Development of an ultrasound-steam process for microbial surface decontamination, enzyme inactivation and heat transfer enhancement

By

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ABSTRACT

The development of a combined ultrasound and steam heating process for surface microbial decontamination, enzyme inactivation, heat transfer enhancement and pasteurization of high acid and acidified foods represents a significant advancement in novel food processing technology. This study was aimed at investigating the effectiveness and feasibility of utilizing combined ultrasound and steam process to achieve these objectives in separate studies.

The study involved subjecting fresh vegetables (carrots, cauliflower, broccoli, & zucchini) to combined ultrasound and steam treatment and comparing them with traditional heating method (steam alone) under various operating conditions (in presence or absence of air). Initial and residual microbial enumeration was carried out to evaluate the decontamination efficacy using non pathogenic *Escherichia coli* K-12 (and compared against common pathogens), while the enzyme activity was evaluated to assess the degree of enzyme inactivation achieved by the treatment. Additionally, heat transfer enhancement by ultrasound and steam combination heating was analyzed by monitoring temperature profiles in foods and by simulating the food particles during the treatment process.

The results demonstrated that the ultrasound process effectively enhanced the microbial decontamination on the surface of fresh vegetables as compared to heating in steam or steam/air media directly. The ultrasound wave likely disrupted insulation effect of the surface air layer thereby improving the condensation heat transfer from steam leading to more effective and enhanced microbial destruction. The treatment achieved significant reductions in bacterial load (>4 logarithmic cycles) to facilitate improved food safety and quality.

Furthermore, the ultrasound and steam combination process successfully established the vegetable blanching process by inactivating the oxidative enzymes (peroxidase used as the index) present in fresh vegetables. The ultrasound waves enhanced the effectiveness of both steam and steam/air heating conditions for effective inactivation of oxidative enzymes, a necessary requirement for successful freezing and storage of vegetables. This enzyme inactivation also contributed to better retention of nutrients and delayed enzymatic deterioration, enhancing the overall freshness and quality attributes of vegetables. Blanching time was established for different vegetables (carrots, cauliflower, broccoli, green bell peppers and zucchini) based on a ~95% reduction in peroxidase activity. The vegetables blanched using combined ultrasound and steam or ultrasound and water technology were

compared with traditional hot water and steam blanching and demonstrated to have better quality retention during frozen storage.

Moreover, the combined ultrasound and steam heating process demonstrated notable improvements in heat transfer efficiency in foods packaged in flexible pouches. The application of ultrasound waves presumably promoted container agitation and liquid/particle mixing thereby facilitating uniform distribution of heat within the flexible pouches. Tests were carried out with equivalent pasteurization treatments for different foods (mango pieces, apple slices, pineapple wedges and acidified carrots). The process resulted in enhanced heat transfer leading to reduced processing times which further resulted in improved energy efficiency and product quality.

The findings highlight the potential of the combined ultrasound and steam process as a promising alternative for surface microbial decontamination and enzyme inactivation in fresh vegetables, and heat transfer enhancement in high acid and acidified low acid foods packaged in flexible pouches. This innovative approach offers a comprehensive solution for ensuring food safety, extending shelf life, and maintaining better product quality. Further optimization of process parameters and scale-up studies are warranted to facilitate its practical implementation in the food industry. Overall, the combined ultrasound and steam heating process holds great promise for transforming food processing practices and contributing to safer, healthier, and more sustainable food systems.

RÉSUMÉ

Le développement d'un procédé combiné d'ultrasons et de vapeur pour la décontamination microbienne de surface, l'inactivation enzymatique, l'amélioration du transfert de chaleur et la pasteurisation des aliments très acides et aliments acidifiés représente une avancée significative d’une la nouvelle technologie de transformation des aliments.

Cette étude visait à évaluer l'efficacité et la faisabilité de l'utilisation d'un processus combiné d'ultrasons et de vapeur pour atteindre ces objectifs dans des études distinctes.

L'étude consistait à soumettre des légumes frais (carottes, chou-fleur et brocoli) à un traitement combiné par ultrasons et vapeur et en les comparant au mode de chauffage traditionnel (vapeur seule) dans différentes conditions de fonctionnement (en présence ou en l'absence d'air). Dénombrement microbien initial et résiduel a été réalisé pour évaluer l'efficacité de la décontamination à l'aide *d'Escherichia coli* non pathogène -K 12 (et comparée à des agents pathogènes courants), tandis que l'activité enzymatique a été examinée pour évaluer le degré d'inactivation enzymatique atteint par le traitement.

De plus, l'amélioration du transfert de chaleur par le chauffage combiné aux ultrasons et à la vapeur a été analysée en surveillant les profils de température dans les aliments et en simulant les particules alimentaires pendant le processus de traitement.

Les résultats ont démontré que le procédé par ultrasons améliorait efficacement la décontamination microbienne à la surface des légumes frais par rapport au chauffage à la vapeur ou à la vapeur/air directement. L'onde ultrasonore a probablement perturbé l'effet d'isolation de la couche d'air de surface, améliorant ainsi le transfert de chaleur de la vapeur conduisant à une destruction microbienne plus efficace et améliorée. Le traitement a permis d'obtenir des réductions significatives de la charge bactérienne (> 4 cycles logarithmiques) pour améliorant la sécurité et la qualité des aliments.

De plus, le processus de combinaison des ultrasons et de la vapeur a établi avec succès le processus de blanchiment des légumes en inactivant les enzymes oxydatives (peroxydase utilisée comme indice) présentes dans les légumes frais. Les ondes ultrasonores ont amélioré l'efficacité des conditions de chauffage à la vapeur et à la vapeur/air pour une inactivation efficace des enzymes oxydatives, une condition nécessaire pour une congélation et un stockage réussi des légumes. Cette inactivation enzymatique a également contribué à une

meilleure rétention des nutriments et à la détérioration enzymatique retardée, améliorant la fraîcheur globale et les attributs de qualité des légumes. Le temps de blanchiment a été établi pour différents légumes (carottes, chou-fleur, brocoli, poivrons verts et courgettes) sur la base d'une réduction de 95 % de l'activité de la peroxydase. Les légumes blanchis à l'aide de la technologie combinée ultrasons et vapeur ou ultrasons et eau ont été comparés au blanchiment traditionnel à l'eau chaude et à la vapeur et ont démontré une meilleure conservation de la qualité pendant la congélation.

De plus, le processus combiné de chauffage par ultrasons et vapeur a démontré des améliorations notables de l'efficacité du transfert de chaleur dans les aliments emballés dans des sachets souples. L'application d'ondes ultrasonores a vraisemblablement favorisé l'agitation du récipient et le mélange liquide/particule, facilitant ainsi une distribution uniforme de la chaleur à l'intérieur des sachets souples.

Des tests ont été réalisés avec des traitements de pasteurisation équivalents pour différents aliments (morceaux de mangue, quartiers d'ananas et carottes acidifiées). Le processus a permis d'améliorer le transfert de chaleur, ce qui a réduit les temps de traitement, ce qui a encore amélioré l'efficacité énergétique et la qualité du produit. Les résultats mettent en évidence le potentiel du procédé combiné ultrasons et vapeur comme alternative prometteuse pour la décontamination microbienne de surface et l'inactivation enzymatique de légumes frais, et l'amélioration du transfert de chaleur dans des aliments très acides et – faiblement acidifiés emballés dans des sachets souples. Cette approche innovante offre une solution complète pour garantir la sécurité alimentaire, prolonger la durée de conservation et maintenir une meilleure qualité des produits. Une optimisation plus poussée des paramètres du processus et des études de mise à l'échelle sont justifiées pour faciliter sa mise en œuvre pratique dans l'industrie alimentaire. Dans l'ensemble, le processus combiné de chauffage par ultrasons et vapeur est très prometteur pour transformer les pratiques de transformation des aliments et contribuer à des systèmes alimentaires plus sûrs, plus sains et plus durables.

STATEMENT FROM THE THESIS OFFICE

According to the regulation of the Faculty of Graduate Studies and Research of McGill University, Guidelines for Thesis Preparation include:

Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted for publication, or the clearly duplicated text of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" and must be bound together as an integral part of the thesis.

The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. To ensure that the thesis has continuity, connecting texts that provide logical bridges between the different papers are mandatory.

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CONTRIBUTION OF AUTHORS

Several parts of this research have been presented at national and international conferences and submitted for publication in journals. Some authors have been involved in manuscripts.

Their contributions are as follows:

Regina Basumatary is the PhD candidate who planned and conducted experiments, gathered, and analyzed data, and wrote all manuscripts under the supervision of Professor Ramaswamy.

Dr. H.S. Ramaswamy is the thesis supervisor, under whose guidance the research was planned and conducted. In addition, submission of all the manuscripts was carried out by him after his careful correction, edition, and reviewing.

Dr. Hamed Vantankhah helped in equipment setup and data analysis of the microbial studies and preliminary heat transfer studies.

Dr. Ali Taherian offered technical advice and instructions regarding the textural measurements in enzyme inactivation and storage studies and helped in the frozen storage studies in Chapter 5.

Mr. Maheshwor Bastakoti helped in the frozen storage studies - in Chapter 5 and conducted part of heat transfer studies in Chapter 6.

Mr. Ali Asgar Rampurwala helped in the frozen storage studies in Chapter 5.

Mr. Neelakanth Lamani helped in the frozen storage studies in Chapter 5.

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LIST OF PUBLICATIONS AND PRESENTATIONS:

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Basumatary R, and Ramaswamy H.S., 2023. Comparison of ultrasound steam, steam/air, and ultrasonic hot water bath pasteurization processes for acidified carrots. (Ready to be submitted to Innovative Food Science & Emerging Technologies Journal).

Basumatary R, and Ramaswamy H.S., 2023. **U**ltrasound enhanced steam heating process for surface microbial decontamination of fresh vegetables and thermal destruction kinetics of microorganisms. (In preparation to submit to Journal of Food Process Engineering).

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Conference Presentations:

Basumatary R, Vantankhah H, and Ramaswamy H.S. 2019. "Evaluation of heating characteristics of porous materials in the presence of steam and sound combinations". Poster presentation in ASABE NABEC Section Meeting June 15th-June 19th, Quebec City, Canada

Basumatary R, Vantankhah H, Dwivedi M, John D, and Ramaswamy H.S. 2020. "Ultrasound-steam combination process for microbial decontamination and heat transfer enhancement: Overview and Concept Testing". Poster presentation in IFTPS Annual Meeting in San Antonio (March 3-5, 2020). Won 3rd prize in 2020 IFTPS Charles R. Stumbo Student Paper Competition.

Basumatary R, Vantankhah H, and Ramaswamy H.S. 2020. Evaluation and modelling of peroxidase inactivation kinetics using piezo steam. Northeast Agricultural and Biological Engineering Conference (NABEC) 2020. July 28-29, 2020, two-day online event.

Basumatary R, and Ramaswamy HS. 2021. "Evaluation of ultrasound-steam blanching process for enhancing the effectiveness of enzyme inactivation and quality retention in vegetables". Poster presentation in IFT FIRST 2021 Virtual Program July 18th -July 21st, 2021.

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Basumatary R, and Ramaswamy H.S. 2023. “Use of ultrasound-steam processing in Food industry – a bibliometric analysis.” Poster presentation in ASABE NABEC Section Meeting July 31st -August 3rd, University of Guelph, Canada.

CONTRIBUTION TO KNOWLEDGE

- The study provided a physical alternative for microbial decontamination of vegetables which is mostly done with chemical treatments.
- The study helped establish novel processing methods for surface decontamination of pathogens in vegetables and for enzyme inactivation of vegetables prior to freezing.
- Combined ultrasound steam study is new and novel, largely unexplored. Results provide application of novel physical processing methods for microbial decontamination and enzyme inactivation.
- This is the first major studies on the ultrasound-steam application for microbial decontamination of vegetable surfaces and enzyme inactivation, as well as, blanching prior to freezing and storage, and pasteurization of acid and acidified foods in flexible pouches.
- This provides an effective and environmentally friendly physical method for microbial decontamination of vegetables which can be successfully used for surface microbial decontamination studies.
- While steam and ultrasound have been independently used successfully for various food processing applications, their combination has not been widely evaluated either to improve the efficacy of surface decontamination, enzyme inactivation or improve overall rate of heat transfer.
- This study showed combined ultrasound-steam can be successfully implemented for effective blanching with reduced treatment time to achieve enzyme inactivation of vegetables prior to freezing.
- The results are potentially useful in the food processing industry (canning, drying, and freezing) for better quality retention and process turn over as effective blanching treatment for frozen vegetables.
- Potential economic benefits: Reduction in treatment time to achieve microbial lethality and enzyme inactivation and retention/Increase in product quality can lead to potential economic benefits.
- Due to the reduction in process time, ultrasound-assisted steam blanching may be considered as a green technology and limits the use of steam when compared to conventional steam and hot water blanching especially in reducing effluent waste generation.

Dedicated to

My lovely parents Mr. Kalendra Basumatary and Mrs. Sumitra Basumatary

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1

1. 0. Introduction

In recent years, the food industry has become more competitive and dynamic due to the increasing awareness of consumers in terms of what they eat. In the past, the overall aim of food processing was to provide food products that are safe to consume and to extend their shelf life; however, today's consumers demand for food products that are safe to consume and provide significant nutritional contribution, bioactive compounds, and good sensory properties. The type of food processing involved affects the important food quality attributes such as taste, texture, appearance, and nutritional content. Thermal processing is one of the most common and cost-effective methods for achieving safe foods with an extended shelf life. However, it is well known that exposing food to high temperatures can cause nutritional degradation, physical and chemical changes, and ultimately organoleptic changes (Majid et al., 2015). Due to this, novel technologies have emerged to meet the consumer needs by either improving or replacing conventional technologies. The food industry has created conservation strategies that offer higher yields, cheaper prices, and enhanced food safety in response to consumer requests for minimally processed, wholesome, and nutritious meals. Ultrasound or sonication applications stand out among emerging food preservation technologies for being sustainable, non-destructive, effective, and having a short processing time, in addition to minimising losses of nutritional and bioactive components, causing fewer changes in the sensory characteristics, and lengthening the shelf life of food products (Alenyorege et al., 2019; Tremarin et al., 2019; Xu et al., 2021b). In contrast to conventional heat treatment, ultrasound treatment has been investigated as a preservation technique with considerable potential for extending the shelf life of foods, guaranteeing food safety, and minimising the loss of nutritional value and sensory quality. The germicidal action of ultrasound over a wide range of microorganisms in many liquid foods has been clarified by numerous investigations (Nunes et al., 2022).

1.1. Microbial decontamination (short time exposure)

Fruits and vegetables in their freshly harvested form are most appealing to consumers from sensory and quality perceptions. However, most fruits and vegetables, as they are procured after harvesting, are likely contaminated with field bacteria, some of which could be pathogenic like *Escherichia coli*, *Salmonella* spp. etc. The contaminants could be air borne or could find their way from contaminated water used in the field or processing facility. Processers need to make sure these products are decontaminated prior to the marketing of these commodities. Sanitation using chlorine and other chemicals like Cetylpyridinium chloride (CPC) is the most common treatment used to tackle the problem; however, residue problems have caused a great concern to consumers. Physical treatments are better choices because they don't suffer from residue concerns. Low dose irradiation, pulsed light, plasma are among the physical treatments practiced.

Thermal processing has been the most successful application used for food preservation. The most widely and successfully used medium effectively for thermal processing is perhaps steam with its associated highest level of heat transfer coefficient. Air trapped in steam greatly reduces the condensation heat transfer associated with steam. Entrapped air in retort operations is generally removed by venting treatments. Short term exposure to mild steam and hot water treatments have been used for surface microbial decontamination in some studies. Hot water immersion of inoculated whole broiler thigh pieces at temperatures of 80°C and 85°C for 10 seconds led to significant reductions of 1.09 and 1.25 cfu/g in total viable bacteria ($P \leq 0.05$). Furthermore, the immersion in water at 75°C, 80°C or 85°C for 10 seconds showed significant decrease in thermophilic *Campylobacter* on artificially contaminated broiler carcasses ($P \leq 0.05$). Exposure to atmospheric steam at 90°C for 24 seconds significantly reduced the counts with 0.75, 0.69 and 1.3 $\log_{10}$ cfu/g in total viable bacteria, *Enterobacteriaceae*, and thermophilic *Campylobacter* (Whyte et al., 2003). Efforts have also been made to solve the problem of presence of air by subjecting the food to prior vacuum. Vacuum-steam-vacuum (VSV) process studies have been introduced to increase the efficiency of heat transfer for decontamination of different types of food products (Hassan et al., 2015; Hormansperger et al., 2016; Shah et al., 2017). Though VSV process efficiently decontaminates many food products, the presence of air that comes with the product when introduced in a continuous system can still pose the same problem.

In the above applications, there is always air entrapment as the product is brought for treatment. During the short heating time, the presence of air along the surface and internal tissues of food products, might pose some serious difficulties for rapid steam heating. Without rapid heating, the treatment times are likely to be long resulting in quality damage. The heat and mass transfer mechanisms are affected by the presence of air which slows down the effective heating at the product surface. Adding an ultrasound environment during steam heating can help to remove this surface resistance barrier contributed by air and promote a better heat transfer. Microbial decontamination of food surface can then be achieved in a more effective manner with a short-duration heat treatment provided by the combination of ultrasound and steam. A limited success has been achieved by a commercial process called SonoSteam® for killing microorganisms such as *Campylobacter, Salmonella, Listeria and E.coli* on the skin and surface cavities of poultry and other food products. Some propriety type of research has been published demonstrating some microbial destruction during such a process (Musavian et al., 2014, 2015; Morild et al., 2011; Anderson et al., 2011). A study by Moazzami et al., 2020, assessed the efficacy of ultrasound and steam combination heating (SonoSteam®) on naturally contaminated chicken carcasses in a large-scale Swedish abattoir. Ultrasound at 30-40 kHz and steam at temperatures of 84-85°C or 87-88°C were applied during slaughter, with a line speed of 18,000 birds per hour. The study showed that SonoSteam® treatment led to reduction of log 0.5 ± 0.8 for *Campylobacter jejuni*, log 0.6 ± 0.6 for *Enterobacteriaceae,* log 0.5 ±0.6 for *E. coli* and log 0.4 ±0.7 CFU/g for total aerobic bacteria. Another study by Musavian et al., 2022, showed significant bacterial reduction achieved in three different broiler surface areas (back, breast and neck) at a slaughter speed of 10,500 birds per h at temperatures more than 80°C under the SonoSteam® treatment. The rapid treatment of less than 1.5s exposure time inside the chamber makes the combined ultrasound and steam technology potentially suitable for modern and fast poultry processing lines. The technology has gained FDA recognition although the reduction in microbial count reported has been relatively small (~2 log kill). The combined ultrasound and steam technology has potential to rapidly heat and reduce microbial loads in short heating cycles of <15s. The process is effective for steam heating of food surfaces, which can be slow at start due to the presence of air. The penetration power and heating efficiency of steam treatment is enhanced significantly by sound waves in the ultrasound and steam combination heating process. There is a large volume of information on the use of ultrasound treatment or pre-treatment for enhancing heat and mass transfer in drying, freezing and cleaning applications (Rodriguez et al., 2018; Musielak et al., 2016; Cheng et al., 2015); however, these are limited to sound propagation in *liquid* phase. Very limited data are available on the application of ultrasound to solid foods (Piyasena *et al*., 2003) and almost no such heat transfer studies, and microbial surface decontamination studies have been done on ultrasound and steam combination heating of fresh vegetables.

1.2.Enzyme inactivation (medium time exposure)

Thermal blanching is an important unit operation for processing of many fruits and vegetables. It is given as a short heat treatment for the purpose of inactivating oxidative enzyme present in fruits and vegetables whose activity can result in quality change during storage, especially after freezing and drying. The conventional water and steam blanching methods are most used by many food processors. Water blanching is efficient, but uses a lot of energy, produces large volumes of effluent (loss of energy and production of waste), and promotes nutrient leaching into the water used for blanching. The steam blanching is relatively less effluent producing, more effective and retains better product quality as compared to hot water blanching. This is generally carried out in tunnels or steam boxes at atmospheric pressures where the presence of air during the steam blanching process reduces the associated heat transfer from steam and the prevailing low steam velocity results in longer blanching times leading to higher energy consumption and product quality loss. Ultrasound and steam technology has the potential to enhance the heat transfer by reducing the influence of air and providing some structural vibration agitation thereby, enzyme inactivation could be achieved faster by ultrasound- steam blanching and result in better product quality.

1.3.Acidified low-acid and acid food thermal processing (longer time exposure)

Acid and acidified foods ($pH < 4.6$) are processed at <100°C requiring less energy demanding technologies for pasteurization. Hot water and steam have been used for pasteurization of acid and high acid foods ($pH<4.6$). Low acid foods ($pH>4.6$) can be acidified and then treated like acid or high acid foods at pasteurization conditions, and these will gain the same advantage of lower process temperatures and less severe heat treatment as compared to low acid foods. Novel thermal processing technologies such as microwave, radio-frequency, ohmic heating and high-pressure processing can also be used for high acid food pasteurization (Lau and Tang 2002; Tola and Ramaswamy 2018). Medium and container agitation during processing can enhance the overall rate of heat transfer to the contents and help reduce the heating time there by improving product quality. Both ultrasound - water and ultrasound - steam combination treatments could increase the medium heat transfer rate into containers for enhancing the heat transfer to the product. This concept can be used for both pasteurization and commercial sterilization of foods giving better product quality compared to conventional thermal processing methods.

Ultrasound-steam and ultrasound-water based technologies can also be used to induce agitation within flexible thin profile packages which enhances the mixing and heat transfer within the package. Thermal processing of foods in flexible packages has been well researched. For agitation, mostly external mechanisms involving container movement like end-over-end, axial and reciprocal agitation have been researched (Sablani and Ramaswamy, 1996; Singh et al., 2017). However, there are no studies on the induced agitation in flexible packages under the influence of sound in steam and water.

1.4. Research hypothesis

The thesis research was based on the following hypothesis:

- Ultrasound and steam combination heating will overcome the surface resistance barrier contributed by vegetable tissue air and promote a better heat transfer.
- Combining ultrasound with steam provides enhanced heat transfer and permits shorter time exposure needed for microbial decontamination and the somewhat medium time exposure for blanching (enzyme inactivation) prior to frozen storage.
- Ultrasound and steam combination heating also enhances heat transfer associated with fruits and vegetables packaged in thin profile flexible containers for providing more efficient pasteurization conditions.

1.5. Research objectives

Therefore, the overall objective of this thesis was to study the efficacy of combined ultrasound and steam heating technology for vegetable surface microbial decontamination, enzyme inactivation and pasteurization heat transfer enhancement in flexible pouches.

The specific objectives were:

I. Establish a decontamination process using ultrasound and steam combination technology for surface microbial decontamination of different vegetables demonstrating the advantages over conventional steam.

II. Establish the blanching treatment schedule for fresh vegetables using ultrasound and steam heating process for efficient inactivation of enzyme in different vegetables and compare with conventional treatment.

III. Compare ultrasound blanching with conventional blanching for quality and stability of frozen vegetables during storage.

IV. Evaluate ultrasound and steam heating, and ultrasound and water combination treatment processes for enhancing heat transfer.

V. Developing ultrasound and steam, and ultrasound and water combination treatment processes for pasteurizing naturally acid and acidified foods in flexible pouches.

Chapter 2

2.0. Literature Review

2.1. Microbial Decontamination

Food borne diseases continue to be a global public health and economic burden. According to 2018 World Bank report, US$ 110 billion per year is associated with total productivity loss and medical expenses resulting from unsafe foods in low and middle - income countries. Recently (30th April 2020), WHO reported that every year almost 33 million people die and an estimate of 600 million people (almost 1 in 10 people in the world) fall ill after eating contaminated food.

With the increase in consumption of fresh produce, outbreaks of food borne illness have also increased. Food surface contamination is one of the major causes for these outbreaks. Food can be contaminated through direct sources - during production (fertilizer or raw sewage), during processing (contaminated water used for irrigation, cooling or washing) and during distribution (contaminated ice used for storage or transport, unhygienic handling) or indirect sources (cross-contamination while food preparation). Food contamination can be classified into biological, physical, and chemical contamination. Biological contamination is the major cause for food borne illness and food poisoning. Biological contamination occurs when food is contaminated by humans, rodents, insects, and microorganisms. Various pathogens and spoilage bacteria are responsible for hampering food safety and quality and risking public health safety. Most common pathogens responsible for outbreaks of food borne illness as per Centers for Disease Control and Prevention (CDC) and Food and Drug Administration (FDA) in the recent years (2018-2023) are *E. coli, Salmonella, Listeria, and Cyclospora species.* Examples of some recent outbreaks (2018-2020) caused by pathogens as per CDC in different types of fruits and vegetables are shown in Table 2.1.

The increase in reported food borne illness associated with consumption of raw produce can be attributed to improved surveillance systems as well as insufficient hygiene practices, changes in global trade, increased frequency of consuming meals at food service establishments and produce production, processing, and marketing practices (Park et al., 1999). The increase consumption of fresh and minimally processed produce results in the increased interest in fresh produce safety.

Table 2.1: Recent outbreaks (2018-2023) of food borne illness caused by fruit and vegetable consumptions as per CDC

Pathogen responsible	**Food product**	**Total illness**	**Hospitaliza-tion**	**Deaths**	**Outbreak year**	**Reference**
Hepatitis A Virus infections	Frozen organic strawberries	9	3	0	May 2023	CDC 2023
Salmonella typhimurium	Alfalfa sprouts	63	10	0	December 2022	CDC 2022
Hepatitis A Virus Infections	Fresh organic strawberries	19	13	0	March 2022	CDC 2022
Listeria monocytogenes	Packaged salads	10	10	1	December 2021	CDC 2021
E.coli O157:H7	Packaged salads	10	4	1	November 2021	CDC 2021
E.coli O157:H7	Baby spinach	15	4	0	October 2021	CDC 2021
Listeria monocytogenes	Packaged salads	18	16	3	October 2021	CDC 2021
Salmonella typhimurium	Packaged salad greens	31	4	0	June 2021	CDC 2021
Salmonella oranienburg	Onions	1040	260	0	May 2021	CDC 2021
E.coli O157:H7	Leafy greens	40	20	0	August 2020	CDC 2020
Salmonella newport	Red onions	396	59	0	July 2020	CDC 2020
Cyclospora	Bagged salads	641	37	0	June 2020	CDC 2020
Listeria monocytogenes	Enoki mushrooms	36	31	4	March 2020	CDC 2020
E. coli 0103	Clover sprouts	51	3	0	Feb 2020	CDC 2020
Salmonella javiana	Cut Fruits	165	73	0	December 2019	CDC 2019
E.coli O157:H7	Salad mix	10	4	0	December 2019	CDC 2019
E.coli O157:H7	Romaine lettuce	167	85	0	November 2019	CDC 2019
Cyclospora	Fresh basil	241	6	0	July 2019	CDC 2019
Salmonella uganda	Fresh papayas	81	27	0	June 2019	CDC 2019
Salmonella carrau	Pre-cut melon	137	38	0	April 2019	CDC 2019
E.coli O157:H7	Romaine lettuce	62	16	0	October 2018	CDC 2018
Cyclosporiasis	Fresh salad mix	511	24	0	September 2018	CDC 2018
Cyclosporiasis	Vegetable tray	250	8	0	September 2018	CDC 2018
Salmonella adelaide	Pre-cut melon	77	36	0	July 2018	CDC 2018
Salmonella sandiego	Spring pasta salad	101	25	0	June 2018	CDC 2018
E.coli O157:H7	Romaine lettuce	210	96	5	April 2018	CDC 2018
Salmonella infantis	Vegetable trays	5	0	0	Spring 2019	CDC 2018

Various microbial decontamination techniques have been practiced in the food industry. The current decontamination techniques can be classified into two a) Chemical and b) Physical methods.

2.1.1. Chemical methods

Different chemicals are added in the wash water or process water to reduce the potential spread of microorganisms. Use of antimicrobial chemicals in wash waters of fresh produce is practiced in some countries while others do not permit its use. For example, regulation on antimicrobial treatment of fresh produce does not exists in the European Union even though Commission Regulation (EC) No 2073/2005 on microbiological criteria for foods clearly describes the microbiological limits to which certain types of produce need to comply. The local authority in different countries determines the use of chemical decontaminants in water used for processing of the fresh produce. Thorough evaluation of any antimicrobial chemical before approval is necessary for potential toxicological issues.

Effectiveness for pathogen reduction in fresh produce has been evaluated on a wide range of antimicrobials including ozone, peracids (e.g., peroxyacetic acid), chlorine dioxide, chlorinated trisodium phosphate, hypochlorite and hypochlorous acid (including electrolyzed oxidizing water), and acidified sodium chlorite (Beuchat et al., 1998; Stopforth et al., 2008). The various studies on the use of different antimicrobials such as chlorine and electrolyzed oxidizing water, chlorine dioxide gas and acidified sodium chlorite (ASC), organic peroxides, hydrogen peroxide, ozone, Cetylpyridinium chloride (CPC) and organic acids have been summarized in a book (Dimirci and Ngadi, 2012). The book also explains about the disadvantages of antimicrobial treatments as no standardized protocol is available to evaluate the effectiveness of antimicrobial treatments which makes it difficult to compare the relative effectiveness of different antimicrobial treatments through a review of the literature. Also, with the increase in interest for healthy lifestyle and awareness from consumers the use of chemicals in fresh produce treatment is discouraged.

