the pasta based on multi-way ANOVA. There was no significant difference between pasta containing crab mince with either 0.08% diacetyl plus two percent lactic acid or five percent rosemary plus two percent lactic acid throughout storage.

Table 30. Yeast Counts (CFU/ g) of Fresh Pasta during Refrigerated (5°C) Storage

Treatment Code**	Week 1	Week 2	Week 3	Week 4	Week 5
CON	n.d.*	n.d.	n.d.	< 100	< 100
10CR	n.d.	n.d.	n.d.	< 100	4498 \pm 6946
10CR-R-L	n.d.	< 100	n.d.	< 100	< 100
10CR-D-L	n.d.	n.d.	n.d.	< 100	188 \pm 300
20CR	< 100	n.d.	n.d.	507 \pm 878	8500 \pm 6322
20CR-R-L	< 100	< 100	< 100	187 \pm 82	988 \pm 609
20CR-D-L	n.d.	n.d.	< 100	140 \pm 122	342 \pm 592
p value	0.522	0.479	0.109	0.621	0.076

* n.d. = No cells detected.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 20% crab mince with 0.08% diacetyl plus 2% lactic acid.

Molds

Mold counts for pasta with and without crab mince ranged from 0 to 2.0 $\times 10^4$ CFU/g (Table 31). From week one to week three of refrigerated storage, all mold counts were below 100 CFU/g except pasta containing 20% crab mince and pasta containing 20% crab mince with 5% rosemary and 2% lactic acid although it was not significantly different based on one-way ANOVA. Pasta containing 20% crab mince with 5% rosemary and 2% lactic acid also had significantly ($p < 0.05$) higher mold counts at week three of storage compared to other samples based on multi-way ANOVA. As days storage increased from week four to week five, there was significantly ($p = 0.000$) more mold on the pasta samples based on multi-way ANOVA. In fact, small spots of white fuzzy mold growth were observed when removing the samples out of refrigerated storage at week five. The control pasta had the lowest mold counts throughout storage compared to pasta containing crab mince without additives although it was not significantly different. Pasta containing 20% crab mince had higher mold counts than pasta containing

10% crab mince although it was not significantly different. The addition of 0.08% diacetyl plus two percent lactic acid to crab mince resulted in lower mold counts in the pasta than pasta containing crab mince without additives although it was not significant based on multi-way ANOVA. The addition of five percent rosemary plus two percent lactic acid resulted in higher mold counts in the pasta than pasta containing crab mince without additives although it was not significant based on multi-way ANOVA.

Table 31. Molds (CFU/ g) of Fresh Pasta during Refrigerated (5°C) Storage*

Treatment Code***	Week 1	Week 2	Week 3	Week 4	Week 5
CON	< 100	< 100	< 100"	< 100	132 ± 71
10CR	< 100	< 100	< 100"	< 100	7585 ± 12055
10CR-R-L	< 100	< 100	< 100"	< 100	3667 ± 5658
10CR-D-L	n.d.**	< 100	< 100"	< 100	1247 ± 1917
20CR	< 100	< 100	< 100"	125 ± 1067	12067 ± 6481
20CR-R-L	< 100	< 100	52 ± 44 ^b	347 ± 328	19785 ± 26716
20CR-D-L	< 100	< 100	< 100"	< 100	537 ± 917
p value	0.244	0.930	0.038	0.107	0.364

* Each value is the average of three replications ± standard deviation analyzed in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** n.d. = No cells detected.

***CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

Physical Analyses of Pasta Containing Crab Mince with Additives

Cooking Loss

The higher the percentage crab, the higher the cooking loss, based on one-way and multi-way ANOVA. This was true for each time period evaluated. There was a Significant ($p < 0.01$) effect of treatment on cooking loss for all sampling periods based on one-way ANOVA. At week three and five, the addition of crab mince resulted in higher cooking loss based on one-way ANOVA. From week one to week three there was no significant difference in cooking loss based on multi-way ANOVA (Table 32). However, from week three to week five of refrigerated storage, the pasta samples had a significant ($p < 0.05$) increase in cooking loss based on multi-way ANOVA. Based on multi-way ANOVA, the addition of 20% crab mince without additives to pasta resulted in significantly ($p = 0.01$) higher cooking loss than pasta containing 10% crab mince without additives or the control. There was no significant difference between the control and pasta containing 10% crab mince without additives. The addition of five percent rosemary plus two percent lactic acid or 0.08% diacetyl plus two percent lactic acid to the crab mince resulted in a significantly ($p = 0.000$) greater cooking loss by the pasta than pasta containing crab mince without additives based on multi-way ANOVA. There was no significant difference between pasta containing crab mince with five percent rosemary plus two percent lactic acid or 0.08% diacetyl plus two percent lactic acid.

Table 32. Percent Cooking Loss of Fresh Pasta during Refrigerated (5°C) Storage*

Treatment Code***	Cooking Loss (%)**		
	Week 1	Week 3	Week 5
CON	4.0 ± 0.3"	3.7 ± 0.4"	4.1 ± 0.5"
10CR	3.9 ± 0.4"	3.9 ± 0.1 ^{ab}	4.2 ± 0.1"
10CR-R-L	4.4 ± 0.2 ^{ab}	4.4 ± 0.3 ^{abc}	4.4 ± 0.2"
10CR-D-L	4.3 ± 0.1 ^{ab}	4.2 ± 0.4 ^{abc}	4.4 ± 0.2"
20CR	4.2 ± 0.4 ^{ab}	4.5 ± 0.2 ^{bc}	4.5 ± 0.3 ^{ab}
20CR-R-L	4.8 ± 0.1 ^b	4.7 ± 0.2"	5.3 ± 0.4 ^b
20CR-D-L	4.8 ± 0.3 ^b	4.5 ± 0.3 ^{bc}	4.7 ± 0.4 ^{ab}
p value	0.005	0.005	0.006

* Each value is the average of three replicates ± standard deviation, each analyses conducted in duplicate. Means in the same column not sharing a superscript are significantly (p < 0.05) different based on one-way ANOVA followed by Tukey's post hoc test.

** Percent cooking loss = {(jar wt. + cooking loss wt.) – jar wt.} * 4.

*** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

Cooking Weight

The percent increase in weight of pasta samples after being cooked ranged from **124.4%** to **156%** (Table 33) of the raw pasta sample weight. At week one there were significant differences among treatments based on one-way ANOVA, with the control pasta having the lowest and the pasta containing crab mince with **0.08%** diacetyl plus **2%** lactic acid having the highest cooking weight. However, there was no significant effect of percent crab, additives, or weeks storage on cooking weight of the pasta based on multi-way ANOVA.

Table 33. Percent Cooking Weight of Fresh Pasta during Refrigerated (5°C) Storage*

Treatment Code***	Cooking Weight (%)**		
	Week 1	Week 3	Week 5
CON	124 ± 14 ^a	139 ± 18	138 ± 10
10CR	137 ± 4 ^{ab}	142 ± 9	146 ± 12
10CR-R-L	138 ± 8 ^{ab}	136 ± 11	134 ± 16
10CR-D-L	140 ± 5 ^{ab}	138 ± 12	139 ± 13
20CR	132 ± 12 ^a	137 ± 10	146 ± 12
20CR-R-L	136 ± 4 ^{ab}	133 ± 2	138 ± 3
20CR-D-L	156 ± 3 ^b	141 ± 4	149 ± 12
p value	0.037	0.944	0.686

* Each value is the average of three replicates ± standard deviation, each analyses conducted in duplicate. Means in the same column not sharing a superscript are significantly (p < 0.05) different based on one-way ANOVA followed by Tukey's post hoc test.

** Percent cooking weight = {(cooked wt. – raw wt.) / raw wt.} * 100.

*** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

Firmness

Firmness of the cooked pasta ranged from 0.0014 g* cm to 0.0027 g* cm (Table 34). Over the course of the study, there was a significant decrease (p = 0.000) in firmness of cooked pasta samples based on multi-way ANOVA (Figure 9). Additives utilized in the mince and percent crab mince added to the pasta had no significant effect on the firmness of the pasta.

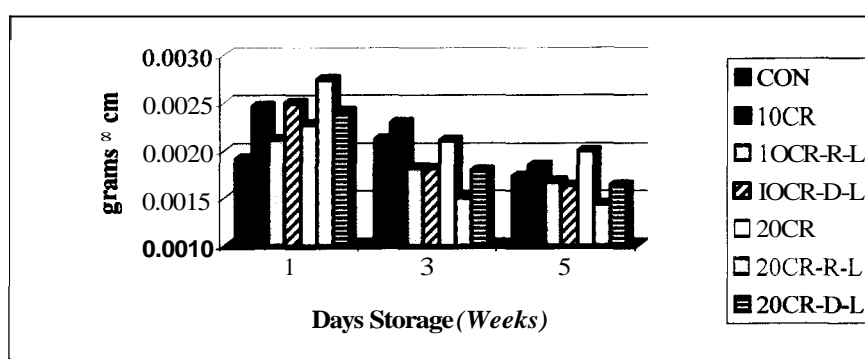
Table 34. Firmness of Fresh Pasta during Refrigerated (5°C) Storage*

Treatment Code**	Firmness (g * cm)		
	Week 1	Week 3	Week 5
CON	0.0019 ± 0.0005	0.0021 ± 0.0002	0.0017 ± 0.0002
10CR	0.0025 ± 0.0002	0.0023 ± 0.0000	0.0018 ± 0.0003
10CR-R-L	0.0021 ± 0.0003	0.0018 ± 0.0005	0.0017 ± 0.0001
10CR-D-L	0.0025 ± 0.0000	0.0018 ± 0.0002	0.0016 ± 0.0002
20CR	0.0023 ± 0.0005	0.0021 ± 0.0005	0.0020 ± 0.0003
20CR-R-L	0.0027 ± 0.0010	0.0015 ± 0.0002	0.0014 ± 0.0002
20CR-D-L	0.0024 ± 0.0003	0.0018 ± 0.0002	0.0016 ± 0.0004
p value	0.500	0.059	0.291

* Each value is the average of three replicates, each analyses conducted in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

Figure 9. Firmness (grams * cm) of Pasta*



*Firmness values are the average of three replications, each analyzed in triplicate.

Width and Thickness

Width and thickness of raw pasta ranged from **7.23 mm** to **7.81 mm** and **0.74 mm** to **1.04 mm**, respectively (Table 35, 36, and 37). At week one of refrigerated storage, there were significant differences in width ($p = 0.029$) and thickness ($p = 0.000$) among raw pasta treatments. The control pasta was significantly wider and thicker than the pasta containing crab mince with **0.08%** diacetyl plus **2%** lactic acid. As weeks storage increased, both width and thickness of raw pasta significantly ($p < 0.05$) increased based on multi-way ANOVA. The addition of crab mince without additives had no effect on the width of the pasta but slightly decreased the thickness of the raw pasta although it was not significant. The addition of additives significantly ($p < 0.05$) decreased the width and thickness of the raw pasta. However, there were no significant differences in width and thickness of pasta noodles containing crab mince with **0.08%** diacetyl plus lactic acid versus those containing five percent rosemary plus two percent lactic acid.

Width and thickness of cooked pasta ranged from **10.17 mm** to **10.78 mm** and **0.93 mm** to **1.24 mm**, respectively (Tables 35, 36, and 37). There were no trends observed among treatments for width and thickness of cooked pasta based on one-way analysis of variance. As weeks storage increased, there was a significant ($p = 0.000$) increase in the thickness of cooked pasta based on multi-way ANOVA. The addition of crab mince without additives had no effect on the thickness of the cooked pasta but did significantly ($p = 0.01$) increase the width of the cooked pasta based on multi-way ANOVA. **An** increase in percent crab **from** 10% to **20%** to the pasta had no significant effect on the cooked pasta's width and thickness. The addition of additives significantly ($p < 0.01$) decreased the thickness of the cooked pasta compared to pasta containing crab mince

without additives although there were no significant differences observed for width based on multi-way ANOVA. There were no significant differences in width and thickness of pasta noodles containing crab mince with 0.08% diacetyl plus lactic acid or five percent rosemary plus two percent lactic acid.

After ~~being~~ cooked, the pasta noodles increased in size by an average 37.9% for width and 27.1% for thickness. The width and thickness of cooked pasta samples were statistically ($p = 0.000$) larger than width and thickness of the raw pasta except for pasta containing 10% crab analyzed at week 1.

Table 35. Width and Thickness of Raw and Cooked Pasta after One Week Refrigerated (5°C) Storage*

Treatment Code**	Raw		Cooked	
	Width (mm)	Thickness (mm)	Width (mm)	Thickness (mm)
CON	7.68 ± 0.23^b	1.04 ± 0.06^b	10.49 ± 0.30	1.24 ± 0.09^b
10CR	7.67 ± 0.09^b	0.98 ± 0.12^{ab}	10.78 ± 0.17	1.09 ± 0.11^{ab}
10CR-R-L	7.41 ± 0.10^{ab}	$0.76 \pm 0.05''$	10.68 ± 0.25	$0.93 \pm 0.06''$
10CR-D-L	7.43 ± 0.12^{ab}	$0.76 \pm 0.04''$	10.35 ± 0.39	$1.01 \pm 0.04''$
20CR	7.49 ± 0.06^{ab}	$0.81 \pm 0.02''$	10.62 ± 0.27	1.09 ± 0.03^{ab}
20CR-R-L	7.58 ± 0.09^{ab}	$0.74 \pm 0.03''$	10.48 ± 0.38	$0.97 \pm 0.08''$
20CR-D-L	7.23 ± 0.37^a	0.77 ± 0.03^a	10.26 ± 0.36	1.01 ± 0.03^a
p value	0.029	0.000	0.434	0.002

* Each value is the average of three replicates \pm standard deviation, each analyses conducted in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary + 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl + 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary + 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl + 2% lactic acid.

Table 36. Width and Thickness of Raw and Cooked Pasta after Three Weeks Refrigerated (5°C) Storage*

Treatment Code**	Raw		Cooked	
	Width (mm)	Thickness (mm)	Width (mm)	Thickness (mm)
CON	7.51 ± 0.38	0.85 ± 0.05	10.17 ± 0.17"	1.08 ± 0.03 ^{ab}
10CR	7.60 ± 0.06	0.83 ± 0.02	10.41 ± 0.08 ^{ab}	1.06 ± 0.02"
10CR-R-L	7.67 ± 0.08	0.83 ± 0.06	10.34 ± 0.08 ^{ab}	1.07 ± 0.02"
10CR-D-L	7.56 ± 0.15	0.85 ± 0.05	10.48 ± 0.07 ^{ab}	1.04 ± 0.01"
20CR	7.81 ± 0.24	0.86 ± 0.06	10.70 ± 0.11 ^b	1.14 ± 0.04 ^b
20CR-R-L	7.52 ± 0.05	0.77 ± 0.02	10.69 ± 0.38 ^b	1.08 ± 0.01 ^{ab}
20CR-D-L	7.73 ± 0.04	0.87 ± 0.06	10.55 ± 0.13 ^{ab}	1.10 ± 0.04 ^{ab}
p value	0.381	0.669	0.025	0.009

* Each value is the average of three replicates ± standard deviation, each analyses conducted in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary + 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl + 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary + 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl + 2% lactic acid.

