A SIMULATION MODEL OF QUANTITATIVE MICROBIAL RISK ASSESMENT MODEL DURING PROCESSING OF FRESH CUT LETTUCE

By

HAREGEWOIN T WOLDEMESKEL

Bachelor of Science in Agriculture
Addis Ababa University
Addis Ababa
1982

Master of Science in Food Science
Oklahoma State University
Stillwater, OK
2002

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
July, 2009

A SIMULATION MODEL OF QUANTITATIVE

MICROBIAL RISK ASSESMENT MODEL DURING

PROCESSING OF FRESH CUT LETTUCE

Dissertation Approved:

Dr. William McGlynn
Dissertation Advisor

Dr. Stanley Gilliland

Dr. Niels Maness

Dr. Lynn Brandenberger

Dr. A. Gordon Emslie
Dean of the Graduate College

Dedication

This work is dedicated to my beloved daughter, Keisha Munsanje without, whose caring support wouldn't have been possible. I earned this degree, but we did it together.

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr William McGlynn for his guidance, encouragement, patience and support throughout my graduate program. I would like to thank Dr. Stanley Gilliland, Dr. Niels, Maness and Dr. Lynn Brandenberger for serving on my committee. Dr. Gilliland, thank you for your support in the laboratory work of the research. I owe a special thanks to Dr. Yan Bin Li , Professor, and his graduate student at a time, Abani K. Pradhan from University of Arkansas for the technical support you gave me in converting the research idea into a mathematical model. I wouldn't have done this if it weren't for you. Thanks to all my friends and close family members who were there to encourage and make me happy during my graduate studies.

My parents Mrs Ejigayehu Bedasso and my father Mr. Takele Woldemeskel: you are my inspiration. Thank you for your love and encouragement. My brothers and sisters thank you for your moral support and encouragement. Also, I would not pass without thanking my brother, Mesfin Woldemeskel and his beloved wife Woinshet Abera for their encouragement. I wouldn't have made it if it weren't for your encouragement I get every day I wake up. I owe a special thanks to my daughter Keisha for her patience, while I was at school when she needed me.

Finally, I want to thank you, GOD, my gracious father, for the strength you gave me and for providing all that I needed in accomplishing this study.

TABLE OF CONTENTS

Chapter Page

1. LITERATURE REVIEW 1
References 14

2. INFLUENCE OF INCUBATION TEMPERATURE, PUMMELING TIME, AND LEAF SAMPLING PROTOCOL ON THE RECOVERY OF AEROBIC BACTERIA FROM FRESH-CUT SALAD MIX AND MINIMALLY-PROCESSED WHOLE-HEAD LETTUCE 18

Abstract.....18
Introduction 19
Materials and methods.....22
Impact of incubation temperature on total recovery of aerobic microorganisms in fresh cut in fresh cut salad mix 22
Effect of pummeling time on microbial recovery of aerobic bacteria in fresh cut salad mix 23
Effect of leaf-sampling protocol on microbial recovery of aerobic bacteria from minimally-processed whole-head iceberg lettuce 24
Results and discussion.....26
Conclusion.....27
References……. 28

3. SIMULATION MODEL OF QUANTITATIVE MICROBIAL RISK ASSESSMENT DURING PROCESSING OF FRESH CUT LETTUCE 33

Abstract.....33
Introduction 35
Materials and methods.....38
Results and discussion.....44
Conclusion.....52
References 54

4. APPENDIX 70

LIST OF TABLES AND FIGURES

Table Page

1.1. Sampling points and temperatures in a study of predicted *Listeria monocytogenes* contamination of lettuce and peppers in a model processing and distribution…..8
2.1. Comparison of incubation temperature for aerobic bacteria enumeration on ready to eat vegetables……..30
2.2. Effect of pummeling time on recovery of aerobic bacteria in ready to eat vegetables.....31
2.3. Effect of sampling method on enumeration of aerobic micro-organisms on minimally processed whole head iceberg lettuce.....32
3.1. Population data used as inputs into the quantitative risk assessment model for aerobic bacteria in fresh cut lettuce 57
3.2. Cell addresses and formulas used in the quantitative risk assessment model of @Risk in fresh cut lettuce processing.....58
3.3. Predicted effects of processing lettuce on microbial load of aerobic bacteria as simulated by @Risk from 10,000 randomly selected data points.....59
3.4. Population data used as inputs into the quantitative risk assessment model for coliform bacteria in fresh cut lettuce 60
3.5. Predicted effects of processing lettuce on microbial load of coliform bacteria as simulated by @Risk from 10,000 randomly selected data points.....61
3.6. Population data used as inputs into the quantitative risk assessment model For yeasts and molds in fresh cut lettuce.....62
3.7. Predicted effects of processing lettuce on microbial load of yeasts and molds as simulated by @Risk from 10,000 randomly selected data points 63
3.8. processing steps ranked as predicted for their impact on aerobic bacterial load in shredded lettuce as following 10,000 iterations.....64
3.9. Processing steps ranked as predicted for their impact on coliform bacterial load in shredded lettuce as following 10,000 iterations.....65
3.10. Processing steps ranked as predicted for their impact on yeasts and molds load in shredded lettuce as following 10, 000 iterations.....66

Figure Page

1.1. Steps of microbial food safety risk assessment ……8

3.1. Flow diagram of fresh cut lettuce 67

3.2. Comparison of micro-organisms load during fresh shredded lettuce production with data from a randomly selected iteration.....68

3.3. Relative comparison of microorganisms load from output results with a randomly selected iteration during fresh cut lettuce processing.....69

Chapter I

Introduction

In today's food industry, food safety and quality has reached high standards. Hazard Analysis Critical control Point (HACCP) systems and Good Manufacturing Practices (GMPs) are set for most processed food products. Temperature, humidity, packaging and use of modified atmosphere are some of the factors that have been shown to influence microbial growth in food (40) and many control measures address these factors. But, regardless of the best control measures applied, food products are still exposed to a risk of contamination leading to disease outbreaks (4).

Outbreaks of human illness due to consumption of raw fruits and vegetables in particular have increased in recent years. Epidemiological surveillance programs indicate that the potential for outbreaks has been enhanced due to changes in dietary habits, increases in the number of immune-compromised consumers, changes in agronomic and processing practices, and increases in the rate of consumption (6).

Agronomic and processing practices may affect the microbial quality and shelf life of raw and processed fruits and vegetables as a result of contamination with pathogenic microorganisms in the field, while harvesting, and during post harvest handling. In particular, the quality of wash water, packaging methods and materials, and transportation/storage temperatures may be significant issues (32).

In general, microbial populations are usually higher in fresh cut vegetables than in raw ones. There may be a number of reasons for this. Prolonged processing times, such

as a delay in refrigeration after pre-washing and trimming has been found to cause elevated microbial counts (32). The commercial shelf-life of fresh-cut vegetables depends on the ingredients and manufacturing methods and typically varies from 7 to 14 days. In addition to lactic acid bacteria, which are responsible for spoilage, the predominant microbiological population in fresh cut vegetables consists of psychrotrophs such as *Pseudomonas spp.* and *Erwina spp.* (15). Vankerschaver and others (34) measured the microflora of fresh leafy vegetables, consisting predominantly of the psychrotrophic bacteria P*seudomonas* and *Erwinia* spp., with an initial count of 10^5 CFU/g. Microbial counts of minimally processed vegetables for soup packed in modified atmosphere were found to be approximately 10^8 CFU/g, $5.6x10^6$ CFU/g, $1.5x10^7$ CFU/g and 10^6 CFU/g for aerobic bacteria, coliforms, *Pseudomonas spp*. and lactic acid bacteria respectively. (25).

Shelf-life studies on ready to eat vegetable salad mix with a ratio of 75% lettuce, 15% carrot and 10% cabbage stored at 4 °C yielded initial counts of lactic acid and psychrotrophic bacteria at $8 x10^2$ CFU/g and $1.07 x 10^5$ CFU/g respectively (15). At the end of the observation, after 7 days of storage, the population of psychrotrophs rose by 2 log cycles. Similarly, studies on the effect of sanitizing treatment on shelf-life of fresh cut iceberg lettuce found that, regardless of different sanitizing treatments, the population of psychrotrophs and *Enterobacteriaceae* bacteria increased as storage temperature increased from 5°C to 15°C (38). Dipping of lettuce in water with chlorine was found to reduce the initial population of mesophilic aerobic organisms. Even so, the same treated samples were found to have a rapid increase in population of aerobic microorganisms, when they were stored at 15 °C rather than at 5°C(38). A hot water treatment (50°C) has

been shown to delay browning of iceberg lettuce and control the population increase of naturally occurring microorganisms for few days, though as the storage progresses, the population increases (10).

Type of spoilage and quality deterioration largely depends on the type of vegetables being processed. Studies on the level of contamination of fresh leaf vegetables show that cabbage, artichoke, and celery are exposed to a higher level of contamination compared to lettuce and spinach (14). The survival and growth of a pathogen on or in raw produce may also be influenced by metabolic activity and natural constituents associated with a specific commodity. For example, mixed lettuce and chicory endive, which naturally contain low quantities of sugar, have been found to support the growth of large numbers of Gram-negative microorganisms which do not produce non-volatile compounds. On the other hand, mixed pepper and celery products, which are relatively higher in sugar content, have been shown to support the fast growth of different spoilage microorganisms (20). The expression of these capabilities may largely be due to intrinsic and extrinsic ecological factors present in the produce or imposed at different points during the entire production system, including processing and distribution.

The rate of increase of microbial growth in fresh cut vegetables has been shown to be dependent on the form of the product, storage conditions and composition of the product. Studies on shelf life of fresh cut vegetables (17) showed that rapidity of processing, the effectiveness of washing, and continuity of refrigeration are significant factors controlling level of contamination in fresh-cut salads. Choice of salad

constituents, their ratio, and the presence or absence of washing after cutting also seemed to be influential factors in controlling microbial propagation during storage.

Individual processing steps can influence microbial populations on fresh-cut produce. Studies on the microbial quality of fresh-cut red lettuce processed using standard industrial practices have found the shelf-life of lettuce did not exceed 7 days. Considering production processes, counts of psychrotrophic bacteria, coliform and lactic acid bacteria were influenced by all steps in the production process with a pronounced effect seen at shredding, rinsing, and centrifuging steps (3). Peeling, cutting and shredding, exposes a product to air and possible contamination. Higher humidity and larger cut surfaces create ideal conditions for the growth of microorganisms (2). Shredding destroys surface cell and allows juices to leak from the inner tissues onto equipment and the produce itself. Similarly, slicers and cutters may also be powerful sources of microbial contamination due to the inaccessible sites that harbor microorganisms (9). Considerable contamination by *Listeria monocytogenes* was discovered during chopping, mixing and packaging and *Listeria monocytogenes* was found regularly on shredded cabbage in a study by Nguyen and Carlin (28).

Contamination of lettuce during trimming depends on a number of factors. Handling of a head of lettuce to remove the core and the top layers of leaves is usually done by humans and it is believed that poor sanitation practices by human handlers in a processing plant can be a source of contamination (5).

Microbial control efforts for fresh-cut produce have typically focused on washing/sanitizing, controlled or modified atmosphere packaging, and temperature control. Several studies have demonstrated the effectiveness of controlled atmosphere

storage for increasing the shelf-life and maintaining the quality and safety of minimally processed fresh produce (28, 21). Carbon dioxide enrichment has been observed to delay deterioration and microbial growth in diced onion, particularly the growth of psychrotrophic microflora (8). But clearly these treatments are not sufficient to completely control pathogens.

Washing/sanitizing treatments are not sufficient either. Researchers have found that the efficiency of sanitizers used for killing pathogens on fruits and vegetables may be compromised because the sanitizers are unable to reach locations within tissues that may harbor pathogens (6).

Considering all these microbial issues, production of safe food has been an increasing concern. In order to be able manage food safety risks, there is a need to identify the conditions that are more likely to lead to contamination of produce with particular pathogens and to determine the level of impact such contamination is likely to have on human health (23). Developing risk-predicting models for the organisms which often occur as contaminants in minimally processed vegetables is considered to be a way of predicting the microbiological food safety of minimally processed vegetables. Risk analysis is one of the fastest growing food safety activities in recent years. The major objective of risk analysis is to provide scientific and experimental-based risk estimates that enable producers and processors to manage food safety. It is also known to be a necessary component to assist in selecting priority hazards and identifying hazardous scenarios.

