NOVEL AND ALTERNATIVE PROCESSING TECHNIQUES TO IMPROVE THE QUALITY OF THERMALLY PROCESSED FOODS

YETENAYET BEKELE TOLA

Department of Food Science and Agricultural Chemistry

Macdonald Campus, McGill University

Montreal, Canada

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SUGGESTED SHORT TITLE

NOVEL AND ALTERNATIVE FOOD PROCESSING TECHNIQUES

This thesis is dedicated to my wife Meskerem and sons Naol and Eba

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ABSTRACT

Low acid vegetables are commonly subjected to intensive thermal treatments. But such treatments significantly damage quality of the products. So far different attempts have been made to improve the quality of canned foods either through modification of processing methods or the use of novel processing technologies. Both approaches have their own limitations to achieve required quality improvement or are hindered by practical applicability in terms of food safety. However so far, limited attempts were made in terms of modification of product property and combined use of alternative processing techniques. Therefore the over goal of this work was to improve quality of canned vegetables, through achieving the following objectives.

The first part of this study was focused on searching novel acid infusion mechanisms to modify product pH. Acid infusion kinetic study results showed that pressure assisted approach showed faster, uniform and dependable pH reduction. The associated decimal pH reduction times were 2.4 to 4.4 times higher in conventional method. Furthermore, optimized acid infusion conditions were determined and predictive models were developed using CCD of RSM.

The second and third parts of the study investigated the resistance of *Bacillus licheniformis* spores under different treatments combination. In the second part, the combined effects of different heating methods, types of acidifying agents and pH levels on resistance of the spores were studied. Temperature and pH showed highly significant ($p \leq 0.01$) effect with the lowest *D* value at pH 4.5. The overall range of *D* and *z* values were 1.1 to 11.2 min and 12.6 to 17^{o}C, respectively, regardless of heating methods, type of acidifying agent and pH levels. Likewise, the third part, investigated the resistance of the spores under pressure-thermal-pH combinations. Conventional log-linear and Weibull models were used to evaluate survivor curves and certain-log cycle reduction of spores. Survivor curves were better described by the latter model. Pressure-temperature combinations showed significant effects on *D* and Weibull rate (α) parameters. The destruction pattern was also dependent on pH, with lower *D* and α values at lower pH. Pressure-thermal death times estimated using Weibull model parameters were higher than values determined by log-linear model, with the latter showing adequacy and the former demonstrating over-treatment.

The fourth part of this work was focused on studying the influence of acid infusion on quality retention of carrot. Texture degradation kinetic rate of acid infused (pH 4.5) and control (pH

6.2) samples were investigated at different processing methods. Results showed that on average a 1.7 (conventional thermal), 1.4 (ohmic heating), 1.2 (high pressure-thermal) faster texture degradation rate was observed on controls. Further microscopic and molecular studies of cell wall showed that acid infused samples exhibited intact cell wall structure with lower β- elimination reaction products.

Finally, a validation study of different processing methods was conducted using an inoculated pack study. Delivered pasteurization values for each treatment conditions showed more than 7 log reduction of spores for initial inoculum concentration of 10^8 spores/container. This confirmed the adequacy of the designed processing schedules to inactivate *B. licheniformis* to insure food safety. Therefore, through combined use of developed novel acid infusion technique and determined inactivation kinetic data, quality of canned vegetables can be significantly improved with use of moderate alternative processing methods with required food safety.

RÉSUMÉ

Les légumes peu acides (pH> 4,6) sont généralement soumis à des traitements thermiques relativement intensifs. Jusqu'ici, divers procédures ont été développé pour améliorer la qualité des aliments en conserve, soit par modification des méthodes classiques ou par l'application de technologies novatrices. Ces deux approches présentent des avantages mais aussi leurs propres limites. Un nouveau concept dans ce domaine est la modification d'une propriété du produit et ce combiné avec des traitements alternatifs pour améliorer le processus ainsi que la qualité des légumes en conserve.

Le point central de le première partie de cette étude été l'évaluation des nouveaux mécanismes de l'infusion d'acide pour modifier le pH du produit. Les résultats de l'étude cinétique ont montré que l'acidification haute pression offre une approche plus rapide, plus uniforme et qui produit une réduction de pH fiable. Les valeurs *D* étaient de 2.4 à 4.4 fois plus élevées âne la méthode conventionnelle par rapport à l'acidification haute pression. En plus, les conditions optimales ont été réalisées avec la MSR.

Les deuxième et troisième sections de l'étude ont examiné la résistance des spores de *Bacillus licheniformis* sous différentes combinaisons de traitements. La température et le pH ont présenté un effet hautement significatif ($p < 0,01$) avec la valeur D la plus basse à pH 4,5. La variation des valeurs D et z est de 1.1 à 11.02 minutes et de 12,6 à 17°C, respectivement, indépendamment des méthodes de chauffage, d'agent acidifiant et les niveaux de pH. De même, la résistance des spores dans les combinaisons pression-thermique-pH (400-600MPa, 40-60°C, pH 4.5-6.2) a été étudié les modèles logarithmiques-linéaires et Weibull ont été utilisés pour évaluer les courbes de survie et les réductions logarithmiques des spores. Les courbes de survie ont été mieux décrites par le modèle Weibull. Les combinaisons pression-température ont montré des effets significatifs ($p \leq 0,05$) pour les valeurs D (modèle log-linéaire) et les paramètres de taux Weibull (α). La tendance de la destruction était également dépendante du pH où les valeurs inférieures D et α ont correspondu à un pH plus bas. Les temps de mort pression-thermique (5D et 12D) estimées en utilisant les paramètres du modèle Weibull étaient plus élevés que les valeurs déterminées par le modèle log-linéaire, nous montrant un traitement adéquat et une situation de surtraitement, respectivement.

La quatrième partie de ce travail a porté sur l'étude de l'influence de l'infusion d'acide sur la rétention de la qualité des carottes. Le taux cinétique de la dégradation de la texture dans les échantillons acidifiés (pH 4,5) et non-acidifiés (contrôle; pH 6,2) à été étudié sous différentes méthodes de traitement. Les résultats ont montré une accélération de la dégradation de la texture de 1,7-fois (chauffage conventionnel), 1.4-fois (chauffage ohmique, OH), 1.2-fois (traitement à haute pression, HP-T) comparée aux échantillons contrôles. D'autres études microscopiques et moléculaires de la paroi cellulaire ont montré que les échantillons acidifiés ont maintenu leurs structure cellulaire intacte avec des produits de réaction β-élimination inférieurs que la contrôle.

Finalement, une validation des méthodes de traitement a été effectuée à l'aide du paquet inoculé. Les valeurs de pasteurisation pour chaque condition de traitement ont montré une réduction de spores d'un facteur au moins de 7-$\log_{10}$ car aucun survivant n'a été détecté à partir de l'inoculum initial de 10^7 spores/conteneur. Cela confirme l'adéquation des temps de traitement conçus pour inactiver les spores de *B. licheniformis* pour assurer la sécurité alimentaire. Par conséquent, l'utilisation combinée des nouvelles techniques d'acidification et des données cinétiques d'inactivation, la qualité des légumes en conserve pourrait être considérablement améliorée par l'utilisation de méthodes de traitement alternatives.

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CONTRIBUTIONS OF AUTHORS

Parts of the thesis research have been presented at international conferences and some manuscripts have been published and prepared for publication. Principally two authors have been involved in the thesis and their levels of contributions to the various articles are as follows:

Yetenayet B. Tola is the PhD candidate who, under the guidance of his supervisor, planned and conducted all the experiments, gathered and analyzed the results, and drafted all the manuscripts for scientific publications.

Dr. Hosahalli S. Ramaswamy is the thesis supervisor, under whose guidance the research plan was carried out, and who assisted the candidate in planning and conducting the research with his expertise in areas of food processing and quality analysis. He was also responsible for the final correction, edition, revision and processing of the manuscripts for publications.

LIST OF PUBLICATIONS AND SCIENTIFIC PRESENTATIONS

I. Part of this thesis has been published, submitted or in preparation

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Hussein Hassan, **Yetenayet** B. Tola and Hosahalli S. Ramaswamy. Radiofrequency and Microwave Heating: Similarities, Advantages and Limitations. In: Georg Awuah, Hosahalli S. Ramaswamy and Juming Tang (eds), Radio Frequency: Principles, Practices and Applications, Volume 3 (submitted to the editors).

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Yetenayet B. Tola and Hosahalli Ramaswamy.2011. Simplified Method in Extraction of Lycopene and ß-carotene Rich Powder of Watermelon Juice as a Food Colorant and Antioxidant Source, IFT annual conference, June 11-14, 2011, New Orleans, USA.(oral and poster).

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Yetenayet B. Tola and Hosahalli Ramaswamy.2010. Going Beyond Conventional Osmotic Dehydration for Quality Advantage and Energy Saving, Presented on Inaugural workshop of Ethiopian Journal of Applied Science and Technology, June 2010, Jimma, Ethiopia.(oral)

CONTRIBUTION TO KNOWLEDGE

i. In conventional production of acidified low acid foods, acidification or pH reduction of low acid foods is done using one of conventional acidification techniques. Conventional acid infusion methods commonly use acetic acid in high concentrations which can have detrimental effect on the flavor and taste characteristics of the product. In addition, it takes longer time to reach finished equilibrium pH, exhibits lack of uniform pH reduction, and lacks accurate and dependable pH reduction models (since pH reduction doesn't consider the slowest pH reduction part). Furthermore, in conventional methods, Food and Drug Administration of USA doesn't recommend type and concentration of acidifying agents to be used. In this study a novel pH reduction technique (high pressure assisted acid infusion) was evaluated and optimized. The pH reduction capacity of different types of acidifying agents, at different acidification conditions, were determined and predictive models were developed. Acid infusion kinetic parameters were determined and predictive quadratic models were developed to forecast pH reduction for other similar type of vegetables.

ii. The role of *B. licheniformis* in production of acidified low acid foods and as a surrogate food microorganism has not been considered in food industry. Inactivation kinetic study of this microorganism were mostly limited to high pH foods. In this study, heat resistance behaviors of the spores of *B. licheniformis* at different pH levels and processing methods were determined for different types of acidifying agents. The kinetic parameters for these studies are additional data for design and optimization of acidified thermal processing sechedules.

iii. In addition to the above, the resistance of spores of *B. licheniformis* for combined effects of pressure, temperature and pH was investigated. This was a pioneer study on the inactivation kinetics of *B. licheniformis* spores under the combinatory conditions to show the synergetic effect of combined lethal agents. Inactivation behavior of spores to these agents were evaluated according to log-linear and Weibull models. First order inactivation kinetic and Weibull model parameters were used to determine decimal reduction time and pressure-thermal death times. So far no agreement has been reached either to use the Weibull or classical first-order models to describe the inactivation behavior of spores in pressure

assisted thermal processing methods. The discrepancy between the two models, in terms of decimal reduction/pressures-thermal death time, to achieve required degree of lethality were identified in this study. The study also showed the discrepancy between the two models using experimental data to determine thermal death times.

iv. Attempts were also made, for the first time, to show the importance of acid infusion in low acid vegetables to improve their texture retention through retarding the rate of β-elimination reaction which is the responsible reaction process for cell wall degradation and texture softening. The study was well supported through microscopic study of cell wall and texture retention of carrot tissue. Attempts were made to relate molecular, microscopic and macroscopic modifications of cell wall components of carrot to investigate in detail the benefits of controlled acid infusion on retention of texture of thermally processed low acid vegetables. The combined application of controlled pH reduction-alternative processing methods on quality retentions were demonstrated and their commercial applications were validated.

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NOMENCLATURE

^{o}C	degree celcius
s	Second
cm^3	cubic centimeter
cm	Centimeter
H^+	hydrogen ion
k	rate constant (per min or per second)
t	Time
D	decimal reduction time (min)
z	temperature (^{o}C) or pressure (MPa) sensitivity value
Ea	Activation energy (J/mole or kJ/mole)
^{o}K	degree Kelvin
R	molar gas constant (8.3145 J/mole ^{o}K)
Va	activation volume (ml/mol)
P_{ref}	reference pressure (MPa)
β_o	interception coefficient
β_{ii}	coefficient of quadratic effect
β_{ij}	coefficient of interaction effect
x_i	variables
pKa	acid dissociation constant
p	Probability
kHz	kilo Hertz
A	Amper
V	Volt
Hz	Hertz
µs	micro second
N_t	survivor spores after a specific time *t* (CFU/ml)
N_o	initial viable cell or spore count at time zero (CFU/ml),
R^2	regression coefficient
g	gravitational acceleration (m/s^2)
h	Hour
α	characteristic time or scale parameter (min) of Weibull model
β	shape parameter (non-dimensional)of Weibull model
t_R	reliable life equivalent to decimal reduction time (min)
t_d	time to certain number of log reduction (min)
a	coefficient of regression line represent intercept of a line
	coefficient of regression line represent slope of a line

b	
$\sum$	summation operator
n	number of values of a given parameter
O_i	i^{th} observed value
Pi	i^{th} predicted value
T_2	initial temperature of the sample to reach target temperature
T_3	target temperature
HC	heat of compression of sample during pressurization
ΔP	processing pressure during holding time
ΔTH	temperature gain by the sample from surrounding medium
N	Newton
H_o	initial hardness of sample at time zero
H_t	hardness measured at a given time t,
H_∞	nonzero equilibrium hardness property after prolonged heating
f	residual fraction
T	temperature (^oK or ^oC) at time t
µl	micro liter
P_T^z	pasteurization value at reference temperature (min) and z value (^oC)
L*	initial lightness value
a*	initial redness value
b*	initial yellowness value
L	final lightness value
a	final redness value
b	final yellowness value
ΔE	total color change

LIST OF ABREVATIONS

FDA	Food and Drug Administration
CFR	Code of Federal Regulation
CA	Citric Acid
MA	Malic Acid
GDL	Glucono-Delta-Lactone
BC	Buffering Capacity
MPa	Mega Pascal
HP	High Pressure
CCRD	Central Composite Rotatable Design
RSM	Response Surface Methodology
ANOVA	Analysis of Variance
ns	Non-Significant
OH	Ohmic Heating
USA	United State of America
CH	Conventional Heating
AC	Alternating Current
DC	Direct Current
CFU	Colony Forming Unit
CIDA	Canada International Development Agency
HPP	High Pressure Processing
kPa	kilo Pascal
RMSE	Root Mean Square Error
DNA	Dinuclotid Nucleic Acid
PME	Pectinmethylesterase
PG	Polygalacturonase
AI	Acid Infused
RT	Retort Temperature
AIR	Alcohol Insoluble Residue
DM	Degree of Methylation

UG	Unsaturated Galacturonides
WSF	Water Soluble Fraction
HP-T	High Pressure Thermal
DM	Degree of Methylation
WHO	World Health Organization
NACMCF	National Advisory Committee on Microbiological Criteria for Foods
TPA	Texture Profile Analysis
CV	Coefficient of Variation
CIE	Commission Internationale de L'Eclairage
ST-P	Steam Pasteurization
ST-S	Steam Sterilization
HTST	High-Temperature-Short-Time
WIM	Water Immersion Mode
SK-V	Shaking Vertical
SK-H	Shaking Horizontal
HPHT	High-Pressure-High-Temperature

CHAPTER ONE

1 INTRODUCTION

On the question of survivors on this planet, there is always a competition between human being, pests and microorganism for food. Microorganisms are the major competitors for our food and are the main cause for food spoilage. The influence of microbial agents is predominant for high moisture products which support the growth of microorganisms and biochemical reactions eventually making the food inedible or unsafe for human consumption. The term "food preservation" refers to any one or more of several techniques available to promote food safety and extend the shelf-life of foods. Preservation methods used are all designed to reduce, control or eliminate one or more of food spoilage causative agents. Among many available food preservation mehods, thermal processing is one of the most commonly used technique (Teixeira and Tucker 1997). Thermal processing implies that the use of all forms of heat treatments in which food spoilage agents are controlled or inactivated by heat. Among different thermal processing methods, canning of foods is the most widely used technique in the food industry. In conventional thermal processing, prepared foods are first placed in hermetically sealed containers and heated in pressurized vessels at high enough temperatures for long enough predetermined time to ensure the destruction of spoilage and pathogenic microorganisms. In-container thermal processing of foods has been utilized long way since Nicholas Appert first discovered canning in 1809 and microbiologically scientific methodologies were commercially adopted in the 1920s to determine minimum safe sterilization process (Ball 1923; 1928; Bigelow 1920;1921). The classification of foods into different pH groups was a milestone in these studies. For low acid foods (pH>4.6), the heat treatment given is commonly termed "commercial sterilization" and is aimed at eliminating the most resistant pathogenic spores of *Clostridium botulinum* (Esty and Meyer 1922). *C. botulinum*, a mesophilic, gram posative rod shaped anaerobic spore forming bacteria, can easily germinate under the conditions that exist in canned foods and therefore produce a potent neurotoxin, that can lead to lethal conditions commonly referred to as botulism. In commercial canning of low acid foods, it was therefore assumed that a high degree of safety can be achieved if the process is designed to eliminate any survivor of *C. botulinum* (Stumbo 1953; 1975). A commercial thermal processing method designated to reduce

the population of *C. botulinum* by 12 logarithmic cycles (i.e., equivalent time to reduce the population from 10^{12} to 1) was considered safe and termed as 'botulinum cook' or a 12 decimal reduction process (12D process). Based on the decimal reduction time of 0.25 min at 121^{o}C, the standard process for low acid foods was therefore to achieve at least 3 min of effective treatment at 121^{o}C at the slowest heating point in the can. This effective time was designate as the process lethality (F_o value; hence for minimum commercial sterility F_o = 3 min). However in most canned meats and vegetables (pH>4.6), the 'botulinum cook' process, although safe from public health point of view, may not prevent spoilage since some of the spoilage causing bacterial spores are much more heat resistant (with D values as high as 1.0-5.0 min at 121^{o}C (Stumbo 1950) than *C. botulinum*. To reduce spoilage to low levels (example one can in a million cans), these products are processed for longer time intervals to result in much higher Fo values as high as 13 - 31 min (Donald 1996). This intensive heat treatment for extended period of time in static retort not only kills food microorganisms but also permanently damages important food quality components. Particularly the slow heating of canned foods accounts for thermal lag yielding significant overcooking of products by the time the required lethality is delivered to the central cold location in the can. Today, with improved living standard of people and better consciousness for healthy quality foods, the demand of food processing goes beyond the fundamental requirements of safety and shelf stability. More emphasis is being placed on better quality products. Because of this the consumption of canned vegetables and fruits is declining in the food market (USDA 2010).

In order to minimize the impact of intensive thermal treatments on the quality of canned foods, different attempts have been developed. The efforts can be broadly grouped into two. Efforts in the first category has been focused on improving quality of canned foods through modification of conventional processing methods or packaging materials. The second catagory focused on the use of novel thermal and non thermal processing methods. In the first category efforts have been made to reduce process time by enhancing heat transfer rate to the product and optimizing the time-temperature combination for better quality retention with required degree of safety. These could be achieved through use of agitation of containers in the retort during heating (Clifcorn et al., 1950; Berry et al., 1979; Berry and Bradshaw 1980; Berry and Kohnhorst 1985; Sablani and Ramaswamy 1996; Dwivedi and Ramaswamy 2010), high temperature short time processing (Mansfield 1962; Lund 1977), thin profile packaging (Lampi 1980; Mermelstein

1978) and optimizing process conditions using variable retort temperature concept (Durance et al., 1996). Even though these modifications ensured relatively better quality retention as compared to conventional static retort heating, however, the intensity of heat treatment is still high enough to cause significant damage to quality attributes. In addition, the practical applicability of most of these methods is limited to liquid foods or liquid containing small particulate (except the thin profile processing which can be used for solid foods). In the second category, the practical use of novel heating methods (microwave and radio-frequency) is limited due to problems associated with heterogeneity in heating of foods, runaway heating and problems associated with packaging materials as well as cost (Buffler 1992; Buffler 1992; Fito et al., 2005). In non-thermal processing, the use of high pressure processing alone, is somewhat limited due to its inability to inactivate spores of microorganisms at ambient temperature. However, these days, Ohmic heating (OH) and high-pressure-high temperature (HPHT) processing are gaining popularity in food industry for better quality retention. These methods are relatively robust and more applicable if they are combined with other alternative preservation methods to circumvent their limitations. OH has a volumetric heating behaviour and commonly used to sterilize liquid or small sized particulate foods relatively at high temperature (Fryer and de Alwis 1989; Sastry and Palaniappan 1992), but it could be adapted to large sized high percentage particulate foods. HPHT is also often used at elevated temperature and pressure levels which has still impose significant effects on quality even though it demonstrated better quality retention as compared to conventional thermal method. Therefore moderate temperature (OH or conventional) or moderate-temperature-moderate-pressure combinations could overcome impacts of high temperature /temperature-pressure effect on quality of products. However, extensive and optimum use of these processing methods could be achieved through modification of product property before actual processing. This mainly because pH modofocation of low acid foods provoides an opportunity to exploit the benefit of such processing methods to process under moderate processing conditions without compormising food safety issues.

In thermal or pressure assisted thermal processing methods, the intensity of a given process is determined based upon pH of a food. Foods having pH>4.6 are subjected to commercial sterilization schedules to inactivate spores of *C. botulinum*. However, products having pH<4.6 do not support the germination and growth of *C. botulinum* spores, and hence can be processed at moderate processing conditions (<100^{o}C). In conventional thermal processing,

pH reduction is done using conventional acidification techniques, which have several limitations. Therefore, a novel acid infusion mechanism should be sought to modify pH of low acid products. Novel acid infusion techniques that could help to achieve controlled, rapid and more uniform pH reduction of low acid vegetables with a minimum change on flavour and taste of a product are desirable. Besides this, such a modification creates an opportunity to use moderate processing methods to produce products with better quality and required degree of safety but with reduced cost.

Once the pH of low acid vegetables modified, processing schedule should be established according to resistance of target food microorganism for lethal agents. However, from a food safety point of view, *C. botulinum* cannot grow in foods with low pH (pH<4.6) and is not considered a microorganism of concern as long as proper pH reduction is done and maintained during production, storage and distribution. Target food microorganisms for low pH foods should be tolerant to acid or acidified environments with relatively better heat and pressure resistance as compared to other aicd tolerant bacilli species. Because of this, FDA (2010) suggested *Bacillus licheniformis* as a target microorganism for production of acid or acidified low acid foods. This is mainly because, when *B. licheniformis* survives a given treatment condition and co-exists with *C. botulinum* spores it has the capability to neutralise growth medium low pH to higher level (pH>4.6) in which *C. botulinum* would be able to grow and produce toxin (Rodriguez et al., 1993). This shows that the co-existence of *B. licheniformis* indirectly imposes food safety issues for insufficiently acidified and/or improperly processed acidified low acid foods. In addition to this, the bacterium is frequently involved in the spoilage of canned vegetables (Fields et al., 1977) and it is more heat (Janštová and Lukášová 2001) and pressure (Nakayama et al., 1996) resistant than other *bacillus* species. Complete inactivation of this bacterium in acidified foods is crucial to ensure food safety and product stability. The overall goal of this work was to improve quality of canned vegetables through different alternative preservation methods as compared to conventional approaches with required degree of safety. To achieve this goal, the study was conducted in sequential manner addressing the following objectives.

Therefore the objectives of this work were as follows with a general plan of work illustrated in Figure 1.1:

i. To investigate and optimize high-pressure assisted acid infusion conditions as a novel acidification method for rapid, uniform, more dependable and controlled pH reduction of low acid vegetables

ii. To determine thermal resistance parameters of *B. licheniformis* spores under different types of acidulates, pH levels and heating methods

iii. To determine pressure-assisted thermal inactivation kinetic parameters of *B. licheniformis* spores under different pH conditions and to evaluate predicting power of different models for spore inactivation

iv. To investigate the influence of acid infusion on quality of carrot processed under different alternative processing methods

v. To carryout microbial validation study and assessment of quality retention of selected processing methods

GENERAL PLAN OF RESEARCH WORK

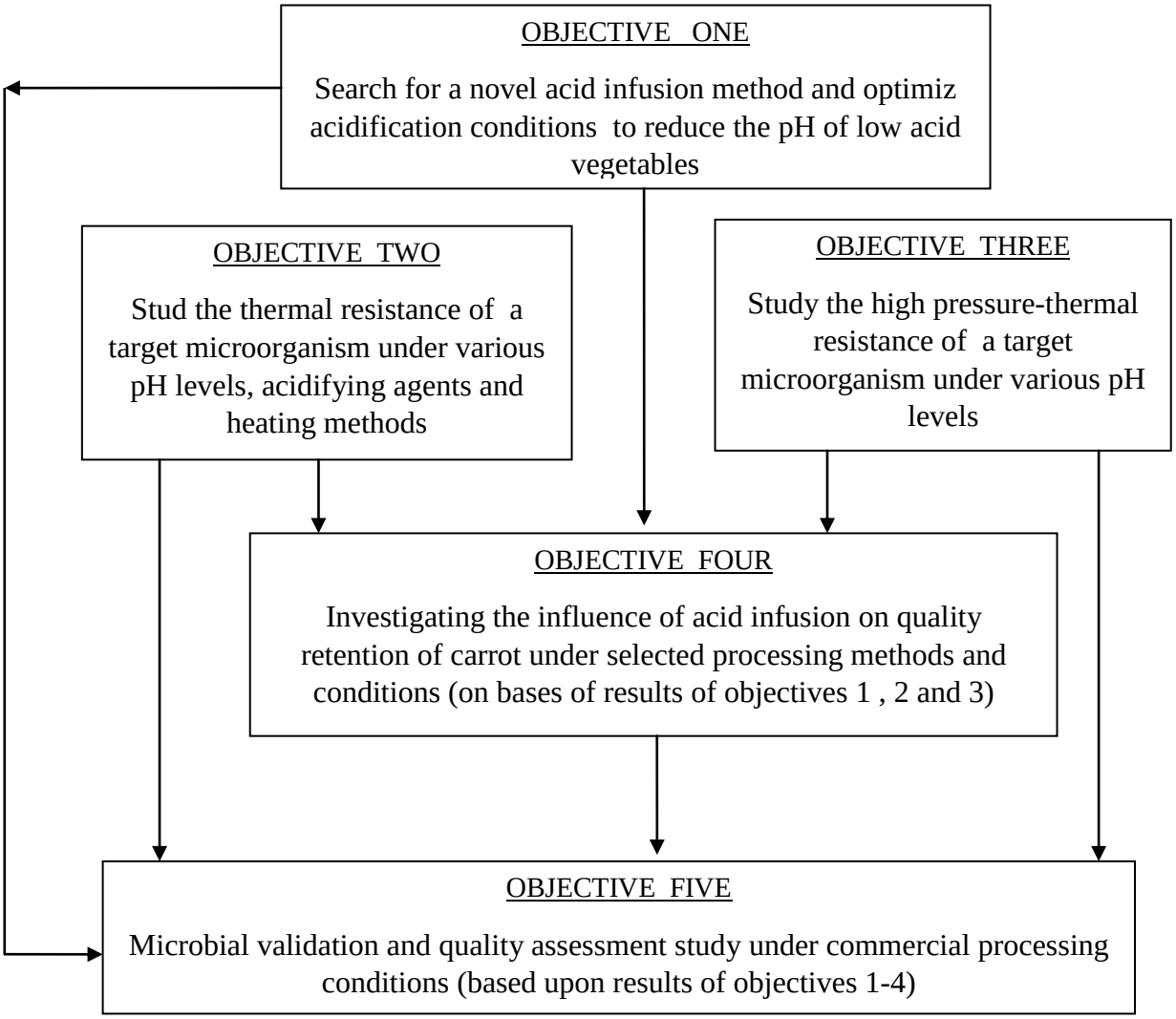

Figure 1.1 Schematic representation of the general plan of the research work

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Introduction

Thermal processing is defined as the combined use of temperature and time to eliminate or reduce the concentration of target food microorganisms and ensure products safety and shelf stability during storage and distribution. Time and temperature combination required for thermal processing of foods are influenced by several factors, like heat resistance of target food microorganism, pH of food, nature of food, size and type of container, heating method and post process storage and distribution conditions. In commercial canning of vegetables sterilization process schedules are designed to eliminate thermally resistant spore forming *C. botulinum.* C. *botulinum* (commonly type A, B and E) organisms are gram positive, motile, anaerobic rods, 0.5-2 μm in width, with oval, subterminal spores which causes botulism (Cato et al., 1986). Botulism is a neuroparalytic illness resulting from the action of a potent toxin produced by the action of C. *botulinum.* The ability of this bacterium to cause food poisoning in humans is directly related to the production of heat resistant spores that survive the preservation methods that kill non spore forming organisms (Kim and Foegeding 1992). Acidity, water activity and food composition also determine the resistance of spores to lethal agents. Commonly the thermal resistance of spores also increases with higher pH and lower salt content of the medium in which the spores are suspended (Zezones and Hutchings 1965). For low acid foods (pH>4.6) sterilization schedule, *C. botulinum* spores is chosen as a reference microorganism, which is characterized by a z-value of 10°C and a $D_{121.1^oC}$-value of 0.21 min (Van Loey et al., 1995). Based upon this criterion the desired degree of lethality at reference temperature (121.1^{o}C) for canning of vegetables ranges from 3-6 min (Smith 2003). In commercial canning of vegetables, the sterilization process has to be done at least at a temperature of 110-130^{o}C for time interval of 13-31 min (Donald 1996) to inactivate spores of other more heat resistant spoilage microorganisms (Table 2.1). This intensive processing schedule significantly affects quality of canned vegetables. In addition to this, excess heating schedule has an economic implication in terms of energy consumption and processing cost (Durance 1997). To overcome such limitations in canning industries, so far various efforts have been made to explore moderate and more efficient processing methods. Therefore in the following sections, attempts were made to indicate efforts so far done to improve quality of

canned vegetables and existing limitations to use alternative processing methods to improve quality of canned vegetables.

2.2 Limitations and advantages of conventional canning

The concept of in-container sterilization involves the application of high temperature thermal treatment for a sufficiently long time to destroy microorganisms of public health and spoilage concern. According to CODEX ALIMENTARIUS (1993) "commercial sterility of thermally processed food" means "the condition achieved by the application of heat, sufficient, alone or in combination with other appropriate treatments, to render the food free from microorganisms capable of growing in food at normal non-refrigerated condition at which the food is likely to be held during distribution and storage". Commercially sterile products, apart from their record history of safety, are also free from spoilage microorganisms and commonly have a shelf life up to 2 years at ambient storage condition. Heat treatments further inactivate endogenous spoilage enzymes and the cooking effect has also improve palatability of the products, like the case of canning beans. Furthermore, canning as compared to other preservation methods (drying and freezing) has low cost of production due to production capacity of large number of cans per unit time. However, commercial sterilization or canning has also the tendency to induce permanent changes to the nutritional and sensory properties of canned products (Priestley 1979; Jen 1989). In addition to quality losses, retort energy as a function of process temperature and time, has significant implication in terms of energy consumption and processing cost (Durance 1997).

The quality parameters of vegetable crops can be expressed in terms of their sensory properties (appearance, aroma and taste), nutritive value, chemical constituents, mechanical properties (texture), functional properties and defects (Ahmed and Shivhare 2006). During thermal processing of vegetables these properties are altered and both, over and under cooking are undesirable (Kasai et al., 1994). For processed vegetable products, texture is one of the main attributes that governs the acceptability of the product by a consumer. The texture of canned vegetables is often softer than desired (Durance 1997). For canned vegetables, changes in texture are strongly associated with the transformation in cell wall polymers due to enzymatic or non-enzymatic reactions.

Table 2.1- Selected vegetables for canning , associated pH levels, weight of product and canning time at retort temperature of 121^{o}C for can size 211x304 and initial product temperature of 21^{o}C (Donald 1996)

Vegetable	pH	Weight of product (g)	Sterilization Time (min)
Asparagus	5.0-6.0	150	13
Lima bean	5.4-6.0	130	19
Beets	5.3-6.6	159	23
Carrot	5.2-6.2	167	23
Peas	5.7-6.0	156	31

In thermal processing, the loss of firmness can be attributed to loss of turgor and chemical changes in the cell wall matrix polysaccharides due to effect of intensive heat treatment. Among many carbohydrates in cell wall, cellulose, hemi-cellulose and pectin are the major ones. The former two are resistant to heat up to 150^{o}C and do not show significant structural and compositional change during heating (Williams and Besler 1996). However most of changes associated with texture of plant based foods are mainly related with change and modification of pectin which depends upon nature of the plant tissue, pre-processing and processing conditions (Sila et al., 2008). During thermal processing of vegetables, pectin degradation may undergo either acid (pH $\leq$ 3.5) catalyzed depolymerisation (Krall and McFeeters 1998; Van Buggenhout et al.,, 2009) which is not common in case of canned low acid vegetables or base catalyzed (pH$\geq$4.5) depolymerisation through β-elimination reaction which is prevalent in canned low acid vegetables (Albersheim et al., 1994; Van Buggenhout et al., 2009)(Figure 2.1). The degradation of pectin is parallel with tissue softening due to separation or loss of integrity of adjacent cells which ultimately results in loss of desirable textural properties (Lecain et al., 1997).

The other quality evaluation criteria for processed vegetables is their color. Consumers take the product color into consideration as a criterion, because it has a key role in food choice, preference and acceptability (Kays 1999). Change in color of vegetables could happen either due to enzymatic or non-enzymatic degradation of color responsible components. Natural color components of vegetables, like chlorophyll, carotenoids, betanins and anthocyanin's are degraded during intensive thermal processing. Even mild heat treatment can trigger Maillard

reaction, a reaction between proteins and reducing sugar which ultimately convert to insoluble brown or black components known as melanoidins.

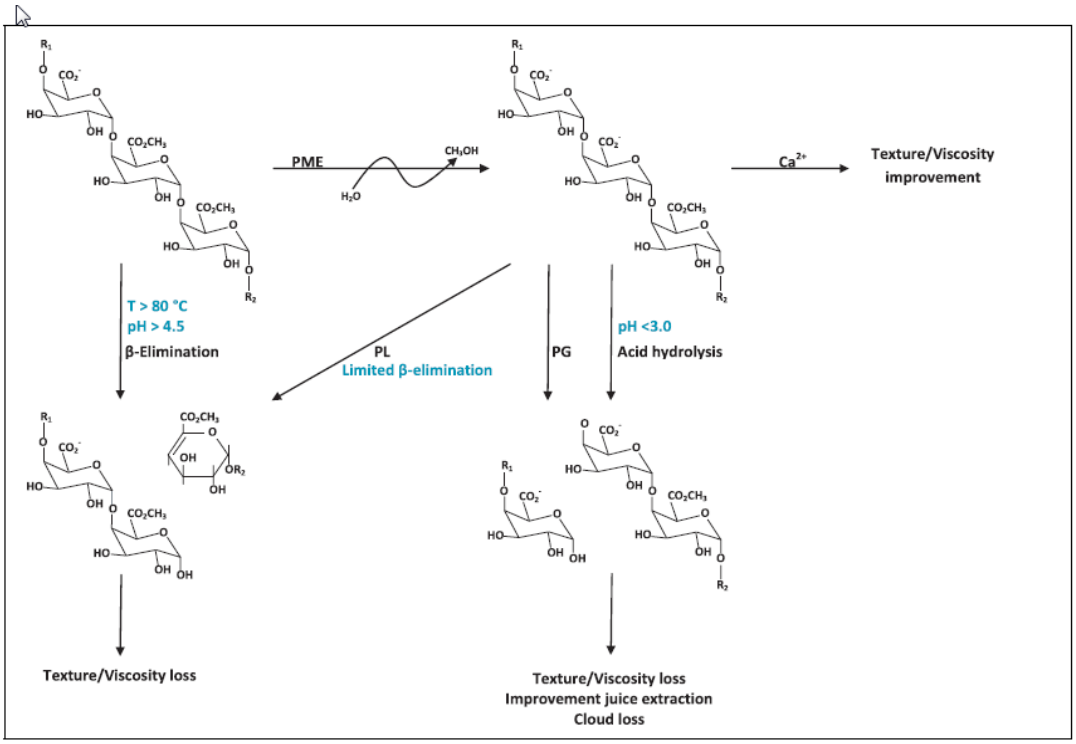

Figure 2.1 Schematic presentation of the most frequently studied pectin changes in the context of process-induced texture changes: PME = pectin methylesterase, PG = polygalacturonase, T = high temperature, OMe = methoxylesters (Van Buggenhout et al., 2009).

Heating of foods can be both beneficial and detrimental to the nutrient content of foods. Nutrient loss is another quality factor during thermal processing of vegetables. Vitamins are among the most sensitive and severely affected nutrient components during canning. Significant loss of vitamin C is commonly observed during heating of foods due to its solubility in water and high degree of oxidative nature (Fennema 1985). Like ascorbic acid, thiamin and folate are also heat sensitive and degraded during thermal processing (Lathrop and Leung 1980). However, vitamin A is relatively heat stable and insoluble in water, but up to 30% loss during thermal processing is reported (Howard et al., 1999). Furthermore, β -carotene is one of the carotenoids which can be affected during thermal processing of vegetables through isomerization and oxidation (Lemmens et al., 2009). The level of β-carotene in processed vegetables is essential in nutritional and commercial terms, since it is the principal precursor of vitamin A. Carotenoids

are present in nature in the trans configuration, which is more stable. However the cis-isomers may occur and increase during methods as well as during industrial sterilization processes (Sweeney and Marsh 1971). In vegetables, the cellular structure and its complex combination with protein confer carotenoids some stability. However, during several processing phases, the ultra-structure of carotenoids and their complexes can be broken, exposing the pigments to adverse factors that can lead to more extraction or destruction. Generally their stability relies on the extent of processing and storage conditions, carotenoid structure in tissue, presence of oxygen, light exposure, water activity level, pH of environment, presence of metal ions and anti-oxidants (Britton 1991;1992;Odriguez-Amaya 1993b). Thermal processing of vegetables has also a beneficial effect in terms of improving the digestibility of foods through modifying some nutrients by enhancing their availability to digestive enzymes. It can unfold secondary, tertiary and quaternary structures of protein and improve their bioavailability since peptide bonds are easily accessible by protein based digestive enzymes. Furthermore processing maks proteins of legumes more digestible through inactivation of anti-nutritional components like trypsin inhibitors. However modifications of primary protein structure has a contradicting effect because heating affects their native structure and their bioavailability (Swaisgood 1985). Generally to achieve a balance between food quality and safety, there is a need to optimize conventional processing methods or need to search alternative processing approaches to improve quality of canned vegetables without compromising food safety.

2.3 Opportunities in quality optimization of canned vegetables

Quality maximization is a major challenge in thermal processing of foods. An important objective in designing a thermal process is to manipulate the process in such a way as to optimize quality, while maintaining safety requirements. The effect of the thermal sterilization process on quality has been of major concern for food processors since the invention of canning. In order to improve quality of canned vegetables there are two key focus areas which can be explored. Quality improvement can be achieved either by optimizing process temperature and reducing processing time through modification of conventional processing methods and packaging materials or through use of novel and alternative combinations of processing methods.

2.3.1 Conventional approaches to optimize canned foods quality

These days, the major goal in processing of vegetables is to keep food pathogenic and spoilage microorganisms to a safe level (Ahmed and Shivhare 2006). Process schedules are established based upon kinetic data of reference microorganism and relevant quality factor. Studies on kinetics of quality loss and microorganisms inactivation are usually indicated by first-order reactions kinetic model with respect to time. Kinetic data showed that, thermal death rates of bacteria generally undergo at a greater acceleration rate with increase in temperature than concurrent reactions that lead to quality loss. For instance, different studies confirmed that, the thermal death rate (z-values implies temperature increase required to reduce decimal reduction time by a factor of 10) of food microorganisms (z = 3.5-15^{o}C) for an increase in temperature of heating medium is much faster than spoilage enzymes (z =10-34.5^{o}C) and quality factors (z=13-72^{o}C) (Table 2.2). This difference in heat sensitivity vulnerable factors gave an opportunity to apply the principles of High Temperature Short Time (HTST), and Ultra High Temperature (UHT) processing for better quality retention (Mansfield 1962; Lund 1977), since an increase in heating medium temperature significantly inactivate microorganisms than quality factors. These methods have a success story for liquid foods or liquid foods with small particulates. Unfortunately, the HTST and UHT concepts are severely limited for large size particulate-liquid and high viscous foods (Holdsworth 1985; Saguy 1988). Due to this reason commercialization of HTST for large particles-liquid foods not yet approved by regulatory agencies due to absence of clear demonstration of achievable processes lethality for large particles in motion during HTST/UHT treatment. However, other alternatives attempts have been made to improve quality of particulate canned products through modification of retort process through enhancing movement of cans and agitation of foods in retort and containers respectively. In the conventional still retort, slow heat transfer causes the product to be subjected to more heat than is necessary to achieve commercial sterility (Eisner 1988). Increasing consumer demand for better quality products provoked food manufactures to focus on rotary sterilization as an alternative method to enhance heat penetration and decrease process times. Clifcorn et al. (1950) for the first time suggested the use of end-over-end rotation of cans in heating medium in order to increase heat transfer in canned food products, as the can rotates, the headspace bubble moves along the length of the can resulting in the agitation of the can content.

Table 2.2 Temperature sensitiveness of food microorganisms, enzymes and quality factors

Vulnerable Factors	z(^{o}C)	References
Vegetative bacteria	3.5– 9.4	Byrne et al., 2006; Murphy et al., 1999; Sarker et al., 2000
Bacterial spores	4.2 – 15	Byrne et al., 2006; Gaze and Brown 1990; Sarker et al., 2000; Stumbo et al., 1950
Enzymes	10– 34.5	Anthon and Barret 2002; Park et al., 1988; Ramaswamy and Abdelrahim 1991
Color	20 – 74	Ramaswamy and Abdelrahim 1991; Silva and Silva 2000
Vitamins	44 -72	Ramaswamy and Abdelrahim 1991

Agitation processing played a critical role to shorten processing time through enhancing heat transfer rate to the product (Willhoft 1993). Unlike to conventional static retort the form of mechanical agitation of cans (end-over-end for vertically oriented cans or fixed/free axial for horizontally oriented cans) in the retort fasten heat transfer through increasing the rate of heat transfer to the product (Sablani and Ramaswamy 1996; Meng and Ramaswamy 2005; Awuah et al., 2007; Dwivedi and Ramaswamy 2010) shorten processing time, better quality retention and lower energy consumption. For instance in a 603 x 700 can, cream type corn may receive a 20 min process at 126.7^{o}C in agitation retort compared to 200 min in static retort (Gravin and Wedding 1995) , a 10 fold reduction in overall processing time. Awuah et al. (2007) also indicated that agitation allows higher temperatures up to 137.8^{o}C and reduces process times for particulate foods. A number of studies have been published that to evaluate physical parameters that influence the bubble motion, including: rotational speed, system geometry, headspace volume, product viscosity, off-center axis of rotation, particle density and presence of particulates for different types of agitations (Naveh and Kopelman 1980; Anantheswaran and Roa 1985; Sablani and Ramaswamy 1996). However such type of processing conditions for products in container with limited head space and mobility in cans, like canned vegetables, tuna, salmon, tightly packed products, and ham cannot benefit as such more from enhanced heating through container agitation. Sablani and Ramaswamy (1998) for instance reported that at 40% particle concentration in tightly packed container the heat transfer coefficient was low with poor particles mixing. Literature information also indicate that agitation retorting is more suitable for semi fluid or particulate foods than conductive foods. For instance in-container sterilization of fluid or semi-fluid foods can be accelerated by so-called forced convection processes in which

containers are agitated by axial rotation (Berry and Kohnhors 1985), end-over-end rotation (Abbatemarco and Ramaswamy 1994; Sablani and Ramaswamy 1995) or other movement during the cook phase (like recent research activities on shaking system with reciprocating motion). Therefore, unfortunately, many of our most popular canned vegetables do not benefit from agitation as such because they have no sufficient head space and fluid to permit convective heat transfer with in the can.