Table 37. Width and Thickness of Raw and Cooked Pasta after Five Weeks Refrigerated (5°C) Storage*

Treatment Code**	Raw		Cooked	
	Width (mm)	Thickness (mm)	Width (mm)	Thickness (mm)
CON	7.61 ± 0.06	0.90 ± 0.02	10.24 ± 0.14	1.07 ± 0.04
10CR	7.61 ± 0.13	0.90 ± 0.09	10.29 ± 0.31	1.13 ± 0.08
10CR-R-L	7.59 ± 0.04	0.83 ± 0.05	10.56 ± 0.08	1.07 ± 0.03
10CR-D-L	7.66 ± 0.04	0.83 ± 0.07	10.61 ± 0.39	1.09 ± 0.02
20CR	7.68 ± 0.11	0.89 ± 0.04	10.62 ± 0.24	1.12 ± 0.04
20CR-R-L	7.62 ± 0.11	0.84 ± 0.03	10.31 ± 0.04	1.10 ± 0.06
20CR-D-L	7.64 ± 0.02	0.86 ± 0.03	10.23 ± 0.33	1.10 ± 0.03
p value	0.822	0.371	0.334	0.713

Each value is the average of three replicates ± standard deviation, each analyses conducted in duplicate. Means in the same column not sharing a superscript are significantly (p < 0.05) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary + 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl + 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta Containing 20% crab mince with 5% rosemary + 2% lactic acid; 20CR-D-L = Pasta containing 20% crab mince with 0.08% diacetyl + 2% lactic acid.

Instrumental Color

Instrumental color analyses were conducted on raw and cooked pasta samples. “L” values indicate a range of black = 0 to white = **100**. The “a” values indicate a range of negative numbers = green to positive numbers = red. The “b” values indicate a range of negative numbers = blue to positive numbers = yellow. Overall, the addition of rosemary significantly ($p = 0.000$) decreased “L”, “a”, and “b” values based on multi-way ANOVA. As percent crab increased from **10** to **20%** “L” and “b” values significantly ($p = 0.000$) decreased based on multi-way ANOVA. Raw pasta samples ranged from **49.37** to **66.83**, **-0.07** to **1.50**, and **13.47** to **19.90**, for “L”, “a”, and “b”, respectively (Tables **38**, **39**, and **40**). Weeks storage did not significantly affect “L” and “a” values. From week one to week three of refrigerated storage, there was a significant ($p < 0.01$) decrease in “b” values although from week three to week five there was no change based on multi-way ANOVA. The addition of **20%** crab mince resulted in significantly ($p < 0.05$) lower “L” values compared to the control pasta based on multi-way ANOVA. There was no significant difference in “L” values between pasta containing **10%** crab mince without additives and the control. There was no significant difference in “a” values between pasta containing crab mince without additives and the control. The addition of **20%** crab mince to pasta significantly ($p = 0.000$, $p < 0.01$, respectively) reduced the “L” and “b” values compared to pasta containing 10% crab mince. The addition of five percent rosemary plus lactic acid to crab mince significantly ($p < 0.01$) reduced “L”, “a”, and “b” values of raw pasta compared to raw pasta containing crab mince without additives based on multi-way ANOVA. The addition of **0.08%** diacetyl plus two percent

lactic acid to crab mince increased the “L” values of the raw pasta compared to raw pasta containing crab mince without additives although it was not significant.

Overall, after being cooked, pasta with rosemary had significantly ($p = 0.000$) lower “L” and “b” values and significantly ($p = 0.003$) higher “a” values compared to other samples. After being cooked, pasta with higher percentages of crab had significantly lower “L” ($p = 0.000$) values but significantly ($p = 0.000$) higher “a” and “b” values based on multi-way ANOVA. There was a significant ($p = 0.001$) decrease in “L” values of the cooked pasta samples as storage time increased. Of the cooked pasta samples “L”, “a”, and “b” values ranged from **58.71** to **72.91**, **-1.85** to **-0.51**, and **11.57** to **15.05**, respectively (Tables **38**, **39**, and **40**). As weeks storage increased, there was a significant ($p = 0.001$) decline in the “L” value of cooked pasta samples from an average of **69.29** at week one to **66.90** at week five based on multi-way ANOVA. There was no significant change in the “a” values as weeks storage increased. From week one to week three of storage, there was a significant ($p < 0.05$) decrease in “b” values in pasta containing crab mince from an average **13.85** at week one to **12.99** at week 3 based on multi-way ANOVA. From week three to week five, there was no significant change in the pasta sample’s “b” values. Pasta containing crab mince without additives had significantly ($p = 0.001$) lower “L” values and significantly ($p = 0.000$) higher “b” values compared to the control based on multi-way ANOVA. Pasta containing **20%** crab mince without additives had significantly ($p = 0.000$) higher “a” values compared to the control. Pasta containing **10%** crab mince without additives was not significantly different from the control. After being cooked, pastas containing **20%** crab mince had significantly

($p = 0.000$) lower “L” values and significantly ($p = 0.000$) higher “a” and “b” values compared to pasta containing 10% crab mince (Figures 38, 39, and 40). After being cooked, pasta containing crab mince with five percent rosemary plus two percent lactic acid had significantly ($p = 0.000$) lower “L” and “b” values and significantly ($p < 0.01$) higher “a” values compared to pasta containing crab mince with 0.08% diacetyl plus two percent lactic acid or the control based on multi-way ANOVA. With the addition of 20% crab mince, the difference between pasta containing five percent rosemary plus two percent lactic acid and the other pastas containing crab mince was more apparent ($p < 0.05$) than pasta containing 10% crab mince. After being cooked, pasta containing crab mince with 0.08% diacetyl plus two percent lactic acid had significantly ($p = 0.000$) lower “b” values compared to pasta containing crab mince without additives based on multi-way ANOVA. There was no significant difference between “L” or “a” values of pasta containing crab mince without additives and pasta containing crab mince with 0.08% diacetyl plus two percent lactic acid.

After being cooked, the pasta samples average “L” value was 13.7% higher than the raw pasta “L” value (Figures 10, 11, 12). The average “a” value was 244% more green than the raw pasta “a” value. The average “b” value was 19.8% less yellow than the raw pasta samples.

Table 38. Colorimetric Values of Raw and Cooked Pasta after One Week Refrigerated (5°C) Storage*

Treatment Code**	Raw			Cooked		
	L	a	b	L	a	b
CON	66.83 ± 5.23 ^b	1.25 ± 0.67	19.41 ± 1.29	71.47 ± 3.69	-1.37 ± 0.26	13.80 ± 0.96
10CR	64.53 ± 5.55 ^{ab}	0.79 ± 0.69	18.69 ± 2.65	71.03 ± 1.48	-1.45 ± 0.29	14.38 ± 0.86
10CR-R-L	55.34 ± 0.98 ^{ab}	0.55 ± 0.24	17.10 ± 1.33	66.48 ± 3.11	-1.41 ± 0.04	12.76 ± 0.41
10CR-D-L	66.55 ± 2.23 ^b	1.16 ± 0.74	19.91 ± 2.90	71.86 ± 1.96	-1.45 ± 0.37	13.70 ± 1.30
20CR	59.38 ± 5.94 ^{ab}	1.10 ± 0.96	17.55 ± 2.44	70.47 ± 1.28	-1.13 ± 0.14	14.67 ± 0.13
20CR-R-L	52.17 ± 4.55 ["]	-0.07 ± 0.65	14.40 ± 0.82	64.98 ± 4.99	-0.89 ± 0.27	13.45 ± 0.65
20CR-D-L	62.07 ± 1.42 ^{ab}	1.50 ± 0.68	17.70 ± 1.97	68.77 ± 4.15	-0.89 ± 0.23	14.20 ± 0.99
p value	0.009	0.248	0.117	0.228	0.070	0.286

* Each value is the average of three replicates ± **standard deviation**, each analyses conducted in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

Table 39. Colorimetric Values of Raw and Cooked Pasta after Three Weeks Refrigerated (5°C) Storage*

Treatment Code**	Raw			Cooked		
	L	a	b	L	a	b
CON	64.76 ± 2.00 ^b	1.16 ± 0.65	17.02 ± 2.27	72.91 ± 0.98 ^d	-1.63 ± 0.25 ^b	12.18 ± 0.88 ^d
10CR	61.07 ± 2.49 ^b	1.03 ± 0.38	16.83 ± 1.57	69.89 ± 1.37 ^{cd}	-1.57 ± 0.15 ^b	13.69 ± 0.53 ^{cd}
10CR-R-L	54.50 ± 2.06 ^{ab}	0.80 ± 0.29	15.76 ± 0.95	65.10 ± 1.28 ^b	-1.35 ± 0.25 ^b	12.08 ± 0.83 ^b
10CR-D-L	61.80 ± 1.88 ^b	1.32 ± 0.43	16.975 1.86	71.52 ± 1.18 ^{cd}	-1.47 ± 0.26 ^b	12.30 ± 0.84 ^d
20CR	59.34 ± 2.39 ^b	0.98 ± 0.66	16.34 ± 1.43	68.56 ± 0.76 ⁱ	-0.90 ± 0.26 ⁱ	14.33 ± 0.61 ⁱ
20CR-R-L	49.86 ± 3.57 ["]	0.37 ± 0.49	13.47 ± 1.37	58.71 ± 2.23 ["]	-0.51 ± 0.36 ["]	12.40 ± 0.48 ["]
20CR-D-L	64.80 ± 3.43 ^b	0.95 ± 0.56	14.56 ± 1.15	68.98 ± 0.91 ^e	-0.76 ± 0.32 ^b	13.94 ± 1.42 ["]
p value	0.000	0.380	0.053	0.000	0.000	0.002

Each value is the average of three replicates ± standard deviation, each analyses conducted in duplicate. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

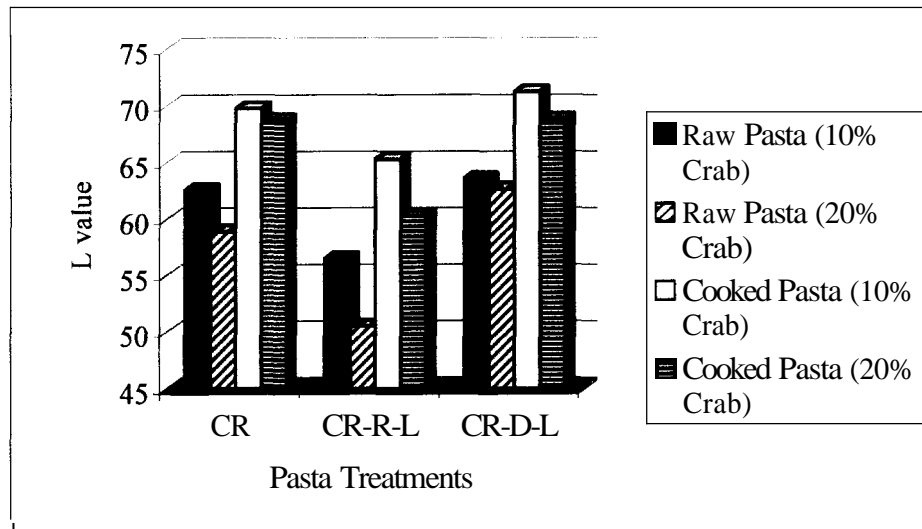
Table 40. Colorimetric Values of Raw and Cooked Pasta after Five Weeks Refrigerated (5°C) Storage*

Treatment Code**	Raw			Cooked		
	L	a	b	L	a	b
CON	61.21 ± 2.72 ^b	0.81 ± 0.41	18.11 ± 1.04	72.58 ± 0.64 [']	-1.85 ± 0.138 [']	11.57 ± 1.32 ^d
10CR	62.06 ± 1.92 ^b	0.80 ± 0.66	16.57 ± 2.17	68.37 ± 0.73 ^d	-1.70 ± 0.26 [']	13.62 ± 1.06 ^{ab}
10CR-R-L	59.72 ± 3.90 ^b	0.03 ± 0.57	14.45 ± 2.31	64.23 ± 0.96 ^b	-1.39 ± 0.068 ["]	12.31 ± 0.71 ^a
10CR-D-L	62.35 ± 1.57 ^b	0.89 ± 0.20	17.36 ± 1.33	70.22 ± 1.04 ^d	-1.55 ± 0.23 ^{cd}	12.81 ± 0.79 ["]
20CR	57.78 ± 2.59 ^b	1.24 ± 0.45	16.67 ± 1.18	67.12 ± 1.30 ^b	-1.06 ± 0.22 ^d	15.05 ± 0.29 ^b
20CR-R-L	49.37 ± 4.56 ["]	0.46 ± 0.58	14.08 ± 1.5	57.15 ± 1.79 ["]	-0.55 ± 0.27 ^b	12.81 ± 0.39 ["]
20CR-D-L	61.05 ± 2.34 ^b	1.03 ± 0.44	16.71 ± 2.46	68.59 ± 1.04 ^b	-1.07 ± 0.30 ^d	14.21 ± 0.92 ^{bc}
P value	0.001	0.098	0.099	0.000	0.000	0.002

* Each value is the average of three replicates ± standard deviation, each analyses conducted in duplicate. Means in the same column not sharing a superscript **are** significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

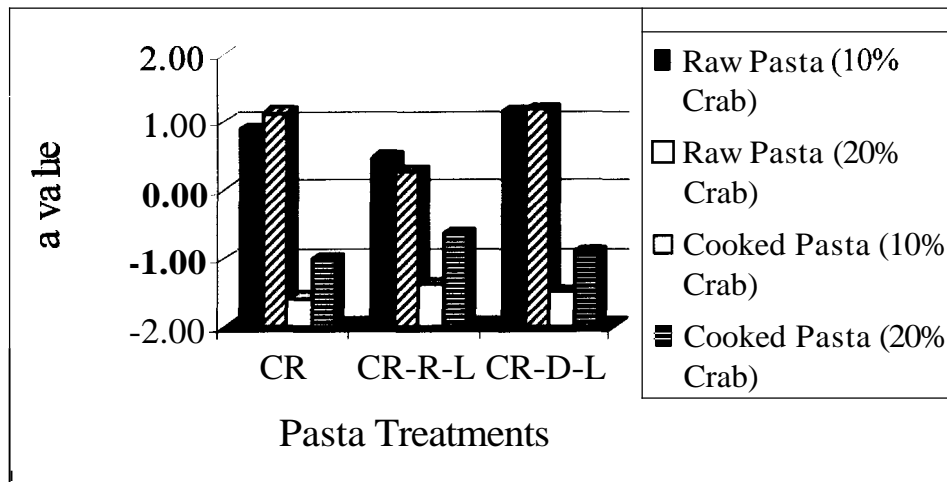
** CON = Control; 10CR = Pasta containing 10% crab mince; 10CR-R-L = Pasta containing 10% crab mince with 5% rosemary plus 2% lactic acid; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR = Pasta containing 20% crab mince; 20CR-R-L = Pasta containing 20% crab mince with 5% rosemary plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid.