According to the United Nations (UN) Food and Agriculture Organization (24) and, the UN World Health Organization (WHO) (37), risk analysis is composed of three

elements: risk assessment, risk management and risk communication. The first step in this series of activities, risk assessment, is a process of scientific evaluation of the probability of occurrence of adverse health effects from a potential human exposure to food-borne hazards. During risk assessment, factors contributing to risk are prioritized and limits are set to meet the standards (18). The terminology of risk assessment for microbial food safety is not definitive and there are differences among different international agencies. Nevertheless, the key elements in the series of activities are the same (11). Application of quantitative risk assessment to microbial food safety can help to identify those stages in the manufacture, distribution and handling of foods that contribute to an increased risk of food-borne illness. This helps focus resources and efforts on most effectively reducing the risk of food-borne pathogens (11, 24). Once comprehensive risk analysis data has been validated, this system can easily be used with confidence to predict responses of microorganisms under different conditions (26). Quantitative risk assessment may also serve as a foundation for future food safety control systems by minimizing our dependence on the microbiological examination of foods, particularly difficult-to-assays products such as foods in international trade (38, 39).

Quantitative risk assessment may be more or less systematic. More systematic methods include assessment of non-pathogenic spoilage microorganisms, which provide a foundation for the prediction and extension of shelf-life and gives an overall look at potentially-interlocking issues related to food safety and food spoilage.

The four steps of microbial food safety risk assessment are hazard identification, exposure assessment, hazard characterization and risk characterization (Fig. 1.1). These steps systematically identify and evaluate the significance of microbial hazards in food of

concern. The result of the process is a risk estimate and a measure of risk magnitude, based on current scientific knowledge and understanding.

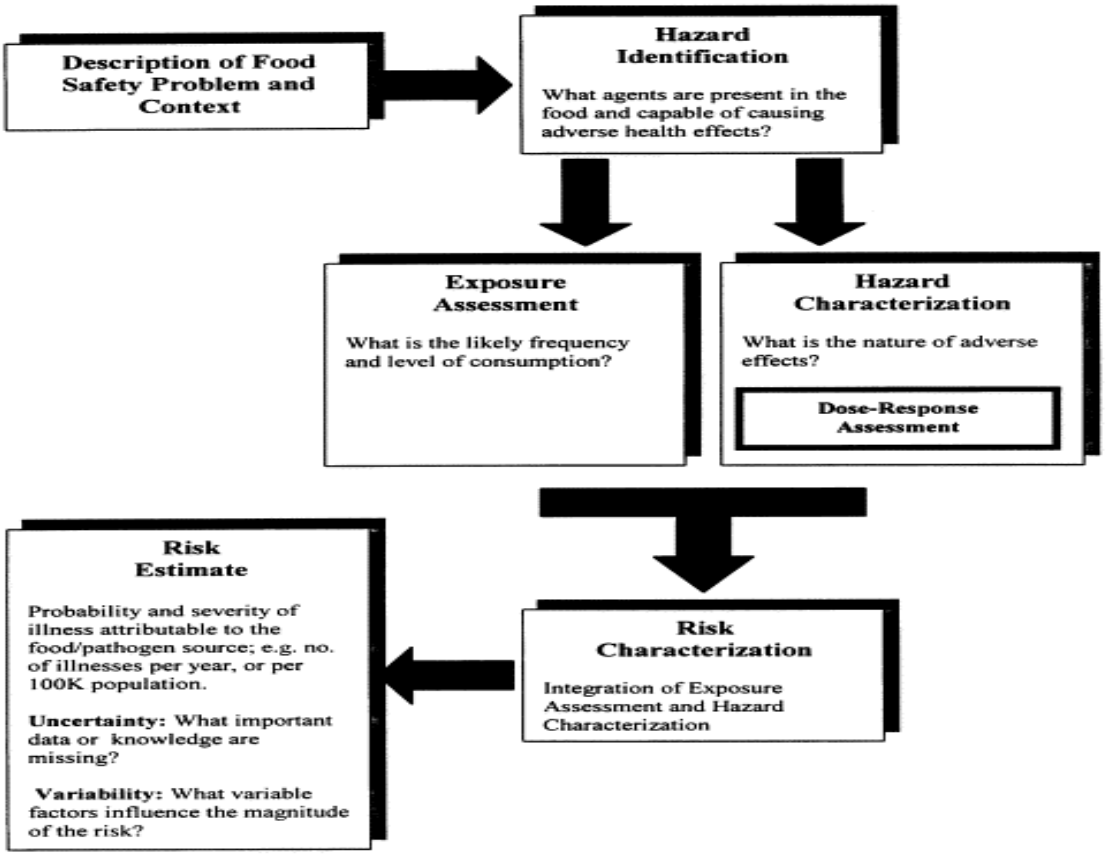


Figure 1.1: Steps of microbial food safety risk assessment (24).

Sampling standards

In most of the studies reviewed, sampling numbers for determining risk of contamination from fruits and vegetables have varied widely. Studies with different vegetable products have used different sample populations. One shelf-life study on ready-to-eat vegetables (15) used a total of 144 samples of mixed vegetable salad. Another study on contamination of fresh vegetables in the field and during marketing used population samples of 80, 41, and 38 for lettuce, cabbage and spinach respectively (14). In fact, many studies have used quantitative data from previous studies. But in general studies have used base line quantitative microbial population data to predict risk of contamination by simulation and probability distribution (12).

Assessment of risk from microbial contamination

In recent years, predictive microbiology has been used as an alternative to the traditional microbiological assessment of food quality and safety (27). Most risk assessment models are built based on quantitative data from literature, and expert opinions are often added to fill in the data gaps (12). Unlike epidemiologists, whose starting point for risk assessment is disease, food scientists start with the food and reason towards the circumstances of illness. There are different types of models available in predictive microbiology. Polynomial, empirical models can be used easily without applying detailed knowledge of the process, but their predictive power is limited to the specific experimental conditions and original data collected (1). Other models also enable prediction of risk from microbial contamination of processed produce, and provide many possibilities for quantitative estimation of spoilage and food safety. These estimations, which are based on quantitative and qualitative information, give an

overview of the effect of important processes on risk, which would help determine the rate-determining steps for microbial contamination (39).

Microbial risk assessment studies usually use Monte Carlo simulation, which bases its predictions on probability frequency distributions used as input parameters. This method takes into account variability and lack of precise knowledge about input conditions; it also describes the actual system being modeled in a sensible, relatively complex manner (35).

Population curve fitting by multivariate analysis is also one of the many models being used. This was used to predict the growth pattern of *Escherichia coli* in minimally processed vegetables (7). Population-fitting curves derived from the growth data of *Escherichia Coli* on minimally processed cucumber, carrot and tomato showed that microbial safety of minimally processed vegetables depends mainly on initial population of microbial contaminants, storage temperature, and storage time (7). Under this set of influencing factors, predicted population numbers derived from population curve fitting were compared to experimentally-measured population numbers; the actual and predicted values were found to be in agreement. The statistical values for the model fit were also evaluated (7).

Monte Carlo simulation is a computerized mathematical technique which uses statistical sampling techniques and allows one to account for risk in quantitative analysis and in decision making. Based on the range of data of the actual situation, Monte Carlo analysis generates random samples for input distribution and frequency distribution as input parameters (11, 35, 36). Monte Carlo simulation has been used to create many quantitative risk assessment models (QRAM) for food pathogens; most of the studies

have been on S*almonella enteritidis*, other *Salmonella* spp., and *E. coli* O157:H7 (18, 12, 11). For example, a QRAM for the survival/growth of salmonella in whole chicken was built by simulating data using @Risk software (Version 4.0, Palisade, Newfield, NY). The incidence and minimum and maximum potential growth of salmonella on whole chicken during consumer transport was predicted using Monte Carlo simulation and PERT distribution – a probability distribution calculated from minimum, maximum, and most-likely population values – was used to model the extent of pathogen contamination events (29).

So-called "worst case" statistical analysis is sometimes used to quantitatively determine how risky any given factor influencing a particular system might be (18). This approach evaluates a chain of extreme situations in a process, describing the most unlikely unfavorable conditions that could occur and possibly compromise product safety. A worst-case analysis can be useful in that if product safety is shown to be within the acceptable limits even given "worst-case" assumptions, then the products can be classified as safe and the risk associated with that process can be assumed to be minimal. The results of "worst-case" analyses are sometimes subjected to further analysis, as by definition a "worst-case" analysis always overestimates the likely actual risk.

To give a better idea of the possible use of "worst-case" analysis, we may examine a study that used predictive modeling to examine packaging design for fresh cut mixed lettuce and mixed peppers. A "worst-case" cold chain simulation was used to evaluate possible concern related to temperature abuse ($T > 10°C$) of minimally processed vegetables during transport and unloading at the super market, as well as during storage and display while on sale (19). This study used seven steps and eight

moments of sampling in the simulated distribution chain. The time-temperature combinations used to simulate the "worst case" cold chain distribution of fresh cut-vegetables are presented in table 1.1. The study showed that yeast spoilage could negatively impact shelf-life, but no growth of *L. monocytogenes* was predicted in either the mixed lettuce or the mixed bell peppers.

Step in distribution chain	Time–temperature combination	Moment of sampling
Processing–packaging	**T<12 °C**	moment of sampling 0
Storage at producer	**T=4 °C, $t_{maximum}$=24 h**	moment of sampling 1
Transport from producer to distribution centre	T=2–3 °C, $T_{maximum}$=5 °C, t=2 h	
Storage at distribution centre	**T=10 °C, $t_{maximum}$=24 h**	moment of sampling 2
Transport from distribution centre to supermarket	T=2–3 °C, $T_{maximum}$=5 °C, t=2 h	
Unloading at the supermarket and first storage	**T=10 °C, t=1 h, $t_{maximum}$=8 h**	moment of sampling 3
Storage at chilled counter	**T=7 °C, $t_{maximum}$=48 h**	moment of sampling 4
Purchase by the consumer and transport at domestic refrigerator	**T=20 °C, t=2 h**	moment of sampling 5
Storage in domestic refrigerator	**T=7 °C, t=?**	moments of sampling 6, 7 and 8

Table 1.1 – Sampling points and temperatures in a study of predicted *Listeria monocytogenes* contamination of lettuce and peppers in a model processing and distribution chain (20).

To be most effective and accurate, risk assessment should make use of results from "worst-case" studies, expert knowledge, data from the literature, and sensible assumptions about the various risk factors associated with a given system that is being modeled. All of these empirical data as well as values derived from probability distribution functions may be drawn together by Monte Carlo simulations. Monte Carlo simulation can provide an important analysis of the model and various statistical software

packages, such as @RISK by Palisade, are capable of using Monte Carlo simulations to link empirical data with probability distribution functions for the different processing steps in the manufacture of fresh-cut produce. Model parameters may also be adjusted based on various assumptions. Thus, these packages can help create complex, accurate and sensitive predictions that can be used to assess risk of microbial contamination of fresh-cut produce during processing.

References

1. Aantrekker E.D., Boom R. M., and M. H. Zwietering. 2003. Quantifying recontamination through factory environments. *Int. J. Food Microbiol.* 80: 117-130.

2. Ahvenainen M. 1999. New approaches in improving the shelf-life of minimally processed fruits and vegetables. *Trends in Food Science & Technol*. 7: 179-187.

3. Allende A., Aguyano E., F. Artes. 2004. Microbial and sensory quality of commercial fresh processed red lettuce throughout the production chain and shelf-life. *Int. J. Food Microbiol.* 91:109-117.

4. Beuchat, L. R., 1996. Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* 59: 204-216.

5. Beuchat L. R. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Unit, World Health Organization. WHO/FSF/FOS/ 98: 42 pp.

6. Beuchat L. R., 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infect***.** 4: 413-423.

7. Bharathi S., Ramesh M. N., and M. C. Varadaraj. 2001. Predicting behavioral pattern of Escherichia coli in minimally processed vegetables. J. *Food Control*, 12: 275-284.

8. Blanchard M., Cstaigne F., Willemot C., and J. Makhlouf. 1996. Modified atmosphere preservation of freshly prepared diced yellow onion. *Post harvest Biol. and Technol.* 9: 173-183.

9. Bracket R. E. 1987. Microbiological consequences of minimally processed fruits and vegetables. *J. Food Quality*. 10: 195-206.

10. Brackett Y. L., Shewfelt R. L., and L. R. Beuchat. 2001. Changes in appearance and natural micro-flora on iceberg lettuce treated in warm, chlorinated water and then stored at refrigeration temperature. *Food Microbiol*. 18: 299-308.

11. Cassin M. H., Lammerding A. M., Todd E. C. D., Ross W., and S. R. McColl. 1998. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef humbergers. *J. Food Microbiol*. 41: 21-44.

12. Duffy, S., and D. W. Schaffner. 2002. Monte Carlo simulation of the risk of contamination of apples with Escherichia coli O157:H7. *Int. J. Food Microbiol*. 78: 245-255.