The other alternative strategy explored to improve quality of canned products was through modification of packaging material of products. In this regard the primary objective of processing of foods in thin profile forms is to improve better retention of product quality through minimizing the process time while achieving the required commercial sterility. The use of thin profile processing using flexible pouch was developed during the 1960s in USA, by a consortium of food packaging companies working in conjugation with the US army Natick Laboratories (Herbert and Bettison 1987). In this type of packaging, retort pouches are used, where the heat transfer is faster because containers have larger surface area compared to the ones used in conventional canning (cylindrical metal cans or glass jars of equal volume) and the distance for the heat to get the coldest point is shorter. Despite its disadvantages of slow line, labor-demanding operations and fragile packages, thin profile packaging is considered a potential alternative to aseptic processing (Ramaswamy and Marcotte 2005). Furthermore pouches can withstand sterilization temperatures up to 130^{o}C, making it amenable to HTST operations (Awuah et al., 2007). In addition to this, there is chance of deformation, loss of seal integrity and explosion of containers if in case the internal pressure in containers is not counterbalanced with the external one during heating and cooling phases (Sablani 1996). Because during heating the internal pressures might exceed the saturation pressure of the steam due to presence of residual air in containers and the internal pressure might be higher than the external during the cooling phase. Additional bottlenecks identified with pouches include product entrapment at the seal interface and micro-leak channels that could allow microbial invasion (Awuah et al., 2007). Berry and Bush (1988) also indicated that in most instances, semi-rigid plastic containers heat more slowly than comparably sized and shaped metal containers under identical processing conditions.

Commonly in canning for packed solid foods, very high temperatures will cause severe thermal degradation of the food near the surface long before the food at the center of the container has risen in temperature. On the other hand, a relatively low retort temperature (slow rate of heating) will cause a greater quality loss because of longer time it will take to obtain commercial sterility (Ohlsson 1980). This concern leads to several tries at developing computer simulation studies to investigate the effect of thermal sterilization on quality of canned foods. The first attempt on computer aided optimization of nutrient retention in thermaly processing of foods was published by Teixeria and others (1969). The experimental evidence to support the accuracy and validity of developed computer model was shown in subsequent works by Teixeira et al. (1975a). The same research group extended their computer model to evaluate the effects of variable retort temperature (VRT) profiles on thiamin degradation (Teixeira et al., 1975b). Practically almost all commercial retorts processes for canned foods employ only one retort temperature throughout the cooking which make them constant retort temperature (CRT) process, in terms of modification of processing environment to reduce sterilization process a VRT concept was explored. VRT processing as one of the potential approach to improve both cost of production and quality of canned products has been receiving increasing attention (Banga et al., 1991; Almonacid-Merino et al., 1993; Durance et al., 1997). For each combination of retort temperature, container and food material, only one process exists for each sterilization value. In essence, the only question is which retort temperature is best for a particular product. With the help of an Integrated Control Random Search (ICRS) algorithm (Banga et al., 1991), large number of different VRT's are possible for a given product, and selection of an optimum process is most easily found with a computerized experimental search technique than actual experiment.

Earlier researches on VRT processing mainly focused on optimizing the overall or volumetric nutrient retention (Teixeira et al., 1975b; Saguy and Karel 1979; Nadkarni and Hatton 1985) of products. Quality improvement reported in these works as such rather minimal as compared to CRT processes. However, different data indicated that VRT improves surface quality of conductive heating foods up to 20% as compared to CRT and helps reduction of processing time (Noronha et al., 1993; Durance et al., 1996; Chen and Ramaswamy 2002). In other wors, VRT minimized process time, reduced heat damage to product surface, maximized

overall nutrient retention with lower energy costs (Banga et al., 1991; Noronha et al., 1993; Almonacid-Merino et al., 1993; Durance et al., 1996). But the practical applicability of VRT in various processing industries is hindered by several factors. One of the possible drawbacks for the implementation of VRT profiles is the fine temperature control necessary to achieve the maximum quality retention and at the same time to reach correct lethality value at the cold spot. It needs to have very accurate temperature measurement during heating and cooling phases to find optimum time-temperature combination for better quality retention (Durance et al., 1997). A small difference in temperature inside the retort can lead to a large variability in the lethality value. Furthermore the operation is cumbersome and unreliable when retort operation was strictly manually controlled and needs also a case by case determination of processing time. Even though VRT approach showed good potential in terms of surface quality improvement, reduction of processing time and energy conservation, still the temperature level used to achieve commercial sterility inflicts significant change on quality parameters of canned vegetables, since processes are optimized at sterilization temperature.

2.3.2 Novel processing technologies to improve quality of foods

The current attempt to improve quality of thermally processed products is focused on the use of alternative or novel processing technologies. Invention or discovery of new technologies are key activities behind the increasing competiveness among food industries to supply safe and better quality products. Based up on this, various novel thermal and non-thermal technologies are emerging to address the need of great nutritional and sensorial quality of industrially processed foods.

The main goal of using novel processing technologies is to reduce intensive and extensive processing effects on foods, which are generated by high temperature and long processing time. According to Roose and Karel (1991*)* the major factor for the development and modification of food processing technology is the desire to minimize effect of processing, in order to get products of relatively better quality. To achieve this objective, food processing equipment now have sophisticated technological levels of control to minimize costs, shorten production time, and improve product quality. Currently, two broad researches are going on in areas of novel processing technologies. The first group encompasses the use of electro-heating technologies or novel thermal processing technologies (microwave, radio-frequency and ohmic

heating) and the second category is accentuated on use of non-thermal technologies (high pressure processing, ultrasound, pulsed electric field etc.) alone or in combination with other preservation methods to preserve fresh like characteristics of processed foods.

2.3.2.1 Novel thermal technologies to improve food quality

As indicated in the above sections, most of foods available in the market are produced by conventional intense thermal processing technologies. Although heat is the most common method for transformation, preservation and sanitation, it is well known today that the consequences of conventional intensive heating are not necessarily good for products in terms of consumers' acceptability. Because of this, in developed nations the market for canned foods from such an intensive processing method is changing. However, rapid and increasing developments in various branches of physics lead to considerable improvement on appearance of novel food processing and preservation methods. In novel thermal technologies, the generation and application of heat into foods is different as compared to conventional thermal processing. In novel thermal or electroheating technologies, volumetric heating occurs quickly to target temperatures with reduced holding time which is significantly reducing come up time effect and ultimately improving quality retention. Furthermore electroheating technologies transfer energy from their source directly to the food without heating up the heat transfer surface of processing equipment. Radiofrequency (RF), Microwave (MW), and Ohmic heating (OH) are examples of novel thermal food processing technologies. These technologies are subdivided into either indirect one, when the electrical energy is first converted to electromagnetic radiation and subsequently generates heat within a product (e.g. MW or RF heating) or direct electro-heating where electrical current is applied directly to the food (e.g. OH). In the following sections the application and limitations of these technologies in terms of processing of safe and shelf stable foods are indicated.

2.3.2.1.1 Radio-frequency and microwave heating

Electromagnetic waves are formed when an electric field couples with a magnetic field. The two fields of an electromagnetic wave are perpendicular to each other and to the direction of the wave. The frequency range of 10-60MHz and 1-3GHz are the working ranges for RF and MW heating applications respectively. Ionic polarization of ions and dipole rotation of polar molecules in a food are the two mechanisms of RF and MW heating (Decareau and Peterson

1986; Sahin and Sumnu 2006). RF heating has been used in the food processing industry for many decades. In current trends it shows promising potential to replace the conventional heating methods in various food processing and preservation industries. The technology has been successfully applied for drying (Rice 1993; Mermelstein 1998; Piyasena 2003a), baking (Tang et al., 2005; Tewari 2007) and thawing of frozen products (Anon. 1992; Zhao et al., 2000), and cooking of meat (Laycock et al., 2003; Orsat et al., 2004; Guo et al., 2006; Marra et al., 2009). Many promising applications of RF dielectric heating also exist in the food industry; with several limitations to be solved. The major technical challenges are dielectric breakdown (arcing) and thermal runaway heating, which can lead to both packaging failure and product destruction (Tang et al., 2005; Zhao et al., 2006; Ramaswamy and Tang 2008). In addition to these limitations, economically RF heating is more expensive than other forms of heating and that inhibits the use of the technology in its present form. Rowley (2001) reported that for equivalent power output, RF heating is more expensive than conventional convection, radiation, steam or ohmic heating system. Estimated high operational cost of RF and technical problems such as dielectric breakdown and thermal runaway heating, which can be damaging to both the product and packages, have delayed the full commercialization of this technology in food processing industries (Piyasena et al., 2003a).

Microwave radiation (at 915 or 2450 MHz) is non-ionizing, and in a sufficient intensity will simply cause the molecules to vibrate, thereby causing friction, which produces the heat that cooks food. Water molecules and other polar molecules align to the MW electric fields that are rotating very quickly inside the product and create volumetric heating. Since the heat is produced directly in the food, the thermal processing time is sharply reduced. The color, texture and other sensory attributes of foods processed by microwave sterilization are often better compared with those of conventionally retorted foods. Commercial equipment for microwave sterilization are currently available in Belgium (e.g. TOP's Foods), Holland, and Italy. Microwave sterilized products like different pasta dishes, pasta sauces, rice dishes; main dishes, microwave sterilized salmon and rice are available in Europe (Ramaswamy and Tang 2008). In terms of quality, MW processed foods have better qualitative traits than conventionally processed foods. In terms of microbial safety, MW sterilization can achieve the same reduction of bacterial population as conventional retorting, because products such as salmon fillets, macaroni and cheese, mashed

potato and beef in gravy have been processed with proven safety (Guan et al., 2003; Tang et al., 2008). Successful sterilization of homogenous liquid and particulate high and low acid foods are also reported (Coronel et al., 2005; Kumar et al., 2007). MW heating technology is studied also for pasteurization of soft cheeses (Burfoot and James 1992), milk (Sierra and Vidal 2001) and fruit juices (Fox 1994; Nikdel et al., 1993). Use of MW energy for baking of cereals is also reported (Decareau 1986; Regier and Schubert 2001; Vicente and Castro 2007). It can also be coupled with different drying methods to accelerate the rate of drying process and enhance quality of dried products (Vega-Mercado et al., 2001; Fito et al., 2005). Advantages associated with the application of MW energy in vegetables and fruits dehydration are associated with more efficient drying in the falling rate period, and reduction of drying time (Araszkiewicz et al., 2004; Askari et al., 2006). Even though the technology has all these wide range of applications, it has certain limitations for its enhanced commercialization and application in North America food industries. The major limitation arises due to its heterogeneous heating behavior in non-homogenous foods. The waves penetrate unevenly in thick pieces of food and there are also "hot spots" caused by wave interference. This can be thought as a non-homogenous heating in some parts of the food and this might create in spots for survival of food borne pathogens (Buffler 1992). The other limitation of MW heating is associated with packaging materials for bulk production and commercialization of foods. It is limited to small sized food packages because of relatively small penetration depth wave into dielectric materials. Solid products are usually sterilized after being packed, so no metallic materials can be used in packaging when MW is used. Furthermore, the commercialization of MW heating is hindered due to its high cost of energy (Fito et al., 2005). Furthermore there are uncertainties associated with microbial inactivation because of its heterogeneity in product heating, which doesn't ensure that all points of the food reach the required temperature to induce microbial death (Buffler 1992). These limitations delay commercial use of microwaves in USA and Canada to produce pre-packaged shelf stable foods. Because of this, commercial MW processed food supply is pending upon Food and Drug Administration (FDA) and Canadian Food Inspection Agency (CFIA) acceptance.

2.3.2.1.2 Ohmic/electric resistance heating

During Ohmic heating of foods, an alternating electric current (AC of 50/60 Hz) is passed through the food, and the electric resistance of the food for flowing current causes the power to be translated directly into volumetric heat (Vicente et al., 2006). Heating of the food in OH systems (Figure 2.2) follows Ohm's law which relates amount of current passes through the food with existing potential difference between the two electrodes and resistance of the material. Electrical conductivity of the food system is the main parameter which determine the heating rate of the food than thermal conductivity. The food acts as a conductor of electricity when it is sandwiched between the electrodes of the ohmic heating system (Awuah et al., 2007). Since most foodstuffs are naturally resistant to the passage of electrical current and, for this reason, are able to convert electrical energy into thermal energy. Therefore heating occurs in the form of internal energy transformation in the food from electrical to thermal (Sastry and Barach 2000). Fast heating happens due to the movement of charged ions to opposite charged electrodes in very short period of time. Because of this, the technology can be considered as a high temperature short time process (Zareifard et al., 2003) with reduced energy consumption.

The technology provides good potential for thermal processing of foods without relying on a mechanism like conduction of heat from the surface into the fluid (Fryer 2003). Since it is a bulk heating method, it does not need a heat transfer surface hotter than the bulk food going to be processed. In addition to this, in OH the temperature of particulates increases without the need of heat transfer through solid liquid interfaces. Generally, unlike conventional heating methods, OH is simple and easy to use technology, delivers fast and uniform heating, allow to start/stop heating process instantaneously and works independent of the thermal conductivity of a food (Bansal and Chen 2006). The technology currently is being utilized in processing of liquid foods, liquid-solid food mixtures, meats, fruits and vegetables. Particularly for particulate foods, they don't experience large temperature gradient within the food system, and most commonly, the solids heat equally or faster than the liquid (Fryer and de Alwis 1989; Sastry and Palaniappan 1992) unlike unequal rate of heating of particles during conventional heating. In a commercial OH system for liquid foods, the maximum dwell time is about 1.5 min followed by heating in holding tube or tank at the target temperature for required heating time followed by cooling and aseptic packaging (Yang et al., 1997).

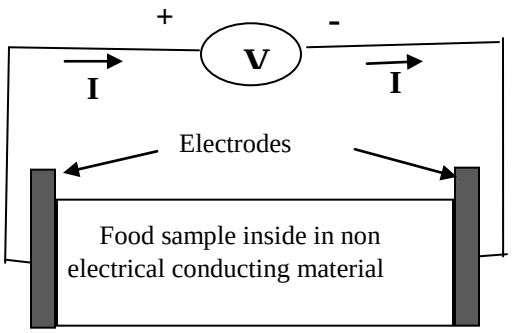

Figure 2.2 Schematic representation of simplified version of ohmic heating system

Several applications have been proposed and developed for OH in relation to microbial control and quality improvement to replace the conventional heating methods of food pasteurization or sterilization at industrial scale (Vicente et al., 2006). Concerning microbial inactivation, the principal mechanism of OH is thermal. Previously, successful studies demonstrated the lethality of the process to spores of *Bacillus stearothermophilus* in alginate beads (Brown et al., 1984). In addition to thermal effect, the presence of non-thermal effect has also been reported (Pereira et al., 2007; Sun et al., 2008) in terms of enhanced effect on spores inactivation. FDA (2000) recommended a more conservative approach only by considering the contribution of thermal effect from the holding tube (Dignan et al., 1989) with a minimum sterilization value of 3 min (lethality equivalent to 3 min of heating at 121.1^{o}C).

In terms of quality improvement different studies showed that OH contributes to better texture retention of fruits and vegetables. Farahnaky and others (2012) studied effects of OH, MW and conventional heating methods of different vegetables after exposing to the same treatment time. Their result demonstrated that equal exposure time resulted in over cooking effect in case of OH, and hence the overall processing time and texture degradation can be reduced by reducing the OH treatment with required degree of safety. Yang and others (1997) also studied the microbial safety and quality of 6 different types of ohmically sterilised foods after 3 years of storage. They reported that all the products were demonstrated to have better sensory quality with no post process contamination. Currently OH is widely used in pasteurization and sterilization of liquid or pumpable foods such as fruit juices, soup, purees, milk, soya milk, ice cream mix, egg, stew like products, fruit syrup, heat sensitive liquids, etc (Mizrahi 1996; Lima and Sastary 1999; Icier 2004; Leizerson and Shimoni 2005; Icier 2009) to

produce better quality products. Furthermore with existing literature information, FDA and USDA recommended the use of OH for pasteurization and sterilization of such type of foods in aseptic processing (Zell et al., 2009; Icier and Bozkurt 2010). Low acid foods in OH still should be done at very high temperatures ($\geq 130^{o}C$) (Yang et al., 1997), and hence quality degradation is likely while better retention of quality is observed in OH as compared to conventional method. Scientific data related to processing and preservation of acid infused solid foods or solid-liquid food with large percentage of particles is very limited. In addition to this, the synergetic effect of OH with other preservation methods (eg. pH reduction) are not yet studied well in terms of inactivation of food microorganisms and quality aspects. Therefore the effect of this technology in combination with other alternative processing methods should be studied with emphasis on large portion of particulate or solid foods to improve quality of canned vegetables.

2.3.2.2 Improving food quality through high pressure based processing

High pressure processing (HPP) is one of a leading novel non thermal food processing technology where food is subjected to elevated pressures (up to 1000 MPa) with or without the addition of heat, to achieve microbial inactivation or to alter the food attributes in order to achieve consumer-desired qualities. HPP acts instantaneously and uniformly throughout a mass of food independent of size, shape, volume, and food composition (FDA 2010). The technology has little effect on covalent bonds (Tauscher 1998; 1999), thus, foods subjected to HPP treatment at or near to room temperature will not undergo significant chemical transformations due to the pressure treatment itself.

Exposing of high acid chilled liquid foods to pressure level of 400-600 MPa for 1 to 15 min can inactivate vegetative food microorganisms and ensure food safety and ensure better quality (Smelt 1998). But like other food preservation methods, inactivation of bacterial spores is a very challenging task in HPP alone. As it is confirmed by various, works inactivation of dormant spores by sole application of HP is difficult unless and otherwise combined with high initial temperature to reach target sterilization temperature (Margaret et al., 2007; Peter 2007). At ambient temperature, bacteria spores can survive up to 1000 MPa (Patterson 2005). Because of this, commonly high pressure is combined with elevated temperature to inactivate pressure and heat resistant spores (de Heij et al., 2005; Raja et al., 2006; Wilson et al., 2008). Elevated

pressure with combined application of initial heating (90-110°C) can inactivate spore forming bacteria (Master et al., 2004; Rajan et al., 2006; Ahn et al., 2007; FDA 2010) for commercial sterilization of low acid foods. Application of a pasteurization pressure up to 600 MPa can induce the germination of bacteria spores (Wuytack et al., 1998; Knorr 1999; Raso and Barbosa-Canovas 2003) and hence germinated spores are relatively easy to kill with application of heat, since germinated spores are more susceptible to temperature than other spores (Hayakawa et al., 1994). Such type of effects can be significant if the pH of the food reduced below 4.6 through acidification step and germination of resistance spores can also be inhibited. Furthermore as pH is lowered most microbes become more susceptible to HP inactivation, and recovery of sublethally injured cells is reduced (Garcia-Graells et al., 1998; Pagan et al., 1999; Margaret et al., 2007).

In terms of preservation of quality attributes, small molecules such as vitamins, and flavour compounds remain unaffected, since the covalent bonds remain intact during HPP, rather the process affects non-covalent bonds (Rastogi et al., 2007). Many articles were published to show the impact of HPP or pressure assisted thermal processing on quality of foods and HPP foods exhibited better quality retention as compared to thermally processed ones (Kim et al., 2001; Master et al., 2004; Nguyen et al., 2007; Trejo et al., 2009; Vervoort et al., 2011). Technically during a high-pressure process, the food material undergoes physical compression. For example, a volume of 1 liter of water may be reduced to 0.85 liter while under 600 MPa pressure (approximately 15% reduction in volume)(Ting et al., 2002). Therefore such a compression (decrease in volume with an increase in pressure but increase in temperature) results in a fast and uniform compression heating with initial temperature of 70-90^{o}C, a sterilization temperature of 110-125^{o}C could be achieved very shortly (Barbosa-Cánovas and Juliano 2008). With fast compression heating to reach target temperature, reduced holding and fast decompression cooling, the overall processing time is reduced and quality gain advantage is significantly high as compared to conventional canning. Although HP pasteurized products for high acid and chilled foods have already been successfully introduced to the market, however, implementation of high-pressure-high-temperature (HPHT) sterilization in food industry still remains a challenge. In case of HPHT sterilization the processing load on certain type of foods is sometimes as severe as conventional thermal sterilization to affect product quality (Vervoort et

al., 2012). Leadly and others (2008) also indicated that conventional sterilized (Fo= 3 min) and pressure assisted thermally sterilized (700MPa, 117^{o}C, 2 x 2 min for 2 cycle interrupted by 1 min hold) of beans exhibited the same classic olive brown color change in bean which is typically the same with heat preserved beans. They also reported that a considerable texture loss was observed on beans treated under pressure assisted thermal sterilization. In different work, combining pressure-heat treatments between 500-700 MPa and 90-115^{o}C showed that enhanced texture loss due to chemical pectin conversions (de Roeck et al., 2009) which manifested on degradation of texture of carrot tissue. Willson et al. (2008) indicated that both high pressure and high temperature may cause loss of hardness of vegetables through cell wall breakdown and turgor pressure. The other limitation of pressure assisted thermal treatment is that it needs equilibration of samples to relatively higher initial temperature of 70-110^{o}C to reach elevated target sterilization temperature of 115-125^{o}C or more (Barbosa-Cánovas and Juliano 2008; Leadly et al., 2008; Shao et al., 2010). In conventional thermal processing of acid or acidified foods, this equilibration temperature might be sufficient enough to kill less heat resistant food microorganisms. In most common thermal preservation methods, foods having pH of less than 3.5, 3.5 < pH < 4 and 4 < pH < 4.3 can be pasteurized at temperatures of 80, 85 and 90^{o}C for 2-10 min respectively (William 2013).

Studies conducted so far by HPHT sterilization might work well to process low acid animal products like poultry, red meat, and milk. However, in terms of the processing of vegetable, the quality gain advantages as such were not remarkable even though it demonstrated relatively better quality retention as compared to conventional method. Since HPHT sterilization is done at elevated pressure and temperature levels, degradation of sensitive quality parameters is inescapable. Furthermore the cost of heavy duty high pressure equipment (>700MPa) together with required a high temperature heating system, limit commercialization and wide utilization of the technology. At moderate pressure levels in combination with moderate heating, the technology has very limited effect on color responsible compounds (Oye et al., 2008). But bacteria spores are highly resistant to even elevated pressure levels and it is difficult to produce shelf stable low acid foods (Heinz and Knorr 2005; Black et al., 2007). However, so far very limited or no sufficient data is available to combine pH as a third factor with moderate pressure (300-600MPa) and temperature (40-70^{o}C) conditions to overcome the above limitations. The pH

of most of canned vegetables is 4.8-5.8. Vegetables in this category are equally categorized as low acid products and subjected to intensive canning schedules. However a 0.3 to 1.3 unit reduction on their pH allowed less intensive processing conditions which gives an opportunity to employ the use of controlled acid infusion of low acid vegetables. Since *C. botulinum* spores cannot germinate and grow in foods at pH value below approximately 4.8 (FDA 2010), moderate pressure-heat treatment for acid infused vegetables could play a role for better quality retention with minimum change on taste and flavour. It has been reported that more spore inactivation of *C. botulinum* was observed in mashed carrot than buffers suspension which was ascribed with shift in pH in meshed carrot contributed for more lethality (Margosch et al., 2004). In addition to external lethality factors due to moderate pressure-thermal treatment, the nature of food matrix and its environment (pH<4.6) could also play significant role to enhance inactivation of food microorganisms (Ramaswamy and Marcotte 2006). Therefore moderate pressure-thermal treatment could be enough to destroy pressure-heat intolerant pathogenic and spoilage microorganisms which are critically important in acidified low acid foods. The growth of other food microorganisms can be controlled by low pH (pH<4.6) environment.

2.3.3 Acid infusion and its role on quality of canned vegetables

Most vegetables are low acid foods and require intensive thermal processing schedule in order to kill microorganisms responsible for spoilage and safety issues. As indicated above, this type of processing in conventional canning method significantly affects texture, color and nutrient contents of canned vegetables. Food preservation techniques either use solitary or combination of methods to minimize the extent of quality degradation of low acid products. One of the common preservation method is reduction of pH of low acid foods through acidification steps. Different methods of food preservation use the application of scientific and engineering principles to protect foods from deterioration. Production of acidified low acid foods in combination with conventional thermal processing method is a common practice for certain type of products. Most of acidified foods produced in such conventional approach have very low pH (pH< 4.0) and only limited to few products (Table 2.3). In this regard acidification or acid infusion is considered as a means to reduce the extent of processing condition through reduction of pH of low acid foods. However so far no data is reported in terms of combined use of acid infusion with novel processing technologies.

The combined use of conventional preservation alternatives with novel processing methods result in, less loss of flavor, texture, color and other nutrients as compared to conventional approach. Optimal processing of foods always requires a compromise between the beneficial and destructive influences of processing methods. One of the challenges for the food industry is to minimize these quality losses, meanwhile providing an adequate process to achieve the desired degree of safety. In the above sections benefits and limitations of available novel food processing methods are discussed. Among them, OH and HP based processing are gaining popularity and applicability in food processing industries. The current application of OH is mainly for liquid or pumpable foods. So far the application of this technology in combination with controlled acid infusion for solid foods has not yet explored. Furthermore, the use of HP with moderate heat for processing of low acid foods is limited due to resistance of bacteria spores. Use of HPHT sterilization approach also resulted in significant effect on quality of low acid vegetables. But the use of moderate-pressure-moderate-heat with low pH can enable to overcome the above limitations and ensure better quality products with required degree of safety.

Table 2.3 Common types of acid preserved or acidified foods (NSW 2014)

Acidification method	Products
Fermented and high salt (often hot pack)	Olives, sauerkraut, pickled cucumbers and bell pepper. Lactic fermentation under high salt condition
Fermented and high salt (often hot pack)	Soy sauce, Asian fish sauces. Complex lactic fermentation under high salt conditions
Vinegared products (often filled with hot brine)	Pickled onions, gherkins
Products complying with the CIMSCEE Model (1985) or 21 CFR 169.	Mayonnaises, salad dressings, sauces (some). Inherently stable due to aqueous phase with low pH and adequate acetic acid, salt and sugars
pH, acetic acid, hot pack, (Refrigerate after opening)	Sauces, ketchup, condiments
pH, hot pack (Refrigerate and use promptly after opening)	Cook-in sauces, pasta sauces, salsas, fruit, fruit juices, marinades, commercial vegetables in oil
pH, preservative(s)	Fruit juice cordials
pH and high sugar, pasteurized (Refrigerate after opening)	Jams

Most of the time food microorganisms are sensitive to pH changes. The effect of low pH environment in terms of inhibiting germination of growth of spores or vegetative cells are well studied and documented (Roberts and Hoover 1996; Dogan and Erkmen 2004; Reddy et al., 2006; Buzrul et al., 2008). It is also well known that low pH environment enhances the lethality of food microorganisms for a given lethal agent and hence acidified foods are commonly processed under a reduced processing schedule which in turn improves quality of thermally processed foods.

2.4 Conventional acid infusion methods and their limitations

According to FDA (21 CFR 114.3 (b)) acidified foods shall be so manufactured, processed, and packaged that a finished equilibrium pH value of 4.6 or lower is achieved for all ingredients within the time designated in the scheduled process and maintained throughout storage and distribution. One of the mechanisms in a hurdle concept to control the risk of botulism is reducing pH of low acid foods below 4.6 to control its growth and toxin production. Presence of viable spores of *C. botulinum* in properly preserved acidified low acid doesn't constitute public health hazard because of the inability of this microorganism to grow in foods whose pH≤4.6. Proper and more efficient pH reduction methods of low acid vegetables is a critical step in the production of safe acidified low acid foods. FDA (21 CFR 114.8 (3)) recommends the following acidification methods:

i. Blanching of the food ingredients in acidified aqueous solutions.
ii. Immersion of the blanched food in acid solutions.
iii. Direct batch acidification, which can be achieved by adding a known amount of an acid solution to a specified amount of food during acidification.
iv. Direct addition of a predetermined amount of acid to individual containers during production and
v. Addition of acid foods to low-acid foods in controlled proportions to conform to specific formulations.

However, there are several limitations associated with above conventional acidification methods. FDA (undated) report indicated that a history of botulism was reported from products produced by 29 firms due to inadequate acidification of low acid foods using conventional acidification methods. In addition to this, in conventional methods, most of the time pH

equilibration to achieve finished equilibrium pH takes long time (as long as 10 days in foods with very large particulates) (FDA 1998) after processing. For acid soaking methods, vegetables are commonly maintained at refrigerated condition for more than 24 hr to reduce the microbial growth. This also compels additional cost and time in production line. Furthermore, blanching in acid solution imposed also additional heating effect on the product which could contribute to additional loss of quality parameters, but HP can remve air in tissues and filled with liquid. Addition of acid foods to low acid foods commonly works for liquid and perfectely mixed foods. In addition to this, in conventional methods finished equilibrium pH is measured after taking the bulk pH than pH at the slowest pH reduction part which may not actually insure required pH reduction in all spots of a food. Besides these limitations, pH uniformity between the surface and slowest acidification part is also high. Furthermore, according to FDA (21 CFR 114.8 (3(iii))) direct addition of acid into each container of a production line is the least accurate and less dependable method to achieve finished equilibrium pH< 4.6. When process deviations occure because of these limitations, during thermal processing of acidified foods, the product is either reprocessed or destroyed. On top of these, the conventional methods lack the power to predict the concentration of acids and acidification conditions required to achieve required pH reduction for further optimization steps. Therefore, novel acid infusion mechanisms should be sought to overcome the above limitations for dependable, fast and uniform pH reduction of low acid vegetables.

So far vacuum acidification efforts were made to overcome those limitations. For instance Derossi et al. (2011) in their vacuum acidification work (for 2 min holding in vacuum and 720 min (still longer time) at ambient atmosphere) they achieved 28 and 24 % reduction in pH at 400 and 200 mbar pressure levels respectively. But in terms of concentration of acidifying agent and other acidification conditions they did not get significant differences as compared to conventional method. However the importance and potential of high pressure infusion of acids into low acid products has not yet been studied in terms of overcoming the above limitations. So far the technology has been widely used to inactivate pressure intolerant microorganisms (Muss et al., 1999a,b; Noma 2002; Ramaswamy and Shao 2010), modification of functional properties of foods (Messen et al., 1997; Paul 1999) and extraction of important bioactive compounds (Zhang et al., 2004; Nagendra et al., 2009). Since high pressure acts instantaneously and uniformly throughout a mass of food, independent of size, shape, and food composition (isostatic

effect) (FDA 2010) it could allow to infuse acids into low acid foods in a very controlled manner for variety of products. Furthermore it permits an opportunity to work below or at ambient temperature condition without any heating effect.

2.5 *Bacillus licheniformis*: as a reference microorganism for production of acidified low acid foods

Bacillus licheniformis is a gram-positive, motile and spore-forming rod, bacterium belonging to the group of Bacilli. Unlike other bacilli that are typically aerobic, *B. licheniformis* is a facultative anaerobe. The genus *Bacillus* are capable of producing endospores that are resistant to adverse environmental conditions (Claus and Berkeley 1986). It is most frequently involved in the spoilage of canned vegetables (Fields et al., 1977), cooked meats, raw milk as well as industrially produced baby foods (Lund 1990). Particularly the role of *B. licheniformis* in production of acidified low acid foods is very important from a food safety point of view. It has the ability to grow in very acidic media with a good capability of elevating low pH of the growth medium to higher pH's(>4.6) in which *C. botulinum* could be able to grow and produce toxins (Rodriguez et al., 1993). Hence survival spores from heat processes may increase the risk of botulinium intoxication. It is well known that *C. botulium* will not grow on foods when the pH < 4.6. However, Raatjes and Smelt (1979) reported that the bacterium can grow and produce toxin if it is co-cultured with acid tolerant pH elevating bacteria of Bacilli. Furthermore it is relatively more heat (Janštová and Lukášová 2001) and pressure (Nakayama et al., 1996) resistant as compared to other Bacillus species. Because of these features it can be considered as a surrogate bacterium for production of acid or acidified low acid foods. But so far only limited or no data has been reported in terms of inactivation kinetic parameters of the bacterium under reduced pH environment in combination with effects of different types of acidifying agents and processing methods.

2.6 Kinetic considerations for microbial inactivation and quality degradation

2.6.1 First order kinetic or log linear model

Inactivation of food microorganisms, spoilage enzymes and quality factors commonly modeled based on mechanistic concept, which is considering a given population of microorganism, enzyme or quality factors has similar or homogenous resistance to stress condition . This concept implies that the destruction of microorganisms, spoilage enzymes or quality factors may be regarded as a reaction of the first or higher order kinetic considering similarity of resistance of molecules or microorganisms in population to stress conditions (Cerf 1977). Therefore in this case process of destruction of microorganisms is believed as analogues to chemical reaction and can be expressed as either in first or higher order kinetic model though fitting of experimental data to destruction curves. Most of inactivation or destruction works have been explained based on this concept. Hence first order kinetic model assumes a linear relationship between the declines in the logarithm of the number of survivors/concentration over treatment time (Schaffner and Labuza 1997), and hence the model is expressed as:

$$\log\frac{N_{(t)}}{No} = -\frac{t}{D};\ (t \geq 0);\ slope = -\frac{1}{D} \tag{2.1}$$

where, N_0 is the initial viable cell or spore count (CFU/ml), C_o for enzyme or nutrient, N_t is the number of survivors remaining after exposure to a lethal treatment for a specific time t (CFU/ml), C_t for enzyme or nutrient and D is the time required to destroy 90% of the organisms or enzyme concentration or nutrient component which is estimated from $\log_{10}$ (N/N_0) *vs.* treatment time (min).

The temperature (Eq. 2.2) and pressure (Eq. 2.3) dependency of D values are expressed as through the kinetic parameters z_T (at constant pressure) and z_P (at constant temperature), respectively:

$$z_T = \frac{T_2 - T_1}{\log_{10}\left(\frac{D_1}{D_2}\right)} = -\frac{1}{Slope} \tag{2.2}$$

$$z_P = \frac{P_2 - P_1}{\log_{10}\left(\frac{D_1}{D_2}\right)} = -\frac{1}{Slope} \tag{2.3}$$

The z_T and z_P values represent the respective increases in °C and MPa required to reduce the value of *D* by one $\log_{10}$ unit or factor of 10. In a conventional approach, they are determined as the negative reciprocal of the slope of $\log_{10}$ *D* versus temperatures/pressure within the study range.

2.6.2 Two-parameter Weibull model to predict microorganism inactivation

As indicated in above section, in many of the cases, inactivation of food microorganisms using a given lethal agent are commonly described using first order inactivation model assuming that all cells or spores in a population have the same resistance to lethal agents. However for different processing methods deviations from linearity (logarithmic), like upward or downward concavity of curves, shoulder, sigmoidal, or biphasic curve with tailing effect have been reported (Cerf 1977). Such a deviation can be expressed based on vitalistic concept which indicates that individuals in genetically homogenous population possesse different degree of resistance for lethal agents (Lee and Gilbert 1918). Based upon this concept, microorganisms possessing the average degree of resistance are the majority and with those having the maximum and minimum resistance are the minority which seems that the resistance of microorganisms is normally distributed in the population (Withell 1942; O'Connorf 1974; Levinsonh and Hyatt 1971). This prompted a question about universality of log-linear model to determine inactivation kinetic parameters.

To describe variation from linearity and for accurate determination of the behavior of inactivation curves, different types of nonlinear models have been proposed. Commonly used nonlinear models are Weibull (Peleg and Cole 1998; Van Boekel 2002; Ahm et al., 2007; Wang et al., 2009; Hereu et al., 2011), biphasic (Xiong et al., 1999; Hereu et al., 2011; Pacheco et al., 2011), log-logistic (Cole et al., 1993; Little et al., 1994, Anderson et al., 1996; Chen and Hoover 2003) and modified Gomptertz model (Bhaduri et al., 1991; Linton et al., 1995). However, among all these models, Weibull model is popular because of its simplicity and flexibility to describe various types of inactivation curves (Peleg and Cole 1998; Fernandez et al., 1999; Peleg

and Penchina 2000). The cumulative form of the Weibull model, which has been successfully used in modeling of microbial inactivation (Van Boekel 2002), is given as:

$$\log_{10}\left(\frac{N_t}{N_o}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \tag{2.4}$$

where, α is the characteristic time or scale parameter (min), β is the shape parameter (non-dimensional), and all other parameters are as previously defined.

In a semi-log plot the Weibull distribution corresponds to a concave upward survivor curve when $\beta < 1$, a concave downward curve if $\beta > 1$, and is linear if $\beta = 1$. The first order kinetics approach (straight line in a semi-log plot when $\beta=1$) is therefore considered a special case of the Weibull model (Van Boekel 2002).

2.6.3 Determination of certain-log reduction using Weibull model parameters

In a conventional first order kinetic model, once the D value of a given microorganism is determined at a given specific condition, a certain $\log_{10}$ reduction on microorganism population to targeted level can be estimated by considering multiple D values (nD, e.g for 5-$\log_{10}$ reduction it is 5D). However, to determine certain $\log_{10}$ (n) reduction of bacterial cells using Weibull distribution parameters, an equation (Eq. 2.5) originally was used in failure engineering to describe time to failure in electronic and mechanical systems was suggested by Van Boekel (2002). The 90% percentile of the failure time distribution is termed the reliable life (t_R), and can be calculated from the parameters α and β (Eq. 2.4). In this case the t_R is analogous to the classic *D*-value when only a one $\log_{10}$ reduction is considered (Van Boekel 2002).

$$t_R = \alpha(\ln 10)^{\frac{1}{\beta}} \tag{2.5}$$

Therefore for example for 5 log reduction, Eq. (2.5) can be modified as:

$$t_d = \alpha(-\ln(10^{-d})^{\frac{1}{\beta}} \tag{2.6}$$

where, *d* is the number of decimal reduction which is equivalent to *n* in case of log-linear model.

Given the exponential relationship between α and treatment temperature, α can be used to determine the classical z' value(Eq. 2.8) in similar fashion with conventional TDT-curve (Van Boekel 2002):

$$\log_{10}\alpha = a - bT \quad or \quad \log_{10}\alpha = a - b'P \tag{2.7}$$

$$z'_T = -\frac{1}{b} \quad or \quad z'_P = -\frac{1}{b'} \tag{2.8}$$

where, *a* and *b* are coefficients of the regression line of a curve for $\log_{10}$ α *vs.* temperature.

2.6.4 Texture degradation kinetic study

Texture softening kinetic of treated samples of vegetables commonly explained by fractional conversion model (Rizvi and Tong 1997; Stomeham et al., 2000). The model is a convenient to describe fraction of change of physical property of a reaction as a function of time. For texture kinetic study, fraction of texture (*f*) measured as a function of time (*t*) and can be expressed as:

$$f = \frac{H_o - H_t}{H_O - H_\infty} \tag{2.9}$$

where H_o is initial hardness of sample at time zero, H_t is hardness measured at a given time *t*, and H_∞ is the nonzero equilibrium hardness property after prolonged heating.

To account for nonzero equilibrium hardness, fraction conversion in the following (Eq. 2.10) was used to determine texture degradation kinetic parameters (Rivzi and Tong 1997).

$$\ln(1-f) = \ln\left(\frac{H_t - H_\infty}{H_o - H_\infty}\right) = -kt \tag{2.10}$$

Residual hardness of carrot cubes as a function of treatment time at constant temperature /pressure can be expressed using Eq. 2.11 by rearranging Eq. 2.10. The softening rate constant (*k*) and hardness at prolonged treatment time (H_∞) can be estimated from Eq. 2.11.

$$H_t = H_\infty + (H_o - H_\infty)e^{-kt} \tag{2.11}$$

However, temperature dependence of the texture degradation rate constant (*k*) can be expressed by the Arrhenius equation (Eq. 2.12).

$$\ln(k) = \ln k_{ref} + \left[\frac{E_a}{R} \left(\frac{1}{T_{ref}} - \frac{1}{T} \right) \right] \tag{2.12}$$

where, k_o is texture degradation rate constant at reference temperature, *Ea* is the activation energy (J mole^{-1}), *R* molar gas constant (8.3145 J mole^{-1} ^{o}K^{-1}), *T* temperature (^{o}K) at time *t* (min).

The graph *ln k* versus *1/T* would give a linear plot line from which the inverses slope *–Ea/R* used to calculate the *Ea* required to express temperature dependency of the rate constant.

2.7 Inoculated pack study to validate processing methods

In food processing industries microbial process validation is a key step to assure food safety. FDA defines microbial process validation as "collection and evaluation of data in a more scientific way (objective evidence) from the process design stage through commercial production steps to establish scientifically approved and capable process schedule to meet predetermined specifications to guarantee food safety and better quality."

According to NACMCF (2010) food processing procedures can be validated in either of the following method. (i) pathogen growth inhibition study, the method is used to evaluate the ability of given type of food product formulation in combination with specific type of processing method and packaging material to inhibit the growth of certain food pathogens, (ii) pathogen inactivation study, which is used to evaluate the ability of a given type of food formulation in combination with a given processing method to inactivate food pathogens (iii) combined growth inhibition and inactivation study, which is commonly used to evaluate the ability of a given food formulation or processing method or combination of the two to inactivate certain food pathogens and inhibit the growth of others. The last one is a suitable method of validation in production of acidified low acid foods by inactivating low pH tolerant pathogens and inhibiting those spores which cannot grow in low pH (pH<4.6) environment.