Figure 10. Average “L” Values of Raw and Cooked Pasta Containing 10 or 20% Crab Mince with Additives*



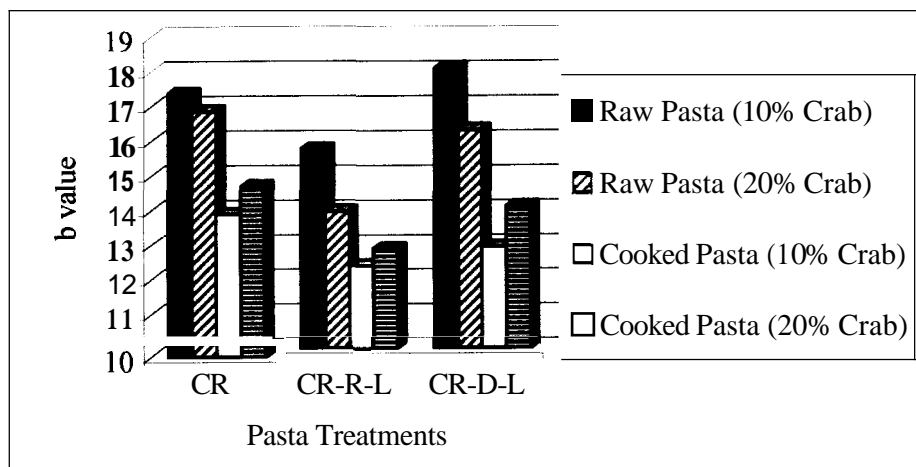
* Color values are the average of week 1, 3, and 5. The color values for each week were the average of three replications, each analyzed in triplicate. CR = Crab mince; CR-R-L = Crab mince with 5% rosemary + 2% lactic acid; CR-D-L = Crab mince with 0.08% diacetyl + 2% lactic acid.

Figure 11. Average “a” Values of Raw and Cooked Pasta Containing 10 or 20% Crab Mince with Additives*



*Color values are the average of week 1, 3, and 5. The color values for each week were the average of three replications, each analyzed in triplicate. CR = Crab mince; CR-R-L = Crab mince with 5% rosemary + 2% lactic acid; CR-D-L = Crab mince with 0.08% diacetyl + 2% lactic acid.

Figure 12. Average “b” Values of Raw and Cooked Pasta Containing 10 or 20% Crab Mince with Additives



* Color values are the average of week 1, 3, and 5. The color values for each week were the average of three replications, each analyzed in triplicate. CR = Crab mince; CR-R-L = Crab mince with 5% rosemary + 2% lactic acid; CR-D-L = Crab mince with 0.08% diacetyl + 2% lactic acid.

RESULTS: SENSORY ANALYSES AND EVALUATION OF PASTA CONTAINING CRAB MINCE WITH ADDITIVES

Chemical and Microbial Analyses of Crab Mince with Additives

Mechanical separation of crab mince resulted in ~~an~~ average meat recovery of 65.86% from the starting by-product. The crab mince contained 80.7% moisture, 4.6% ~~ash~~, and 1.3% fat (Table 41). Protein content of the crab mince ~~was~~ not determined because of equipment failure. The mechanically separated crab mince contained calcium, potassium, magnesium, phosphorus, and sodium concentrations of 5,094, 2,338, 887, 2,139, and 1,891 mg/ ~~kg~~, respectively (Table 42).

**Table 41. Percent Moisture, Ash and Fat of
Crab Mince and Pasta Samples"**

Treatment	Moisture (%)	Ash (%)	Fat (%)
CR	80.7	4.6	1.3
10CR	27.0	1.0	2.4
20CR	24.4	1.5	2.7

Each value is the average of three analyses. Values are given on a wet weight basis.

CR = Crab mince; 10CR = ~~Pasta~~ containing 10% crab mince; 20CR = Pasta containing 20% crab mince.

Table 42. Selected Mineral Concentrations of Crab Mince and Pastas Containing Crab Mince*

Treatment Code**	Mineral Concentration (mg/ kg)				
	Calcium	Potassium	Magnesium	Phosphorus	Sodium
CR	5094 \pm 126 ^c	2338 \pm 167	887 \pm 65 ^b	2139 \pm 106 ^b	1891 \pm 56 ^c
10CR	11329 \pm 139 ^a	1942 \pm 270	526 \pm 64 ["]	1496 \pm 152 ["]	241 \pm 20 ^a
20CR	2199 \pm 284 ^b	2099 \pm 313	584 \pm 99 ["]	1669 \pm 213 ^a	432 \pm 54 ^b
p value	0.000	0.244	0.003	0.007	0.000

* Each value is the average of three analyses. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** Values are given on a wet weight basis.

CR = Crab mince; 10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince.

The average TVBN concentration of crab mince without additives was 10.3 mg N/ 100 g (Table 43). The addition of diacetyl and lactic acid resulted in an average TVBN concentration of 10.8 mg N/ 100g, which was higher than the batch of crab mince without additives, but the differences were not significant. The pH of crab mince without additives was 8.48 (Table 43). The addition of diacetyl and lactic acid to the crab mince resulted in a significantly ($p = 0.000$) lower pH (7.92) compared with the crab mince without additives based on one-way ANOVA. Adding diacetyl and lactic acid to the crab mince resulted in lower aerobic plate counts compared to the crab mince control. The control treatment had an average aerobic plate count of 4×10^5 CFU/g, approximately a half a log higher than the treatment with additives, however, that difference was not significant based on one-way ANOVA.

Table 43. pH, TVBN, and APC of Crab Mince*

Treatment Code**	pH	TVBN (mg N/ 100g)	APC (log CFU/ g)
CR	8.48 ± 0.01^b	10.3 ± 0.2	$4E+05 \pm 5E+05$
CR-D-L	7.92 ± 0.03^a	10.8 ± 1.0	$9E+04 \pm 2E+04$
p value	0.000	0.420	0.254

* Each value is the average of three analyses \pm standard deviation. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

**10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince; IOCR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L = Pasta containing 20% crab mince with 0.08% diacetyl plus 2% lactic acid; 10CR-D-L-R = Pasta containing 0.05% red colorant and 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L-R = Pasta containing 0.05% red colorant and 20% crab mince with 0.08% diacetyl plus 2% lactic acid.

Chemical Analyses of Pasta Containing Crab Mince with Additives

Proximate Analyses

The moisture content of pasta containing 10% and 20% crab mince was 27% and 24.4%, respectively. The more crab mince added to the pasta, the higher the ash and fat content. Pasta containing 10% crab mince had 1.0% ash content and pasta containing 20% crab mince had 1.5% ash content. Pasta containing 10% crab mince had 2.4% fat content and pasta containing 20% crab mince had 2.7% fat content. Protein content of the crab mince was not determined because of equipment failure. The addition of crab mince to the pasta resulted in increases of calcium, potassium, magnesium, phosphorus, and sodium contents, although all were not a significant, based on one-way ANOVA (Table 42). Crab mince had significantly higher levels of calcium, magnesium, phosphorus, and sodium than pasta based on one-way ANOVA. As percent crab increased, calcium and sodium levels significantly ($p = 0.000$) increased in the pasta based on one-way ANOVA. The addition of crab mince to the pastas resulted in higher phosphorus and magnesium

contents in pasta containing 20% crab mince compared to pasta containing 10% crab mince, although the difference was not significant. There was no significant difference between the potassium content in crab mince and the potassium content in the pasta, although with increasing levels of crab mince the potassium level did increase in the pastas.

Water Activity

Overall, the differences in water activity among treatments were very slight. Percent crab, additives, and the combination of percent crab and additives, all had a significant ($p < 0.05$) effect on the water activity of the pastas based on multi-way ANOVA (Table 44). There were no trends apparent which showed the effects of crab percentage on the water activity of the pastas. However, pasta containing 20% crab mince with additives had a significantly ($p < 0.05$) lower water activity than pasta containing 10% crab mince with additives based on multi-way ANOVA. When additives were utilized, the difference in water activity between pasta containing 10% and 20% crab mince was greater ($p < 0.01$) compared to when additives were not utilized. The addition of colorant to the pasta did not result in a significant effect on the water activity, however, a combination of percent crab and the addition of red colorant did have a significant ($p < 0.01$) effect on the water activity of the pasta based on two-way ANOVA. Pastas that contained red colorant had smaller differences in water activity between treatments than pastas without red colorant.

pH

Percent crab mince and additives had a significant effect on the pH of the pasta samples based on multi-way ANOVA (Table 44). The addition of 20% crab mince to pasta resulted in significantly ($p = 0.000$) higher pH than the pasta containing 10% crab mince. Pasta containing crab mince with additives had a significantly ($p = 0.000$) lower pH than pasta containing crab mince without additives. The difference in pH between pasta containing 10% or 20% crab mince was larger ($p < 0.05$) when the crab mince did not contain additives compared to crab mince which contained additives. The addition of red colorant had no significant effect on the pH of the pastas.

TVBN

When comparing TVBN concentrations of pasta containing 10% or 20% crab mince with or without additives, pasta with 20% crab mince had higher TVBN levels compared to the pasta containing 10% crab mince but the differences were not significant based on multi-way ANOVA (Table 44). When comparing TVBN concentrations of pasta containing 10% or 20% crab mince with or without red colorant, pasta with 20% crab mince had significantly ($p = 0.000$) higher TVBN levels compared to the pasta containing 10% crab mince based on multi-way ANOVA. Pasta containing crab mince with additives had a slightly lower TVBN concentration compared to pasta containing crab mince without additives although the difference was not significant. The addition of red colorant to pasta resulted in significantly ($p < 0.05$) lower TVBN concentrations compared to pasta without red colorant.

Table 44. Water Activity, pH, and TVBN of Fresh Pasta*

Treatment Code**	a_w	pH	TVBN (mg N/ 100g)
10CR	0.94 ± 0.00 ^b	7.01 ± 0.11 ^b	4.80 ± 0.66 ^{ab}
20CR	0.94 ± 0.00 ^b	7.57 ± 0.02 ^d	5.02 ± 0.31 ^b
10CR-D-L	0.95 ± 0.00 ^b	6.87 ± 0.05 ["]	4.47 ± 0.23 ^{ab}
20CR-D-L	0.93 ± 0.00 ["]	7.20 ± 0.05 ["]	5.13 ± 0.19 ^b
10CR-D-L-R	0.94 ± 0.00 ["]	6.90 ± 0.01 ^{ab}	3.92 ± 0.16 ^a
20CR-D-L-R	0.93 ± 0.00 ["]	7.21 ± 0.01 ^c	4.89 ± 0.29 ^b
p value	0.000	0.000	0.012

* Each value is the average of three analyses ± standard deviation. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** 10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L = Pasta containing 20% crab mince with 0.08% diacetyl plus 2% lactic acid; 10CR-D-L-R = Pasta containing 0.05% red colorant and 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L-R = Pasta containing 0.05% red colorant and 20% crab mince with 0.08% diacetyl plus 2% lactic acid.

Microbial Analyses of Pasta Containing Crab Mince with Additives

Pasta containing 20% crab mince had higher microbial counts than pasta containing 10% crab mince, although the difference was not significant (Table 45). Pasta containing crab mince with additives had significantly ($p < 0.05$) higher microbial counts than pasta containing crab mince without additives. Pasta containing red colorant had higher microbial counts than pasta without red colorant although the difference was not significant. Yeast and mold counts for all pasta samples were less than 100 CFU/g (Table 45). There were no significant effects of treatment of yeast and mold growth.

Table 45. APC, Yeast, and Mold Counts of Fresh Pasta*

Treatment Code**	APC (log CFU/ g)	Yeasts (CFU/ g)	Molds (CFU/ g)
10CR	2E+04 ± 3E+03	< 100	< 100
20CR	3E+04 ± 4E+03	< 100	< 100
10CR-D-L	4E+04 ± 2E+04	< 100	< 100
20CR-D-L	4E+04 ± 1E+04	< 100	< 100
10CR-D-L-R	2E+04 ± 1E+04	< 100	< 100
20CR-D-L-R	1E+05 ± 1E+03	< 100	< 100
p value	0.538	0.458	0.389

* Each value is the average of three analyses ± standard deviation.

** 10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L = Pasta containing 20% crab mince with 0.08% diacetyl plus 2% lactic acid; 10CR-D-L-R = Pasta containing 0.05% red colorant and 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L-R = Pasta containing 0.05% red colorant and 20% crab mince with 0.08% diacetyl plus 2% lactic acid.

Physical Analyses of Pasta Containing Crab Mince with Additives

Cooking Loss

There were significant ($p < 0.05$) differences in cooking loss based on a one-way ANOVA, however, none were observed based on a Tukey's post hoc test (Table 46). However, multi-way ANOVA indicated that the pastas containing 10% crab mince had significantly ($p < 0.05$) lower cooking loss than pastas containing 20% crab mince. There were no significant effects of additives or colorant on cooking loss of pasta. Cooking loss ranged from 3.3 – 3.9%.

Cooking Weight

There were no significant differences in cooking weight among treatments based on one-way ANOVA. Cooking weight ranged from 95 to 111% (Table 46).

Firmness

There were no significant differences in firmness among treatments based on one-way ANOVA (Table 46). Neither percent crab, additives, nor red colorant had a significant effect on the firmness of the cooked pasta based on multi-way ANOVA. However, there was a trend for the firmness of the pasta to decrease slightly throughout storage.