13. Del Rosario B. A., L. R. Beuchat. 1995. Survival and growth of entero-haemorrhagic *Escherichia coli* O157:H7 in cantaloupe and watermelon. *J. Food Prot*. 58: 105-107.

14. Garcia-Vilanova Ruize B., Galvez Vargas R., and R. Garcia-Vilanova. 1987. contamination of fresh vegetables during cultivation and marketing. *Int. J. Food Microbiol*. 4: 285-291.

15. Garcia- Gimeno, R. M., and G. Zurera–Cosana. 1997. Determination of ready to eat vegetable salad shelf-life. *Int. J. food Microbiol*. 36: 31-38.

16. Golden D. A., Rhodehamel E. J., and D. A. Kauter. 1993. Growth of S*almonella* spp. in cantaloupe, watermelon, and honeydew melons, *J. Food Prot*. 56: 194-196.

17. Guerzoni M. E, Gianotti A., and M. R. Corbo. 1996. Shelf life modeling for fresh cut vegetables. *Postharv. Biol. & Technol*. 9: 195-207.

18. Hoornestra E., and S. Notermans. 2001. Quantitative microbiological risk assessment. *Int. Food Microbiol*. 66: 21-29.

19. Jacxsens L., Devliegher F., and J. Debevere. 2002. Predictive modeling for packaging design: equilibrium modified atmosphere packages of fresh-cut vegetables subjected to a simulated distribution chain. *Int. J. Food Microbiol*. 73: 331-341.

20. Jacxsens L., Devliegher F., and Ragaert P., Vanneste E., J. Debevere. 2003. Relation between microbiological quality, metabolite production and sensory quality of equilibrium modified atmosphere packaged fresh-cut produce. *Int. J. Food Microbiol.* 83*:* 263-280.

21. Kelly W. J., Davey G. P., and L. J. H. Ward. 1998. Characterization of lactococci isolated from minimally processed fresh fruit and vegetables. *Int. J. Food Microbiol*. 45: 85-92.

22. Kenny S. J., L. R. Beuchat. 2002. Survival of *Escherichia coli* O157:H7 and *Salmonella* Muenchen on apples as affected by application of commercial fruit wax. *Int. J. food Microbiol.* 77: 223-231.

23. Lammerding A. M. 1997. An overview of microbial food safety risk assessment. *J. Food Protect*. 60: 1420-1425.

24. Lammerding A.M., A. Fazil. 2000. Hazard identification and exposure assessment for microbial food safety risk assessment. *Int. J. Food Microbiol*. 58: 147-157.

25. Manzano, M., Citterio B., Maifreni M., Paganessi. M., and G. Commi. 1995. Microbial and Sensory quality of vegetables for soup packaged in different atmospheres. *J. Sci Food Agric* . 67:521-529.

26. McClure P. J., Backburn C. D., Cole M. B., Curtis p. S., Jones J. E.,Legan J. D., Ogden I. D., Peck M. W., Roberts T.A., Sutherland J. P., S. J. Walker. 1994. Modeling the growth , survival and death of organisms in foods: The UK Food Micromodel approach. *Int. J. Food Microbiol*. 23: 265-275.

27. McMeekin T. A., Ross T. and J. Olley. 1992. Application of predictive microbiology to assure the quality and safety of fish and fish products. *Int. J. Food Microbiol.* 15: 13-32.

28. Nguyen C. and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr*. 34: 371-401.

29. Oscar T. P. 2004. Quantitative risk assessment model for salmonella and whole chickens. *Int. J. Food Microbiol*. 93: 231-247.

30. Palisade Corporation. 2009. Monte Carlo Simulation. http://www.palisade.com/risk/monte_carlo_simulation.asp

31. Rocha Bastos M. S., Ferreira Soares N. F., Andrea N. J., Arruda A. C., and R. E. Alves. 2005. The effect of the Association of sanitizers and surfactant in the microbiota of the Cantaloupe (*Cucumis melo* L.) melon surface. *Food Control.* 16: 369-373.

32. Sinigaglia M., Albenzio M., and R. Carbo. 1999. Influence of process operations on shelf-life and microbial population of fresh cut vegetables. *J. Industrial Microbiol. Biotechnol.* 23: 484-488.

33. Vanderzant C. and D. F. Splittstoesser. 1992. Compendium of methods for the microbiological examination of foods. American Public health Association. pp. 920.

34. Vankerschaver K. , Willcox F., Smout C., Hendrickx M. and P. Tobback. 1996. The influence of temperature and gas mixture on the growth of intrinsic micro-organisms on cut-endive: predictive versus actual growth. *Food Microbiol*. 13: 427-440.

35. Vose, D. J. 1998. The application of quantitative risk assessment to microbial food safety. *Journal of Food Prot.*. 61: 640-648.

36. Vose, D. J. 2008. Risk Analysis: A quantitative Guide. pp.57.

37. WHO/FAO Joint consultation on the application of Risk management to Food safety matters. 1997. FAO-HQ, Rome, 27-31. Jan. 1997.

38. Y. LI., Bracket R. E., Shewfelt R. L. and Beuchat L. R 2001. Changes in Appearance and natural microflora on iceberg lettuce treated in warm water, chlorinated water and the stored at refrigerated temperature. *Food Microbiol*. 18: 299-308.

39. Zoltan S. Oliver R., and M. Laszlo. 2005. Microbial risk Assessment in the Beverage industry. *Food Control*. 16: 515-521.

40. Zwietering M.H., and S. J. C Van Gerwen. 2000. Sensitivity analysis in quantitative microbial risk assessment. *Int. J. Food Microbiol*. 58: 213-221.

CHAPTER II

INFLUENCE OF INCUBATION TEMPERATURE, PUMMELING TIME, AND LEAF SAMPLING PROTOCOL ON THE RECOVERY OF AEROBIC BACTERIA FROM FRESH-CUT SALAD MIX AND MINIMALLY-PROCESSED WHOLE-HEAD LETTUCE

Abstract

The objective of this study was to determine an appropriate incubation temperature, pummeling time, and sampling method for the total recovery and quantification of aerobic micro-flora found in fresh produce. Microbial analysis for aerobic bacterial count in fresh cut salad mix was conducted and samples were incubated at 21 ° C, 32°C or 35°C for 48 ± 3 hours. Counts for aerobic bacteria recovered from salad mix incubated at 21 °C were found to be significantly ($P<0.05$) higher than those recovered from samples incubated either at 32°C and or 35°C. Aerobic bacterial counts recovered from fresh cut salad that was pummeled for two minutes weren't significantly ($P<0.05$) different from those observed following a one minute pummeling time. Also, recovery of aerobic microorganisms from whole head iceberg lettuce wasn't significantly different ($P<0.05$) from that obtained from the outer layer of leaves alone.

Introduction

The ecosystem of microorganisms on the surface of fruits and vegetables is diverse. The presence and numbers of microorganisms differ depending on the type of produce, agronomic practices, geographical location of production and weather condition before harvest (5, 16). Many of these microorganisms are benign, but some are pathogenic. Thus, sanitizing washes are commonly used in the industry to decontaminate produce prior to further processing or consumption. Research has shown that the efficacy of decontamination of produce can be increased by up to 100-fold by adding disinfectants to wash water (3). However, the ability of sanitizers to disinfect raw produce varies greatly. The type of produce as well as the nature and location of microorganisms on or possibly in the produce makes some pathogens inaccessible and thus influences the efficacy of decontamination treatments. The composition of washing solutions and the intensity and duration of washing also vary, and these variations may influence the efficiency of bacterial removal. The inability of sanitizers to access and remove all microorganisms on the surface of raw produce suggests that they are ineffective in removing cells more intimately associated with morphological structures (3, 8, 18, 4). According to Beuchat (3) microbial cells can be harbored in discontinuities and biofilms formed on the surface of produce. Therefore, cells may be protected from contact with sanitizers and from physical removal, resulting in an increased possibility of pathogens being present in vegetables and fruits at the time of consumption. All of this has implications for the measurement of potential pathogens that may be present on fresh produce in that different washing methods may influence the recovery of cells present on the leaves.

Studies on recovery methods confirm that bacterial recovery from oat and bean leaves was significantly better with pummeling using a mechanical stomacher than with pureeing (7). Comparing the different methods of removing bacteria from leaves, pummeling using a mechanical stomacher for a period of at least one minute was found to have an advantage over washing and sonication. Unlike sonication, pummeling using a mechanical stomacher allowed recovery of essentially all bacteria from the leaves' surface. The effectiveness of the different methods of bacterial removal was confirmed by quantification of the recovered groups of microorganisms. During a study on the influence of operations on microbial populations and changes in a natural microflora fresh produce, Li and others (20) and Sinigaglia and others (17) prepared samples of salad mix and cut carrots for microbial analysis by pummeling sample materials in a mechanical stomacher for two minutes. Thus, although pummeling has been found to be an effective method for removing bacteria from plant material for enumeration, various pummeling times have been employed in different studies.

In addition to washing techniques, incubation temperature can have an effect on the accuracy of microbial enumeration. Garcia and others (9) used an incubation temperature of 37°C to conduct aerobic bacterial counts on leafy vegetables, including lettuce and cabbage. Y. Bin and others (20) used an incubation temperature of 30°C to quantify aerobic mesophilic bacteria during a study on appearance and natural microflora changes in iceberg lettuce. A study of microbial quality of artichoke by Gimenez and others (11) also used 30°C for total plate counts. Thus, previous studies have not always been consistent in terms of preferred incubation temperatures.

The protocol used to sample produce has also been shown to influence microbial counts. Variation in microbial population densities have been shown to be affected by accessibility of leaves to airborne microbes and microclimate (2). Leaf age and position have also been found to influence the frequency distribution and variability of bacterial population sizes associated with leaves of broad-leaved endive. During harvest of endive the linear decrease in density of epiphytic bacteria from outer (older) to inner (younger) leaves of the head was significant (13). However, researchers sometimes continue to sample leaves randomly and do not take into account the physiological stage of the leaf (20).

Many factors have been found to influence the recovery and successful enumeration of bacteria on produce. On the other hand, some factors that might be assumed to be significant have not always proven to be so, at least for other types of food products. For example, incubation time using selective enrichment broths and plating methods were found to have no effect on the performance of the media and the final counts obtained for *vibrio cholerae* in oysters (6). Reviewing preceding research work, there is clearly contradictory information and no standard protocol for optimal sampling protocols, pummeling time, and incubation temperatures to be used during microbial analysis of produce to insure optimal recovery of different groups of microorganisms.

The purpose of this study was to optimize sampling and microbial quantification protocols in fresh produce. This study focused on the impact of pummeling time, and incubation temperature on the recovery and quantification of aerobic bacteria from fresh-cut salad mix and on the impact of leaf sample selection on the recovery and quantification of aerobic bacteria from whole-head iceberg lettuce. Determining the

appropriate pummeling time, incubation temperature, and sampling method will help assure the best recovery and quantification of aerobic micro-flora found in fresh produce.

This study consisted of three different but interrelated experiments:

1. Determination of incubation temperature that is best for enumeration of total plate count in fresh-cut salad mix.
2. Establishment of appropriate pummeling time for recovery of aerobic bacteria from fresh-cut salad mix.
3. Determination of sampling method that maximizes the recovery of aerobic bacteria from whole-head iceberg lettuce.

Materials and methods

Impact of incubation temperature on total recovery of aerobic microorganisms in fresh-cut salad mix

Three 345g bags of salad mix (iceberg lettuce, red cabbage and carrots) were collected each week from a local retail store each week for a total of three weeks. In order to maintain the storage temperature, bags of salad mix were placed in an ice chest and transported to the Oklahoma State University Robert M. Kerr Food and Agricultural Products Center (FAPC) laboratory facilities in Stillwater, OK within an hour and microbial analysis was conducted the same day. Twenty five grams of salad mix were randomly drawn from each of the three bags of salad mix and were aseptically transferred into separate stomacher bags. Diluent consisting of 225 ml of 0.1% peptone water was then added to each stomacher bag and samples were pummeled for one minute in a

mechanical stomacher (IUL Instruments, model CE 2003). A 1 ml aliquot was then removed from each stomacher bag and additional appropriate dilutions were prepared in accordance with standard procedures described in the Compendium of Methods for the Microbial Examination of Foods (19). Aerobic bacterial counts were determined using the pour plate method. Three sets of plates for each dilution were plated on standard plate count agar. After the agar solidified, the plates were inverted and duplicate plates from each sample were incubated in three different incubators set at 21 °C, 32°C and 35°C for 48 ± 3 hours. Colony counts were conducted using a colony counter and data were subsequently analyzed to evaluate the effect of incubation temperature.