Based upon this, to validate food processing methods from safety point of view, the thermal resistance/pressure-thermal resistance of target microorganism or its surrogate should be

characterized in terms of decimal reduction time (D-value (min)) and thermal resistance constant (z-value (^{o}C)) for a specific food formulation and processing method (Larousse and Brown 1997). In thermal processing the same degree of pasteurization value (*P*-value, for temperature levels < 100^{o}C) can be achieved for different time-temperature conditions. The *P*-value implies that duration (min) required to achieve a given reduction ratio in number of food microorganisms at a given constant reference temperature for a certain *D* and *z* values. This can be expresses according to Eq. 2.13:

$$P_T^z = D_T * \log\left(N_o / N\right) \tag{2.13}$$

where, P_T^z is pasteurization value at reference temperature and certain *z* value; *T* is temperature of the product at time *t*, (°C); T_T is reference temperature; z is temperature change required to effect a 10-fold change in the *D* value (^{o}C) .

If for example, log (*No/N*) = 5 specified, then *P*=5*D* commonly considered sufficient processing time for commercial sterility for acidified low acid foods (Pflug and Odlaug 1978). However during actual commercial production condition target temperature level cannot be reached instantaneously. Rather the food or microorganism experiences an individual time temperature history which results in a given food quality parameter or microorganism integrates the impact of different heating times encountered at different temperatures, ultimately will give singular integrated impact of the process. Considering *D* and *z* values of reference microorganism at reference temperature, integrated *P*-value from gathered time-temperature data at the slowest heating point can be determined using Eq. 2.14:

$$P_T^z = \int_0^t 10^{(T-T_r)/z} * dt \tag{2.14}$$

where; all variables are as indicated in Eq. 2.13.

The designed *P*-value calculated using Eq. 2.13 is theoretically equivalent to integrated *P*-value of Eq. 2.14. Through equating the two equations and required rearrangement the predicted microorganism $\log_{10}$ reduction from a given processing schedule at specific condition can be determined using Eq. 15.

$$\Pr edicted \quad \log reduction = \log\left(N_o / N\right) = P_T^Z / D_T \tag{2.15}$$

2.8 Conclusions

Commercially sterilization of low acid foods (pH< 4.6) (canned vegetables) are commonly subjected to 12*D* process schedule at 121.1^{o}C due to concern of *C. botulinum* in food safety. This intensive heat treatment significantly affects quality of canned vegetables and market demand of such type of products is declining. Different attempts have been made to improve quality of these products through modification of conventional processing methods and packaging materials. However most of the modifications might work well for liquid or liquid-particulate foods or at relatively high temperature. In addition to this, efforts in use of novel processing technologies have been explored to produce fresh like products. Most of the time the use of novel processing methods are either limited from a food safety point of view (limitation of spore inactivation eg. HP) or may not work well for tightly packed or conductive foods. However so far attempts not yet exerted in terms of controlled modification of product pH with combined use of novel processing methods to improve quality of canned vegetables. Conventional acidification methods are associated with various limitations in terms of pH variability within the product, extended period of pH equilibration time and lack of dependence and predictability to achieve required finished equilibrium pH. Furthermore their use is only limited to few vegetables with significant effect on flavor and taste of a product. To overcome these limitations a novel acid infusion methods should be sought to use in combination with various alternative processing methods. Furthermore thermal resistance of reference food microorganism should be known and required inactivation kinetic parameters should be accurately determined with relevant kinetic model. The influence of infused acid on quality of the product should be known to scale up designed processing methods to commercial scales. Therefore, the overall goal of this study was to shift thermal processing parameter of low acid canned vegetables from the current more intensive condition to moderate levels through modification of product property and combined use of alternative processing methods for better quality retention.

PREFACE TO CHAPTER THREE

Products produced with addition of acid and preservatives are generally known as pickles or fermented products. Acetic acid and sodium chloride are usually added as preservative to reduce pH or softening of vegetable tissues respectively. The pH of most pickeled products is below 4.0. The combined effect of low pH and preservatives commonly affect taste and flavor and the method is only limited to a few type of products. Furthermore conventional acidification methods commonly used have their own limitations. On top of this FDA, doesn't recommend type and concentration of acidifying agents to be used in production of acidified foods. In this chapter, attempts are made to develope a novel acid infusion method to overcome limitations of conventional methods. High pressure assisted (200-300MPa) acid infusion as a novel technique was compared with conventional method (36-48^{o}C) using first order kinetic model to determine rate of pH reduction of three food grade acids. Based upon data from the kinetic work, an optimization study was conducted using CCRD of RSM. Kinetic data and optimized conditions showed that faster and uniform pH reduction could be achieved using pressure assisted infusion method. Furthermore pressure assisted acid infusion is more dependable to achieve required pH reduction than conventional methods. Developed predictive models and infusion kinetic data can be used to forecast optimum conditions of acidification. The same procedure can be expanded to other vegetables, other acidulants, and processing conditions to estimate the required acidification conditions. This novel method generally overcomes limitations of conventional methods without imposing significant change on taste and flavor of products. The method can be used as controlled pH reduction technique to modify products property in terms of modification of pH profile for production of acidified low acid foods.

CHAPTER THREE

EVALUATION OF HIGH PRESSURE (HP) TREATMENT FOR RAPID AND UNIFORM PH REDUCTION IN CARROTS

ABSTRACT

Acidification (pH < 4.6) of marginally low acid foods permits them to be treated like high acid foods and, hence, have the potential to improve their quality and reduce energy costs by lowering severity of processing conditions. The objective of this study was to evaluate high pressure (HP) treatment for rapid and uniform pH reduction of low acid foods using carrot. Three organic acids (citric, malic and glucono-delta-lactone) were used in the study. Conventional acidification tests were carried out at atmospheric pressure at different temperatures (36-48^{o}C) and different treatment times (0-36 min). HP treatments were given at room temperature (maximum process temperature < 32^{o}C) with different pressures (200-300 MPa) and treatment times (0-14 min). Time dependent acid infusion and the resulting pH reduction were used to evaluate the acidification kinetics. Results showed that the pH reduction rates were described by a first order kinetic model. No significant differences (p>0.05) in acidification rates were observed between the acidifying agents either in conventional or HP acidification process. Pressure (HP acidification) and temperature (conventional acidification) were significantly (p<0.05) reduced decimal reduction time (D) for pH drop. The associated D values were 2.4 to 4.4 times higher in conventional (slower) as compared with HP acidification. For conventional acidification, the z values (temperature sensitivity) were 34-44.8^{o}C and for HP acidification, the z values (pressure sensitivity) were 206-222 MPa. HP acidification provided more rapid and uniform pH reduction as compared to conventional method.

3.1 INTRODUCTION

Microorganisms have a minimum, optimum and maximum pH for their growth and spoilage/pathogenic activity. Yeasts, molds and acid tolerant bacteria can grow at low pH (pH<4.6); but the growth, activity and thermal resistance of most of spore forming spoilage and pathogenic bacteria are suppressed when pH<4.6 (Ramaswamy and Marcotte 2005). On the basis of pH, foods are classified into high acid (pH < 4.6) and low acid (pH > 4.6) groups. Acidity of a food can have critical implication on its microbial ecology to influence the growth of spoilage and pathogenic microorganisms. If the external pH in food environment is sufficiently low (for instance if pH < 4.6 in case of *Clostridium botulinium*), the burden on acid intolerant microorganisms is high and drops their cytoplasm pH to the level where growth can no longer be possible and eventually the cell dies (Adams and Moss 2008).

Foods belonging to the high acid group can be processed with desired degree of safety, better quality and stability by heating up to 100^{o}C. The low acid foods, however, need to be processed at higher temperatures (110-130^{o}C) for longer times in order to make them free of anaerobic heat resistant pathogen *C. botulinum* which, if present, can grow and produce the botulism toxin (Ramaswamy and Marcotte 2005). This higher temperature processing significantly degrades the quality of foods. By reducing the pH of marginally low acid vegetables below 4.6, they can be treated as acid foods and can be thermal processed at reduced severity levels. In addition, these acid/acidified foods are good candidates for high pressure processing, because yeasts and vegetative bacteria are very susceptible to high pressure (Mussa et al., 1999 a,b; Basak et al., 2002). Acidification has been traditionally used as a means of microbial inhibition, which control the germination and growth of acid intolerant microorganisms. Acidified vegetables further have also been shown to have better texture (Heil and McCarthy 1989) and shelf stability as compared to non-acidified low acid foods, and the process saves significant amount of energy.

Generally “acidified foods” are low-acid foods to which acid(s) or acid food(s) are added; they have a water activity greater than 0.85 and have a finished equilibrium pH of 4.6 or below (FDA 21 (CFR 114.3(b)). According to FDA, acidified foods shall be so manufactured,

processed, and packaged that a finished equilibrium pH value of 4.6 or lower is achieved within the time designated in the scheduled process and maintained in all finished foods. Acidified foods shall be thermally processed to an extent that is sufficient to destroy the vegetative cells of microorganisms of public health significance and those of non-health concern capable of reproducing in the food under the conditions in which the food is stored, distributed, retailed and held by the user (FDA 21 CFR 114.80)). This is because they can pose a risk of botulism if pH and other critical factors are not carefully controlled during processing to prevent the germination and growth of viable spores of *C. botulinum* (44 CFR 16204 and 16204). When critical factors are not carefully controlled, the vegetative cells of some microorganisms of non-health significance (such as some spoilage bacteria, yeasts, and molds) can grow in an acid environment and, in so doing, cause the pH of the food to increase. For instance some spoilage spore forming bacteria produce acid-tolerant spores that can germinate, grow, and cause the pH to increase. Therefore, thermal processing that is sufficient to destroy vegetative cells of such microorganisms may not be sufficient to destroy their spores (Fields et al., 1977; Odlaug and Pflug 1978; Montville 1982; Young-Perkins and Merson 1987; Al Dujaili and Anderson 1991). However, when the pH of a food is 4.6 or below, spores of *C. botulinum* will not germinate and grow (FDA 41 CFR 30442 and 30442).

Different acidification methods have been recommended to achieve the required pH reduction in low acid foods (FDA 21 CFR 114.80):

a) Blanching of the food ingredients in acidified aqueous solutions.

b) Immersion of the blanched food in acid solutions. Although immersion of food in an acid solution is a satisfactory method for acidification, care must be taken to ensure that the acid concentration is properly maintained.

c) Direct batch acidification, which can be achieved by adding a known amount of an acid solution to a specified amount of food during acidification.

d) Direct addition of a predetermined amount of acid to individual containers during production. Liquid acids are generally more effective than solid or pelleted acids. Care must be taken to ensure that the proper amount of acid is added to each container.

e) Addition of acid foods to low-acid foods in controlled proportions to conform to specific formulations.

However, these methods have some limitations in terms of establishing required pH reduction. In conventional methods, the pH equilibration takes a long time (FDA 1998). Non uniformity in pH reduction between internal and external part of acidified products is another common problem. Addition of acid to each container in a production line is the least accurate and uncertain method to achieve pH < 4.6. Conventional methods also have poor predictive capabilities to forecast the required pH reduction. When test samples of large size and relatively high solid-to-solution ratios are employed, acid diffusion into sample tissue is generally very slow and requires overnight soaking under reduced temperature condition to control the growth of food microorganisms (Derossi et al., 2010) which add additional cost. The different practices will have a major implication in the production of acidified thermal processing of foods, and methods that accelerate the acidification kinetics will be welcome. Therefore, the objective of this work was to elucidate the potential of high pressure treatment for rapid and uniform acidification of low acid foods for lowering their pH below 4.6 for acidified food thermal processing.

3.2 MATERIALS AND METHODS

3.2.1 Acidifying agents

Three food grade organic acids, citric acid (CA), malic acid (MA) and glucono-delta-lactone (GDL) as a source of gluconic acid were obtained from a local source (Fisher Scientific, Montreal, Canada).

3.2.2 Preparation of carrot sample

Carrots were purchased from a local market and stored under refrigerated (4^{o}C) condition until used. Both the top and bottom parts of the carrot were removed and the middle section was used for the study. Samples were peeled, cut into 1.0 cm cubes and blanched in tap water at 90^{o}C for 3 min. Blanched cubes were immediately cooled in cold water and used for the experiments.

3.2.3 Acidification kinetics

Prior to the acidification kinetics, the pH reduction potential of acidifying agents were evaluated by determining the buffering capacity (BC) of carrot puree according to McCarthy et al. (1991). However, applying this principle using concentrations determined from BC to acidification kinetics study was impractical. Because the process required more than 24 hr to achieve finished equilibrium pH of 4.4±0.1 at the slowest pH reduction part of the cubes at room temperature condition. A preliminary study showed that elevated concentrations around ten times the concentration used in BC studies was required in order to reduce treatment times. In addition, as shown in many previous conventional acidification studies, higher temperatures were considered helpful for more rapid acidification. Therefore, based upon BC results, concentration levels of 1.92 % w/v (pH 2.25) for CA, 1.12% w/v (pH 2.24) for MA and 4% w/v (pH 2.3) for GDL were used. If long equilibration times (>24hr) are available, even one tenth of these concentrations might be sufficient to yield the necessary equilibrium pH at room temperature. The central core part of carrot cube was considered the slowest acidification part to achieve required target pH of 4.4±0.1.

For the acidification kinetics study, 40 g blanched carrot cubes were soaked (1:3 w/v solid: solution) in acidulants of different concentrations and subjected to conventional and high pressure (HP) acidification processes. For conventional acidification, the acidulant solution at a specific concentration taken in a beaker was equilibrated to the desired temperature in a shaker water bath (Julabo Model SW22, Germany) before subjecting the carrots to the treatment. The shaker platform was maintained at 20 rpm during the test to maintain good mixing conditions. Selected temperatures (36, 42, and 48^{o}C) and treatment times (0 to 36 min) were used for the conventional acidification.

High pressure acidification was carried out in high pressure equipment (ACIP 6500/5/12 VB, capacity with working pressure range of 100-650 MPa and temperature range of -20 to +80^{o}C). Water was used as the pressurization medium, the pressurization rate was kept at 4 MPa/s and the depressurization time was < 10 s. For pressure treatment, samples were heat-sealed in polyethylene bags with a specific acidulant (1:3 w/v solid: solution). The samples were subjected to pressure treatments at 200, 250 or 300 MPa for 0 to 14 min, depending on the pressure level. The sample was introduced at room temperature (24±1^{o}C). The average observed

temperature increase per 100 MPa was 2.4^{o}C. The maximum sample temperature during pressure treatment (adiabatic heating) was between 28 and 32^{o}C depending on pressure level employed (Figure 3.1).

Test samples after treatment were quickly removed at predetermined times and briefly rinsed in distilled water to remove acidifying solutions from external surfaces of cubes. In order to exclude the effect of temperature (conventional acidification) and pressure (HP acidification) come-up phases, only holding times were considered as treatment times. Samples subjected to the come-up period were considered zero time treatment. The pH of samples were measured after separating the external and internal (core) parts (about 0.15 cm was cut from each side of the cube to approximately leave an internal core cube of 0.3-0.4 cm^{3}). Then core parts of several cubes were blended together using a small coffee grinder to make a puree. The puree pH was then measured at 25^{o}C using pH meter (Accumet Basic AB15, Fisher Scientific, Montreal, Canada) with required calibration.

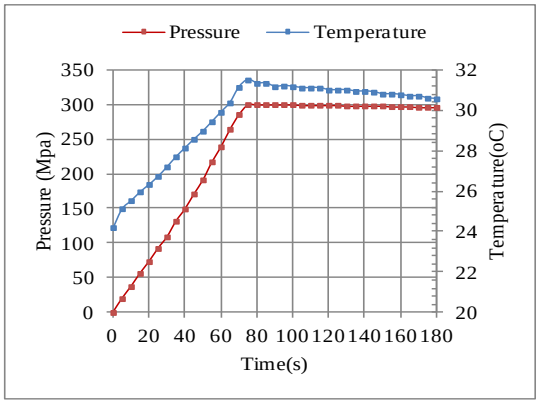

Figure 3.1 Pressure and temperature profiles during HP acidification process for the first 3 min at maximum pressure level.

3.2.4 Data analysis

A first order kinetic model with some modifications was used to fit the rate of pH reduction associated with different acidifying agents and treatment methods. pH is defined as the hydrogen ion concentration on a negative logarithmic scale:

$$pH = - log [H^{+}] \quad (3.1)$$

Higher H^+ concentration means lower pH. A plot of decrease in pH versus time during acidificstion would represent an apparent first order reaction with increasing concentration of H^+ in a sample on log scale with time. By changing the sign (i.e., plotting pH vs time), it can be converted to a regular first order type kinetics. The modified form can be represented with equation (3.3) after required integration of Eq. 3.2.

$$\frac{d[H^+]}{dt} = k[H_o^+] \quad \text{or} \quad -\frac{d[H^+]}{dt} = -k[H_o^+] \tag{3.2}$$

where k is the rate constant; $[H^+]$ is the H^+ at time t; $[H_O^+]$ initial H^+ of carrot (at time zero).

$$-\log[H^+] = -\log[H^+] - \frac{k}{2.303}t \quad \text{or} \quad pH = pH_o - \frac{k}{2.303}t \tag{3.3}$$

Although it would look like a linear zero order model, the above plot will represent H^+ concentration changes on the semi-logarithmic scale as indicated in equation 3.1. The plot of pH versus time would then give a straight line and the acidification rate (k) can be calculated from the slope of the curve. The decimal pH reduction time or D value of acidification can be obtained from Eq. 3.4. D value, in this context, is defined as time required to reduce the sample pH by one unit.

$$D = \frac{2.303}{k} \tag{3.4}$$

Temperature or pressure dependency of acidification rate could be explained either through z value model (D-z) or Arrhenius kinetic model (k-Ea). In this study, both models were used to describe acidification kinetics parameters. In the former case, the temperature or pressure sensitivity of acidification rate at different temperature or pressure levels can be represented by log D versus temperature (conventional acidification) or log D vs pressure (HP acidification). From inverse slope of the linear curve, the temperature or pressure sensitive indicator (z value) can then be calculated. The z value represents the temperature or pressure range required to achieve a ten-fold change in D values.

In the Arrhenius model, the specific acidification rate, k (min^{-1}) is plotted against the reciprocal of the absolute temperature, and the temperature sensitivity parameter is given by the activation energy (Equations 3.5 and 3.6).

$$k = k_o e^{-Ea/RT} \tag{3.5}$$

$$\ln k = \ln k_o - \frac{Ea}{RT} \tag{3.6}$$

where, k_o is acidification rate constant at reference temperature, *Ea* is the activation energy (kJ/mole), *R* is the molar gas constant (8.3145 J/mole ^oK), *T* is temperature (^oK) at time *t.*

The graph *ln k* versus *1/T* would give a linear plot line from which the inverse slope (-E_a/R) used to calculate the *Ea* required for acidification. Furthermore, the pressure dependence of acidification rate constant could be described by the activation volume (V_a, ml/mol) which was derived from the slope of a curve of natural logarithm of rate constant against pressure as indicated in Eq. (3.7):

$$\ln k = \ln k_{\Pr ef} - \left(\left(\frac{V_a}{RT} \right) * \left(P - P_{ref} \right) \right) \tag{3.7}$$

where, P_{ref} is the reference pressure (250 MPa), k_{Pref} is the inactivation rate constant at P_{ref} (min^{-1}) and *T* is the temperature (K), V_a is the activation volume, *R* the molar gas constant (8.3145 J/mole ^oK), *T* is temperature (^oK) at time *t.*

3.2.5 Optimal conditions within the experimental range

Acidification kinetics study was used to generate important kinetic data for determining the optimum acidification conditions to achieve pH of 4.4±0.1. For the purpose of optimization, a central composite rotatable design (CCRD) of the response surface methodology (RSM) was employed. RSM is widely used in food science and engineering areas to evaluate the process performance. In this study, 20 experiments were performed with each acidifying agent according to CCRD using a Design Expert computer software program (version 6.0.2 State-Ease, Inc., Minneapolis, MN) with three variables, each having five levels (Khuri 1989) (Table 3.1). Selected data from previous kinetic studies were considered as a center point for the optimization study. The independent variables and their levels are indicated in coded and natural values in Table 3.1. A second order polynomial model (Eq. 3.8) including the linear, quadratic and an interaction term was proposed to establish fuctional relationship of independent variables on response (pH).

$$Y = \beta_O + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{j=i+1}^{n+1} \beta_{ij} x_i x_j \quad (3.8)$$

where, Y represents response variable pH, β_0 is the interception coefficient, β_i coefficient of the linear effect, β_{ii} the coefficient of quadratic effect and β_{ij} the coefficient of interaction effect, x_i represents the variables.

A numerical optimization procedure was used for identifying the best conditions incorporating appropriate constraint to establish the pH in the range of 4.4-4.5 within the experimental range of processing conditions. The optimization study was conducted to investigate pH uniformity in acidified samples for each treatment condition. The analysis of variance (ANOVA) included the significance of the models and their terms, the estimated model coefficients, the coefficient of determinations, as well as the lack of fit test were performed in order to determine the adequacy of the model to represent the data (Montgomery 1991). Three dimensional surface plots were plotted using the software to evaluate the effect of each variable and their interaction in reduction of pH of the sample.

Table 3.1. Coded and natural values of CCRD for temperature and high pressure acidification

Factors	Code and natural levels of each factor				
	-1.68	-1	0	1	1.68
	Temperature and time for conventional treatments				
Temperature (^oC)	36.95	39	42	45	47.05
Time (min)	16.59	20	25	30	33.41
	Pressure and time for high pressure acidification treatments				
Pressure (MPa)	233	250	275	300	317
Time (min)	3.32	4.00	5.00	6.00	6.68
	Concentrations for temperature and high pressure acidification conditions				
CA	1.08	1.42	1.92	2.42	2.76
MA	0.35	0.66	1.12	1.56	1.87
GDL	3.16	3.50	4.00	4.50	4.84

3.3 RESULTS AND DISCUSSION

3.3.1 Acidification kinetics of carrot cubes

Acidification in this context was defined as time dependent reduction of pH achieved by acid infusion to the central parts of carrot cube after treating it in a given acid solution. The pH reduction is governed by the fundamental mass transfer diffusion kinetics of acid (contributing the hydrogen ion) from the solution to the product. The acidification time to reach a target pH (or the rate of acidification) is dependent on several factors like type of the pre-treatment steps (eg. blanching), acidifying agent, solid to solution ratio, temperature, agitation, acidification methods, and type and nature of the product etc (Salaün et al., 2007; Rastogi and Niranjan 1998; McCarthy et al., 1991).

3.3.2 Conventional acidification knetics

The experimental data on pH reduction for different acids as a function of temperature and time were fitted to the first order kinetic model and the associated acidification rate constant were determined. The first order plots are shown in Figure 3.2 and the computed rate constants from the slopes along with the associated statistical parameters are summarized in Table 3.2. For majority of food components, the time dependency relationship appears to be described by first order kinetic model (Lenz and Lund 1980). The D_T value was calculated from the rate constant (Eq. 3.3) as time required for reducing pH of a sample by one pH unit. Acidification rate increased with an increase of temperature for all acidifying agents and decimal pH reduction time decreased (Table 3.2).

The three acidifying agents showed no significant ($p>0.05$) differences in pH reduction despite their concentration differences at any specific temperature (36 to 42^{o}C). However, temperature level significantly ($p<0.05$) reduced decimal acidification time, except for the non-significance ($p>0.05$) effect for MA between 36 and 42^{o}C. At the highest temperature (48^{o}C), GDL showed a more pronounced ($p<0.05$) pH reduction as compared to other acidulants. This might be because of high concentration of GDL with higher temperature effects for enhanced diffusion of hydrogen ions.

Even though pH reduction increased with an increase in temperature for all acidifying agents, relatively the same pH reduction was achieved with lower concentration of MA (1.12%)

as compared to higher concentrations of CA (1.92%) and GDL (4%). This showed that MA at relatively lower concentration had better diffusivity to achieve the required pH reduction.

The average D_T values for the acidifying agents varied from 22.7 to 9.4 min (Table 3.2) in the temperature range studied. On average, the acidification rates with the different acids were found to double at 48^{o}C as compared to 36^{o}C. Therefore, temperature plays a critical role in enhancing acid diffusion. Increase in temperature might facilitate more molecular random kinetic motion in solutions and promote tissue relaxation in the sample for enhanced diffusion phenomenon.

Table 3.2. Acidification rate and corresponding D_T values, activation energy (*Ea*) and z_T values of different acidulants from heat assisted acidification method

Temperature (°C)	k (min^{-1})	R^2	D_T (min)
		CA (1.91%)	
36	0.122±0.0025[†]	0.94	18.9±0.54
42	0.1501±0.003	0.98	15.3±0.42
48	0.2008±0.0001	0.99	11.5±0.004
	Ea = 34.5±1.5(kJ/mole)	0.97	z_T=55.2±2.3(°C) (R^2=0.97)
		MA (1.12%)	
36	0.1018±0.0053	0.96	22.7±1.66
42	0.1503±0.0011	0.98	15.3±0.15
48	0.2203±0.004	0.99	10.5±0.25
	Ea =53.15±2.42 (kJ/mole)	0.99	z_T=35.85±1.65(°C)(R^2=0.99)
		GDL (4%)	
36	0.1085±0.0025	0.95	21.2±0.69
42	0.1600±0.0011	0.96	14.4±0.13
48	0.2444±0.0026	0.98	9.4±0.14
	Ea = 55.90±1.0 (kJ/mole)	0.99	z_T=34.10±0.5 (°C) (R^2=0.99)

[†]*Standard error of mean*

Furthermore, temperature dependency of the decimal acidification time (D_T) can be expressed by z_T values. The z_T values determined for each acidifying agent are shown in Table 3.2, with the highest for CA. Higher z_T means lower temperature dependency, and therefore CA

diffusion into the samples was less temperature sensitive as compared to diffusion of MA and GDL. The z_T values for the latter two acidifying agents were found to be very close even though they varied in terms of their original concentration (Table 3.2). This result somewhat contradicts with what is reported in Derossi et al. (2011). In their review, they related the pH lowering capacity of acids with a number of acid functions and their *pKa* values. According to the report, citric acid (triprotonic), commonly used in industries to reduce pH of vegetable pieces, had a stronger influence on pH reduction of vegetables as compared to malic (diprotonic) and gluconic (monoprotonic) acids. The discrepancy might be associated with differences in the concentration levels of acidulants used in this study.

The activation energy also explains the temperature sensitivity of acidification rate constant for each type of acidifying agent. The higher activation energy implies the more temperature sensitivity of the agent. According to Arrhenius theory in chemical reaction kinetics there is an exponential relationship between reaction rate and temperature, which means, a small increase in temperature can cause significant change on reaction rate. Based on this the energy is used to explain temperature sensitiveness of acidification rate. The activation energies were calculated as 34.5, 53.2 and 55.9 kJ/mole for CA, MA and GDL respectively (Table 3.2). Therefore, MA and GDL are more sensitive for increase in temperature (36 to 48^{o}C) and hence a faster pH reduction using these acidulants can be achieved by increasing soaking temperature than for CA. However, so far no literature data is available to compare our results with others report. This shows that still there is a limitation of data in terms of expressing acidification kinetics of various acidifying agents using different kinetic models.

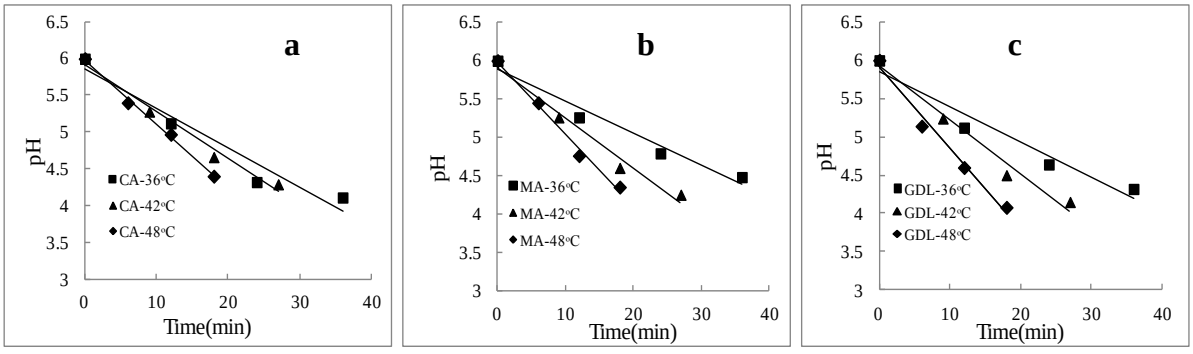

Figure 3.2. Conventional acidification kinetics at 36, 42 and 48^{o}C with (a) citric acid (CA), (b) malic acid (MA) and (c) glucono-delta-lactone (GDL)

At lower temperatures, it was observed that there was no significant difference (p>0.05) among types of acidifying agents in reducing pH of the sample. Therefore, in such cases, the choice of acidulate must consider the cost, effect on sensorial property and capacity to inhibit the growth of microorganisms. For instance CA was found more effective in reducing heat resistance of spores of *Clostridium sporogenes* PA 3679 as compared to GDL (Silla and Zarzo 1995) but it relatively imparts a sour taste to the product. On contrary to this, GDL is a weak acidulant which seems to give very smooth flavor with little or no effect on original flavor of products (Kaercher 1989; McCarthy et al., 1991) even though its microbial growth inhibition capacity is limited.

3.3.3 High pressure acidification kinetics

High pressure acidification data were also fitted to the first order kinetic model. Figure 3.3 shows the rate of pH reduction of the samples for different acidulants under different pressure levels and the computed pressure acidification kinetic parameters are also shown in Table 3.3. High pressure processing technology has been widely used to inactivate pressure intolerant microorganisms (Mussa et al., 1999a; Noma 2002; Ramaswamy and Shao 2010), modification of functional properties of foods (Messen et al., 1997; Paul 1999) and extraction of important bioactive compounds (Zhang et al., 2004; Nagendra et al., 2009). However, so far no study has published on the high pressure acidification kinetics of low acid particulate foods.

Acidification rates were significantly (p<0.05) increased for all acidifying agents with an increase in pressure. The D_p value represents time required to reduce the pH of the sample by one logarithmic unit at a given pressure level. A decrease in decimal acidification time with an increase in pressure signifies enhanced acidification rate with application of pressure.

At 300 MPa, the acidification rate of all acidulants more or less converged to equal values and on average it was found to be 2.9 times faster than the rates observed at 200 MPa (Table 3.3). This shows that there might be rapid and instantaneous diffusion and saturation of hydrogen ions when the pressure level increased to 300 MPa. Maria and Barrett (2010) in their review paper indicated that high pressure levels between 100 to 200 MPa can cause reversible phase transition of lipid bilayer of plant cells from their liquid crystalline form to gel phase, but when the pressure level is above 200 MPa, it destroys and makes fragmentation of membrane structure. Therefore this might contribute to the formation of channels across the cell membrane

and facilitate rapid diffusion of acidulants into the cells. In addition to this, different theories indicated that the relaxation of tissues of biological materials with high pressure treatment (Rastogi and Niranjan 1998) might facilitate a fast and uniform diffusion of acidifying agents during depressurization time. Nevertheless, food samples subjected to high pressure show a minor decrease in volume as a function of the imposed pressure but an equal expansion occurs during decompression. Therefore both the compression and decompression steps also might contribute in tissue structure modification and enhanced diffusion of acidulants in the samples. Moreover, rapid pH reduction in high pressure acidification might not only be associated with diffusion of acids, however, pressure by its nature might have an effect to reduce pH of fresh samples.

Table 3.3. Acidification rate and corresponding D_P values, activation volume (Va) and z_P values of different acidulants in high pressure assisted acidification method

Pressure (MPa)	k (min^{-1})	R^2	D_P (min)
		CA(1.91% w/v)	
200	0.2863±0.01	0.98	8.05±0.27
250	0.6483±0.021	0.99	3.56±0.11
300	0.8747±0.0021	0.95	2.63±0.01
	Va= -28.22±0.76 ml/mole	0.93	z_P=206.4±6.4(MPa) (R^2=0.93
		MA(1.12% w/v)	
200	0.3066±0.011	0.98	7.52±0.28
250	0.6368±0.012	0.98	3.62±0.07
300	0.8032±0.015	0.96	2.55±0.04
	Va= -27.21±1.26 ml/mole	0.96	z_P=213.2±9.1(MPa)(R^2=0.96)
		GDL (4% w/v)	
200	0.3098±0.0017	0.97	7.44±0.04
250	0.6126±0.023	0.95	3.76±0.14
300	0.8786±0.027	0.95	2.62±0.08
	Va= -26.3±0.63 ml/mol	0.97	z_P= 222.3±5 (MPa) (R^2=0.97)

†Standard error of mean

Pressure sensitivity of acidification rates was described by the z_P model normally employed in thermal processing and using the Arrhenius concept traditionally used in chemical reactions. The z_P values for CA, MA and GDL were 206, 213 and 222 MPa, respectively (Table 3.3). The values associated with the different acidulants are in a fairly narrow range (206-222 MPa) which means the pressure influence on HP acidification rates associated with the different acidulants were similar. This is in contrast to the conventional acidification in which CA had a distinctly higher z_T value (Table 3.2) (meaning it was least influenced by temperature changes).

Analogue to activation energy, the effect of pressure on acidification rates can be described by the activation volume model. Estimated activation volumes under HP acidification conditions were -28.2, -27.2 and -26.3 ml/mole for CA, MA and GDL, respectively. A negative activation volume implies that acidification was favored by pressure. A lower value also means the acidification is more sensitive to pressure effects. Further, the HP treatment, due to its isostatic and instantaneous effect, offers potential for more uniform pH reduction in the solid foods. In terms of uniform pH reduction capacity, the pH variation between central and external part of acidified samples in conventional acidification was 1.2±0.3 after a treatment for 20.0-22.7 min (Table 3.5), while the range found with HP acidification was 0.5±0.16 pH unit after a treatment time of 4.3-5.0 min (Table 3.6). With both conventional and HP treatment conditions, longer treatment times resulted in an enhanced reduction of sample pH. The acidification rates under HP conditions (especially at the higher pressure levels), on an average, were found to be about four times faster than with conventional acidification (Table 3.2 and 3.3). Hence, HP acidification helps to establish rapid and uniform pH reduction even when carried out at lower temperature conditions.

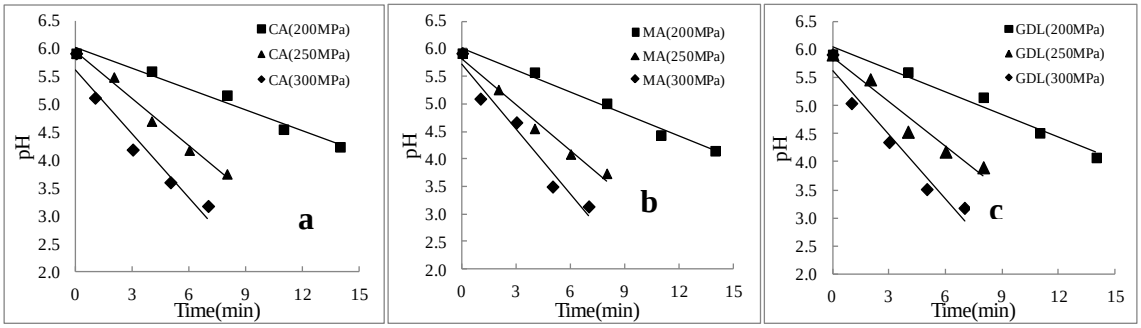

Figure 3.3. HP acidification kinetics at 200, 250 and 300 MPa with (a) citric acid (CA), (b) malic acid (MA) and (c) glucono-delta-lactone(GDL)

3.3.4 Response surface analysis

Since the rate of acidification and pH reduction at the center part of the product depends on many factors, optimization of the process is important to achieve the target pH in an efficient manner. In this study, optimum acidification conditions were determined using a CCRD model and numerical optimization was performed in order to establish target pH of 4.4±0.1 in the central part of carrot cubes. Multiple regressions were performed using the Design Expert software (version 6.0.2 State-Ease, Inc., Minneapolis, MN) to estimate coefficients of significant quadratic models for predicting the effects of acidification variables on pH reduction. The ANOVA results are summarized in Table 3.4 including the significance of model and the various terms, estimated model coefficients, coefficients of determinations, and the significance of the lack of fit. The coefficients of determinations and the lack of fit test were considered important in order to determine the utility of the model and representation of the data (Montgomery 1991). Second-order polynomials response surface models (Eq. 3.9-3.14) were fitted to determine the effect of independent process variables in pH reduction of carrot cubes. The following quadratic equations developed were also used for searching the direction in which to change independent variables in order to reach required pH level:

RSM quadratic equations for conventional acidification

$$pH_{(CA)} = 4.53 - 0.12A_T - 0.075B - 0.15C - 0.077B^2 - 0.054C^2 + 0.035A_T B \quad (3.9)$$

$$pH_{(MA)} = 4.38 - 0.099A_T - 0.098B - 0.24C + 0.084C^2 \quad (3.10)$$

$$pH_{(GDL)} = 4.45 - 0.11A_T - 0.061B - 0.18C - 0.053B^2 \quad (3.11)$$

RSM quadratic equations for high pressure acidification

$$pH_{(CA)} = 4.15 - 0.021A_P + 0.011B - 0.15C + 0.047A_P^2 + 0.12B^2 + 0.062C^2 + 0.031A_P B - 0.036A_P C + 0.093BC \quad (3.12)$$

$$pH_{(MA)} = 4.01 - 0.031A_P - 0.052B - 0.25C + 0.16A_P^2 + 0.23B^2 + 0.152C^2 - 0.063A_P C \quad (3.13)$$

$$pH_{(GDL)} = 4.17 - 0.042A_P - 0.076B - 0.074C + 0.11A_P^2 + 0.075B^2 + 0.054C^2 - 0.059A_P B - 0.039A_P C - 0.056BC \quad (3.14)$$

where, $pH_{(CA)}$, $pH_{(MA)}$ $pH_{(GDL)}$ are responses (pH values of acidified carrot sample) by CA, MA and GDL respectively; A_T and A_P are temperature and pressure levels respectively, *B* and *C* represents time and concentration respectively (all parameters in coded values).

From the analysis of variance results (Table 3.4), pressure acidification conditions were found to be significant ($p<0.05$) to highly significant ($p<0.001$) for CA and GDL, but not significant ($p>0.05$) with MA. For conventional acidification, mainly the linear terms were significant ($p<0.05$) for pH reduction of the samples while quadratic and interaction effects were non- significant ($p>0.05$). The interactions and quadratic terms were significant ($p<0.05$) in HP acidification conditions.

3.3.5 Optimal conditions within the experimental range

Numerical optimization of acidification processes, within the experimental range of conditions, was carried out with the objective of finding optimum levels of independent variables (concentration, contact time and soaking temperature/pressure levels). Design–Expert software (version 6.0.2 State-Ease, Inc., Minneapolis, MN) was used to search the optimum desirability of the response variable. Temperature, pressure, time and concentrations were programmed to be minimized and the target pH was set in the range of 4.4 - 4.5. Optimum results determined under the above constraints for conventional and HP acidification conditions are summarized in Tables 3.5 and 3.6, respectively. In the same tables, verification results (column 6) from the recommended acidification variables are also indicated. The pH values of internal core part and external parts of the cubes were measured to check pH uniformity under conventional and pressure acidification conditions (column 6 and 7). In processing of carrots as acidified low acid vegetable, these conditions can be used as a starting point to establish the required pH reduction. The same procedure can be expanded to other vegetables, other acidulants, and processing conditions to estimate the required acidification conditions.