2.1.2. Physical methods

The physical methods for microbial decontamination can be further classified as:

2.1.2.1. Traditional methods

Use of heat, hot water and steam for pasteurization, cooking, sterilization, frying, baking etc. are most common traditional methods used for making the foods palatable as well as for achieving safety through microbial decontamination. The process of application of heat is not same for all these different applications, some require dry heat and other require wet heat, some short treatment, and others long, some carried out at lower temperatures while others at higher temperatures, some at atmospheric pressure while others at higher pressures, etc. Even mild heating applications may not be suitable for sensitive products such as leafy greens which are intended to be consumed as a fresh product. Mild heat treatment such as immersing the produce in hot water or pasteurization has been studied for some vegetables. *E. coli* O157:H7 and *Salmonella* in inoculated mung beans were undetectable by immersing mung bean seeds in hot water at 85°C for 40 s, followed by dipping in cold water for 30 s, and soaking in chlorine water (2000 ppm) for 2 h (Bari et al., 2010). But this process is time consuming. Mild heat treatment is used for unripe fruits (vapor heat or double dip in water as used for mango, papaya, banana and other fruits for disinfestations) as they are more resistant to the adverse effects of the heat treatment.

Microbial decontamination using steam has been comparatively more effective than hot water pasteurization as the product quality is better retained. Steam surface pasteurization is a promising decontamination technology for destruction of bacteria at different stages of food production (Skåra et al., 2014). Steam is used under vacuum and operated at temperatures above and below 100°C in vacuum-steam pasteurization. Application of vacuum first is to remove air from the chamber. Hassan et al., 2015, found vacuum - steam pasteurization applied at a temperature of > 82°C for a duration of 10 s on sheep and lamb carcasses to be efficient in reducing *E. coli, Enterobacteriaceae* and total plate count (TPC). Shah et al., 2017, also found that vacuum-steam pasteurization can be effectively used to reduce pathogens such as *Salmonella PT 30, E. coli O157:H7* and *E. faecium* on low moisture foods (flaxseed, quinoa, sunflower kernels, milled flaxseed and black peppercorns) at temperature as low as 75°C and 85°C. Efficiency of vacuum-steam-vacuum (VSV) process for surface decontamination of different foods has also been studied. In this process, product is first exposed to vacuum to remove entrapped air followed by a short treatment with steam and a second vacuum treatment to cool the surface and remove the condensed steam through evaporation. During the VSV process, heat is transferred directly on the surface by steam condensation. The amount of time the food is exposed to heat is reduced (0.5 to 1.2 s) which results in a better-quality product. Kozempel et al. (2002) studied the VSV surface intervention process to remove mass transfer barriers (air and water) from the surface of products to allow a better heat transfer. The process was found to be effective to destroy bacteria, *L. innocua* and total aerobic plate count (3 to 5 log cfu/ml). However, the treatment process was effective only for specific products (papayas, mangoes, avocados, kiwis, carrots, cucumbers, and peaches) and thermal or mechanical damage was observed in bananas, cauliflower, broccoli and peppers. The VSV process has been successfully applied for treating certain vegetables, fruits, and meat at 100 to 158 (°C) for 0.03 to 20 (s) and achieved a bacterial destruction ranging from 2 to 5 (log 10 CFU) (Homansperger et al., 2016). The effectiveness of steam heating can be most efficient when combined with other techniques.

In contrast, steam heating has been successfully used as a commercial thermal process (Ball and Olson, 1957). Some early studies have recognized that addition of air to steam as in overpressure processing of flexible containers may retard heat transfer from steam, but still with a powerful turbo fan used for rapid circulation of the heating medium, a 75%/25% (v/v) steam air mixture can be effectively used (Pflug et al., 1963; Ramaswamy et al., 1983; Tung et al., 1984; Ramaswamy and Grabowski, 1996).

2.1.2.2. Novel Methods

The increasing demand of quality product apart from just being safe to consume has led to the development of novel processing methods. Many novel methods have been studied for microbial decontamination of fresh produce, meat, pork, poultry and seafoods. Novel technologies such as pulsed light, pulsed electric field, plasma, UV radiation, high pressure processing and ultrasound are gaining interest for their use in microbial surface decontamination of food products. The various studies on these emerging novel methods for microbial decontamination in food industry have been very well documented in the book "*Microbial Decontamination in the Food Industry - Novel Methods and Applications*" (Dimirci and Ngadi, 2012). Of the emerging novel technologies for microbial decontamination, ultrasound has been widely studied and has been found effective when combined with other methods for microbial decontamination. As the focus of this project is on ultrasound technology a detailed study on the use of ultrasound for microbial decontamination is explained later in this chapter.

2.2. Enzyme inactivation

Enzymes are proteins that enhance the chemical reactions by catalysis. In the catalysis process, substrates are converted into products by the action of enzymes. These products formed in the food may be desirable or undesirable which significantly effects nutritional composition and quality of the product. The well-known enzymes like polyphenol oxidases (PPO) and peroxidases (POD) which are involved in enzymatic browning reactions result in the loss of nutritional quality. Other enzymes like pectin methyl esterase, poly-galacturonase, lipases, and lipoxygenase, among others, cause a detrimental effect on the food organoleptic quality (Surowsky et al., 2015; Khani et al., 2017).

PPOs are the enzymes containing dicopper-proteins groups, which catalyze the oxidation of phenolics, a major cause of brown coloration in fruits and vegetables. These enzymes involve the hydroxylation of monophenols and oxidation of O-diphenols to O-quinones, which undergoes polymerization to form dark color melanin pigments. The other important thermostable enzymes related to enzymatic browning are peroxidases. Peroxidases are the glycoproteins with a heme as the cofactor; the primary function is oxidation of a wide variety of compounds in the presence of hydrogen donating compounds like hydroperoxides. Pectin methyl esterase causes demethylation of pectin resulting in separation of serum and cloudiness in the case of juices (Tajchakavit and Ramaswamy, 1997). Most enzymes cause deterioration and loss of quality of foods during processing, ripening and storage. In order to prevent such changes in the food quality, enzyme inactivation is necessary.

2.2.1. Blanching

The special heat treatment to inactivate enzymes in fruit and vegetable processing is known as blanching. It is a mild heat treatment unit operation prior to freezing, canning, or drying where fruits or vegetables are mildly heated to inactivate enzymes responsible for degradation. Blanching is not indiscriminate heating. Too little is ineffective, and too much damages the produce by excessive cooking, especially where the fresh character of the fruits and vegetables are subsequently to be preserved by processing. This heat treatment is applied according to and depends upon the specificity of fruits and vegetables, the objectives that are followed and the subsequent processing / preservation methods. It helps in modifying texture while maintaining the nutritional value of food (Manpreet et al., 2000). It also helps in preserving color and flavor, while removing trapped air (Bahceci et al., 2004). Blanching is responsible for inactivating enzymes that cause the development of off-flavors and off-colors (Manpreet et al.,2000). Blanching removes volatiles and metabolic gases within vegetable cells and replaces them with water, forming a semi continuous water phase that favours a more uniform crystal growth during freezing (Bahceci et al., 2004). Gas removal by blanching before canning makes the process easier, reduces strain on the can during heating and reduces can corrosion (Lenz and Lund 1977).

2.2.1.1. Blanching methods

Thermal blanching has been practiced widely in the food industry; water and steam blanching being the most traditional heating methods. Ranjan et al, 2017 categorized blanching into dry and wet blanching based on requirement of water for the process (Figure 2.1).

Wet blanching is the technique in which water or steam is used to blanch samples. It can be further categorized based on the type of water used, as hot water blanching, steam blanching and hot gas blanching (Figure 2.1).

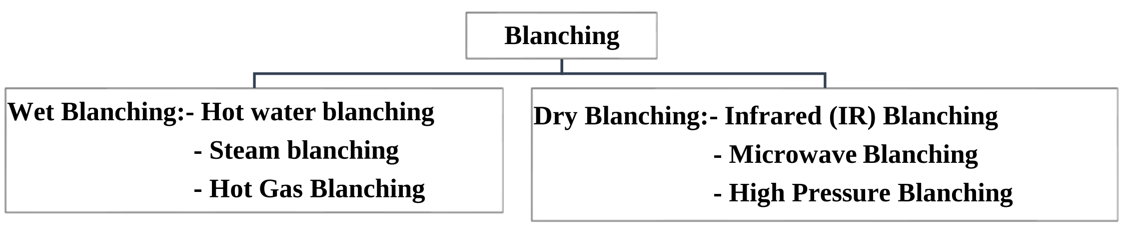

Figure 2.1. Types of Blanching based on requirement of water during processing.

(Ranjan et al., 2017)

Hot water blanching is performed at temperatures ranging typically from 70 to 100^{o}C. Techniques used for hot water blanching are: LTLT (low temperature for longer time), HTST (high temperature for shorter time) or a combination of both (Agblor and Scanlon 2000). Steam blanching has been recommended for a few vegetables like broccoli, pumpkin, sweet potatoes, and winter squash (Ranjan et al., 2017). Steam blanching, however, takes about 1.5 times longer than hot water blanching as it is carried out in tunnels or steam boxes at atmospheric pressures where the presence of air during the steam blanching process reduces the associated heat transfer from the steam. Another technology called individual quick blanching (IQB) was developed to maintain the uniformity of the process throughout the sample. In IQB, a single layer of product is conveyed through the steam chamber and each “individual” piece of product immediately comes in contact with the steam (Jose et al., 2010). In hot gas blanching, combustion of flue gases and steam is used to increase humidity and prevent product dehydration. It has the advantage of reducing waste production and is comparable to conventional blanching with respect to nutrient retention. However, this often results in product weight loss. This approach is not currently used in industries and need to be further investigated and optimized (Jack et al., 1973). Castro et al., 2008, mentioned that wet blanching is associated with disadvantages like longer processing time, more energy consumption, leaching of nutrients (which is more in hot water blanching) and producing more effluents. The alternative to overcome these disadvantages would be dry blanching.

Dry blanching is achieved using microwaves (MW) (Cano et al.,1998; Devece et al., 1999; Ramesh et al., 2002; Severini et al., 2003), infra-red (IR) radiation (Zhu and Pan 2009; Kalathur et al., 2013) or high-pressure treatment (Matser et al., 2000; Terefe et al., 2017). It has been reported that dry blanching has many advantages over conventional blanching. These primarily include lower time required for the inactivation of enzyme complexes that cause degradation in quality and little or no leaching of vitamins, volatiles, pigments, carbohydrates, and other water-soluble components (Ancos et al., 1999).

Nowadays, with the consumers being more concerned about the quality of the food and increase in preference of minimally processed foods, the demand for innovative and novel food processing technologies have increased. Apart from the above-mentioned dry blanching methods some of the non-thermal technologies that have been previously tested for enzyme inactivation are ultrasound (O’Donnell et al., 2010), pulsed electric fields (Andreou et al., 2016), pulsed light (Pellicer and Gomez -Lopez, 2017) and cold plasma (Thirumdas and Annapure, 2020) to mention some. Misra, (2015), reported some of the limitations related to these technologies such as high initial investment (in HPP) and incompatibility for whole solid foods (in PEF).

Non-thermal blanching methods could be an ideal alternative, but they are usually expensive. Though interest in novel methods has been increasing, improvement in traditional methods continues to be an interesting topic of research. The literature shows lack of studies for the combination of traditional with novel methods for blanching. No studies have been conducted to study the use of steam as a medium of heat for blanching combined with ultrasound to enhance heat transfer during blanching.

2.3. Thermal processing

2.3.1. General overview

The use of high temperatures in food preservation with an aim to reduce the number of micro-organisms (either vegetative cells, bacteria or spores) present in food by subjecting it to heat for sufficient time to ensure food safety or to reduce spoilage and increase shelf life is termed as thermal processing. Thermal processing is a traditional, yet among the most effective methods to preserve foods. Thermal processing is also defined in relation to the heat treatment of foods to bring about microbiological safety. Much of the analysis of thermal processing has been developed for foods placed in metal containers, usually the cylindrical metal can made from thin tin-plated steel or aluminum and heated by steam. This process is often referred to as 'canning'. However, the principles of thermal processing apply equally to a variety of packages including cans, bottles, and flexible and semi-rigid plastic pouches. When referring to thermal process, the term "sterile" is often used; however, the food does not become completely sterile since sterility implies the full destruction of all microorganisms. That is why the term "commercially sterile" is more appropriate to use as the thermal process is designed to kill the microorganisms of public health concern and those causing spoilage (Ramaswamy and Marcotte, 2005). Thermal processing technology is a very mature and scientifically based process as it has come a long way from being invented at the beginning of the 1800s and scientifically established by the early 1900s. Thermal processing in cans/glass jars was discovered by Nicholas Apert in 1809 and since then, food canning industry has witnessed remarkable changes in terms of processing methodology, equipment's, energy efficiency and product quality and safety (Judge, 2001). In 1864, the scientific foundation for thermal processing was established when Louis Pasteur discovered that the microorganisms and enzymes cause the food to spoil and make one sick and can be destroyed by heat. In 1998, twenty-four billion food cans were produced, and more than eleven billion cans were used to ship fruits, vegetables, and juices. The first method to calculate minimum process conditions for sterilization was given by Bigelow in 1920 and was called "General method". In 1923, Ball presented a "Formula method", which is a mathematical method to determine the sterilization process. In 1950s, Stumbo revised the "formula method" and made the process calculation more accurate. In 1957, Ball and Olson published an important book on thermal processing. Since then, the mathematics of heat process determination concepts and application are being refined (Dwivedi, 2008).

Until the 1950s, food safety was the major concern of thermal processing and very less importance was given to the loss of product quality. Common thermal processing techniques are still considered effective for preserving foods and ensure that the product is safe, shelf-stable, and devoid of any harmful bacteria (Pratap et al., 2017). However, in recent years due to the increase in consumer awareness about the quality of the food products, efforts have been made to create technology that can improve the product quality without compromising the safety. Several commercially adapted thermal processes like high-temperature short-time processing, ultra high temperature processing, aseptic processing, agitation thermal processing, and retort pouch/thin profile processing, etc., have largely emerged to meet consumer demands for high-quality products. Thermal processing involves heating foods in hermetically sealed containers for specific time and temperature combinations to destroy pathogenic and spoilage microorganisms (Ramaswamy and Marcotte, 2005). Proper prediction/gathering of time and temperature is necessary to provide a safe canning process. For this, it is important to be able to predict/obtain the transient temperatures using models based on a thorough understanding of the mechanisms involved in heat transfer process. Further, the applied thermal treatment not only destroys microorganisms, but also simultaneously results in loss of many nutrients and degrades quality of products appreciably (Awuah et al., 2007). With the development of knowledge and affordable technological concepts, there is a considerable interest in the food industry for adaptation of technologies to satisfy consumer demand for producing high-quality products without compromising safety and shelf stability. Therefore, heat transfer phenomenon associated with thermal processing is an important subject to study and understand in order to develop a process that not only imparts a minimal thermal treatment for ensuring safety and stability, but also result in minimal overcooking (Singh et al., 2017).

2.3.2. Principles of thermal processing

To successfully establish a thermal process for a canned food, understanding the heat transfer and food microbiology are the essential basics. During thermal processing, most spoilage-causing and pathogenic microorganisms are destroyed in a container, where an environment is created to inhibit the growth of remaining pathogens and/or their spores. For instance, low oxygen level in the hermetically sealed cans creates a non-supporting environment of the aerobic microorganisms; thus, the target should be the anaerobic microorganisms, which are pH dependent. From a thermal processing point of view, foods are divided into three groups: high-acid, medium-acid and low-acid foods. Examples of each group are presented in Table 2.2.

Table 2.2. Classification of foods based on pH (Ramaswamy and Abdelrahim, 1991)

pH class	Typical foods
High Acid pH <3.7	Apple, apple juice, apple cider, apple sauce, berries, cherry, cranberry juice, cranberry sauce, fruit jellies, grapefruit juice, grapefruit pulp, lemon juice, orange juice, pineapple juice, sour pickles, vinegar.
Acid 3.7 <pH< 4.5	Fruit jams, fruit cocktails, grapes, tomato, tomato juice, peaches, pineapple slices, potato salad, prune juice, vegetable juice
Low Acid pH > 4.5	All meats, fish, vegetables, mixed entrees (beans and pork, chicken with noodles, etc), most soups.

In low-acid foods, the target pathogenic microorganism is *C. botulinum*, which, in case not destroyed, can produce the deadly *botulinum* toxin. *C. botulinum* can neither grow nor produce *botulinum* when the food pH is lower than 4.5; therefore, it has been generally recognized that the dividing pH between the low and high acid groups is 4.5 as when the pH is less than 4.5, it is not necessary to worry about *C. botulinum*. Other more heat resistant microorganisms may include *B. stearothermophilus*, *B. thermoacidurans* and *C. thermosaccolyaticum*, but these are of little concern if the storage temperature of the processed cans is less than 30°C as these are thermophilic bacteria (optimal growth temperature: 50-55 °C). Pasteurization can be enough for the low-acid foods (pH > 4.5), but in this case, only vegetative microorganisms are destroyed, and the spores remain active; thus, products can be stored for short-term under refrigerated conditions only. In medium-acid and acid (pH < 4.5) foods, *C. botulinum* cannot grow, and the target microorganism is usually the heat resistant, spoilage type vegetative bacteria or enzymes, which can easily be destroyed by pasteurization (Ramaswamy and Abdelrahim, 1991). Determining the proper sterilization process time and temperature for a can is a complex procedure as there are numerous factors involved. These factors include the type and resistance of the target microorganism, pH of the food, and storage conditions after the process, heating conditions, thermophysical properties of the food and container shape and size (Sablani, 1996). To produce a commercially sterile canned food, authorities require establishing a process schedule that includes thermal process parameters, namely product initial temperature, process temperature, process time and any critical factors that might influence the achievement of commercial sterility (Sablani, 1996).

2.3.3. Microbial destruction kinetics

To establish an appropriate thermal processing schedule at a specified temperature, it is essential to know beforehand the thermal destruction rate of the test microorganism or enzyme under the existing conditions in the can. Earlier evidence supports that the inactivation of microorganisms follows the first order reaction kinetics:

$$\ln (N / No) = -kt \qquad (2.1)$$

which can be also expressed as:

$$\log10 (N / No) = -kt / 2.303 \qquad (2.1a)$$

or

$$N / No = e\text{-}kt \qquad (2.1b)$$

where *No* is the number of microorganisms at time zero and *N* at time *t*.

Survivor curve, decimal reduction time and thermal death time: The survivor curve is a straight-line plot of the logarithm of the number of surviving microorganisms against the heating time at a particular temperature (Figure 2.2)

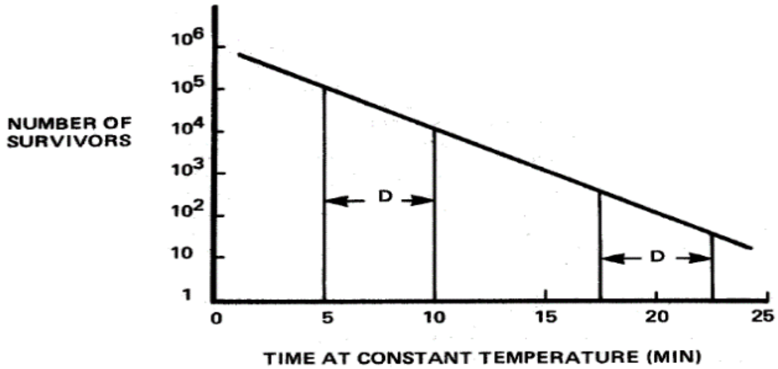

Figure 2.2. A typical survivor curves

At any given temperature, the microbial destruction rate can be defined as decimal reduction time, D, which is the heating time needed to decrease the number of microorganisms by one log cycle i.e. by one decimal reduction (Equation 2.2)

$$D = \frac{t2 - t1}{\log\frac{N1}{N2}} \qquad (2.2)$$

where:

N1: number of survivors after heating treatment for time t1 (min)

N2: number of survivors after heating treatment for time t2 (min)

Complete destruction of any bacterial population is not possible due to the logarithmic nature of the survivor curve. In other words, after an infinite number of D-values, a decimal fraction of the population will remain. Thermal death time (TDT) (Equation 2.3) is a term used in food microbiology pointing out to the heating time required to cause a complete destruction of microorganisms, which can be demonstrated by the failure of a given population to grow in culture media after the heat treatment.

$$TDT = nD \qquad (2.3)$$

where n is the number of decimal reductions.

For example, if TDT represents time to reduce the population from 10^{10} to 10^{0}, TDT would be a measure of 10 D-values.

Temperature dependence and z-value: The temperature sensitivity of D-values is expressed in the form of z-value, which is the temperature range resulting in one log reduction in

D-values (Equation 2.4). Graphically, z-value is the temperature range through which D-value passes though one log cycle (Figure 2.3).

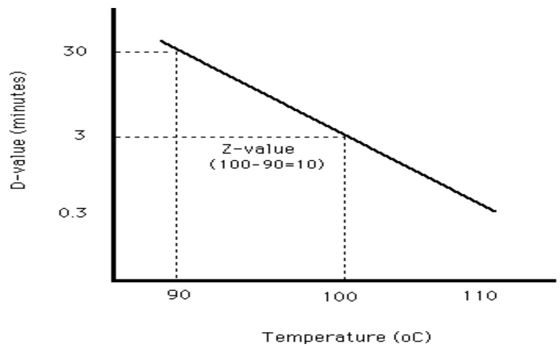

Figure 2.3 A typical thermal resistance curve

Mathematically:

$$z = \frac{T2 - T1}{\log\frac{D1}{D2}} \tag{2.4}$$

where:

D1: D value at temperature T1

D2: D value at temperature T2

2.3.4. Process Lethality

In a real process, the food passes through a time-temperature profile and the lethal effect of temperatures are integrated over the heating time in order to determine the process lethality, Fo. The sterilization process is measured using Fo value. A unit of lethality is equivalent to heating 1 min at a reference temperature T_o – 121°C / 250°F. At To, the integrated process lethality can be obtained using Equation 2.5:

$$F_o = F * 10^{(T-To)/z} \tag{2.5}$$

where:

To: reference temperature

F: heating time at temperature T

Therefore, at a z-value of 10°C, an F value of 10 min at 115°C is equivalent to a delivered F_o value of 2.78 min, whereas an F value of 10 min at 125°C is equivalent to a delivered F_o value of 27.8 min. The minimal F_o must be based on two microbiological considerations. First consideration is the decline in the number of spoilage causing bacteria. Second one is destruction of pathogens of public health concern. In low-acid foods, the microorganism of concern is *C. botulinum*, and it has been randomly established that the minimum process should reduce the *C. botulinum* contamination by 12D. A D-value of 0.21 min at 121°C is assumed for *C. botulinum*; thus, 12D equals to 2.52 min, which is the minimum process lethality (F_o) required. However, for low acid foods, it is more common to have a F_o of 5 min so that more heat resistant microorganisms, which are not of public health concern, will be destroyed and a F_o of 5 min would achieve 5 decimal reductions with reference to those spoilage microorganisms. That's why, the quality of raw materials must be controlled so that the initial count of these microorganisms remains below 100 in each container to keep the spoilage rate below one can in a thousand (Meng, 2006).

2.3.5. Characterization of heat penetration data

To establish a thermal process schedule, heat penetration data must be obtained using copper-constantan thermocouples that are inserted into the product centre through the can wall. An example of product time temperature profile is shown in Figure 2.4.

The accuracy of the time temperature profile depends on the thermophysical properties of the food product, size and shape of the container and the operating conditions of the retort; therefore, it is essential to have cans that are as close as possible to the commercial product. Process time can be calculated by determining heating and cooling rate indices (f_h and f_c) and lag factors (j_{ch} and j_{cc}) from the heat penetration data. It was proved that on a linear scale, when the logarithm of the retort-product centre temperature difference (Tr-T), known as temperature deficit, is plotted against time, a straight line is created after an initial lag. By extending this straight line to the Y axis (Tr-T), the intercept, T_{pih}, is obtained and j_{ch} can thus be calculated, as follows:

$$jch = \frac{Tr - Tpih}{Tr - Tih} \tag{2.6}$$

where j_{ch}, called heating lag factor, is a measure of the delay of the beginning of uniform heating in the product.

The cooling lag factor is called j_{cc} and is the corresponding value for jch during cooling.

$$jcc = \frac{Tw - Tpic}{Tw - Tic} \tag{2.7}$$

Part of the lag is due to the slow come-up time of the retort, and this is accounted for by determining the new zero time for the process. Ball and Olson (1957) used 58% of the come-up time as useful input to the process and it is widely accepted (Holdsworth, 1997). In other words, 42% of the come-up time should be added to the process time at retort temperature.

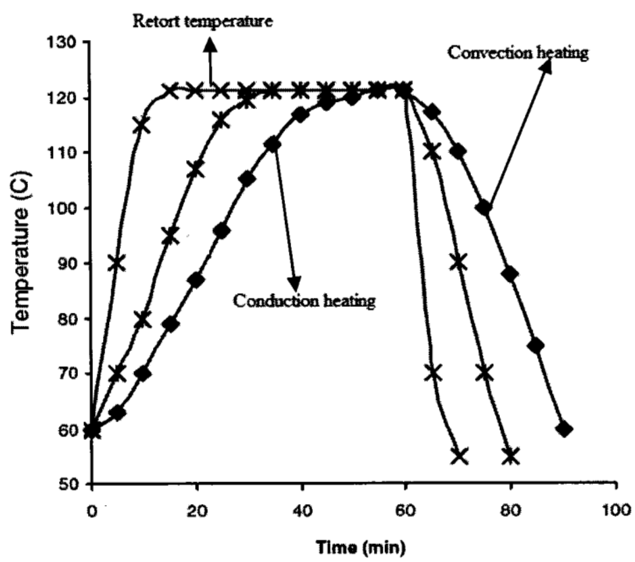

Figure 2.4. Example of time temperature profile in the retort, liquid and particle centre

The heating rate index, f_h, is the negative reciprocal of the slope of the line in Figure 2.4. It represents time required for the curve to pass one log cycle and it is an indicator of the heating rate. Higher f_h implies longer time for the log to traverse one log cycle: therefore, a lower heat penetration rate into the product.

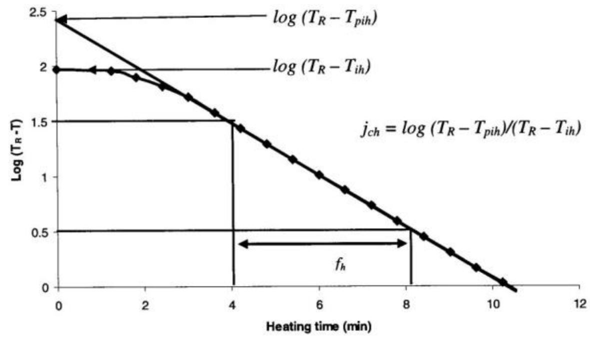

Figure 2.5. Heating curve

The corresponding value for the cooling period is f_c, the cooling rate index (Figure 2.6)

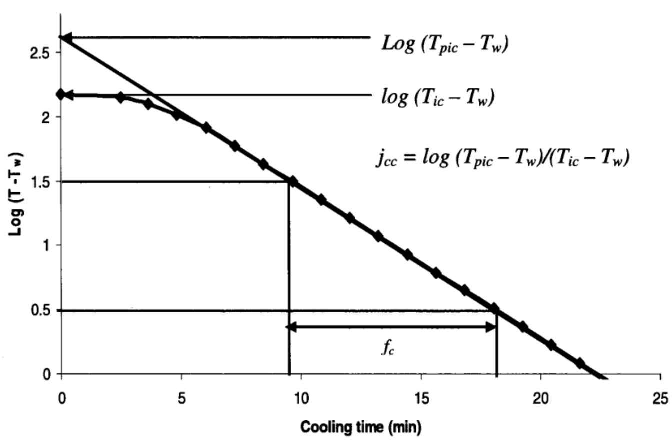

Figure 2.6 Cooling curve

2.3.6. Thermal process calculations

To understand the thermal process, heat penetration data of a specific food product and thermal destruction kinetics of target microorganisms are used (Figure 2.7). The objective of the thermal process calculations is to establish the required process time under a given set of heating conditions, resulting in required process lethality, or to approximate the lethality achieved by a process. In a processing situation, the product goes through a temperature ramp and the process calculation methods integrate the lethal effects of the transient temperature profile. In general, the desired degree of lethality F_o is pre-established, and the processes are designed to bring the minimum of this value to the thermal centre. The process calculations are divided into two groups: General methods and Formula methods. In the General methods, the lethal effects are integrated by either a graphical or numerical integration procedure, based on time-temperature data obtained from test containers under real commercial processing conditions. On the other hand, in the Formula methods, the parameters obtained from time-temperature data along with many mathematical procedures are used to integrate the lethal effects (Ramaswamy et al., 1992).