Effect of pummeling time on microbial recovery of aerobic bacteria in fresh cut salad mix

Experiments to determine optimal pummeling time for a complete recovery of aerobic bacteria from fresh-cut salad mix (iceberg lettuce, red cabbage and carrots) were conducted following the determination of appropriate incubation temperature. Three 345 g bags of salad mix were collected each week from a local retail store for a total of three weeks. In order to maintain the storage temperature, bags of salad mix were place in an ice chest and transported to the Oklahoma State University Robert M. Kerr Food and Agricultural products Center (FAPC) laboratory facilities in Stillwater, OK within an hour and microbial analysis was conducted the same day. Twenty five grams of salad mix were randomly drawn from each of the three bags of salad mix and aseptically transferred into separate stomacher bags. Diluent consisting of 225 ml of 0.1% peptone water was then added to each stomacher bag and samples were initially pummeled for

one minute in a stomacher (IUL Instruments, model CE 2003). A 1 ml aliquot was then withdrawn from each stomacher bag and held aseptically for further dilution and plating. The remaining sample mixture was then pummeled for an additional one minute. One milliliter aliquots were then withdrawn from each stomacher bag as before and additional appropriate dilutions were prepared in accordance with standard procedures described in the Compendium of Methods for Microbiological Examination of Foods (19). Aerobic bacterial counts were determined using the pour plate method. Duplicates of plates for each dilution were plated on plate count agar. After the agar solidified, the plates were inverted and incubated in an incubator set at 21 ^{o}C for 48 ± 3 hrs. Colonies were counted using a colony counter and statistical analyses were conducted on the data in order to evaluate the effect of pummeling time.

Effect of leaf-sampling protocol on microbial recovery of aerobic bacteria from minimally-processed whole-head iceberg lettuce

Two minimally-processed whole heads of iceberg lettuce were collected each week from a local retail store for three weeks. Each week samples were transported to the Oklahoma State University Robert M. Kerr Food and Agricultural products Center (FAPC) laboratory facilities in Stillwater, OK within an hour and microbial analysis was conducted within 24 hours. Each head of lettuce was cut into two equal parts. The cutting knife was sanitized between uses and all handling was done using sanitized gloves, which were changed between samples, and all cutting was done on a sanitized cutting surface, which was freshly sanitized between samples. After the outermost

wrapping leaves of each of the two halves were removed to simulate normal hand trimming, the three most outer layers leaves from one half head were shredded by hand with a stainless steel knife. Again, the cutting knife was sanitized between samples. Twenty five grams of the shredded pieces were then weighed and placed in a stomacher bag. The entire remaining half of head of lettuce was then shredded and 25 grams sample were transferred into a stomacher bag. The cutting knife was sanitized between uses. Diluent consisting of 225 ml of 0.1% peptone water was then added to each stomacher bag and samples were pummeled for one minute in a stomacher (IUL Instruments, model CE 2003). A 1 ml aliquot was then withdrawn from each stomacher bag and additional appropriate dilutions were prepared in accordance with standard procedures described in the Compendium of Methods for Microbiological Examination of Foods (19). Duplicates of plates for each dilution were plated on plate count agar. After the agar solidified, the plates were inverted and incubated in an incubator set at 21 ^{o}C for 48 ± 3 hrs. Aerobic bacterial count was determined using a colony counter and the data were analyzed in order to evaluate the effect of leaf sampling protocol.

Statistical Analysis

Proc mix was used to determine the quantitative difference on bacterial recovery of the different incubation temperatures, pummeling times and methods of sampling. Data was analyzed using Statistical Analysis System (SAS Institute, Carry, NC) to find differences among means of log colony forming units.

Results and Discussion

Recovery of aerobic bacteria from fresh cut salad mix was significantly impacted by incubation temperature (Table 2.1). Aerobic bacteria counts recovered from salad mix incubated at 21 °C were significantly ($P<0.05$) higher than those recovered from samples incubated either at 32°C and or 35°C. Microbial analysis with incubation temperature of 21°C resulted in 6.9 log colony forming units per gram (CFU/g) compared to 6.1 and 6.0 log CFU/g at incubation temperatures of 32°C and 35 °C respectively. There was no significant difference ($P<0.05$) observed in the recovery of aerobic bacteria from fresh cut salad mix and incubated at 32 and 35°C. Thus, we determined that 21°C was an appropriate incubation temperature to use for maximizing recovery of aerobic bacteria from fresh produce.

Results of the statistical analysis on the recovery of aerobic bacterial population presented in Table 2.2 shows that the aerobic bacterial load (6.5 log CFU/g) recovered from fresh cut salad that was pummeled for two minutes wasn't significantly ($P<0.05$) different from that observed following a one minute pummeling time (6.5 log CFU/g). Therefore we conclude that a one-minute pummeling time is adequate to obtain maximum recovery of aerobic bacteria from our samples.

Recovery of aerobic microorganisms from whole head iceberg lettuce as influenced by sampling method is shown in table 2.3. Previous work has shown that the initial aerobic mesophilic load of lettuce usually ranged between $\sim$4 and $\sim$6 log CFU/g and occasionally even more, and the counts from the outer most layer of lettuce exceeded those from interior layers by one to more than log CFU/g (1, 10, 15). But in our study, recovered aerobic bacterial population was 9.2 CFU/g for the whole head lettuce and 8.8

log CFU/g for the outer three layers of leaves. The microbial load on whole head lettuce wasn't significantly different ($P<0.05$) from that observed on just the outer three layers of leaves alone.

Conclusion

Based on our statistical analyses, the two methods of sampling tested – whole head versus three outermost leaves – did not significantly affect the observed recovery of aerobic bacteria in minimally-processed whole-head iceberg lettuce. We also did not observe any difference in aerobic bacterial counts between samples pummeled for either one or two minutes using a mechanical stomacher. We did observe higher counts in samples incubated at 21°C versus those incubated at 32°C and 35°C. Accordingly, samples in subsequent experiments were incubated at 21°C for aerobic bacterial counts.

References

1. Adams, M. R., Hartley, A. D., & L. J. Cox, 1989. Factors affecting the efficacy of washing procedures used in the production of pre-pared salads. *Food Microbio.* 6: 69-77.

2. Andrews, J. H., C. M. Kenerley, and E. V. Nordheim. 1980. Positional variation in phylloplane microbial populations within an apple tree canopy. *Microbiol. Ecol*. 6:71–84.

3. Beuchat L R. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Unit, World Health Organization. WHO/FSF/FOS/ 98.2. 42 pp.

4. Beuchat L. R. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. *Microbes and Infect***.** 4: 413-423.

5. Bracket. R. E. 1999. Incidence, contributing factors and control of bacterial pathogens on produce. *Post harvest Biol. Technol.* 1: 305-311.

6. Depaola A., and Hwang G. C. 1995. Effect of dilution, incubation time, and temperature of enrichment on cultural and PCR detection of *Vibrio cholerae* obtained from the oyster *Crassostrea virginica*. *Molec. and Cellular probes*. 9: 75-81.

7. Donegan K., Matyac C., Siedler R. and Porteous A. 1999. Evaluation of methods for Sampling, recovery and enumeration of bacteria applied to phylloplane. *Applied Environ. Microbiol.* 57: 51-56.

8. Frank J.F. 2001. Microbial attachment to food and food contact surfaces. *Adv. Food Nutr. Res*. 43: 319– 370.

9. Garcia-Vilanova Ruize B., Galvez Vargas R., and R. Garcia-Vilanova 1987. contamination of fresh vegetables during cultivation and marketing. *Int. J. Food Microbiol*. 4: 285-291.

10. Garg, N., Churey, J.J. and D.F. Splittstoesser. 1990. Effect of Processing Conditions on the Microflora of Fresh-cut Vegetables. *Food Protect.* 5:701-703.

11. Gimenez M. ; C. Olarte, S. Sanz, C. Lomas, J.F. Echiavarri, and F. Ayala. 2003. Relation between spoilage and microbiological quality in minimally

12. processed artichoke packaged with different films. *J. Food Microbiol.* 20:231-242.

13. Jacques, M., A. Kinckel, L. L. and Morris. C. E. 1995. Population sizes, immigration and growth of epiphytic bacteria on leaves of different ages and

positions of field-grown endive(*Cichorium endivia var. latifolia*). *Appl. Environ. Microbiol.* 61: 899-906.

14. Jaques M. A., and Morris. C. E. 1995. Bacterial population dynamics and decay on leaves of different ages of ready-to use broad leaved endive. *Int. J. Food Sci. and Technol.* 30: 221-236.

15. Jacques M. A. 1996. The Effect of Leaf Age and Position on the Dynamics of Microbial Populations on Aerial Plant Surfaces. *Aerial Plant Surface Microbiology*, Plenum Press, New York.

16. King A. D., Jr., J.A. Magnuson, , T. Torok and N. Goodman 1991. Microbial Flora and Storage Quality of Partially Processed Lettuce. *J. Food Science* 56: 459-461.

17. Nguyen C. and Carlin F. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr*. 34: 371-401.

18. Sinigaglia M., Albenzio M., and R. Carbo. 1999. Influence of process operations on shelf-life and microbial population of fresh cut vegetables. *J. Industrial Microbiol. Biotechnol.* 23: 484-488.

19. Seo K.H., and J.F. Frank 1998. Attachment of Escherichia coli O157:H7 to lettuce leaf surface and bacterial viability in response to chlorine treatment as demonstrated by using Confocal scanning laser microscopy. *J. Food Prot*. 62: 3-9.

20. Vanderzant C. and D. F. Splittstoesser. 1992. Compendium of methods for the microbiological examination of foods. American Public Health Association. pp. 920.

21. Y. Li., Bracket R. E., Shewfelt R. L. and Beuchat L. R 2001. Changes in Appearance and natural microflora on iceberg lettuce treated in warm water, chlorinated water and the stored at refrigerated temperature. *Food Microbiol*. 18: 299-308.

Table 2.1. Comparison of Incubation Temperature for aerobic bacteria Enumeration On ready to eat Vegetables

Incubation temperature (degree celsius)	Population mean $\log_{10}$ CFU g^{-1}
21	6.9[a]
32	6.1[b]
35	6.0[b]

NB: Mean values followed by the same letter are not significantly different ($P \leq 0.05$). Mean is the average of three experiments, each with three samples in duplicate.

Table 2.2. Effect of pummeling time on recovery of aerobic bacteria in ready to eat vegetables

Pummeling time (minutes)	Population mean $\log_{10}$ CFU g^{-1}
1	6.7[a]
2	6.5[a]

NB: Mean values followed by the same letter are not significantly different ($P \leq 0.05$). Mean is the average of three experiments, each with three samples in duplicate.

Table 2.3. Effect of sampling method on enumeration of aerobic micro-organisms on minimally processed whole head Iceberg lettuce.

Sampling Method	Mean $\log_{10}$ CFU g^{-1}
Whole head	9.2[a]
Outer leaves	8.8[a]

NB: Whole head- lettuce (composite of surface and tissue of the produce)
Outer leaves- the three most outer layers of the same head of lettuce
Mean is the average of three experiments, each with two samples in duplicate.
Mean values followed by the same letter are not significantly different ($P \leq 0.05$).

CHAPTER III

A SIMULATION MODEL OF QUANTITATIVE MICROBIAL RISK ASSESMENT DURING PROCESSING OF FRESH CUT LETTUCE

Abstract

This study used a quantitative risk assessment model to predict the occurrence, death, and/or survival of groups of microorganisms during commercial fresh cut lettuce processing. The specific objectives of this study were to build a quantitative risk assessment model, develop a computer simulation program, and model the occurrence, death, and/or survival of groups of microorganisms in lettuce from trimming all the way through de-watering and packaging. The model inputs included bacterial counts taken at the unit processing operations of trimming, shredding, washing and de-watering (centrifugation). A predictive model was built using this microbial data, which was collected from a local produce processing plant.

The microbial population change was simulated in a sequence for each unit operation using death and survival models, and the model built was then used to evaluate these processing steps so as to determine the overall impact of processing on the microbial load of fresh cut lettuce.

The simulated model predicted that the process of shredding is the biggest contributor to the total aerobic and coliform bacteria estimates in fresh cut lettuce production. Washing with chlorinated water was not predicted to greatly reduce the

microbial load for all three groups of microorganisms. In fact, sensitivity analysis predicted that washing actually contributed to an increase in yeast, mold, and coliform counts in the lettuce. The process of centrifugation was predicted to contribute the least to both the aerobic and coliform bacteria estimates. On the other hand, it was predicted to contribute the most to yeast and mold counts in lettuce processing.