Table 3.4. Estimated regression coefficients, degree of significance, lack of fit and statistical parameters for the model equations

Source	CA		MA		GDL	
	Coefficients		Coefficients		Coefficient	
	Temperature	Pressure	Temperature	Pressure	Temperature	Pressure
Model	***	***	***	***	***	***
Intercept	4.53	4.15	4.38	4.01	4.45	4.17
A (Temp/Pressure)	-0.12***	-0.021*	-0.099***	-0.031*	-0.11***	-0.042**
B (Time)	-0.075***	+0.011*	-0.098***	-0.052**	-0.061**	-0.076***
C (Concentration)	-0.15***	-0.15***	-0.24***	-0.25***	-0.18***	-0.074***
A^2	0.0044^{ns}	+0.047***	$+0.012^{ns}$	0.16***	-0.009^{ns}	+0.11***
B^2	-0.077***	+0.12***	$+0.035^{ns}$	0.23***	+0.053*	+0.075***
C^2	-0.054***	+0.062***	+0.084**	0.15***	-0.020^{ns}	+0.054***
AB	0.035*	+0.031*	-0.0025^{ns}	-0.005^{ns}	-0.021^{ns}	-0.059**
AC	0.00^{ns}	-0.036**	-0.027^{ns}	-0.063**	$+0.034^{ns}$	-0.039**
BC	0.012^{ns}	0.093***	0.005^{ns}	0.030^{ns}	$+0.011^{ns}$	-0.056**
Lack of Fit	0.2150	0.6219	0.8679	0.4549	0.9789	0.8420
R-Squared	0.98	0.99	0.97	0.99	0.95	0.98
Adj R-Squared	0.96	0.97	0.93	0.98	0.91	0.95
Pred R-Squared	0.91	0.91	0.86	0.93	0.87	0.89

*ns= non-significant difference (p> 0.05) *=p< 0.05 ** = p< 0.01 and *** p< 0.001*

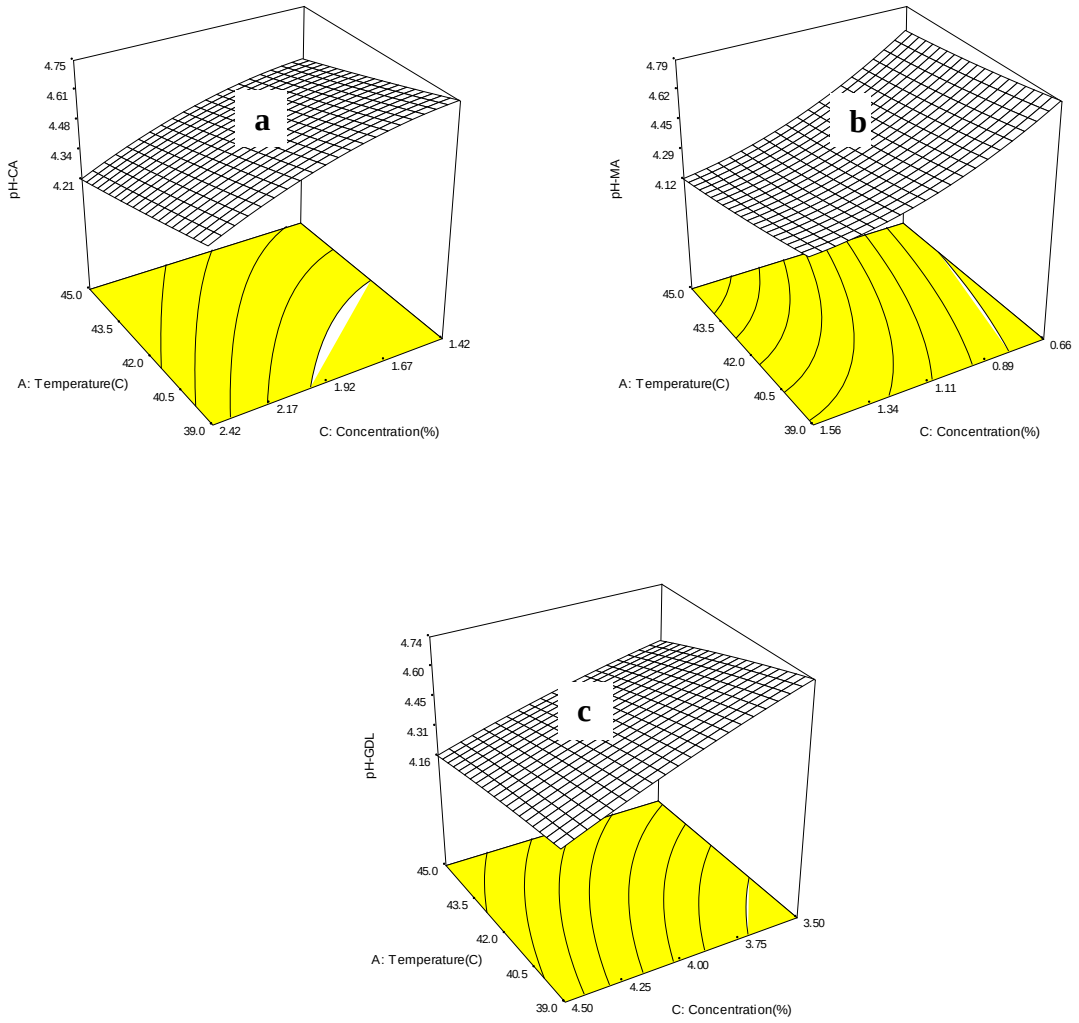

Figure 3.4. Response surface plots of heat assited acidification showing the effect of temperature and concentration at midpoint of time (25 min) for (a) CA, (b) MA and (c) GDL

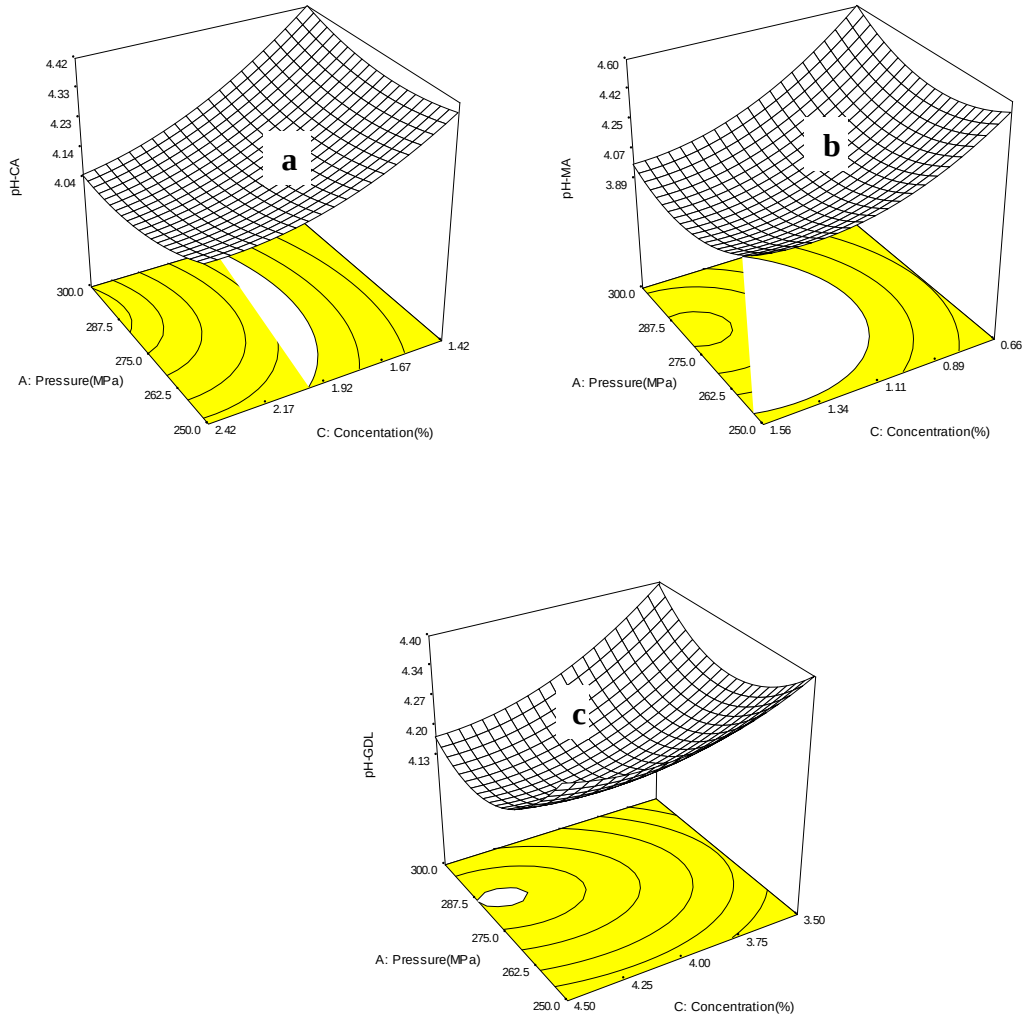

Figure 3.5. Response surface plots of high pressure assisted acidification showing the effect of concentration and pressure at midpoint of treatment time (5 min) for (a) CA (b) MA (c) GDL

Table 3.5. Optimal conditions for heat assisted acidification of carrot cubes with the three acidifying agents to achieve required pH reduction

Type of Acidulate	Temperature (^{o}C)	Time (min)	Concentration (%)	Inside pH CCRD	Inside pH Verified[a]	Outside pH Verified[b]	pH difference (a-b)	Desirability
CA	42±1.75[†]	20±1.3	1.9±0.4	4.5±0.24	4.37±0.064	3.08±0.04	1.29±0.04	0.590
MA	40±1.4	22.6±1.7	1.14±0.15	4.5±0.14	4.34±0.13	3.13±0.15	1.21±0.03	0.664
GDL	40±1.5	22.7±1.3	4.13±0.42	4.5±0.32	4.27±0.09	3.29±0.26	0.98+0.25	0.620

[†] *95% confidence interval*

Table 3.6. Optimal conditions for high pressure acidification of carrot cubes with the three acidifying agents to achieve required pH reduction

Type of Acidulate	HP (MPa)	Time (min)	Concentration (%)	Inside pH CCRD.	Inside pH Verified[a]	Outside pH Verified[b]	pH difference (a-b)	Desirability
CA	250±5[†]	4.3±0.6	1.15±0.5	4.5±0.3	4.39±0.06†	3.89±0.07	0.5±0.02	0.940
MA	254±4	4.4±0.3	0.87±0.1	4.4±0.7	4.35±0.08	3.79±0.05	0.56±0.04	0.860
GDL	260±5	5.0±0.1	3.60±0.4	4.5±0.5	4.29±0.05	3.84±0.08	0.45±0.05	0.698

[†] *95% confidence interval*

3.4 CONCLUSIONS

Acidified thermal processing is one of the thermal preservation methods used to control the growth and activity of microorganisms under milder processing conditions by modifying the pH of low acid foods. Acidification rate can be enhanced by elevating the temperature, pressure, acid concentration and time in either conventional/heat assieted or pressure assited acidification methods. However, pH reduction under pressure assited condition is 4 to 5 times faster than conventional method. In addition to this, HP acidification provides relatively more uniform pH reduction with much lower concentration of acidifying agent. The pH reduction capacity of different acidulants was found different. Much lower concentrations of MA could be used as compared to CA and GDL. GDL required the highest concentration, time, temperature and pressure level as compared to other acidulants to reach target pH of 4.4-4.5. HP assisted pH reduction can be used as a novel approach in the production of acidified foods overcoming limitations of conventional methods.

PREFACE TO CHAPTER FOUR

In previous study, acid infusion conditions were determined and optimized. The ultimate goal of acid infusion and controlled pH reduction of low acid vegetables is to reduce natural pH of vegetables below 4.6 to reduce the need for intensive processing conditions to improve product quality during thermal processing. In such low pH conditions the concern of *C. botulinum* is not an issue since its spores do not germinate, grow and produce toxin. Rather in low pH foods the role of *B. licheniformis* is very important because of its indirect effect on food safety. Survivor spores of *B. licheniformis* have the ability to grow in low pH foods and have the capacity to neutralise growth medium low pH to higher level in which *C. botulinum* would be able to grow and produce toxin. It is also most frequently involved in the spoilage of canned vegetables. In addition to these, it is also more heat and pressure resistant as compared to other *Bacillus* species. Therefore these behaviours make the bacterium to be considered as a reference or target food microorganism in production of acid or acidified low acid foods. Proper design and validation of thermal processing methods need accurately determined inactivation kinetic data at a relevant medium environment condition. Therefore, in this part of study, heat resistance of *B. licheniformis* spores was studied and relevant inactivation kinetic parameters were determined. Determined kinetic data were used in designing of thermal processing methods and validation study to assure food safety and product quality optimization. In previous optimization study, determined acidification conditions of glucono-delta-lactone (GDL) were higher than other acidifying agents and hence excluded from this study.

CHAPTER FOUR

THERMAL DESTRUCTION KINETICS OF BACILLUS LICHENIFORMIS SPORES IN CARROT JUICE EXTRACT AS INFLUENCED BY pH, TYPE OF ACIDIFYING AGENT AND HEATING METHOD

ABSTRACT

The combined effects of different heating methods (conventional (CH-T) vs Ohmic (OH-T), types of acidifying agent (citric and malic acid) and pH levels (pH 4.5, 5.5 and 6.2) were investigated to study their influence on destruction kinetics of spores of *Bacillus licheniformis*. The ultimate aim was to generate relevant kinetic data on an indicator microbial spore, useful for assessing the efficacy of thermal processes for acid or acidified low-acid foods. Kinetic parameters were evaluated according to the first order kinetic model. Temperature and pH showed a highly significant ($p<0.01$) effect on the spore destruction kinetics. Ohmic heating showed marginally better rate of bacterial destruction than conventional heating. The overall range of *D* and z values were 1.1 to 11.2 min and 12.6 to 17^{o}C, respectively, depending up on the pH and temperature levels, method of heating as well as the type of acidulate used. The shortest D_{97^oC}=1.1 min was obtained at pH of 4.5 when citric acid was used as an acidifying agent in OH. No significant difference ($p>0.05$) was observed with respect to *D* and z-values between the two types of acidifying agents.

4.1 INTRODUCTION

Heat resistance of pathogenic and spoilage food microorganisms during thermal treatment can be influenced by several factors. These factors can be intrinsic and/or extrinsic in their nature and can either suppress or support the survival and growth of microorganisms during and after a given heat treatment. Most food preservation methods are based on manipulation of one or more of these factors based on the specific need. Effects of combined intervention strategies can be additive, synergetic or antagonistic based on factor interactions and are chosen according to the specific objectives (to accelerate or suppress the factor effects). The medium pH influences the survival sensitivity of bacterial spores, and at present, it is considered to be the most important factor determining the heat resistance. The acidification canning of some vegetable, with its normal pH's lowered to below 4.6, is gaining considerable commercial importance, and FDA has established standard guidelines for such processes. Acidification helps to reduce not only heat resistance of microorganisms but also prevent germination and growth of surviving spores.

In production of acidified low acid foods, the pH of the original product is reduced to below pH< 4.6 to take advantage of the reduced thermal treatment to help retain better quality in canned vegetables. The pH reduction by itself doesn't insure food safety of acidified low acid foods. Achieved pH reduction should be complemented with a proper thermal treatment schedule to inactivate acid and heat tolerant food microorganisms. The incomplete destruction of spores of these microorganisms may pose food safety problems. Surviving spores of *Bacillus* species are problematic in pasteurized acidified or acidic foods because of their ability to survive and multiply even under moderately low pH conditions. *Bacillus licheniformis* together with *B. subtilis*, *B. pumilus*, *B. cereus* are able to grow and even cause food borne diseases (Christiansson 1992). *B. licheniformis* is most frequently involved in the spoilage of canned vegetables, with ability to grow in acidic media (pH < 4.5) and is reportedly capable of elevating the supporting medium pH to levels at which *Clostridium botulinum* would be able to grow and produce toxin (Mallidis et al., 1990; Rodriguez et al., 1993). Furthermore, its heat resistance capacity has been found to be higher than other acid tolerant *Bacillus* species (Janštová and Lukášová 2001). Therefore, *B. licheniformis* can be considered as a reference or surrogate microbial spore to determine thermal inactivation kinetic parameters for establishing processing

schedules for acidified low acid foods. Data available on the heat resistance of *B. licheniformis* are scarce and variable. Particularly, the influence of pH and acidifying agents on the spore heat resistance is not well studied. Most studies are conducted at higher pH and for strains of lower heat resistance mainly focusing on the effect of temperature on the bacterial heat resistance (Palop et al., 1996; Pereira et al., 2006).

Ohmic heating, achieved through electrical resistance of the food as part of an electrical AC circuit, is a novel thermal treatment for inactivation of bacterial spores. The method, has been gaining industrial application from its energy efficiency and rapid heating behavior of food samples as compared to conventional thermal method (Sastry 2008). It is also believed that, OH predominantly has thermal effect, but there could be also be contributory non-thermal effects, due to passage of electric current through the food. The non-thermal effect has been linked primarily to the applied electric field which allows cell walls to build up charges resulting in the formation of micro-pores across cell membrane (FDA 2000). However, so far no study has been carried out on the comparative evaluation of OH-T and CH-T with respect to the pH effects on inactivation of spores of *B. licheniformis*. Therefore, the objectives of this work were (i) to investigate the combined influence of acidulates, pH and heating methods on thermal destruction behavior of *B. licheniformis* spores, and (ii) to generate relevant destruction kinetic data for use in acidified thermal processing.

4.2 MATERIALS AND METHODS

4.2.1 Microbial spore preparation

Agar slants of *B. licheniformis* species were obtained from culture collections of Health Canada, Ottawa. A stock culture was maintained on nutrient agar slants at 4°C with transfers made every 3 months. A subset of the culture was used in the study period. Preparation of spore suspensions was based on the method detailed in Pereira et al. (2006) with minor modifications. The overnight cultures of *B. licheniformis* grown in nutrient broth, supplemented with 1 ppm of manganese (added as $MnSO_4.H_2O$), was transferred to nutrient agar plate (EMD Chemicals Inc., Köln, Germany) and incubated at 37^{o}C for 14 days. Sporulation was checked by malachite green staining technique for light green stained spores. When sporulation reached approximately 90%, spores were collected by flooding the surface of the agar culture with sterile distilled water and sterile bent glass roads. The spore suspensions from plates were washed three times in sterile

distilled water and separated by centrifugation at 4000×g for 10 min at 4^{o}C (Refrigerated Centrifuge Thermo IEC, Model 120, USA). In between the second and third centrifugation, the suspensions were pasteurized at 80^{o}C for 10 min to reduce concentration of vegetative cells. Eventually the spore suspensions in 0.1% sterile peptone were stored at 4ºC until used.

4.2.2 Preparation of heating medium

Carrot was obtained from the local market and carrot juice was extracted using an electric juice extractor (Hamilton Beach/Proctor-Silex, Inc., Model 67900, Type CJ13, China) and its pH adjusted to 4.5 or 5.5 using citric acid monohydrate or DL-malic acid (supplied from ACROS ORGANICS, USA) as acidifying agent. The natural pH of the juice was pH 6.2 and used as the control. All prepared juices were steam sterilized at 121^{o}C for 15 min. No pH variation was observed for carrot extracts acidified to pH 4.5 or 5.5 after sterilization. However, on an average, a 0.4 unit decrease in pH was observed with natural carrot juice and was adjusted back to the original carrot juice pH of 6.2 using sterile 0.1 N NaOH. This might be associated with degradation of chemical components of the juice which might reulted in the juice extract having acidic propetry.

4.2.3 Conventional heat (CH-T) treatment

Ten ml aliquot of sterile homogenized carrot juice extract was inoculated with 2 ml of spore suspension in order to get an initial concentration of 10^6 to 10^7 spores/ml. These were filled into sterile glass capillary tubes (100 mm long, 1.3-1.5 mm id and 0.2 mm wall thickness, KIMBLE CHASE, Mexico) and flame sealed on both sides under a laminar flow hood. Thermal inactivation treatments were given by immersing the sample filled capillary tubes in a temperature-controlled water bath (Thermo Electron Corporation, HAAKE P5, and Germany) for selected heating temperature combinations: 87^{o}C (0, 6, 12, 18 and 24 min), 92^{o}C (0, 4, 8, 12 and 16 min) or 97^{o}C (0, 2, 4, 6 and 8 min). These temperature levels were selected in order to determine heat resistance kinetic parameters under pasteurization conditions as applicable for acid or acidified low-acid foods. Timing was started (time = 0 min) when the tubes reached the test temperature (10-15s, depending upon target temperature, as determined in a dummy capillary filled with the test sample). At each time interval, four tubes were removed and cooled in ice-water to stop further heating effect.

4.2.4 Ohmic heating (OH-T) treatment

OH-T treatment was achieved using a custom made static Ohmic heating unit. A schematic of the set-up is shown in Figure 4.1. Briefly, the unit consisted of a treatment chamber (a cylindrical glass beaker, 12 cm long, outer diameter of 9.0 cm, and wall thickness of 4 mm) and two stainless steel electrodes (15 cm long and 20 mm thick and the distance between them was 7.5 cm) bent to the same contour of the cylindrical glass beaker. The system included a 110V 60 Hz AC input power supply, an AC/DC transformer and a variable frequency generator (Scientifix Ltd, Guelph, ON, Canada) to generate alternative current at specific frequency for Ohmic heating. The unit was operational up to 170 V and the adjustable frequency range was 1-30 kHz. A frequency of 4 kHz square-wave electrical power was maintained with the help of an oscilloscope (Tektronix TPS2012, China) and with maximum applied current of 9 A.

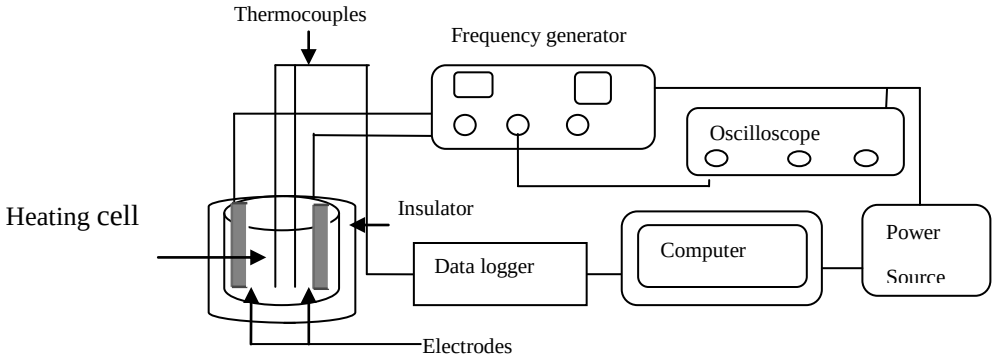

Figure 4.1. Experimental setup of the static OH system

Preliminary results (Figure 4.2) showed that a square wave with applied electric field and time division of 1 V cm^{-1} and 500 µs gave the required heating condition equivalent to CH-T without adding an ionic component to avoid their interference on heat resistance of the spores. Voltage and time divisions were kept the same for all experiments. Sample temperature during OH-T treatment was monitored using k-type thermocouples (Omega Engineering Corp., Stamford, USA) inserted in to sealed sterile glass capillary tube to electrically isolate the thermocouple during data acquisition steps. For inactivation studies, 150 ml of carrot juice extract was aseptically transferred to the Ohmic heating cell and heated to target temperature. Once the desired conditions were achieved, the spore suspension (30 ml) was added to the

heating medium and well mixed throughout the heating period by continuously stirring the medium with a glass magnetic stirrer bar. Sample temperature was maintained constant at testing conditions by controlling the power supply using on/off switch (10 s switch on, to apply electric field and maintain test temperature level and 60 s switch off to avoid temperature overshoot). Due to low ionic solute content of the samples the average come up time of the extract was 4 min. From preliminary work, it was found that a 4 min come up time for inoculated sample resulted only in about one $\log_{10}$ cycle reduction in spore concentration. Even so, to avoid the variation effect of the 4 min come-up period, the heating medium was inoculated with spores suspension (10^7 to 10^8 CFU/ml) only after reaching the test temperature (after 4 min) (Figure 4.2) and the first sample (time zero) was withdrawn immediately after mixing of the sample with the glass magnetic stirrer.

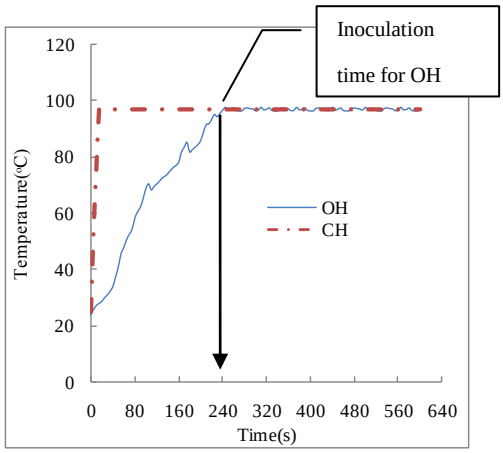

Figure 4.2. Heating profile of CH (broken line) and OH (solid line)

4.2.5 Microbiological analysis

Survivor counts of *B. licheniformis* spores were determined using a spread plate technique using nutrient agar (EMD chemicals Inc., Köln, Germany) as the recovery medium. For CH-T, capillary tubes were washed with 70% ethanol and samples were collected aseptically in sterilized eppendorf tubes. In case of OH-T, samples were removed with sterile micropipette at the established time intervals and immediately transferred to sterile cooled Eppendorf tubes to cool in ice-water mixture. From the cooled spore suspensions, appropriate serial dilutions were made using 0.1% sterile peptone water. Cultures were applied on to the agar plate with 0.1 ml

inoculums volume levels and spread with a sterile bent glass rod. The plates were then incubated at 37ºC and colonies were counted after 24 hr of incubation. No increase in number of colonies was observed with an extended incubation period of the samples.

4.2.6 Data analysis

Data were analyzed for thermal destruction kinetic parameters based on the following assumptions: (i) inactivation of *B. licheniformis* spores at a given temperature in carrot extract happened at random and followed first-order kinetic model (thermal death of the bacterium was exponential with time at constant temperature), (ii) since thermal inactivation was performed in capillary tubes in small volume (0.7- 0.9 ml) for CH-T and due to volumetric and uniform heating nature of OH, critical locations were identical and had equal heat resistance, and (iii) the microorganism was homogenous in heat resistance.

Based on the above assumptions, the *D*-value was estimated from the negative reciprocal of the slopes of the straight portions of survival curves of log (*N/No*) versus time (Eq. 4.1).

$$\log_{10}\left(\frac{N_t}{N_o}\right) = -\,t/D \qquad \text{Slope=-1/D} \qquad (4.1)$$

where, N_t is the heat survived spore concentration (CFU/ml) at time *t* (min); *No* is initial spore concentration (CFU/ml) at time zero (*t* = 0 min); *D* (min) is the decimal spore reduction time which implies minutes of treatment at constant temperature for 90% destruction of existing spores.

The other kinetic parameter, z-value (the temperature sensitivity parameter which indicates a temperature difference that results in 10 times reduction of in D values) was determined as the negative reciprocal of the slopes of the thermal death time plots ($\log_{10}$ *D*-values versus temperatures in study range) (Eq. 4.2).

$$z = (T_2 - T_1)/\log\left(D_1/D_2\right) = -\,1/Slope \qquad (4.2)$$

All experiments were carried out in duplicates and statistical analysis was carried out using Minitab 16.1.0.0 (Minitab Inc., USA) computer software program. Required Box-Cox power transformation was conducted to improve normality of the distribution and equalize

variance to meet the assumptions. For significant results mean separation was conducted using Tukey is pair wise mean comparison at 5% significance level.

4.3 RESULTS AND DISCUSSION

4.3.1 Effects of pH on thermal resistance of *B. licheniformis* spores

The survivor curves of *B. licheniformis* at different temperatures, pH levels and type of acidifying agents are shown in Figures 4.3 and 4.4. Surviving fractions represent the portion of recovered spores after each treatment condition as compared to the original count at time zero. Decimal reduction times obtained from the survivor curves are summarized in Tables 4.1 to 4. 4. Results demonstrated that the survival fraction of spores decreased with time, temperature and reduction in pH for both acidifying agents and heating methods. For instance, *D* values (at 87^{o}C) at pH 4.5 for MA were 35% (CH-T) and 41% (OH-T) lower than at pH 6.2. Further, at 92^{o}C, one unit reduction in pH (pH 5.5 to 4.5) for samples acidified with CA resulted in 18% reduction in *D* values for CH-T, while with OH-T resulted in 21% reduction. This confirmed that the combined effect of low pH and temperature significantly ($p\leq 0.05$) enhance susceptibility of spores to heat treatment conditions and faster inactivation rate.

Table 4.1 Decimal reduction time (*D* (min)) and z-values (^{o}C) for *B. licheniformis* spores as affected by combined exposure to temperature and pH levels in carrot extract acidified with MA in CH-T condition

Temperature (^{o}C)	Acidified with MA to pH					
	pH 4.5		pH 5.5		pH 6.2	
	D (min)[‡]	R^2	*D* (min)	R^2	*D* (min)	R^2
87	7.2±0.35^{c}	0.99	9.5±0.90^{cd}	0.98	11.2±1.5^{d}	0.98
92	4.8±0.55^{b}	0.98	6.6±0.35^{bc}	0.97	7.5±0.11^{c}	0.97
97	1.6±0.05^{a}	0.98	1.9±0.10^{a}	0.98	2.2±0.07^{a}	0.97
z-value (^{o}C)	15.1±0.81^{a}	0.93	14.5±1.34^{a}	0.91	14.2±1.44^{a}	0.92

[‡]*Standard error of mean; Means that do not share a letter are significantly different ($p\leq 0.05$).*

So far several papers have been published on evaluating the influence of pH on heat resistance of different strains of *Bacillus* spores (Mazas et al., 1998; Palop et al., 1999). The maximum heat resistance has been generally associated at neutral pH, and acidification of the

heating medium has been reported to decrease the heat resistance of the spores of genera *Bacillus* (Behringer and Kessler 1992).

Table 4.2 Decimal reduction time (*D* (min)) and z-values (^{o}C) for *B. licheniformis* as affected by combined exposure to temperature and pH levels in carrot extract acidified with MA in OH-T condition

Temperature (^{o}C)	Acidified with MA to pH					
	pH 4.5		pH 5.5		pH 6.2	
	D (min)[ǂ]	R^2	D (min)	R^2	D (min)	R^2
87	5.8±0.92^{cd}	0.99	7.9±0.52^{de}	0.97	9.8±0.41^{e}	0.97
92	3.8±0.57^{b}	0.99	5.1±0.13^{bc}	0.97	5.8±0.21^{cd}	0.97
97	1.5±0.02^{a}	0.96	1.6±0. 05^{a}	0.99	1.7±0.01^{a}	0.97
z-value (^{o}C)	17.0±2.1^{a}	0.96	14.7±0.9^{a}	0.94	13.2±0.30^{a}	0.95

[ǂ]*Standard error of mean*; *Means that do not share a letter are significantly different($p \leq 0.05$)* .

The present study confirms that higher temperature results in a more rapid inactivation of spores with *D* values at 97^{o}C found to be 3.9-6 times lower than those at 87°C. *B. licheniformis* is predominantly heat resistant among other *Bacillus* species (Wang et al., 2003). Janštová and Lukášová (2001) evaluated thermal resistances of 21 strains of *B. licheniformis* heated in cow's milk in conventional heating method and reported $D_{95^{o}C}$ values between 4.5 and 11.8 min which encompassed the range of results of this work regardless of pH level, type of acidifying agent and heating method used. However, the influence of pH on heat resistance of the spores decreased with an increase in heating medium temperature (Tables 4.1-4.4). Similar results have been reported from combined effect of lower pH and heat on inactivation of spores of *B. licheniformis* (Palop et al., 1996). Reduced effect of pH at highest temperature might be due to shorter span exposure times of spores at low pH and the temperature effect could possibly over-dominate the pH effect.

Other views have been provided by different authors related to the decrease in heat resistance of bacteria spores at lower pH of heating medium. Igura and others (2003) related the influence of low pH on heat resistance with exchange of proton with spore mineral content. They indicated that in low pH environments, the spores might be demineralised and charged to H-spores, which are replaced by their minerals with protons leads to weaken thermal resistance of

spores. Other researchers (Bender and Marquis 1985; Marquis and Bender 1985) also reported that demineralization markedly reduced heat resistance of bacterial spores. Furthermore, low pHs are also known to activate spores and stimulate them to germinate, and hence germinated spores are more heat susceptible at higher temperature.

Temperature sensitivity of *D* value is commonly indicated by the z-value. The influence of pH of the heating medium on z values is an important parameter to evaluate spore survival behavior at pH levels. Estimated z values at different pH are also summarized in Tables 4.1 - 4.4 and ranged between 13.2 to 17.0^{o}C and 12.6 to 16.3^{o}C for samples acidified with MA and CA respectively. These values were marginally different and showed to be statistically not significant ($p>0.05$) with respect to pH and type of acidifying agents used. These results are in close agreement with those reported in Rodriguez et al. (1993) for two *B. licheniformis* strains in tomato (z = 14.2^{o}C, pH 4.4). However they are higher than those reported in Behringer and Kessler (1992) in milk (z = 7.5-8.0^{o}C), Nakayama et al. (1996) in distilled water (z=10.8 ^{o}C), Palope et al. (1996) in tomato (z= 10.6 ^{o}C, pH 4) and asparagus puree (z=11.5 ^{o}C, pH 4). These differences could be associated with type of strain, heating medium, pH and temperature levels used to inactivate spores. Janštová and Lukášová (2001) determined the z-values (z=11.6 to 22.1^{o}C) for wide range of *B. licheniformis* strains (21 strains) in temperature range of 95 to 135^{o}C which covered results of this work and reported by others.

The influence of pH of heat treatment medium on the temperature sensitivity of D values (i.e., z-value) is still not clear. Some authors found that pH reduction give rise to higher z-values (Silla et al., 1992; Palop et al., 1996; 1999) which means that at high temperatures pH may even have no effect on heat resistance (Ocio et al., 1994) or that heating surpasses the combined effects of temperature and low pH. However others reported a decrease in z-values with decrease in pH (Mallidis et al., 1990; Silla et al., 1993) which showed the synergetic effect of temperature and low pH to reduce heat resistance of bacteria spores. It has also been found that pH levels showed no influence on the z-value (López et al., 1996; Rodrigo et al., 1997) which might be associated with the wide range of pH adaptability of the spores in a given heating condition. Generally according to available literature data and results of this work, z values for different pH levels showed inconsistency with a decrease in pH levels. This discrepancy might be associated

with type of microorganism studied, nature of heating medium (type of acidulate, pH, composition) used and heating method applied.

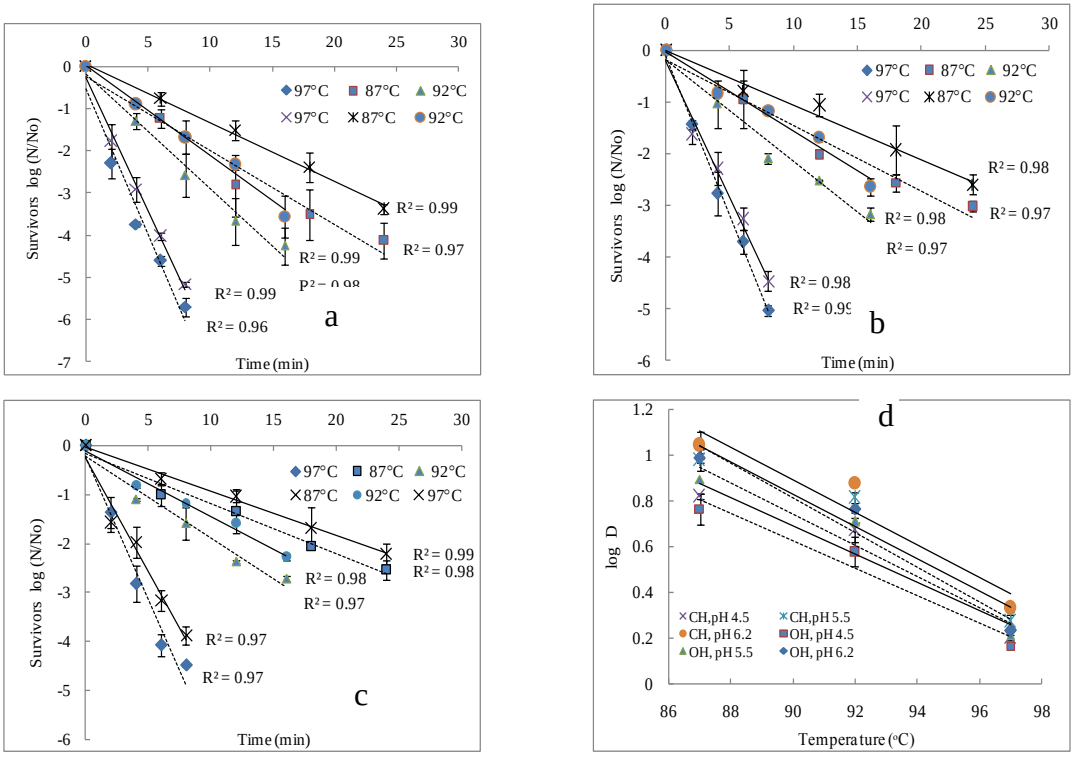

Figure 4.3. Survivor curves for spores of *B. licheniformis* thermally treated using OH (broken lines) and CH (solid lines) for samples acidified with MA (a) pH 4.5, (b) pH 5.5, (c) pH 6.2), (d) Thermal death time curves for both OH-T(broken line) and CH-T(solid line).

Table 4.3 Decimal Reduction Time (*D* (min)) and z-values (^{o}C) for *B. licheniformis* spores as affected by combined exposure to temperature and pH levels in carrot extract acidified with CA in CH-T condition

Temperature (^{o}C)	Acidified with CA to pH					
	pH 4.5		pH 5.5		pH 6.2	
	D (min)[‡]	R^2	*D* (min)	R^2	*D*(min)	R^2
87	7.2±0.08^{de}	0.96	8.4±0.36^{ef}	0.99	9.1±0.13^{f}	0.98
92	4.2±0.26^{c}	0.98	5.1±0.66^{cd}	0.99	5.6±0.69^{de}	0.99
97	1.2±0.04^{a}	0.98	1.5±0.10^{ab}	0.98	2.0±0.12^{b}	0.97
z-value (^{o}C)	12.6±0.31^{a}	0.94	13.3±0.19^{a}	0.94	15.3±0.73^{a}	0.96

[‡]*Standard error of mean; Means that do not share a letter are significantly different($p \leq 0.05$) .*

Table 4.4. Decimal Reduction Time (*D* (min)) and z-values (^{o}C) for *B. licheniformis* spores as affected by combined exposure to temperature and pH levels in carrot extract acidified with CA in OH-T condition

Temperature (^{o}C)	Acidified with CA to pH					
	pH 4.5		pH 5.5		pH 6.2[b]	
	D (min)[‡]	R^2	D (min)	R^2	D (min)	R^2
87	5.4±0.1[cd]	0.92	6.5±0.3[d]	0.95	9.2±0.7[e]	0.95
92	3.3±0.3[b]	0.95	4.2±0.13[bc]	0.96	5.9±0.2[d]	0.96
97	1.1±0.07[a]	0.98	1.4±0.05[a]	0.96	1.6±0.02[a]	0.94
z-value (^{o}C)	16.3±0.74[a]	0.97	15.1±0.8[a]	0.94	13.0±0.7[a]	0.92

[‡]*Standard error of mean*; *Means that do not share a letter are significantly different($p \leq 0.05$).*

Therefore establishing thermal processing schedules for acid or acidified low acid foods needs a case by case accurate determination of z values to insure food safety. Further, since z value is obtained from *D* value plots as the slope, variations in *D* will influence the z values obtained. The range of temperature employed also plays a role since the magnitude of *D* is dependent on the temperature and incorporation of high versus low temperature ranges will influence the sensitivity of z values as well.

4.3.2 Effect of heating methods on thermal resistance of *B. licheniformis* spores

Figures 4.3 and 4.4 illustrate survivor curves of *B. licheniforms* spores subjected to different heating methods for samples acidified with MA and CA respectively. Heat resistance kinetic data for spores subjected to different heating methods and pH levels is shown in Table 4.5. Heating methods were compared only for samples subjected to a selected acidulate, temperature or pH condition. No comparison of heating methods was made between type of acidulate or among temperature levels (Table 4.5) because of possible mixed influence. Result (Table 4.5) showed that although OH-T was marginally better with respect to inactivation rates, in most of the cases, the heating methods had statistically no significant (p>0.05) effect on spores inactivation.

It must be recognized that, the OH was not executed at full power, the actual OH-T treatment duration during the kinetic period is relatively small and essentially represents a predominant conventional thermal hold (intermittent application of 10 s "on" but 60 s "off", of the pulse during the treatment). Therefore, even the small influence improvement demonstrated with OH-T should be viewed with interest.

From Figures 4.3 and 4.4, the relatively better effect of OH-T at all temperature levels confirms the supplemental effects of OH-T on spores inactivation. Therefore, even though no statistical difference was observed when majority of experiments were considered between two heating methods, differences in *D* value existed and are recommended to be considered when designing processes involving multiple *D* values. For instance considering a 5*D* process schedule at 87^{o}C (pH 4.5) (Tables 4.3 and 4.4) a 36 min process would be required with CH-T as compared to a 27 min process with OH-T.

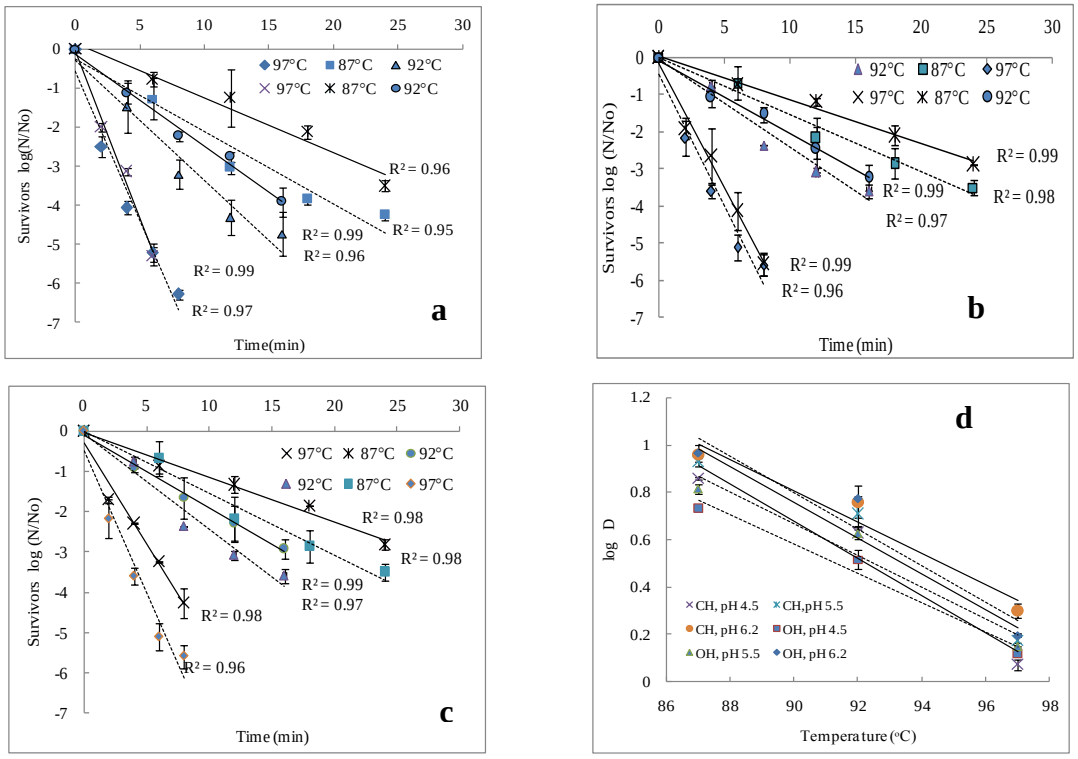

Figure 4.4 Survivor curves for spores of *B. licheniformis* thermally treated using OH (broken lines) and CH (solid lines) for samples acidified with CA (a) pH 4.5, (b) pH 5.5, (c) pH 6.2 (d) Thermal death time curves for both OH-T (broken lines) and CH-T (solid lines).

In further observation in OH-T (Tables 4.2 and 4.4) lower pH results in significant effect at lower temperatures (87 and 92 ^{o}C) than higher one (97 ^{o}C). This could be associated with longer exposure time for the spore suspensions to OH-T at lower temperatures. Furthermore, combined low pH-thermal effect could surpass or mask the electric effect of OH-T on the spores' heat resistance at 97^{o}C. For these reasons it ,generally, is believed that destruction of microorganisms using OH-T predominantly through thermal effect and hence the contribution of electric effect may not be significant to lethality (Palaniappan et al., 1990).