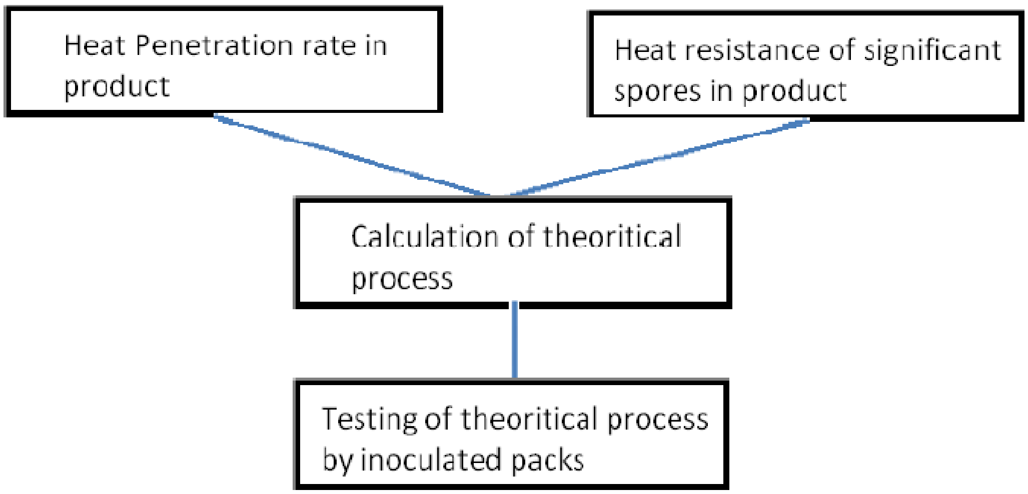

Figure 2.7 Establishment of adequate thermal process (Lopez, 1987)

2.3.7. Optimization of thermal processing

The main objective of the thermal processing is to destroy the pathogenic and spoilage causing microorganisms; however, there has been an increasing concern among consumers regarding the lower quality of thermally processed canned products. This concern has resulted in numerous studies on minimizing this quality degradation in canned foods (Sablani, 1996).

Exploiting the higher sensitivity of microorganisms than quality factors, such as colour, texture, flavour and nutrients, to the elevated temperatures, high temperature short time (HTST) and ultra-high temperatures (UHT) processes have been developed in order to minimize quality degradation of canned food products while ensuring safety (Reuter, 1993). HTST and UHT concepts have been successfully applied in liquid foods and not in liquid particulate canned food products because of the relatively slow rate of heat transfer and the existence of large temperature gradients between the surface and centre of the container (Teixeira et al., 1975).

Aseptic processing consists of heating the food to a high temperature, holding for a short time, cooling down and packaging into a sterilized container in a sterile chamber. Since foods are sterilized without any package in aseptic processing, measures can be taken to improve the heat transfer. These methods include high efficiency heat exchangers, such as plate heat exchangers, scraped surface heat exchangers and tubular heat exchangers. The application of this type of HTST processing is currently limited to liquid and not for liquid particulate canned food products due to remaining problems, such as heat transfer coefficients of the particles and residence time distribution (Ramaswamy et al., 1997). To solve these problems, other technologies, such as microwave heating and ohmic heating, have been tested. Aseptic processing has been used successfully in convective heating of liquid food products and not in conductive heating of solid ones as the heat transfer in the latter is relatively slower and large temperature differences exist between the surface and centre of the container. Alternatives include thin profile packaging and agitation processing (Willhoft, 1993).

Thin profile packaging has also gained popularity. In this type of packaging, retort pouches and semi-rigid containers are used, where the heat transfer is faster because containers have larger surface area compared to the ones used in conventional processing 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). During the thermal process, internal pressures of these containers might exceed the saturation pressure of the steam due to the residual air. Also, during the cooling phase, the internal pressure might be higher than the external one resulting in sudden pressure drop in the retort; thus, deformation of containers, loss of seal integrity and chance of explosion of containers will take place, in case the internal pressure is not counterbalanced with the external one. The counterbalancing is achieved through compressed air (Sablani, 1996).

In agitation processing, rotary retorts can improve the heat transfer in liquid-particulate canned food products, resulting in shorter processing time, better quality retention and lower energy consumption. This type of processing is another alternative to aseptic processing for liquid particulate canned food products.

The increase in awareness among the consumers to consume better quality products apart from just being safe has led to modifications and advancements in thermal processing methods like aseptic processing, thin profile, and agitation processing. The non-thermal processing techniques could be an ideal alternative, but they are expensive. To achieve higher quality products, products should be minimally processed but this would result in short shelf life under refrigerated conditions. Another alternative is to add a food grade acid to the low acid products, move the food to high acid category and process them as a high acid product. This is termed as "Acidified thermal processing". For such products, if sensory property is not adversely affected by the added acid, pasteurization heat treatment under 100°C can result in both product safety and shelf stability. Tola and Ramaswamy (2018) summarized the overall approach to improve quality of low acid vegetables with reduced energy cost through modification of product property and use of alternative thermal process technologies (Figure 2.8).

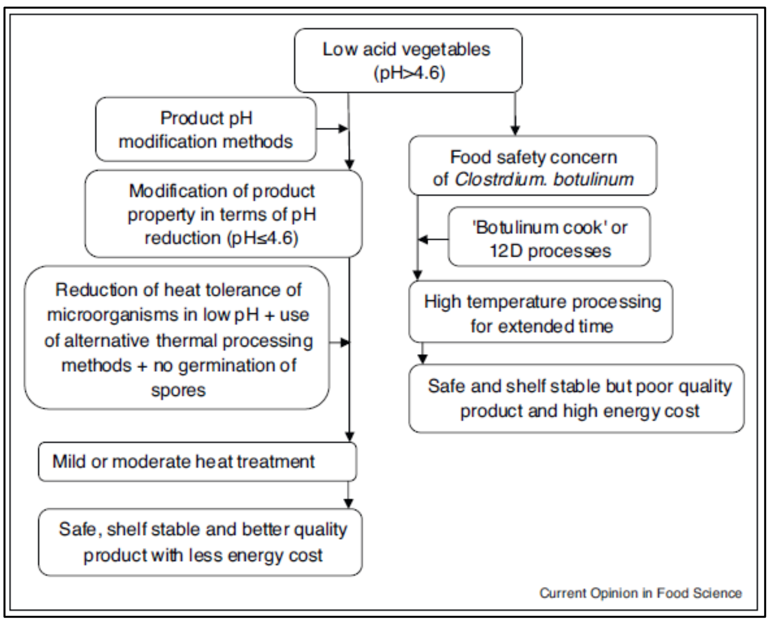

Figure 2.8. Schematic diagram shows opportunities to improve quality of low acid vegetables through product pH modification and use of alternative novel thermal processing methods (Tola and Ramaswamy 2018)

The modification of pH of low acid products can be achieved in different ways. Traditionally it can be achieved through addition of food grade acids which takes almost 10 days (in solid foods) to achieve the required pH (<4.6). As the traditional method is inefficient alternative pH reduction methods - vacuum or high pressure assisted acids infusion methods have been studied in recent years to achieve more efficient pH modification of solid foods (Zhao and Xie 2004, Derossi et al., 2010, Tola and Ramaswamy 2013).

2.3.8. Pasteurization / thermal processing of acid or acidified foods

In thermal processing methods, the intensity of a given process is determined based upon the pH of a food. Acid and acidified foods can be processed at moderate processing conditions (<100°C). Thermal processing of acidified foods is generally established according to specified guidelines from US FDA which need to be adhered to even with the newer quality improvement techniques done either through introducing higher rate of heat transfer or through enhancing more homogenous heat distribution within the product. In general, only small improvements have been demonstrated to improve quality of canned vegetables. In terms of modification of product property where possible, pH of a food plays a critical role as mentioned above. Acidification of food to pH < 4.6 has multiple benefits: (i) pathogenic *Clostridium botulinum* and other spore formers do not germinate, grow, and produce toxin or spoil the food, (ii) pH reduction reduces heat resistance of microorganisms and (iii) acidified foods (pH < 4.6) are processed at <100℃ with more efficient and less energy demanding technologies. Although the pH reduction has potential for lower energy and shorter process time processes, not all foods can benefit from this approach because the added acid can result in unacceptable sensory properties (Tola and Ramaswamy 2018).

With the increase demand of consumers for better product quality, efforts have been continuously made to replace the conventional thermal processing methods with novel technologies. Lau and Tang (2002) compared pasteurization of pickled asparagus with conventional hot water heating and microwave pasteurization (915 MHz) and found that microwave pasteurization resulted in a uniform heating and reduced process time by at least one-half compared to water-bath heating and markedly reduced the thermal degradation of asparagus.

Koskiniemi et al., 2011, studied the effect of salt addition and distribution during dielectric heating of acidified vegetables using a 915 MHz continuous microwave system to produce shelf-stable acidified vegetables. Tola and Ramaswamy (2014) compared different heating methods -conventional heating vs ohmic heating for acid and acidified foods and found ohmic heating to be more effective compared to conventional heating process. They also mentioned that to get 5D process schedule for inactivation of *B. licheniformis spores* at 87°C (pH 4.5), a 36 min process would be required with conventional heating as compared to a 27 min process with ohmic heating.

Tola and Ramaswamy (2018) reviewed the different novel thermal processing methods (microwave (MW), radiofrequency (RF), ohmic heating, and pressure assisted thermal processing) applied on acid and acidified vegetables and compared with conventional thermal processing. Literature has shown that only MW, RF, ohmic heating and pressure assisted thermal processing have been studied as alternatives to conventional thermal processing of acid and acidified foods and no literature is available on application of piezo-steam and ultrasound-water combination for pasteurization of acid and acidified foods.

2.4. Ultrasound

2.4.1. Historical overview

Developments in the application of ultrasound in processing began in the 1930s in the years preceding the Second World War when it was being investigated for a range of technologies including emulsification and surface cleaning. By the 1960s the industrial uses of power ultrasound were accepted and being used in cleaning and plastic welding which continue to be major applications (Mason, 2003). The possibility of using low-intensity ultrasound to characterize foods was first realized over 60 years ago; however, it is only recently that the full potential of the technique has been realized (Povey and McClements 1988). Ultrasound has been widely used for food analysis because of its non-destructive and non-invasive characteristic. (Dolatowski et al., 2007).

In recent years, use of power ultrasound in food processing has gained the attention of food technologists. There is a wide range for possible applications of power ultrasound in the food industry. It was first used in emulsification. Emulsions generated with ultrasound are often more stable than those produced conventionally and often require little, if any, surfactant (Mason et al., 1996). Investigations have shown that the use of ultrasound as a processing aid can reduce the production time of yoghurt up to 40% (Dolatowski et al., 2007).

Various studies have proved that ultrasound has been used successfully as a processing aid in many food processing unit operations such as ultrasound assisted -drying, freezing, filtration, extraction of flavors, mixing and homogenization (Paniwnyk, 2014). A large volume of information is available on the use of ultrasound for enhancing heat and mass transfer in drying, freezing & cleaning applications (Rodriguez et al., 2018; Musielak et al., 2016; Cheng et al., 2015; Leal et al., 2013. Otto et al., 2011; Legay et al. 2011). Ordonez et al., (1987) first introduced the concept of ultrasound-assisted thermal processing, with a prediction for its applicability for the support of conventional thermal treatments. This concept, which makes use of the synergy between ultrasound and heat for bacterial inactivation has proven to be of potential interest in food preservation. In subsequent years, lot of research and development has been done and papers have been published for the use of ultrasound for enhancing heat and mass transfer in different food processing unit operations.

2.4.2. Principles of ultrasound

Sound waves beyond the audible frequency range (in general, >20 kHz) is referred as Ultrasound. Short waves with wavelengths in the range of centimeters to nanometers are the general features of ultrasound waves. Short wavelength indicates a high degree of discrimination and a high concentration of energy which allows it to be used as a diagnostic tool and source of energy. This application of ultrasound involves different frequency ranges (Figure 2.9) and the uses of these ranges in food industry has been an ongoing subject of interest.

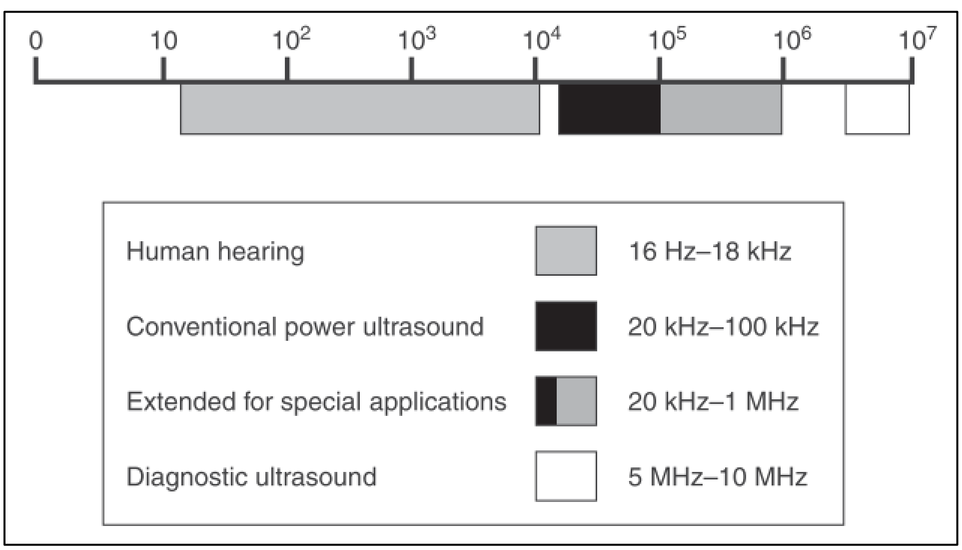

Figure 2.9. Frequency range of sound

2.4.3. Ultrasound generation

Ultrasonic wave producing system comprises the generator, transducer and the application system. Generator produces electrical or mechanical energy and transducer converts this energy into the sound energy at ultrasonic frequencies. Fluid-driven, magnetostrictive and piezoelectric transducers are the three main types of transducers that have been reported (Mulet et al., 2003).

Electro restrictive transformer principle which is based on the elastic deformation of ferroelectric materials within a high frequency electrical field and is caused by the mutual attraction of the molecules polarized in the field is mostly used for the generation of ultrasound (Raichel, 2000).

A high-frequency alternating current will be transmitted via two electrodes to the ferro electrical material for polarization of molecules. Then, after conversion into mechanical oscillation, the sound waves will be transmitted to an amplifier, to the sound radiating sonotrode and finally to the treatment medium.

2.4.4. Mechanisms and effects of ultrasound

A continuous wave-type motion, longitudinal waves is generated when sound energy passes to the medium which results in alternative compression and rarefaction of the medium particles (Povey and Mason, 1998). Many phenomena (hydrodynamic effects) may ensue from propagation of an ultrasonic wave into a fluid and more particularly into a liquid medium (Figure 2.10). Two of them, of major importance for heat transfer enhancement, are acoustic cavitation and acoustic streaming (Legay et al., 2011; Chemat et al., 2017). Acoustic streaming ensues from the dissipation of acoustic energy which permits the gradients in momentum, and thereby the fluid currents (Wu, 2018; Bach et al.,2019). The speed gained by the fluid allows a better convection heat transfer coefficient near the solid boundaries, sometimes leading to turbulence and promoting heat transfer rate. The phenomena of generation, growing and eventual collapse of the bubbles (Figure 2.11) which occurs when ultrasound is applied to liquid systems is referred to as acoustic cavitation. Depending on the frequency used and the sound wave amplitude applied several mechanical, chemical, biochemical and thermal effects can be observed which enables a variety of applications. Mechanical effects include collapse pressure, turbulences, and shear stresses (Yusaf et al., 2014), while the chemical effects include generation of free radicals (Lateef et al., 2007). In the cavitation zone extremely high temperature (5000°K) and pressures (1000 atm) (Soria and Villamiel, 2010) are generated. The temperature and pressure indicated are generated during a very short period of time. These shock waves generated contribute to the ultrasound effect and can be widely used for food processing purpose.

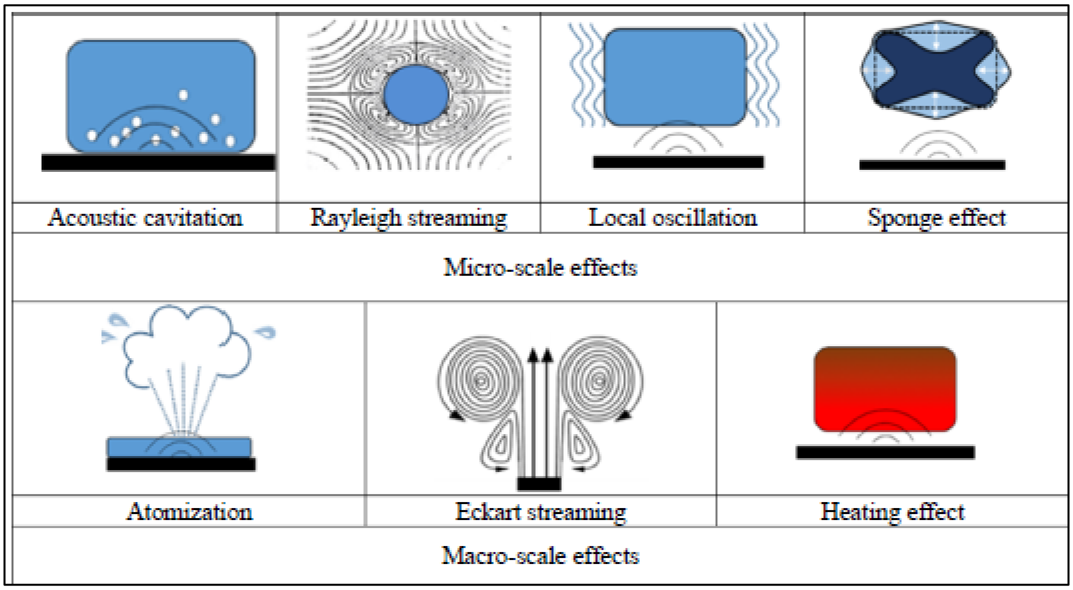

Figure 2.10. Different ultrasound phenomena

Source : Zhang et al. (2020).

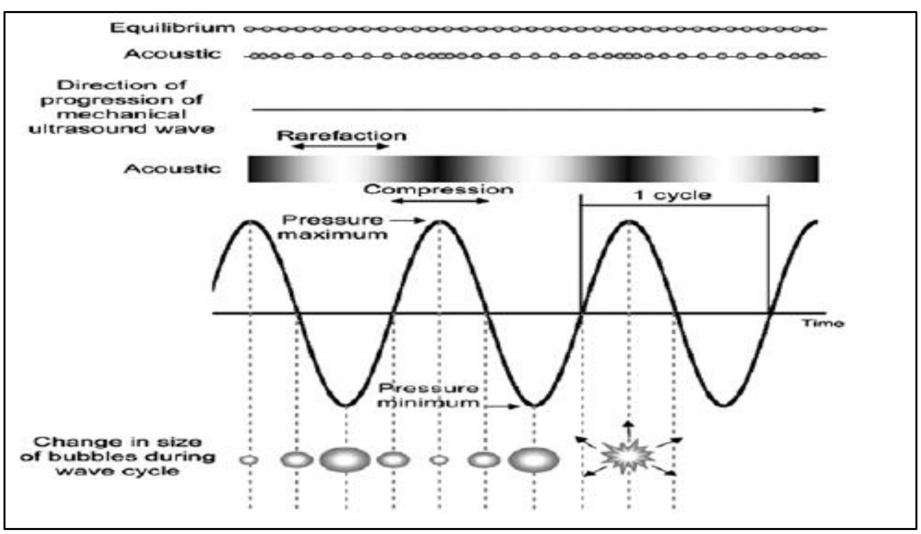

Figure 2.11. Cavitation caused by ultrasonication
Source: Soria and Villamiel (2010).

Alternating positive and negative pressures produced due to frequency of the ultrasound causes expansion or compression of the material, resulting in cell rupture. Ultrasound causes hydrolysis of water inside the oscillating bubbles leading to formation of H^+ and OH^- free radicals that can be captured in some chemical reactions. This disruption effect of sonication is significantly resisted by homogenous liquids (Ercan and Soysal, 2013). During sonication treatment, bubbles produced are divided into two types based on their structure:

(1) Non-linear, forming large bubble clouds with equilibrium size during pressure cycles are known as stable cavitation bubbles.
(2) Non-stable, rapidly collapsing and disintegrating into smaller bubbles are known as internal (transient) cavitation bubbles.

These small bubbles quickly dissolve, but during bubble stretching, the mass-transfer boundary layer is thinner, and the interfacial area is greater than during bubble collapse which implies that more air transfers into the bubble during the stretching phase than leaks out during the collapse phase (Tiwari and Mason, 2012).

According to certain experimental findings and photographic investigations, a collapsing cavitation bubble's impact might last 10-7 seconds and result in a local pressure of up to 193 MPa. This clarifies a variety of phenomena that are related to chemistry, biology, engineering, and other fields. It also explains why acoustic cavitation is thought to be the primary result of enhanced ultrasonic heat transfer. As shown schematically in Figure 2.12, a bubble implosion close to a solid-liquid interface breaks thermal and velocity boundary layers, lowering thermal resistance and generating microturbulence (Legay et al., 2011).

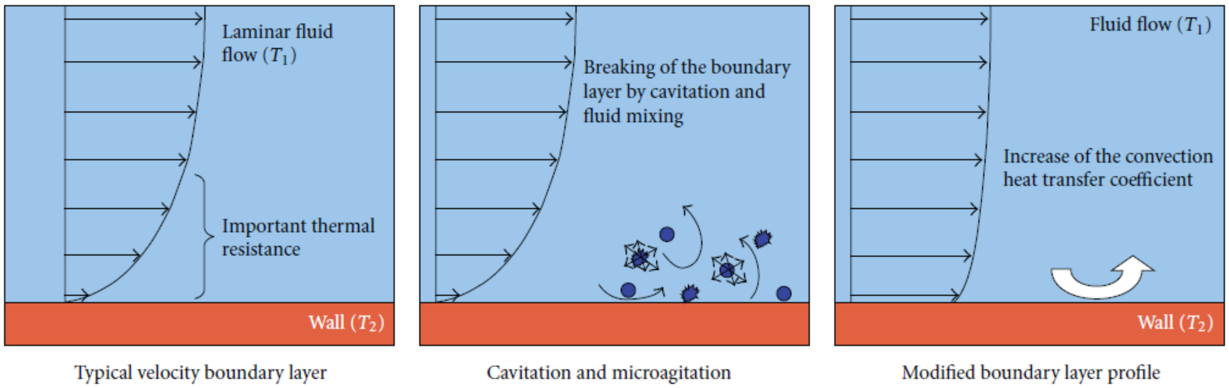

Figure 2.12: Explanation of heat transfer enhancement by acoustic cavitation Source: Legay et al. (2011).

Acoustic streaming, a property of low intensity ultrasonic waves, is another significant hydrodynamic phenomenon of ultrasound propagation. The velocity of acoustic streaming varies from 0.01 to 1 m/s, generating turbulence through the medium, depending on the parameters of the ultrasound and the distance to the transducer. In both liquid and gaseous fluids, acoustic streaming is produced and travels through the medium in two different ways: (1) bulk-driven streaming, also known as Eckart streaming, occurs a few centimeters from the ultrasound transducer as a result of a momentum gradient brought on by energy dissipation, and (2) boundary-driven streaming, also known as Rayleigh streaming, occurs close to the solid boundary as a result of friction between that boundary and the surrounding fluid (Wu 2018.; Manasseh 2016; Bach et al., 2019). By lowering the thickness of the stationary boundary layer, Rayleigh streaming, also known as microstreaming, effectively improves heat and mass transmission at the solid boundary (Orbay et., al 2017; Hyun et al.,2005). Acoustic energy is lost during the process of acoustic streaming, allowing momentum gradients and consequently fluid currents. Riley (1998) further distinguished between the Rayleigh streaming found at boundary layers and solid-liquid interfaces and the quartz wind streaming occurring in the fluid bulk. The fluid's increased speed enables a stronger convection heat transfer coefficient close to the solid boundaries, which might occasionally cause turbulence and accelerate heat transfer (Figure 2.13). Since ultrasound is regarded as non-thermal energy, the heating effects of ultrasound propagation generated by mechanical vibrations are often negligible (Zhang et al., 2020). When calculating the ultrasonic energy delivered to the medium using the calorimetric method, this impact in heat transfer systems is considered negligible.

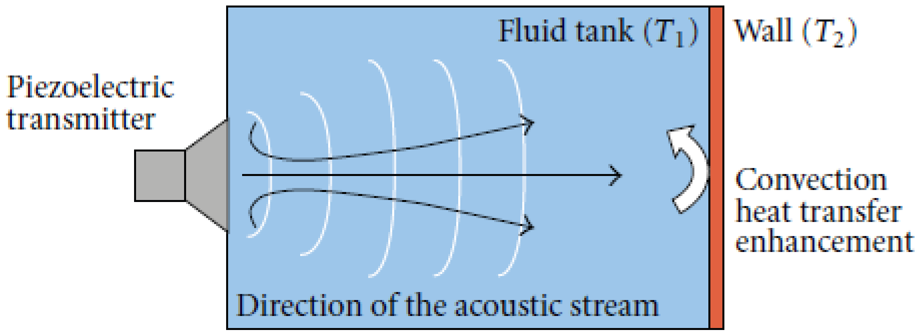

Figure 2.13. Acoustic streaming—enhancement of convection heat transfer
Source: Legay et al. (2011).

2.4.5. Classification of ultrasound application

The most important criteria for the classification of ultrasound applications is the amount of energy generated by the sound field (Knorr et al., 2004).These are low and high energy ultrasounds which are classified by their sound power (W), sound energy density (Ws/m^3) and sound intensity (W/m^2).

The low energy (low power, low intensity) ultrasound applications are performed at frequencies higher than 100 kHz and below 1 W/cm^2 intensities (Knorr et al., 2004). They are successfully used for analytical applications to get information about the physicochemical properties of foods such as composition, structure and physical state (Jayasoorya et al., 2004, McClements, 1995; Knorr et al., 2004).

The high energy ("Low frequency ultrasound" or "power ultrasound") waves are those with a frequency between 20 and about 100 kHz and are intensities higher than 1 W/cm^2 (typically in the range 10 - 1000 W/cm^2) (McClements, 1995). Indeed, ultrasound can change the medium in which it propagates because it is typically transmitted at a high power level (a few tens of Watts). Powerful macroscopic effects for enhancing heat transmission can be produced by cavitation or acoustic streaming, two phenomena created by disrupting a fluid bulk using power ultrasound. Power ultrasound is therefore used in a variety of procedures, including cleaning, plastic welding, sonochemistry (Legay et al., 2011), and others.

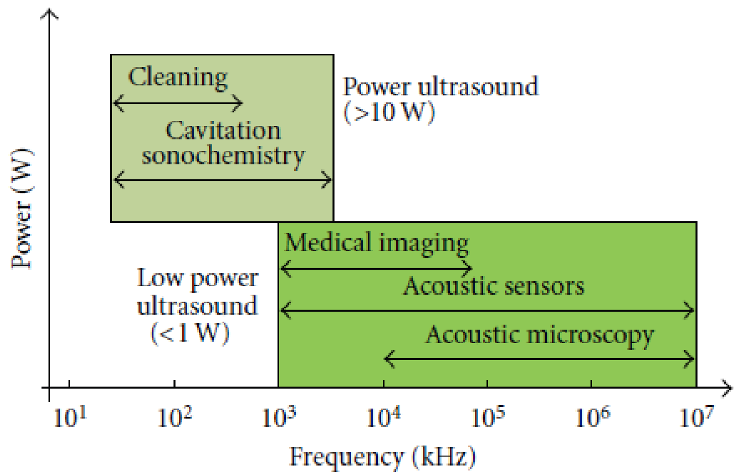

Figure 2.14. Utilizations of ultrasound according to frequency and power
Source Legay et al. (2011)

Additionally, it is frequently employed to speed up mass and heat transfer operations. Due to the physical, mechanical or chemical effects of ultrasonic wave's destruction (e.g. physical disruption, acceleration of certain chemical reactions) can be observed at this power. High-intensity ultrasound has been widely used to generate emulsions, disrupt cells, disperse aggregated materials, modification and control of crystallization processes, degassing of liquid foods, enzyme inactivation, enhanced drying and filtration and the induction of oxidation reactions (Knorr et al., 2004, Zheng et al., 2006).

The "High frequency ultrasound" is found in the intermediate frequency range of 100 kHz to 1MHz. Power ultrasound is more frequently employed to facilitate heat transfer. Figure 2.14 displays a few common applications for ultrasound based on frequency and power.

Methods of ultrasound applications can be divided into three: 1) Direct application to the product, 2) Coupling with the device, 3) Submergence in an ultrasonic bath (Chemat et al., 2011).