Table 46. Percent Cooking Weight, Percent Cooking Loss, and Firmness of Fresh Pasta*

Treatment Code****	Cooking Weight (%)**	Cooking Loss (%)***	Firmness (g * cm)
10CR	95 ± 4	3.4 ± 0.3 ^a	0.0024 ± 0.0000
20CR	111 ± 10	3.7 ± 0.2 ["]	0.0024 ± 0.0003
10CR-D-L	111 ± 8	3.3 ± 0.3 ^a	0.0028 ± 0.0002
20CR-D-L	105 ± 2	3.8 ± 0.1 ^a	0.0026 ± 0.0006
10CR-D-L-R	103 ± 3	3.3 ± 0.4 ^a	0.0028 ± 0.0003
20CR-D-L-R	104 ± 3	3.9 ± 0.1 ^a	0.0025 ± 0.0006
p value	0.057	0.039	0.698

* Each value is the average of three analyses ± standard deviation. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** Percent cooking weight = $((\text{cooked wt.} - \text{raw wt.}) / \text{raw wt.}) * 100$.

*** Percent cooking loss = $\{(\text{jar wt.} + \text{cooking loss wt.}) - \text{jar wt.}\} * 4$.

**** 10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L = Pasta containing 20% crab mince with 0.08% diacetyl plus 2% lactic acid; 10CR-D-L-R = Pasta containing 0.05% red colorant and 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L-R = Pasta containing 0.05% red colorant and 20% crab mince with 0.08% diacetyl plus 2% lactic acid.

Width and Thickness

The longer the pasta formulations were extruded from the pasta maker, the more the width and thickness of the noodles decreased (Table 47). The pasta formulations were extruded in the following order: 10CR, 20CR, 10CR-D-L, 20CR-D-L, 10CR-D-L-R, and

20CR-D-L-R. The 20CR-D-L-R treatment had a significantly ($p < 0.05$) smaller thickness than 10CR when they were raw and a significantly ($p < 0.01$) smaller width than 20CR after being cooked. 10CR-D-L-R had a significantly ($p < 0.01$) smaller width than 10CR and 20CR after being cooked based on one-way ANOVA. Percent crab had no effect on the width and thickness of the raw or cooked pasta batches. Pasta containing crab mince with additives had smaller raw and cooked width and thicknesses than pasta without additives, although the differences were not significant. Pasta containing red colorant had smaller width and thicknesses than pasta without red colorant, which was significantly ($p < 0.01$) different when comparing the width of the cooked noodles.

Table 47. Width and Thickness of Raw and Cooked Fresh Pasta*

Treatment Code**	Raw		Cooked	
	Width (mm)	Thickness (mm)	Width (mm)	Thickness (mm)
10CR	7.72 ± 0.11	0.94 ± 0.02 ^b	10.19 ± 0.15 ^{bc}	1.17 ± 0.03
20CR	7.72 ± 0.00	0.88 ± 0.03^{ab}	10.32 ± 0.17 ^c	1.10 ± 0.09
10CR-D-L	7.73 ± 0.02	0.83 ± 0.03 ^{ab}	10.15 ± 0.21 ^{abc}	1.05 ± 0.03
20CR-D-L	7.70 ± 0.08	0.85 ± 0.06^{ab}	10.07 ± 0.25 ^{abc}	1.11 ± 0.07
10CR-D-L-R	7.60 ± 0.22	0.84 ± 0.04 ^{ab}	9.66 ± 0.18 ["]	1.08 ± 0.07
20CR-D-L-R	7.61 ± 0.10	0.83 ± 0.04 ["]	9.79 ± 0.09 ^{ab}	1.10 ± 0.07
p value	0.555	0.038	0.006	0.429

Each value is the average of three analyses ± standard deviation. Means in the same column not sharing a superscript are significantly ($p < 0.05$) different based on one-way ANOVA followed by Tukey's post hoc test.

** 10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L = Pasta containing 20% crab mince with **0.08%** diacetyl plus 2% lactic acid; 10CR-D-L-R = Pasta containing 0.05% red colorant and 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L-R = Pasta containing 0.05% red colorant and 20% crab mince with 0.08% diacetyl plus 2% lactic acid.

Instrumental Color

Percent crab, additives, and the addition of colorant to the pasta had a significant effect on the color of the raw and cooked pastas based on one-way ANOVA (Table 48). Pasta containing 20% crab mince with additives had significantly ($p < 0.05$) lower "L" values than pasta containing 10% crab mince with additives after being cooked based on multi-way ANOVA. Pasta containing 10% crab mince and red colorant had significantly ($p < 0.01$) lower raw "L" values and significantly higher ($p < 0.05$) cooked "L" values based on multi-way ANOVA. The addition of diacetyl to the crab mince had no effect on the raw and cooked "L" values of the pasta based on multi-way ANOVA. Raw and

cooked pasta containing red colorant had significantly ($p = 0.000$) lower “L” values than pasta without red colorant based on multi-way **ANOVA**.

Raw and cooked pasta containing 10% crab mince with additives had lower “a” values than pasta containing 20% crab mince and the difference was significant ($p < 0.01$) after being cooked. Pasta containing 10% crab mince with additives and colorant had significantly ($p = 0.000$) higher raw “a” values and significantly ($p = 0.05$) lower cooked “a” values than pasta containing 20% crab mince and colorant. There was no significant effect of additives alone, however, the combination of percent crab and additives had a significant ($p < 0.05$) effect on the “a” value based on multi-way **ANOVA**. As the percentage of crab increased, the difference in “a” values between pasta containing 10% or 20% crab mince with additives were smaller than pasta containing 10% or 20% crab mince without additives. Raw and cooked pastas that contained red colorant had significantly ($p = 0.000$) higher “a” values than pasta without red colorant based on one-way and multi-way **ANOVA**. Raw pasta containing red colorant and 10% crab mince had a significantly ($p = 0.000$) higher “a” values than pasta containing red colorant and 20% crab mince based on multi-way **ANOVA**. However, after being cooked, pasta containing red colorant and 20% crab mince had a significantly ($p = 0.000$) higher “a” values than pasta containing red colorant and 10% crab mince.

Raw pasta containing 20% crab mince had higher “b” values than pasta containing 10% crab mince and the differences were significant ($p = 0.001$) after being cooked based on multi-way **ANOVA** comparing pasta containing crab mince with and without additives. Raw and cooked pasta containing 20% crab mince had significantly

($p = 0.000$) higher “b” values than pasta containing 10% crab mince based on multi-way ANOVA comparing pasta containing crab mince and colorant and the difference was significant ($p = 0.001$) after being cooked. There was no significant effect of additives on “b” values of raw or cooked pastas. Raw and cooked pasta containing red colorant had significantly ($p = 0.000$) lower “b” values than pasta without red colorant. As percent crab increased, the difference between pasta containing 10 or 20% crab mince was significantly ($p = 0.01$) apparent in raw and cooked pasta containing red colorant compared to pasta without red colorant.

Table 48. Colorimetric Values of Raw and Cooked Fresh Pasta*

Treatment Code**	Raw			Cooked		
	L	a	b	L	a	b
10CR	50.1 ± 0.2 ^c	1.0 ± 0.2 ^a	18.4 ± 0.4 ^{cd}	66.4 ± 0.7 ^b	-1.9 ± 0.3 ^a	14.8 ± 1.1 ^b
20CR	49.7 ± 0.6 ^c	1.1 ± 0.1 ^a	18.0 ± 0.6 ^{cd}	64.5 ± 0.5 ^b	-0.7 ± 0.4 ^a	17.9 ± 0.9 ^c
10CR-D-L	49.1 ± 0.4 ^c	1.1 ± 0.1 ^a	17.6 ± 0.4 ^c	66.3 ± 1.1 ^b	-1.6 ± 0.1 ^a	14.7 ± 0.8 ^b
20CR-D-L	50.0 ± 0.6 ^c	1.2 ± 0.1 ^a	19.1 ± 0.4 ^d	65.1 ± 1.0 ^b	-1.3 ± 0.4 ^a	16.6 ± 0.5 ^{bc}
10CR-D-L-R	33.7 ± 0.4 ^a	23.2 ± 0.4 ^c	10.6 ± 0.3 ^a	50.1 ± 1.3 ^a	18.1 ± 1.3 ^b	7.6 ± 0.5 ^a
20CR-D-L-R	35.2 ± 0.3 ^b	20.0 ± 0.5 ^b	14.1 ± 0.3 ^b	47.1 ± 2.5 ^a	20.6 ± 1.6 ^c	18.3 ± 1.1 ^c
p value	0.000	0.000	0.000	0.000	0.000	0.000

* Each value is the average of three analyses ± standard deviation.

** 10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince; 10CR-D-L = Pasta containing 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L = Pasta containing 20% crab mince with 0.08% diacetyl plus 2% lactic acid; 10CR-D-L-R = Pasta containing 0.05% red colorant and 10% crab mince with 0.08% diacetyl plus 2% lactic acid; 20CR-D-L-R = Pasta containing 0.05% red colorant and 20% crab mince with 0.08% diacetyl plus 2% lactic acid.

Sensory Analyses of Pasta Containing Crab Mince with Additives

Panel members were asked their age before conducting the taste test. The majority of the panelists fell into one of three age groups (Figure 13). Thirty percent were between the ages of 18 – 30 years old, 26% were between the ages of 31 – 43, and 37% were between the ages of 44 – 56. Seven percent of the panelists were between 57 – 69 years. The panel was comprised of approximately 2/3 women and 1/3 men (Figure 14).

Figure 13. Participation by Age (Years)

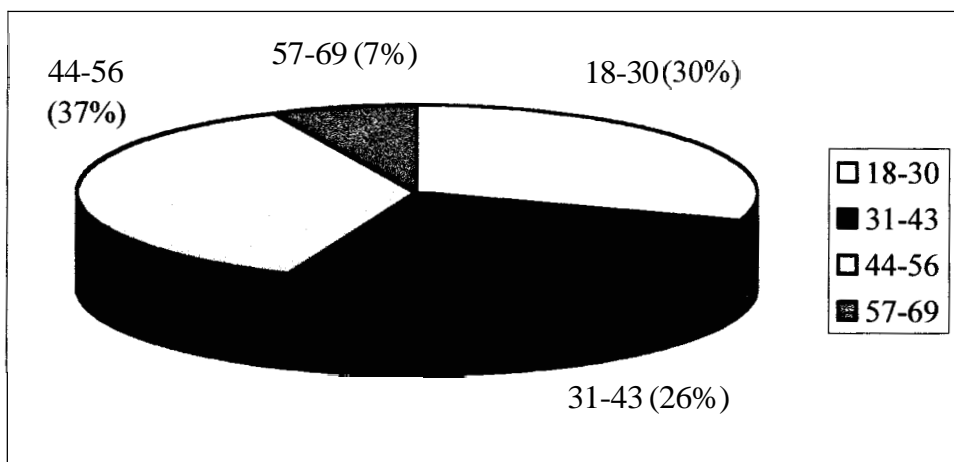
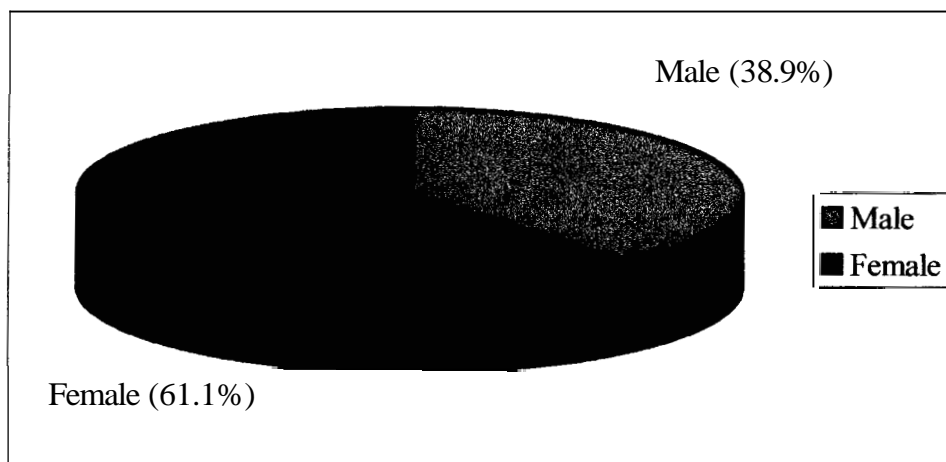


Figure 14. Participation by Gender



When asked how often they ate pasta, the majority of panel members (61%) responded that they ate pasta more than once per week, while 33% of the panel members ate pasta once per week (Figure 15). No one stated that they ate pasta every day or never and 5.6% stated that they ate pasta once per month. When asked what they considered the single most important quality characteristic for pasta 53.7% said flavor while 42.6% said texture (Figure 16). Only 1.9% of the panelists considered nutrition, cost, or **aroma** as the single most important quality characteristic of pasta.