Table 4.5 Effect of heating methods and pH levels on heat resistance (D value in min) of *B. licheniformis* spores compared for each temperature level and type of acidulate

Heating Method	pH	Temperature levels*		
		87^{o}C	92^{o}C	97^{o}C
		Acidified with MA		
CH	6.2	11.2±1.50^{b}	7.5±0.11^{c}	2.2±0.07^{c}
OH	6.2	9.8±0.41^{b}	5.8±0.21^{bc}	1.7±0.01^{ab}
CH	5.5	9.5±0.90^{ab}	6.6±0.35^{bc}	1.9±0.01^{bc}
OH	5.5	7.9±0.52^{ab}	5.1±0.13^{ab}	1.6±0.05^{ab}
CH	4.5	7.2±0.35^{ab}	4.8±0.55^{ab}	1.6±0.05^{a}
OH	4.5	5.8±0.92^{a}	3.8±0.57^{a}	1.5±0.02^{a}
		Acidified with CA		
CH	6.2	9.1±0.13^{d}	5.6±0.69^{c}	2.0±0.12^{b}
OH	6.2	9.2±0.7^{cd}	5.9±0.2^{cd}	1.6±0.02^{a}
CH	5.5	8.4±0.36^{bcd}	5.1±0.66^{bc}	1.5±0.10^{a}
OH	5.5	6.5±0.3^{abc}	4.2±0.13^{ab}	1.4±0.05^{a}
CH	4.5	7.2±0.08^{ab}	4.2±0.26^{ab}	1.2±0.04^{a}
OH	4.5	5.4±0.1^{a}	3.3±0.30^{a}	1.1±0.07^{a}

*Means that do not share a letter are significantly different ($p \leq 0.05$). *Heating methods were only compared at each temperature level for a given type of acidulate*

But sublethal electrical injury or additional lethal effect of electricity on spores might be manifested at higher pH and higher temperature levels. So far there were limited reports on inactivation of *B. licheniformis* spores using OH-T under reduced pH conditions. Due to this reason it is difficult to compare results of this work with other literature information. However data reported on inactivation of *B. subtilus* spore in 0.1 % NaCl solution (Hyung-Yong et al.,

2000) showed close agreement on *D* values at higher pH level. For the majority of the above reports and this work, maximum heat resistance was obtained at relatively higher pHs for both types of heating methods, which is in agreement with most published data on *B. licheniformis* (Nakajo and Moriyama 1993; Palop et al., 1996).

4.3.3 Effect of acidifying agents on thermal resistance of *B. licheniformis* spores

In this work, the two acidulates used showed no significant ($p > 0.05$) difference on heat resistance of *B. licheniformis* spores. The *D* values obtained for spore suspensions treated using CA and MA were very close in all treatment conditions (Tables 4.1- 4.4). This might be because of the small volume of acidulates added to adjust the pH of the juice extracts which might be insufficient to demonstrate a significant effect on reducing spores resistance to treatment conditions. Similar results were reported on heat resistance of different bacteria spores when they were heated in presence of different types of acidifying agents (Palop and Martinze 2006; Tsang et al., 1995). Fernández et al. (1994) found no effects between citric and glucono-delta-lactone on heat resistance of *B. stearothermophilus* spores. Similarly, Brown and Martínez (1992) and Ocio et al. (1994) indicated that there were no difference on heat resistance of *C. sporogenes* and *C. botulinum* spores heated with the same type of acidulate respectively. Palop et al. (1996) evaluated the effect of different acidifying agents on heat resistance of spores of *Bacillus subtilis* and *Bacillus coagulans* sporulated at 35 and 52^{o}C and reported that, spores sporulated at 35^{o}C showed no difference in heat resistance among type of acidulates used (lactic, acetic, citric and hydrochloric). However, Leguérinel and Mafart (2001) showed that spores of *Bacillus cereus* were found less heat resistant in a thryptone salt broth acidified with citric acid as compared to malic acid. Furthermore, in this study, types of acidulate showed no significant ($p > 0.05$) difference on z-values. However, when the z-values evaluated against pH level, carrot extract acidified with both acidulates showed an increasing trend in z values with decrease in pH, except the reverse effect of samples acidified with CA in CH-T (Figure 4.5); reasons not clear. Therefore the spores inactivating effect from the combined treatment conditions in this study mainly depends on the pH of heating medium used and the heating method rather type of acidulates.

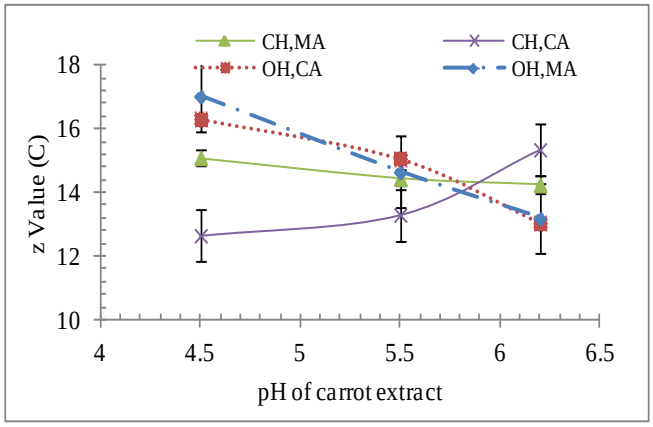

Figure 4.5 Influence of pH on z-values of *B. licheniformis* spores heat treated (87-97^{o}C) at different heating methods (OH-T broken lines vs CH-T solid lines) in carrot extract acidified with MA and CA.

4.4 CONCLUSIONS

In thermal processing of acid or acidified low acid foods, it is common to select a representative reference microorganism that best fit for selected processing condition and food type to be produced. Due to its thermal resistance nature and pH elevating capacity, *B. licheniforms*, among other *Bacillus* genera, can be used as a reference microorganism. The influence of lowering pH was high in the lower temperature range and diminished with an increase in temperature due to dominance of thermal effect over the combined effects of heat and pH. Decimal reduction times calculated for both acidifying agents under OH-T method were found slightly lower than with CH-T method with a significant value at higher pH and temperature. When multiple *D* values were considered in thermal processing schedule, even small difference in *D* values could result in a significant reduction in processing times. Temperature dependency of *D* value (z value) showed mixed results in relation to pH and the type of heating medium used.

PREFACE TO CHAPTER FIVE

In chapter four, heat inactivation parameters were determined with different heating methods to design and validate thermal processing methods for the production of acidified low acid foods. Meanwhile in this study pressure assisted thermal inactivation kinetic parameters were determined at different pH levels for the same purpose. Pressure assisted thermal pasteurization method is one of potential novel processing methods for the production of better quality acidified low acid foods. Proper design and use of the technology in commercial production steps requires data about resistance of target food microorganism (*B. licheniformis*). Since the mechanisms of spores inactivation using pressure assisted thermal treatment is different from the sole effect of heat, an independent inactivation kinetic study of the spores is necessary. In a former study, the type of acidifying agents showed insignificant effect on inactivation of the spores. Due to this reason citric acid was used in this study to reduce pH of samples since it is widely used in the food processing industries. Both first order kinetic (log-linear) and Weibull (non linear) model were used to determine spore inactivation kinetic parameters and describe survivor curves. The latter model was included because of its versatility to describe various shapes of pressure assisted thermal inactivation survival curves. Most of previous studies using the Weibull model were mainly described pressure and thermal effects on Weibull model parameters without showing the practical significance of the parameters in terms of establishing processing schedules. Therefore, in this study the relevance and discrepancy of the two models in terms of decimal reduction time and pressure-thermal death time were elucidated. Furthermore inactivation kinetic parameters determined at different pH levels were used in subsequent chapters as an input to design and validate pressure assisted thermal processing method.

CHAPTER FIVE

COMBINED EFFECTS OF HIGH PRESSURE, MODERATE HEAT AND pH ON THE INACTIVATION KINETICS OF BACILLUS LICHENIFORMIS SPORES

ABSTRACT

The goal of this work was to investigate the combined effect of high pressure (HP) processing (temperature, pressure and time) and product (pH) related variables on destruction of spores of *Bacillus licheniformis* in carrot juice. A 3-level factorial experimental design was used with the microbial culture inoculated into the carrot juice extracts at control pH 6.2 and acidified pH of 4.5 and 5.5 conditions of pressure (400, 500, 600 MPa), temperature (40°, 50°, 60°C) and time (0-40 min). Conventional log-linear (first order) and Weibull (non linear) models were used to describe survivor curves and achieving a certain-log cycle reduction criteria, tested based on the models. Results showed that survivor curves exhibited slightly upward concavity and were better described by a Weibull than a log-linear model with lower RMSE and higher R^2 values (when the destructions were limited to 3-4.9 log cycles). Treatment combinations showed significant ($p \leq 0.05$) effects on *D* and z values (log-linear model) and Weibull rate (α) parameter while the shape (ß) parameter was not significant. 5*D* and 12*D* pressure-thermal death times estimated using Weibull model parameters were higher than those from the log-linear model, with the latter showing adequacy while the former demonstrating over-treatment. The destruction pattern was also dependent on pH, with lower pH contributed to higher degree of destruction. Therefore pH reduction can be used as an effective factor combined with high pressure processing, analogues to acidified thermal processing.

5.1 INTRODUCTION

Thermal processing is a widely used preservation method to inactivate and destroy pathogenic and spoilage microorganisms of foods and to ensure safety and shelf life stability. Although heat treatment is an effective means of preservation, it significantly affects heat-labile quality components. The use of heat as a sole method for destroying bacteria spores significantly affects the texture, color and nutritive value of foods. Because of this, the food industry needs to develop alternatives to conventional processing technologies in response to the growing consumer demand for higher-quality and minimally processed foods. Different novel thermal and non-thermal technologies have been emerging to address the need of minimizing losses of nutritional and sensorial properties. Among non-thermal technologies, high hydrostatic pressure processing (HPP) has significantly overcome from the limitations of conventional heating effects. Studies have confirmed that HP can effectively inactivate vegetative cells of pathogenic microorganisms (Basak et al., 2002; Riahi et al, 2003; Dogan and Erkmen 2004; Buzrul et al., 2008; Ramaswamy et al., 2008; Gill and Ramaswamy 2008; Hiremath and Ramaswamy 2012), but HPP alone cannot inactivate bacterial spores unless combined with high temperature. As this processing technology is regulated by three process variables — time, temperature and pressure, the opportunity arises to combine pressure with moderate heating to overcome bacterial spore inactivation limitations associated with HPP. Many studies have demonstrated that the combined use of high pressure with moderate heat can inactivate heat-resistant spores and enzymes (Reddy et al., 2003; Koutchma et al., 2005; Shao et al., 2010; Ramaswamy and Shao 2010b). Additional spore inactivation can be achieved by combining pressure-heat treatment and another hurdle factor, such as low pH or nisin (Kalchayanand et al., 1994). The present study sought to investigate the combined effects of pressure, temperature and low pH on the level of bacterial spore inactivation during production of acidic or acidified low-acid foods. Furthermore information related to inactivation kinetics of bacterial spores under such treatment conditions is very limited. In order to assess the safety of high-pressure-treated acidified or acidic foods it is also necessary to develop more inactivation kinetics data for spore producing bacteria.

Bacillus licheniformis is most frequently involved in the spoilage of canned vegetables (Fields et al., 1977), cooked meats, raw milk and industrially-produced baby foods (Lund 1977). The role of *B. licheniformis* in acidic or acidified foods is very important from a food safety point of view, as it can growing in acidic media and raise the pH to levels where *C. botulinum*

could grow and produce its toxin (Rodriguez et al., 1993). Furthermore *B. licheniformis* is relatively more pressure-resistant (Nakayama et al., 1996) than other *Bacillus* species.

In studying thermal inactivation kinetics of microorganisms, it has been assumed that all cells or spores in a population have an equal resistance to lethal treatments. Such an assumption is commonly modeled by a first-order kinetic model which postulates a linear relationship between the logarithm of the number of survivors and treatment time. However, a number of studies have shown that significant deviations from the log-linearity occur (Peleg and Cole 1998; Van Boekel 2002), resulting in an upward or downward concavity of survivor curves. Such deviations from linearity render it irrational to use the same assumptions and model for all survivor curves, lest an under- or over-estimation of kinetic parameters result in under- or over-estimation of processing schedules. Therefore, to study and accurately determine kinetic parameters under different treatment conditions various models have been recommended (Peleg and Cole 1998). Among the non-linear models developed, the Weibull model has gained popularity due to its simplicity and flexible nature in describing both upward and downward concavity of survival curves (Buzrul et al., 2008). Furthermore it has been successfully used to model inactivation of microorganisms (Chen and Hoover 2004; Buzrul et al., 2008) under the combined effects of high pressure and temperature (Buzrul and Alpas 2004; Buzrul et al., 2005). Furthermore, the model takes into account the biological variation within a population of spores with respect to inactivation processing conditions (Dagmara and Stefan 2009).

The objectives of this study were therefore to: (i) study the combined effects of treatment conditions on survival of spores and determine the inactivation kinetic parameters using both models (ii) evaluate the effect of treatment conditions on log-linear and Weibull model parameters, (iii), compare the appropriateness of log-linear and Weibull models for describing the inactivation of *B. licheniformis* spores under combined HP-temperature-pH conditions, and finally (iv) compare a certain-log reduction criterion test for *B. licheniformis* spores in HP-thermal-pH processes using log-linear and Weibull models.

5.2 MATERIALS AND METHODS

5.2.1 Preparation of spore suspension

Preparation of the spore suspension was the same as indicated in section 4.2.1 of chapter four.

5.2.2 Preparation of heating medium

The preparation of the heating medium and pH adjustment followed the same procedures as indicated in section 4.2.2 of chapter four.

5.2.3 High pressure unit

A multi-vessel high-pressure unit (Model U111, Unipress, Warsaw, Poland), capable of operating at pressures up to 700 MPa, within a temperature range of - 40 to 100°C was used to carry out the inactivation studies. The unit functioned with a low viscosity heat and pressure transferring liquid (silicone oil M40.165.10, Huber GmbH, Offenburg, Germany). The unit was equipped with 5 beryllium-copper alloy pressure chambers, each connected with a k-type thermocouple (Omega Engineering Corp., Stamford, USA) and a pressure sensor to monitor the temperature and pressure history of the sample over the treatment cycle. Each chamber was independently connected to an oil driven intensifier (U111, Unipress, PL) through one of five high-pressure valves (SITEC, Maur/Zurich, Switzerland). The pressure chambers were immersed in a silicone oil bath equipped with a thermostat (CC245, Huber GmbH, Offenburg, Germany). This design allowed for the simultaneous treatment of five different samples in one pressure build-up close to iso-bar, iso-thermal conditions.

5.2.4 Spore inoculation and high pressure-thermal treatment

A 5 ml aliquot of acidified and sterilized carrot juice extract (from section 5.2.2), was thoroughly homogenized and inoculated with a spore suspension in order to achieve an initial spore concentration of 10^8 to 10^9 ml^{-1}. Then the suspension (1.8 ml) was filled to the brim in to sterilized cryogenic vials (Nunc Cryo Tubes Nr. 375299) in an aseptic manner leaving almost no air gaps. Prior to the compression phase, the samples were left in the pressure chamber for 5-8 min (the time required to attach the cells to the equipment) to equilibrate at the initial temperatures which resulted in the desired processing temperatures of 40±0.5, 50±0.5, and 60

±0.5°C, respectively, after compression. Combined pressure and temperature treatments of the samples were done at pressure levels of 400, 500, and 600 MPa. The pressure build-up rate was adjusted to approximately to 3.7 MPa s^{-1} and decompression occurred in less than 5 s. After decompression, the sample tubes were immediately withdrawn and stored in an ice-water mixture. Culturing was done within 1 hr after decompression. For each experiment, samples treated with pressurization followed by immediate depressurization at the same temperature were considered time zero samples and served to quantify the initial number of surviving spores. Pressurization time reported in this study does not include pressure come-up or release time.

5.2.5 Microbial assay

The numbers of surviving spores were determined using a spread plate method on nutrient agar (EMD chemicals Inc., Germany). Before serial dilution, the cryogenic vials were surface washed with 70% ethanol, serial dilutions were made in 0.1% sterile peptone water, and 0.1 ml inoculums were spread on plates using a sterile bent glass rod. The plates were incubated at 37ºC, and colonies were counted after 24 hr. No increase in colony number was observed with an extended incubation period of the samples.

5.2.6 Data analysis

Survivor counts were transformed to $\log_{10}$ values and the level of spore reduction was defined as $\log_{10}(N/N_0)$, where N_0 was the initial concentration of spores after pressurization and immediate depressurization (excluding holding time) and N_t the number of surviving spores after a given treatment time. All treatments were repeated at least twice and those showing a variability coefficient higher than 15 % were tested for one additional time.

5.2.7 Modeling of pressure-thermal inactivation kinetics

5.2.7.1 Log-linear model

For the log-linear model, one assumes that microbial cells or spores within a population have a similar resistance to applied heat and/or pressure treatment. Hence the model assumes a linear relationship between the declines in the logarithm of the number of survivors over treatment time, *i.e*. a first order kinetic model:

$$\log \frac{N_{(t)}}{No} = -\frac{t}{D};\ (t \geq 0);\ slope = \frac{-1}{D} \tag{5.1}$$

where, N_0 is the initial viable cell or spore count (cfu/ml), N_t is the number of survivors remaining after exposure to a lethal treatment for a specific time t (cfu/ml), and D is the time required to destroy 90% of the organisms, or decimal reduction time, estimated $\log_{10}$ (N/N_0) *vs.* treatment time, (min).

Temperature and pressure dependency of D values are expressed in terms of z-values. The z-values implies temperature or pressure levels increase required to decrease the D value by 90%. The temperature (Eq. 5.2) or pressure (Eq. 5.3) dependency of D values is expressed as through the kinetic parameters z_T and z_P, respectively:

$$z_T = \frac{T_2 - T_1}{\log_{10}\left(\frac{D_1}{D_2}\right)} = -\frac{1}{Slope} \tag{5.2}$$

$$z_P = \frac{P_2 - P_1}{\log_{10}\left(\frac{D_1}{D_2}\right)} = -\frac{1}{Slope} \tag{5.3}$$

The z_T and z_P values represent the respective increases in °C and MPa required to reduce the value of D by one $\log_{10}$ unit. T_1 and T_2 or P_1 and P_2 represents, temperature and pressure levels at D_1 and D_2. In a conventional approach, z_T and z_P are determined as the negative reciprocal of the slope of $\log_{10} D$ *vs.* temperatures/pressure within the study range.

5.2.7.2 Two-parameter Weibull model

The non-linear Weibull model is based on the assumption that cells or spores in a microbial population have different resistances to treatment conditions and the curve of their survival to a lethal agent represents a cumulative exponential distribution. The cumulative form of the Weibull model, which has been successfully used in modeling microbial inactivation (Van Boekel 2002), is given as:

$$\log_{10}\left(\frac{N_t}{N_o}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta} \tag{5.4}$$

where, α is the characteristic time or scale parameter (min), β is the shape parameter (non dimensional), and all other parameters are as previously defined.

In a semi-log plot the Weibull distribution corresponds to a concave upward survivor curve when $\beta < 1$, a concave downward curve if $\beta > 1$, and is linear if $\beta = 1$. The first order kinetics approach (straight line in a semi-log plot when β=1) is therefore considered a special case of the Weibull model.

5.2.7.3 Determination of decimal and certain-log reduction times using Weibull model parameters

To determine certain-$\log_{10}$ reduction of bacterial cells using Weibull distribution parameters, an equation suggested by Van Boekel (2002), and originally used in failure engineering to describe time to failure in electronic and mechanical systems was employed. The 90% percentile of the failure time distribution is termed the reliable life (t_R), and can be calculated from the parameters α and β (Eq. 5.4). In this case the t_R is analogous to the classic *D*-value when a one $\log_{10}$ reduction is considered (Van Boekel 2002).

$$t_R = \alpha(\ln 10)^{\frac{1}{\beta}} \tag{5.5}$$

Therefore for a certain number of log reduction, Eq. 5.5 can be modified as:

$$t_d = \alpha(-\ln(10^{-d})^{\frac{1}{\beta}}) \tag{5.6}$$

where, d is the number of decimal reductions; d = 5 gives the time required for five log reduction and d = 12 gives a twelve log reduction as in the traditional thermal processes based on the criterion of destruction of *C. botulinum* spores.

Given the exponential relationship between α and treatment temperature, α can be used to determine the classical z' value in similar fashion with conventional TDT-curve (Van Boekel 2002):

$$\log_{10}\alpha = a - bT \tag{5.7}$$

$$z'_T = -\frac{1}{b} \tag{5.8}$$

where *a* and *b* are coefficients of the regression line of a curve for $\log_{10}$ α *vs.* temperature at different pressures. z'_P values can be obtained using similar relationship with $\log_{10}$ α *vs.* pressure at different temperature .

5.2.7.4 Model fitting and evaluation

Log linear models were fitted to the inactivation data using Microsoft Excel 2007. Curve fitting and estimation of Weibull model parameters were conducted by minimizing the sum of squares error between the observed and predicted data using nonlinear least-squares regression analysis with the help of the Solver option in Microsoft Excel 2007.

The goodness of fit of the models was assessed using the regression coefficient (R^2), and the root mean square error (RMSE):

$$RMSE = \sqrt{\frac{\sum_{i=1}^{i=n}(O_i - P_i)}{n}} \tag{5.9}$$

where, *n* is the number of values of a given parameter which are compared, O_i is the i^{th} observed value, and Pi is the i^{th} predicted value.

R^2 measures how well a linear or a non-linear model fits the data, where the greater the R^2 value, the better is the model describes the data. The RMSE measures the average deviation between the observed and fitted values of a given parameter, and thus a smaller RMSE value indicates a better fit of data to the model.

5.3 RESULTS AND DISCUSSION

5.3.1 Temperature and pressure profiles

Sample temperature increase during pressure treatment due to adiabatic heating is shown in Figure 5.1. In the figure, solid lines show pressure profiles to reach pressure levels of 400 MPa (A), 500 MPa (B) and 600 MPa (C), respectively. At each pressure, temperature profiles to reach test temperatures are indicated in broken lines. Pressure and temperature profiles for 1000s only are included in the figure. Profile levels above 1000s are excluded from the figures since both pressure and temperature reached equilibrium levels at which they were maintained during treatment times. Due to small sample size, high thermal conductivity of pressure vessel made

from beryllium-copper alloy, small pressure holding chambers and low pressurization rates allowed rapid dissipation of the compression heat, thus allowing the HPP to proceed at the process temperatures selected for the experiments.

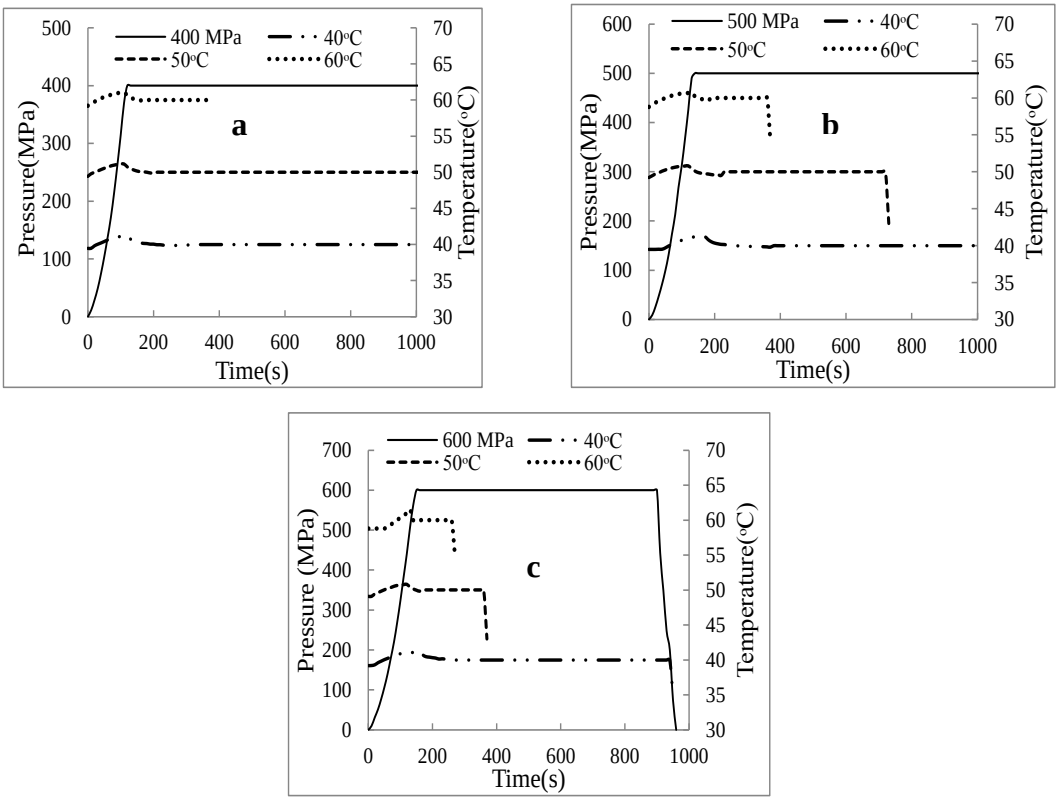

Figure 5.1 Temperatures and pressure profile of samples treated at combined pressure and temperature levels. Temperature profiles (broken lines) at 400 (A), 500(B) and 600 (C) MPa pressure levels (solid lines).

5.3.2 Comparison of the models to fit survivor curves

A valid statistical model of microbial destruction kinetic behavior would help food professionals to predict process schedule, optimize processing conditions and decrease cost of target microorganisms inactivation. Based on its low RMSE values and higher regression coefficients ($R^2 \geq 0.92$) (Table 5.1), the log-linear model showed a reasonable fit to describe survivor data for all pressure × temperature combinations at a given pH level. However, the Weibull model showed a much superior fit. This discrepancy in model accuracy occurred mainly because, except under moderate temperature moderate pressure combinations (or after long treatment times), the linear model was not capable of describing the slight upward concavity of the survivor curves (Figures 5.2 and 5.3). Good fit of the Weibull model to survivor curves and

high correlation between the predicted and experimental values of survival ratio were observed in all combined treatment conditions with a better fit of observed and predicted values. Though Weibull model showed better fit to individual survivor curves, the log-linear model also showed a reasonable fit particularly at higher pressure and temperature combinations, where the effect of temperature over pressure would be intensified (Table 5.1). The log-linear model is based on one parameter (*D* value) while Weibull model is based on two, making the former simpler to handle. Therefore both models were considered in this study for further considerations.

5.3.2.1 Log-linear model and effects of process variables

Table 5.2 summarizes associated inactivation kinetic values determined according to log linear model. The evaluated kinetic parameters excluded effects of pressure come-up and come-down times, and hence estimated that the kinetic parameters adequately describe combined iso-baric pressure and iso-thermal heat resistance of *B. licheniformis* spores at the pH levels studied.

An increase of both pressure and temperature levels, reduced decimal reduction time through acceleration of inactivation rate of spores. For instance, an increase in temperature from 40 to 60^{o}C at pH 4.5 resulted in 82% and 87% reduction on *D* values at 400 and 600 MPa, respectively. Meanwhile at the same pH level an increase in pressure from 400 to 600 MPa contributed 62 and 71% reduction on *D* values, at 40 and 60^{o}C respectively. Therefore an increase on both temperature and pressure combinations contributed to significant spores inactivation. The synergetic effect of the combined use of these variables on inactivation of food microorganisms has been demonstrated in different studies (Maggi et al., 1996; Ramaswamy and Shao 2010). The inactivation of bacterial spores by pressure assisted treatment is generally considered to rely on pressure-induced spore germination, followed by inactivation of germinated spores (Gould and Sale 1970; Dirk et al., 2004). Apart from external effect of pressure and temperature, modifying the medium environment condition also played a role to inactivate the spores. In addition to synergetic effect of pressure and temperature, low pH (pH 4.5) environment was added as a third factor to investigate the combined effect of the three variables on lethality of the spores. It is believed that high pressure in combination with other treatment conditions steadily accelerate the inactivation of bacteria spores while simultaneously bringing down the number of survivors (Raso et al., 1998; Reddy et al., 2003).

Table 5.1 Comparison of log-linear and Weibull models for the survivor curves of *B. licheniformis* spores treated at different combined pressure, temperature and pH levels

Temperature (^oC)	Pressure (MPa)	pH 4.5 RMSE* (R^2)*		pH 5.5 RMSE* (R^2)*		pH 6.2 RMSE* (R^2)*	
		Weibull	log-linear	Weibull	log-linear	Weibull	log-linear
40	400	0.098(0.997)	0.498(0.97)	0.204(0.985)	0.400(0.97)	0.159(0.989)	0.422(0.96)
	500	0.186(0.991)	0.522(0.97)	0.120(0.995)	0.566(0.95)	0.169(0.988)	0.371(0.97)
	600	0.141(0.993)	0.525(0.97)	0.152(0.994)	0.762(0.94)	0.135(0.997)	0.450(0.96)
50	400	0.158(0.992)	0.710(0.94)	0.141(0.993)	0.402(0.96)	0.142(0.992)	0.318(0.97)
	500	0.152(0.997)	0.920(0.92)	0.169(0.991)	0.602(0.94)	0.222(0.990)	0.400(0.95)
	600	0.170(0.993)	0.562(0.96)	0.126(0.995)	0.446(0.95)	0.083(0.996)	0.408(0.96)
60	400	0.007(0.999)	0.180(0.99)	0.103(0.997)	0.380(0.97)	0.029(0.999)	0.648(0.98)
	500	0.047(0.999)	1.190(0.96)	0.122(0.995)	0.610(0.97)	0.143(0.988)	0.308(0.97)
	600	0.054(0.999)	0.476(0.98)	0.002(0.999)	0.463(0.97)	0.015(0.999)	0.474(0.97)

**Results calculated from average values of two independent replicates*

Table 5.2 *D (min)* (Eq. 5.1) and t_R *(min)* (Eq. 5.5) values determined for different combination of treatments on bases of log-linear and Weibull models respectively

Pressure	Temperature	pH 4.5		pH 5.5		pH 6.2	
(MPa)	(°C)	*D*	t_R	*D*	t_R	*D*	t_R
	40	11.9±0.8	7.1±0.1*	13.6±1.4	8.1±0.3	14.1±0.7	9.9±0.9
400	50	7.5±0.5	3.0±0.7	7.7±0.7	5.1±0.4	8.6±0.5	5.8±0.3
	60	2.1±0.1	1.7±0.01	2.5±0.1	1.7±0.2	2.8±0.2	2.1±0.02
	40	7.9±0.3	4.5±0.2	9.1±0.8	5.1±0.3	10.3±0.1	7.2±0.09
500	50	3.7±0.1	1.2±0.1	4.0±0.3	1.9±0.05	4.5±0.1	3.2±0.07
	60	1.7±0.1	0.9±0.3	2.0±0.1	1.3±0.2	2.5±0.01	1.8±0.1
	40	4.5±0.2	2.2±0.01	4.7±0.1	2.1±0.1	5.0±0.1	2.6±0.4
600	50	1.6±0.01	0.8±0.10	1.9±0.01	0.9±0.04	2.2±0.1	1.2±0.03
	60	0.60±0.04	0.50±0.02	0.70±0.03	0.40±0.03	0.70±0.02	0.40±0.04

**Standard error of mean*

Results (Table 5.2) showed that at 400 MPa, reducing pH of the medium from 6.2 to 4.5 resulted in a reduction of *D* value by 16% (40^{o}C) and 28% (60^{o}C). But at 600 MPa, the pH effect on resistance of spores was 10 and 14% at the same temperature levels, respectively. The lower pH effect at higher pressure-temperature combination might be due to dominance effect of pressure-temperature combination over pH effect. So far no sufficient literature data were available to compare to our results, however Taisuke et al. (2003) reported that spores of *B. licheniformis* JCM2505 suspended in distilled water and treated at 200 MPa and 65^{o}C showed a *D* value of 8.5 min which has almost equivalent *D* value (8.5 min) with *B. licheniformis* spore used in this study in treatment combination of 400 MPa, 50^{o}C at pH 6.2.

With the combined increase of both pressure and temperature, the destruction rate of spores was increased at pH 4.5 with a significant increase in $\log_{10}$ cycle reduction (Table 5.3). A 4.9-$\log_{10}$ reduction in cfu/ml of sample was observed after holding the pressure for 3 min at 600MPa, 60^{o}C and pH of 4.5. Extending the same treatment combination for an additional 5 min resulted in no survivor spores (data not shown). Moderate treatment level of 500 MPa at 50^{o}C for 12 min resulted in a 3.4-$\log_{10}$ cycle reduction at the same pH level. However, a cycle reduction of only 2.9-$\log_{10}$ was observed in a mild treatment combination (40 min holding time at 400 MPa, 40^{o}C and pH 6.2). In similar work, but with a different strain (*B. licheniformis strain* SSICA DA2) and close to neutral pH, survivor spore concentration was lowered by 5-$\log_{10}$ after treatment at 50^{o}C and 700 MPa for 10 min (Zanichelli et al., 1998), clearly showing the role of pH reduction in enhancing the combined effects of temperature and pressure to inactivate bacterial spores.

The increase in sensitivity of spores with acidification of carrot extract might be due to the low-pH environment promoting reversible exchange of endogenous cations (Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, and Mn^{2+}) with exogenous protons from the acidified environment, thus rendering the native spore proton charged and significantly reducing its resistance to additional stress conditions (Marquis and Bender 1985). Furthermore simultaneous use of pressure and temperature might also enhance spore inner membrane permeability, leading to greater ions and

protons exchange, thus altering the spores into to H-charged forms (Paredes-Sabja et al., 2007). This might further enhance the destruction power of treatment combinations.

In addition to this, Berg and Sandine (1970) reported that low pHs could activate and stimulate spores to germinate, and hence germinated spores become more sensitive to external environmental conditions. Bergerova et al. (2011) further showed the combined effects of pressure, temperature and low pH on the integrity of DNA in biological systems, which might lead to damage in the spore DNA which would ultimately hinder the normal replication process and viability. Therefore in agreement with studies with other *Bacillus* species (Roberts and Hoover 1996), appreciable inactivation of *B. licheniformis* spores was observed when the pressure exceeded 500 MPa and the temperature was above 50°C (higher severity processes). A spore suspension at pH 4.5 treated at 600 MPa exhibited better spore destruction rate at all temperature levels than higher pH/lower pressure treatment combinations.

Temperature and pressure dependency of the *D* value were expressed in terms of z_T (^oC) and z_P (MPa) values. The values of z_T or z_P are, the temperature or pressure increases required to reduce the decimal reduction time by one $\log_{10}$ unit respectively. For the classical log-linear model these values were determined from Eq. 5.2 and 5.3. The z_T values also showed a general similarity of decreasing trend as the processing pressure levels increased (Table 5.4). The associated z_P values were more variable and did not demonstrate specific trends except that they were generally either lower at 50^oC as opposed to 40 or 60^oC. Such inconsistent trend of z_P values with temperature has been reported in earlier study(Ramaswamy and Shao 2010). The likely reason for this behavior is the relative effectiveness of temperature and pressure with respect to the microbial destruction. At non-lethal temperatures (lower temperatures), the pressure effect is presumably to be higher, while when the temperatures at the lethal level, the temperature effect surpasses the pressure effect. This again is due to the fact that higher pressures result in a volume decrease as opposed to higher temperatures contributing to volume increase there by contributing to opposing trends. Because of these opposing trends, treatment conditions showed statistically insignificant ($p>0.05$) effects and inconsistancy on these parameters. With respect to the pH, the sensitivity at lower pH was generally at higher than higher pH for carrot juice extract.

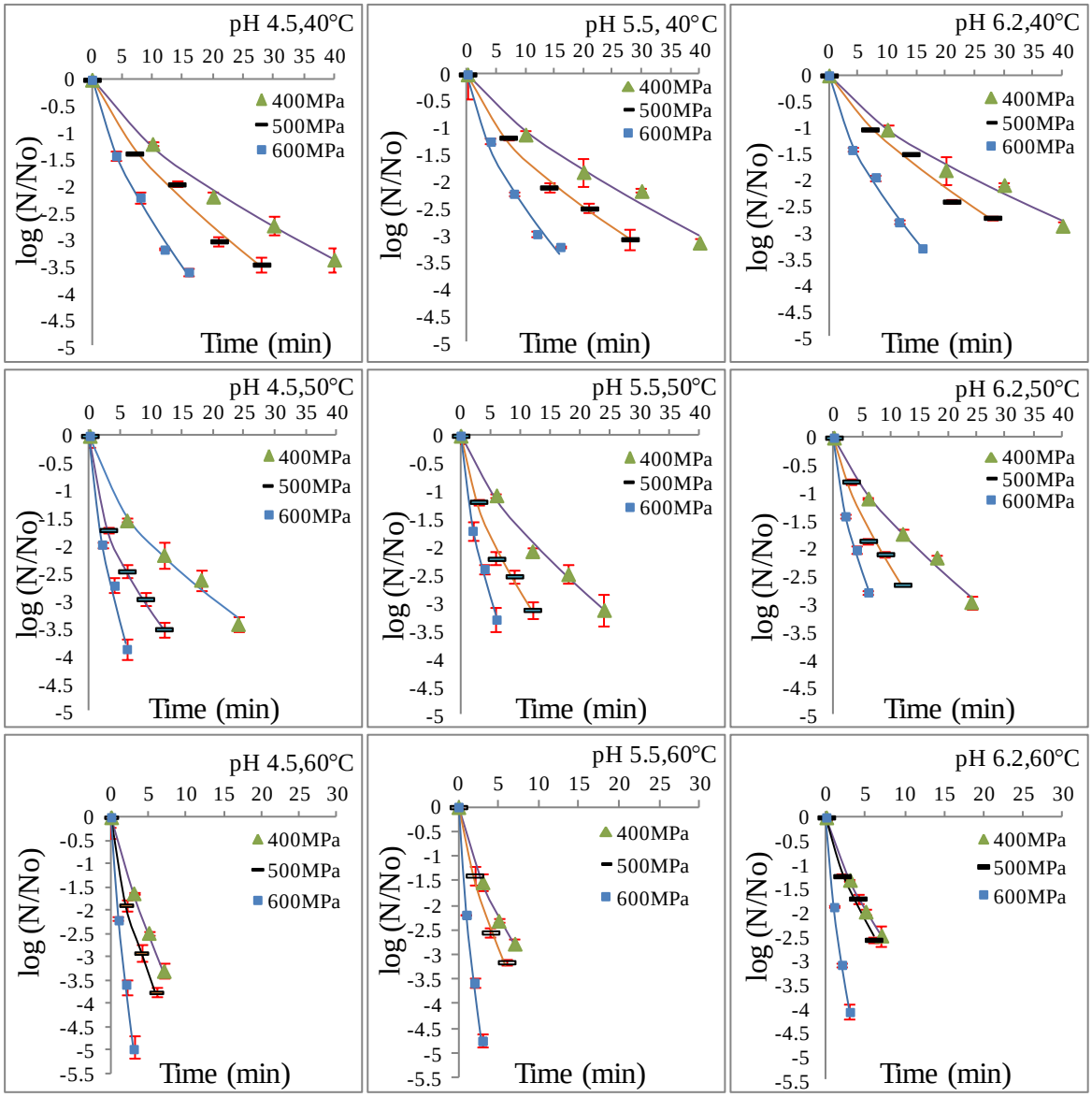

Figure 5.2 Effect of pressure on survivor curves of *B. licheniformis* spores in carrot extract at constant pressure and pH levels. Symbols are experimental values and lines are curves fitted using Weibull model

5.3.2.1 Weibull model and effect of process variables

Weibull model parameters α and ß were determined based on Eq. (5.4). For all treatment combinations tested in the present study, ß <1 (Table 5.5), leading to the inference that the a sub-population of spores may have had the ability to survive the stresses applied or showed a decrease of inactivation rate over time due to adaptability of survivor spores fraction to stress. In

support of reliability engineering, the Weibull model takes into consideration how parameters α and ß vary with treatment conditions like pressure, temperature and pH levels (Van Boekel 2002). When the Weibull parameter β < 1.0, survivor curves show an upward concavity, with first a rapid rate of decline followed by a slower rate. Such a survivor curve is indicative of the presence of a subpopulation (strains) in the spore suspension (Peleg and Cole 1998).

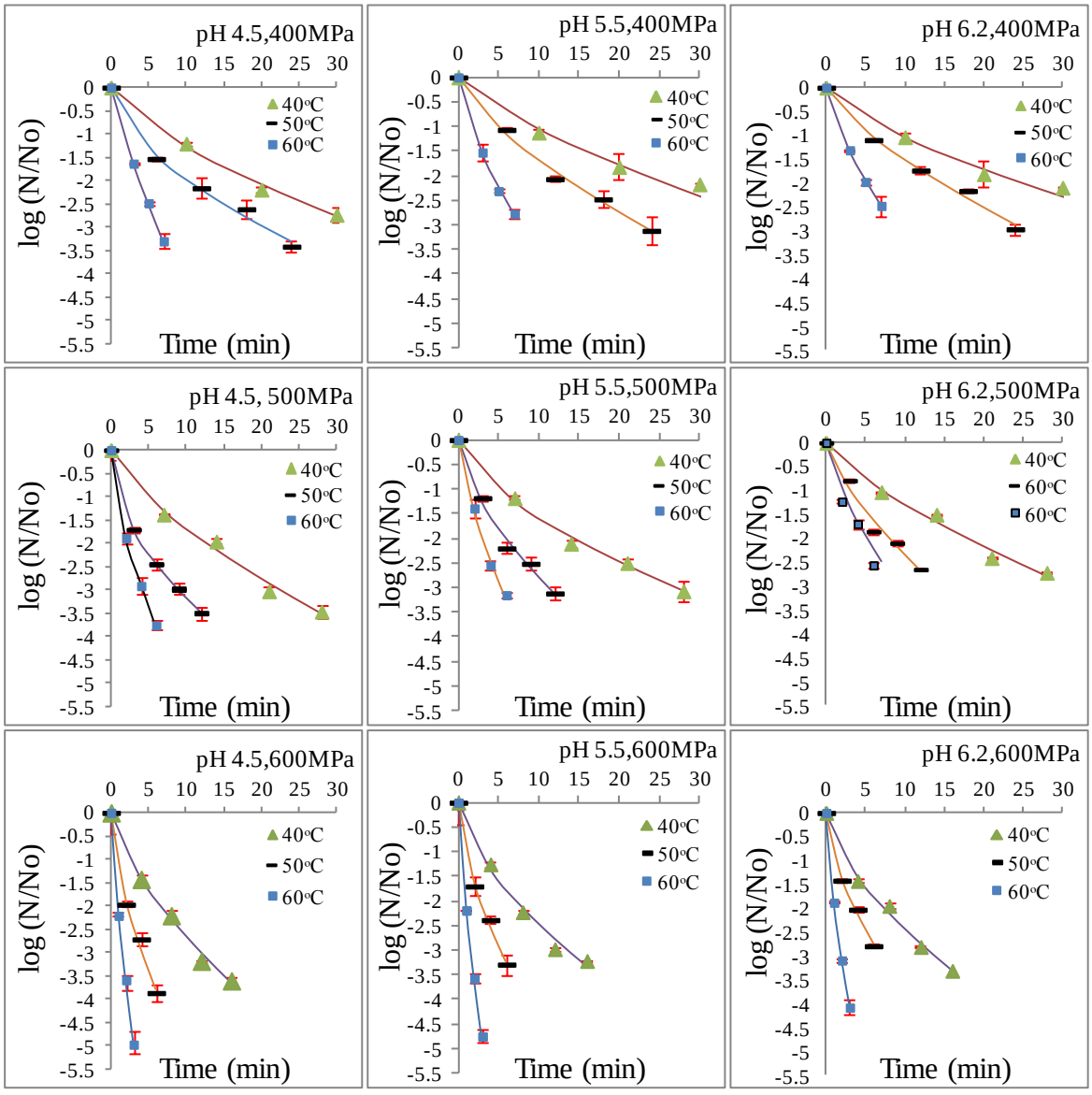

Figure 5.3 Effect of temperature on survivor curves of *B. licheniformis* spores in carrot extract at constant temperature and pH levels. Symbols are experimental values and lines are curves fitted using Weibull model

Since this work was performed using *B. licheniformis spp.* (*i.e.*, might not be a single pure strain) spores having different resistances to treatment conditions could have been present. However, none of the treatment parameters had a significant effect ($p > 0.05$) on ß, which concurs with a number of different studies (Fernández et al., 2002). In his review paper, Van Boekel (2002) also evaluated data from 55 different studies of thermal inactivation of vegetative cells, and in 48 of them he reported ß values to be independent of temperature. Moreover Cunha et al. (1998) indicated that the independence of ß on external factors, occurred because the parameter was a behavior index which showed the kinetic pattern of the mechanism controlling the process studied.