To transfer ultrasonic vibrations to the sample a coupler device is used in the application system. This is mostly obtained by ultrasonic bath and probe system (Figure 2.15a). In ultrasonic baths, generally the transducers are fixed to the underside of the tank and most of the baths are operated at around 40 kHz (Leadley et al., 2006; Mason, 1998). Transducers, which are often found at the device's base, are where the acoustic waves are transmitted (Chemat et al., 2017). The ability to simultaneously treat many samples of various sizes is this sort of device's key benefit. However, there are a lot of restrictions when it comes to microbial inactivation. For instance, the application of acoustic energy in ultrasonic baths is typically low, and wave propagation is uneven due to reflection on the bath walls. This indicates that the cavitation microbubbles do not collapse uniformly across the bath, resulting in a treatment that is not equally effective everywhere. Additionally, ultrasonic processing can only be done in batch mode (Santos et al., 2008; Rodriguez et al., 2018).

In probe systems the horns or probes are used to transmit or to amplify the ultrasonic signal (Figure 2.15b). Their lengths must be half the wavelengths, or multiple, to maintain the resonant conditions of the system (Mason, 1998). The horn shape defines the amplitude gain of ultrasonic signal. If the probe is the same diameter along its length, then no gain in amplitude will occur but the acoustic energy will be simply transferred to the media. It is crucial to consider the probe's location within the ultrasonic reactor as well as its geometry and design (Lee et al., 2009b). The length, geometry, design, and tip diameter of the probe are among its crucial characteristics in terms of the effectiveness of microbial inactivation (Awad et al., 2012; Zupanc et al., 2019). In actuality, the cavitation just beneath the product is produced by the acoustic energy that is transferred to it through the probe's tip. Since this technology is more potent and capable of achieving more effective microbial inactivation than the ultrasonic bath, it is the one that is most frequently employed in the USA (Chemat et al., 2017; Rodriguez et al., 2018). However, with time, the cavitation microbubbles' implosion may cause the probe tip to become pitted, which could lead to the discharge of metal powder into the treated media (Palma et al., 2017).

There are two distinct modes that can be discussed when discussing US application modes: continuous and pulsed. When the transducer is continually activated by an electrical sine wave, acoustic waves are continuously emitted across the medium. The treated medium has a noticeable temperature increase as a result of the constant flow of acoustic energy, which could harm the food product. On the other hand, this application mode results in greater performance in terms of microbial inactivation (Bermudez-Aguirre and Barbosa-Cánovas, 2012).

Contrarily, in the pulsed mode, also known as "duty-cycles," acoustic waves are intermittently released for a predetermined period of time, exciting the transducer and causing the discharge of very brief electrical signals. As a result, the temperature increase of the treated sample during the US treatment is smaller than in the continuous mode discharge. Even though duty-cycles are repeated throughout the treatment, the efficacy of microbial inactivation in the pulsed mode is lower than in the continuous mode (Bermudez-Aguirre and Barbosa-Cánovas, 2012).

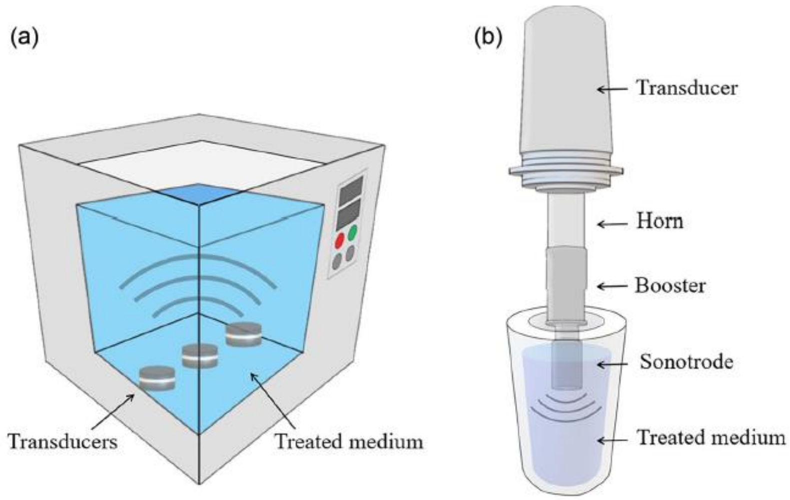

Figure 2.15: Ultrasound application systems: (A) ultrasonic bath; (B) probe system
Source: Beiti et al. (2023)

2.4.6. Heat transfer enhancement by ultrasound

Legay et al. (2011) conducted a thorough assessment of the studies on the impact of ultrasonic waves on the enhancement of heat transport, including convection. Numerous intentional studies have been conducted in the last ten years to determine the impact of various ultrasonic properties and other key variables that may affect the improvement of convective heat transfer. Frequency, power, propagation medium, geometry of the heat transfer system, placement of the heating surface in relation to the ultrasonic transducer surface, and flow rate in the case of forced convection are some of the variables connected to the thermal enhancing effect of ultrasound. Several scholars have investigated the influence of these variables both separately and in combination to increase heat exchange.

The use of ultrasound as a heat transfer enhancement technique was the subject of sporadic research from the 1960s until 2000, almost all of which concentrated on boiling and spontaneous convective heat transfer. Since that time, there have been an increasing number of studies on various phase transition processes as well as convective heat transfer. Due to the distinctive effects, it causes inside the propagation medium, the use of ultrasound is increasingly recognized as a potential and effective tool for improving heat and mass transfer.

Dehbani et al., (2022) offered a thorough overview of the mechanisms and contributing elements in the increase of convective heat transfer utilizing ultrasound. The comprehensive review on heat transfer enhancement by ultrasound was summarized with most recent research on applications of ultrasound interference in free and forced convection heat transfer systems.

2.4.7. Ultrasound - liquid applications for microbial decontamination

Ultrasound application is a non-thermal technology that contributes to the increase in the shelf life and microbial safety especially in food with heat sensitive, nutritional, sensory, and functional characteristics (Bhat et al., 2011; O'Donnell et al., 2010). Various studies published indicate that the effectiveness of microbial decontamination by ultrasound is relatively higher when combined with other combination treatments or techniques compared to the use of ultrasound or other technique alone (Rivera et al., 2011; Sagong et al., 2013).

2.4.7.1. Ultrasound (US) treatment of fresh produce

Fresh produce can easily get contaminated with air, water and soil borne microorganisms. The main step for reducing microbial load is washing with sanitizing solutions which is the traditional method for minimally processed fruits and vegetables. But it has always been a challenge for the food industry to achieve adequate level of removal and/or inactivation of pathogens such as *Salmonella* from the surfaces of fresh products. Surface decontamination of fresh produce with ultrasound has been a topic of research for many years (Zhou et al., 2012). Several fruits and vegetables have been evaluated for the effectiveness of US treatments in food preservation by being submerged in a liquid medium, typically water, where the US is emitted. For instance, Cao et al., (2010), evaluated the inactivation of total coliform counts, and mold and yeast counts, in strawberry fruits. After 10 min of treatment (20°C), the inactivation with US (ultrasonic bath, 40 kHz, 350 W, continuous mode) was 2.43±0.02 and 3.02±0.02 Log_{10} CFU/g in the counts of total coliform and mold and yeast, respectively, and caused a decrease of 13±0.24 % in the decay index in comparison to the untreated samples after 8 days of storage at 5°C. To enhance the microbiological and storage quality of fresh-cut lettuce, a combined treatment using ultrasonic and polylysine was examined by Fan et al., 2019a and Fan et al., 2019b. After being treated with ultrasound (20 kHz, 17–29 W/L), polylysine (0.1–0.6 g/L), and their combination at 20 °C for 10 min, fresh-cut lettuce was packaged and kept at 4 °C for 12 days. The findings demonstrated that 23 W/L ultrasound in combination with 0.4 g/L polylysine treatment was more effective than 23 W/L ultrasound or 0.4 g/L polylysine treatment alone at preventing the growth of microorganisms like total colonies, mould and yeast, and total coliform counts of fresh-cut lettuce during storage. Many studies have focused on ways to increase the efficacy of microbial inactivation in fresh produce by combination of ultrasound with chemical sanitizers (Bilek and Turantaş, 2013). Bang et al., 2017 found synergistic effects of combined US and peroxy acetic acid treatments against *Cronobactoer sakazakli* biofilms on fresh cucumber. Duarte et al., (2018), found US treatment to improve antimicrobial effect of sodium dichloro isocyanurate in reducing *Salmonella typhimurium* on purple cabbage. Alenyorege et al., (2019b) also found sweeping frequency US+NaOCl washing treatment to be effective for the reduction of *Listeria innocua*.

More recently, Alenyorege et al. (2019b) found that 40 kHz sweep frequency ultrasound in combination with 100 mg/L sodium hypochlorite resulted in maximum reduction and inhibition of mesophilic counts, yeast, and molds in fresh-cut Chinese cabbage. Beiti et al. (2023) gave a thorough overview of how well US technology eliminates microorganisms in various food matrices and model systems. The review paper summarized that US is specifically highlighted together with the two physical processes that are the most industrially feasible, namely heating at low temperatures and/or treatments at high pressure, leading to the processes known as thermosonication, manosonication, and manothermosonication. Most early studies (up to 2012) have been summarized in a book (Dimirci and Ngadi, 2012) and more recent data are included in Table 2.3.

Table 2.3. Application of ultrasound for microbial decontamination in fresh produce

Food Product	**Technique**	**Major outcome**	**Reference**
Truffles	70% Ethanol + US	Achieved 4 log reductions for *Pseudomonads*, reductions greater than 2 logarithmic units for *Enterobacteriaceae*, lactic acid bacteria and moulds and 1.5 logarithmic reductions for yeasts	Rivera et al. (2011)
Cherry Tomatoes	US alone US + peracetic acid (40 mg/L)	*S. enterica typhimurium*: 0.8 log10 CFU/g sample reduction *S. enterica typhimurium*: 3.9 log10 CFU/g sample reduction	São José and Vanetti (2012)

Spinach	chlorine +US	additional log reductions of 1.0 and 0.5 CFU/g for *E. coli* cells	Zhou et al. (2012)
Lettuce	US	2-log CFU/g reduction of *E. coli* and *S. Enteritidis*	Birmpa et al. (2013)
Strawberries	US	3.04, 2.41, 5.52 and 6.12 log CFU/g reduction of *E. coli, S. aureus, S. Enteritidis* and *L. innocua*, respectively.	Birmpa et al. (2013)
Lettuce & Carrots	0.1% Tween 20 + US	Reductions of 2.49 and 2.22 log CFU/g *B. cereus* spores on lettuce and carrots, respectively, without causing deterioration of quality. These reductions were 1 log greater than those obtained by immersion in 200 ppm chlorine for 5 min.	Sagong, et al., (2013)
Peas	US + NaOCl	Reduced total aerobic counts on sprouts by 5.86 and 5.51 log units	Chiu and Sung (2014)
Green peppers and Melons	ultrasound and 1% lactic acid	Reduced *E. coli* and *Salmonella* by 2.9 and 2.8 log CFU/cm^2 in green peppers & 2.5 and 3.1 log CFU/cm^2 E. coli and *S. Enteritidis* in melons	Sao José et al. (2014)
Lettuce	US + Essential oil of Oregano	Reduction of 2.65 ± 0.23 log CFU/cm^2 of *E. coli*	
Lettuce	US + Essential oil of Oregano and thyme	*Salmonella enterica Abony* reduction. Control ~ 1 log CFU/cm^2. Ultrasound continuous mode: 2.23 ± 0.29 log CFU/cm^2. Ultrasound pulsed mode: between 1.68 and 1.78 log CFU/cm^2.	
Alfalfa and mung bean Sprouts	US	Reduction of *Salmonella* by 1.40 ± 0.40 and 1.89 ± 0.51 log CFU/g and *E. coli* by 1.06 ± 0.32 and 1.23 ± 0.40 log CFU/g for Alfalfa & Mung respectively	
Fresh Arugulas	US + sodium hypochlorite (100 mg/L)	Reduction of 1.46 log CFU/g in aerobic mesophilic microflora	Francisco et al. (2018)
Chinese cabbage	Sweep frequency ultrasound (SFUS), sodium hypochlorite (NaOCl) and (SFUS + NaOCl)	SFUS + NaOCl treatment reduced mesophilic counts by an additional 2.7 log CFU/g, yeasts and molds by an additional 2.0 log CFU/g with the combination treatment	Alenyorege et al. (2019a)
Fresh cut celery	Thermo-US	Decontaminate Escherichia coli O157:H7 and Salmonella enterica serovar Typhimurium (S. Typhimurium): > 5 log	Kwak et al. (2011)
Fresh-Sliced Button Mushrooms	Low conc. acidic electrolyzed water + US	Inhibition of enzyme activity and extension of shelf life (1.24 -1.49 times)	Wu et al. (2018)
Non-heading Chinese cabbage	US washing with different frequencies	Escherichia coli >3 log CFU/g reduction	Alenyorege et al. (2018)
Dried Black pepper grains and tapioca starch	Airborne ultrasonic technology - 30-120 min at room temperature	Inoculated with Bacillus subtilis vegetative cells and spores. 2.19 log CFU/g and 2.01 log CFU/g after 30 min of treatment for black pepper and tapioca starch respectively	Charoux et al (2019)

Non-heading Chinese cabbage	Combined power US and chlorine disinfection treatment	Additional 0.5-2.5 log CFU/g in general microflora obtained as compared with NaOCl treatment alone	Irazoqui et al. (2019)
Fresh cut Chinese cabbage	US washing with different frequencies	Escherichia coli and Listeria innocua. E. coli more sensitive than L. innocua, achieving >3.0 log reductions after 10 min	Alenyorege et al. (2019b)
Lettuce leaves	Low-intensity electrical current + US	Total coliform, the total yeast & mold inactivation rate 2.891og, > 3log, and 2.98log, respectively.	Kilicli et al. (2019)

*US -Ultrasound

2.4.7.2. Ultrasound treatment of meat, pork, poultry and seafood

Application of ultrasound as a potential microbial decontamination tool in poultry, meat, pork and sea foods has also been reported in various studies (Morild, 2011; Turantaş et al., 2015; Pedros-Garrido et al.,2017; Alarcon-Rojo et al., 2018). Haughton et al., 2012, studied the application of sonication and “thermo-sonication” (53±1°C) for reducing numbers of *Campylobacter, Enterobacteriaceae* and total viable counts (TVC) on raw poultry and found the thermos-sonication to be more effective. Smith, 2011 mentioned that combination of high intensity ultrasound with antimicrobials resulted in higher reduction of pathogens in meat. Recently Pedros-Garrido et al., 2017, evaluated high intensity ultrasound for surface decontamination of salmon, mackerel, cod and hake fillets, and demonstrated it to be effective. Some results related to US applications as applied to meat and fish are summarized in Table 2.4.

Table 2.4. Application of ultrasound for microbial decontamination in poultry

Product	Microbial Decontamination Technique	Major outcome	Reference
Broiler breast	Ultrasound + margination	*E. coli* reduction of 3.3/ml log CFU and 2.5/ml log CFU reduction of *Salmonella typhimurium*	Smith (2011)
Chicken wing skin	US + water, and US + 1% Lactic acid	Gram negative bacteria reduction - 1.0/cm2 ≤4.0/cm2 log CFU	Kordowska-Wiater and Stasiak (2011)
Chicken Drumstick	Ultrasound	No significant reduction of *Campylobacter, Enterobacteriaceae,* total viable count	Haughton et al. (2012)
Chicken Breast	Ultrasound	0.2 CFU /g log reduction of Psychrophilic bacteria	Piñon et al. (2012)
Salmon, mackerel, cod, hake	High intensity US in water 5-15 min	1.1 -1.5 log reduction in oily fish and 0.5 log reduction in white fish	Pedros-Garrido et al., (2017)

*US-Ultrasound

2.4.8. Ultrasound application for enzyme inactivation

As in the case of microbial species, enzymes also show a huge variety of sensitivities toward ultrasonic irradiation. A variety of ultrasonic combination treatments such as manosonication, thermosonication, and manothermosonication are possible, with treatments such as manothermosonication known to be able to inactivate enzymes at lower temperatures and/or in a shorter time than the comparable thermal process (Rastogi, 2011). Inactivation is thought to occur via a range of mechanisms such as thermal effects, with thermolabile enzymes being more sensitive to ultrasound than those that are heat resistant, free radical inactivation and impairment of substrates as examples. The action of some enzymes can result in the deterioration of food flavours and changes in food colour, e.g., browning. Whilst enzyme inactivation can easily be achieved by heating, this can often be detrimental to the food itself. As a result, the use of ultrasound has become of more interest.

Awad et al., (2012), stated in their review that enzyme inactivation is an important process for enhancing the stability, shelf life, and quality of many food products, and went on to give many examples of this from recent literature. There are studies on the deactivation of lipoxygenase, peroxidase, and polyphenol oxidase in model buffer systems using manothermosonication, the inactivation of proteases and lipases from psychotropic Pseudomonas ,the inactivation of pectin enzymes in tomato juice ,peroxidase inactivation by combinations of heat and ultrasound at neutral or low pH (Paniwnyk, 2014). Studies show that use of ultrasound for blanching or processing of juice etc. to inactivate enzymes saves time and energy by giving better results (Paniwnyk, 2014). The inactivation time was shortened and inactivation efficiency of PPO and POD in bayberry juice was enhanced when subjected to ultrasound treatment as compared with thermal treatment (Cao et al., 2018).

Ultrasonic deactivation of enzymes linked to the browning or spoilage of food has proven to be successful over a wide range of processes and food products. The quality of the food, its texture and flavors, and its nutritional value can be maintained and retained if process times are kept to a minimum and treatment temperatures are reasonably low. This therefore allows potential for widespread use of ultrasound treatments; however, this is still much dependent on the enzyme and processing system in use.

2.4.9. Ultrasound-steam combination treatments

Microbial decontamination of food surface can be achieved in a more effective manner by using a short-term heat treatment provided by combination of ultrasound and steam. SonoSteam^{R} (European Patent EPO: 02 722 020.1 2113) is a patented process for steam and sound combination process treatment developed by Force Technology, Brøndby, Denmark (Andersen et al., 2011). In this technique, high power ultrasound (30-40 kHz) is combined with high pressure steam to decontaminate food surfaces. The microbial reduction effect is caused by steam while ultrasound enhances heat transfer by allowing steam to reach the microorganisms in the microstructure and cavities on the food surface more efficiently. The product is exposed to steam only for a very short duration which results in maximal microbial reduction and minimal quality changes.

Musavian et al., (2014), studied the industrial scale application of the SonoSteam treatment on broiler carcasses contaminated by naturally occurring *Campylobacter spp*. They reported that, microbial reductions of approximately 1.0 $\log_{10}$ CFU in *Campylobacter* and 0.7 $\log_{10}$ CFU in TVC was achieved during the process. Another study on the application of combined steam (95°C) and ultrasound as a short treatment (1 to 2 s) for microbial decontamination (*Enterobacteriaceae spp*.) of transportation boxes and crates (Musavian et al. 2015). In another study, steam (94°C -95°C) and ultrasound (25-40 kHz) combination was used to treat mozzarella cheese inoculated with *Pseudomonas fluorescens* and *Enterobacteriaceae* for a short time (Lacivita et al., 2018) demonstrating a microbial reduction of 0.8 to 2 log cycles. Morild et al., 2011, found a similar trend in inactivation of pathogens on pork using the steam-ultrasound combination (1 to 2 s). Schultz et al., (2012), studied the effect of steam-ultrasound treatment on *Norovirus*-contaminated raspberries and found that it does not appear to be an appropriate method to achieve sufficient decontamination. Although the level of microbial destruction achieved was relatively small (1-2 log reduction) the technology has been acknowledged as concept and has gained FDA recognition. Some results on short time heating using steam and sound are included in Table 2.5.

Table 2.5. Application of ultrasound and steam for microbial decontamination

Food Treated	**Treatment**	**Time**	**Microbial Reduction**	**Reference**
Pork	Steam (130°C) + US (30 to 40 kHz)	0.5 to 2s	0.9 to 1.5-log reductions of E. coli 0.4- to 1.1-log reductions for *Salmonella* & *Y. enterocolitica*	Morild et al. (2011)
Broiler Carcasses	Steam (90–94°C) + US 30–40 kHz (SonoSteam®)	15-20 min (8500 birds/hr)	1.0 $\log_{10}$-*Campylobacter* & 0.7 $\log_{10}$ CFU TVC	Musavian et al. (2014)
Industrial fish & meat transportation boxes and crates	steam (95°C) + US 20 to 40 kHz	1-2 s	Aerobic counts &*Enterobacteriaceae* spp. below detection limit (<10 CFU)	Musavian et al. (2015)
Mozzarella cheese	steam (94-95°C) + US (25-40kHz)	0.5 to 6 s	0.8 -2 log cycle reduction in *Pseudomonas* fluorescens & *Enterobacteriaceae*	Lacivita et al. (2018)
Chicken carcasses	US (30-40 kHz) + steam (84-85°C or 87-88°C) (SonoSteam®)	<1.5 seconds (18000 birds/hr)	log 0.5 ± 0.8 for *Campylobacter jejuni*, log 0.6 ± 0.6 for *Enterobacteriaceae*, log 0.5 ±0.6 for *E. coli* and log 0.4 ±0.7 CFU/g for total aerobic bacteria.	Moazzami et al., (2020)
Broiler surface areas- (back, breast and neck)	US (25 to 40 kHz) + steam 90°C (SonoSteam®)	<1.5 seconds (10,500 birds/hr)	0.8,1.1 and 0.7 log *Campylobacter* reductions; 1.6,1.9 and 1.1 log *Enterobacteriaceae* reductions and 2.0,2.4 and 1.3 log reductions of Total viable counts respectively in back, breast & neck.	Musavian et al., (2022)

*US-Ultrasound

According to the literature surface microbial decontamination studies, using combined ultrasound and steam technology have made significant progress in recent years. But these studies are mostly proprietary research using the commercial setup -SonoSteam® and all these studies have been conducted on poultry. Few more studies have been conducted on other foods like pork and mozzarella cheese and decontamination of crates in meat and fish industry. Despite the progress a huge research gap can be seen on surface microbial decontamination studies using ultrasound and steam technology. No studies have been conducted on use of this technology for surface decontamination of fresh vegetables. Literature also shows how ultrasound have been successfully used to enhance different unit operations in food processing like drying, freezing etc. But no studies have been conducted to tap on the potential of ultrasound and steam combination heating for enzyme inactivation in fresh vegetables for its use in heat transfer enhancements. Addressing these gaps will contribute to development of more efficient and sustainable processes for food and industrial applications, ensuring improved safety and quality of products. In this regard an effort has been made to study the microbial decontamination, enzyme inactivation and heat transfer enhancement efficiency of combined ultrasound and steam technology.

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Connecting Statement to Chapter 3

As discussed in Chapter 2 steam is the most effective heating medium used in thermal processing applications and ultrasound can be used to enhance heat transfer. Literature has lack of studies conducted about the use of ultrasound and steam technology for surface microbial decontamination, enzyme inactivation and heat transfer enhancement. In this chapter a study was conducted to see the effectiveness of ultrasound and steam technology for microbial surface decontamination in fresh vegetables.

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Results have been presented as posters/ oral presentations in conferences:

Basumatary, R., Vantankhah, H., Dwivedi, M., John, D., and Ramaswamy, H.S., 2020. Ultrasound-steam combination process for microbial decontamination and heat transfer enhancement: Overview and Concept Testing. Poster presentation in IFTPS Annual Meeting in San Antonio (March 3-5, 2020). Won 3rd prize in 2020 IFTPS Charles R. Stumbo Student Paper Competition.

Basumatary, R., Vantankhah, H., and Ramaswamy, H.S., 2021. "Evaluation of surface microbial destruction of vegetables using ultrasound to enhance heat transfer from steam and steam/air mixtures". Poster presentation in NABEC 2021 Virtual Program July 26th -July 27th.

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Contribution of authors

Basumatary is the PhD candidate and carried out most of the experimental work with Vatankhah, Dwivedi and John providing some technical assistance in planning, execution, and analysis of the experiments. The research was carried out under the direction of Professor Ramaswamy.

Chapter 3

Evaluation of combined ultrasound and steam heating process for fresh vegetables surface microbial decontamination and thermal destruction kinetics of microorganisms

Abstract

In commercial thermal processing applications, steam is the most effective heating medium used. However, for rapid short time direct heating of food products it poses some serious difficulties because of the presence of air along the surface and internal tissues of the food products. The entrapped air interferes with the heat transfer mechanism and slows down the effective heating of the surface. Addition of an ultrasound environment to steam can assist in removing this surface barrier and promote better heat transfer. The objectives of this research were to evaluate the suitability of ultrasound and steam combination heating for surface microbial decontamination. Experiments were carried out with different vegetables surface impregnated with non-pathogenic *Escherichia coli* as the test microorganism, and subjected to different steam, air, and ultrasound combination heating conditions. It was demonstrated that a short-time (<30 s) heating with combined ultrasound and steam could result in 3-6 logarithmic cycle destruction of *E. coli* population on carrots, cauliflower, broccoli, and zucchini surfaces compared to steam or steam/air only treatment where only 2-5 log CFU reduction was achieved in 30s. The focus of this study was to demonstrate the success of an ultrasound-steam combination process for surface microbial decontamination.

3.1. Introduction

Microbial contamination causes spoilage of freshly produced fruits and vegetables, meat and poultry, fish etc. The surface of fresh produce or meat, poultry and fish can be contaminated by pathogenic and spoilage bacteria to different degrees. The source of these microbial contamination is generally wastewater, animal or human feces, soil and harvesting, and storage conditions. Food safety and quality can be improved by reducing the surface contamination of these food products. Various physical and chemical microbial decontamination techniques have been implemented to render food safe.

Thermal processing is one of the most common methods for achieving safe foods with an extended shelf life but, in general, it also leads to some loss in nutritional and sensory quality of the foods (Ball and Olson, 1957). Steam has been the most effective medium for heating with perhaps the highest level of heat transfer coefficient associated with it and is used successfully in most commercial thermal processing operations. Surface decontamination of foods requires that a brief exposure to steam is employed and is intended to not result in any significant temperature increase in the produce bulk which can result in quality change. A lot of information is available in the published literature on the use of steam as a heating medium for decontamination in thermal processing of beef, raw poultry meat, fruits and vegetables and fish (Peterz et al., 2016; Bezanson et al., 2018). However, the presence of air along the surface and internal tissues of the food products makes it difficult for rapid short time steam heating of the food products. Heat transfer rates are degraded by the presence of air as it interferes with the condensation mechanism and as a result diminishing the effective heating of the food surface. Efforts have been made to solve this problem by subjecting the food to a prior vacuum. Vacuum-steam-vacuum (VSV) process has been introduced to increase the efficiency of heat transfer for decontamination of different types of food products (Hassan et al., 2015; Hormansperger et al., 2016; Shah et al., 2017). This process involves subjecting the food to vacuum first to remove the surface air and then to steam treatment for efficient condensation heat transfer and finally a second vacuum treatment to remove the condensed water through evaporation. Though VSV process can efficiently decontaminate many food products, the presence of air that comes with the product when introduced in a continuous system can still pose the same problem.

Adding an ultrasound environment in combination with steam can help to remove this surface barrier contributed by air and promote a better heat transfer. Ultrasound is one of the widely used technologies for many commercial applications. Ultrasound refers to sound waves with frequencies higher than 16 kHz and most of the applications range between 20 kHz to 100 MHz (Yusaf and Al-Juboori 2014). The basic mechanism postulated for microbial inactivation is the acoustic cavitation. Cavitation is the formation of low-pressure voids in the liquid which grow, briefly oscillate, and then asymmetrically implode with great intensity. This generates extremely high local temperature zones, heating/cooling rates and pressure waves, giving rise to many chemical reactions (sono-chemical). Many studies show the successful application of ultrasound for enhancement of food processing efficacy and preservation methods with minimal or no damage to the product quality (Ercan and Soysal, 2013; Tao and Sun, 2013; Kentish and Feng, 2014; Turantaş et al., 2015; Musielak et al., 2016; Gallo et al., 2018; Rodríguez et al., 2018). While steam and ultrasound have been independently used successfully for various food processing applications, their combination has not been widely evaluated to improve the efficacy of surface decontamination. The objective of this study was to evaluate the effectiveness of ultrasound-steam combination process over conventional steam or steam/air process for microbial surface decontamination and to characterize, to model the kinetics of microbial destruction using combined ultrasound steam and demonstrate the effectiveness for microbial decontamination over conventional steam processing using the destruction rate as the parameter.

3.2. Materials and Methods

3.2.1. Raw materials

Fresh carrots (*Daucus carota*- Farmers market Canada), cauliflower (*Brassica oleracea var. botrytis*), broccoli (*Brassica oleracea var. italica*) and zucchini (*Cucurbita pepo var. giromontiina*) were purchased from a local supermarket (Maxi & Cie, QC, Canada) one day before the experiments were planned and stored at 4ºC.

For surface decontamination studies in the pilot plant, the microorganism used was a non-pathogenic strain *Escherichia coli* K-12 (ATCC-29055), which has been used in other studies as a surrogate for *E. coli* O157:H7 (Awuah et al., 2005). *Escherichia coli 30800472* and *Listeria monocytogenes* 1870 were the pathogenic strains used in thermal destruction kinetics studies (lab scale) to compare with the destruction kinetics of the non-pathogenic strain to ensure that *E. coli* K-12 represented a good surrogate for pathogenic control.