Introduction

In today's food industry, high standards have been set for food safety and quality. Hazard Analysis Critical control Points (HACCP) systems and Good Manufacturing Practices (GMP's) have been developed and are in some cases mandated for most processed food products. But, regardless of the best control measures applied, food products are still exposed to risk of contamination that may lead to disease outbreaks (6). These risks may be hard to assess because they are not uniform. For example, studies on the level of contamination of fresh vegetables show that cabbage, artichoke, and celery may be exposed to a higher level of contamination than lettuce and spinach (15). Production location, which includes the climatic and environmental conditions of production fields, plays a big role in the microbial safety of fresh cut produce. Irrigation and harvest practices are some of the common sources of contamination. Decay and injury caused by plant pathogens can also act as an entry port for human pathogens. Farm workers' personal hygiene also has an influence on the transmission of pathogenic bacteria to produce that is being harvested (FDA Commodity specific guidelines, April 2006). During vegetable production, farm implements come in direct contact with the produce and therefore serve as a vehicle of contamination. Another important aspect to consider is that wash water in tanks is generally reused and may be contaminated with pathogens if a contaminated product coming from the field is washed in that water. Therefore, if not properly sanitized, wash water can become a source of microbiological contamination for every piece of product that passes through that water (17).

Aside from the variable risks described above, production processes may also influence risk for any given product. Producing fresh-cut leafy vegetables usually involves trimming, coring, slicing, shredding, washing, centrifugal drying, and packaging. In fresh bagged lettuce, higher microbial counts of psychrotrophic bacteria, coliforms, and lactic acid bacteria were influenced by all steps in the production process with a pronounced influence being seen at the shredding, rinsing and centrifuging steps in a study conducted by Allende and others (2). This is not difficult to understand as peeling, cutting, and shredding exposes a product to possible contamination.

Chlorinated water is often used to provide some disinfection of fresh-cut produce. The US FDA recommends 50–200 mg l^{-1} total chlorine at pH 6.0–7.5 and contact times of 1–2 min, (24) for washing/sanitizing fresh produce. The International Fresh-Cut Produce Association (17) Model HACCP Plan for shredded lettuce suggests a maximum chlorination of 100–150 mg l^{-1} total chlorine at pH 6.0–7.0 (13). Free residual chlorine concentrations between 2 and 7mg kg^{-1} are required to ensure a complete destruction of microorganisms in water (Food Processors Institute, 1980). However, several reports suggest that the effectiveness of a chlorinated wash in reducing microbial populations on shredded lettuce is minimal at best (1, 4, 8, 11). Washing shredded lettuce in chlorinated water resulted in a decrease of the initial counts by 0.7–1.5 $\log_{10}$ units (3). Washing time had no effect on microbial reduction. This is in accordance with other researchers who found that increasing the washing period above 1 or 2 min showed no improvement on the reduction of mesophilic bacteria (1, 6).

There is evidence to suggest that the effectiveness of chlorinated water for removing microorganisms from the surface of cut lettuce can be improved by increasing process temperatures. Delaquis and others (11) showed that residual microbial populations were 2 log CFU/g lower on cut lettuce washed in chlorinated water at 47 °C compared with product washed at 4°C. Y. Li and others, (26) also suggested that the heat treatment employed (50°C) may have reduced initial populations of some groups of microorganisms naturally occurring on iceberg lettuce, but enhanced microbial growth overall during subsequent storage. Overall, the effectiveness of washing with chlorinated water for eliminating possible pathogens appears to be limited.

The purpose of this study was to develop a model system to simulate produce contamination and cross-contamination for the purpose of determining the level of microbial risk on fresh and minimally processed vegetables at different stages during processing. Determining the level of contamination and/or cross contamination will enable prediction of the critical points to be controlled at different stages in the production system and help set criteria for regulation. Application of microbial risk assessment also provides an additional tool to food safety professionals. This study focused on assessing the realistic hazards present during lettuce processing; it developed a model to predict quantitative microbial load of aerobic bacteria, hygiene-indicator bacteria (coliforms), and yeast and mold on fresh cut lettuce.

Quantitative microbial risk assessment models have previously been used for microbiological assessment of food quality and safety (19). These models were used to enable prediction of risk of microbial contamination and cross contamination during commercial processing. These predictions, which are based on quantitative information, gives an overview of the impact of important process steps on microbial populations, which can help determine levels of relative risk.

Material and Methods

Samples of Iceberg lettuce were collected aseptically from a fresh cut vegetable Processing Plant in Oklahoma City, Oklahoma.

Unit operations sampled during fresh cut lettuce production

The flow diagram of fresh cut lettuce processing as employed by our industrial cooperator is presented in Figure 3.1.

Reception: Heads of lettuce were transported to the Processing Plant and were stored at the reception port. It was assumed that retailers followed standard produce handling and transportation procedures.

Trimming: Three outer most layers of leaves and the core were removed manually from each head of lettuce using a knife and a corer.

Shredding: Lettuce was shredded into pieces of about four cm in size, using an industrial rotary stainless steel bladed shredder.

Washing: Shredded lettuce pieces were washed in chlorinated water for about one to two minutes.

Centrifugation: The washed shredded lettuce was centrifuged for about a minute to remove the free water.

Samples were taken immediately after the lettuce completed each processing step. At trimming, samples were collected after trimming and coring. During shredding, samples were taken after heads of lettuce passed through the shredding machine and the shredded lettuce dropped on a conveyor. Samples were also taken from the conveyor belt immediately after passing through the chlorinated water wash tank. The final sampling point was immediately after the shredded and washed lettuce was removed from the dewatering centrifuge.

Samples were originally also taken from the "Reception" step, prior to any trimming and handling. However, these data were found to fluctuate widely and to not correlate well with data obtained further along in the process. This may be due to the fact that lettuce heads at the "Reception" step were still intact. As it was not feasible to sample multiple heads of lettuce, we determined that it was impossible for us to obtain a sufficiently representative sample with which to enumerate microbial counts at the "Reception" step. Therefore, this data was not used in our model.

Samples were collected at four different points in the production process (Figure 3.1). A trimmed, intact head was collected after trimming (Point 1) and approximately

200 grams of shredded lettuce was collected at subsequent steps (Points 2-4). Duplicate samples were collected at each processing step to give a total of 8 samples collected per visit. Samples were collected on eight different days over a period from June to November, 2005. Thus, a total of 64 samples were collected altogether, 16 from each sampling point in the process.

Because the initial microbial populations in different batches of produce were observed to vary a great deal, perhaps because the produce was obtained from widely varying production areas, population data from the processing step "Trimming" were used as a baseline population level from which to calculate the relative impact of further processing.

Samples were transported to the Robert M. Kerr Food and Agricultural Products Center (FAPC) at Oklahoma State University in Stillwater, OK within one hour after sampling was complete and microbial analyses were conducted within 24 hours. Samples were kept on ice or under refrigeration (<5°C) until they were analyzed.

Microbial analysis

Standard microbial enumeration methods developed by the AOAC (Association of Official Analytical Chemists) modified as described in Chapter 1 were used for microbial quantifications. Approximately 25 gm of shredded lettuce was pummeled with a Stomacher IUL Instruments (CE 2003) in 225 ml of sterile 0.1 % peptone water

(Difco). Additional appropriate dilutions were prepared in accordance with procedures described in the Compendium of Methods for the Microbiological Examination of Foods (25). Aerobic bacterial count was determined following the pour plate method. Plate Count Agar (Difco) was used for plating and duplicate plates were incubated at 21°C for 48±2 hrs. Coliforms group count was determined using Violet Red Bile Agar (VRBA) with incubation temperature of 35°C for 24±2 hrs. The same plates used for coliforms were overlayed with 5ml Violet Red Bile Agar with MUG (4-methylumbelliferyl-beta-D-glucuronide) for enumeration of generic *E. Coli*. Potato Dextrose Agar with 3% tartaric acid (Difco) was used for enumeration of yeast and molds. These plates were incubated for 5 days at room temperature.

Model design

Our model was similar to those used by other researchers in previous work with other food products. Specifically, a Quantitative Microbial Risk Assessment model (QMRA) was created in an Excel spread sheet (Microsoft, Richmond, WA) for prediction and quantification of incidence, distribution and inactivation of aerobic bacteria, coliforms, and yeast and molds. The model then was simulated using @Risk Professional Version 4.5 (Palisade, Newbury, N.Y). Monte Carlo simulation was used for identification of critical points. Lettuce processing was modeled as a series of unit operations: trimming, shredding, washing, and de-watering (centrifugation). Sensitivity analysis of Monte Carlo simulation was performed to provide a quantitative measure for determining the most important factors affecting the risk of microbial contamination

during fresh cut lettuce processing. Sensitivity analysis measures the importance of each unit operation(10). Unlike QRAM by Oscar (22), sensitivity analysis was considered in this model because all randomly-selected values from the normal distribution were used to calculate the model output.

Other models, for example the quantitative risk assessment model of Oscar (21), have used input settings empirically derived from various pre-existing experimental data sets. However, no assumptions and/or data from previous studies were data were used in this simulation. Rather, the input settings in this model were based entirely on locally-collected microbial data. During QRAM simulation, @Risk sampled collected data for all unit operations randomly from within the calculated normal distribution.

Input setting

As noted above, our quantitative risk assessment model was based on population data obtained from microbial analysis of lettuce processed at a local processing company. These values served as a baseline for aerobic bacteria, coliforms and yeast and mold populations. The relationship between input and output variables was constructed in such a way that the difference in microbial load was added to the following processing step. Shredded lettuce after centrifugation was taken to be the final output.

Our model assumed that heads of lettuce were from the same lot and that the microbial population was evenly distributed throughout the lettuce after shredding. It was also assumed that processing a batch of heads of lettuce was completed within twenty

minutes and that there was no growth in the population of indigenous microorganisms during processing.

Input values used by @Risk for predicting aerobic bacteria, coliforms and yeast and molds population at different stages of processing were calculated as follows:

- Average microbial populations for a given type of microorganisms were calculated for lettuce after trimming. The log values of these counts were used as the input population value for @Risk calculations for the process of trimming.
- The average log value changes observed in microbial populations after a given processing step were used to calculate the input population values for subsequent processing steps.

Table 3.1 gives the empirical population data that was input into the model used by @Risk to predict population changes as a result of processing for aerobic bacteria in fresh cut lettuce.

Impact of lettuce processing on aerobic bacteria, coliforms, and yeast and mold population distributions in fresh cut lettuce processing was simulated based on the values of our sixteen baseline sample data points. These values were fitted to a step-wise simulation model and data was simulated from 10,000 iterations. The information in table 3.2 is presented to show the cell addresses and formulas used by @RISK during simulation of the different microbial population data. In this process @Risk chose risk

normal distribution function for all groups of microorganisms of interest during fresh cut lettuce production.

For example, note that the first node in the quantitative risk assessment model for coliforms (Table 3.2) simulated the impact of process of trimming, cell D6, and was created with @Risk using normal distribution with a mean of 3.04 log units and a standard deviation of 0.72. The impact of shredding in cell D7 was modeled using normal distribution as a best choice of @Risk, with a setting of mean value difference of -0.15 log units and a standard deviation of 0.77. Mean value difference of -0.3 and 0.29 log units and standard deviations of 0.58 and 0.55 were used in the input settings of coliforms during model creation for washing and centrifugation processes respectively. At the input settings for both washing and centrifugation steps, @Risk chose normal distribution.

Results and discussions

Aerobic bacteria

The microbial load of aerobic bacteria on lettuce after heads of lettuce following the unit operation of trimming was predicted to be 4.6 log CFU/g (Table 3.3). This load was within the range found by other scientific studies (1, 16, 18). As the same trimmed heads of lettuce pass the next step of processing, shredding, the model which simulated

aerobic bacterial population predicted an increase of 0.8 log units. This relative increase of the simulated aerobic bacterial load was also in agreement with other studies (2, 9).

Besides eliminating plant debris, soil and nutrient rich cellular fluids, the operation of washing was believed to reduce the initial microbial load (16). Others have reported a reduction of approximately 2.7 log units of aerobic mesophilic microflora when lettuce was washed by chlorine solution (1). Washing lettuce immediately after shredding also decreased the initial aerobic bacterial load by 0.7-1.5 log 10 CFU/g in another study (3). In addition, fresh cut vegetables have sometimes been observed to harbor lower microbial populations compared to whole vegetables, presumably due to washing in chlorinated water, (9, 20). Washing shredded lettuce usually is aimed at removing soil and debris and at reducing microbial loads (23).