Figure 15. Pasta Consumption Frequency

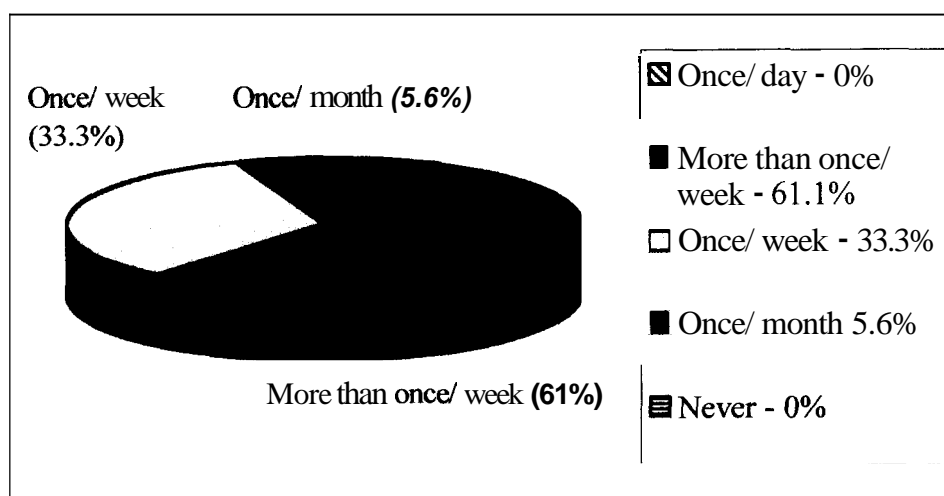
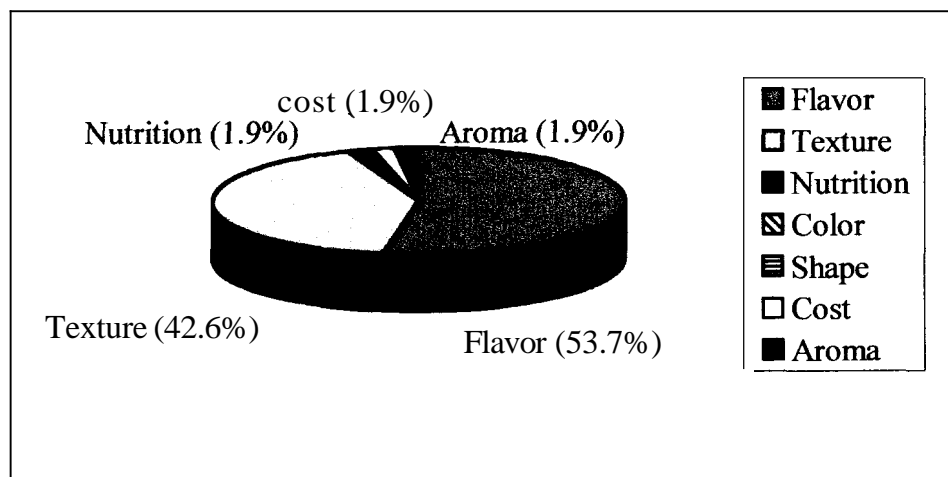
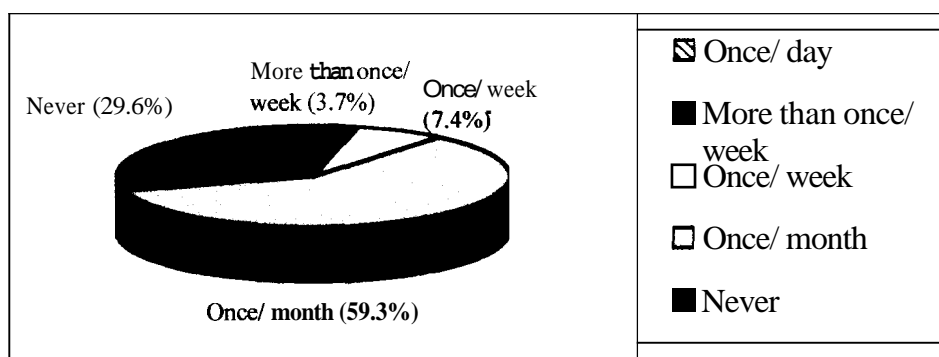


Figure 16. Single Most Important Quality Characteristic for Pasta



Color and shape were not considered the most important attribute by any of the panel members. Panelists were asked how often they purchased colored pasta (Figure 17). The majority (59.3%) of the panel members stated that they bought colored pasta once per month, 29.6% stated that they never bought colored pasta, 3.7% stated that they bought colored pasta more than once per week and 7.4% stated they bought colored pasta once per week. None of the panel members bought colored pasta daily.

Figure 17. Colored Pasta Purchasing Frequency



Panelists were also asked if they liked seafood-flavored pasta (Figure 18). Approximately 91% of the panelists stated that they had never tried seafood pasta, and 9.3% said that they liked it. No panel members said they did not like seafood-flavored pasta. When panelists were asked if they would be interested in purchasing a pasta product that contains seafood, 46.3% said yes, 53.7% said maybe, and 0% said no (Figure 19).

Figure 18. Do You Like Seafood-flavored Pastas?

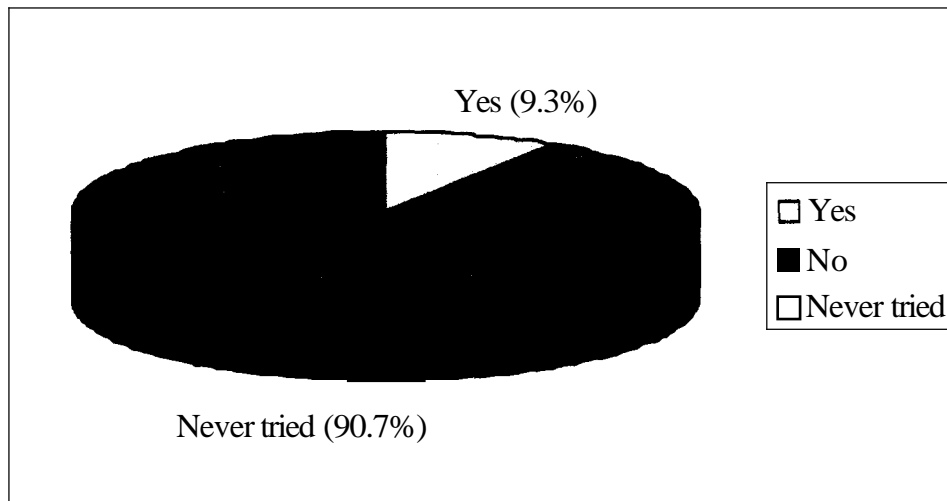
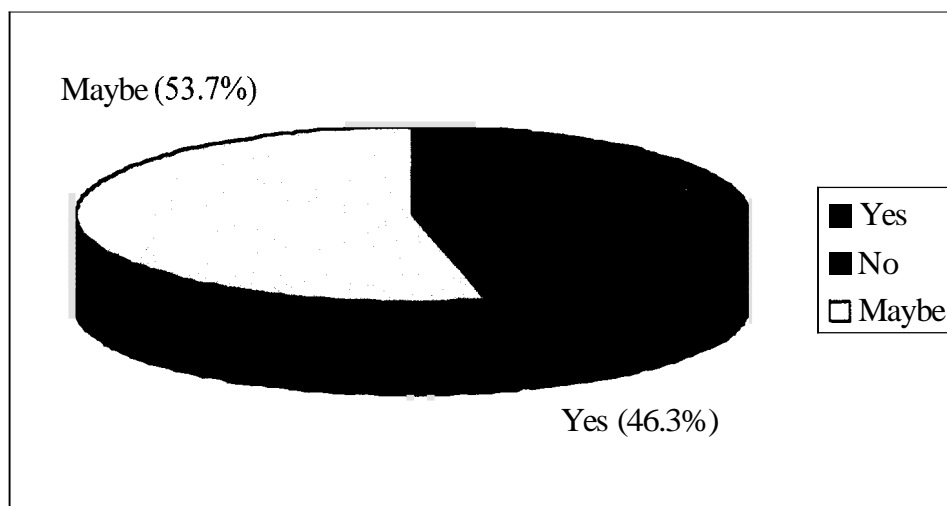


Figure 19. Would You Be Interested in Purchasing a Pasta Product that Contains Seafood?



Panel members were given six pasta samples to evaluate which included: pasta containing 10% crab mince (10CR), pasta containing 20% crab mince (20CR), pasta containing 10% crab mince with 0.08% diacetyl plus two percent lactic acid (10CR-D-L), pasta containing 20% crab mince with 0.08% diacetyl plus two percent lactic acid (20CR-D-L), pasta containing 0.5% red colorant and 10% crab mince with 0.08% diacetyl plus

two percent lactic acid (**10CR-D-L-R**), and pasta containing 0.5% red colorant and 20% crab mince with **0.08%** diacetyl plus two percent lactic acid (**20CR-D-L-R**). The panelists were asked to rank each sample's color, aroma, flavor, texture, and overall acceptability on a 9-point hedonic scale (Table **49**). Neither percentage crab mince, nor presence of additives significantly affected the characteristics of the pasta as determined by sensory analyses. **All** of the samples received scores of **6.7** for color, with the exception of **10CR-D-L-R** treatment, which had a significantly ($p < 0.05$) lower color score of **5.3**. The **10CR** and **10CR-D-L** pasta treatments both received the highest score for aroma, which was **6.7**, although there were no significant differences among the samples. The **10CR-D-L** treatment received the highest score for flavor, which was **6.8**, although there were no significant differences among the samples. Scores for texture ranged from **6.1 – 6.8**, again no significant difference among treatments. The “overall acceptability” scores for the pasta ranged from **6.1 – 6.3**, which corresponded with “like slightly” on the ballots. Overall, the highest average hedonic score (mean = **6.5**) was obtained for flavor while the characteristic texture received the lowest average score (mean = **6.4**.) Panel members were also asked to give comments on the pasta sample's attributes so that the product could be further developed to the consumer's liking. **As** far as texture was concerned, many of the panel members were able to detect crab shell particulate in the pasta and commented that the pasta had a “gritty texture”. However some panel members thought the texture was great while others thought that it had a “mushy” or “chewy” consistency. In general, most panelists commented that they liked the flavor of the pasta samples, however, the pastas did not have a seafood flavor and tasted more like “plain pasta”. One panelist who commented about **10CR** stated, “This sample is definitely my favorite. It

smells and tastes like regular pasta.” **Most** of the comments about color were given for the 10CR-D-L-R and 20CR-D-L-R treatments, which had red colorant in the formulations. Based on the panelists comments, more panel members disliked the color of 10-CR-D-L-R than those who liked the color. This agreed with the very low numerical score received by that treatments. One panel member commented, “The color needs to look a little darker instead of pale red.” However, quite a few panelists liked the color and one panel member stated, “Like the pink color. Not much for colored products, but this one matches up well with the seafood concept.” Of the panel members who commented about the color of the 20CR-D-L-R treatment, more panel members liked the color than those who did not. One panel member stated that the “color was very appealing”. There were very few comments regarding aroma or overall acceptability of the pasta products.

Table 49. Mean Scores of Pasta Characteristics Based on a 9-Point Hedonic Scale*

Treatment Codes**	Pasta Characteristics**				
	Color	Aroma	Flavor	Texture	Overall
10CR	6.6 ± 1.6 ^b	6.7 ± 1.5	6.7 ± 1.7	6.8 ± 1.5	6.3 ± 1.8
20CR	6.7 ± 1.5 ^b	6.3 ± 1.6	6.5 ± 1.7	6.3 ± 1.7	6.2 ± 1.7
10CR-D-L	6.7 ± 1.6 ^b	6.7 ± 1.3	6.8 ± 1.4	6.3 ± 2.0	6.1 ± 1.9
ZOCR-D-L	6.7 ± 1.3 ^b	6.5 ± 1.4	6.6 ± 1.3	6.1 ± 1.6	6.3 ± 1.5
10CR-D-L-R	5.3 ± 2.2 ^a	6.5 ± 1.2	6.5 ± 1.5	6.4 ± 1.6	6.2 ± 1.6
20CR-D-L-R	6.7 ± 2.3 ^b	6.4 ± 1.6	6.2 ± 1.9	6.5 ± 1.6	6.2 ± 1.9

** 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely

Each value is the mean score of 53 panelists ± standard deviation. Means in the same column not sharing a superscript are significantly

*** (p < 0.05) different based on one-way ANOVA followed by Tukey's post hoc test.

10CR = Pasta containing 10% crab mince; 20CR = Pasta containing 20% crab mince; 10CR-D-L = Pasta containing 10% crab mince with **0.08%** diacetyl plus 2% lactic acid; 20CR-D-L = Pasta containing 10% crab mince with **0.08%** diacetyl plus 2% lactic acid; 10CR-D-L-R = Pasta containing 0.05% red colorant and 10% crab mince with **0.08%** diacetyl plus 2% lactic acid; 20CR-D-L-R = Pasta containing 0.05% red colorant and 20% crab mince with **0.08%** diacetyl plus 2% lactic acid.

DISCUSSION

Mechanical Separation of Crab Mince

Due to technical difficulties, mechanical separation of mince from crab legs resulted in smoke formation and overheating of the machine while mechanical separation of the crab carapace did not. This was probably due to the harder and thicker nature of the leg shell **as** compared to the shell of the carapace. Therefore, mechanical separation of legs is not recommended. Deboning the crab carapace, however, was also somewhat challenging. Due to the smooth oval structure of the crab carapace, the cylinder teeth were not always able to grip the body to push it around the cylinder for crushing. Thus, the bodies had to be broken into smaller, non-uniform shaped pieces to facilitate grinding and meat separation. Another technical difficulty of using the mechanical separator was that the crabs had to be continually fed into the hopper. In the initial testing phase some of the crab carapaces had to be scraped out of the cylinder because a lag time of 30 minutes had passed while additional crab was being prepared for the separator. During that 30 minute time period, meat and shell dried in the cylinder microslits, thereby blocking minced product from passing through. With continual operation of the mechanical separator, the meat and shell within the microslits did not have a chance to *dry* and harden because new "wet" product was constantly being ground. Since continual operation was needed, two workers were required to maintain the flow of the crabs into the hopper. Although the processing was continuous, as processing time increased, the machine did have to be shut off occasionally **as** semi-ground crab by-product collected on top of the rotating cylinder without subsequent grinding and meat-shell separation. This may have been due to the end plate being only one-quarter open. If opened further the

collection of semi-ground crab by-product may not have occurred. If the end plate were open wider, however, there would likely be a decrease in minced meat yield, although the separator may operate more smoothly.

Meat yield is dependent on the bar breaker setting, end plate setting, and amount of meat available in the crabs. In an initial study, optimal yield was recovered with an end plate setting of one-quarter open and a bar breaker setting of 0.120. These parameters were used for all subsequent studies. Mechanical separation of crab bodies resulted in minced meat yield ranging from 44 - 69%, an average of 70%, and an average of 66% for the second, third, and fourth studies, respectively. The lower average meat yield achieved in the second study may have been due to the collection and measurement of the initial crab mince coming out of the machine. The manufacturers of the Paoli One-Step Deboner indicated, through personal communication, that optimal meat yield occurs after at least 10 pounds of product have passed through the machine. This allows the microgrooves to become filled with meat and the yield to reach equilibrium. The lower yield may also be attributed to seasonal differences in the crab. Crabs utilized for the second study, "Effects of Additives on the Shelf-life of Mechanically Separated Crab Mince" were harvested in late August compared to the crabs utilized for the third and fourth study, which were harvested in January and April. Gates and Parker (1992) were able to mechanically extract 22% crab meat based on the uncooked Blue crab weight. Lee et al. (1993) were able to extract 50% meat from composite blue crab processing by-product when gills were removed. The difference between their meat yield data and ours is probably due to the starting product. The crab bodies, in our study, had been delimbed, debacked, eviscerated, and degilled. If our yield were based on the uncooked weight of the crabs it

would be much lower. The differences in meat yield may also be due to the different crab species utilized. Both Lee et al. (1993) and Gates and Parker (1992) used Blue crabs while our study utilized Jonah crabs.

The average meat yield from all studies obtained by mechanical separation was 64%. Feasibility studies would need to be conducted to determine the total cost associated with mechanical separation of crab "waste" to ensure that it would result in a net profit.