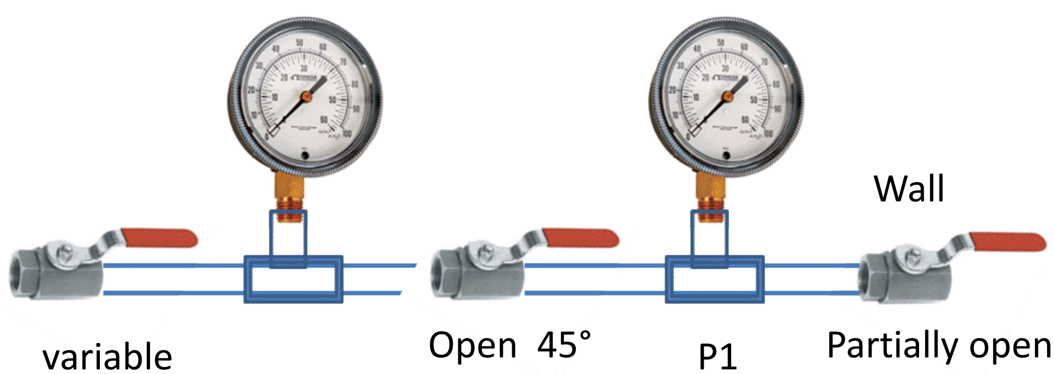
For microbial analysis the media and solutions used were: Tryptic soy broth (TSB), Tryptic soy agar (TSA), Brain heart infusion (BHI) and 0.1% sterile peptone water from Sigma-Aldrich, Difco Laboratories, Detroit, MI, USA.

3.2.2. Ultrasound -steam heating equipment setup

The ultrasound-steam equipment comprising of steam chamber, horn, transducer, and ultrasound power source - a laboratory-scale ultrasonic processor, LSP-500 was used for the studies involving microbial surface decontamination, enzyme inactivation and heat transfer enhancement. The steam chamber was modified to ensure better product treatment and better application of ultrasound to the treated product under a confined chamber volume. To ensure the quantity of steam supplied to the treatment chamber, studies were also conducted after installing a pressure gauge and various downstream valves to control the amount of steam flow. Steam flow from the boiler was scaled down to be introduced at atmospheric pressure into the steam chamber. Air supply was similarly introduced into the chamber and an air flow meter was installed to quantify the airflow. The different stages of equipment setup are described below in detail.

a. Steam Chamber

b. Protractor to measure valve opening

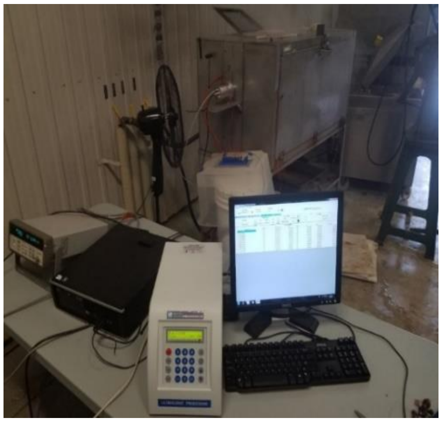


c. Valves to control steam flow.

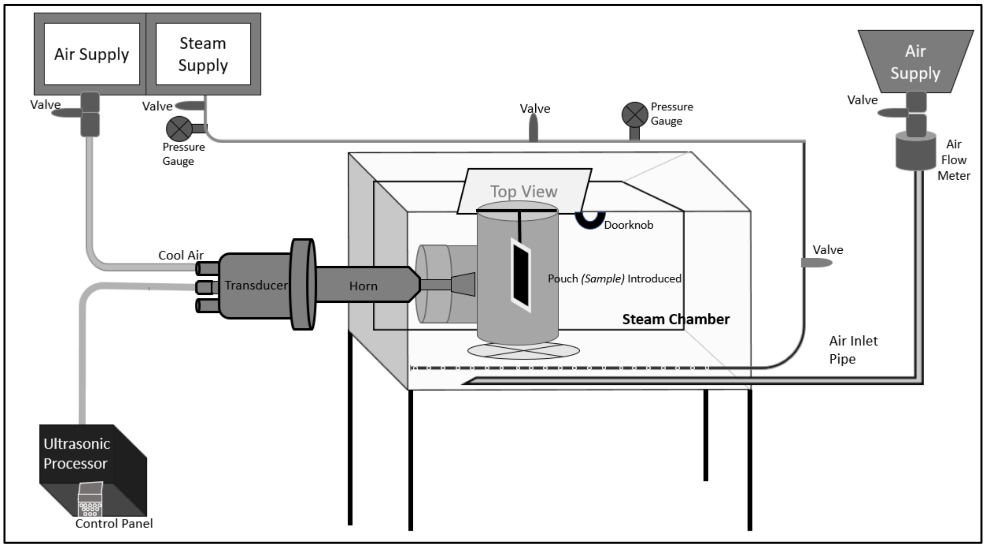
Figure 3.1: Steam Flow measurement setup (a to c)

The different parts and schematic diagram of the ultrasound-steam setup are shown in Figure 3.2.

a. Steam Chamber (closed) **b. Inside view of steam chamber with horn and duct**



c. Ultrasound transducer **d. Ultrasound Horn** **e. Ultrasound generator/controller**



f. Schematic diagram of ultrasound steam treatment set up.

Figure 3.2. (a-f) Ultrasound -steam equipment setup

The treatment setup consists of two main components:

a) The steam chamber/ steam cabinet to bring steam and air at specific rates.

b) The ultrasound system for application of ultrasound in the steam/air environment

Steam Chamber:

The steam chamber was a rectangular shape box made of stainless steel. Stainless steel was used as it was compatible for food products. It was connected to the source of steam supply from the boiler which is delivered at 15 psig pressure (i.e. 30 psia steam inlet). Steam was injected into the chamber through a plurality of nozzles from the bottom and delivered at atmospheric pressure (15 psia or 0 psig). The nozzles were designed to help in uniform steam distribution inside the chamber. After 10 min purging nearly saturated steam heating conditions with temperature close to 100°C could be established in the chamber. The pipeline that connected the steam chamber and steam supply source was fitted with 3 ball valves and 2 pressure gauges as shown in Figure 3.2 for facilitating calibration and measurement of steam flow rate at atmospheric pressure. The purpose of the valves was to have control over the amount of steam flowing into the chamber (kg/h) as per the experimental requirements. The gauges helped to read the line pressure and determine the pressure difference caused due to the different valves opening angles and how it affected the mass flow rate of steam.

Test samples were introduced into the steam chamber from the top. A small opening was cut on top of the steam chamber for introducing the samples to be treated and then partially covered to allow gradual purging of the medium. The steam chamber was quite large (119 cm x 60 cm) for the size of ultrasound transducer and horn. Since we had only one small capacity ultrasound horn mounted in the steam chamber, to provide the maximum effect of ultrasound waves a T-shape circular large diameter metal duct was used and placed inside the steam chamber as shown in Figure 3.2. The sample to be treated was then introduced from the top and placed very near to the horn. The dimensions of the duct are 20.5 cm diameter and 37 cm length in each direction of the T opening. This should be kept in mind for scale up purposes. A front door was provided to open and close the steam chamber and for any modification of the system.

During the treatment, both the front door and top opening (made for sample introduction) were fully closed, to contain the maximum amount of steam inside the chamber and facilitate purging the steam through the small port at the top without building up pressure inside.

The steam chamber was connected to the ultrasound system through the transducer and horn mounted on it (Figure 3.2). The ultrasound equipment (LSP 500, Industrial Sonomechanics, LLC, Miami, Florida) consisted of a 500 W ultrasonic generator (500 W LSP-500 generator) which transformed the 50 Hz AC line power to a 20 kHz signal that drove the piezoelectric transducer (ACT-19-LSP). The amplitude of oscillation (power) could be varied between 20 to 100 %. The transducer was coupled to a horn (CH-type horn with 12.7 mm tip**)** which amplified and transmitted the vibration down its length to the steam in the treatment chamber. The vibrating surface of the horn promoted acoustic cavitation in the steam/liquid medium. This phenomenon can typically be seen as a cloud of bubbles forming in the vicinity of the tip and heard as an intense hissing noise. The system was operated using the software driven instrument controller and temperature data were gathered using an Agilent data acquisition system. The set-up is illustrated as shown in Figure 3.2. Figure 3.2a is the steam chamber in which the vegetables were subjected for treatments. Figure 3.2b shows the inside view and the top view of the steam chamber when its open with the duct which is the treatment area. Figure 3.2c is the ultrasound horn; Figure 3.2d is the ultrasound transducer and Figure 3.2e is LSP-500 - laboratory scale ultrasonic processor. Figure 3.2f is the schematic diagram of overall experimental setup.

The main components are discussed below in detail:

1. **Ultrasound generator:** The 500 W LSP-500 generator is designed to supply a continuous resonant frequency lock and a constant amplitude during operation. The generator's LCD display can be used to change the settings for the ultrasonic amplitude (20% - 100%) or the duration of the ultrasonic output. Additionally, this generator has the capability of 'pulsed' operation. The generator supplies constant amplitude to the sample by automatically adjusting the power delivered to the transducer as a function of variable loading conditions. This capacity generator was chosen to make it suitable for pilot scale processing. The ultrasonic generator transforms the 50 Hz AC line power to a 20 kHz signal that drives the piezoelectric transducer.

2. Ultrasound transducer: The LSP-500 processor has an air-cooled piezoelectric transducer, ACT-19-LSP. This transducer has a power rating of 500 W and can supply ultrasonic energy to both batch and continuous processes. It is necessary that the transducer be cooled with clean and dry compressed air if operating for more than 30 min. A supply of cool dry air is provided to cool.

3. Ultrasonic horn: Full-wave Barbell Horn (FBH) with 21 mm tip **(**286 mm L x 21 mm tip dia) made of titanium alloy with maximum amplitude of 90 microns was attached to the transducer. The horn amplified and transmitted the vibration down its length to the steam in the treatment chamber. The shape and size of the horn can control the intensity of radiation and can determine the amount of amplification. This can be modified as per the requirement during scale up but the same is not the case for the CH-type horn, so FBH horn was chosen for design of this system.

4. Steam flow measurement: To quantify the amount of steam used for the experiments, steam flow measurement was measured. Figure 3.1. shows the experimental setup for steam flow measurement. The opening of the first valve (near the wall) was kept constant (marked). 2nd valve was opened at 45° angle and kept constants for all the experiments. Steam supply was at 15 psi. All the experiments were conducted for 1 min as it was difficult to contain the steam in the container for longer time. The amount of steam condensed was measured.

3.2.3. Experimental procedure

Microbial cell cultures were prepared for both pathogenic and non-pathogenic strains, and thermal destruction kinetics studies were carried out to ensure that the non-pathogenic strain used for the studies (inoculated on the vegetables) in the pilot plant scale had better heat resistance than the pathogenic strains that can be present in the fresh vegetable produce.

3.2.3.1. Preparation of cell cultures for microbial destruction studies

Escherichia coli K-12 (ATCC-29055), which has been used in other studies as a surrogate for *E. coli* O157:H7 (Awuah et al., 2005; Arya et al., 2017) was used in the pilot plant scale studies. *Escherichia coli 30800472* isolated from bovine clinical mastitis and *Listeria monocytogenes 1870* were the pathogenic strains used in thermal destruction kinetics studies (lab scale) to compare with non-pathogenic strain to ensure that *E. coli K-12* represents a good surrogate for pathogenic control. The cultures were maintained at -80°C for storage. To prepare the inoculum, the cultures were streaked in TSA plates and incubated at 37°C for 24 h. A loop of inoculum was then suspended in 20 ml tryptic soy broth (Sigma-Aldrich, Difco, MO, USA) and incubated in shaker incubator @ 150 rpm for 24 h at 37°C. The bacterial solution was then centrifuged for 10 min at 12500 rpm. The pellets obtained after centrifuging was then washed with 20 mL phosphate buffer solution. The washing-centrifugation procedure was done three times by discarding the supernatant after each centrifugation and re-suspending the pellet in phosphate buffer solution. The final inoculum stock solution contained approximately 10^8 CFU/mL bacterial cell population and was stored at 5°C until required.

3.2.3.2. Thermal destruction kinetics studies in the lab

A temperature-controlled water bath was used for carrying out the thermal treatments. Stainless steel heating tubes with O-ring sealed screw caps were used as the sample holder. The come-up time in the stainless-steel capped test tube was approximately 1 min. Based on the data on thermal destruction kinetics of *E. coli* K-12 from the literature, three temperatures (60, 65 and 70°C) were selected for the thermal treatments. For each temperature, three holding times were used. The holding times did not include the come-up time (about one min). The intervals of holding times were different for each treatment temperature. To ensure the accuracy of results the treatments were carried out in triplicates. For each treatment, 1.5 ml of distilled water was taken into a previously autoclaved stainless steel capped test tube. About 100 µl inoculum of ***E.coli*** K-12, *E. coli 30800472 and L. monocytogenes 1870* was added into each tube, mixed, and the tubes were sealed. The tubes were heated by suspending them in the water- bath for specified times. After each treatment, the tube was cooled by immersing in an ice water bath for 10 min. It was then further taken for microbial enumeration.

3.2.3.3. Sample preparation

The fresh vegetable samples – carrots, cauliflower, broccoli, and zucchini were cut into specific dimensions only 1-2 h before the experiments. Carrots were peeled, washed with tap water to remove any visual impurities, and diced into cubes (1.5 cm x 1.5 cm) using vegetable chopper. Cauliflowers and broccolis were cut into uniform florets (0.90 to 2.2 cm of florets) after removing the leaves and washing. Zucchinis were diced into cubes (1.5 cm x 1.5 cm) using a vegetable chopper. The cut vegetables were inoculated with microbial culture and then subjected to different surface microbial decontamination treatments.

3.2.3.4. Culture preparation and inoculation

The *E. coli* K-12 (ATCC-29055) inoculum stock solution mentioned previously which contained approximately 10^8 CFU/mL bacterial cell population was inoculated on the cut vegetable samples. The inoculation techniques were standardized based on dipping /coating time; amount of inoculum used for dipping or coating and the time required for drying the inoculated samples to give maximum number of colonies count on the vegetables before treatment.

Cut carrots were used to standardize the inoculation techniques. For dipping technique, carrot cubes (10 g) were dipped in 20 mL *E. coli* inoculum for 30 s,1 min and 2 min (Haughton et al., 2012), transferred to sterile petri-dish and air dried inside a laminar flow hood under mild air flow conditions for around 30 min and enumerated. For coating technique, one side of the carrot cubes were placed on sterile petri-dish and coated with 100 µl and 200 µl of inoculum and kept for drying under laminar flow hood under mild air flow conditions for 30 min and enumerated to compare.

After standardization the coating technique with 200 µl of inoculum coated on one side of the carrots showed better results with higher counts compared to 100 µl coating or dipping technique.

Likewise, all the other vegetables (cauliflower, zucchini, and broccoli) were also coated with 200 µl of inoculum, dried for 30 min under laminar flow hood to ensure the vegetables are inoculated with maximum microbial culture and transferred to sterile bags. Sterile bags aided in transferring the samples safely to the treatment chamber. The inoculated vegetables are then subjected to different ultrasound steam treatments for surface microbial decontamination studies.

3.2.3.5. Ultrasound – steam treatments

The inoculated vegetables were subjected to combined ultrasound-steam and steam only treatment both in presence (25% air/75% steam) and absence of air with the coated side facing the ultrasound horn. The system was set to operate in continuous mode and 75% amplitude setting. For subjecting them to ultrasound and/or steam treatment, vegetables were attached at the tip of a specially prepared long metal wire and exposed to the steam and ultrasound -steam treatment environment in presence and absence of air for short intervals of time (5, 15 and 30 s) in continuous mode, with and without ultrasound. Immediately after the treatment, they were removed and dipped in ice cold peptone water solution to cool.

3.2.3.6. Microbial enumeration

From thermal destruction studies the thermally treated samples were poured into presterilized micro centrifuge tubes. Appropriate serial dilutions of samples from thermal treatments as well as control were performed in 0.1% sterile peptone water (Difco Laboratories, Detroit, MI, USA). Both pathogenic and non-pathogenic strains of *E. coli* were spiral plated (spread plate technique) on TSA plates (Difco Laboratories, Detroit, MI, USA) and pathogenic listeria was spiral plated on BHI agar plates (Sigma-Aldrich, Difco Laboratories, Detroit, MI, USA). The plates were incubated for 24 h at 37°C under aerobic conditions and then enumerated.

As per the standard procedure mentioned in Health Canada for microbial enumeration in food samples, 25g of treated or inoculated sample was mixed with 225 g (0.1% peptone water) in stomacher bag and ground in stomacher for approximately 2 min for homogenization. The sample was then serial diluted upto 10^7. As per the treatments and dilutions 100µl of 10^2 to 10^7 dilution were plated on TSA (Difco Laboratories, Detroit, MI, USA) plates in triplicates and incubated for 24 h at 37°C for enumeration. The results were expressed as CFU/mL for thermal destruction studies and CFU/g for the food samples, calculated using Equation 3.1.

$$\frac{CFU}{ml} = \frac{Number\ of\ colonies\ counted\ X\ Dilution\ Factor}{Volume\ of\ sample\ plated} \tag{3.1}$$

3.2.3.6. Kinetic data analysis

Based on the assumptions that (a) the destruction of *E. coli - K12* cells inoculated in distilled water treated at any temperature occurred at random in accordance with the first-order kinetics model and (b) all cells have the same resistance to heat; the microbial count data were analysed to determine the parameters of thermal destruction kinetics (Vatankhah et al., 2019). According to prior hypotheses, survival curves could be used to determine the decimal reduction time (D value) of each treatment condition (Ramaswamy et.al. 2019; Equation 3.2).

$$Log\frac{N_t}{N_0} = \frac{-t}{D_T} \tag{3.2}$$

where t is the exposure time (min), where *N* is the survival number of cells or spores after treatment (CFU/mL), N_0 is the initial count (CFU/mL), and D_T is the decimal reduction time (min) at temperature T (°C), which is equal to the negative reciprocal slope of the semi-logarithmic survival curve and strongly dependent on temperature. As a result, the D value can be calculated as the negative reciprocal of the log (N/N_0) versus time (min) linear regression slope (Equation 3.3)

$$D = -\frac{1}{slope} \tag{3.3}$$

The temperature sensitivity indicator (z value), in addition to the D value, was calculated. The z value was calculated by graphing the linear scale of the treatment temperature with the decimal logarithm of the decimal reduction time (log10 D value). The Z value was calculated as the negative reciprocal of a thermal resistance curve's linear regression slope (log D vs. T; Ramaswamy et al., 2019).

$$z = \frac{(T_2 - T_1)}{[\log(D_1) - \log(D_2)]} \tag{3.4}$$

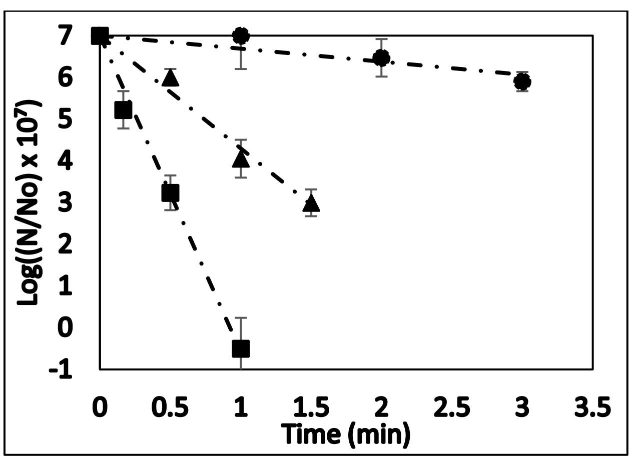
where D_1 and D_2 are *D* values at temperature T_1 and T_2, respectively.

3.3. Results & discussion

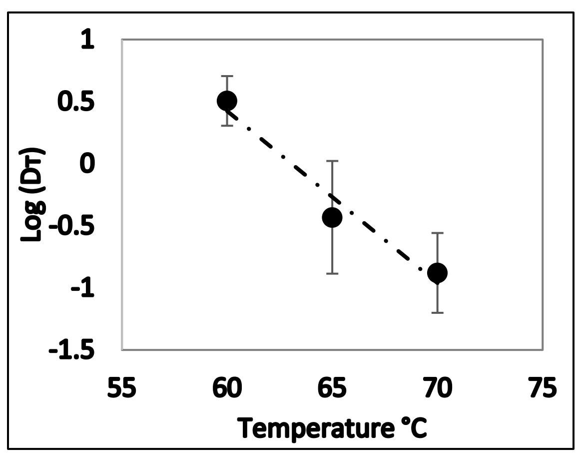
3.3.1. Thermal destruction kinetics

The survival curves of *E. coli* K-12, *E. coli 30800472* and *L. monocytogenes 1870* for thermal destruction kinetics at various temperatures are shown in Figures 3.3, 3.4 and 3.5, respectively. When the temperature used was lower, the destruction slope was less steep, and when the temperature used was higher, the destruction slope and steepness rose, exhibiting higher destruction rates. The semi-logarithmic curves showed a high linearity as a function of processing temperature, confirming the applicability of first-order or log-linear destruction kinetics, as shown in Figures 3.3 (1a), 3.4 (1a) and 3.5 (1a). Using the coefficient of regression (slope), the D values at 60–70°C were estimated to vary from 3.21 to 0.13 min for *Escherichia coli K-12*, 2.88 to 0.12 min for *Escherichia coli* 30800472, and 2.83 to 0.03 min for *Listeria monocytogenes* 1870. (Table 3.1). *Escherichia coli* K-12 had a D value of 4.88 min in water at

55°C, compared to a D value of 4.44 min in margarine at 60°C, (Hamoud- Agha et al., 2014; Vatankhah et al., 2019). According to another study (Jin, 2008), *E. coli* K-12 in the liquid whole egg had a D value of 0.22 min at 60°C and a similar D value of 0.22 min at 70°C. Data from the literature demonstrates that *E. coli* K-12's D value fluctuates according to the products' composition and processing circumstances. Data collection under experimental conditions was therefore crucial. Since this study was focused on surface microbial decontamination of vegetables and the vegetables have high water content the thermal destruction kinetics was carried out in water. The temperature sensitivity values of *L. monocytogenes* 1870, *E. coli* 30800472, and the *E. coli* - K12 were determined using Equation (3.3), which was used to fit the D value data. The range of z values found in this study compares favorably with other papers. (Hamoud -Agha et.al 2014; Jin, 2008; Vatankhah et.al., 2019; Koutchma et.al 2001). Table 3.3 shows the D and z values for the *E. coli-K 12 (non-pathogenic strain) used* for coating the vegetables and those for pathogenic strains *(Listeria monocytogenes 1870 and E. coli 30800472*). The D values for K-12 ranged from 0.13 - 3.21 min between 70°C and 60°C while that for the pathogenic strains ranged from 0.03 to 2.88 min. The non-pathogenic strain of K-12 was observed to be more thermally resistant than the pathogenic strains of both *L. monocytogenes and E. coli*. Therefore, *E coli* K-12 represents a good surrogate for pathogen control and its inactivation can be used as a conservative estimate for pathogen destruction.

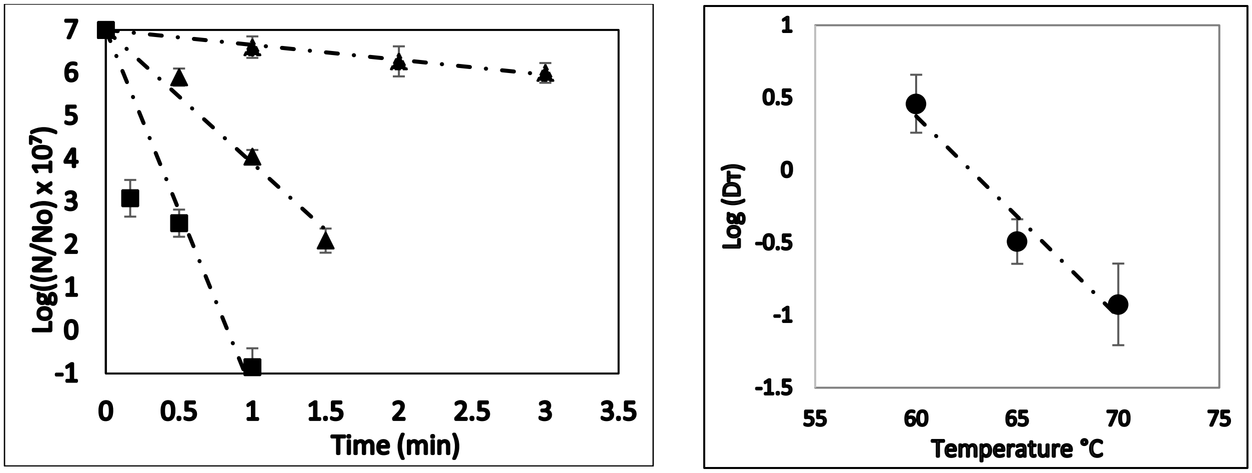


1a.



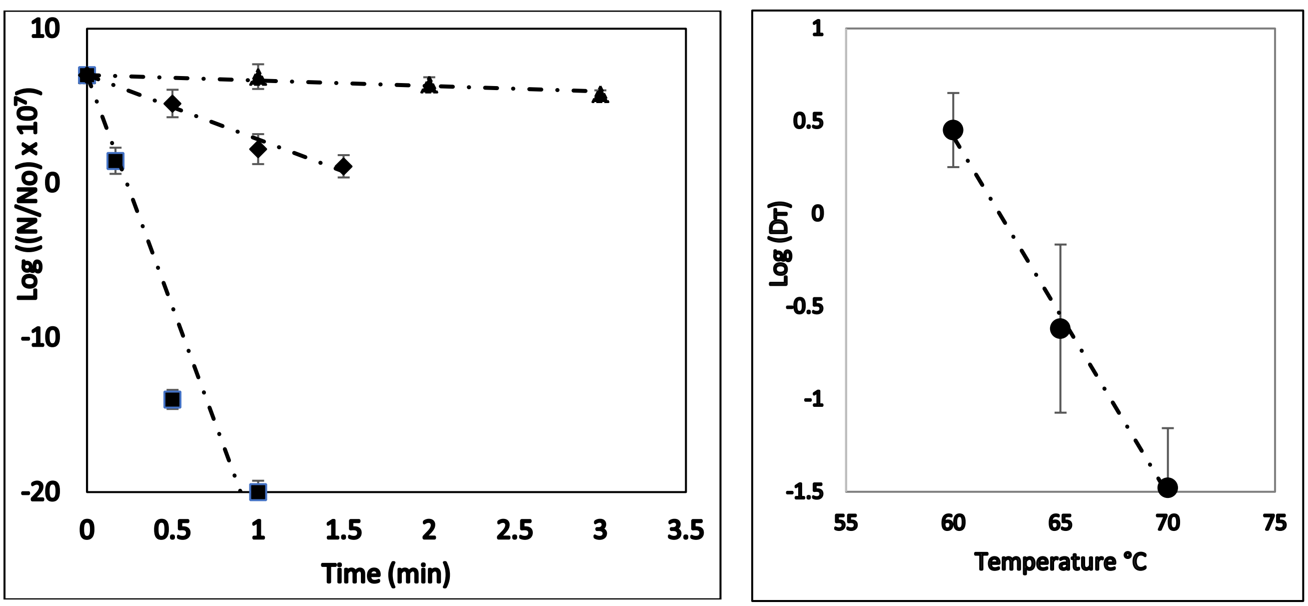
1b.

Figure 3.3. (1a) The semi-log survival curves of thermal destruction of *Escherichia coli* K-12 (ATCC-29055) during pasteurization at 60°C (●), 65°C (▲), and 70°C (■); (1b) the heat resistance parameter estimation using z value concept.



1a. 1b.

Figure 3.4. (1a) The semi-log survival curves of thermal destruction of *Escherichia coli 30800472* during pasteurization at 60°C (●), 65°C (▲), and 70°C (■); (1b) the heat resistance parameter estimation using z value concept.



1a. 1b.

Figure 3.5. (1a) The semi-log survival curves of thermal destruction of *Listeria monocytogenes 1870* during pasteurization at 60°C (●), 65°C (▲), and 70°C (■); (1b) the heat resistance parameter estimation using z value concept.