Table 5.3 Logarithmic reduction of bacterial spores suspended in carrot juice extract during pressure-assisted thermal processing at different pH levels

Pressure (MPa)	Maximum treatment time (min)	pH 4.5	pH 5.5	pH 6.2
		log CFU/ml	log CFU/ml	log CFU/ml
		40°C		
400	40	3.5±0.4	3.1±0.6	2.8±0.1
500	28	3.6±0.1	3.2±0.4	2.7±0.1
600	16	3.7±0.2	3.5±0.2	3.3±0.1
		50°C		
400	24	3.5±0.2	3.2±0.4	3.0±0.2
500	12	3.4±0.1	3.2±0.2	2.7±0.1
600	6	3.9±0.1	3.4±0.1	2.8±0.1
		60°C		
400	7	3.3±0.1	2.8±0.1	2.5±0.1
500	6	3.8±0.3	3.0±0.1	2.6±0.1
600	3	4.9±0.1	4.6±0.3	4.1±0.1

**Standard error of mean*

The scale parameter α represents the first reduction time (min) that would represent a decimal reduction in the population of surviving spores. For the conventional kinetic model, when ß = 1, α corresponds to the classic *D* value. Like the *D* value, the scale parameter α decreases with an increase in temperature and pressure. The ANOVA showed that both

temperature and pressure levels had a significant ($p \leq 0.05$) effect on α values, and pH had no significant effect ($p > 0.05$) (Table 5.5). This implies that unlike pH, temperature and pressure have relationships with the scale parameter, as was reported by Chen and Hoover (2003). Even though there was statistically no significant difference with pH levels, the lowest value of α was observed when the spore suspension was treated at pH 4.5 (600 MPa, 60^{o}C), whereas maximum values were achieved at pH 6.2 (400 MPa, 40^{o}C). This shows the combined effects of low pH with other treatment conditions in minimizing the scale parameter indicating the enhancement of spore destruction susceptibility.

When β is not equal to one in the Weibull model, the two parameters α and β can be used to determine t_R (Eq. 5.5) (time during which one $\log_{10}$ cycle reduction in number of surviving spores will occur) which is analogue to the classical *D* value (Van Boekel 2002). The t_R value calculated for the first $\log_{10}$ reduction of spores are very often used in food processing areas to establish processing schedule (Buzrul 2007). The computed t_R values are also included in Table 5.2. Since the scale parameter (α) is influenced by temperature and pressure, the t_R values are also significantly affected by these variables. Both an increase in pressure and temperature reduced the t_R values. At pH 4.5, an increase in process temperature from 40 to 60^{o}C reduced the t_R value by 76 and 77% at 400 and 600 MPa respectively (Table 5.2). Medium pH value of 4.5 also showed better reduction on t_R at all pressure and temperature combinations as compared to pH 6.2, except almost equivalent t_R value at 600MPa,60^{o}C for both pH levels. The temperature and pressure dependence of the scale parameter (α) could be modelled analogous to a conventional thermal death time model, since a linear log-relationship existed between α and temperature/pressure (Van Boekel 2002). Therefore z'_T and z'_P values for non-linear model were determined from the negative reciprocal of the slopes $\log_{10}\alpha$ *vs.* temperature/pressure (Eq. 5.7 and 5.8).

Temperature dependency of α (z') showed a decreasing trend with an increase in pressure level at pH 4.5. However other z' and z'_P values showed inconsistency with an increase in temperature/pressure. This inconsistency might have originated from the differences in scale parameters for the different treatment conditions determined based up on Eq.5.4. Inconstancy in *z* values between the two models were also reported in Van Boekel (2002) with over and under

estimation of parameters. The z_T values, in general, did not depend on pH which demonstrated some variability except at 600 MPa.

Table 5.4 Temperature (z_T , $z^{'}$) (^{o}C) and pressure (z_P , z'_P) (MPa) dependency of decimal reduction time values of *B. licheniformis* spores under different treatment conditions based upon log-linear and Weibull models respectively

Pressure (MPa)	pH 4.5		pH 5.5		pH 6.2	
	z_T	z'	z_T	z'	z_T	z'
400	31.0±3.0*	36.6±0.5	30.3±2.0	29.2±0.9	28.8±2.0	29.8±2.1
500	29.4±2.0	29.2±2.2	27.5±2.3	29.8±0.3	28.5±2.6	30.5±0.7
600	23.3±2.7	25.6±2.3	23.5±1.8	31.9±1.5	23.2±1.7	27.2±1.1
	z_p	z'_P	z_p	z'_P	z_p	z'_P
40	465±11	370±10	241±13	233±16	446±30	339±6
50	299±14	303±9	330±27	247±9	346±24	347±19
60	370±10	320±24	276±18	271±15	339±16	278±15

**Standard error of mean*

5.3.3 Comparison of models performance through kinetic data

Although both *D* and t_R decreased significantly with an increase in treatment combinations, the calculated t_R values according to Weibull model (Eq. 5.4) were found considerably lower than the classical *D* values (Table 5.2). This apparently shows that the destruction rate of spores computed from log-linear model would be lower than those calculated using the Weibull model. In reality, however, that may not be true. While in the log-linear model the magnitude of destruction is simply a multiple of *D*, with Weibull model it is not computed directly from t_R; instead both α and β parameters need to be used together to compute the value. Peleg and Cole (1998) recognized that, in homogenous population of microorganism and when β=1, the concept of *D* value may be feasible, but when β≠1 the t_R value progressively changes and hence the average or overall t_R value is calculated in which its magnitude depends on number of log cycle reduction that are followed. When upward concavity of survivor curves (β<1) are observed, they would yield a more rapid inactivation rate at the start then slow down to a steady value. In other words, the spores remaining after the quick decline have less probability

of dying and/or they might be adapted to combined treatment effects. In such cases, the Weibull t_R value will be lower than the *D* value because it would include the steeper decline in the first phase. As will be made clear, this does not mean, as with *D* values, the same trend will hold good for multiple t_R values. This again means that *D* values are determined from the linear part of the overall survivor curves whereas t_R takes into account everything that happens up to reaching the survival decimal reduction point. The discrepancy between the two indicates that there is somewhat of a higher degree of microbial destruction at the start (perhaps the more sensitive ones declining). However what is more important in process compilations is the relatively more resistant latter part of inactivation curve. This might sounds like possibility of underestimation of the thermal destruction times when a single t_R value is considered as in the case of conventional *D* value. It should be noted again that as with the log-linear approach where 5*D* is equal to 5 x *D*, t_d is not equal to 5 x t_R (see section 5.3.6 because the former is based on one parameter and the latter on two). The total death time (as 3*D*, 5*D* and 12*D*) values are discussed in the following section.

5.3.4 Comparison of models for predicting 3, 5 and 12-log decimal reduction times

The ultimate aim of investigating the combined effects of treatment conditions on inactivation of bacteria spores is to determine an optimal processing schedule to sterilize or pasteurize foods. Conventional thermal death time or in this case pressure assisted thermal death time is defined as the length of time at different pressure-temperature combination necessary to completely destroy a definite concentration of spores. In the conventional method of thermal process calculation based on kinetic data of log-linear model, a survivor ratio of 10^{-5} (based on for example *B. lichenoformis*) serving as a lethality criterion for pasteurization of acidified or acid foods while a 10^{-12} (commonly based on *C. botulinum*) for commercial sterilization criterion of low acid foods. The concept of 5*D*, (5-$\log_{10}$ cycle reduction in microbial numbers) is considered a sufficient process time to produce safe and shelf-stable acid or acidified products (pH<4.6). Therefore, in first-order kinetic model, once the *D* value under given treatment conditions is known, the total time required to achieve 5*D* is just a multiple of *D* values. The same concept is applied to determine Weibull t_d, to estimate time required to achieve 5-$\log_{10}$ cycle reduction; however in this case according to Eq. 5.6 because it requires both parameters α and β.

Table 5.5 Weibull model parameters as influenced by combined pressure-temperature and pH levels during inactivation of spores of *B. licheniformis* inoculated in carrot juice extract

Pressure (Mpa)	Temperature (^{o}C)	pH 4.5		pH 5.5		pH 6.2	
		α	ß	α	ß	α	ß
	40	2.21±0.06*	0.71±0.03	2.57±0.26	0.73±0.04	3.27±0.35	0.76±0.01
400	50	0.79±0.21	0.59±0.03	1.58±0.01	0.72±0.05	1.86±0.23	0.74±0.06
	60	0.62±0.03	0.84±0.03	0.54±0.12	0.73±0.07	0.67±0.02	0.74±0.03
	40	1.38±0.06	0.71±0.001	1.92±0.49	0.72±0.03	1.93±0.38	0.70±0.04
500	50	0.27±0.01	0.54±0.004	0.54±0.004	0.64±0.02	1.01±0.06	0.73±0.02
	60	0.24±0.13	0.65±0.10	0.41±0.11	0.73±0.09	0.60±0.08	0.75±0.04
	40	0.66±0.01	0.67±0.01	0.56±0.04	0.62±0.01	0.74±0.14	0.66±0.04
600	50	0.24±0.23	0.67±0.07	0.24±0.02	0.63±0.02	0.34±0.01	0.64±0.01
	60	0.16±0.01	0.79±0.05	0.13±0.01	0.73±0.01	0.13±0.02	0.70±0.04

**Standard error of mean*

Table 5.6. 3*D*, 5*D*, 12*D* and t_d (d= 3, 5 or 12) (Eq. 5.6) values to achieve 3-, 5- or 12-log reduction of spores of *B. licheniformis* according to log-linear and Weibull model parameters at pH 4.5 and 6.2

Temp.	pH 4.5						pH 6.2					
(°C)	*3D*	$t_{d\ (d=3)}$	*5D*	$t_{d\ (d=5)}$	*12D*	$t_{d\ (d=12)}$	*3D*	$t_{d\ (d=3)}$	*(5D)*	$t_{d(d=5)}$	*12D*	$t_{d\ (d=12)}$
						400MPa						
40	35.8±2.4*	33.3±2.8	59.6±4.1	68.4±7.7	143.1±9.8	235.2±38.2	42.3±2.1	42.0±2.5	70.5±3.5	82.4±3.8	169.3±8.5	262.2±6.5
50	22.6±1.4	20.2±2.3	37.7±2.3	47.9±3.5	90.5±5.5	210.7±0.1	25.7±1.5	26.8±0.8	42.9±2.4	54.6±3.8	102.9±5.9	184.8±25.4
60	6.4±0.26	6.3±0.3	10.7±0.4	11.7±0.8	25.6±1.0	33.5±3.7	8.5±0.51	9.0±0.7	14.2±0.8	18.1±2.0	34.0±2.06	59.2±9.3
						500 MPa						
40	23.7±0.9	21.2±1.0	39.5±1.5	43.8±2.2	94.8±3.6	151.1±7.7	30.8±0.2	29.3±1.7	51.3±0.3	60.4±1.3	123.0±0.6	208.9±8.5
50	11.2±0.3	9.6±0.5	18.6±0.5	24.7±1.1	44.7±1.2	124.8±4.0	13.6±03	14.0±0.5	22.7±0.6	28.1±1.6	54.5±1.3	93.1±9.1
60	5.0±0.43	4.3±0.8	8.3±0.7	9.4±0.8	19.8±1.7	37.5±4.2	7.5±0.04	7.9±0.1	12.5±0.1	15.6±0.8	30.0±0.2	50.4±5.7
						600 MPa						
40	13.4±0.5	11.7±0.5	22.3±0.8	25.0±1.5	53.4±1.8	92.3±7.9	15.0±0.3	13.9±0.5	25.0±0.5	30.2±0.3	60±1.2	115.1±9.5
50	4.8±0.03	4.2±0.1	8.1±0.1	9.2±0.4	19.4±0.1	35.3±6.5	6.7±0.3	7.0±0.4	11.2±0.4	15.5±1.2	26.8±1.0	61.2±6.1
60	1.9±0.08	1.9±0.08	3.1±0.1	3.6±0.2	7.4±0.3	10.8±0.5	2.2±0.07	2.0±0.07	3.7±0.1	4.3±0.04	8.9±0.3	15.0±0.5

**Standard error of mean*

The thermal death time is commonly determined by extrapolation of the linear $\log_{10}$ survivors versus time relationships. When the relationship is not linear, like when $\beta<1$, a discrepancy will arise between the calculated thermal death time according to log-linear and Weibull model parameters. Calculated times for 3, 5 and 12-$\log_{10}$ reduction of spores determined at pH 4.5 and 6.2 on the basis of log-linear and Weibull models are shown in Table 5.6. The results highlight the discrepancy between the two approaches. It was demonstrated earlier that for one-log reduction in spore population, the Weibull t_R values were relatively lower than *D*. For the *3D* treatment (mild processing), the predicted pressure assisted thermal death times were nearly the same for both models (Table 5.6). But as the process severity increased to 5*D* and 12*D* levels, the difference between the two model predictions deviated with Weibull model giving significantly higher treatment times than the log-linear kinetic model. Since all $\beta < 1$, the survivor curves had slightly upward concavity and therefore started deviating from the first order model obtained from average rate of destruction data over the gathered data. Figures 5.2 and 5.3 demonstrate this trend. The treatment times from Weibull model were 10-30% higher than the first order model for 5*D* predictions, while for 12*D* they were 40-130% higher. For lower range value predictions, which could be verified experimentally (3-4 log reductions), Weibull models were certainly superior. However, for 5*D* and higher, they were very poor. In several verification test runs there were no survivors when processed to first order 7 or 8*D* levels demonstrating an adequate process. However, the Weibull model would require much longer processing times. One could argue it would provide better safety as conservative estimates. However, processing for 235-260 min while 140-170 min (40^{o}C, 400 MPa) are sufficient or 92-115 min (40^{o}C and 600 MPa) while 53-60 min would be enough waste of time and resources lowering the efficiency of the system and deteriorating the product quality. Peleg and Cole (1998) also indicated that, when the purpose of using Eq. 5.4 is to accurately describe the survivor of the most resistant members of the population, then the equation can be used as a model. However when α and β values are used to determine the distribution of cells or spores resistances, the result may be an exaggerated fraction of the most resistant survivors.

Therefore, the Weibull model is a simple yet more robust model to describe microbial inactivation behavior, but it is not free of limitations. The major limitation of the model are the

two model parameters (α and ß), which are determined through non-linear regression analysis. These parameters are strongly dependent, so an error in α is balanced by ß and *vice versa* (Van Boekel 2002). Such type of autocorrection of one with the other may result in parameter instability (Mafart et al., 2002) which could contribute to inaccurate estimation of inactivation kinetic parameters. Furthermore, since Eq. 5.6 was originally developed and widely used in the materials science domain, it might require further evaluation and modification to apply to the biological arena. However, considering the nature of survivor curves pressure assisted thermal death times for 3-4 log reduction predicted based upon Eq. 5.6 were found more reliable than decimal reduction times. Therefore it is difficult to make a generalization, that linear model evaluated in this work could give less estimation to establish processing schedules, since it has proven record history. Determining kinetic data values using Weibull approach should be vigilantly verified with experimental results or the classic approach. In addition to this a case by case determination of thermal death time would lead to a reasonable decision to produce safe and shelf stable product.

5.4 CONCLUSIONS

In the last decade considerable research on HPP technology has been conducted due to advances in equipment, as well as consumer demands for better and more natural-like products. However, the wide adoption of these technologies is limited due to their limitation to inactivate bacteria spores at ambient or low temperature conditions. By combining high pressure with high temperature, spores can be inactivated faster and more efficiently. However, the benefit of a combined pressure-temperature treatment is progressively impaired with increasing temperature. This limitation can be avoided by introducing a low pH environment to inactivate spores and control their further growth. Predictive models are essential to determine inactivation kinetic parameters to establish necessary process schedules. The non-linear Weibull modeling approach is often recommended as a better model than the classic log linear model in describing survivor curves which deviate from linear relationship. This may be true for low severity treatments resulting in a certain level of surviving population rather than for process establishments based on more severe decimal reductions as in 5*D* or 12*D* processes.

PREFACE TO CHAPTER SIX

In previous studies, novel acid infusion mechanism was developed and inactivation kinetic data of *B. licheniformis* spores at different processing methods were determined. These are important input to combine and investigate the impact of controlled pH reduction at certain processing schedules on product quality before actual process design and validation. In the study of conventional acidification of vegetables, commonly pH is reduced to shift intensive heat treatment to moderate processing conditions. However, infused acid either through conventional or novel method could play a role in terms of hastening or delaying quality degradation rates. Therefore the objectives of this study were to answer questions like, could the infused acid play a role either to enhance or delay texture degradation ? if so, what are the mechanisms? and which alternative processing methods could best combine with controlled acid infusion for better quality retention ? The first question was approached by studying texture degradation behaviour of acid infused and non-infused (control) samples under different processing methods. Through studying degradation behaviours, relevant texture degradation kinetic parameters were determined and the influence of infused acid was revealed. The second question attempted by conducting microscopic study of the cell wall structure and molecular study of pectin depolymerisation due to β-elemination reaction and demethoxylation. Eventually, the final question was addressed by comparing different alternative processing methods under acid infused conditions. For this study, three commercial processing schedules (under, average normal and over processing) were selected to investigate the influence of infused acid on quality. Since texture and β-carotene of carrot are important quality parameters, they were used to evaluate the effect of various processing methods.

CHAPTER SIX

EFFECT OF NOVEL PROCESSING TECHNIQUES ON TEXTURE SOFTENING AND BETA-CAROTENE CONTENT OF THERMALLY PROCESSED CARROTS

ABSTRACT

Effect of novel processing methods was evaluated on product texture and β-carotene content of carrots following acidification to reduce pH from 6.0 to 4.4. Thermal treatments under Conventional (CH-T) and Ohmic heating (OH-T) conditions at 87, 92 and 97°C, individually and/or in combination with high pressure processing (HP-T) (400-600MPa/40-60°C), were given up to 90 min. A fractional conversion model was used to compute texture softening rate constant, k and activation energy, E_a. Acid-infused carrot samples had lower k values than the control, implying a better texture retention in acidified products. In order to explore this further, acid infused and control samples were subjected to selected processing methods for 0, 7 and 25 min representing minimal, optimum and over-processing conditions respectively. Texture value, pectin depolymerization by β-elimination, demethoxylation, cell micro-structure modification and β-carotene content were evaluated. Results showed that acid infused samples retained significantly ($p \leq 0.05$) better texture than the untreated ones. Pectin depolymerization by β-elimination was greater ($p \leq 0.05$) in control samples than acid-infused samples. In contrast, pectin depolymerization by demethoxylation showed no such differences ($p > 0.05$) with acid-infused samples. This indicates that pectin degradation was more dominated by β-elimination than demethoxylation, and these results concurred with the cell microstructure observations of processed carrots. Thermal and HP-T processing after acid infusion reduced the β-carotene content of carrots more than in control. However, mild heat treatment of carrots at 97°C under CH-T and OH-T enhanced the β-carotene levels to higher than in raw control carrot samples.

6.1 INTRODUCTION

Various food processing methods have been used to produce safe, better quality and more convenient foods. Conventional thermal processing methods have a proven record for producing safe and shelf-stable canned foods. However, the thermal processing practice traditionally employed for low-acid (pH>4.6) foods significantly damage their quality.

Texture is an important quality parameter of processed fruits and vegetables that dictates their market value and consumer acceptance. According to the USDA (1998), texture accounted for 30% score for the market acceptability of canned carrots. The texture of fruits and vegetables soften due to enzymatic and non-enzymatic changes in pectin and to a much lesser extent on other cell wall polysaccharides (e.g., cellulose and hemicellulose) (Williams and Besler, 1996). Pectin is an interesting cell wall polymer because of its abundance, solubility and sensitivity to biochemical or chemical reactions during processing and storage (Van Buren, 1979). The action of pectin-degrading enzymes such as pectinmethylesterase (PME) and polygalacturonase (PG) are closely tied to texture degradation of fruit and vegetable tissues (Fischer and Bennet, 1991; Houben et al., 2013). Pectin must first undergo PME-mediated demethoxylation as a prerequisite to PG-mediated pectin depolymerization and solubilization which eventually result in softening of tissues. However, demethoxylated pectin can cross-link with divalent ions (e.g., Ca^{2+}) forming super-molecular assemblies which have a firming effect on the tissue (Sila et al., 2008). This property presents an alternative opportunity for improving the texture of thermally processed fruits and vegetables. A number of studies have shown that selective inactivation of PG and controlled action of PME through different pre-processing treatment methods could enhance complex formation of demethylated pectin with Ca^{+2} ions for better post-processing texture retention (Ng and Waldron, 1997; Smout et al., 2005; Vu et al., 2006). Soaking in a calcium chloride solution, high pressure (HP) pre-treatment in calcium chloride solution, and low-temperature long-time blanching with the use of different brine ingredients are different pre-treatment methods demonstrated to improve the texture of thermally processed vegetables (Howard et al., 1994; Sila et al., 2005; Shahidul et al., 2007).

Pectin can also be degraded non-enzymatically by acid hydrolysis under low pH (< 3) or through ß-elimination reactions at higher pH (> 4.6) conditions (Keijbets and Pilnik 1974). At the plant cell wall pH generally ranges between 4 and 6 (Brett and Waldron 1996), the occurrence of acid hydrolysis of plant based foods during thermal or thermal-assisted high pressure processing is minimal as compared to ß-elimination reactions (Sila et al., 2008). Some studies have investigated acid infusion as a method of controlled pH reduction (4.3 < pH < 4.5) designed to improve textural quality of subsequent thermally-processed low acid vegetables. Improvement in texture of thermally processed foods will largely depend on intervention strategies at the molecular or microscopic level. Controlled pH reduction in low acid food systems could limit the effect of heat on pectin by inhibiting certain pectin degradation mechanisms at the molecular or microstructure level. Therefore, controlled reduction in pH of low-acid foods can reduce pectin degradation by minimizing demethoxylation and depolymerization reactions. Such pH manipulation could also enhance the use of novel processing methods and further improve the quality of thermally-processed foods.

The objectives of the present study were to evaluate the: (i) texture softening kinetics of carrots (*Daucus carota* L.) under different processing conditions (thermal and high pressure) with or without (control) acid infusion (AI) pre-treatment, (ii) influence of different processing methods on the texture, pectin and β-carotene content of AI carrots vs. control, and (iii) influence of various processing methods and AI on carrot cell wall microstructure.

6.2 MATERIALS AND METHODS

6.2.1 Carrot preparation and acid infusion

Carrots were purchased from a local grocery store and kept under refrigerated conditions (4^oC) until used. The carrots were peeled after cutting the top and bottom sections, andmade to 1.0 cm cubes, blanched in water (90^oC, 3 min) to inactivate enzymes and were finally cooled. The carrot cubes were then filled and heat sealed into two polyethylene bags: one with 1.15% (w/v) citric acid (AI), the other with distilled water (control), in the proportion of 3:1 (v/v) liquid: solid. The bags were then subjected to a short pre-established HP treatment (255 MPa, 5 min) so as to rapidly infuse the acid solution to achieve a core pH of 4.4±0.1 in the carrot cubes. HP

treatment was carried out with water as the pressurization medium as indicated in Tola and Ramaswamy (2013). Carrot cubes were then removed from the bags, and briefly rinsed with distilled water and used for subsequent tests. No significant pH variation occurred in control samples after high pressure treatment, and the pH remained at 6.0±0.2.

6.2.2 Part A. Texture degradation kinetics under different processing conditions

In the first part of the study, the effect of two thermal processing procedures [conventional (CH-T) and ohmic heating (OH-T) at 87, 92 and 97°C, and a moderate HP treatment (HP-T) (400 to 600 MPa; 40 to 60°C) were given for times ranging up to 90 min. The treatment procedures are schematically illustrated in Figure 6.1.

For conventional heating (CH-T), carrot cubes were filled in retort pouches (nylon/aluminium/polyethylene) (15.5 cm long and 3.5 cm wide) up to a height of 6 cm and then covered with either citric acid solution (pH 4.4) (AI samples) or water (control). The pouches were heat sealed and the conventional thermal treatment (CH-T) was given in a temperature-controlled circulating hot water bath (HAAKE C10, Thermo Electron Corporation, Karlsruhe, Germany).

Ohmic heating (OH-T) was given in a custom-made static ohmic heating unit as indicated in Seyhun et al. (2013) (Figure 4.1), consisting of a cylindrical glass beaker (4 mm thick, 12 cm long, 9.0 cmoutside diamemter) housing two stainless steel electrodes (15 cm long, 20 mm thick) situated 7.5 cm apart and fitted to the chamber's contour. For the operation of OH-T unit, the chamber was filled with 0.5% (w/v) calcium chloride solution at either pH 4.4±0.1 (AI) or pH 6.0±0.2 (control). Test samples were placed in a netted Nylon bag and immersed in the beaker. The OH-T unit was powered by a 110V, 60Hz AC power supply and equipped with an AC/DC transformer and a variable frequency generator (Scientifix Ltd, Guelph, ON, Canada). The unit was operational up to 170 V over an adjustable frequency range of 1-30 kHz. An applied current of up to 9 A, supplied as a 4 kHz square-wave was applied and monitored using a TPS2012 oscilloscope (Tektronix, Hong Kong, China). A preliminary calibration was used to verify that the OH procedure could be used to generate a heating profile in the sample closely matching to that of the CH-T treatment by manually switching the power supply on and off. Time-temperature profiles for CH-T and OH-T were monitored with K-type thermocouples (Omega Engineering Corp., Stamford, USA) covered by sealed glass capillary tubes to avoid electric

interference during data acquisition steps. At pre-set time intervals, samples were withdrawn and cooled immediately in ice-water mixture. To account for variations in come up time, for both the heating methods, the first sample (t = 0) was taken after approximately 6 min of heating. This lag time was experimentally determined by measuring the temperature evolution in the centre of carrot cubes.

The HP-T treatments were given in the HP equipment described earlier. A high capacity water bath (FP45, Julabo Labortechnik GMBH, Eisenbahnstr, Germany) was used to circulate temperature-controlled hot water around the pressure shell in order to regulate the shell temperature +2°C above the target temperature to compensate for heat dissipation and maintaining the target test temperatures. To maintain temperature stability, a thick walled polyoxymethylene insulating chamber was used as sample holder during the treatments. Full details of this chamber and its performance are presented in Shao et al. (2010). Prior to HP-T treatments, the insulator and samples were preheated in a temperature controlled water bath to stabilize initial heating temperature (T_i) (Equation1) (Nguyen et al., 2007) based on the target heating temperature (T_t) of the treatment.

$$T_i = T_t - (H_C * \frac{\Delta P}{100} + \Delta T_H) \tag{6.1}$$

where, T_i initial temperature of the sample required to reach target temperature (T_t), H_C is the heat of compression of the sample (positive ΔT° per 100 MPa pressure build-up), ΔP is the processing pressure during the holding time, ΔT_H is temperature gain by the sample from surrounding medium.

Given the carrot's high moisture content and the fact they were soaked in either a citric acid solution or water, H_C can be assumed to be same as that of water. Moreover, since samples were kept in a well insulated chamber (Shao et al., 2010), one can assume that they gained no heat ($\Delta T_H = 0$) from the surrounding medium before pressurization. Samples were subjected to range of pressure/temperature combinations (400-600MPa/40-60°C) at different intervals over different pressure holding times ranging up to 90 min. After each treatment, samples were immediately withdrawn and cooled in an ice-water mixture. For each experiment, samples that underwent pressurization and immediate depressurization were considered time zero (t = *0*) control samples representing the initial texture.

6.2.3 Part B. Quality retention studies under different processing regimes

In second part of the study (Figure 6.1), AI and control samples were subjected to selected equivalent processing methods tested above for comparative purposes [CH-T and OH-T at 97°, HP-T at 600 MPa/60°C, and a standard retort process (RT) at 121°C], for 0, 7 and 25 min representing minimum, optimum and over-processing conditions respectively. Residual hardness, pectin depolymerization by β-elimination, demethoxylation, and the cell wall structure modification were assessed. Sample β-carotene content was also determined. Texture retention was investigated through exploring the molecular properties of pectin and the microscopic study of cell wall.

Thermal treatments (CH-T 97^{o}C or RT 121^{o}C) were given in a static retort in the water immersion or steam heating mode. AI and control carrot cubes (192 g) were placed in cans (size 306x307) and filled with either citric acid solution (AI, pH 4.4) or a 2% brine (control), respectively. Cans were then subjected to different process holding times: zero (up to the come up time), 7 min and 25 min as optimum and over-processing scenarios. The 7 min process had been previously determined to be sufficient to achieve a 5 $\log_{10}$ reduction in *Bacillus licheniformis* spore population under AI conditions (pH= 4.5). The 25 min processing represented an over-processing and served to evaluate the effect of AI on carrot's texture and pectin after extended treatment times. Under OH-T, the same weights of carrots and liquid were used, except that both the citric acid solution (pH 4.4) and distilled water (pH 6.5) were supplemented with 0.5% calcium chloride to enhance heating medium's electrical conductivity. The HP-T treatments were applied at 600 MPa and 60^{o}C at the same condition.

Time-temperature profiles were recorded using K-type (for OH-T) or T-type (for CH-T and RT) thermocouples (Omega Engineering Corp., Stamford, USA). Multiple thermocouples were located at the core of carrot cubes at the geometric center of cans and near the electrodes which were the locations representing slowest heating points. The OH equipment set up was otherwise identical to that used in the kinetic study work.

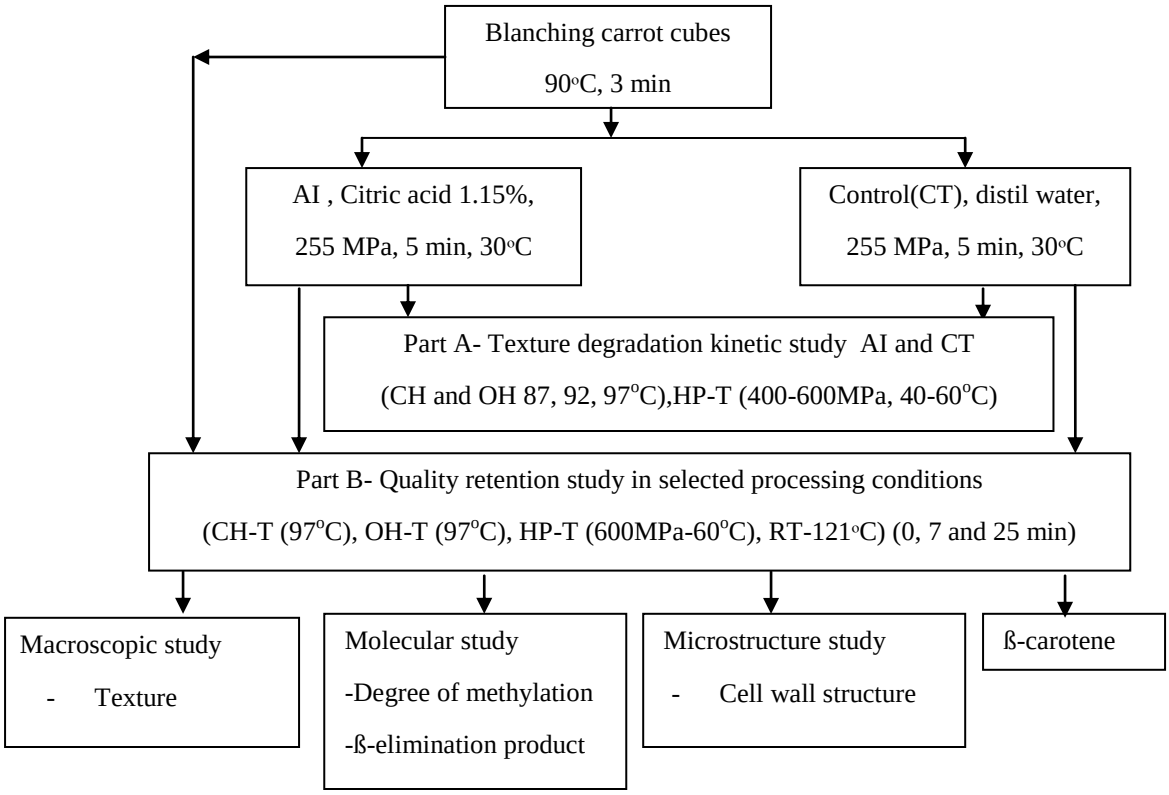

Figure 6.1. Schematic diagram showing general plan of the study

6.2.4 Texture measurement

Sample texture was measured using a Texture Analyzer (TA/XT/PLUS, Stable Micro Systems Ltd., Godalming, UK) equipped with a 25 mm diameter flat head cylindrical compression probe operating at a constant speed of 1 mm s^{-1}. Texture was expressed in term of hardness (N) defined as the maximum force needed to compress the carrot cylinder to 50% of its original thickness in the first cycle of compression. At least eight samples were measured for each treatment combination (×3 replicates) and mean values were reported. Samples from texture measurement were made to paste and stored at -40°C for subsequent experiments.

6.2.5 Texture degradation kinetics study

The texture softening kinetics of processed vegetable samples are commonly explained by a fractional conversion model (Rivzi and Tong 1997; Stoneham et al., 2000). The model accurately corrolate the extent of chemical reaction for texture lose with measurment of physical

properties, like hardness or firminess (Rivzi and Tong 1997). For a texture kinetics study, the fraction of texture (*f*) is measured as a function of time (*t*) and can be expressed as:

$$f = \frac{H_o - H_t}{H_o - H_\infty} \tag{6.2}$$

where, H_o is initial hardness of sample at time zero, H_t is hardness measured at a time *t*, and H_∞ is the non-zero equilibrium hardness property after prolonged heating.

To account for a non-zero equilibrium hardness, Eq. 6.2 was used to determine texture degradation kinetic parameters (Rivzi and Tong 1997):

$$\ln(1-f) = \ln\left(\frac{H_t - H_\infty}{H_o - H_\infty}\right) = -kt \tag{6.3}$$

Residual hardness of carrot cubes as a function of treatment time at a constant temperature/pressure can be expressed through a rearrangement of Eq. 6.3 as indicated in Eq. 6.4.

$$H_t = H_\infty + (H_o - H_\infty)e^{-kt} \tag{6.4}$$

The softening rate constant (*k*) and hardness at a prolonged treatment time (H_∞) were estimated from equation 6.4. The temperature dependence of the rate constant (*k*) can be expressed by the Arrhenius equation (Eq. 6.5).

$$\ln k = \ln k_{ref} + \left[\frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right] \tag{6.5}$$

where, k_{ref} is the rate constant at the reference temperature, E_a is the activation energy (kJ mole^{-1}), *R* molar gas constant (8.3145 J mole^{-1} ^{o}K^{-1}), *T* temperature (^{o}K) at time *t*.

A plot of ln *k* versus 1/*T* gives a linear plot line from which the inverse slope $-E_a/R$ can be used to calculate the E_a required for texture degradation. Texture degradation rate constants (*k*-values) and the final residual hardness value (H_∞) were estimated by plotting the residual hardness (H_t/H_o) against time using the nonlinear least-squares regression analysis of the solver option in Microsoft Excel 2007 (Microsoft Corporation). The second step involved estimating the *Ea* value (kJ mol^{-1}) by plotting the natural logarithm of the *k*-value (per minute) against the reciprocal of the respective absolute temperature (°K^{-1}; e.g., Eq. 6.5).

6.2.6 Alcohol insoluble residues (AIR)

Alcohol-insoluble residues **(**AIR) were prepared by making a fine paste of samples thawed in refrigerator (from -40^{o}C) using mortar and pestle according to McFeeters and Armstrong (1984). Approximately 10 g of paste were homogenized (10 min) in 50 ml of 95% ethanol and the suspension filtered with Whatman No 1 filter paper and the residue was re-homogenized (5 min) in 25 ml of 95% ethanol and the suspension was filtered again. The residue was then mixed with 25 ml of pure acetone and held for 5 min with occasional shaking, followed by filtration. Eventually the sample was then air-dried in an oven at 40^{o}C for 18 hr, then kept in a desiccators until further analysis.

6.2.7 Degree of Methyl esterification (DM)

The degree of methyl esterification (DM) of carrot pectin is expressed as the molar ratio of methoxyl groups to anhydrous galacturonic acid in AIR. Anhydrous galacturonic acid was determined colorimetrically using a method from Blumenkrantz and Asboe-Hansen (1973). First, a 20 mg sample of AIR was hydrolyzed through the drop-wise addition of 8 ml of 98% sulphuric acid and followed by 2 ml deionised water. Samples were held for 5 min and an additional 2 ml of deionised water was added for complete dissolution. A further dilution was made by the addition of 50 ml of deionised water. In a chilled tube, an 0.6 ml aliquot of hydrolyzed AIR received 3.6 ml of chilled tetraborate reagent (0.0125 M $Na_2B_4O_7.10H_2O$ in 96% H_2SO_4). Once sealed tubes were heated to 100^{o}C for 5 min in an oil bath, then immediately cooled in an ice-water mixture. The cooled sample received 0.1 ml of a 0.15% solution of 2,4-metha-hydroxydiphenyl in 0.5% NaOH and was thoroughly mixed for 1 min. After a further 15 min the mixture's absorbance was measured at 520 nm (Novaspec II Visible, England). Blanks were prepared by adding 0.1 ml of 5% NaOH instead of 2,4-metha-hydroxydiphenyl solution. Moles of anhydrous galacturonic acid were calculated from a standard curve derived from absorbance obtained with standard solutions of galacturonic acid ranging from 0 to 100 µg ml^{-1} (R^2= 0.984).

Using a method based on Ahmed and Labavitch (1977), methoxy content was determined by first hydrolyzing the carrot pectin ester bonds with an alkali solution (Na and Waldron 1997). A 20 mg sample of AIR was mixed with 8 ml deionised water and vortexed at high speed for 10 min. To achieve hydrolysis of the pectin's ester bonds, 3.2 ml of 2M NaOH solution was added and the mixture, incubated at 20^{o}C for 1 hr with occasional shaking, then neutralized by the

addition of 3.2 ml of 2M HCl. After a 20 min equilibration samples were diluted with 50 ml of 50 mM phosphate buffer (pH 7.5). Methanol determination involved taking triplicate 1 ml aliquots of the diluted solution, adding 1 ml of alcohol oxidase (1 unit ml^{-1} activity) (Sigma Aldrich, from Pichia pastoris) to each, and incubating for 15 min at 25^{o}C. Colorimetric quantification of the methanol released proceeded by adding 2 ml of 0.02 M Pentadion solution (except for blanks) followed by incubation at 58^{o}C for 15 min (Klavons and Bennet 1986). Once cooled, samples' absorbance was measured at 412 nm using a spectrometer (Novaspec II Visible, England). Moles of methanol were determined from a standard curve of methanol ranging from 0 to 40 µg ml^{-1} methanol, (R^2=0.98), using 50 mM phosphate buffer as a blank.

6.2.8 Determination of unsaturated galacturonides

Pectin glycosidic bond breakage arising from ß-elimination reactions and the resultant formation of 4,5-unsaturated galacturonides (UG) was assessed in diluted samples of the water soluble fraction of AIR. A 0.5 g sample of AIR was mixed with 90 ml of deionised water, the mixture boiled for 5 min, cooled to room temperature in tap water. The pH having been adjusted to 6.5 with 0.1 M NaOH, the mixture was filtered through Whatman paper No. 1, the filtrate constituting the water soluble fraction (WSF) of AIR. The UG concentration was estimated by taking 12.5 ml of WSF, diluting it with 37.5 ml of deionised water and measuring at 235 nm (Model DU 800, Spectrometer, Beckman Coulter, California, USA), with further dilution if necessary. The concentration of UG (mM UG g^{-1} AIR) was calculated based on a mean molar extinction coefficient of 5412 $M^{-1}cm^{-1}$ (Kravtchenko et al., 1992).

6.2.9 Microstructure study of cell wall of carrot

For the microstructure study, carrot cubes from the middle portion of the same carrot were prepared and labelled with thread during the treatments to avoid cell wall structure variation within and between carrot samples. Transverse sections (2 mm thick) of processed carrot cubes were fixed, embedded and sectioned according to Weigel and Glazebrook (2002) with minor modifications. Samples were first treated with FAA solution (Formaldehyde 3.7%, Acetic acid 5% and ethanol 50%) for 4 hr at room temperature, then dehydrated for 30 min in a series of alcohol solutions (50, 60, 70, 85 and 95%). Samples were then treated with absolute acetone for 30 min to remove carotene from the sample to improve staining. Dehydrated slices were stained overnight in 0.1% eosin Y in 95% ethanol. Stained samples were twice washed with 100%

ethanol for 1½ hr. Washed samples treated in a series of xylene:ethanol solutions (25%:75%, 50%:50%, 75%:25%, 100%:0%) each for 30 min, except the last one for 1 hr. This step is important to wash the alcohol since paraplast is not miscible with ethanol. Samples were placed in scintillation vials half-filled with 100% xylene and 30-35 chips of paraplast and incubated overnight at room temperature. Vials then were incubated at 42^{o}C and 30-35 paraplast chips were added every 30 min (repeated 5 times with occasional shaking). The xylene/paraplast solution was then replaced with molten paraplast and incubated at 60^{o}C with changes of molten paraplast in every 4 hr. Infilterated paraplast played a role to make trouble free sectioning of tissue. Samples were then embedded in a histoprep base mold, cooled and kept in a refrigerator at 4^{o}C. Sectioning was conducted using microtome (Model RM 2125 Leica Microsystems Nussloch GmbH, Heidelberger, Germany) at a thickness of 20 µm. Sliced ribbons were starched on 42^{o}C sterile water for 5 min to streach the ribbons and placed on a superfrost plus slide, then warmed overnight on slide warmer (Fisher Scientific, Model 77) at 50±2^{o}C. The next day the paraffin residue was washed with xylene (100%) for 5 min and stained again with 0.1% eosin Y in 95% ethanol for 1 hr to enhance the visibility of cell wall structures. The stained sections were washed with series of 75, 95, and 100% ethanol (30 s each) to remove the dye and sections were evaluated using a light microscope (Carl Zeiss, Axiostar Plus 1169-151) at 40× magnification. Images were taken for each section with a QIMAGE camera (Imaging, MicroPublisher 3.3 RTV).