Shelf-life of Mechanically Separated Crab Mince with Additives

Proximate and Mineral Analyses

Mechanical separation of crab mince resulted in a moisture content of 72 - 78%, a fat content of **0.9** - 1.5%, and an ash content of 4.5 - 9.9%. Moisture content varied only slightly among the studies, however the average ash content obtained for the "Evaluation of Mechanically Separated Crab Mince" was much higher than in the latter studies. There were no notable differences in nutritional content of crab mince obtained from the legs or carapace. The differences among the studies, although slight, may be due to seasonal variation in the crabs. Jonah crab meat that ~~has~~ not been mechanically deboned has been shown to contain approximately 78% moisture, 16% protein, 1 - 2% fat, and 1.5% ash (Krzynowek et al., 1982). These results correlate well with our moisture and fat values for mechanically separated crab mince. The ash values of mechanically separated Jonah crab mince were much higher than for Jonah crab meat that had not been deboned. This increase in ash content was expected. Lee et al. (1993) reported ash values of 7.13 – 11.99% for deboned Blue crab mince. With an increase in ash content, it is expected that there will be an increase in specific mineral contents. Lee

et al. (1993) reported calcium contents ranging from **12.65 - 37.80** mg/g for deboned Blue crab meat. Results from our studies were similar to these findings. Calcium contents of deboned Jonah crab meat ranged from **5013 - 31482** mg/kg making it the most abundant of the selected minerals analyzed. Potassium, magnesium, phosphorus, and sodium contents ranged from **2312 - 2987, 887 - 2013, 2139 - 3047** and **1891 - 3184** mg/kg, respectively. The first study conducted, which was the “Mechanical Separation of Crab Mince”, resulted in mince with much higher mineral content than in the subsequent studies. There were slight differences in mineral concentrations throughout all studies, but this was probably due to seasonal variation in the crab meat and perhaps shell hardness. The higher mineral content from the first study may indicate seasonal variation as well, although it may also be attributed to metals being scraped off the mechanical separator by the shell. This would also increase the ash content slightly. Perhaps, after the machine was “seasoned”, the ash values reflected only the mineral content in the crab mince whereas mince produced during the first use may have reflected additional metals scraped off the machine.

pH

The pH of the crab mince for all studies ranged between **8.3** and 8.6. pH is often used **as** an indicator of seafood quality. During initial storage, meat pH typically drops **as** glycogen stores are converted to lactic acid and then there is typically an increase **as** protein and non-protein nitrogen are degraded, releasing basic components such as ammonia, TMA, free amino acids, and amines. The shelf-life study of crab mince, “Mechanical Separation of Crab Mince”, showed a decrease in pH for both mince extracted from carapace and legs and then an increase in pH for the carapace samples

throughout storage. The differences between the pH of the carapace mince meat and the leg minced meat were not extensive.

In the "Effect of Additives on the Shelf-life of Crab Mince" study the pH of the control mince decreased from **8.5** to 7.4 throughout the eight day refrigerated storage period. The addition of lactic acid to crab mince resulted in significantly lower pHs compared to the pHs of the control crab mince for each study. These results are similar to other reported findings (Dorsa et al., 1997; Marshall and Jindal, 1997). Marshall and Jindal (1997) reported an initial decrease in pH of 0.2 - 0.4 in catfish fillets dipped in 1 - **4%** acetic acid and lactic acid treatments. Our results indicated an initial decrease in pH of 0.9 for crab mince containing additives including lactic acid compared to crab mince with no additives, and 0.5 - 0.7 in crab mince that contained additional additives besides lactic acid. The sodium lactate treatment was not significantly different from the control, which is similar to findings reported for the **use** of sodium lactate in meat products (Williams et al., 1995; Shelef et al., 1997). The addition **of** rosemary to crab mince resulted in significantly lower pH compared to the control crab mince, while the addition of diacetyl resulted in significantly higher pH compared to the control crab mince. However, when a combination of diacetyl and lactic acid **was** incorporated into the crab mince, it resulted in a significantly lower pH than the control crab mince. This was primarily due to the addition of the lactic acid.

TVBN

Total volatile base nitrogen is also **an** important indicator of seafood quality. **As** protein and non-protein nitrogen, such **as** nucleotides, trimethylamine oxide, and peptides are broken down due to microbial degradation, there is an increase in ammonia, TMA

and DMA. During storage, TVBN concentrations increased in minced meat obtained from the carapace and legs of crabs, although the differences between the two were not notable. TVBN concentrations for this study ranged from an average of **16.9 mg N/ 100 g** to **21 1.8 mg N/ 100 g**. Similarly, TVBN concentrations also significantly increased in crab mince with additives during refrigerated storage ranging from an average of **18 mg N/ 100g** to **37mg N/ 100 g**. The difference between the two studies may be due to higher microbial activity throughout the first shelf life study. The TVBN values may also reflect differences in microbial flora within the crab mince. The addition of lactic acid significantly reduced TVBN concentrations compared to crab mince without lactic acid. The addition of sodium lactate and diacetyl also significantly reduced TVBN concentrations compared to the control. The efficacy of these additives in reducing TVBN concentrations, may be due to their inhibition of microbial growth. In contrast, crab mince with rosemary had higher TVBN concentrations than the control. This may be due to bacteria in the rosemary participating in the breakdown of proteins and non-protein nitrogen.

In the third study conducted, which evaluated the shelf-life of pasta containing crab mince, crab mince had lower TVBN concentrations compared to the crab mince from the first two studies. Values from the third study ranged from **17.0 and 20.5 mg N/ 100g** on day two. The addition of additives to the crab mince had no significant effect on the TVBN concentrations of the pasta, which was expected since the additives were applied only two days prior to conducting TVBN analyses and the initial mince quality (based on microbial load) was very high. The crab mince utilized in the fourth study,

“Sensory Analyses and Evaluation of Pasta Containing Crab Mince with Additives” had initial TVBN concentrations of 10.3 mg N/ 100g and 10.8 mg N/ 100 g, respectively, for the crab mince without additives and crab mince with 0.08% diacetyl plus 2% lactic acid. Similar to the third study, there was no significant difference in the concentration of these two mince batches because they were evaluated at day 3 and the microbial load was very relatively low.

TBARS

TBARS levels in crab mince were only measured in the study “Mechanical Separation of Crab Mince” and “Effect of Additives on the Shelf-life of Crab Mince”. TBARS is a measure of malondialdehyde, which is a secondary product of lipid oxidation. TBARS levels ranged from 0.06 - 1.29 $\mu\text{g/g}$ and 0.79 - 1.71 $\mu\text{g/g}$, in both studies, respectively. These low values were expected since the crab mince contained only 1 - 2% fat. The TBARS concentrations were negligible and did not indicate that lipid oxidation was a primary source of degradation. There were no notable differences in TBARS values between crab mince extracted from the carapace or legs.

In the second study, “Effect of Additives on the Shelf-life of Crab Mince”, the addition of lactic acid significantly reduced TBARS concentrations compared to crab mince that was not treated with lactic acid. Crab mince that contained diacetyl and sodium lactate also had significantly lower TBARS concentrations than the control. Williams et al. (1995) also noticed significantly lower TBARS concentrations in catfish fillets treated with 2% sodium lactate. Sodium lactate is recognized as a chelator of metals in foods, may potentially stabilize fatty acids, and reduces lipid oxidation in foods (Shelley, 1994). These results would be expected when using sodium lactate because of its

documented antioxidant properties, however, there has not been a significant amount of research investigating lactic acid and diacetyl **as** antioxidants. It would be interesting to determine the mode of action that these additives use to decrease lipid oxidation in media or seafood systems. Interestingly, dried rosemary did reduce TBARS concentrations although the reduction was not significant. Vareltzis et al. (1997) noted significantly lower **MDA** levels when rosemary extract was incorporated **as** a dip for fillets and mince of mackerel (a fatty fish) and hake (a lean fish). Our TBARS results were not significant, however, this may have been due to the level of fat in crab versus fish. There was not much lipid oxidation in the crab mince *so* any inhibition may have been so slight that it was not significant.

Microbial Analyses

As previously stated in the literature review, processing facilities typically produce crab meat containing approximately 10^5 CFU/ g (Wentz et al., 1985), however, mechanical separation reduces the shelf life **of** minced crab. Raccach and Baker (1978) reported a 10-fold increase in microbial counts after fish by-product was mechanically deboned. Lee et al. (1993) reported that mechanical separation increased the mesophilic and psychrotrophic counts in composite Blue crab by-product between 10^4 - 10^7 CFU/ g and estimated a shelf-life for Blue crab mince of two days. **As** days storage increased, microbial counts of crab mince in both our shelf-life studies evaluating crab mince and pasta containing crab mince significantly increased. This increase in microbial counts has been reported in a variety of meat and seafood products (Sun and Oliver, 1994; Shelef et al., 1997; Williams and Phillips, 1998). Mechanical separation of crab meat for "Mechanical Separation of Crab Mince" resulted in microbial counts of 10^6

CFU/ g on day one and by day four microbial counts had reached 10^7 - 10^9 CFU/ g. If 10^7 CFU/g is considered the maximum microbial count for the acceptable quality of crab meat, the mince in this study had a shelf life of between 2 - 3 days, which is similar to the findings of Lee et al. (1993). Mechanical separation of crab meat for “Effect of Additives on the Shelf-life of Crab Mince” resulted in microbial counts of 10^7 CFU/ g at day one and 10^8 CFU/ g by day eight. This crab mince had a one-day shelf-life. In contrast, microbial sampling of crab mince utilized in the “Development and Quality of Pasta Containing Crab Mince with Additives” on day one indicated that the mince had lower than 10^4 CFU/ g. The crab mince was retested for microbial load and at day four of refrigerated storage the microbial counts were 10^5 CFU/ g. Similarly, batches of crab mince utilized for the “Sensory Analyses and Evaluation of Pasta Containing Crab Mince with Additives” which was tested for microbial counts at day three were 10^5 CFU/ g. The difference in initial microbial load for the first and second study compared to the third and fourth study is likely due to several reasons. In order to improve the sanitary condition of the mechanical separator for the third and fourth study, all parts of the mechanical separator were sanitized with a commercial sanitizer and allowed to dry for about an hour before product was deboned. It has been noted that one of the primary sources of microbial contamination is from the processing equipment (Chiasson et al., 1998). The sanitizer killed viable microorganisms that were on and in the machine. Our microbial counts were lower than those reported by Gates and Parker (1992), who indicated that hourly clean up of the separator improved microbial counts of Blue crab mince, yet after separation counts were still between 10^5 - 10^7 CFU/ g. This difference may be due to the actual cleanliness of the facility during processing, the effectiveness of

the sanitizer, and the initial microbial load of the crabs prior to being mechanically separated.

Additives applied to the crab mince had a significant effect on the microbial load of the mince. The addition of lactic acid reduced the microbial load of crab mince by $\frac{1}{2}$ log although the reduction was not significant. Marshall and Kim (1996) reported that dipping catfish fillets in 3 - 4% lactic acid for 5 - 60 seconds extended their shelf-life up to 12 days and the difference between the mesophilic and psychrotrophic counts of fillets treated with lactic acid and the control was 1.9 log. Perhaps the difference between their results and ours is the high concentration of lactic acid utilized as well as the different types of products, which will affect the distribution and types of microbes. Additionally, the pH of the crab mince was much higher than for typical fish fillets, thus the addition of lactic acid, although it reduced the pH of the mince, was still above neutral. This would still be a comfortable environment for bacteria to grow. Perhaps the increased cell membrane permeabilization by lactic acid theorized by Alakomi et al. (2000) may have resulted in inhibition of bacterial growth throughout storage.

The addition of 5% rosemary plus 2% lactic acid resulted in significantly ($p < 0.01$) lower microbial counts in crab mince during refrigerated storage. The antimicrobial efficacy of rosemary may be due to the activity of polar phenol compounds (Del Campo et al., 2000), although more investigation is needed. It may also be due to the efficacy of lactic acid in invading gram-negative cells and allowing other antimicrobials to enter the cell and disrupt cellular functions (Alakomi et al. 2000). It had been noted that rosemary does not have an antimicrobial effect on gram negative bacteria (Del Campo et al., 2000; Farag et al., 1989) and that this may be due to the lipopolysaccharide

wall that inhibits fatty acids from entering the cell (Ouattara et al., 1997). However, if lactic acid were able to disrupt the cell membrane, permitting compounds in rosemary to enter, then it might be able to inhibit microbial growth. Interestingly, the addition of 2% rosemary plus 2% lactic acid also reduced microbial counts compared to crab mince with 2% rosemary only, however, the reduction was not significant. It has been reported by Shelef et al. (1980) that as the concentration of ground rosemary increases in media, the antimicrobial efficacy also increases. These results were not found in our study which may be due to the different concentrations of rosemary tested between the studies. Shelef et al. (1980) utilized a different range of concentrations, from 0.3% to 2% of finely ground rosemary. Perhaps our range, of 2-5% rosemary, was not wide enough to see a difference between treatments. Additionally, the antimicrobial efficacy of rosemary in Shelef's study was conducted in media, whereas the antimicrobial efficacy of rosemary in our study was conducted in crab mince.

The addition of 0.08% diacetyl to crab mince significantly ($p < 0.01$) reduced the microbial growth of the crab mince to 6.5×10^7 CFU/ g throughout refrigerated storage. The addition of 0.08% diacetyl plus 2% lactic acid to crab mince also resulted in lower microbial counts when the mince was used for the third and fourth studies, "Development and Quality of Pasta Containing Crab Mince with Additives" and "Sensory Analyses and Evaluation of Pasta Containing Crab Mince with Additives". Sun and Oliver (1994) noted that 0.05% diacetyl did not significantly reduce APC counts of oysters. However, Jay (1982b) reported that 400 ppm (0.04%) diacetyl injected into ground beef inhibited growth of lactic acid bacteria, non-lactic gram positive bacteria, nonpseudomonad gram-negative bacteria, yeasts, and molds when stored at refrigerated temperatures up to 8

days. He also stated that pH had a significant effect on the efficacy of different concentrations of diacetyl. At pH **5.0**, microbial growth was substantially inhibited by diacetyl compared to at pH **8.0**. Perhaps the addition of **800** ppm diacetyl in our study was high enough to have **an** antimicrobial effect on the bacteria even at a pH above neutral. No mode of action was reported for diacetyl, however, it **has** been noted to have antimicrobial efficacy especially against gram negative bacteria. It would be interesting to evaluate the mode of action of diacetyl as a permeabilizer of gram-negative bacteria as suggested by Alakomi et al. (2000).