Table 3.1. Estimated D value and z value parameters of pathogenic and non-pathogenic bacteria

Temperature °C	*Listeria monocytogenes 1870*		*E. coli 30800472*		*E. coli K-12 ATCC 25404*	
	D value (min)	R^2	D value (min)	R^2	D value (min)	R^2
60	2.83	0.92	2.88	0.99	3.21	0.90
65	0.24	0.97	0.32	0.99	0.37	0.98
70	0.03	0.92	0.12	0.88	0.13	0.94
	Z value (°C)	R^2	z value (°C)	R^2	z value (°C)	R^2
	5.18	0.99	7.35	0.98	7.22	0.96

3.3.2. Surface Microbial Decontamination

Table 3.2 shows the effect of ultrasound with steam/air heating medium (25% air / 75% steam) with different vegetables (carrots, cauliflower, broccoli, and zucchini) with *E. coli K-12* on surface. Reduction was achieved even *in the presence of air (75/25 steam/air*), the destruction varied from no reduction with 5 s heating, 1 log after 15 s and 2 log after 30 s heating. The destruction however increased to 1, 4 and 5 logs after 5, 15 and 30s when subjected to combined ultrasound-steam/air treatment. Thus, the added ultrasound resulted in a significant 1-3 log extra reduction in microbial population. This level of air inclusion is normally used in thermal processing applications when employing flexible packages and the added air provides the needed overpressure to protect the packages from failure. It also reduces the heat transfer coefficient from steam condensation from about 20,000 to 2,000 W/m^2C due to the insulating effect caused by included air. Addition of ultrasound helps to move the stagnant air layer away from the contact surface providing opportunity for enhancing the condensation of steam on product or container surface.

Table 3.3 shows the results with steam in a well vented chamber – a situation not easy to establish during open steam applications in a steam box tunnel as would normally be carried out in practice. Studies on carrots, cauliflower, broccoli, and zucchini showed that 3-6 log CFU reduction could be achieved within 30 s when these vegetables inoculated with *E. coli K-12* were subjected to combined ultrasound-steam treatment compared to steam only treatment where 2-5 log CFU reduction was achieved in 30 s. The differences were lower because steam is already better than steam/air. These results show that in the absence of air, higher destruction can be achieved. Even so, contribution of ultrasound is evident. In practice, air free steam treatment is not possible as the open entry and exit that are present in these systems always bring air as the vegetables are fed into the system. Moreover, the vegetable also has entrapped air which come out during the treatment; therefore, addition of ultrasound is helpful for enhancing the microbial destruction effect.

Table 3.2. Microbial decontamination of different vegetables under steam/ air (75/25) *vs* steam/air (75/25) plus sound

Treatment	**Exposure Time (s)**	**Residual survival counts *E coli-K 12* counts (CFU/g)**							
		Carrots		Cauliflower		Broccoli		Zucchini	
		Average CFU/g	Std Deviation	Average CFU/g	Std Deviation	Average CFU/g	Std Deviation	Average CFU/g	Std Deviation
Initial Count	0	5.7×10^8	0.14×10^8	5.0×10^8	0.42×10^8	5.3×10^8	0.28×10^8	4.5×10^7	0.32×10^7
Steam/ Air + Sound	5	6.0×10^7	0.56×10^7	7.4×10^6	0.57×10^6	9.7×10^5	0.42×10^5	4.2×10^6	0.36×10^6
Steam /Air	5	4.0×10^8	0.21×10^8	2.5×10^8	0.23×10^8	3.0×10^8	0.28×10^8	3.0×10^7	0.23×10^7
Steam/ Air + Sound	15	9.5×10^4	0.70×10^4	6.5×10^5	0.56×10^5	4.3×10^5	0.42×10^5	8.9×10^4	0.65×10^4
Steam Air	15	1.8×10^7	0.14×10^7	1.0×10^7	0.14×10^6	1.5×10^7	0.95×10^6	1.9×10^6	0.18×10^6
Steam/ Air + Sound	30	1.2×10^3	0.64×10^3	5.7×10^3	0.28×10^3	2.0×10^3	0.14×10^3	1.6×10^2	0.62×10^2
Steam Air	30	6.7×10^5	0.36×10^5	6×10^5	$.77 \times 10^5$	2.8×10^5	0.74×10^5	2.3×10^5	0.46×10^5

Table 3.3. Microbial decontamination of different vegetables under saturated steam vs steam plus sound

Treatment	Exposure Time (s)	Residual survival counts *E coli-K 12* counts (CFU/g)							
		Carrots		Cauliflower		Broccoli		Zucchini	
		Average CFU/g	Std Deviation	Average CFU/g	Std Deviation	Average CFU/g	Std Deviation	Average CFU/g	Std Deviation
Initial Count	0	5.7×10^8	0.14×10^8	5.0×10^7	0.42×10^7	5.3×10^7	0.28×10^7	4.5×10^7	0.32×10^7
Steam + Sound	5	2.5×10^5	0.28×10^5	3.0×10^3	0.28×10^3	2.8×10^4	0.14×10^4	3.9×10^3	0.29×10^3
Steam Only	5	7.8×10^6	0.56×10^6	4.5×10^5	0.14×10^5	6.7×10^5	0.30×10^5	4.8×10^5	0.17×10^5
Steam + Sound	15	1.0×10^3	0.28×10^3	5.0×10^2	0.42×10^2	2.0×10^2	0.16×10^2	7.0×10^2	0.39×10^2
Steam Only	15	8.0×10^4	0.70×10^4	5.9×10^3	0.56×10^3	3.0×10^3	0.15×10^3	6.4×10^3	0.65×10^3
Steam + Sound	30	3.0×10^2	0.14×10^2	8.5×10^1	0.21×10^1	1.7×10^1	0.35×10^1	9.5×10^1	0.24×10^1
Steam Only	30	2.1×10^3	0.49×10^3	2.4×10^2	0.63×10^2	3.2×10^2	0.42×10^2	3.6×10^2	$0.67\ 10^2$

3.4. Conclusions

The study showed that ultrasound-steam combination heating is a promising technology for effective surface microbial decontamination of vegetables. The application of ultrasound demonstrated enhancement of heat transfer to achieve significant enhancement in microbial destruction. The study showed that the presence of air can interfere with the rate/extent of heat transfer to the treated vegetable surfaces thereby reducing the microbial decontamination efficacy. Addition of ultrasound to the steam or steam/air system reduced the surface barrier to heat transfer and enhanced the microbial decontamination efficiency. Surface contamination of vegetables presents a serious problem with respect to public health, safety, and their marketability. The study provided an alternate physical method for microbial decontamination of vegetables which is currently done with chemical treatments. The study helped establish novel processing methods for surface decontamination of pathogens in vegetables.

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Connecting Statement to Chapter 4

The potential of ultrasound-steam technology to enhance the heat transfer by reducing the influence of air was discussed in the previous chapter. Similar studies with slightly longer (moderate) treatment times are discussed with respect to enzyme inactivation in this chapter. Blanching is a short heat treatment given to fruits and vegetables to inactivate the naturally present oxidative enzymes which would otherwise affect the storage stability of canned, frozen and dehydrated foods and cause discoloration and off flavor formation. Enzyme inactivation of food products could be achieved by hot water, steam, microwave and hot gas heating conditions. The hypothesis for this chapter is that more rapid heating and more effective inactivation of enzyme can be achieved for blanching by the ultrasound-steam combination heating which can lead to better frozen food product quality. There is lack of studies in literature about the use of ultrasound–steam technology for enzyme inactivation. This chapter focuses on the use of ultrasound-steam technology for enzyme inactivation in different fresh vegetables. The blanching times for different vegetables under different treatment conditions were established through enzyme inactivation kinetics and vegetables were blanched to achieve 1.5 log reduction (~95% inactivation) of the enzyme peroxidase activity to be given as a pre-treatment prior to freezing and frozen storage.

This study was part of RITA-CTAQ project and funded by RITA Consortium.

Results have been presented as poster presentations in conferences:

Basumatary, R., Vantankhah, H., and Ramaswamy, H.S. 2020. Evaluation and modelling of peroxidase inactivation kinetics using piezo steam. Northeast Agricultural and Biological Engineering Conference (NABEC) 2020. July 28-29, 2020, two-day online event.

Basumatary, R. and H. S. Ramaswamy, H.S. 2021. “Evaluation of ultrasound-steam blanching process for enhancing the effectiveness of enzyme inactivation and quality retention in vegetables”. Poster presentation in IFT FIRST 2021 Virtual Program July 18th -July 21st, 2021.

Contribution of authors

Basumatary is the PhD candidate and carried out most of the experimental work with Vatankhah, providing some technical assistance in planning, execution, and analysis of the experiments. The research was carried out under the direction of Professor Ramaswamy.

Chapter 4

Establish the blanching treatment schedule for fresh vegetables using ultrasound-steam process for enzyme inactivation in different vegetables and compare with conventional treatment.

Abstract

Blanching is a short heat treatment given to fruits and vegetables to inactivate the naturally present oxidative enzymes which can result in undesirable changes in quality of canned, frozen, and dehydrated foods. Enzyme inactivation could be achieved by hot water, steam, microwave, and hot gas heating conditions. This study was aimed at demonstrating the effectiveness of ultrasound-steam heating process for enzyme inactivation as an advantage over conventional steam processing and hot water blanching. Peroxidase inactivation kinetics for different fresh vegetables such as carrots, broccoli, cauliflower, zucchini, and green bell pepper were evaluated, and blanching time was established based on 1.5 decimal reduction of peroxidase activity (~95% inactivation). Blanching times and quality retention based on texture, color and ascorbic acid retentions of different vegetables treated with ultrasound-steam and methods were evaluated and compared. Results showed that ultrasound-steam blanching was superior in terms of quality influence and reduced treatment time for blanching.

4.1. Introduction

Oxidative enzymes like peroxidase, polyphenol oxidase etc. are responsible for deterioration and post harvest loss of quality of foods during processing, ripening and storage. The nutritional quality and appearance are affected due to enzymatic browning caused by the oxidative enzymes in fruits and vegetables. This in turn reduces the consumer’s acceptability and therefore cause significant economic impact, both to food producers and to food processing industries. To prevent such changes in the quality there is a need to inactivate the enzymes in fruits and vegetables prior to processing.

Blanching is a mild heat treatment given to inactivate enzymes in fruit and vegetable processing prior to freezing, canning, or drying. It is an important unit operation in fruit and vegetable processing. This heat treatment is applied according to and depends on the specificity of fruits and vegetables, the objectives that are followed and the subsequent processing / preservation methods. It helps in modifying texture while maintaining nutritional value of food, preserve color and flavor, and inactivates enzymes that cause the development of off flavor and discoloration, especially prior to freezing and dehydration as this is the only major heat treatment given to them prior to processing. During canning, it also serves to remove the entrapped air in internal tissues which otherwise would be released inside the cans after sealing putting strain on the container during heating and defeating the purpose of creating vacuum in cans. It also facilitates better packing of fruits and vegetables in cans.

Thermal blanching has been practiced widely in the food industry; water and steam blanching being the most traditional methods. The commonly used thermal processing technologies for enzyme inactivation always result in some loss of nutritional components due to thermal degradation, color change, texture softening and leaching. There is a necessity for alternative technologies to reduce the enzyme activity without unduly influencing the quality of fruits and vegetables. Though the novel technologies such as pulsed electric fields, high pressure processing, and pulsed light and cold plasma have been tested for enzyme inactivation, they all have some limitations such as high initial investment and incomplete or non-uniform enzyme inactivation, incompatibility with the foods. Hence, it is important to explore alternatives or refinements to improve traditional methods.

Ultrasound or ultrasonic treatment has been recently explored for enzyme inactivation and has been found to be effective (Rathnakumar et al., 2023). Several ultrasonic combination treatments such as manosonication, thermosonication, and manothermosonication are possible, with treatments such as manothermosonication known to be able to inactivate enzymes at lower temperatures and/or in a shorter time than the comparable thermal process (Rastogi, 2011). Inactivation is thought to occur via a range of mechanisms such as thermal, with thermolabile enzymes being more sensitive to ultrasound than those that are heat resistant, free radical inactivation and impairment of substrates as examples (O'Donnell et al., 2010). The action of some enzymes can result in the deterioration of food flavors and changes in food color, e.g., browning. While enzyme inactivation can easily be achieved by heating, this can be detrimental to the food itself. As a result, the use of ultrasound as an aid to enhance a certain process or unit operation has become of more interest. The literature shows lack of studies for combination of traditional and novel methods for blanching. No studies have been conducted to study the use of steam as a medium of heat for blanching combined with ultrasound to enhance heat transfer during blanching.

The objective of this study was to evaluate the blanching kinetics using combined ultrasound and steam or steam/air, hot water heat treatments for different vegetables (carrots, cauliflower, broccoli, green bell pepper and zucchini), target peroxidase enzyme inactivation and compare the different quality parameters with conventional steam or hot water blanching.

4.2. Materials and Methods

4.2.1. Raw Materials

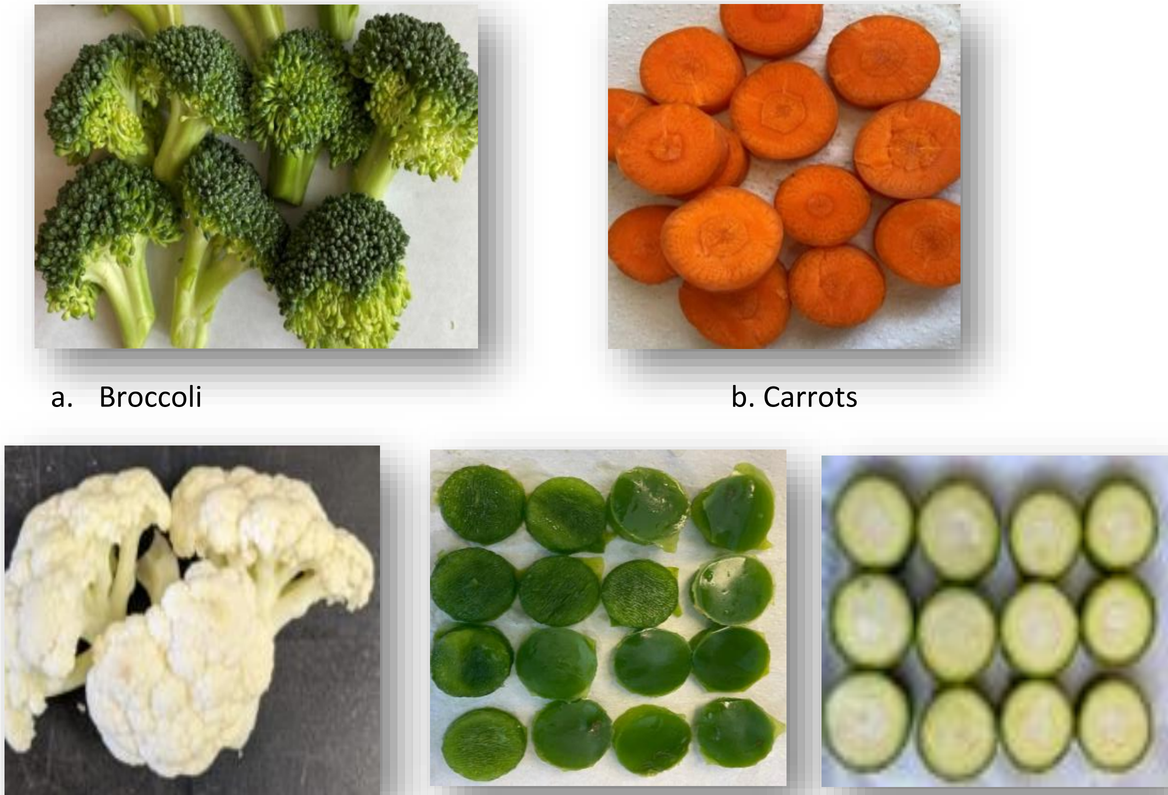
The same batch of *fresh* carrots ***(Daucus carota*- Farmers market Canada),** cauliflower *(Brassica oleracea* var. botrytis), broccoli (*Brassica oleracea* var. italica) zucchini (*Cucurbita pepo* var. giromontiina) and green bell pepper (*Capsicum annuum*) was purchased from a local supermarket (Maxi & Cie, QC, Canada) one day before the blanching and enzyme inactivation experiments were planned and stored at 4ºC.

4.2.2. Chemical reagents and buffer

For enzyme extraction polyvinylpyrrolidone and sodium phosphate buffer solution (pH 6.5) was used. For the POD assays, a substrate solution using 0.1 mL of hydrogen peroxide (30 g/100 mL solution), 0.1 mL guaiacol (99 g/100 mL solution) and 99.8 mL of sodium phosphate buffer solution (pH 6.5) were used. The concentration of buffer solution (sodium phosphate buffer) used for enzyme extraction and POD assay is 0.1 mol/L (pH 6.5).

4.2.3. Blanching treatments

The raw vegetables were peeled and sliced into appropriate dimensions (carrots 10mm thick & 25mm dia; zucchini 10 mm thick & 30 mm dia; green bell pepper -8 mm thick & 15mm dia; broccoli 30 mm thick flower section and flattened on one side; cauliflower 20mm thick flower section and flattened on one side) as shown in Figure 4.1 and known amount (50g each batch) were blanched with different thermal processing techniques.



a. Broccoli b. Carrots

d. Cauliflower d. Green bell pepper e. Zucchini

Figure 4.1. Vegetables cut into specific dimensions for blanching treatments

4.2.3.1. Ultrasound and steam or steam/air blanching

The cut vegetables were placed on perforated basket and subjected to combined ultrasound-steam and steam only treatment both in presence (25% air/75% steam) and absence of added air with the open end of the basket facing the ultrasound horn. The experimental setup for ultrasound-steam treatment was like that described in Chapter 3 with slight modification in the steam chamber as shown in Figure 4.2. The ultrasound system was set to operate in continuous mode and 75% amplitude setting. The vegetables were exposed to the steam and ultrasound - steam treatment environment in presence and absence of air for different intervals of time, 30-300 s in continuous mode, with or without ultrasound. Immediately after the treatment, they were immersed in ice cold water (4-5°C) to cool and reduce the enzyme inactivation process. The blanched vegetables were then taken for quality analysis – color, texture and ascorbic acid content analysis and enzyme inactivation studies.

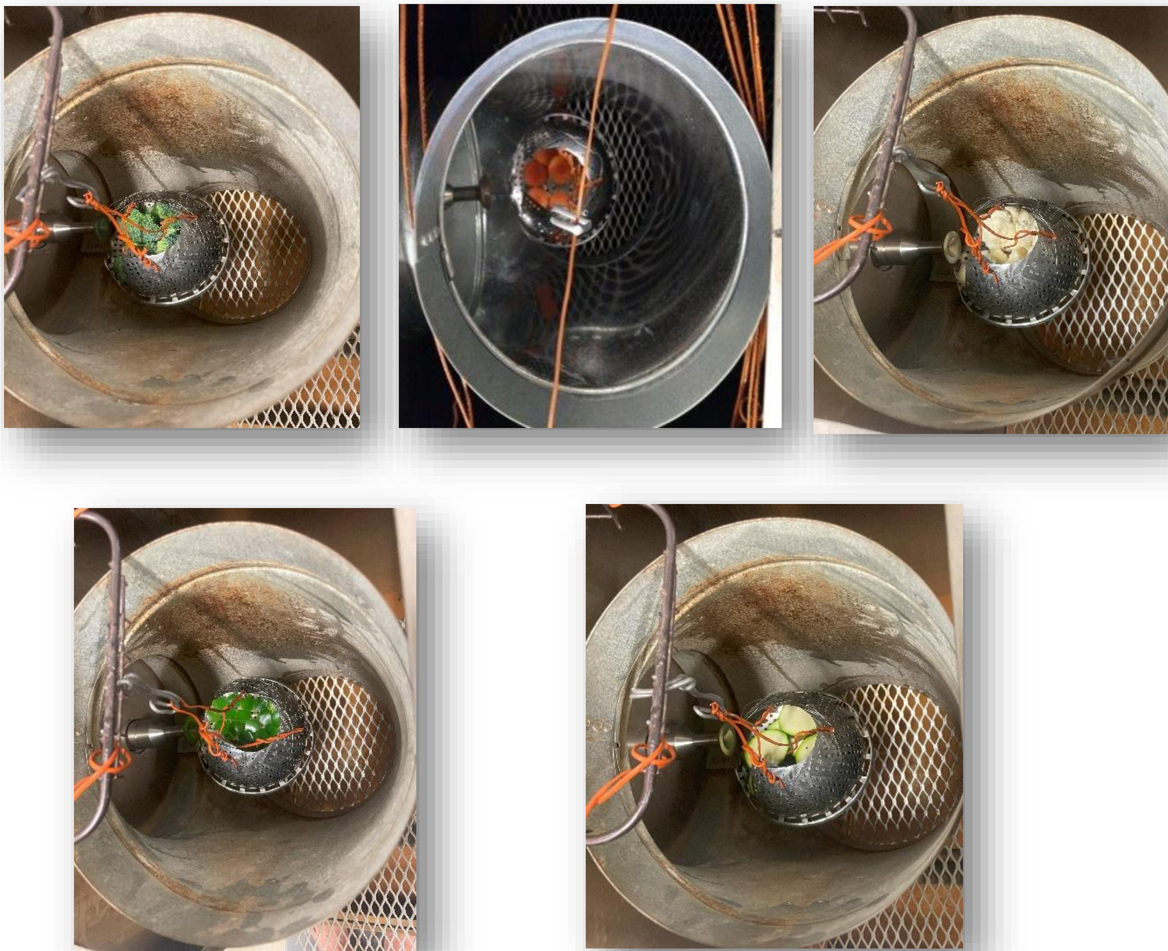


Figure 4.2. Cut vegetables placed in ultrasound-steam chamber for blanching

4.2.3.2. Hot water blanching

A hot water bath maintaining temperature at 95 ±3°C was used for blanching the cut vegetables. Same perforated basket and 50g of the vegetables were used for blanching. The vegetables were immediately cooled in ice cold water (4-5°C) to stop enzyme inactivation process. The blanched vegetables were then taken for quality analysis – color, texture and ascorbic acid content analysis and enzyme inactivation studies.

4.2.3.3. Ultrasound hot water blanching

A digital ultrasound water bath (Model – TH-SPQXJ-40A, made in China) of 10-liter capacity with ultrasonic frequency of 28/40 Hz, ultrasonic power of 240W and heating power of 500W was used for ultrasonic hot water blanching. The chamber used to hold the sample had dimensions of 53 x 32 x 22 cm. An external heating coil was added to the ultrasound water bath to speed up the heating process to the required processing temperatures and adequately maintain them. The ultrasonic waves were employed in full wave mode. Only carrot samples were treated in all the 3 different blanching treatments – hot water blanching, ultrasound hot water and ultrasound-steam blanching and further enzyme inactivation studies were conducted to compare the effectiveness of different types of blanching. We did not further do these comparison studies for other vegetables as our focus was just to understand the difference in blanching times when ultrasound is added to hot water and steam and then, compare it with conventional hot water blanching.

4.2.4. Enzyme inactivation studies

4.2.4.1. Enzyme extraction method

Approximately 20 g of blanched samples was homogenized at 4°C in mixer grinder for 5 minutes with 50 ml phosphate buffer (pH 6.5) and 1 g polyvinylpyrrolidone. Afterwards, the homogenized mixture was centrifuged (15000 g, 4 °C, 45 min), and supernatant was collected as enzyme extract. Enzymatic assays were performed on the same day of extraction.

4.2.4.2. Peroxidase (POD) activity assay

Assay of peroxidase was performed by the method of Morales-Blancas et al., (2002), with slight modifications by mixing 0.120 mL of enzyme extract with 3.5 mL of the peroxidase (POD) substrate solution. Immediately after mixing the extract with the substrate, the final solution was homogenized using a vortex before measuring the activity of the enzyme. POD activity was measured monitoring the change in absorbance during 400 s at 470 nm. The enzyme activity (EA) was defined as the amount of enzyme necessary to produce an increase of one unit in the value of absorbance during 1 min. The residual activity was determined as EA/EAo, where EA is the average EA after thermal treatment and EA_o is the initial enzyme activity (fresh vegetable). EA at t = 0 corresponded to the enzyme activity of extracts without thermal treatment.

4.2.4.3. Kinetics data analysis and blanching time determination

First-order kinetics (Equation 4.1) has been reported to describe the thermal inactivation of enzymes in general:

$$ln\frac{N}{N_\circ} = -\frac{t}{D} \tag{4.1}$$

Where, N represents the enzyme activity (change in optical density/min) at time (t) in min, No is the initial enzyme activity at time zero. D is the decimal reduction time (D-value is time needed to reduce 90% of the activity).

The D value (Equation 4.2) can be obtained as the negative reciprocal of the linear regression slope of log (N/No) versus time (min).

$$\mathbf{D = -\left(\frac{1}{slope}\right)} \tag{4.2}$$

Blanching time was determined as time taken to achieve ~95% inactivation of peroxidase enzyme. It was calculated as 1.5 D as determined above (Ramaswamy and Ranganna, 1981).

4.2.5. Quality analysis

4.2.5.1. Texture profile analysis

The hardness of blanched carrots, broccoli, cauliflower, zucchini, and green bell pepper were obtained using the TA-XT Plus Texture Analyser (Texture technologies corp., Scarsdale, NY, USA). The software used to obtain the texture parameter values was Texture Exponent 32 software (Texture Technologies Corp., Scarsdale, NY/ Stable Micro Systems, Godalming, Surrey, UK). The machine was equipped with 50 cm OD acrylic probe. For each experiment the processed products were placed under the probe.

The pre- and pro-test speeds were 1 mm/s and 0.50 mm/s, respectively and the target mode of the test was set on distance where distance was 2 mm for carrots, 3 mm for zucchini, 4 mm for green bell pepper, 15 mm for broccoli and 10 mm for cauliflower and time was set to 5 s with a trigger force of 5 g. The parameters of probe height calibration were set to return distance 15mm, return speed 1.7 mm/sec and contact force 1 g. For texture assessment, a minimum of 10 samples were tested for each commodity in triplicates. To obtain the TPA, a two-cycle compression test was used that mimicked two bites. The software represents these two bites as two peaks on a force vs distance graph. The maximum force required to compress the sample in the first compression was noted as its hardness.

4.2.5.2. Color analysis:

The CIE L* (lightness), a* (red - green), and b* (yellow - blue) color attributes of the treated samples and raw control were determined using Minolta Tristimulus Chroma Meter

(Minolta Corp., Ramsey, NJ, USA) and displayed by the software (SpectraMagic, Minolta Corp., Ramsey, NJ, USA). The hue angle was calculated based on which quadrant the color is located (Figure 4.3).

Using the standard calculation for hue [Arc tan(b*/a*)], positive signed results are generated for the first quadrant [+a*, +b*] only. The other quadrants should be handled so that a 360° representation is accommodated, and results are expressed as positive signed numbers. Second quadrant [-a*, +b*] and third quadrant [-a*, -b*J calculations should be: hue = 180 + Arc tan (b* / a*). Fourth quadrant [+a*, -b*] calculations should be: hue = 360+Arc tan (b* / a*) (McLellan et al., 1995).

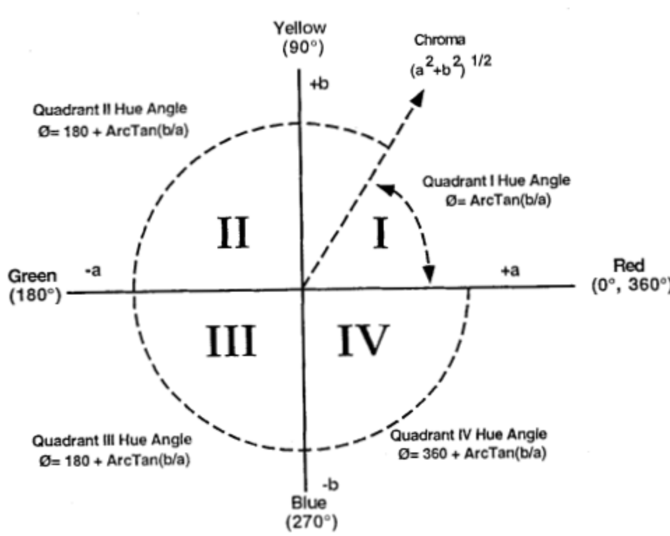


Figure 4.3. The hunter L,a,b system diagram showing only the a-b plane
Source: McLellan et al., 1995

4.2.5.3. Ascorbic acid analysis

Ascorbic acid content of all the vegetables was measured using AOAC Method 967.21 - 2,6 dichlorophenol indophenol visual titration method (Nielsen, 2010).

4.3. Results and discussion

4.3.1. Enzyme inactivation kinetics and blanching time

Figures 4.4 to 4.8 show the residual activity of enzyme peroxidase in broccoli, carrots, cauliflower, green bell pepper and zucchini, respectively, under different blanching treatment conditions. The curves were fitted to a first order log-linear model of log residual activity vs treatment time. In general, the figures show that better enzyme inactivation was achieved when vegetables were subjected to ultrasound treatment combined with steam or steam/air and hot water combination heat treatment. Presence of air in the steam medium resulted in reducing the influence of the condensing steam. This is consistent with the results from Chapter 3 with respect to surface microbial decontamination. The order of inactivation was clearly (starting from the least) hot water, steam/air, steam/air plus ultrasound, steam and finally the best steam and sound. Hot water blanching was least effective. The curves became progressively steeper moving from hot water to steam and ultrasound.