However, the mean aerobic bacteria population numbers for processed lettuce after washing predicted in this study were that same as those after shredding and before washing, namely 5.4 log CFU/g (Table 3.3). Thus, no reduction in population was predicted. The simulated @Risk model also predicted the aerobic bacterial load of centrifuged lettuce to be higher than the washed cut lettuce by 0.4 log units. Published scientific studies have found that fresh cut produce that followed a standard processing procedure had lower microbial loads than that of unwashed whole vegetables (1, 4, 5). But our model indicated that fresh cut lettuce had an aerobic bacterial load that was 1.2 log units higher after shredding, washing and centrifugation compared to the load after

trimming (Table 3.3). These predictions are seen as a trend in most of the 10,000 iterations (Figure 3.2).

Coliform group

The population data used in the quantitative risk assessment model for colifom bacteria in fresh cut lettuce by @Risk is presented in table 3.4. The simulated data for trimming (Table 3.5) predicted a coliform count of 3.7 log CFU/g after trimming. After shredding, the predicted value increased by 0.6 log units. Washing was expected to remove nutrients from the cut produce and reduce microbial load from the surface of shredded lettuce. But the predicted microbial mean value for the number of coliforms increased to 4.4 log CFU/g after the shredded lettuce was washed in chlorinated water, and did not differ much with the count after shredding, which was 4.3 log units. This value instead increased by only 0.1 log units. The centrifugation step was predicted to increase the population of coliforms to a mean value of 5.0 log CFU/g.

Comparison of the predicted simulated mean value which modeled the impact of each processing step on the distribution of coliforms during lettuce processing indicates that coliform load after the process of centrifugation increased by 1.3 log units compared to the initial count after trimming. Shredding contributed to the increased predicted value of 0.6 log units of coliforms. Washing didn't have a significant impact on coliform counts during fresh cut lettuce production. Values in figure 3.2 show one example of the 10, 000 iterations from simulation of QRAM. The coliform load in log units from the

different processing steps in this iteration (Fig 3.2) relates very well to the mean values of the output in table 3.5. This is true both for aerobic bacteria of table 3.3 and figure 3.2 and yeast and molds of table 3.7 and values of a random iteration in figure 3.2.

Yeasts and molds

Yeasts and molds distribution in fresh cut lettuce production was modeled by @Risk in the same manner as for the aerobic bacteria and coliforms. During simulation of the model, @Risk randomly sampled normal distribution, which was calculated from log mean population data. Node 1 (Table 3.2) calculated the yeast and molds distribution on lettuce after heads of lettuce were cored and the top three layers of leaves were trimmed. The load at this level was considered to be the initial load of yeasts and molds during fresh cut lettuce processing. Similar to the simulation for aerobic bacteria and coliforms, empirical yeasts and molds counts were used by @Risk as the inputs for modeling the impact of shredding, washing and centrifugation (Table 3.6).

The model simulating the impact of trimming gave an output of 2.3 mean log CFU/g of yeasts and molds (Table 3.7). Simulation results of the model after heads of lettuce had passed through a shredding machine indicated that the mean yeast and mold load increased by 0.3 log units due to shredding. The model simulating the process of washing predicted an increase in yeasts and molds load of 0.1 log units. At the end of fresh cut lettuce processing, the simulated model resulted a 0.5 log units increase in

yeasts and molds population due to centrifugation compared to a trimmed head of lettuce. The same trend was observed in the different iterations (Figure 3.2).

Sensitivity analysis

Quantitative risk assessment is intended to systematically identify hazards and estimates their risk. But it is generally agreed that it is impossible to determine risk with high accuracy (27, 28). Quantitative evaluation of food safety is complex, since in most cases many variables have a great deal of statistical variability (28). Sensitivity analysis is a way to measure the effect of a parameter's variability on the variability of the predicted output value, which in our case was the variability of microbial load in fresh cut lettuce at the end of processing. It is also of value in determining correlations. Rank correlation determines the degree to which a given variable is implicated when the overall risk is high (10). Correlation coefficients range between -1 and 1. The closer the coefficient is to one, the higher the correlation and thus the more important the factor is for introducing risk into the production process.

Correlation coefficients of Monte Carlo simulations have previously been used to measure the importance of risk determining factors during fresh-cut lettuce processing (10). We also employed sensitivity analysis, specifically ranked correlations, to evaluate the effect of different processing steps on the final microbial loads in fresh cut-lettuce after centrifugation. The model here simulated 10, 000 iterations from randomly selected

data points and higher positive correlation coefficients signified the relative impact of a unit operation to the final predicted microbial load. It is important to note that these comparisons were relative within simulations.

Aerobic bacteria

Results of the sensitivity analysis (Table 3.8) on the impact of processing lettuce indicates that shredding has a correlation coefficient of 0.576, which indicates that it was the biggest contributor to the increased population of aerobic bacteria in fresh cut lettuce. This result was in agreement with other scientific results (16) and also confirms that in this particular processing plant, shredders are the biggest source of contamination. According to a study on the effect of unit operations on counts for aerobic bacteria in fresh cut lettuce, the counts on shredded lettuce increased by two log units compared to a whole lettuce and dipping shredded lettuce in chlorinated water reduced the counts for aerobic bacteria in fresh cut lettuce by three log units (16).

The process of washing, with correlation coefficient value of 0.479, was found to have the second greatest impact. The processes of trimming and centrifugation with correlation coefficient values of 0.468 and 0.388 respectively take third and fourth place in terms of the relative ranking of their contribution to the increased counts of aerobic bacterial in fresh cut lettuce (Table 3.8). Other studies have previously found that conveyors and centrifuges were not a significant source of contamination (16).

Similarly, our results indicated that the process of centrifugation contributed the least to the aerobic bacterial load during production of fresh cut lettuce.

Coliforms group

The process of shredding and trimming (Table 3.9) had correlation coefficient values of 0.567 and 0.527 respectively. This nominally indicates that shredding was predicted to contribute more to the final coliform counts than trimming, which would generally be in accordance with other studies (16). However, because the correlation coefficients are fairly close, it is difficult to conclude authoritatively that there was any practically significant difference in the relative impact of the two processing steps. In any case, @Risk's simulated model predicts that these two processing procedures are very nearly equally important and taken together have the largest impact on final coliform counts in the shredded lettuce. Washing was observed to be the third-ranked contributor (Table 3.9) to the overall counts of coliform estimates. The centrifugation step was predicted to contribute the least to the final coliform counts.

Yeasts and molds

Contrary to the results observed for bacterial populations, our analyses gave a predicted correlation coefficient of 0.620 for the centrifugation process, thus predicting that centrifugation had the highest impact on the counts of yeasts and molds in fresh-cut lettuce (Table 3.10). Washing would ordinarily be expected to reduce microbial load.

However, according to the simulated model, washing was actually predicted to be the second largest contributor to increased yeasts and molds population with a correlation coefficient of 0.538 (Table 3.10). The processes of shredding and trimming were the two smallest contributors to the yeasts and molds load estimate in shredded lettuce with correlation coefficient values of 0.417 and 0.317 respectively (Table 3.10), again in contrast to what we observed with bacterial populations, particularly coliforms.

We can only speculate on why we saw such different predicted effects of processing on bacteria versus yeasts and molds. It may simply be that our model is inaccurate in some respects due to limited sampling. Or it may be that centrifugation served either directly or indirectly to inoculate shredded lettuce with yeast cells and/or mold spores but not with bacterial cells. For example, if the ambient air in the facility was contaminated with yeast cells and/or mold spores, the movement of air through the shredded lettuce during the centrifugation process may have served to inoculate the product. Other studies have found that centrifugation may contribute to increased microbial counts during lettuce processing, though yeasts and molds were not specifically implicated (2).

Comparison of overall predicted population data to a single predicted iteration

Comparison of overall predicted population numbers for the different groups of microorganisms are compared with the numbers from a randomly-selected iteration in

Figure 3.3. From this we can see that the population trends for a given iteration do not differ dramatically from the overall trends as predicted by @Risk for all groups.

Conclusion

Our model, which simulated the impact of lettuce processing on microbial load, predicted that shredding would be the biggest contributor to the counts of aerobic bacterial estimate in fresh cut lettuce. However, for coliform bacteria the processes of trimming and shredding were predicted to be the most important contributors to an increase in microbial numbers estimates. Although our observed population counts for aerobic bacteria in fresh cut lettuce decreased slightly after washing, our model predicted that washing in a chlorine solution doesn't actually help reduce microbial load during shredded lettuce production. The simulation model by @Risk also predicted that the process of centrifugation contributes the least to aerobic and coliform counts estimate. Centrifugation was predicted to contribute the most to for yeasts and molds load estimate during processing.

Although the results from the simulation during fresh cut lettuce processing seem to be reasonable and generally agree with previous studies, there are clearly areas of improvement for the model. As noted previously, we were not able to use data from the "Reception" step in our model because it was not feasible to obtain a sufficiently representative sample with which to enumerate microbial counts that would correlate with data collected at subsequent points in the process.. This made it difficult to establish

a solid baseline from which to estimate actual changes in microbial populations. In addition, we observed a fair amount of variability in the baseline data we used as inputs in our model. Overall, more sampling would probably be needed to refine and validate our model.

In addition, there may be other factors that were not quantified in this study that had a significant influence on the baseline data used to create the predictive model. For example, we did not measure chlorine strength, water temperature, or residence time in the sanitizing wash. Evaluating the effects of these additional factors could be helpful in refining and validating our model. In a typical industrial setting where much more baseline data regarding microbial populations and control factors such as sanitizing wash strength are routinely collected, it should be possible to create and validate a much more robust risk assessment model.

Overall, our model predicted that fresh cut lettuce would have a higher microbial load at the end of processing than at the beginning. Moreover, our model indicated that the sanitizing wash, which according to our baseline data effected a slight decrease in microbial populations across the board, was not predicted to be effective in reducing microbial loads and some cases was predicted to contribute to an increase in microbial counts at the end of processing. This demonstrates the potential utility of risk assessment models for determining critical points and establishing confidence in control measures as they relate to food safety.

References

1. Adams, M. R., Hartley, A. D., & L. J. Cox, 1989. Factors affecting the efficacy of washing procedures used in the production of pre- pared salads. *Food Microbiology*, 6: 69-77.

2. Allende A., Aguyano E., and F. Artes. 2004. Microbial and sensory quality of commercial fresh processed red lettuce throughout the production chain and shelf-life. *Int. J. Food Microbiol.* 91:109-117.

3. Baur S., Klaibern R., Hammes W. P., and R. Carle. 2004. Sensory and Microbiological quality of shredded, packaged Iceberg lettuce as affected by pre-washing procedures, with Chlorinated and Ozonated water., *Innov Food Sci and Emerging Techno.* 5: 45-55.

4. Beuchat, L. R., & R. E. Brackett. 1990. Survival and growth of Listeria monocytogenes on lettuce as influenced by shredding, chlorine treatment, modified atmosphere packaging and temperature. *J. f Food Science*, 55: 755-758.

5. Beuchat, L. R. 1992. Surface disinfection of raw produce. *Dairy Food and Environ. Sanitation*, 12: 6-9.

6. Beuchat, L. R. 1996. Pathogenic microorganisms associated with fresh produce. *J. food Prot.* 59: 204-216.

7. Beuchat L. R. 1998. Surface decontamination of fruits and vegetables eaten raw: a review. Food Safety Unit, World Health Organization. WHO/FSF/FOS/ 98: 42 pp.

8. Bracket R. E. 1987. Microbiological consequences of minimally processed fruits and vegetables. *J. Food Quality*. 10: 195-206.

9. Bracket, R. E. 1992a. Microbiological Safety of Chilled foods. *Trends Food Sci. Technol.* 3: 81-85.

10. Cassin M. H., A. M. Lammerding, C. D. Ewen, T. W. Ross, R. S. McColl. 2004. Quantitative risk assessment for *Escherichia coli* O157:H7 in ground beef hamburgers. *Int. J. food Microbiol*. 41:21-44.

11. Delaquis P. J. S. Stewart, P. M. A. Toivonen and A. L. Moyls. 1999**.** Effect of warm, chlorinated water on the microbial flora of shredded iceberg lettuce. *Food Research International.* 23: 7-14.

12. FDA Commodity specific guidelines, April 2006.

13. Flickinger, B. 1999. Quality drives a 'growing' industry. *Food Qual*. 6: 27–32.

14. Food Processors Institute. 1980. Cleaning and sanitizing. In: Katsuyama, A.M., Strachan, J.P. (Eds.), Principles of Food Processing Sanitation. The Food Processors Institute, Washington, DC (Chapter 4).

15. Garcia-Vilanova Ruize B., Galvez Vargas R., and R. Garcia-Vilanova . 1987. Contamination of fresh vegetables during cultivation and marketing. *Int. J. Food Microbiol*. 4: 285-291.