The addition of sodium lactate significantly reduced the microbial growth of the crab mince. In fact, initial counts at day one were 10^6 CFU/ g, which were lower than control counts and they decreased to 10^5 CFU/ g before increasing to 10^6 CFU/g by day eight. Williams et al. (1995) noted that the addition of **2%** sodium lactate **as** a dip to catfish fillets resulted in **a** shelf-life extension of 4 to 7 days compared to the control. Shelef et al. (1997) noted that the addition of **2%** sodium lactate to ground beef held in refrigerator storage increased the shelf life by **2** - 4 days compared to the control. The addition of **2%** sodium lactate increased the shelf-life of the crab mince in our study by **7+** days. Perhaps one of the reasons for sodium lactate's efficacy is its ability to maintain its antimicrobial efficacy above a neutral pH whereas most antimicrobials work better at a lower pH (Williams and Phillips, 1998). **As** noted in the literature review, sodium lactate is a weak lipophilic acid and it can pass through the cell membrane in the undissociated form and dissociate within the cell to acidify the cell interior.

Molds, yeasts, and coliforms in crab mince were only analyzed in the "Mechanical Separation of Crab Mince" study. Typically, these microbes are not

considered the major spoilers of seafood products and are usually not analyzed. Coliform counts are an indication of fecal contamination and are sometimes analyzed to evaluate contamination of seafood by the water source or the processing facility and workers. Mold and yeast counts were <100 CFU/g throughout the 11-day storage period. Coliform counts varied and the range was between “not detected” to 1,100 MPN/ g. There were no notable differences in yeast, mold, or coliform counts between crab mince obtained from the carapace or the legs.

Development and Quality of Pastas Containing Crab Mince with Additives

Proximate Analyses

As percent crab mince in the pasta formulations increased, **so** too did the moisture, ash, and fat contents of the pasta. The higher ash and moisture contents compared to the control pastas was expected since the crab mince had 4.5% ash content and an average **80%** moisture. **All** pasta formulations were to contain 25% moisture content, however, in the sensory analyses study, pasta containing 10% crab mince had a 27% moisture content. This may have occurred because the moisture content of crab mince was estimated based on prior moisture contents from previous studies, in which the crab mince had less moisture. The addition of crab mince resulted in significant ($p = 0.000$) increases in calcium and sodium contents and higher levels of magnesium and phosphorus contents in the pasta. This was also expected because of the high mineral content in the crab mince. There were also significantly higher concentrations of calcium and sodium in the pastas **as** result of adding the crab mince. However, the addition of crab mince was not high enough to make the pasta a “good source” of calcium or even a high source of calcium.

Protein contents of the pastas were not determined due to mechanical problems with the Kjeldahl machine. However, fresh pasta typically contains 11.3g of protein/ 100g of sample (USDA, 1999). Since crab meat typically contains 16 g of protein/ 100g, incorporations of crab mince into pasta formulations should increase the protein content.

Water Activity, pH, and TVBN

Water activity values ranged from 0.92 - 0.95 and 0.93 - 0.95 for the pasta evaluated in the “Development and Quality of Pasta Containing Crab Mince with Additives” study and the pasta evaluated in the “Sensory Analyses and Evaluation of Pasta Containing Crab Mince with Additives” study, respectively. Our results correlate well with other water activity results reported, which average approximately ~ 0.93 for fresh pasta (Trovatelli et al., 1988). As percent crab increased, there were no significant differences in water activity between pasta containing 10 or 20% crab mince for the “Development and Quality of Pasta Containing Crab Mince with Additives” study. However, there was a significant decrease in water activity of pasta made with 20% crab mince compared to 10% crab mince in the sensory analyses study. The addition of additives to the crab mince reduced the water activity of the pastas, probably due to the “holding” of available water. However, the addition of red colorant to the pastas had no effect on pasta water activity. Although there were significant differences in water activity results, values fell within a narrow range of **0.92** - 0.95, thus these differences would have no real effect on either microbial growth or consumer acceptability of the pastas.

As percent crab increased, the pH of the pastas containing crab mince significantly increased. This was expected since the crab mince had a higher pH than the

flour dough. The addition of additives to the mince significantly decreased the pH of the pastas. For pasta containing crab mince with **0.08%** diacetyl plus **2%** lactic acid, the decrease was probably due to the addition of lactic acid compared to the addition of diacetyl since diacetyl increased the pH of crab mince. **As** storage time increased, the pH of all pasta treatments increased. This was probably due to degradation of the pasta which released some basic compounds **as** well as the degradation of the protein in the crab mince. Other published shelf-life studies of fresh pasta have typically evaluated microbial quality and have only stated the initial pH and water activity of the product being analyzed. Therefore, the water activity and pH changes in pasta products over time need to be more thoroughly investigated. The addition of colorant to the pasta had no effect on the pH. This was expected because the pH of the colorant was 7.0, which was similar to the pH of the flour dough.

As percent crab increased, pastas containing crab mince had significantly higher TVBN concentrations for the shelf-life study; higher TVBN concentrations were not significant in the pastas evaluated for sensory analyses. This increase in TVBN concentrations was due to microbial degradation of the protein and non-protein nitrogen of the crab mince. TVBN concentrations in the pasta increased from week one to week three of storage and then decreased **from** week three to week five. This was not a consistent trend. The range of TVBN concentrations for the shelf-life study was between 3.5 - **4.9 mg** N/ 100g, which were very low. Similarly, the pasta evaluated for consumer acceptance had initial TVBN concentrations between **3.9** - 5.0 **mg** N/ 100g. Thus, the TVBN concentrations in these studies did not indicate substantial protein and non-protein degradation of the crab mince nor a reduction in consumer acceptance of the pasta. In the

consumer acceptance study, pastas containing red colorant and crab mince had significantly ($p = 0.05$) lower TVBN concentrations than pastas without red colorant. There may be some antimicrobial properties of some of the ingredients used to produce the colorant, however, of the ingredients listed on the specifications sheet including carmine, paprika, and caramel, the author could find no information regarding the use of these ingredients as antimicrobials.

Microbial Analyses

During the shelf-life study of pastas containing crab mince, temperature abuse occurred after **21** days of refrigerated storage with an increase of temperature to 13.5°C. This significantly increased APC and yeast counts and increased mold counts although not significantly. Giannuzzi (1998a) also noted an increase in microbial growth at higher storage temperatures. According to his results, ricotta-filled ravioli had a shelf life of **14.0, 7.2, 6.7, and 1.2** hours when held at **0, 4, 8, and 10°C**, respectively. Before temperature abuse occurred in our study, the APC, yeast, and mold counts remained relatively unchanged in the different pasta treatments. From week one to week three the pastas had APC counts of $10^2 - 10^3$ CFU/g with an increase to $10^5 - 10^6$ CFU/g at week four. Additionally, all yeast and mold counts were below **100** CFU/g through week three and then steadily increased at week four.

The International Commission for Microbiological Specifications has set a standard for APC counts for fresh pasta dumplings of 10^6 CFU/g. Microbial growth throughout the shelf-life study ranged from $10^3 - 10^6$ CFU/g, $0 - 10^3$ CFU/g, and $0 - 10^4$ CFU/g, for APC, yeasts, and molds respectively. Studies looking at the aerobic plate counts of various fresh, filled pastas have reported initial aerobic plate counts between

10^8 CFU/g in ricotta filled raviolis and 10^5 - 10^6 CFU/ g for ricotta and vegetable raviolis (Giannuzzi, 1998b; Lopez et al., 1998). These values are much higher than the initial *APC* counts of our pasta with or without crab mince, however, microbial levels are typically higher in filled products due to the incorporation of several different ingredients, which each increase the microbial level within the final product. Our pasta consisted of only flour, water, and crab mince with either 5% fresh rosemary or **0.08%** diacetyl with 2% lactic acid. The pasta dough in the Giannuzzi (1998b) study had an initial microbial count of 10^4 CFU/g, which was higher than our microbial counts. One of the reasons for the differences in the two studies **is** the moisture content of the dough. In the Giannuzzi study, the targeted moisture content of the pasta dough was **30-31%**, whereas the targeted moisture content in our dough, which consisted **of** the flour, water, and crab mince with additives was 25%. Perhaps the higher moisture content in Giannuzzi's dough provided a better environment for the bacteria to grow and multiply during processing. Our processing environment may have also been cleaner, resulting in less microbial contamination during processing.

APC, yeast, and mold counts were lowest in the control pasta and increased with the addition of crab mince, however, the counts were not significantly different up to three weeks of storage. At week four and five, however, *APC* growth was significantly ($p = 0.000$) higher in pasta containing 20% crab mince than pasta with 10% crab mince. Thus, although the addition of crab mince did increase microbial counts, the growth of the bacteria was inhibited when held at refrigerated temperatures. Throughout the five week storage study, it was finally the temperature abuse that reduced the shelf-life of the pasta, not the addition of crab mince.

The addition of diacetyl plus lactic acid lowered **APC**, yeast, and mold counts compared to pasta containing crab mince without additives although it was only significantly ($p = 0.000$) lower for yeast growth after temperature abuse had occurred. Since the microbial load was so low during the first three weeks of refrigerated storage, the additives did not really make a difference between pasta containing crab mince with additives or without additives until microbial growth had significantly increased. Jay (1982b) noted that at **400 ppm** diacetyl was effective in inhibiting the microbial growth of **13** nonpseudomonad gram-negative bacteria, 16 yeasts, and 6 molds in refrigerated ground beef samples. Additionally, it was stated that only four yeasts were able to grow at pH below 7 with a concentration of **300 ppm** diacetyl while they all grew at pH **8.0**. At **300 ppm** all molds were inhibited except at pH **8.0**, at which they all grew. At **400 ppm** all growth was inhibited at a pH between **5.8 - 5.9**. However, at a pH of 8.0, neither yeasts nor molds were inhibited. Our study utilized **0.08% (800 ppm)** diacetyl, which was a higher concentration than reported by Jay, however, the pH of our pasta samples ranged from 6.02 - 7.67. Thus, at possibly a lower pH this level of diacetyl would have been able to inhibit more of the yeasts and molds when temperature abuse occurred. A higher concentration of diacetyl might also have been more effective.

Pastas containing crab mince with 5% fresh, ground rosemary plus 2% lactic acid had higher **APC** and molds counts although not significant and significantly lower yeast counts compared to pasta containing crab mince without additives. Since yeast counts were so low, inhibition of growth was really only seen after temperature abuse had occurred, similar to the findings when diacetyl and lactic acid were used. Our findings are in contrast with Del Campo et al. (2000) who found that Oxy'less, a rosemary extract,

did not inhibit the growth of two yeasts, *R. gluten* and *C. luurentii*. This difference may be because we used fresh rosemary, whereas they used a rosemary extract. As stated in the literature review, spices are thought to have more of an antimicrobial effect than their extracts. Additionally, the yeasts that were found in filled ravioli were *Candida* species, *Rhodotorula* species, and *Pichia membranaefaciens* (Lopez et al. 1998). Thus, perhaps different species of yeasts are more sensitive to rosemary than others. The fact that both the APC and mold counts were higher in pasta containing crab mince with rosemary and lactic acid although not significantly different from pasta containing crab mince without additives suggests that fresh rosemary contained numerous aerobic bacteria and molds or the sample was contaminated during processing. Shelef et al. (1980) noted that finely ground rosemary had microbial counts of 1.1×10^5 CFU/ g. As far as significant differences in microbial inhibition by rosemary between the shelf-life study of crab mince and shelf-life study of pasta containing crab mince are concerned, dried rosemary was utilized in the crab mince study while fresh rosemary was utilized in the pasta study. Perhaps the dried rosemary was a more effective antimicrobial because it had a lower initial microbial load than the fresh rosemary.

Pasta from the sensory analyses study had initial microbial counts of $10^3 - 10^4$ CFU/ g. Pasta containing 20% crab mince had higher APC counts than pasta containing 10% crab mince although the difference was not significant. This increase was expected since the crab mince had a much higher microbial load than pasta dough. Pasta containing crab mince with diacetyl and lactic acid had significantly ($p = 0.05$) higher APC levels than pasta containing crab mince without additives. However, these were the initial microbial counts and the purpose of the antimicrobials was to extend the shelf-life

of products throughout storage, not to reduce the initial microbial load of products. Thus, the additives probably did not have time to demonstrate inhibition against the bacteria. Additionally, the incorporation of additives into the crab mince was an extra processing step that may have also increased microbial contamination. Pasta containing red colorant did have higher APC counts compared to pasta without red colorant although it was not significant. This may have been due to microbial contamination in the color extract or further contamination of the pasta by this extra processing step. Yeast and mold counts were below 100 CFU/ g for all pasta batches. The addition of crab mince, additives, and red colorant had no significant effects on the yeast and mold counts.

Physical Analyses

Cooking Loss

As percent crab increased, there was an increase in cooking loss for pastas evaluated during the shelflife study. These results were similar for the pasta evaluated for consumer acceptance as well. As percent crab mince increased in the pasta, there was a dilution of flour and more importantly, a dilution of gluten protein. With less gluten, a looser gluten matrix was formed and leaching of solids occurred. The solid in most pastas primarily consists of starch, however, the pastas containing crab mince could also have a loss of mince. Pasta containing crab mince with additives also had significantly more cooking loss during the shelf-life study than pasta containing crab mince without additives. No difference was detected between pasta containing crab mince with and without additives for pasta evaluated for sensory analyses. Again, the cooking loss may have been due to a slight dilution in the gluten proteins resulting in more cooking loss. Please note, however, that the range of cooking loss for both pasta studies was very low.

Pastas evaluated for shelf-life and sensory analyses had cooking losses between 3.7 - 5.3% and **3.4 - 3.9%**, respectively. Rayas-Duarte et al. (1996) and Bergman et al. (1994) reported cooking losses of **6.4%** and 5.3%, respectively, for pastas made of durum flour. Pastas made with durum flour should not exceed 7 - 8% cooking loss (Rayas-Duarte et al., 1996). Cooking loss occurs regardless of the ingredients, thus although the crab mince increased the cooking loss compared to the control pasta, the values were still within the acceptable range for good pasta quality. As storage time increased, from week three to week five, cooking loss also significantly increased ($p < 0.05$). This may have been due to general degradation of the pasta over time by enzymatic degradation from amylases and proteases in the flour and crab mince as well as microbial degradation. The gluten matrix may have loosened and broke down allowing more leaching of solids. Again, note that over the five-week period cooking loss in the shelflife study increased by only 2% and was still well below acceptable levels.

Cooking Weight

There was no effect of percent crab, additives, colorant, or weeks storage on the cooking weight of pastas. The cooking weight, or uptake of water, ranged from **124** to **156%** for the pasta shelf-life study. Pasta made for sensory analyses had similar results. The cooking weight, or uptake of water, ranged from **95** to 111%. The lack of effect of crab mince on the cooking weight was unexpected. One would assume that with an increase in crab mince there would be less flour to take up water, thereby reducing the cooking weight. Perhaps **10** and 20% crab mince substitution was not high enough to show a significant decrease in cooking weight, or else the crab mince also took up water.