D values were computed from the slopes of these curves and used to obtain the blanching times calculated as the time to cause ~95% inactivation of peroxidase enzyme (Ramaswamy and Rangana 1989) based on the D values which is 1.5 D are shown in Table 4.1. With respect to the D value the order was reversed, the shortest one associated with steam and sound and the longest with hot water, opposite of the slopes associated. The individual blanching times calculated as 1.5 D values are also summarized in Table 4.2. Blanching times were shorter with steeper curves and therefore they were directly related to D values. Shorter blanching time with smaller D value (faster rate of enzyme inactivation). The computed blanching time was highest for hot water blanching compared to steam, steam/air or hot water in combination with ultrasound for all vegetables. These blanching times were used for testing the subsequent influence on quality parameters.

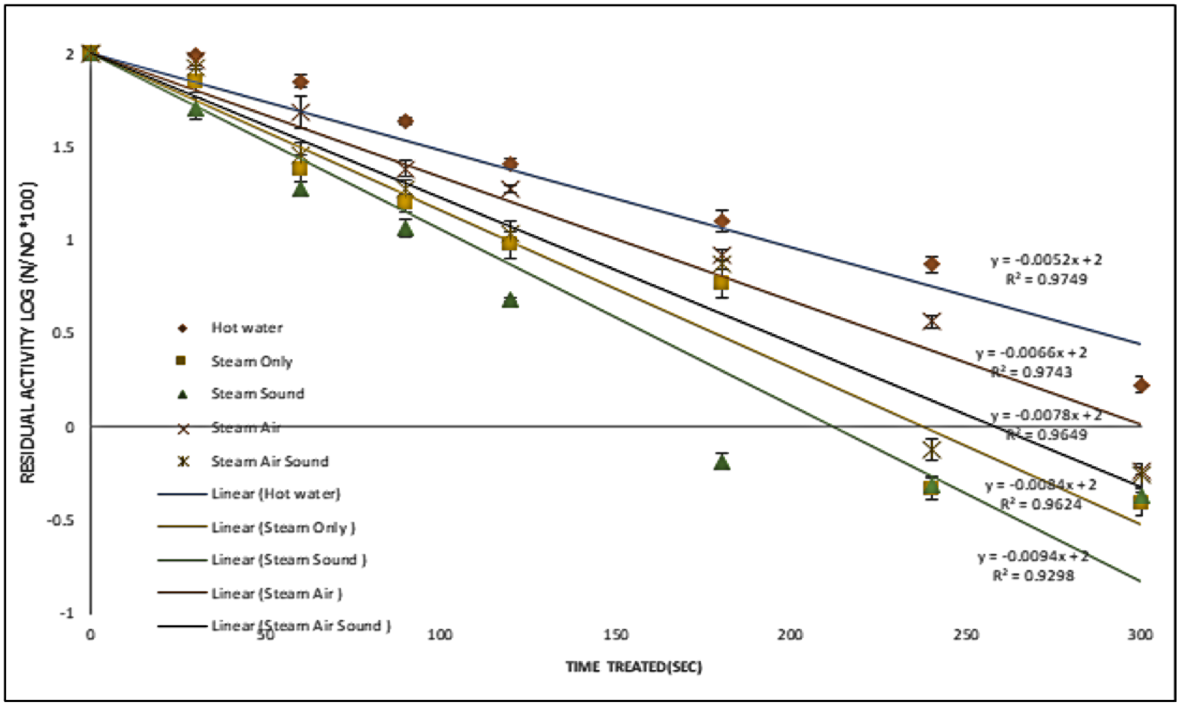


Figure 4.4. Residual of peroxidase activity in broccoli under different treatment conditions

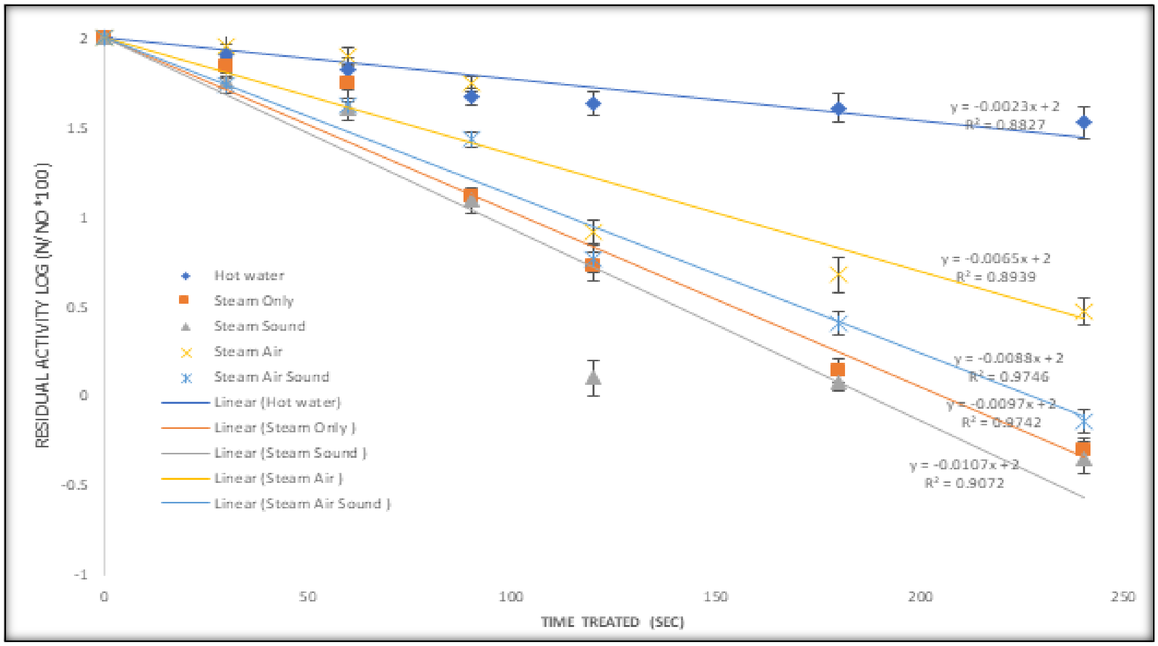


Figure 4.5. Residual peroxidase in carrots under different treatment conditions

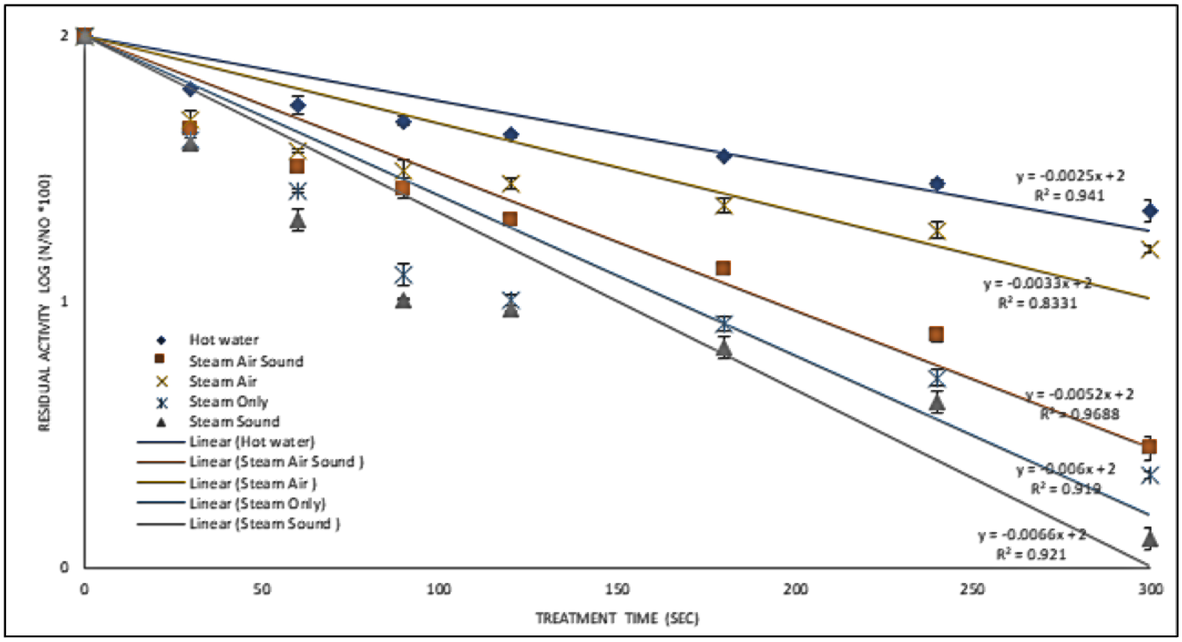


Figure 4.6. Residual peroxidase activity in cauliflower under different treatment conditions

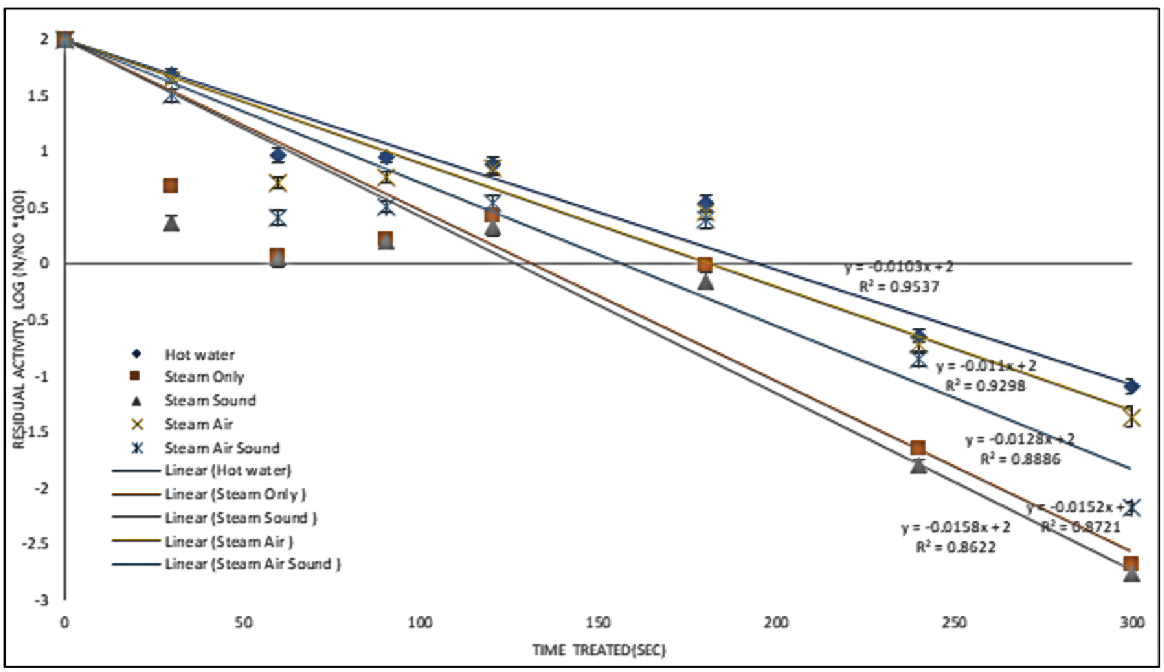


Figure 4.7. Residual activity of peroxidase in green bell pepper under different treatment conditions

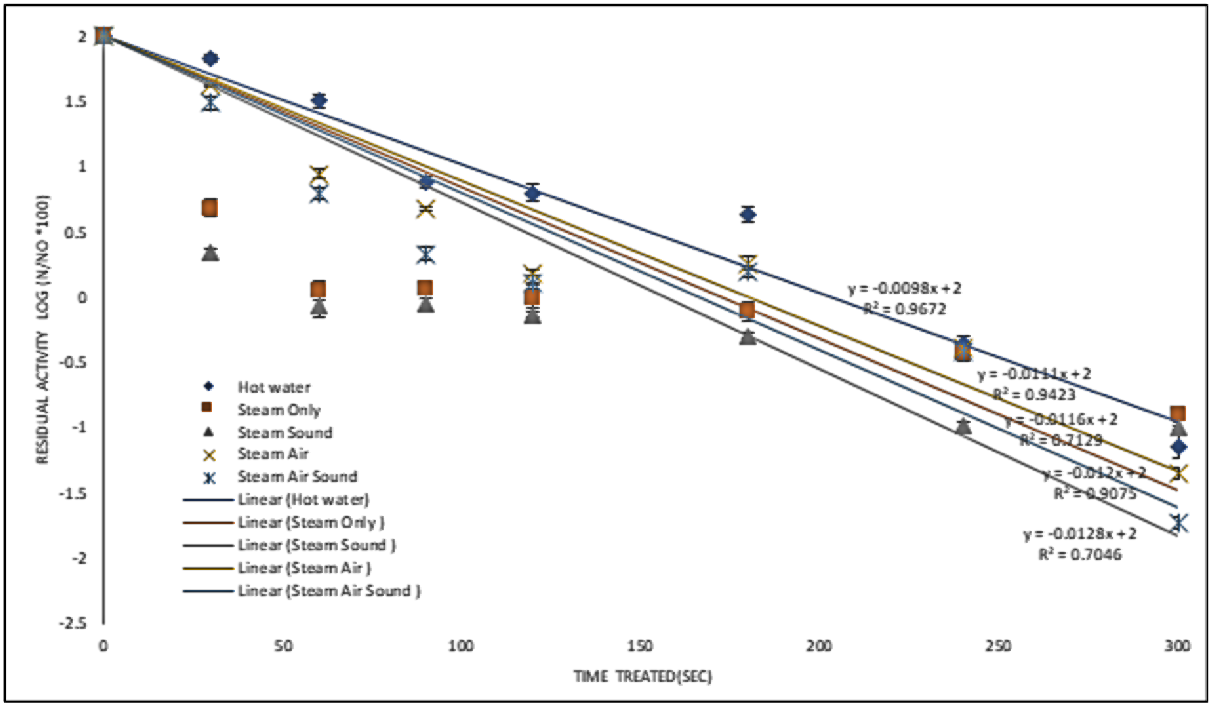


Figure 4.8. Residual activity of peroxidase in zucchini under different treatment conditions

Table: 4.1. Peroxidase activity D value for vegetables under different treatment conditions:

Treatments	D values (min)									
	carrots	R^2	cauliflower	R^2	broccoli	R^2	green bell pepper	R^2	zucchini	R^2
Hot water	7.25	0.88	6.65	0.94	3.20	0.97	1.62	0.95	1.70	0.96
Steam	1.72	0.90	2.77	0.92	1.98	0.92	1.09	0.86	1.44	0.71
Steam Sound	1.55	0.97	2.53	0.91	1.77	0.96	1.05	0.87	1.33	0.70
Steam Air	2.56	0.89	5.05	0.83	2.53	0.97	1.52	0.92	1.50	0.94
Steam Air Sound	1.89	0.97	3.20	0.96	2.14	0.96	1.30	0.88	1.39	0.90

Table: 4.2. Blanching time (min) for vegetables under different treatment conditions:

Treatments	Blanching time (min)				
	carrots	cauliflower	broccoli	green bell pepper	zucchini
Hot water	10.88	9.98	4.80	2.43	2.55
Steam Air	3.84	7.58	3.80	2.28	2.25
Steam Air Sound	2.84	4.80	3.21	1.95	2.09
Steam Sound	2.58	4.16	2.97	1.64	2.16
Steam	2.33	3.80	2.66	1.58	2.00

4.3.1.1. Ultrasound – hot water treatment blanching time comparison

Studies were conducted to compare effectiveness of hot water blanching, steam ultrasound and ultrasound hot water blanching considering carrots for treatment. The blanching time calculated from the D value is shown inclusive in Figure 4.9. The ultrasound inclusion with both steam and hot water blanching were found to be effective for enzyme inactivation, but one needs to recognize that using water leads to lot of effluent waste and leaching of nutrients compared to steam blanching.

4.3.2. Texture profile analyses

Blanching treatment not only resulted in enzyme inactivation, but also in texture softening. The longer the blanching time, higher is the texture loss or softer is the texture. Figures 4.10 to 4.14 show the kinetics of texture softening or reduction in hardness of broccoli, carrots, cauliflower, green bell pepper and zucchini because of blanching treatment time using the five different techniques. The trends were expected to be reversed, the treatment causing highest inactivation rate should cause the highest reduction in hardness. Because such a media would result in greater heat penetration causing maximum softness. But the results were, however, mixed. The hardness of vegetables treated with combined ultrasound with steam/air scored best with steam/air next followed by steam and sound and then steam only, and the last one was hot water. It appears the mechanism causing texture loss is somewhat different than the one causing enzyme inactivation. Texture loss sometimes is associated with a biphasic model (combination of rapid and slow softening rates).

Further it should be noted that these curves only reflect transient changes in residual texture values for different vegetables. As indicated in the table inset for blanching time, the treatment times needed for the different conditions are not same. The curves with a less steep slope (hot water) and the vegetable needs to be treated for ~11 min blanching (heating times not shown on the figure) to achieve 1.5 D reduction in peroxidase activity. While steam and sound heating requires only ~3 min. For comparing the blanching influence on texture, one needs to look at texture loss with hot water treatment after 11 min versus steam and sound treatment for 3 min, which widens the gap between these two treatments. These are highlighted in the next Chapter where the vegetables are blanched for an equivalent level of peroxidase inactivation.

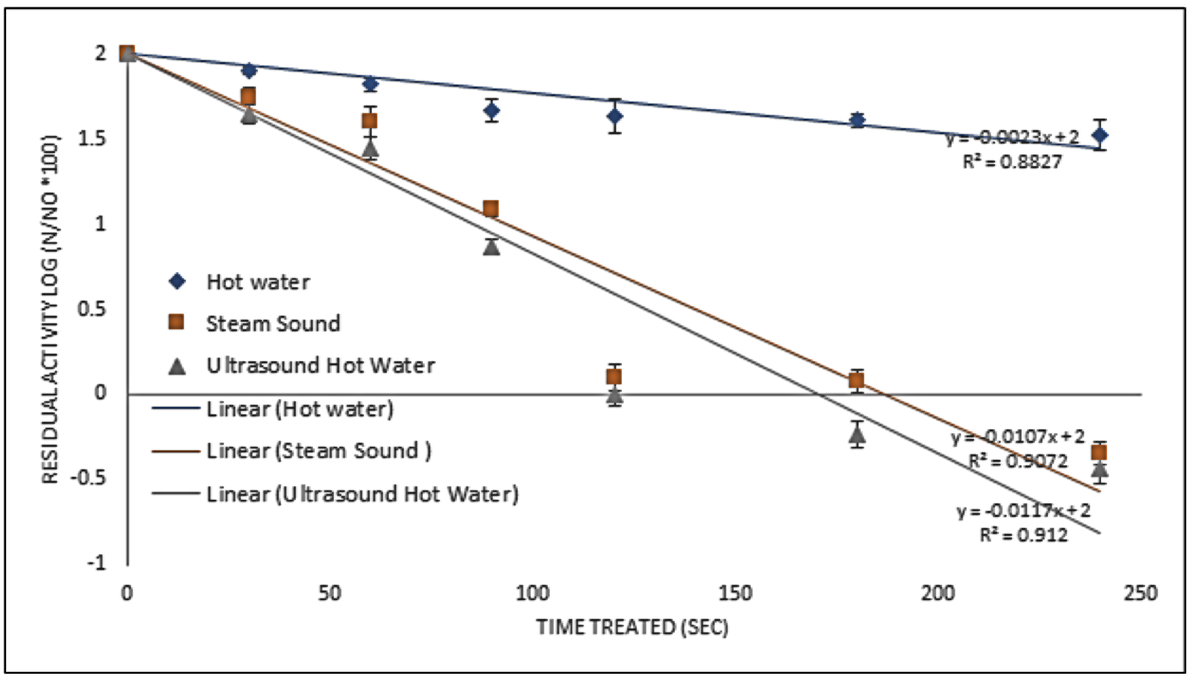


Figure 4.9. Comparison of residual activity of peroxidase in carrots under ultrasound and steam and combined hot water and ultrasound treatment.

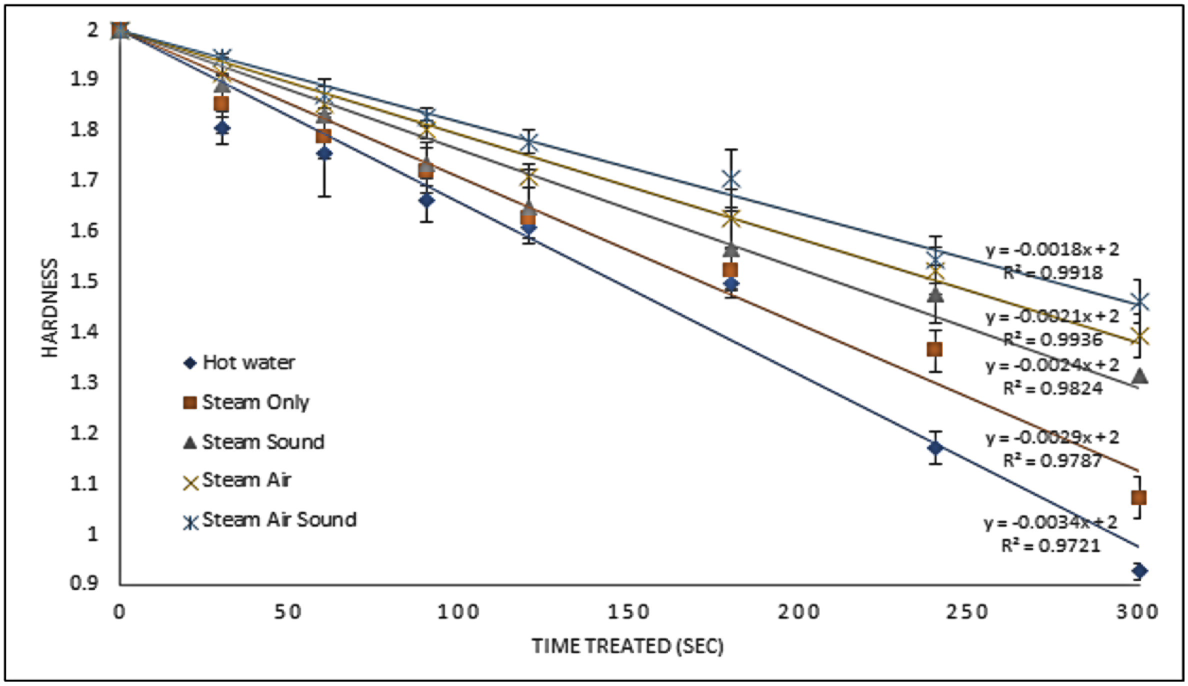


Figure 4.10 Hardness of broccoli

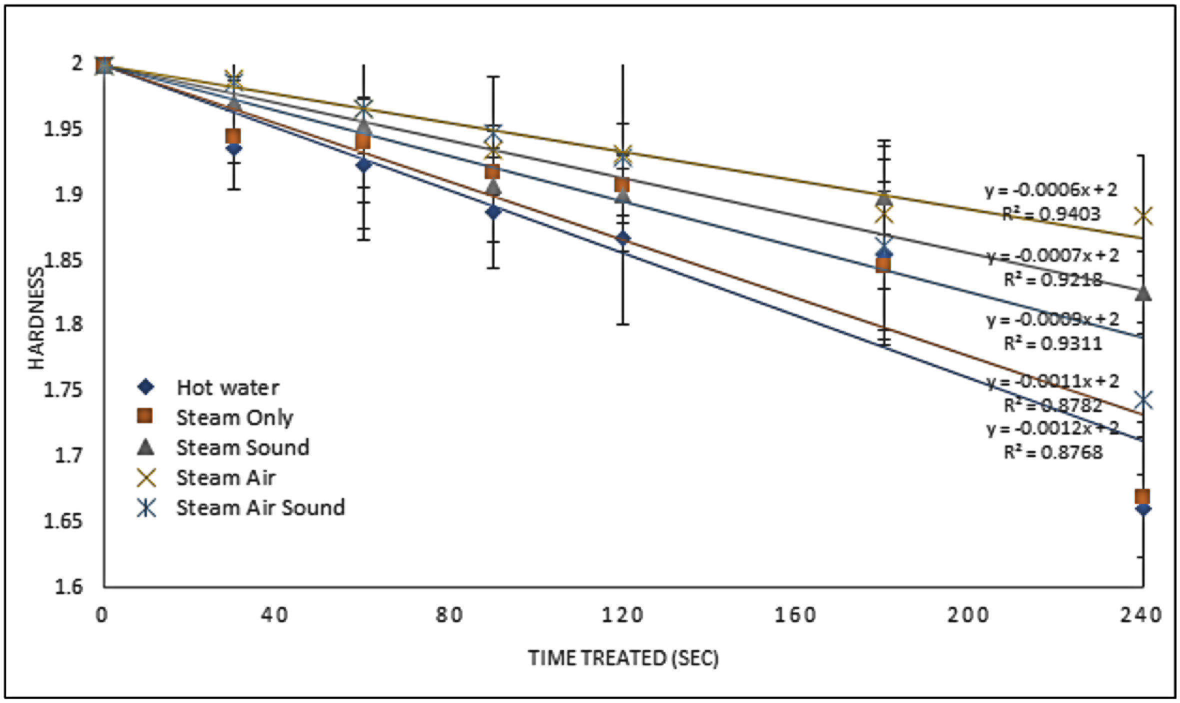


Figure 4.11. Hardness of carrots

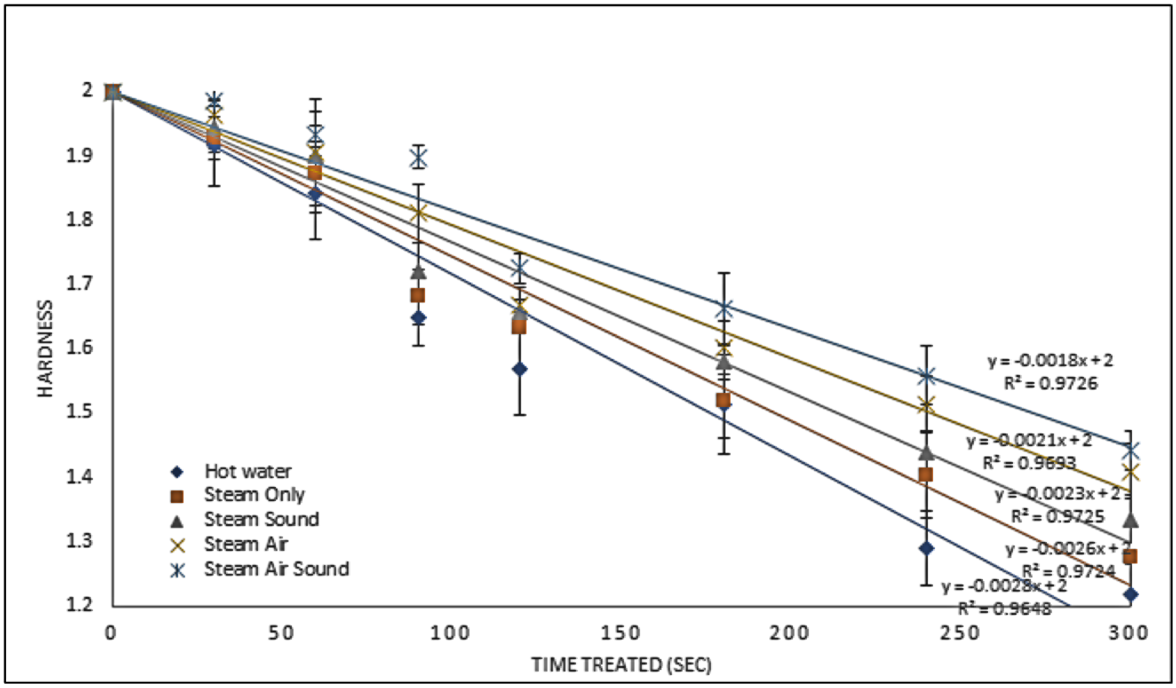


Figure 4.12. Hardness of cauliflower

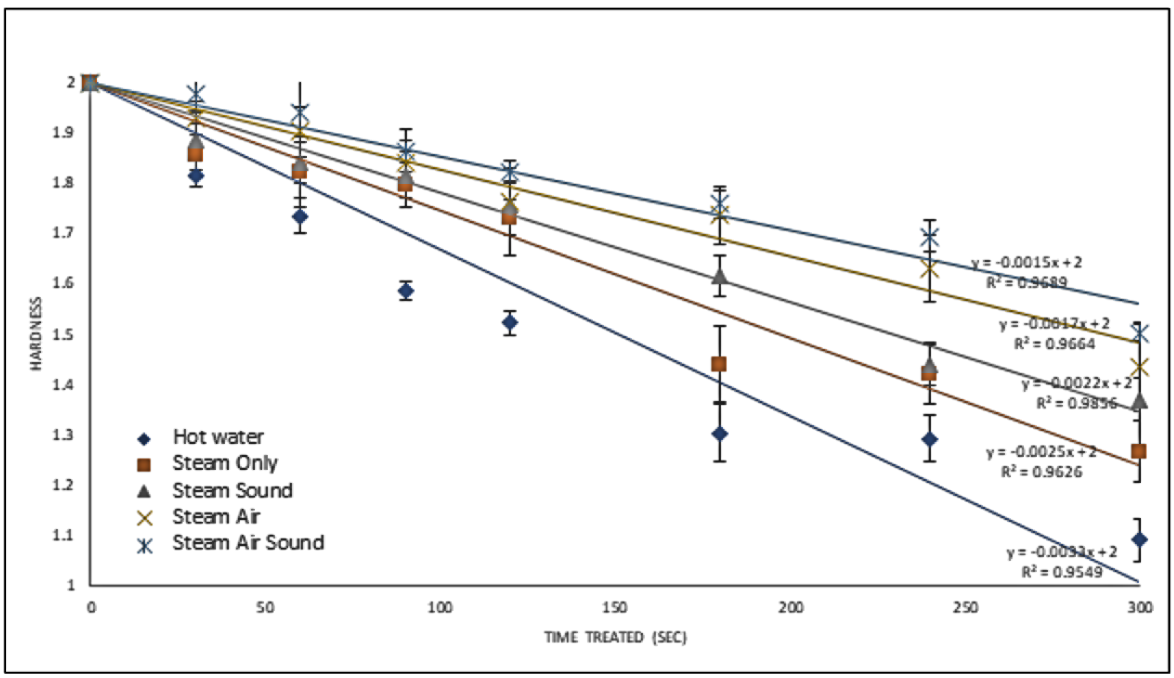


Figure 4.13. Hardness of green bell pepper

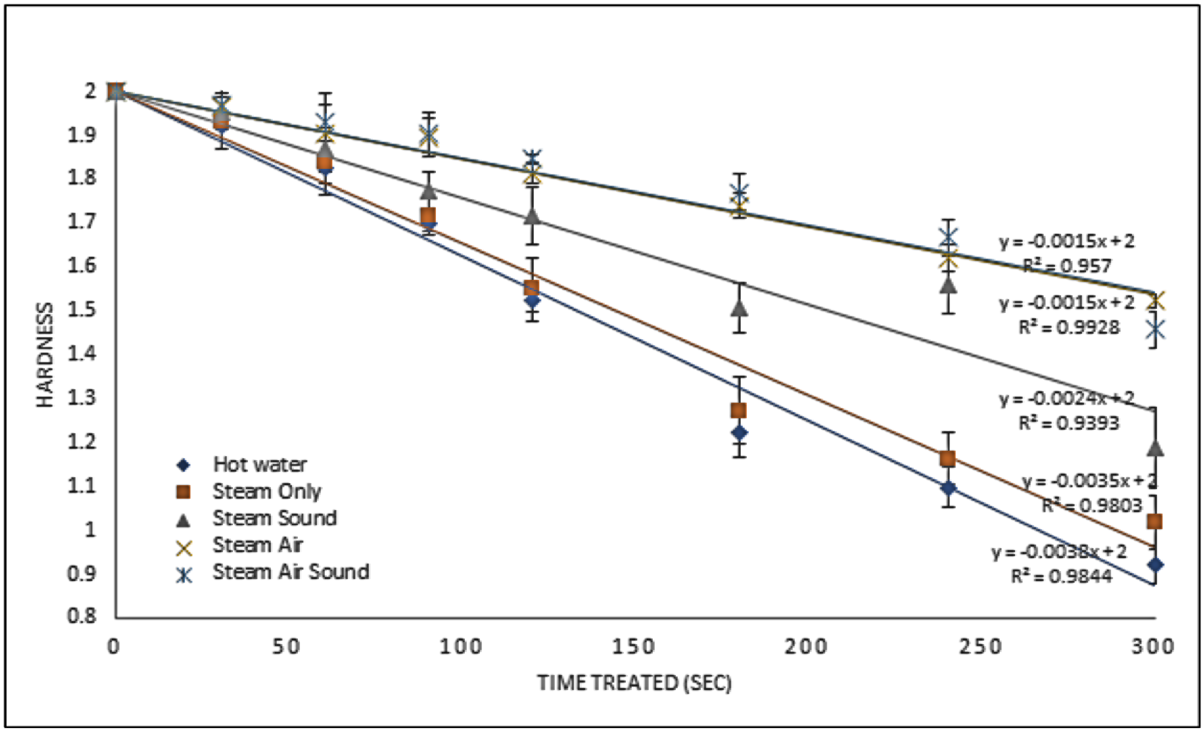


Figure 4.14. Hardness of zucchini

4.3.3. Ascorbic acid retention

The ascorbic acid content retention (Figures 4.15 to 4.19) showed a similar trend as that of texture quality with hot water demonstrating the worst-case scenario presumably contributed by leaching of ascorbic acid in the blanching water. The ascorbic acid content retention of vegetables treated with ultrasound with steam/air combination was the best followed by steam and sound and then steam only, and the last one was hot water.