16. Garg, N., Churey, J.J. and D.F. Splittstoesser. 1990. Effect of Processing Conditions on the Microflora of Fresh-cut Vegetables. *Food Protec.* 5:701-703.

17. IFPA 2001. Food Safety Guidelines for the Fresh-cut Produce Industry, 4th edn. (edited by J.R. Gorny). 216 pp.

18. King A. D., Jr., J.A. Magnuson, , T. Torok and N. Goodman 1991. Microbial Flora and Storage Quality of Partially Processed Lettuce. *J. food Science.* 56:459-461.

19. McMeekin T. A., Ross T. and J. Olley. 1992. Application of predictive microbiology to assure the quality and safety of fish and fish products. *Int. J. Food Microbiol*.15: 13-32.

20. Nguyen-the, C. and F. Carlin. 1994. The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev.Food Sci. Nutr*. 34: 371-401.

21. Oscar T. P. 1998. The development of a risk assessment model for the use in the poultry industry. *J. Food safety*. 18: 371-381.

22. Oscar T. P, 2004. Quantitative risk assessment model for salmonella and whole chickens. *Int. J. Food Microbiol*. 93: 231-247.

23. Simons, L.K., P. Sanguansri. 1997. Advances in the washing of minimally processed vegetables. Food Aust. 49: 75–80.

24. US Food and Drug Administration (USFDA). 1998. Guidance for Industry. Guide to minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables. US Food and Drug Administration, Washington, DC.

25. Vanderzant C. and D. F. Splittstoesser. 1992. Compendium of methods for the microbiological examination of foods. American Public Health Association. pp. 920.

26. Y. LI., Bracket R. E., Shewfelt R. L. and L. R. Beuchat. 2001. Changes in appearance and natural microflora on iceberg lettuce treated in warm water, chlorinated water and the stored at refrigerated temperature. *Food Microbiol*. 18: 299-308.

27. Whting R. C.; R. L. Buchanan. 1997. Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasturised liquid eggs. *Int. J. Food Microbiol*. 36:111-125.

28. Zwietering M. H., and S. J. C Van Gerwen. 2000. Sensitivity analysis in quantitative microbial risk assessment. *Int. J. Food Microbiol*. 58: 213-221.

Table 3.1. Population data used as inputs into the quantitative risk assessment model for aerobic bacteria in fresh cut lettuce.

Step	Sampling point	Measured Input (log CFU/g)	Measured Output (log CFU/g)
1	After trimming	4.2	4.2
2	After shredding	0.3	4.5
3	After washing	-0.3	4.2
4	After centrifugation	0.2	4.4

Note: Output values are mean values of the sampled data. Input values for sampling points 2 – 4 are the mean log difference in microbial load from the preceding step.

Table 3.2. Cell addresses and formulas used in the quantitative risk assessment model of @Risk in fresh cut lettuce processing

Unit Operation	Distribution	Cell	Formula
Aerobic bacteria			
Trimming	Input	C6	RiskNormal(4.21327, 0.54336, RiskFit(64000, 59905, "Best Chi-Sq"))
Shredding	Input	C7	RiskNormal(0.3253, 0.68342, RiskFit(64000, 92720, "Normal"))
washing	Input	C8	RiskNormal(-0.2935, 0.56086, RiskFit(64000, 23058, "Normal"))
Centrifugation	Input	C9	RiskNormal(0.20432, 0.44125, RiskFit(64000, 46820, "Normal"))
Trimming	Output	D6	RiskOutput(,"microbes",1) + ROUNDDOWN(POWER(10,C6),0)
Shredding	Output	D7	RiskOutput(,"microbes",2) + ROUNDDOWN(POWER(10,C7)*D6,0)
washing	Output	D8	RiskOutput(,"microbes",3) + ROUNDDOWN(POWER(10,C8)*D7,0)
Centrifugation	Output	D9	RiskOutput(,"microbes",4) + ROUNDDOWN(POWER(10,C9)*D8,0)
Coliforms group			
Trimming	Input	D6	RiskNormal(3.04113, 0.72392, RiskFit(10610, 61547, "Normal"))
Shredding	Input	D7	RiskNormal(-0.14672, 0.77824, RiskFit(10610, 69574, "Best Chi-Sq"))
washing	Input	D8	RiskNormal(-0.31255, 0.5737, RiskFit(10610, 14540, "Normal"))
Centrifugation	Input	D9	RiskNormal(0.29056, 0.54598, RiskFit(10610, 88773, "Normal"))
Trimming	Output	E6	RiskOutput(,"microbes",1) + ROUNDDOWN(POWER(10,D6),0)
Shredding	Output	E7	RiskOutput(,"microbes",2) + ROUNDDOWN(POWER(10,D7)*E6,0)
washing	Output	E8	RiskOutput(,"microbes",3) + ROUNDDOWN(POWER(10,D8)*E7,0)
Centrifugation	Output	E9	RiskOutput(,"microbes",4) + ROUNDDOWN(POWER(10,D9)*E8,0)
Yeasts and molds			
Trimming	Input	C6	RiskNormal(2.2757, 0.19568, RiskFit(99649, 62476, "Normal"))
Shredding	Input	C7	RiskNormal(0.22914, 0.26134, RiskFit(99649, 23517, "Normal"))
washing	Input	C8	RiskNormal(-0.09704, 0.33383, RiskFit(99649, 92851, "Normal"))
Centrifugation	Input	C9	RiskNormal(0.35239, 0.38091, RiskFit(99649, 91117, "Normal"))
Trimming	Output	D6	RiskOutput(,"microbes",1) + ROUNDDOWN(POWER(10,C6),0)
Shredding	Output	D7	RiskOutput(,"microbes",2) + ROUNDDOWN(POWER(10,C7)*D6,0)
washing	Output	D8	RiskOutput(,"microbes",3) + ROUNDDOWN(POWER(10,C8)*D7,0)
Centrifugation	Output	D9	RiskOutput(,"microbes",4) + ROUNDDOWN(POWER(10,C9)*D8,0)

Table 3.3. Predicted effects of processing lettuce on microbial load of aerobic bacteria as simulated by @Risk from 10,000 randomly selected data points.

Sampling point	Predicted Mean log 10 CFU/g Output
After trimming	4.6
After shredding	5.4
After washing	5.4
After centrifugation	5.8

NB: Output values are mean values of 10, 000 iterations

Table 3.4. Population data used as inputs into the quantitative risk assessment model for the coliforms bacteria in fresh cut lettuce.

Step	Unit operation	Measured Input log CFU/gm	Measured Output (log CFU/gm)
1	After trimming	3.0	3.0
2	After shredding	-0.1	2.9
3	After washing	-0.3	2.6
4	After centrifugation	0.3	2.9

Table 3.5. Predicted effects of processing lettuce on microbial load of coliforms bacteria as simulated by @Risk from 10,000 randomly selected data points

Sampling point	Predicted Mean log 10 CFU/g Output
After trimming	3.7
After shredding	4.3
After washing	4.4
After centrifugation	5.0

NB: Output values are mean values of 10,000 iterations.

Table 3.6. Population data used as inputs into the quantitative risk assessment model for yeasts and molds in fresh cut lettuce.

Step	Sampling points	Mean log 10 CFU/g	
		Measured Input	Measured Output
1	After trimming	2.3	2.3
2	After shredding	0.2	2.5
3	After washing	-0.1	2.4
4	After centrifugation	0.4	2.8

Note: Output values are mean values of the sampled data. Input values for sampling points 2 – 4 are the mean log difference in microbial load from the preceding step.

Table 3.7. Predicted effects of processing lettuce on microbial load of yeasts and molds as simulated by @Risk from 10,000 randomly selected data points.

Sampling point	Predicted Mean log 10 CFU/g Output
After trimming	2.3
After shredding	2.6
After washing	2.7
After centrifugation	3.2

NB: Output values are mean values of the 10,000 iterations.

Table 3.8. Processing steps ranked as predicted to their impact on aerobic bacterial load in shredded lettuce 10, 000 iterations.

Rank	Processing step	Correlation coefficients
#1	After shredding	0.576
#2	After washing	0.479
#3	After trimming	0.468
#4	After centrifugation	0.388

Table 3.9. Processing steps ranked as predicted for their impact on coliform bacterial load in shredded lettuce following 10, 000 iterations.

Rank	Processing step	Correlation coefficients
#1	After shredding	0.562
#2	After trimming	0.527
#3	After washing	0.419
#4	After centrifugation	0.384

Table 3.10. Processing steps ranked as predicted for their impact on yeasts and molds load in shredded lettuce as following 10, 000 iterations.

Rank	Processing step	correlation coefficients
#1	After centrifugation	0.626
#2	After washing	0.538
#3	After shredding	0.417
#4	After trimming	0.317

Figure 3.1. Flow diagram of fresh cut lettuce processing.

Sampling points 1-4 are denoted by number.

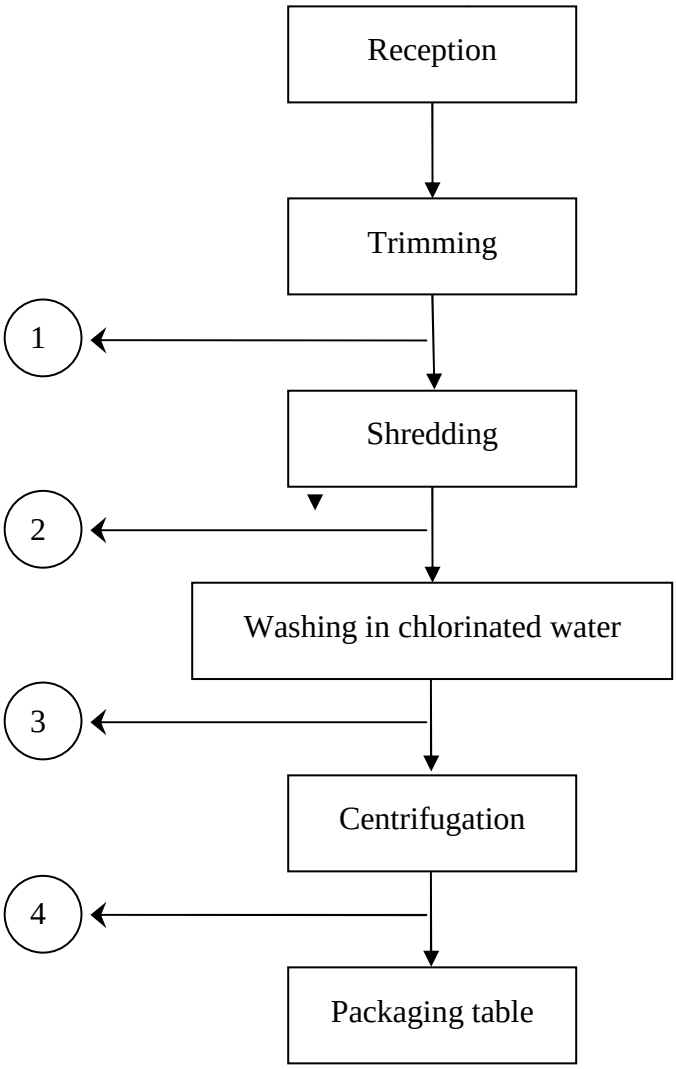


Figure 3.2. Comparison of micro-organisms load during fresh shredded lettuce production with data from a randomly selected iteration