6.2.10 β-carotene determination

Extraction of processed carrots' total ß-carotene content followed the method of Sadler and others (1990), with minor modifications. Carrot puree stored at -40^{o}C was ground to a fine paste with mortar and pestle. A sample of fine paste (1 g) was mixed with of 1 g $CaCl_2.2H_2O$ and 50 ml extraction solvent (50% hexane, 25% acetone, and 25% ethanol, containing 0.1% BHT) and shaken 30 min at 4±1^{o}C. After adding 15 ml of distilled water, the solution was frequently shaken for a further 15 min at 4±1^{o}C. The organic phase, containing the ß-carotenoids, was separated from the water phase, using a separation funnel, and was filtered using Whatman filter paper No.1. The extraction procedure was carried out under subdued light to avoid degradation of carotenoids. Beta-carotene was estimated from a standard curve (R^2=0.997) of β-carotene (Sigma Aldrich) dissolved in the same solvent combination.

6.2.11 Statistical analysis

The goodness of fit of the linear curves of (H_t/H_o) *vs.* time (t) and ln k per minute *vs.* 1/T° were assessed using regression coefficients (R^2). All experiments were carried out at least in triplicates and statistical analyses were conducted using Minitab 16.1.0.0 (Minitab Inc., USA). Pearson product moment correlation coefficients between measured variables (r) were determined using the same software. A Box-Cox power transformation was conducted to improve normality of the distribution and equalize variance to meet ANOVA assumptions for parameters violating the assumptions. Mean separation was conducted using the Tukey pairwise mean comparison at 5% significance level.

6.3 RESULTS AND DISCUSSION

6.3.1 Heating and pressure profiles for kinetic study

As shown in Figure 6.2a, except for a small jump in temperature before reaching processing temperature, adjustments to the OH-T setup resulted in similar heating rates as in the CH-T set-up. The heat/pressure profiles for the first 10 min of HP treatment at 40, 50 and 60°C at 600 MPa HP-T are shown in Figure 6.2b demonstrating the process temperatures to be maintained well at the required levels throughout the treatment period. For this purpose, the pressure vessel's outer jacket temperature was maintained at +2°C (based on a preliminary study) using a circulating water bath which allowed the chamber temperature to be equilibrated to the required level.

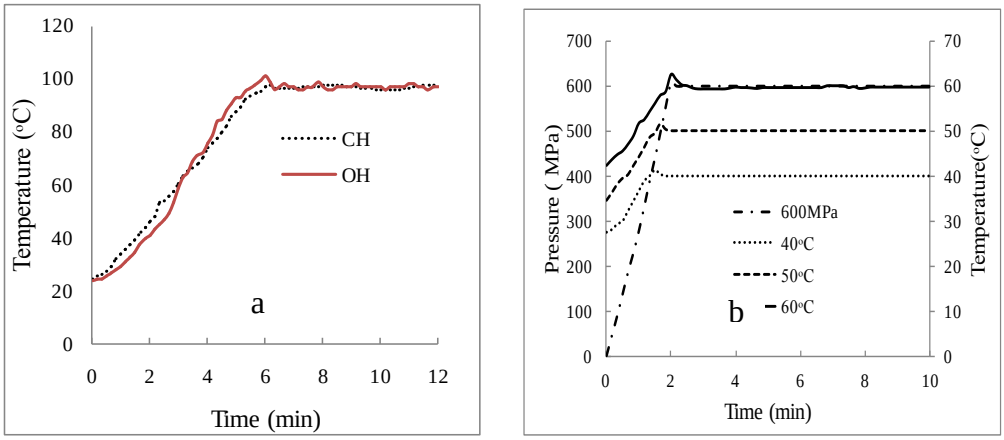

Figure 6.2 Heating profiles of (a) CH-T and OH-T, and (b) HP-T (only temperature levels of 40, 50 and 60°C at 600 MPa are shown for simplicity of the graph)

6.3.2 Part A. Texture degradation kinetics study

Given texture's preeminent importance in consumer acceptance of processed foods (Van Buren 1979), texture degradation of carrot cubes under CH-T, OH-T and HP-T processing methods was investigated. Representative graphs showing the relative hardness (H_t/H_0) of carrot cubes as a function of time under thermal [CH-T and OH-T] and HP processing are shown in Figures 6.3 and 6.4, respectively. In all cases, with an increase in treatment time, temperature or pressure-temperature levels, the residual hardness of samples decreased. Since a rapid softening rate in early phase, and subsequent slower softening pattern was observed for all cases, the fractional conversion kinetic model (Equation 6.4) was deemed appropriate for describing the softening behaviour. Estimated softening kinetic parameters are presented in Table 1 for CH-T and OH-T and Table 2 for HP-T. CH-T was used as the reference processing method to compare the novel processing methods of OH-T and HP-T.

The control samples (no acid infusion) on average softened 1.7 and 1.3 times faster than AI samples under CH-T and OH-T methods, respectively. Heil and McCarthy (1989) similarly reported that pH modified foods retained greater firmness than normally processed controls, showing that controlled acidification of low acid vegetables could reduce texture softening under thermal processing methods. The slower softening is assumed to result from reduced heat-induced chemical degradation of cell wall components of AI samples.

The softening rate was greater with OH-T than CH-T which concurs with the work of Farahnaky et al. (2012) who attributed it to the additional electric current effect on carrot tissue, which could hasten the degradation of cell wall components. In contrast, under HP-T processing, little or no difference in texture degradation was noticed between AI and control samples (Figure 6.4). Therefore, as compared to thermal treatments, the acidification process may not offer significant quality advantage in HP-T processing. However, when HP-T was compared to thermal methods (CH-T and OH-T), a much slower rate of textural degradation was noted. Previous studies also noted that HP treatment reduced the rate of texture degradation as compared to conventional thermal methods (Nguyen et al., 2007). This could be due to use of lower treatment temperatures (40-60^{o}C) under HP-T as compared to thermal treatments (87-97^{o}C). Apart from the moderate effects of HP-T processing, Basak and Ramaswamy (1998) also

suggested that texture improvement under moderate high pressure processing might be due to an increase in compactness of tissue structure as a result of tissue degassing. In high pressure sterilization of low acid foods, both high pressure and high temperature are used which may result in loss of tissue firmness by cell wall breakdown and loss of turgidity (Wilson et al. 2008). For instance Leadley et al. (2008) reported considerable softening of green bean under high pressure sterilization conditions even though a better result was obtained when compared to conventional thermal processing. Therefore in this regard, pH reduction could help to avoid limitations in high pressure sterilization on quality of low acid foods by allowing the use of moderate processing conditions.

Temperature sensitiveness of softening rate constants could be adequately described by the Arrhenius model. Activation energies (Ea) were estimated by plotting the natural logarithm of the texture degradation rate constant versus the reciprocal absolute temperature (Tables 6.1 & 6.2). The Ea found for the thermal treatments are in close agreement with those published for carrot tissues processed similarly, but at different temperature ranges (Sila et al., 2004; Vu et al., 2004; De Roeck et al., 2008). For both thermal treatment methods, AI samples showed higher Ea values than control samples (Table 6.1). The Arrhenius approach suggests that a higher Ea would imply higher temperature sensitivity of texture softening rate of AI samples than control. This, however, is in contrast to the lower softening rate associated with the AI samples. These observations imply that at relatively lower temperatures, better texture retention can be achieved with acidification process while under more severe temperature treatment conditions the opposite may be true.

For thermal treatments, estimated Ea varied from 81.5-132.4 kJ mol^{-1}. The lowest Ea was observed with OH-T control sample, which in fact showed the highest texture softening rate for carrot tissue. This implies that pH reduction of low acid vegetables could have a major effect on the firmness of carrots processed with ohmic heating. Meanwhile the higher Ea for AI samples might be associated with the thermal stability of carrot tissue, where a lower pH at moderately lower temperatures might play a role in slowing down of dissolution of pectin. Ea for the HP-T treatments ranged from 55.7-62.1 kJ mol^{-1} and 30.6-76.2 kJ mol^{-1} for AI and control samples, respectively (Table 6.2). With an increase in pressure, the activation energy decreased, indicating

the reduced temperature effect on texture softening. However, these values do not concur with the results of Sila et al. (2004), which might be due to variation in treatment conditions.

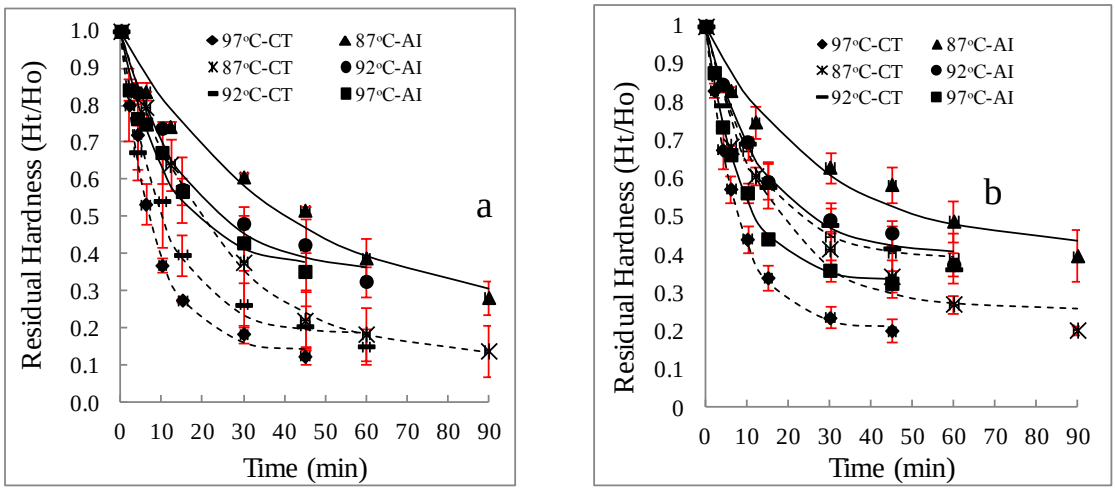

Figure 6.3. Effect of temperature on texture degradation of carrots in AI (solid lines) and CT(control, broken lines) conditions for (a) CH-T and (b) OH-T at 0.1 MPa

Table 6.1 Softening rate constants and activation energy of acid infused (AI) and control(CT), of carrot cubes under conventional (CH-T) and ohmic (OH-T) heating conditions (0.1 MPa, 87°C ≤ T° ≤ 97°C)

Temperature	Kinetic	CH-T		OH-T	
(°C)	parameter	AI	CT	AI	CT
87	k [a]	25.6±3.6[b]	43.6±8.4	36.5±1.2	63.2±4.2
92	k	58.9±0.5	101.9±29.0	70.7±1.4	75.1±0.5
97	k	83.75±3.0	131.3±26.0	118.2±8.2	132.4±12.4
—	E_a [c]	132.5±19.4	122.2±0.8	130.1±4.1	81.5±3.0

[a] softening rate constant k as (10^{-3} min^{-1}), [b] ± standard error of the mean, [c] activation energy E_a as kJ mol^{-1}

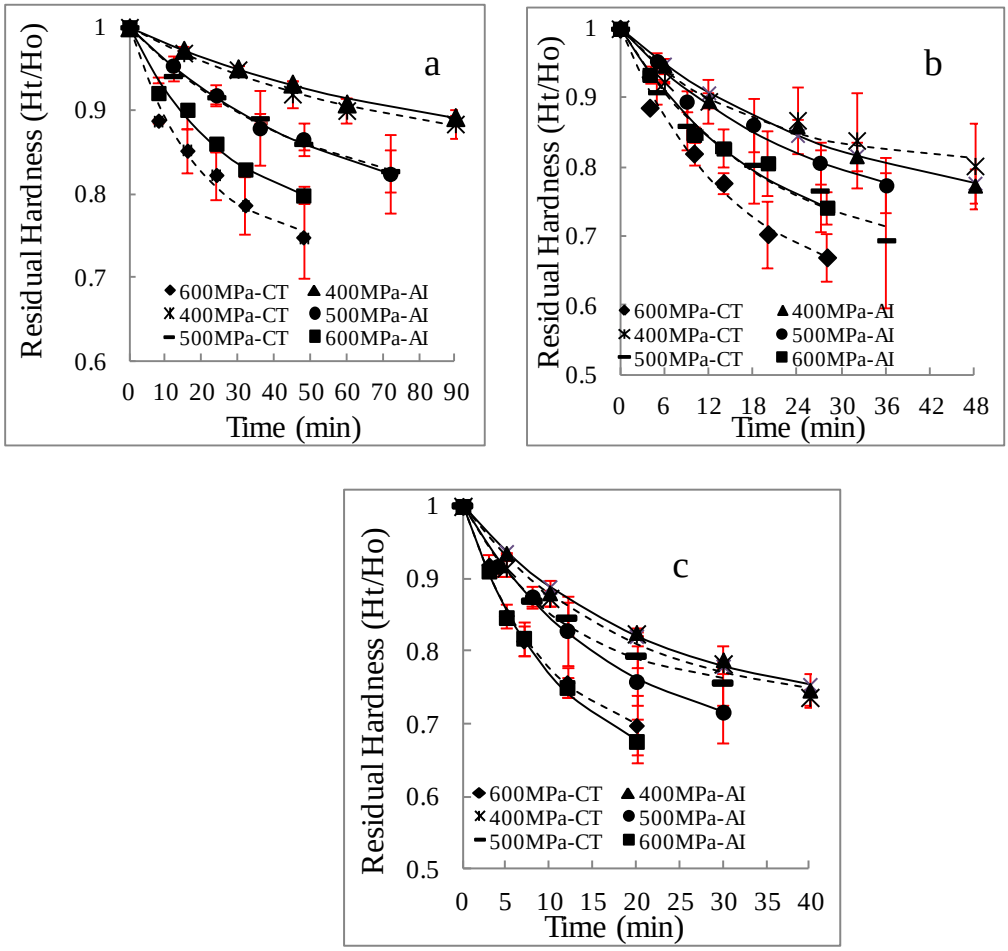

Figure 6.4. Effects of pressure at constant temperature on texture degradation of carrot in AI (solid lines) and CT(control, broken lines) at (a) 40°C, (b) 50°C and (c) 60°C

Table 6.2 Softening rate constants and activation energy for acid infused (AI) and control(CT) of carrot cubes under combined high pressure-temperature (HP-T) treatment conditions

Temperature (°C)	Kinetic Parameter	Pressure (MPa)					
		400		500		600	
		AI	CT	AI	CT	AI	CT
40	k [a]	12.4±0.3[b]	14.0±4.0	18.1±1.6	26.0±3.5	38.5±0.5	50.2±4.6
50	k	34.7±6.4	36.9±4.0	50.4±4.3	62.8±5.0	52.6±5.4	68.3±5.1
60	k	52.0±1.0	52.0±1.0	61.6±4.6	86.2±5.1	101±3.0	108±4.2
—	E_a [c]	62.4±1.0	58.5±1.1	53.8±6.7	52.2±2.0	41.7±1.1	33.1±5.7

[a] softening rate constant k as (10^{-3} min^{-1}), [b] ± standard error of the mean, [c] activation energy E_a as kJ mol^{-1}

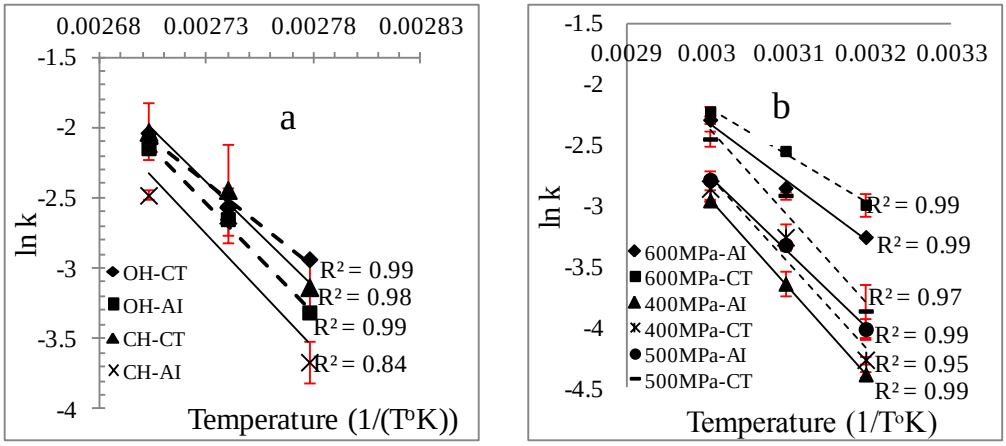

Figure 6.5. Temperature dependency of softening rate constant (*k*) for (a) thermal (CH-T and OH-T at 0.1 MPa), and (b) HP-T (400-600MPa) treatments for AI (solid lines) and CT (control, broken lines) of samples

6.3.3 Part B. Effects of AI on macro, molecular and microstructure of carrot

The kinetic study of texture degradation results showed that AI samples retained better texture than control. However, the mechanism of how acid infusion played a role to lower the texture damage needs further explanation.To address this question, the texture softening of carrot (macrostructure) was compared with pectin degradation (molecular component) and cell wall modification (microstructure component). Equivalent processing conditions of CH-T (97°C), OH-T (97°C) and HP-T (600 MPa-60°C) were selected based on previous inactivation kinetics data for *B. licheniformis* spores. Conventional canning (RT, 121°C) was included to compare effects of selected processing methods with processing schedule of low acid vegetables.

6.3.3.1 Effects of AI on texture of carrot tissue

The effects of AI on firmness and pectin content of carrot tissue following selected processing conditions are summarized in Table 6.3. Treatment prior to the come up time were excluded and hold times (0-25 min) were considered as the treatment times. At zero holding time, HP-T samples showed the greatest retention of texture (≥ 82%) as compared with CH-T (≥ 67.8%) and OH-T (≥ 58.7%) samples, whereas the conventional retort process (RT) showed only 26% retention compared to raw carrots. This indicates that under conventional canning, majority of texture loss occurs even before the retort reaches the operating temperatures. This may have resulted from the slow heating and cooling of the product under studied static retort

operating conditions. However for others, at t = 0 min, AI showed no significant ($p > 0.05$) effect on retention of firmness. However, when the holding time was extended to 7 and 25 min, a better ($p \leq 0.05$) retention of texture was observed for AI samples as compared to control ones under both CH-T and OH-T. This concurs with results of the kinetic study and hence AI could play a role to protect cell wall damage through minimizing heat induced chemical degradations. Greater texture retention was observed under HP-T; however, with no significant difference ($p > 0.05$) between AI and control conditions, which also is in agreement with the kinetic data. Apart from texture improvement, pH reduction certainly minimizes processing intensity, which should significantly reduce the energy cost. Furthermore, it enhances opportunities to exploit merits of novel processing methods to produce safe and better quality products through the use of moderate HP-T treatment as a pasteurization application.

6.3.3.2 Effects of AI and processing conditions on ß-elimination & pectin depolymerisation

Heat induced texture degradation in vegetables occurs through acid- or base-catalyzed pectin depolymerization. Elevated temperature (>80^{o}C) combined with a pH > 4.5 promotes a base-catalyzed depolymerization of pectin which occurs through ß-elimination reaction (Albresheim et al., 1960) and increases in parallel with pH. Therefore, lowering the pH of low acid foods and minimizing the intensity of processing conditions could play a role to reduce the rate of pectin depolymerization due to ß-elimination reactions.

In the present study, significant differences occurred in the formation of unsaturated galacturonides (UG) among the different processing conditions (Table 6.3). The alternative processing methods (vs. conventional canning, RT) resulted in a significant ($p \leq 0.05$) reduction in ß-elimination reaction products. The UG levels found in this study were similar to those reported by Vu et al. (2006). The highest UG concentrations were obtained for samples processed under conventional RT (Table 3, highest severity) which also had the least texture retention. The come up time for the CH-T and OH-T treatment methods had no effect on ß-elimination reaction products regardless of acidification conditions used. The lowest level was associated with HP-T treatments. Significantly higher ($p \leq 0.05$) UG concentrations were found in control than AI for 7 and 25 min CH-T processing, and for the 25 min OH-T processing. No such differences were apparent for HP-T processing. This confirms that controlled reduction of pH of low acid vegetables can slow down pectin depolymerization through ß-elimination

reactions. Several studies have confirmed that ß-elimination depolymerization is mainly responsible for excessive softening of low acid foods (Waldron et al., 2003; De Roeck et al., 2008). This study also confirmed that the existence of strong relationship between formation of UG and extent of texture softening. Strong inverse correlation coefficients were found between UG and residual texture for 7 min (r = -0.883) and 25 min (r= -0.821) treatments (Table 6.4). The depolymerization reaction due to β-elimination leads to pectin solubilisation and loss of cell-to-cell adhesion, ultimately manifesting in a loss in tissue integrity and firmness. However, as indicated in this study, such a degradation process in low acid vegetables can be reduced by limiting the β-elimination reaction through controlled pH reduction. Apart from pH reduction, texture improvement of thermally processed vegetables can be achieved through selective use of moderate processing methods. In the case of HP-T treatments, fewer unsaturated groups were formed, resulting in a better texture (Table 6.3).

During pectin depolymerization, removal of methylated carboxyl groups from pectin molecules is necessary for ß-elimination reactions to occur. Therefore, the rate of ß-elimination-driven pectin degradation should be enhanced when demethylation is greater (Sajjanantakul et al., 1989; Waldron et al., 1997). Different studies have aimed at minimizing the extent of ß-eliminative degradation through manipulation of DM, either through genetic engineering, or by activation of exogenous or endogenous PME using pre-treatment conditions (Vu et al., 2004, 2006). However, in the present work, PME was inactivated through blanching so as to independently investigate the effect of AI on the DM of carrot pectin and its correlation with ß-elimination reaction products. The DM was found to decrease with an increase in the severity of treatment conditions. Compared to 59.8% in raw carrots, the DM after the come up time for the conventional canning process (RT) was 36.5% (Table 6.3). Unlike the influence of UG on texture, AI did not show any significant effect on DM ($p>0.05$). Though not significant, decrease in DM due to chemical demethoxylation resulted in some softening of tissue. Other studies have found a more significant inverse relationship between DM and softening of carrot (Ng and Waldron 1997; De Roeck et al., 2008), and a strong negative correlation with ß-elimination reaction products of r = -0.878 (7 min) and r = -0.961 (25 min). This might be due to the availability of methylated carboxyl groups which could enhance ß-elimination reaction. Kravtchenko et al. (1992) also indicated that chemical demethoxylation can take place at the same time as ß-elimination under the same temperature and pH conditions. Therefore, the non

significance ($p>0.05$) of difference in DM between AI and control samples showed that pH reduction of low acid vegetables largely minimizes extent of depolymerization of pectin by reducing ß-eliminative reactions rather than demethoxylation. Fraeye et al. (2007) also indicated that ß-elimination was more temperature sensitive than demethoxylation. In conclusion, it can be stated that AI treatment does not seem to result in significant effects on DM under moderate processing techniques, but can significantly reduce ß-elimination depolymerization and result in better texture retention of carrot tissue.

6.3.3.3 Microstructure study of cell wall of carrot

Food processing method's impact is largely manifested at the macroscopic level through changes in texture of vegetables. Microstructural changes are the main contributing factors to alter textural properties of vegetables including changes in cell turgidity and cell wall integrity (De Belie 2002). It is generally believed that microstructure changes occur because of process induced biochemical or chemical transformations of cell wall components. Light microscopy was used to assess the impact of different processing methods on cell wall structure of carrots. Figure 6.6 shows micrographs of AI and control samples processed by different processing methods as compared to the raw carrot; 7 min and 25 min treatment times were also included for comparisons. The micrograph of raw carrot shows that the cell walls stained well, showing a strong cell-to-cell binding. However, for samples cooked at 121^{o}C, cells walls' ability to retain the dye was highly diminished and cells lacked their structural integrity and organization, showing distinct cell wall damage which could be associated with pectin solubilisation as indicated by Redgwell et al. (1997). With CH-T and OH-T treatments at 97^{o}C, cells wall of AI samples showed better staining than control. This was particularly true when the treatment time was 25 min with the control samples' cell walls losing their staining capacity. This confirms that AI has a role in protecting cell wall integrity. These micrographs also confirm previous results in Table 6.3. Under HP-T treatment no remarkable changes were observed in cells wall structures. Minor changes like cells deformation and enlargement were observed for samples treated at 600 MPa-60^{o}C for 25 min with a small cell wall disruption due to combined effect of pressure and heat.

Table 6.3 Effects of acid infusion and different processing methods applied for 0 min (come up time only), 7 min (5 log reduction in *Bacillus licheniformis* spores), and 25 min (over processing) on retention of texture of carrot and degradation of pectin as indicated by unsaturated galacturonides (UG) and degree of methylation (DM)

Processing methods*	Residual Hardness(%) (100*(H_t/H_{raw})±SE			UG (mM /g AIR^{-1})±SE			DM (%)±SE		
	Treatment time								
	0 min	7 min	25 min	0 min	7 min	25 min	0 min	7 min	25 min
HP-T-AI	88.0±6.0^{a}	82.6±4.5^{a}	76.1±5.9^{a}	1.1±0.18^{c}	2.4±0.11^{d}	3.3±0.09^{f}	59.1±3.8^{a}	49.6±0.9^{a}	44.4±1.8^{a}
HP-T-CT	82.0±6.2^{ab}	78.4±4.8^{a}	72.1±5.8^{a}	1.2±0.34^{c}	3.8±0.18^{cd}	4.2±0.45^{ef}	55.2±2.9^{a}	46.9±1.7^{ab}	43.3±2.3^{ab}
OH-AI	67.8±2.2^{bc}	48.6±1.8^{b}	32.4±3.4^{b}	2.1±0.20^{bc}	4.0±0.73^{cd}	4.6±0.06^{de}	51.1±1.4^{a}	36.5±2.4^{cd}	32.7±1.6^{bc}
OH-T-CT	69.8±3.9^{bc}	16.2±0.5^{d}	11.2±0.3^{c}	2.0±0.19^{bc}	5.3±0.13^{c}	5.7±0.18^{c}	50.0±1.7^{a}	36.3±2.4^{cd}	32.6±2.8^{c}
CH-AI	58.7±1.8^{c}	46.6±1.1^{b}	31.0±1.0^{b}	1.8±0.22^{bc}	5.2±0.12^{c}	5.6±0.19^{cd}	51.3±2.9^{a}	43.0±1.8^{abc}	32.0±2.4^{c}
CH-T-CT	62.1±1.6^{c}	34.1±1.2^{c}	12.5±1.4^{c}	2.3±0.17^{b}	6.1±0.54^{b}	6.8±0.22^{b}	48.3±2.4^{ab}	38.6±3.1^{bc}	26.8±2.8^{c}
RT	26.0±3.8^{d}	4.6±0.3^{d}	3.7±0.3^{d}	7.5±0.14^{a}	8.7±0.13^{a}	9.6±0.05^{a}	36.5±1.6^{b}	26.2±2.0^{d}	16.1±0.7^{d}
Raw	100	100	100	ND	ND	ND	59.8±1.0	59.8±1.0	59.8±1.0

*HP-T (High Pressure-Thermal, 600MPa-60°C), OH (Ohmic Heating, 97°C), CH(Conventional Heating, 97°C), RT(Retort Temperature, 121°C) , AI (Acid Infused), CT=Control, ND (not determined). Different superscripted letters indicate column-wise (by method), but not row-wise (by processing duration) significant differences ($p \leq 0.05$).

Table 6.4 Pearson product moment correlation coefficients and (*p* values) for correlations between residual hardness, unsaturated galacturonides (UG) and degree of pectin methylation (DM) under different processing methods

	Residual Hardness		Residual Hardness	
	7 min	UG 7 min	25 min	UG 25 min
UG 7 min	-0.883(0.008)	-	-	-
DM 7 min	0.921(0.003)	-0.878(0.009)	-	-
UG 25 min	-	-	-0.821(0.024)	-
DM 25 min	-	-	0.903(0.005)	-0.961(0.001)

6.3.3.4 Effects of AI and processing conditions on ß-carotene of carrot

Carotenoid content of carrots is one of the important quality parameters of raw or processed carrots. Beta-carotene is an important plant pigment and is responsible for the color of carrots. This micronutrient constitutes provitamin A (Bendich and Langseth, 1989) and its nutritional properties and antioxidant capacity have been established (Palozza and Krinsky, 1992). Figure 7 shows that under different treatment conditions, AI (*vs.* control) with CH-T and OH-T processing retained slightly lower concentrations of β-carotene. This indicates that the combined effect of heat and low pH could result in acid-induced β-carotene degradation. However, as compared to the raw sample (65.4 µg g^{-1}), AI samples in CH-T and OH-T methods gave better yields. In addition to this, control samples for all heat treatment methods (CH-T, OH-T and RT) provided better β-carotene yield than raw or HP-T treated carrots. This could be explained by effects of thermal treatment on tissue structure and damage to cell membrane which result in a greater extractability of β-carotene (Rodriguez and Kimura 2004; Sharma et al., 2009). Hedren et al. (2002) also reported a 2-fold increase of β-carotene extraction from processed carrot than a raw one. Furthermore, Holden et al. (1999) also reported that cooked A-plus cultivar carrot exhibited 28.8% increase in β-carotene content as compared to raw sample.

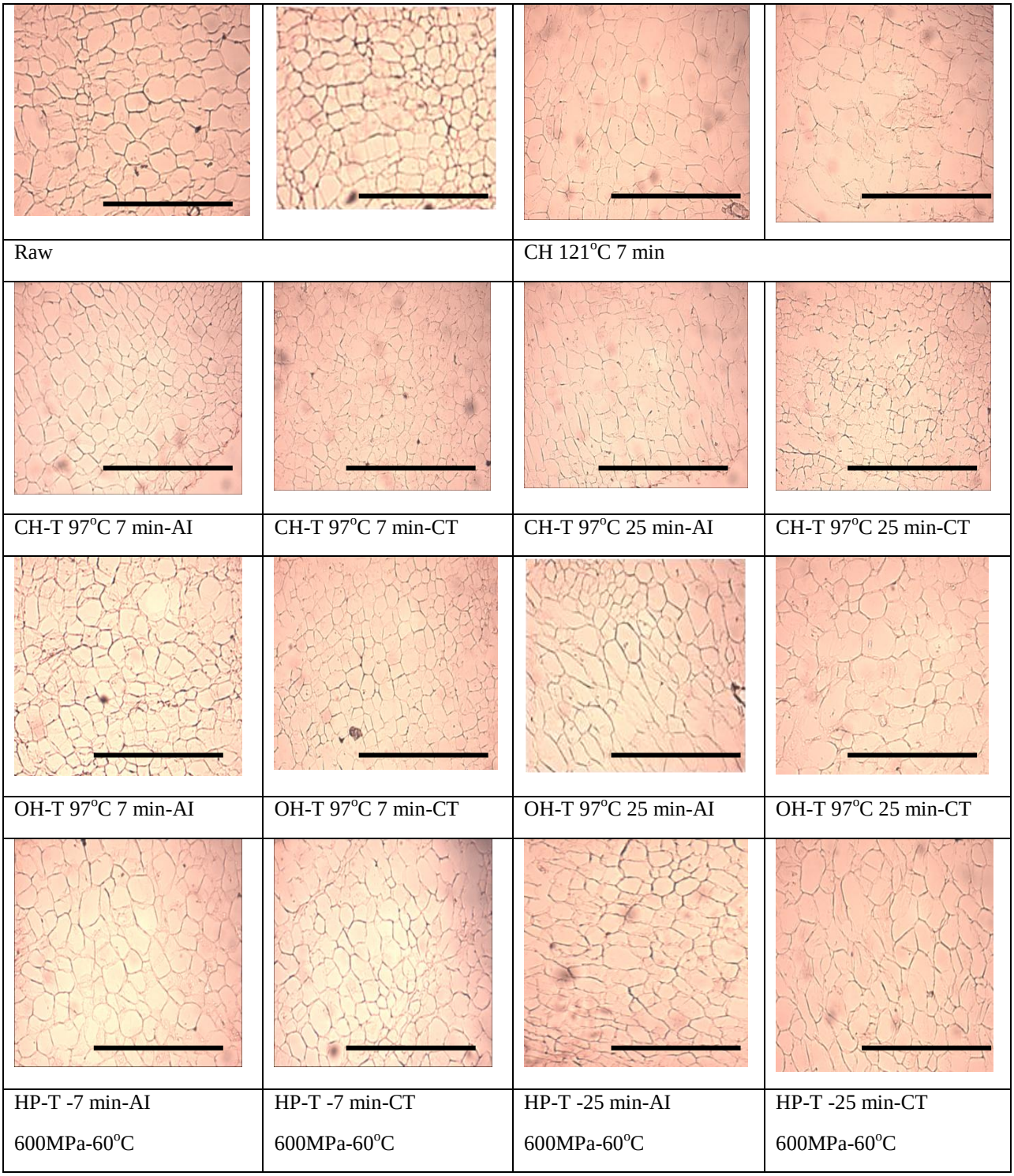

Figure 6.6 Microphotographs of carrot tissue (magnification 40×) including raw and tissues processed by different processing methods (CH= conventional, OH=Ohmic heating and HP-T= High Pressure-Thermal, AI= Acid infused, CT=control samples for 7 or 25 min. Bars = 200 µm)

However, when thermal treatments were compared, better extractability of β-carotene was observed for control (no acid infusion) than AI samples in CH-T and OH-T (97^{o}C) than at sterilization temperatures (121^{o}C for RT). This showed that long heating time at high temperatures could result in enhanced β-carotene degradation. However, samples subjected to HP-T treatments resulted in lower β-carotene yield (not necessarily degradation), even when compared to the raw samples. This could be due to compressing effect of high pressure treatment on tissue structure which might further inhibit extractability of β-carotene from carrot cells. In addition, low temperature (60^{o}C) used in combination with moderate- pressure (600MPa) might not induce a significant damage on tissue or cell structures which eventually limit the extractability of β-carotene.

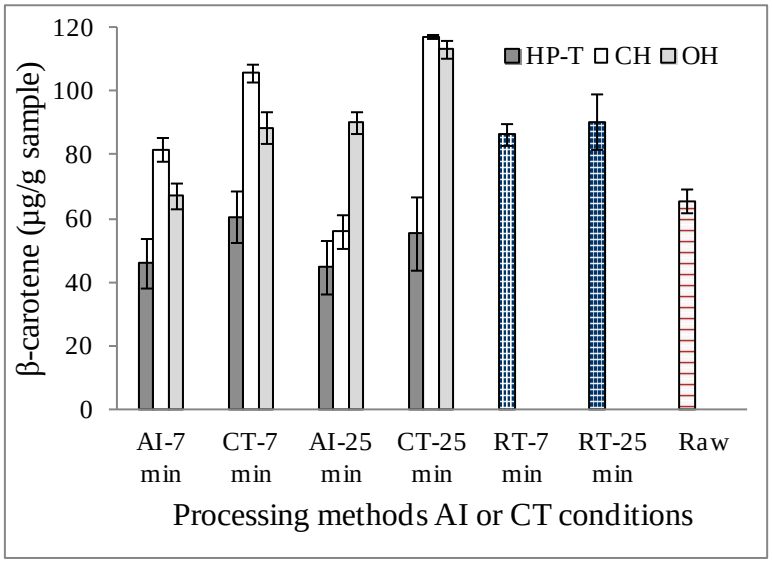

Figure 6.7 β-carotene concentration (± standard error) of samples subjected to different processing methods (CH-T and OH-T at 97^{o}C, HP-T(600MPa-60^{o}C, and RT at 121^{o}C) under AI and WI (control) conditions for 7 or 25 min treatments.

6.4 CONCLUSIONS

The effect of controlled acid infusion pre-treatment in combination with conventional and novel processing methods was investigated to elucidate its impact on quality parameters. Controlled pH reduction of low acid foods has advantages in terms of reducing process intensity which reduces the energy demand. In addition, the controlled pH reduction of carrots ensured better texture retention under different processing conditions. Acid infusion improved retention

of carrot texture by minimizing the rate of β-elimination reactions and allowing a relatively better pectin methoxyl group retention. Results demonstrated that the thermally-induced texture degradation of carrots and the progress of β-elimination are influenced by acid infusion even before the initiation of the actual process. Reduced levels of demethoxylation and depolymerization of pectin resulted, in turn, lowered the pectin solubilisation, led to less cell wall separation, and ultimately to better texture retention. Therefore, texture softening of vegetables can be minimized through controlled acidification as a pre-treatment step, in combination with the use of alternative processing methods. The potential of high pressure thermal processing in combination with acid infusion can overcome the limitations of conventional thermal or high pressure-sterilization on quality of low acid foods.

PREFACE TO CHAPTER SEVEN

In previous chapters, a novel acid infusion method was developed, and thermal and pressure-thermal resistance of reference microorganism were determined and role of acid infusion to improve quality of canned vegetables was investigated. These information are sufficient enough to design a processing schedule for commercial production of acid infused low acid vegetables. A successful process validation program depends upon information and knowledge from the product and process development steps. Therefore the effectiveness and practical applicability of deigned process conditions should be validated to certify food safety and product quality. Since the goals of previous studies were to design different alternative processing methods which are suitable for routine commercial manufacturing of safe and better quality acidified low acid foods, the efficiency of designed processing methods in terms of inactivation of *B. licheniformis* spores were validated using inoculated pack study. Therefore in this study different pasteurization methods were validated to achieve more than 7-$\log_{10}$ cycle reduction of *B. licheniformis* spores. Furthermore quality gain advantages of different pasteurization methods over conventional canning were evaluated.

CHAPTER SEVEN

MICROBIOLOGICAL VALIDATION OF ACIDIFIED THERMAL AND HIGH PRESSURE PROCESSING OF CARROTS AND ASSESSMENT OF PRODUCT QUALITY

ABSTRACT

Thermal and high pressure (HP) processing methods were validated for citric acid infused carrots (pH 4.5) using inoculated *Bacillus licheniformis* spore pack study. Previously established thermal inactivation kinetic data were used to determine the target process times (to achieve 7-log kill of *B. licheniformis*). The microbial spores were inoculated at center of fabricated carrot alginate beads and subjected to different processing methods. Delivered process lethalities evaluated by the microbial count method and measured time-temperature data, were equal to or higher than the targeted values. No survivors were found in any of the processed products regardless of treatment methods demonstrating that the designed processes were adequate. Furthermore, texture, color and β-carotene retention in processed carrots were compared with those processed under conventional canning. Significant (p<0.05) difference in texture retention was observed among processing methods. Residual hardness of carrots were 86% with HP, 70% with ohmic heating and 8% with conventionally canned product. The same trend was observed with chewiness value. However, processing methods showed no differences (p>0.05) with respect to color change. In terms of β-carotene, carrots subjected to relatively more severe heat treatment (water immersion mode in static retort) showed better β-carotene extractability than samples from HP.

7.1 INTRODUCTION

In the food industry, the recent focus has been to produce better quality products without compromising safety. Depending on their intensity, thermal and high pressure-thermal processes are classified into two categories. Pasteurization is carried at a relatively mild temperature (70-100^{o}C) or moderate pressure process (~650MPa), which destroys vegetative cells of microorganisms but has almost no effect on spores. However, sterilization is a thermal process at higher temperatures (above 100^{o}C) or high temperature-high-pressure combination processes (700-900 MPa, with product initial temperature of 70-90^{o}C) with the objective of destroying all forms of microorganisms, including spores. In these processes, production of commercially sterile, microbiologically stable product is the prime goal. Commercial sterilization is defined as the condition achieved by the application of sufficient heat alone, or in combination with other appropriate treatments to render the food free of microorganisms capable of growing in the food at normal non refrigerated conditions at which the food is likely to be held during distribution and storage. Obviously, it means the processed product could have some dormant microbial spores that are incapable of showing their activity during normal storage and distribution conditions.

Different processing technologies can be used to meet such objectives. However, the efficacy of a given process for a specific food product is evaluated on the basis of protocols and/or scientific data to demonstrate that a given process and associated control procedures can reproducibly deliver safe and better quality products. This process is known as process validation and FDA defines validation as a "documented program which provides a high degree of assurance that a specific process will consistently and repeatedly produce a product meeting its predetermined specification and quality attributes" (WHO, 1999). Based upon the scientific validity protocols and methods, as well as scientific validity of the results and conclusions, the producer can reach a sound conclusion about the efficacy (lethality) of a given processing schedule considering a target microorganism. Validation is also a critical step to the assurance of product safety not only for those which receive a thermal process (Leaper and Richardson 1999) but also for other alternative processing methods. In validation studies, microbial inactivation kinetic data are used to determine whether thermal or nonthermal technologies or combinations

of pH, water activity, preservatives, and other treatments will provide sufficient lethality to render a food product safe (NACMCF 2010) or inhibit the germination and growth of other heat resistant spores.

In thermal based food processing methods, pathogen lethality during heat treatments depends on treatment time and temperature to achieve needed lethality. But there are other complementary strategies that could help to control the growth of survivor spores. For instance, Stumbo (1973) indicated that, the probability of most heat resistance dangerous bacteria (Type A and B of *C. botulinum*) to grow and produce toxin in products having pH < 4.6 is very unlikely and the organism is considered to have no public health significance in more acid foods. However this might not work always, if these organisms co-exist with *B. licheniformis* in high acid foods (pH<4.6). If a given pasteurization treatment is not sufficient enough to inactivate the spores of *B. licheniformis*, it has the capacity to elevate the pH above 4.6 and could create a condition conducive for germination and growth of *C. botulinum* spores (Rodriguez et al., 1993). Therefore a given pasteurization process schedule for high acid or acidified foods should be sufficient enough to inactivate spores of *B. licheniformis.* Once such a process carefully designed and sufficiently achieved in such products (pH<4.6), a pasteurization process would allow long shelf-life stability at room temperature (Ramaswamy and Abbatemarco 1996) without compromising public health. In addition to this, evaluation of the impact of various processes on quality attribute of interest is theoretically and practically feasible when the impact of a given processing method is quantified from a safety point of view. This allows a processing method that will ensure both safety and quality. Therefore, in this study the combined use of controlled acid infusion and different thermal/pressure assisted thermal pasteurization methods were validated.

7.2 MATERIALS AND METHODS

7.2.1 Preparation of carrot samples and acid infusion

Preparation of carrot cubes and acid infusion to reduce pH of the cubes were done according to the protocols presented in Chapter 6.