Firmness

As days storage increased throughout the shelf-life study, firmness of the pasta decreased. Again, the protein matrix may have been degraded resulting in a less firm pasta after cooking. The addition of crab mince and additives had no effect on pasta firmness based on one-way and multi-way ANOVA. The firmness of cooked pasta evaluated for sensory analyses was also not affected by the addition of crab mince, additives, or colorant. Perhaps the addition of 10 and 20% crab mince was not sufficient to significantly affect firmness. If higher additions of crab mince were added to the pasta, it would be interesting to see the effect it would have on firmness. When Matsuo et al. (1972) added 1 and 10% fish protein concentrate to a pasta formulation, the addition resulted in lower pasta quality as measured by tenderness and compressibility scores.

Our firmness values, which ranged from 0.0014 to 0.0028 g^{*} cm were much lower than those reported by others. For example, Rayas-Duarte et al. (1996) reported firmness values of 4.9, 4.1, 3.5, 3.3, and 5.6 g*cm for dried spaghetti made from durum flour, light buckwheat, dark buckwheat, amaranth, and lupin, respectively. These differences may be due to processing methods, the type of attachment (plastic tooth) utilized, or the shape of the pasta. One of the limitations of measuring firmness in pasta products is that the results cannot always be compared due to the variation of pasta sizes and shapes (Cole, 1991). Thus, a lot of researchers also prefer to utilize sensory analyses as a means of determining firmness. These scores can be based on a number of ranges, therefore, comparing those results are difficult as well. Our sensory analyses, however, indicated that panel members liked all pasta samples slightly regardless of the ingredients that were added.

Width and Thickness

In the shelf-life study, width and thickness of raw pasta samples increased by 37.9% and 27.1%, respectively, after cooking. Width and thickness of pasta from the sensory study increased by 30.5% and 27.9%, respectively, after cooking. During cooking both the protein and starch components swelled with water thereby increasing the size of the pasta noodles as noted by Smewing (1997).

As weeks storage increased, both width and thickness of the raw pasta, and the thickness of the cooked pasta increased. Due to slight degradation of the protein matrix, the noodles may have expanded slightly as the protein film started to degrade and separate. Raw pastas containing crab mince had no significant differences in width and thickness compared to the control pasta, however, cooked pastas containing higher percentages of crab mince were significantly ($p = 0.01$) wider than the cooked pasta control. Raw pastas containing crab mince with additives had significantly smaller width and thickness than pastas containing crab mince without additives and significantly smaller thickness after being cooked. These variations in significance should hold true whether the noodles are raw or cooked, however, the noodle sampling method may account for some of the differences. When selecting samples to be analyzed, nine pieces of pasta out of a raw 25-g sample were measured for width and thickness, then put back with the rest of the noodles in that sample and cooked, and then another nine pieces were removed for analyses. Thus, the cooked noodles that were measured were probably not the same noodles that were selected when raw. Additionally, variation within the noodles occurred during extrusion through the pasta maker. While the samples were being extruded through the die, some bits of shell and dried pasta from the previous batch

became stuck in the die holes, thereby decreasing the width or thickness of the next noodles. Thus, the significant differences between the pastas with and without crab mince or additives were probably due to processing and sampling methods, not due to effects of these ingredients on the width and the thickness. Similar results were obtained when measuring width and thickness of raw and cooked pasta samples that were evaluated for sensory analyses. There was a general trend that the longer the pastas were extruded from the machine, the narrower and thinner the raw and cooked pasta became. Again, this was due to particulate shell and dried pasta from the previous pastas batch becoming stuck in the die holes. Note that the width and thickness of raw pasta samples for the shelf-life study ranged from **7.23 - 7.81** mm and **0.74 - 1.04** mm, respectively, and after being cooked ranged from **10.17 - 10.78** mm and **0.93 - 1.24** mm, respectively. The width and thickness of the raw pasta batches for sensory analyses ranged from **7.60 - 7.73** mm and **0.83 - 0.94** mm, respectively, and after being cooked ranged from **9.66 - 10.32** mm and **1.08 - 1.17** mm, respectively. Thus, although there were increases in pasta width and thickness during storage, and decreases in width and thickness due to processing or sampling methods, the variation in size was minimal. In fact, during a sensory evaluation it is unlikely that panelists would notice a difference of less than two millimeters. Unfortunately, no peer reviewed papers on pasta quality have width and thickness information available to confirm our findings.

Instrumental Color

Percent crab, additives, and storage time had a significant effect on the color of the raw and cooked pasta evaluated for the shelf-life study. As percent crab mince increased, "L" and "b" values significantly decreased. These lower values were due to the

addition of crab mince, which appeared to have a medium brown color. The addition of 10% crab mince was too small to significantly effect the lightness or the yellowness of the pasta, however an increase to 20% crab mince did have a significant effect. After cooking, pastas with higher concentrations of crab mince had significantly lower “L” values but higher “a” and significantly higher “b” values. During boiling, the pigments in the pasta, which consist of the xanthophylls lutein and taxanthin (Walsh et al., 1970), were probably bleached by the heat resulting in a more white, less red, less yellow pasta. The water uptake of the pasta affect cooking may have also diluted the color of the pasta. Additionally, one of the components in the crab mince is shell, which typically has a reddish, orange color after being cooked. Particulate flour could have maintained its reddish tones throughout cooking so that the pasta with higher percentages of crab mince had higher “a” values. As storage time increased, there was a decrease in both raw and cooked “L” values and a decrease in “b” values from week one to three, however “a” values were not significantly effected by storage. This color loss over time may have been due to oxidative degradation of the carotenoid pigments by lipoxygenases during storage. Pasta containing diacetyl and lactic acid had higher raw “L” values and lower cooked “b” values than pasta containing crab mince without additives. The diacetyl was a very bright yellow color, which may have increased the lightness of the pasta to a certain extent and then possibly leached out in the cooking water while being cooked resulting in lower “b” values than pasta containing mince without diacetyl. Pasta containing crab mince with rosemary and lactic acid had significantly lower “L”, “a”, and “b” values than pasta containing crab mince without additives. These results were expected because the dried, ground rosemary was an olive green color which gave the crab mince a dark,

brownish-green appearance. After being cooked, the pasta containing crab mince with rosemary and lactic acid still had lower “L” and “b” values but significantly higher “a” values. This higher “a” value was not expected, however, after cooking, the pastas containing rosemary had a brownish color. Perhaps some of the pigments within the rosemary were degraded by the heat, as well as being leached out into the cooking water resulting in a dull brown, colored pasta. As far as differences within pasta treatments that contained rosemary are concerned, pastas containing 20% crab mince had significantly lower “L” values and significantly higher “a” and “b” values after being cooked than pastas containing 10% crab mince. These higher “a” and “b” values may be due to a combination of both pigment denaturation and leaching, as well as higher mince and shell flour content within the pasta containing 20% crab mince.

Pasta with crab mince and additives for the sensory analyses study had similar color results to pasta treatments evaluated for the shelf-life study. Pasta containing higher percentages of crab had lower “L” values and higher “a” and “b” values than pasta containing lower percentages of crab mince and these results were significant after the pastas were cooked. Again, the addition of more crab mince made the pasta a bit darker, and after boiling, the flour in the pastas became bleached and less yellow due to heat denaturation of the pasta carotenoids. Additionally, with an increase of crab mince from 10% to 20% there was a larger ratio of shell flour in the pasta, which may account for the higher “a” values. The addition of diacetyl and lactic acid had no effect on the raw or cooked color values of the pasta. Since replicate pasta batches for sensory analyses were not made, one cannot say for sure why there was not a significant effect of additives on the color of the batches, however, it may have been due to seasonal differences in crab

color, as well as differences in the color of the food grade diacetyl that was purchased for this sensory study. Perhaps the crab mince had a more yellow tone or the diacetyl was actually less yellow or had a lower “b” value. For the sensory analyses test, red colorant was put into two of the pasta formulations. Raw and cooked pastas containing red colorant had significantly lower “L” and significantly higher “a” and “b” values than pasta without red colorant. These results were expected because upon visual inspection the colorant was a dark red almost purple color, not orange. Within pasta batches that had colorant, raw pasta containing 10% crab mince had significantly higher “a” values than pasta containing 20% colorant, however, after being cooked it had significantly lower “a” values than pasta containing 20% mince. This was probably due to the colorant leaching out of the pasta into the cooking water. The colorant was 100% water-soluble and it was readily distributed throughout the cooking water after boiling the pasta. Since pasta containing 20% crab mince had more shell it had a larger “a” value after being cooked than pasta containing 10% crab mince. Additionally, when the colorant was added to the pasta batches it was not shaken before use, so the color between the two pasta batches was very different. After being cooked, pasta containing 10% crab mince had a light pink color while pasta containing 20% crab mince had a light orange-red color. This occurred probably because the various ingredients in the colorant have different densities. If the colorant is not mixed or the whole bottle is not used at once, color variations may occur. Fortunately, this gave the consumers two different shades of red color to evaluate for acceptability in pasta containing crab mince.

Sensory Analyses

Sensory panel members were primarily from the University of Maine campus. The majority of these panel members were women, while men made up **38.9%**. Sixty nine percent of the panel members were older than **30** and, thus, **less** likely to be college students. Perhaps the reason why the majority of the panel members were older than **30** is because the advertisement of a gourmet fish pasta containing seafood attracted people with a higher disposable income who can afford to buy both fish pasta and seafood on regular **basis**. When panelists were asked how often they ate pasta, 94% of the respondents stated they ate pasta either more than once per week or once per week. Thus, the panel members may have also been interested in the product because they eat pasta frequently. When asked what they considered was the single most important quality characteristic for pasta, **53.7%** said flavor while 42.6% said texture. Very few panelists considered nutrition **as** the single most important quality characteristic. One of the "angles" that could be used to advertise a pasta containing seafood is its enhanced nutritional value, however, from panelists' responses it appears that people are less concerned with nutrition and more concerned about taste and texture.

The addition of color to a pasta containing crab mince may be one way to attract the consumer's eye when shopping. When asked how often panel members purchased colored pasta, 70% stated they bought colored pasta either once per month, once per week, or more than once per week, but no panel members chose color **as** the single most important quality characteristic. Utilizing a colorant may increase the sales of pasta containing seafood to the **70%** of people who purchase colored pasta on a regular basis.

When panel members were asked if they like seafood-flavored pastas, 91% stated they had never tried it. Additionally, **46.3%** said they would be interested in purchasing a pasta that contains seafood while **53.7%** said they might be interested. There are several filled pasta products on the market today that contain seafood, however, from the panelists' responses not many people have tried these products. Since the panel members were interested in evaluating a seafood-flavored pasta, a lack of exposure may be the reason why these products have not become popular. If this pasta containing crab mince were produced on a commercial scale, taste testing in grocery stores and a more intense level of advertising than has been done for these already commercial produced seafood pastas may increase the knowledge of this type of product.

Average sensory scores for flavor, texture, aroma, and overall acceptability were between **6 – 7**, and there was no significant difference between any of the pasta samples tested for these attributes. Panel members did like pasta containing 10% crab mince with and without additives slightly more than the other samples although there was no significant difference. A large number of panel members commented on the lack of seafood flavor in the pasta. Panel members liked the texture of pasta containing 10% crab mince more than the other samples, however, it was not significantly different. Quite a few panel members commented on the grittiness detected in the other samples. This was due to residual shell flour in the crab mince after being mechanically separated. Pasta containing 10% crab mince and colorant did receive significantly lower color scores than the rest of the samples. After being cooked, the pasta had a dark pink color. The average color score for all pasta treatments excluding pasta containing crab mince and colorant was **6.7**.

Sensory analyses indicate that further development of this product is needed before it can go to full-scale commercialization. One of the primary goals of this project was to produce a gourmet seafood-flavored pasta, however, due to the number of processing steps needed prior to consumption, the seafood flavor was minimal. Future steps to increase the seafood flavor of the pasta may include using stock in place of water or adding a seafood flavored powder. The texture of the pasta also needs to be improved. Consumers detected a grittiness that was unappealing. Lower concentrations of crab mince could reduce this, however, there would be little if any seafood flavor in the product. Utilizing a low percentage such as 10% crab mince combined with a flavor enhancer such as a base or stock may increase consumer acceptability of the pasta.

CONCLUSIONS

Mechanical separation of crab mince resulted in an average yield **of 64%** from the starting by-product. The crab mince had a shelf-life of one to two days based on microbial counts. However, the addition of sodium lactate, lactic acid, rosemary, and diacetyl were effective in extending the shelf-life **of** the crab mince. Combinations of additives may prove most effective since each additive improved only some aspects **of** quality. Results indicate that high crab mince quality can also be maintained by proper sanitation practices. Proper sanitization **of** the mechanical separator just prior to operation will help to ensure a longer shelf-life of the crab mince.

Pastas containing 10 or **20%** crab mince with additives were successfully extruded. The pasta had a shelf-life of a minimum three weeks based on microbial counts. To ensure extended high quality shelf-life of the fresh pasta, it must be kept at refrigerated temperature, or microbial counts will increase to unacceptable levels.

The physical quality of the pasta, **as** measured by firmness and cooking loss, decreased slightly with the addition **of** crab mince and length of storage.

Sensory analyses indicated that further development of the pasta is needed prior to commercialization. Most panelists stated that the pasta samples tasted like regular pasta and had **minimal** seafood or crab flavor. Some panel members also detected a grittiness within the pasta noodles. Perhaps incorporating a low percentage of crab mince while utilizing a crab flavored stock or base to replace the water in the pasta formulation **will** increase the seafood flavor and reduce shell flour. Incorporating the mince into a filled pasta product **may** also help concentrate the seafood flavor while reducing the grittiness

due to shell flour. Finally, future research should be conducted to investigate and develop other types of value added foods that could be enhanced by the addition of crab mince.

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Biography of the Author

Barbara Gillman was ~~born~~ in Newport, Rhode Island on February 12, 1977. She was raised in Hiram, Maine and graduated from Sacopee Valley High School in 1995. She attended The University of Maine and graduated in 1998 with a Bachelor's degree in Zoology. In the fall of 1999 she entered the graduate program in the Department of Food Science and Human Nutrition at The University of Maine.

While at The University of Maine, Barbara ~~has~~ become a member of Phi Tau Sigma honorary society, the Institute of Food Technologists and the Northeast section of the Institute of Food Technologists. Barbara is a candidate for the Master of Science degree in Food Science and Human Nutrition from The University of Maine in August, 2001.