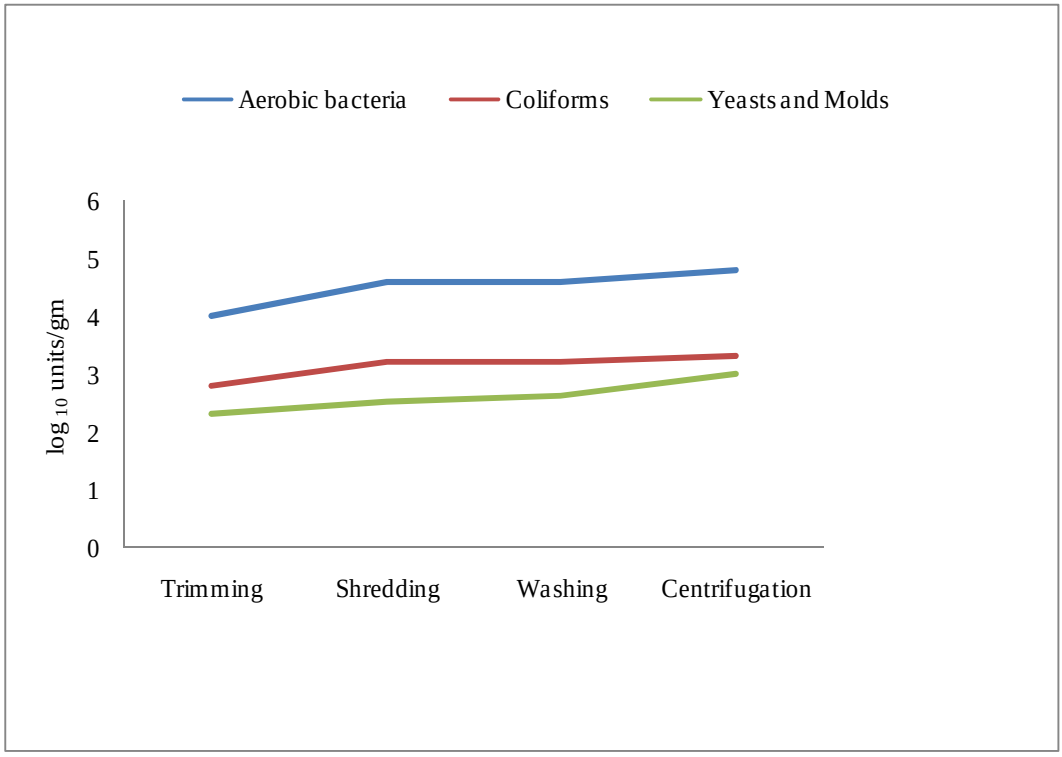
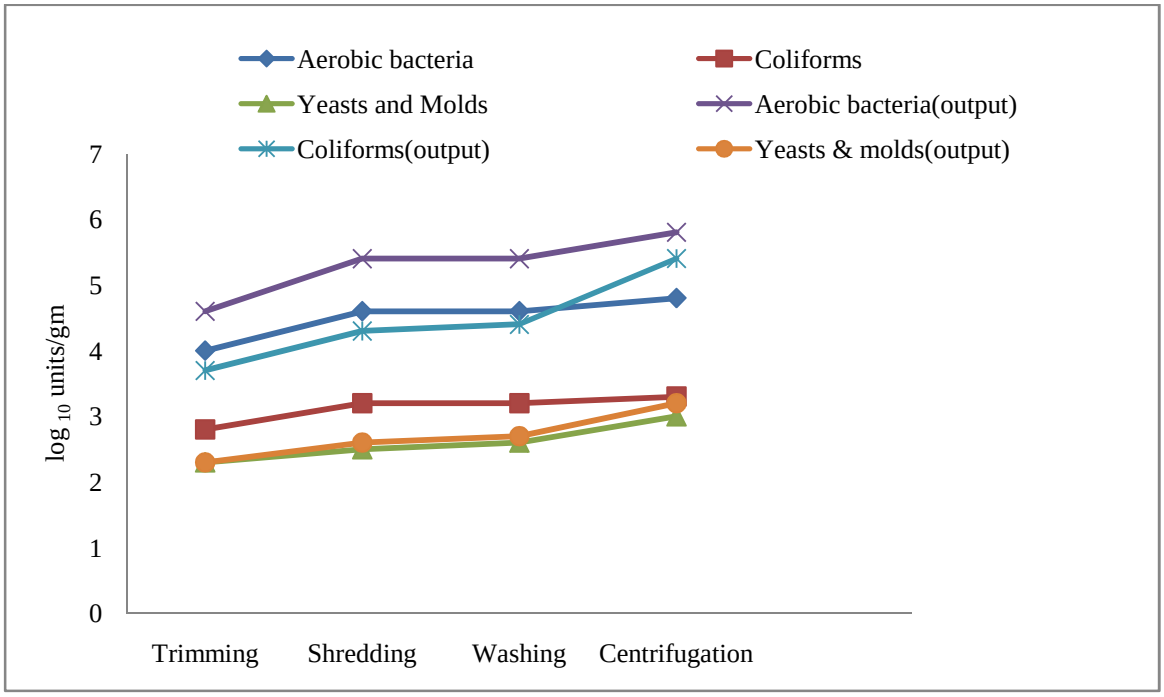


Figure 3.3. Relative comparison of microorganism load from output results for one randomly selected iteration.



Appendix

Experimental microbial data for comparison of incubation temperature for aerobic bacteria Enumeration On ready to eat salad mix

Incubation temperature (degree celsius)	Sample	Population mean $\log_{10}$ CFU g^{-1}
21	1	7.1
21	2	6.6
21	3	7.1
21	4	7.1
21	5	7.5
21	6	7.3
21	7	6.1
21	8	6.8
21	9	6.4
32	1	5.8
32	2	5.5
32	3	6.0
32	4	6.0
32	5	6.4
32	6	6.3
32	7	5.8
32	8	6.3
32	9	6.3
35	1	6.0
35	2	5.6
35	3	5.9
35	4	6.1
35	5	6.2
35	6	6.2
35	7	5.7
35	8	6.6
35	9	6.3

NB: Values are counts from mean of duplicate plates and of nine samples

Experimental microbial data used to measure the effect of pummeling time on recovery of aerobic bacteria in ready to eat salad mix

Stomaching time (minutes)	Sample	Population mean $\log_{10}$ CFU g^{-1}
1	1	6.3
1	2	6.1
1	3	6.3
1	4	6.9
1	5	6.4
1	6	6.5
1	7	6.7
1	8	7.0
1	9	6.3
2	1	6.5
2	2	6.3
2	3	6.3
2	4	7.1
2	5	6.8
2	6	6.5
2	7	6.9
2	8	7.3
2	9	5.5

NB: Values are counts from mean of duplicate plates and of nine samples

Experimental microbial data used to measure the effect of sampling method on enumeration of aerobic micro-organisms on minimally processed whole head Iceberg lettuce.

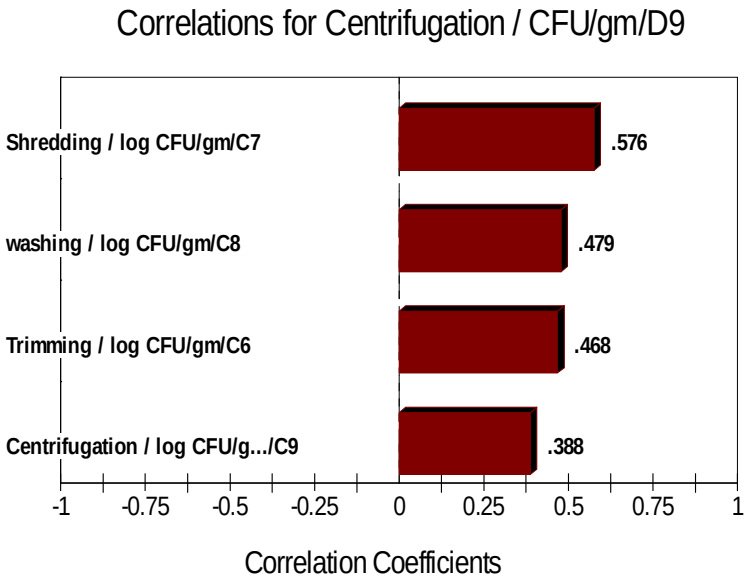
Sampling Method	Sample	Mean $\log_{10}$ CFU g^{-1}
Whole head	1	9.3
	2	9.2
	3	9.1
	4	9.0
	5	10.1
	6	8.5
Outer leaves	1	9.3
	2	8.9
	3	8.8
	4	9.1
	5	8.4
	6	8.3

NB: Values are counts from mean of duplicate plates and of six samples

Lettuce

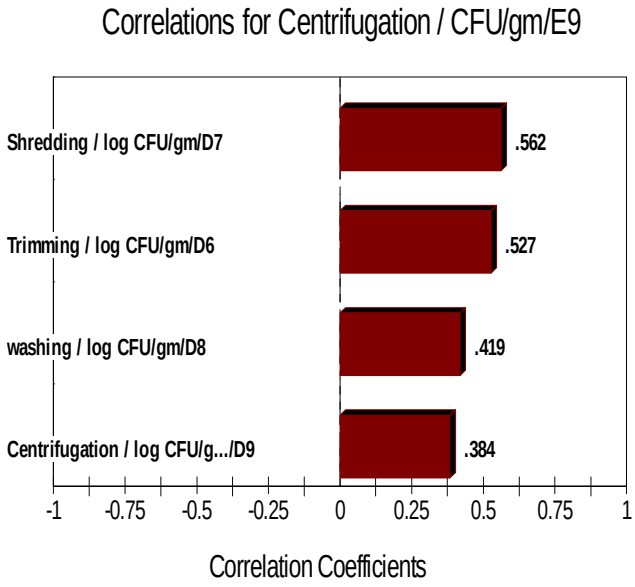
Aerobic bacteria

Tornado graph for correlation between the risk estimates for the aerobic bacterial load in fresh cut lettuce and the four predictive factors during processing.



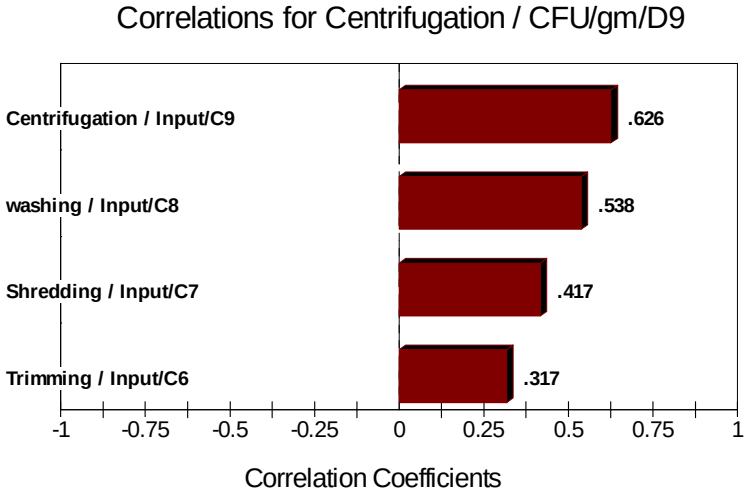
Coliforms group

Tornado graph for correlation between the risk estimates for coliforms in fresh cut lettuce and the four predictive factors during processing.



Yeasts & molds

Tornado graph for correlation between the risk estimates for the yeasts and molds load in fresh cut lettuce and the four predictive factors during processing.



VITA

Haregewoin Takele Woldemeskel

Candidate for the Doctor of Philosophy

Dissertation: A SIMULATION MODEL OF QUANTITATIVE MICROBIAL RISK ASSESMENT DURING PROCESSING OF FRESH CUT LETTUCE

Major Field: Food Science

Biographical:

Personal Data: Born in Wonji, Ethiopia, on February 5, 1966.

Education:

Received Bachelor of Science in Agriculture, from Addis Ababa University, Ethiopia in 1982.
Received Master of Science in Food Science, from Oklahoma State University, USA in 2002.
Completed the requirements for the Doctor of Philosophy in Food Science with emphasis on Horticulture at Oklahoma State University, Stillwater, Oklahoma, in July 2009.

Experience:

Agricultural Extension Officer, Ministry of Agriculture, Ethiopia from 1995-1999. Horticulturist, Ministry of Agriculture, Ethiopia December 1993 to September 1995.

Professional Memberships:

Institute of Food Technologists (IFT)
American Cereal Chemists Association (ACCA)
Sigma XI Scientific Research Society
Pi Alpha Xi Honor Society

Name: Haregewoin T. Woldemeskel Date of Degree: July, 2009

Institution: Oklahoma State University Location: Stillwater

Title of Study: A SIMULATION MODEL OF QUANTITATIVE MICROBIAL RISK ASSESMENT DURING PROCESSING OF FRESH CUT LETTUCE

Pages in Study: 76 Candidate for the Degree of Doctor of Philosophy

Major Field: Food Science

Scope and Method of Study:

The objectives of this study were to build a quantitative risk assessment model, develop a computer simulation program, and model the occurrence, death and/or survival of groups of microorganisms in lettuce from trimming all the way through de-watering and packaging. Baseline microbial data was used and 10, 000 iterations were simulated using Monte Carlo simulation by @RISK soft ware. Risk estimates and sensitivity analysis were used to determine the relevance of each processing unit during fresh cut processing.

Findings and Conclusions:

The simulated model predicted that the process of shredding is the biggest contributor to the total aerobic and coliform bacteria estimates in fresh cut lettuce production. Washing with chlorinated water was not predicted to greatly reduce the microbial load for all three groups of microorganisms. In fact, sensitivity analysis predicted that washing actually contributed to an increase in yeast, mold, and coliform counts in the lettuce. The process of centrifugation was predicted to contribute the least to both the aerobic and coliform bacteria estimates. On the other hand, it was predicted to contribute the most to yeast and mold counts in lettuce processing.

ADVISER'S APPROVAL: Dr. William McGlynn