7.2.2 Carrot alginate beads preparation, acid infusion and sterilization

Leaching of inoculated spores from real food particles during processing is a major limitation to biological validation of thermal processing and often results in under estimation of delivered lethality. In order to overcome this limitation, simulated food particles like those fabricated using alginates, egg albumin etc., are commonly used in validation studies. Following this trend, alginate particles were prepared using carrot puree according to method indicated by Brown et al. (1984) with under the optimum conditions established in Hassan and Ramaswamy (2011). Briefly, 300 g peeled carrot was boiled for 20 min and then blended with 100 ml distilled water to make a fine puree. The puree was thoroughly mixed with 0.225 g tri-sodium citrate and 14.1 g sodium alginate. A quantity of 0.9 g of calcium sulfate suspended in 30 ml distilled water was added to the puree and mixed thoroughly. The puree was then rolled into balls of approximately 2 cm diameter and soaked for 32 hr in 2.6% calcium chloride solution. Hard enough beads can save their integrity till end of a given process and will have similar thermo-physical properties to a real carrot particles (Marcotte et al., 2000; Hassan and Ramaswamy 2012). Soaked balls were withdrawn and rinsed with distilled water and then cut into 1 cm cube sizes. The cubes were then infused with citric acid solution (1.15% w/v) with the help of high pressure (255 MPa, 5 min) to reduce their pH to 4.4±0.1 (as detailed in Chapter 6). The cubes were then autoclaved at 121^{o}C for 15 min for inoculated pack study.

7.2.3 Chemical sterilization of real carrot cubes used as filler

For validation study and to simulate actual processing condition, sterile cans/heating cell/polyethylene bags were filled with sterile carrot cubes and sterile liquid (water acidified with citric acid to pH 4.4±0.1). Real carrot cubes were chemically sterilized using 2.5% sodium hypochlorite solution. Cubes first thoroughly washed with soap and tap water and continuously washed under running tap water for 1 hr. Washed cubes were aseptically transferred to sterile beaker and soaked in sufficient volume of sodium hypochlorite solution for 5 min. Soaked sample again washed three times with sterile distilled water and further soaked for 20 min (two times) in sterile water to remove residues of hypochlorite solution. Sterile carrot cubes from this step were acid infused using sterile 1.15% citric acid solution according to previous protocol to adjust the pH to 4.4±0.1 and then were used with sterile alginate beads from previous step for validation study.

7.2.4 Target microorganism and inactivation kinetic parameters

Bacillus licheniformis spores are used as surrogates for acid tolerant, pH elevating and heat resistant *Bacillus* species to validate processing methods of acidified low acid vegetables (FDA 2010). *B. licheniformis* can grow in pasteurized acidified foods because of its capability to survive and grow at low pH. It has higher heat (Janštová and Lukášová 2001) and pressure (Nakayama et al., 1996) resistance as compared to other *Bacillus* species. Lack of complete inactivation of these spores creates indirect food safety risk by creating conducieve environment for germination, growth and toxin production of *C botulinum.* Due to this reason complete inactivation of spores of this bacterium in acid or acidified foods is a critical step to ensure food safety. Therefore for this study, the bacterium was selected as a target surrogate for validation of acidified low acid foods. Inactivation kinetics of *B. licheniformis* spores at different conditions, as determined previously and detailed earlier (Chapters 4 and 5) are summarized in Table 7.1 and were used to determine pasteurization values (*P*-values).

Table 7.1 Inactivation kinetic parameters of *B. licheniformis* spores determined in carrot juice extract acidified to pH 4.5 with citric acid (parameters were compiled from our previous works in chapters 4 and 5)

Heating/processing method	Treatment combinations	D value (min)	z value (^{o}C
Conventional heating (CH-T)	97^{o}C, 0.1 MPa, pH 4.5	1.2	12.6
Ohmic heating (OH-T)	97^{o}C, 0.1 MPa, pH 4.5	1.1	16.3
High pressure-Thermal (HP-T)	600MPa, 60^{o}C, pH 4.5	0.6	23.3

7.2.5 Processing methods

In this study six alternative pasteurization methods and one conventional sterilization method were selected as indicated in Table 7.2 to conduct microbial validation study and product quality retention assesment. Among six pasteurization methods, four of them were conventional thermal methods and the remaining two were novel food processing methods (ohmic heating and high pressure-thermal processing). All processing methods were validated mimicking actual commercial processing conditions. Selected processing methods were: (i) pasteurination in cans

(307x306) in water immersion mode (WIM) in static retort at 97°C, (ii) pasteurization in cans in water immersion mode in a shaking water bath to simulate agitation heating (100 strokes per min at 97°C), orienting cans either in horizontal (SK-H) or vertical (SK-V) position (iii) pasterurization in cans in static retort using steam (97°C) as a heating medium (ST-P), (iv) pasteurization using ohmic heating in custom made ohmic heating unit at 97°C, (v) high pressure pasteurization (HP-T) at 600 MPa at 60°C, and (vi) commercial sterilization in cans in static retort (121°C) using steam as a heating medium (ST-S) as a control to compare quality gain advantages of other pasteurization methods. Except the last method all samples of other processing methods were infused with 1.15% citric acid to reduce pH of carrot to 4.4±0.1 for pasteurization treatments and cooling was done by immediately removing the containers and immersing them in cold water. Cooling for sterilization treatment follwed the same commercial collong procedure in the static retort. All pasteurization conditions were selected based upon microbial inactivation results of chapters 4 and 5.

For thermal treatment a pilot scale static retort, a water bath (Julabo, model SW22, Germany) and a custom made OH unit were used. Cans (307 x 306 , Home Canning Co., Montreal, QC), ohmic heating cell (12 cm L x 9 cm internal diameter) for OH and polyethylene bag (12 cm L x 12 cm width) for HP were filled with equal weight (200 g) of carrot and liquid(160 g). Both OH and HP treatment setups were the same as indicated in Chapter 6. For thermal processing methods the tips of two thermocouples (type-T, Omega Engineering Corp., Stamford, CT) were located at the center of the alginate beads and carrot cubes (two for each) and placed close to each electrode (preliminary study showed that samples placed close to the electrodes heated slower than other cubes and geometric center of cans respectively. Thermocouples attachment was made for those non-inoculated samples to gather temperature/time data. Output was recorded using data acquisition system (HP34970A, Hewlett Packard, Loveland, CO) at 10 s intervals.

7.2.6 Determination of process lethality

In order to compare different processes regarding their lethality, the concept of the "*P*-value" was used. The *P*-value is defined as the duration (min) required to achieve a given reduction ratio in the number of microorganisms at a given constant temperature. Designed *P*-

values were calculated from the initial and final spore counts and available inactivation kinetic data of target microorganism using Eq. 7.1, assuming the traditional thermal death time model. This is a first order reaction model that is defined by the decimal reduction time (D_T) and a temperature sensitivity kinetic parameter (z-value). This equation indicates the severity of a given thermal pasteurization method with respect to microbial destruction and it is a key element to establish processing time at constant temperature.

$$P_T^z = P - value = D_T \log\left(N_o / N\right) \quad (7.1)$$

where; P_T^z is *P*-value at 97°C and certain z value (Table 7.1); N_t is the final count of recovered spores after a specific time-temperature history, N_o is the initial spore count at time zero, and D_T is the decimal reduction time (min) at a fixed temperature to reduce the spore concentration by a factor of 10.

Meanwhile, the improved general method on designing of process schedule allows comparisons to be made between different processes at a given reference temperature. Due to temperature gradient or residence time distributions, every spatial element of the food product will experience an individual time temperature history which results in a given food quality parameter or microorganism integrates the impact of different heating times encountered at different temperatures, ultimately will give singular integrated impact of the process. By integrating these point values (from collected time-temperature profile at the slowest heating and cooling point) an actual average process value can be obtained using Eq. 7.2.

$$P_T^z = \int_0^t 10^{(T-T_r)/z} * dt \quad (7.2)$$

where; P_T^z is *P*-value at 97°C and certain z value (Table 7.1); T is temperature of the product at time *t*, (°C); T_r is reference temperature (97°C); z is temperature change required to effect a 10-fold change in the D_T value (°C) (Table 7.1).

The *P*-value calculated using Eq. 7.1 will be the equivalent as that calculated from time-temperature integration (Eq. 7.2), provided that first order kinetics have been followed for the spores destruction throughout the heating process. By combining the above two equations

predicted log reduction from a given integrated pasteurization treatment can be estimated using Eq. 7.3

$P_T^z = \int_0^t 10^{(T-T_r)/z} * dt = D_T * \log\left(N_o / N\right)$, through rearranging this equation

$$\text{Predicted log reduction} = \log\left(N_o / N\right) = P_T^z / D_T \quad (7.3)$$

Before processing, all samples were test-run to ensure that consistent processing conditions were maintained and to establish required *P*-values (i.e. time (min) required to achieve 7-log reduction on *B. licheniformis spores*). The *P*-value, is an integrated lethality measure to quantify the thermal processing of foods (Holdsworth 1985). The above equations are widely used for canned food thermal process evaluation and calculation, and equally applicable for samples subjected to OH-T, sicne FDA considered only the thermal effect is responsible to inactivate microorganisms. The efficacy of HP treatment to inactivate the spores is evaluated using Eq. 7.1 considering specific D_T value as indicated in Table 7.1.

7.2.7 Microbial validation study

7.2.7.1 Bacterial spore preparation, inoculation and recovery

Preparation of spore suspension of *B. licheniformis* for validation study was the same as indicated in Chapters 4 and 5. For inoculated pack study initial spore suspension was first adjusted to approximately 10^9 CFU/ml concentration. Then suspension was inoculated at the center of 5 sterile carrot alginate beads (20 µl spore suspension per bead, and 5 beads per package to give a cumulative volume concentration of 100 µl per package) using sterile syringe targeted to give approximate inoculation level of 10^8 spores/can (or spores/ heating cell for OH-T or spores/ polyethylene bag for HP). The center of the cube was considered as the slowest heating part and spores inactivation at this spot ensured total spore inactivation. In this study, carrot cubes, alginate beads, liquid, cans, heating cell and electrodes of OH, thermocouples, polyethylene bags (for HP) and other equipment were kept sterile and all can seaming and thermal sealing of polyethylene bags were done under aseptic conditions. OH-T processing was done under laminar flow hood to avoid any contamination. Sterile condition was maintained to exclude contamination of other heat resistant spores since the validation studies in terms of inactivation of *B. licheniformis* spores were conducted below 100^{o}C.

The survival of *B. licheniformis* spores in processed samples were analyzed by count reduction method. In this method the log reduction of the spores in processed products was determined by counting the survivors after incubation. All inoculated alginate beads from each can (all 5 beads) were collected aseptically and crushed with 20 ml sterile 0.1% peptone water in a stomacher (Interscience, Model W, France) for 6 min to release the spores from the alginate beads matrix. Four 1 ml portion of homogenate from each container were spread plated (from 10^0 to 10^{-3} serial dilution) on nutrient agar as the recovery medium and incubated at 37^{o}C for 48 hr. Survival colony counts from containers were expressed as average viable CFU/cans/heating cell/bag. If no viable spores were detected in the processed samples, the survival number of spores in a sample was recorded as below the detection limit. Three samples subjected to each processing condition were evaluated in the study.

In this study, processing methods were custom designed through preliminary tests to yield equivalent effect of inactivation of spores of *B. licheniformis* (7 log CFU/container) in acid infused carrot cubes (Table 7.2). For each run of conventional thermal treatment, two containers per treatment were run simultaneously. One was filled with sterile carrot cubes but with inoculated alginate beads for microbial validation study. In this case inoculated beads (5 per container) were placed at the center of the sterile can filled with sterile carrot and liquid and then hermetically sealed under aseptic condition. However the other container was filled with acid infused non sterile carrot, liquid and non inoculated alginate beads (2 beads per container) for the purpose of quality assessment study. Time temperature profiles from the second can were collected from two carrot and two alginate beads attached with thin flexible thermocouples (type-T, Omega Engineering Corp., Stamford, CT) at the center of the can and assumed that both inoculated and non-inoculated cans experienced the same time/temperature profile since they were exposed to the same processing conditions. Therefore, the validation was evaluated from inoculated alginate bead test cans while quality retention studies were completed with the control samples.

7.2.8 Quality evaluation study

7.2.8.1 Texture evaluation

A texture profile analysis (TPA) of samples was measured using a Texture Analyzer (TA/XT/PLUS Stable Micro. System Ltd., Godalming, UK, Stable Micro Systems, England) using cylindrical compression flat head probe of 25 mm diameter. Compression was performed twice (up to 50% sample thickness) at a compression rate of 1 mm/s. Each measurement was repeated 10 times and 8 data points (with CV less than 10%) were selected to estimate mean values. TPA involves analyzing the force-time curve for a product to evaluate various textural parameters. However in this study, hardness and chewiness values of samples were considered. Hardness measures the residual amount of firmness left at the end of the first compression. Chewiness is defined as the product of hardness, cohesiveness, and springiness results of TPA.

7.2.8.2 β-carotene extraction and measurement

Extraction and measurement of total ß-carotene content of samples were performed according to procedures indicated in Chapter 6.

7.2.8.3 Color measurement

Visual total colour of samples was determined using CIE (Commission Internationale de L'Eclairage) L*a*b* color space to evaluate the effect of processing methods on color change of processed samples using tri-stimulus colorimeter (Minolta camera CM-500d, Japan), which was calibrated using white tiles. The measurements were taken using D-65 illuminant and 10° observer. Total color change was expressed in terms of "L*" value (lightness, ranging from zero (black) to 100 (white), "a*" (redness) value and "b*" (yellowness) value. Color measurement was made after making carrot puree and filling in small petridish. Raw carrot was considered a target sample and color changes were evaluated as compared to the raw color. At least three measurements were taken at different locations on the surface of each puree and the procedure was repeated three times. Total color change (ΔE) between raw and final color of processed carrot was calculated using Hunter-Scotfield's equation Eq. 7.4

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (7.4)$$

where, ΔE represents the total color change as compared to raw; L^* *and* L are initial and final lightness values respectively; a^* *and* a are initial and final redness values respectively; b^* *and* b are initial and final yellowness values respectively.

7.2.9 Statistical analysis

Data are presented as means ± standard deviation of three observations. ANOVA was performed with general linear model using Minitab 16.1.0.0 (Minitab Inc., USA) computer software program. For significant results mean separation was conducted using Tukey pair wise mean comparison at 5% significance level.

7.3 RESULTS AND DISCUSSION

7.3.1 Heating profiles of carrot and carrot alginate particles

Biological validation of thermal processing can be categorized based on whether or not the bacterial spores are in contact with a food (Dignan et al., 1989). In the contact method, a given thermal process is evaluated through inoculating the bacterial spore suspension in food particles (Berry et al., 1985). Direct inoculation of spore suspension into food particle has some limitations in terms of possible spore leaching, but it offers many advantages including uniform inoculum and controlled particle size. In earlier studies, Perspex (poly-methylacrylate) beads containing spores were used to monitor processing of particles in continuous flow systems (Hunter 1972). Later attempts involved spores immobilized in calcium alginate gel beads (Dallyn et al., 1977) and large food alginate cubes containing spores (Brown et al., 1984). Sodium alginate based food particles are more commonly used to validate current thermal processing methods. In the presence of calcium ions, alginate based particle has the ability of forming thermally stable hard gels (Phillips and Williams 2000). Texture integrity of these particles during thermal processing minimizes the leaching of inoculated spores to the surrounding liquid. Therefore, inoculated carrot alginate fabricated particles can be used as a carrier of spores to determine the process lethality considering initial and final counts (Abdelrahim 1994; Walsh et al., 1996; Marcotte et al., 2000). Based upon this, carrot based alginate particles were produced and their heating profile was compared with carrot cubes both in ohmic and conventional heating (water immersion) methods. The heating rate of alginate beads was found almost the same with

real carrot particles (Figure 7.1). Particularly identical heating behavior was observed during ohmic heating and conventional heating. This might be because of very low percentage of sodium alginate added (4.7%) in carrot pure during particles preparation which did not impose a significant change on thermal property of alginate beads. In addition to this, Hassan and Ramaswamy (2012) reported that both calcium chloride concentration and immersion time had insignificant effect on the thermo-physical properties of alginate based particles. This confirmed that alginate based beads and carrot cubes had the same thermal diffusivity. Furthermore, structural integrity of carrot alginate beads during heating and cooling phases remained intact which is an important property to avoid leaching of inoculated spores during lethality study. Therefore carrot based alginate particles were used as a carrier of spores to represent carrot during validation study of various processing methods (Table 7.2).

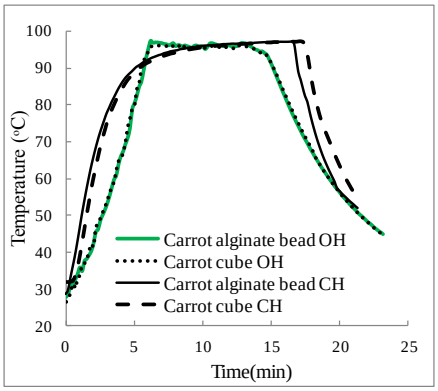

Figure 7.1 Heating profiles of acid infused carrot cubes (broken) and carrot alginate beads (solid) for ohmic and conventional heating in water immersion mode

7.3.2 Heat penetration data and process lethality verification

In thermal processing, heat penetration parameters together with microbial inactivation kinetic data are commonly used for establishment, validation and optimization of thermal processes. Temperature/time profiles to validate different processing methods are indicated in Figure 7.2. Sterilization temperature (steam, at 121^{o}C) was included to compare the quality advantages of other pasteurization methods as compared to conventional commercial canning. Preliminary tests were made to calculate the duration in minutes (the *P*-value) of an equivalent process time (min) at a given constant temperature (retort temperature = T_r = 97^{o}C) that would result in the same reduction ratio in microbial count. Because lethality is additive, the integration lethal effect of different time-temperature profiles can be combined to get the effective time as is

commonly done in thermal process calculations. Based on data collected from the heating curves, the integrated *P*-values (Eq. 7.2) of each processing method calculated according to improved general method are presented in Table 7.3.

Designed *P*-values (Eq. 7.1) were established based upon *B. licheniformis* spores inactivation kinetic parameters as indicated in Table 7.1 is also shown as decimal reduction time (D_T), which shows that time for a number of decimal reductions required (n) at a constant temperature. The thermal processing methods yield the same *P*-value of 8.4 minutes (Eq. 7.1), while the OH-T yields a slightly lower value (7.7 min) due to some electrical effects of OH on inactivation kinetics and HP process has a relatively much different value (4.2 min) due to HP effects on microbial destruction. The conventional processing value is based on commercial sterility (Fo 10.6 min at 121°C) (Eq. 7.2). Approximate come up times for the particle to reach target temperature of 97 (pasteurization) or 121°C (commericial sterilization) are also indicated in Table 7.3. The fastest was for ohmic heating (6.2 min) and followed by steam heating in static retort at 97°C(ST-P) (7.8 min).

Table 7.2 Types of pasteurization methods validated for inactivation of spores of *B. licheniformis* and evaluation of quality retention advantages

No. Treatments	Cans/heating cell/ Bags orientation during processing	*P*-values* based upon kinetic data Eq. 7.1
1. Water immersion mode at 97°C in static retort (WIM)	Vertical	$P_{97^{O}C}^{12.6} = 8.4$ min
2. Steam heating mode at 97°C in static retort (ST-P)	Vertical	$P_{97^{O}C}^{12.6} = 8.4$ min
3. Shaking of cans in water immersion mode at 97°C in water bath (SK-V)	Vertical	$P_{97^{O}C}^{12.6} = 8.4$ min
4. Shaking cans in water immersion mode at 97°C in water bath (SK-H)	Horizontal	$P_{97^{O}C}^{12.6} = 8.4$ min
5. Ohmic heating in heating cell at 97°C (OH-T) (static heating)	Vertical	$P_{97^{O}C}^{16.3} = 7.7$ min
6. High pressure assisted thermal treatment (HP-T) (600MPa-60°C)	Polyethylene bag	$P_{60^{O}C\ at\ 600MPa}^{23.3} = 4.2$ min
7. Commercial sterilization in steam static retort at 121 °C (ST-S)	Vertical	$F_{121^{O}C}^{10} = 3$ min**

**Based upon determined kinetic data, combined pressure and heat treatment at pH 4.5 of medium a 7 log reduction can be achieved for 4.2 - 8.4 min holding time, ** literature values to deliver minimum process lethality to achieve 12 D reduction for C. botlinum spores.*

In case of OH-T, heating of carrot cubes and carrot alginate beads takes place via volumetric resistance heating (Fryer 1995) of both particles and liquid and hence shorten the come-up-time. Steam retorting had a lower come-up-time due to high heat transfer rates associated with condensation of steam on cans. The others methods, except water immersion mode (under static condition) (19.5 min), exhibited similar come up times (11.2-12.3 min). This showed that OH-T and steam heating resulted in shorter come up time which could contribute to quality improvement through reducing processing time. *P*-value (Eq. 7.2) obtained from integration of time-temperature profiles from different thermal processing method are also indicated in Table 7.3. On an average a *P*-value of 10.1±0.4 min was observed for all thermal pasteurization treatments, except for OH, due to difference on spore inactivation kinetic data. Equivalent *P*-values implied that the same degree of pasteurization even if different processes produce different heat penetration curves or different temperature/time histories (Figure 7.2).

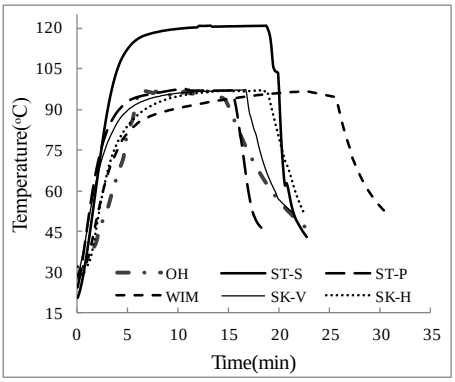

Figure 7.2 Heating profile of carrot particles in different thermal treatment methods, ST-S= steam sterilization (121^{o}C) in static retort, ST-P= steam pasteurization (97^{o}C) in static retort, WIM= water immersion mode (97^{o}C) in static retort, SK-V= Shaking mode of heating (97^{o}C) in water bath cans in vertical orientation, SK-H= Shaking mode of heating (97^{o}C) in water bath cans in horizontal orientation, OH= Ohmic heating (97^{o}C)

In general the integrated *P*-values (Eq. 7.2) are higher than those required for achieving the appropriate processes (due to accommodation of additional effects during come-up and come-down periods of retorts). Further, these could not be compared for commercial sterilization and HP processing due to conditions outside the normal range of temperature conditions employed for acidified thermal processing.

Ultimately, the effectiveness of a given thermal/pressure assisted thermal treatment is evaluated in terms of log reduction of spores which gives the equivalent biological lethality. Selected processing method should be capable to produce safe product to avoid issues of public health safety. To achieve such objectives, thermal or HP processes are commonly planned on a more conservative procedure. In this study microbiological validation study was conducted to achieve 7 or more log reduction of *B. licheniformis* spores under worst pasteurization conditions (high level of inoculum, considering the slowest heating spots both in the product and container). Designed (Eq. 7.1) and delivered (Eq. 7.2) process schedules as well as predicted log reduction (Eq. 7.3) and actual log reduction achieved from the processes are presented in Table 7.3.

Table 7.3 Different thermal processing methods and their corresponding pasteurization value (*P*-value) determined from *B. licheniformis* spores inactivation kinetic and heat penetration data

Treatment	Inoculated level (spores/can /heating cell/bag	Come up time(min) to $97^oC/60^oC$	*P*-value (min) required to achieve 7-log reduction Eq. 7.1	*P*- value (min) delivered Eq. 7.2	Predicted log reduction (CFU/container) Eq. 7.2 & Eq. 7.3	log CFU/container observed from actual experiment*
WIM	$1x10^8$	19.5	8.4	9.7±0.4†	8.1±0.7	> 7***
SK-V	$1x10^8$	11.2	8.4	10.5±0.5	8.8±0.5	> 7
SK-H	$1x10^8$	12.3	8.4	10.3±0.4	8.6±0.5	> 7
ST-P	$1x10^8$	7.8	8.4	9.8±0.5	8.2±0.6	> 7
ST-S	$1x10^8$	12	8.4	10.6±0.6	8.8±0.6	> 7
OH	$1x10^8$	6.2	7.7	8.3±0.1	7.5±0.1	> 7
HP-T	$1x10^8$	2**	4.2	nd	nd	> 7

† *standard deviation, *no colony was observed after 48 hr culturing on nutrient agar medium, and nd is not determined, **samples preheated to 42°C to reach target temperature of 60°C during pressure buildup step, *** no colony growth observed from cultures samples after each processing treatment*

Delivered *P*-value ranged from 8.3 to 10.6 min with a corresponding predicted minimum log reduction of spores from 7.5 to 8.8 CFU/can/heating cell/bag, which was slightly more than targeted 7 log reduction of spores (Table 7.3). The number of surviving spores in processed samples after incubation were monitored and no growth of colonies (> 7 log) were observed for any of the treated product (Table 7.3). The same efficacy was observed in HP processing with no spore recovery after delivered process. This showed that delivered process schedules were

sufficient enough to inactivate spores of *B. licheniformis*. From a safety point of view, process schedules greater than these could enable to produce *B. licheniformis* free acidified foods. Therefore the concern of pH elevation due to the action of *B. licheniformis* and creation of optimum germination and growth environment for spores of *C. botulinum* could be controlled, since growth and toxin production of *C. botulinum* under reduced pH (<4.6) environment is very unlikely.

7.3.3 Quality retention evaluation

The purpose of a scheduled process is to deliver a predetermined amount of lethal agent (varying depending on the type of heat, pressure), in a given time under the right conditions, to ensure eradication of target micro-organisms of concern with minimal damage to quality properties. In this study, the effect of different type of pasteurization methods for acidified thermal processing are compared relative to conventional canning for low acid vegetables. Quality parameters included were texture, color and β-carotene content.

7.3.3.1 Texture retention

Human perception of canned vegetables palatability is derived from a complex interaction of sensory and physical properties during chewing. The objective mechanism used to evaluate the effect of a given food preservation method on texture properties of a product varies. The development of texture profile analysis (TPA) is an important milestone in food science to understand how and to what extent processing methods influence texture of foods (Pons and Fiszman 2007). In most cases, a linear correlation has been observed between TPA results and subjective texture measurements (Meulleneti et al., 1998).

In this work, the processing effects of thermal and HP processing methods on textural properties of carrot are compared. Hardness and chewiness are the two textural properties that were evaluated. The hardness value was measured by the peak force of the first compression of the sample simulating the first bite during chewing. Chewiness is literally defined as energy required to chew a solid food to a state where it is ready for swallowing, but mathematically expressed as a product of hardness, cohesiveness and springiness. Figure 7.3 shows that there is a significant difference (p<0.05) in retention of hardness and chewiness among processing methods. Better hardness and chewiness were obtained from samples subjected to HP and OH-T

treatments. When the residual hardness was compared with the raw, 86 and 70% of the hardness was preserved in HP and OH-T treatments, respectively. The chewiness values were also superior in these methods as compared to others. These results are also in agreement with Knockaert et al. (2011) study on carrots. This quality advantage mainly originated from inherent processing nature of the two methods. In OH, the heating is rapid and volumetric which reduces total processing time and its impact. The food responds to the passing current by generating volumetric heat internally due to its inherent resistance (Sastry and Barach 2000). Such a heating behavior resulted in uniform and fast heating throughout the food and reduced the overall processing time with reduced come up time as indicated in Table 7.3 and hence contributed to better quality retention (Palaniappan and Sastry 1991). In addition to this the energy conversion efficiency and fast heating behavior of OH-T is very high since no packaging material is heated with the food.

HP treatment, considered as the non-thermal alternative to conventional thermal processing, allows uniform and rapid pressure treatment (at relatively low or moderate temperatures) which ultimately minimizes the effects of conventional heating (Nuguyen et al., 2007). The advantage of pressure processing is also confirmed by other studies (McClements et al., 2001; Nuguyen et al., 2007). These studies reported that the process can be considered equivalent to a high-temperature-short-time (HTST) treatment (Leadley 2005) without significantly affecting the quality of the product.

The benefit of combining use of moderate pressure and temperature in terms of production cost effectiveness and safety is also indicated in de Heiji et al. (2005). Because of these behaviors of the two processing methods better texture retention could be achieved. When other pasteurization methods were compared, no significant (p>0.05) difference was observed with respect to the texture of carrots processed in ST-P, WIM, SK-V, SK-H. Since equal volume of solid and liquid were used during all treatment conditions, samples in the shaking water bath might not get enough headspace during shaking to enhance better mixing of samples. However, texture retention from these processing methods was considerably higher than samples processed in conventional canning for non-acidified carrot. Residual hardness and chewiness observed from conventional canning (ST-S) using steam as a heating medium were only 8% and 2.6%,

respectively (Figure 7.3). This shows that an intensive and extensive thermal treatment in conventional canning causes serious damage to quality attributes of low acid canned vegetables.

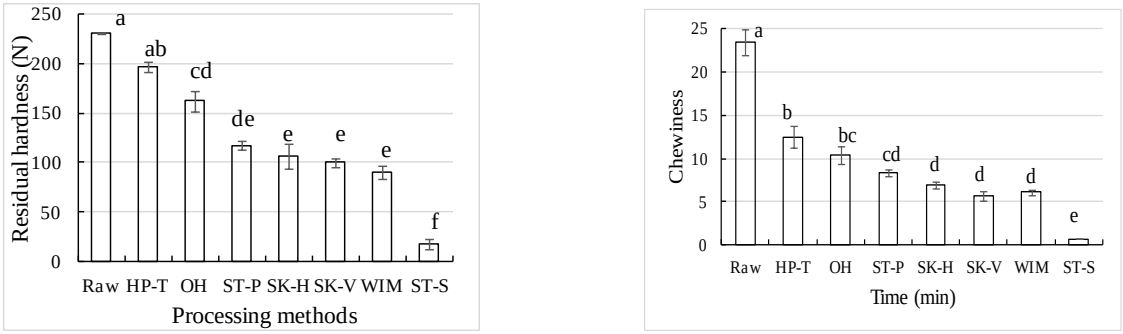

Figure 7.3 Effect of different processing methods on residual hardness and chewiness of carrot. , ST-S= steam sterilization (121^{o}C) in static retort, ST-P= steam pasteurization (97^{o}C) in static retort, WIM= water immersion mode (97^{o}C) in static retort, SK-V= Shaking mode of heating (97^{o}C) in water bath cans in vertical orientation, SK-H= Shaking mode of heating (97^{o}C) in water bath cans in horizontal orientation, OH= Ohmic heating (97^{o}C)

Therefore the application of moderate processing conditions with strategy of using different complementary preservation methods could be an important alternative to improve quality of canned vegetables. Therefore, the combined use of moderate processing methods (thermal/pressure assisted thermal) with novel pH reduction strategy of low acid vegetables, could significantly contribute to minimize the impact of intensive thermal or high-pressure-high temperature (HPHT) sterilization process. Barbosa-Cánovas and Juliano (2008) also indicated that synergetic approaches through pH reduction or use of antimicrobial preservatives can help to reduce limitations of intensive processing methods but ensure safety and better quality product with reduced processing cost.

7.3.3.2 Color retention

Color change of many foods after processing is an important quality attribute in marketing. Though it does not reflect nutrition value, it is important as it relates to consumer preference based on appearance. Color measurement is one of the objective parameters that can be used as a quality index of foods in quality control of processed foods (Giese 2000). The color of the food material could change during processing due to certain enzymatic and/or non-enzymatic reactions. The effect of various processing methods on carrot color was evaluated in

terms of total color change observed according to Eq. 7.4. The effect of processing methods was insignificant (p>0.05) in terms of total color change of carrot, except sample treated in conventional canning method (ST-S) which showed more color change as compared to others (Figure 7.4).

As indicated also in Liesbeth et al., (2012) no noticeable difference in total color was observed from mild (600MPa, 25°C) and severe (600 MPa, 61.3°C) pasteurization treatments, even though better retention of color was observed for samples in high pressure treatments than thermal. The insignificant difference in color change among pasteurization methods might be due to reduced impact of treatments on color responsible microstructure of carrot tissue. Even though, statistically no difference was observed among pasteurization methods, slightly better retention of color was observed for samples from HP and OH-T (Figure 7.4). Color change observed in case of conventional canning (ST-S) could be due to Maillard reaction because of intensive heat treatment or due to loss of certain color components.

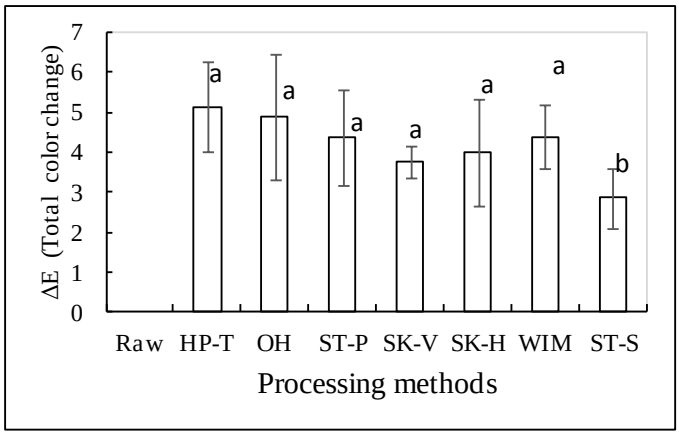

Figure 7.4. Effect of different processing methods on total color change of carrot. , ST-S= steam sterilization (121°C) in static retort, ST-P= steam pasteurization (97°C) in static retort, WIM= water immersion mode (97°C) in static retort, SK-V= Shaking mode of heating (97°C) in water bath cans in vertical orientation, SK-H= Shaking mode of heating (97°C) in water bath cans in horizontal orientation, OH= Ohmic heating (97°C)

7.3.3.3 β-carotene content

Carrots are the richest source of α- and β-carotene of common fruits and vegetables consumed all over the world. Beta-carotene is an important nutrient that is responsible for the orange color of carrots. It can act as a pro-vitamin A and as an antioxidant, by which it can help to protect humans from several diseases (Britton 1995). The impact of thermal and HP

processing on the carotenoid content of carrots was largely dependent on the processing intensity applied. Results in Figure 7.5 show that samples in water immersion mode (WIM) resulted in better β-carotene yield. This might be that more β-carotene could be released from carotene responsible matrixes of carrot tissue during relatively extended heat (come up time of 19.5 min, Table 7.3) treatment. The more severe treatment enhanced the extractability of β-carotene as compared to the less severe treatment in case of HP which is in agreement with results indicated in chapter 6. Different researchers (Lopez et al., 2008; Vásquez-Caicedo et al., 2006) reported that β-carotene exist in crystalline form in carrot chromoplast. This intracellular localization while bounded with other macromolecules implies that the compound should be released from such a matrix for easy extractability through relatively more severe treatments. Aguilera (2005) and Waldron et al. (2003) also reported that the cell walls could influence the release of β-carotene from tissue matrix, evidanced in the case of HP, since better texture retention was observed as compared to low β-carotene content (Figure 7.5). As processing can affect the food matrix and cell wall, it can also influence the accessibility of carotenoids. However, with more intensive thermal treatment (ST-S) increase in β-carotene extractability was not observed. This might be associated with enhanced degradation of the compound due to oxidation and other mechanisms than mechanisms enhancing extractability (Lemmens et al., 2009). But in general no considerable difference was observed in terms of β-carotene extractability among other thermal pasteurization methods which is in agreement with Liesbeth and others (2012) results.

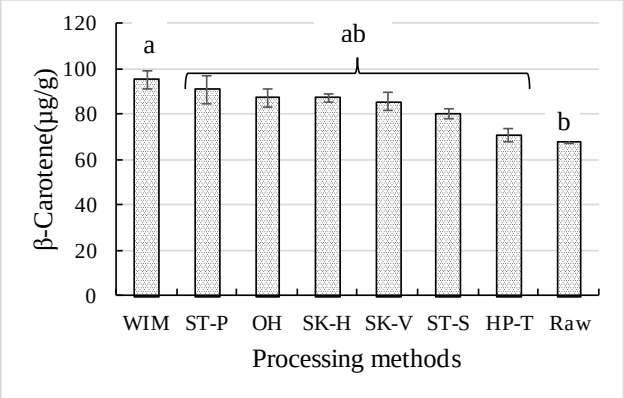

Figure 7.5. Effect of processing methods on β-carotene content of processed carrot. , ST-S= steam sterilization (121^{o}C) in static retort, ST-P= steam pasteurization (97^{o}C) in static retort, WIM= water immersion mode (97^{o}C) in static retort, SK-V= Shaking mode of heating (97^{o}C) in water bath cans in vertical orientation, SK-H= Shaking mode of heating (97^{o}C) in water bath cans in horizontal orientation, OH= Ohmic heating (97^{o}C)

7.4 CONCLUSIONS

In conventional production of acid or acidified low acid foods (pH < 4.6) the presence of *C. botulinum* (type A and B) spores might not be a health concern and moderate processing conditions are commonly practiced. However the co-existence of spores of *B. licheniformis* in such type of foods with spores of *C. botulinum* could endanger public health, because the former has the capacity to grow in acid or acidified foods and hence elevate the pH above 4.6 in which the latter can grow and produce toxin. Therefore in these types of foods, complete inactivation of spores of *B. licheniformis* is a critical step to ensure food safety. Designed processing schedules were found sufficient enough to achieve required degree of spore inactivation to minimize food risk issues associated with spores of *B. licheniformis* in production of acid or acidified low acid foods.

CHAPTER EIGHT

8 GENERAL CONCLUSIONS

In this study, attempts were made to improve quality of canned vegetables through combined action of pH modification of products and novel processing methods as alternative processing approaches. Microbial safety and quality retention advantages of combined acid infusion-novel processing methods were based on *B. licheniformis* as a target microorganism which is a good marker for assuring the safety of acidified low acid foods. The following findings were the specific highlights of the study:

i. Novel acid infusion method was designed, evaluated and optimized to reduce pH of low acid vegetables below 4.6 which could allows the use of moderate processing conditions for better quality retention and energy savings. High pressure assisted acid infusion method overcomes limitations associated with conventional acidification techniques. The method achieves more uniform and rapid pH reduction of low acid foods. Furthermore the determined acidification kinetics parameters and developed predictive models in this study could be used as an input to optimize acid infusion conditions for other high pH vegetables. In addition, this approach provides ample opportunities to use different novel and conventional thermal or pressure assisted thermal processing methods as a moderate processing approach for better quality retention.

ii. One of the basic requirements in thermal processing is the availability of thermal inactivation kinetics data of target microorganisms. Inactivation kinetic data of *B. licheniformis* as a target food microorganism were determined for the first time at different heating methods (conventional heating versus ohmic heating), type of acidifying agents and pH levels. Determined kinetic data in this study can be used to design, develop and optimize food processing conditions to ensure food safety and better quality retention. Kinetic data at pH 4.5 could be used as an input to design and validate different thermal processing methods in production of acid or acidified low acid foods.

iii. Likewise thermal processing methods, pressure assisted thermal inactivation kinetic data of *B. licheniformis* spores were determined at different pressure-temperature combinations and pH levels. Experimental results were evaluated based upon first order kinetic and Weibull models. Pressure and temperature dependency inactivation parameters were determined at different pH levels. Based on determined kinetic parameters pressure assisted death times of spores were determined and evaluated using both models. Results showed that convergence of the two models to predict the death time in lower degree of lethality (3-log cycle reduction) and a divergence at higher degree of lethality (> 4 log cycle reduction). The impact of using either of one of the model for pressure assisted-thermal inactivation of spores of *B. licheniforms* is clearly demonstrated in terms of food safety and quality.

iv. Acidification of low acid vegetables in conventional ways is mainly done to shift extent of heat treatment from intensive to moderate processing conditions for better quality retention. However infused acid in carrot tissue further played a critical role in terms of minimizing the rate of cell wall degradation. Macroscopic (texture softening), microscopic (cell wall integrity) and molecular (pectin depolymerization) studies were employed to elucidate the mechanism on how infused acid retards or protects texture degradation during processing. Acid infused samples showed better texture retention with more cell wall integrity and less β-elimination products as compared to non-infused ones. This showed that infused acid retards the rate of cell wall degradation through minimizing or slowing down the rate of β-elimination reaction, which is the main cause for pectin depolymerization and loss of texture than demethoxylation reaction. Therefore combined use of acid infusion with moderate processing methods (ohmic heating or moderate pressure-modrate-heat treatment) could result in more and more quality improvement advantages as compared to sole use of novel processing methods. This could be considered as an alternative approach to improve quality of low acid vegetables through modifying the product property and use of alternative processing technologies.

v. The validity of designed processing methods in terms of food safety and quality retention advantages were conducted under commercial processing conditions for different

processing methods. Validation study was conducted considering worst case scenario (high inoculum concentration, slowest heating points in the product and container). Results confirmed that minimum delivered process lethality were higher than required process lethality to achieve more than 7-log reduction in spores count. In terms of quality retention among evaluated processing methods, acid infused samples from moderate pressure-moderate heat (600MPa, 60^{o}C, and 4.4 min) treatment and ohmic heating (97^{o}C, 7.7 min) exhibited superior quality retention. Samples from conventional canning (121^{o}C, 10 min) showed inferior results in terms of texture retention and color.

RECOMMENDATIONS FOR FUTURE STUDY

i. It is recommended to conduct pressure assisted acid infusion in meat and meat based products to reduce their pH for mild processing conditions. The effect of different acidifying agents and acid infusion parameters needs further study to enhance the wide use of the technology for different type of foods.

ii. Combined use of acid infusion and novel thermal processing methods (microwave and radio frequency) needs further study and optimization to benefit from combined use of pH reduction and electro-heat technologies.

iii. The flexibility of Weibull model in terms of describing inactivation behavior of lethal agents on vegetative cells or spores is indicated in many studies. However the use of Weibull model parameters in terms of describing decimal reduction time and thermal death time needs further study. Revision or modification of so far recommended equations to determine decimal reduction time or thermal death time are important to use the model in biological areas and to establish processing schedules.

iv. *B. licheniformis* is a potential candidate to be used as a surrogate reference food microorganism for acid elevating Bacillus species. But further studies in different types of acid or acidified foods and type of processing methods are required to generate

sufficient inactivation kinetic data in order to get approval from reualtory bodies to use the bacterium as a reference microorganism .

v. Infusion of acid in low acid foods improves texture of thermally processed vegetables through reducing the softening rate. This advantage is gained due to reduced pectin dissolution because of reduced β-elimination reaction. However the mechanism on how the rate of β-elimination reaction is reduced is not yet known. This needs further study to maximize the benefit of controlled pH reduction in low acid foods during thermal processing.

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