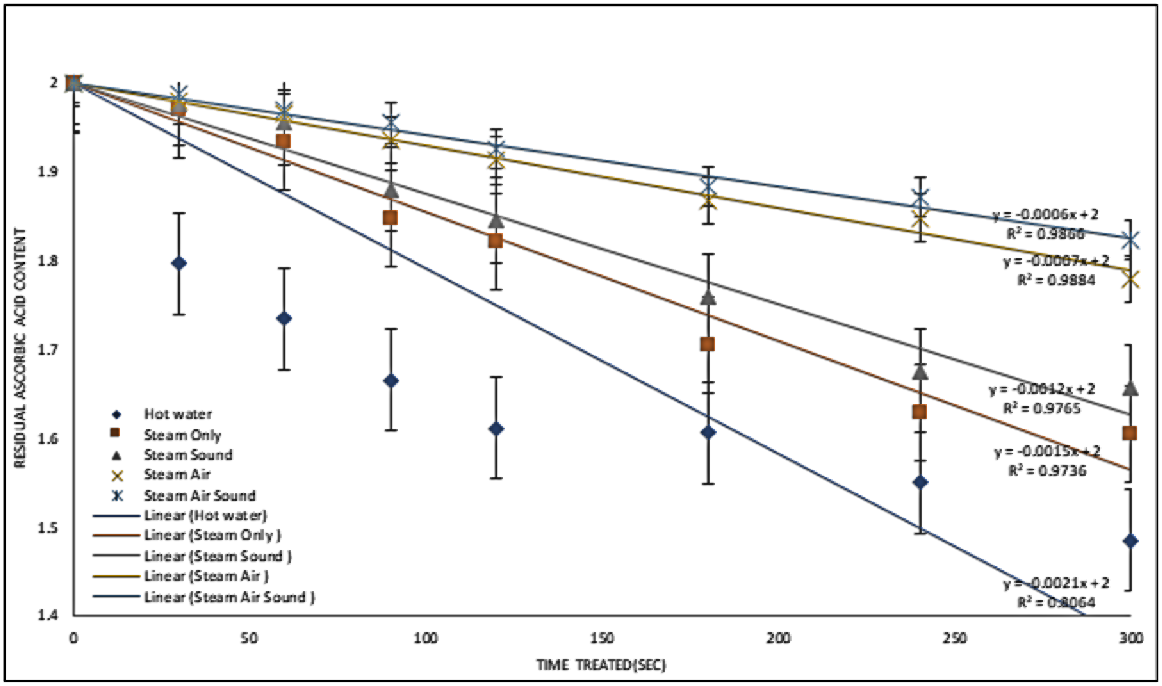


Figure 4.15. Ascorbic acid content of broccoli under different treatments

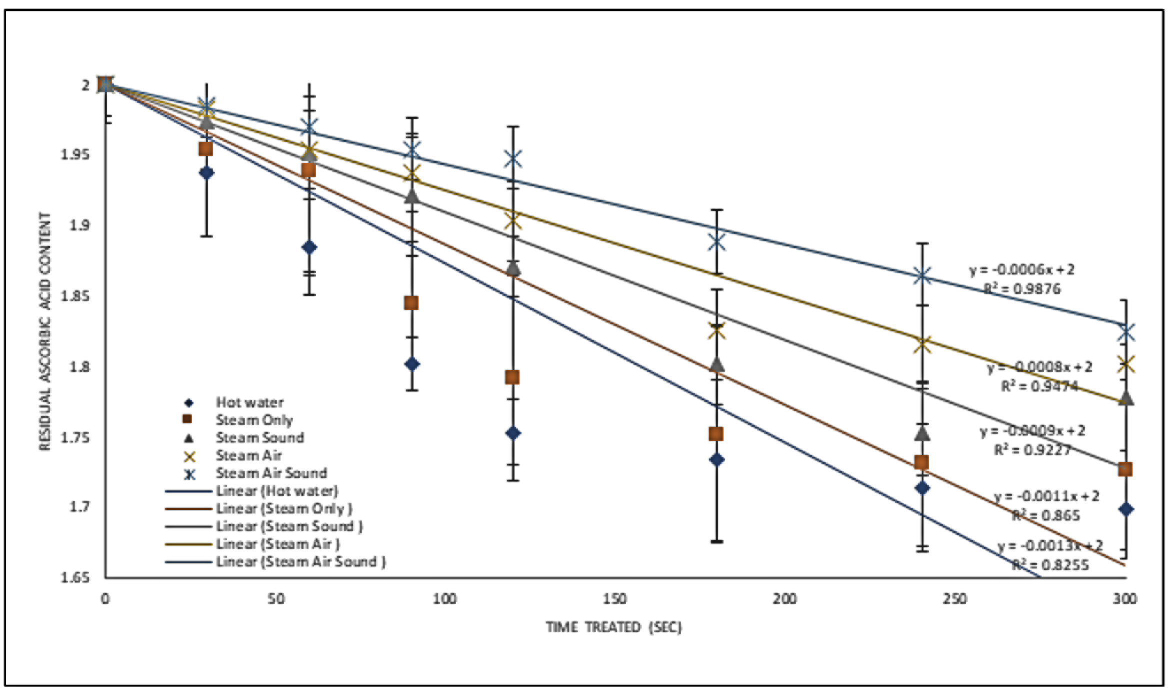


Figure. 4.16. Ascorbic acid content of carrots under different treatments

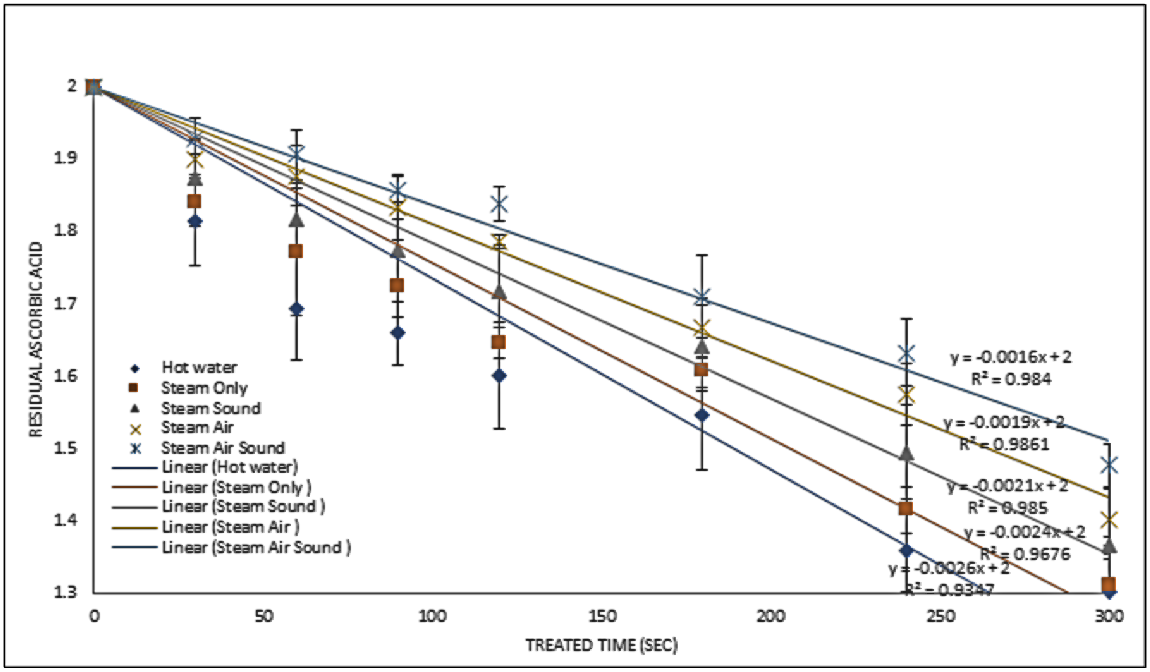


Figure 4.17. Ascorbic acid content of cauliflower under different treatments

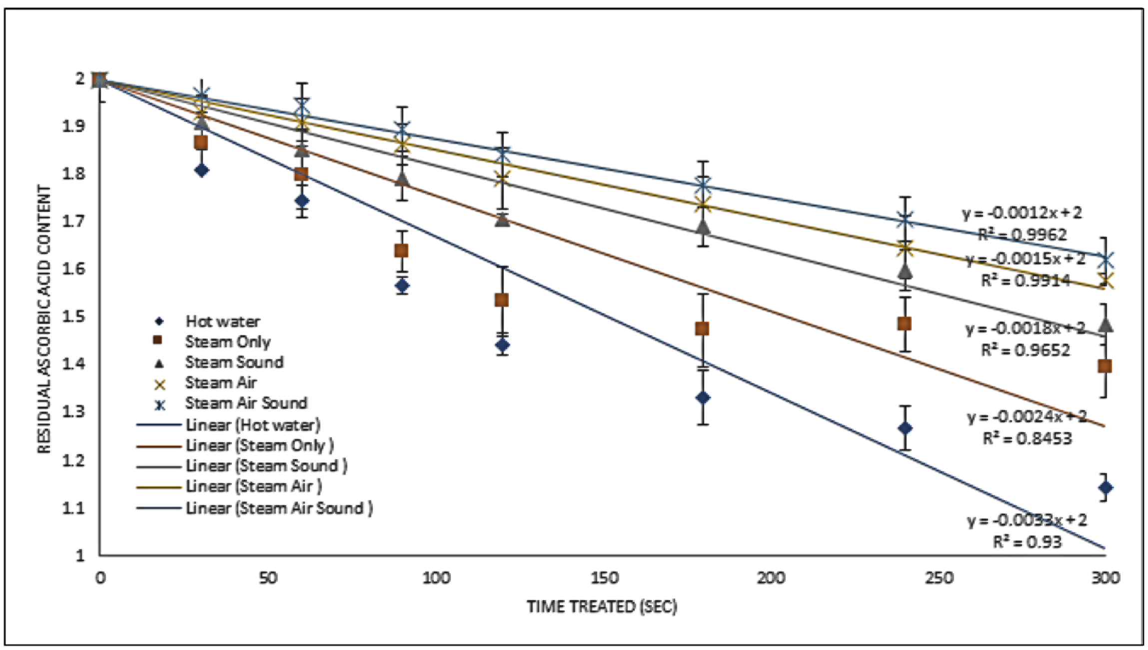


Figure 4.18. Ascorbic acid content of green bell pepper under different treatments

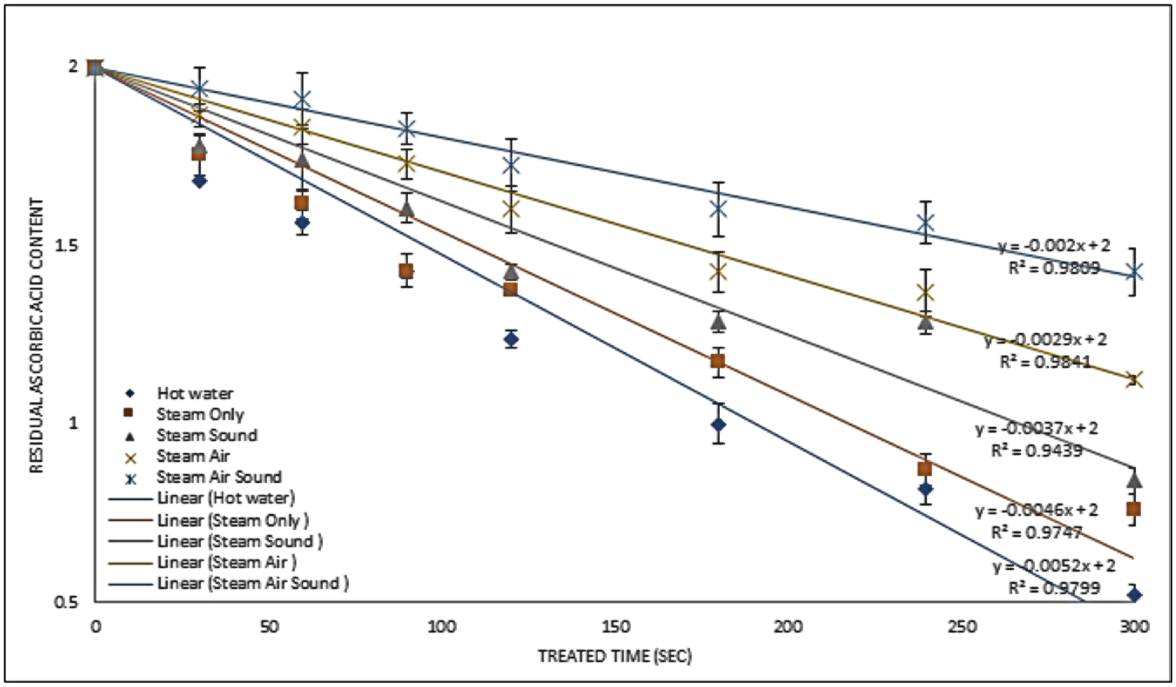


Figure 4.19. Ascorbic acid content of zucchini under different treatments

4.3.4. Color

The hue angle of different vegetables namely, broccoli, carrots and green bell pepper are shown in Figures 4.20 to 4.24. These vegetables have colors that show redness, greenness, and yellowness range because of which the hue angle was chosen to compare the color parameter. For broccoli the green color retention would identify better color quality retention. In Figure 4.20 the hue angle is highest at 120 s treatment under steam treatment in presence and absence of air and is in quadrant II showing retention of more greenness compared to steam ultrasound treatment and hot water treatment. But in 30 s retention of greenness in broccoli was better when treated under steam ultrasound in presence of air. With increase in treatment time the retention of green color in broccoli was seen to reduce. But overall, we see least retention of greenness in hot water blanching.

For carrots the redness color retention would show better color quality retention. From Figure 4.21. a 90 s treatment of carrots had higher retention of redness under ultrasound-steam treatment compared to other blanching treatments. As the blanching time increases the color retention of carrots varies without following a specific trend.

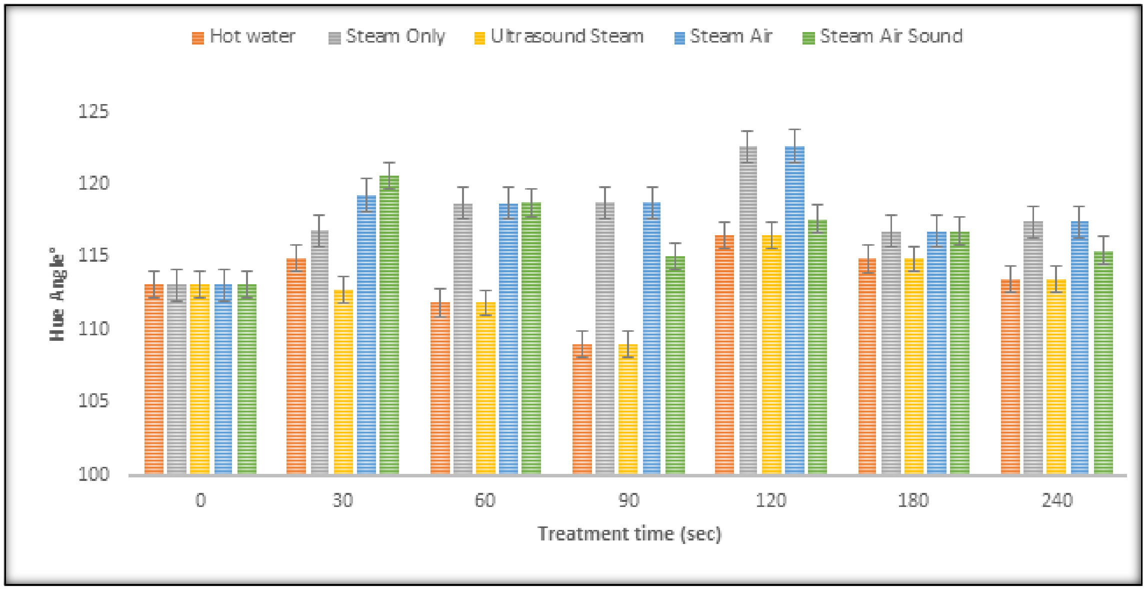


Figure 4.20 Hue angle of broccoli

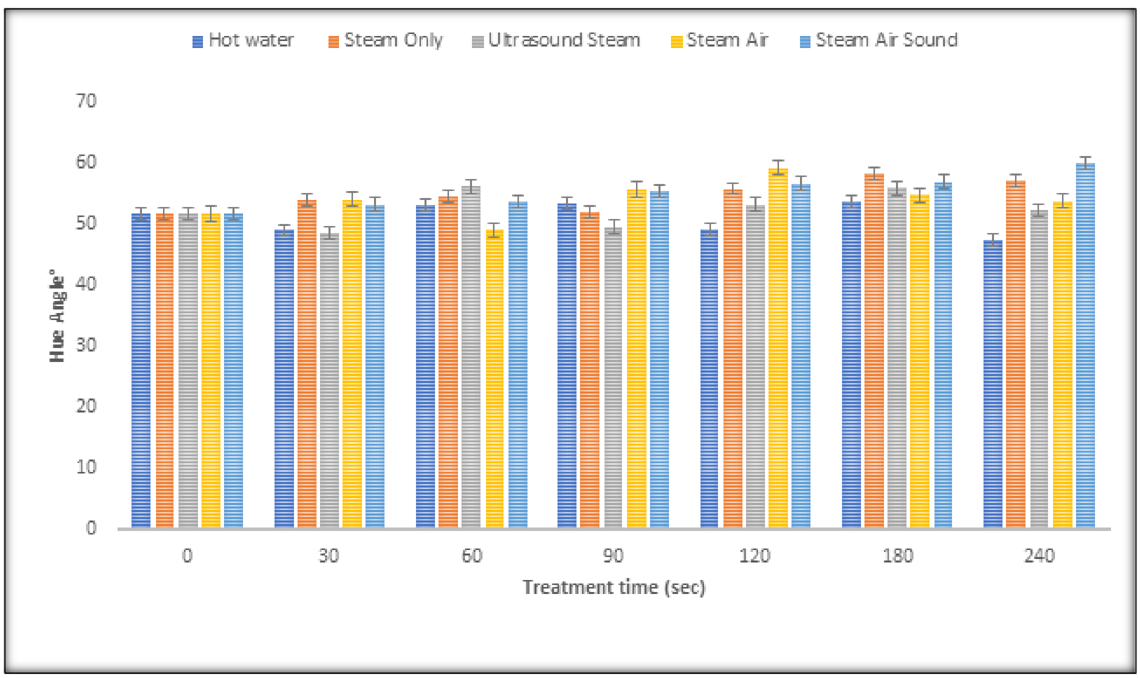


Figure 4.21. Hue angle of carrots

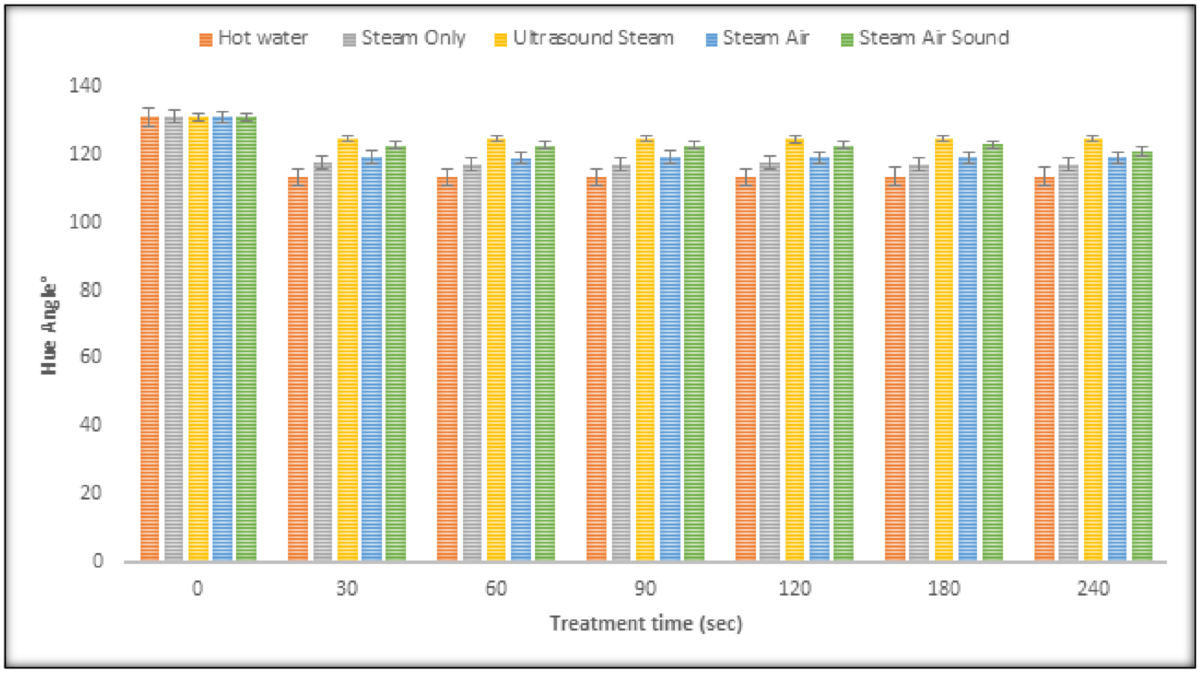


Figure 4.22. Hue angle of green bell pepper

For green bell pepper, the green color retention will identify better color quality retention. In Figure 4.22, it can be observed that the samples treated under ultrasound steam both in presence and absence of air have better retention compared to steam only or hot water blanching. It can also be observed that the treatment time has not much effect in the color retention.

For cauliflower and zucchini, the L* value was considered to compare color retention after treatments (Figure 4.23 and Figure 4.24). L* value shows the retention of whiteness/brightness. Figure 4.23 shows that the whiteness of cauliflower was better retained when blanched under ultrasound and steam treatment compared to steam alone and hot water blanching. The result showed variation at 120 s treatment where better retention of whiteness was seen when treated with steam alone in presence of air. Similar results were observed in blanched zucchini (Figure 4.24). Zucchini were sliced in cylindrical dices as shown in Figure 4.1. e, and the white portion in the middle was used for color measurements. Figure 4.24 shows that combined ultrasound steam treatment both in presence and absence of air showed better retention of brightness compared to steam or hot water blanching.

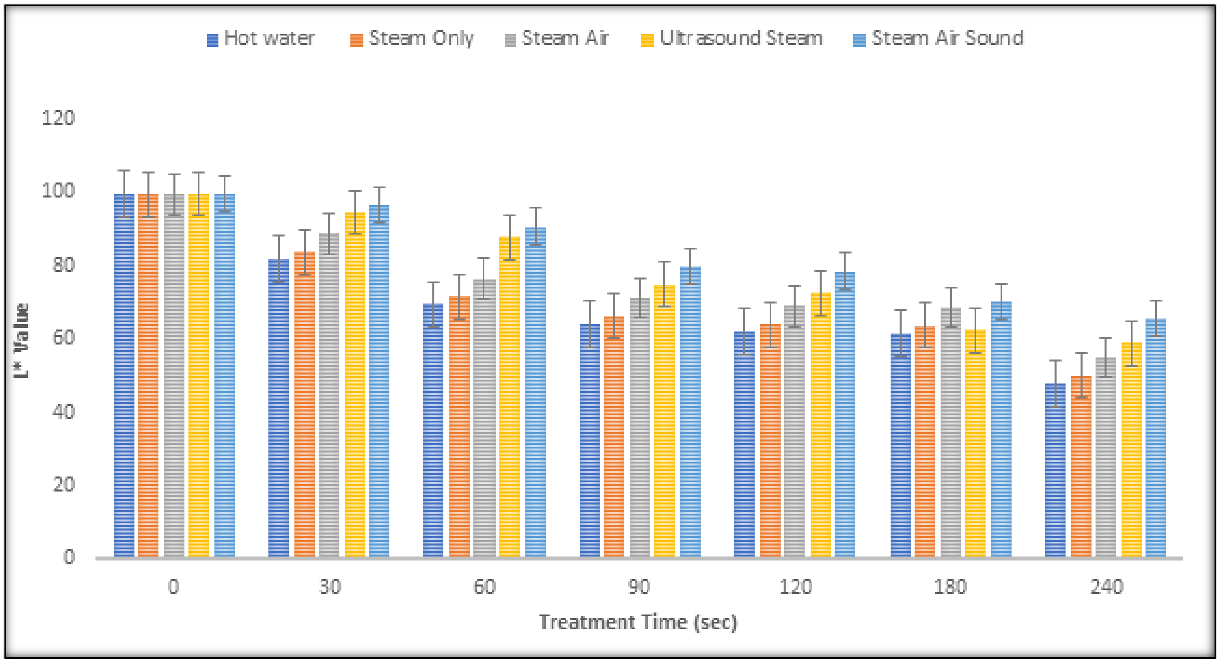


Figure 4.23. L* value of cauliflower

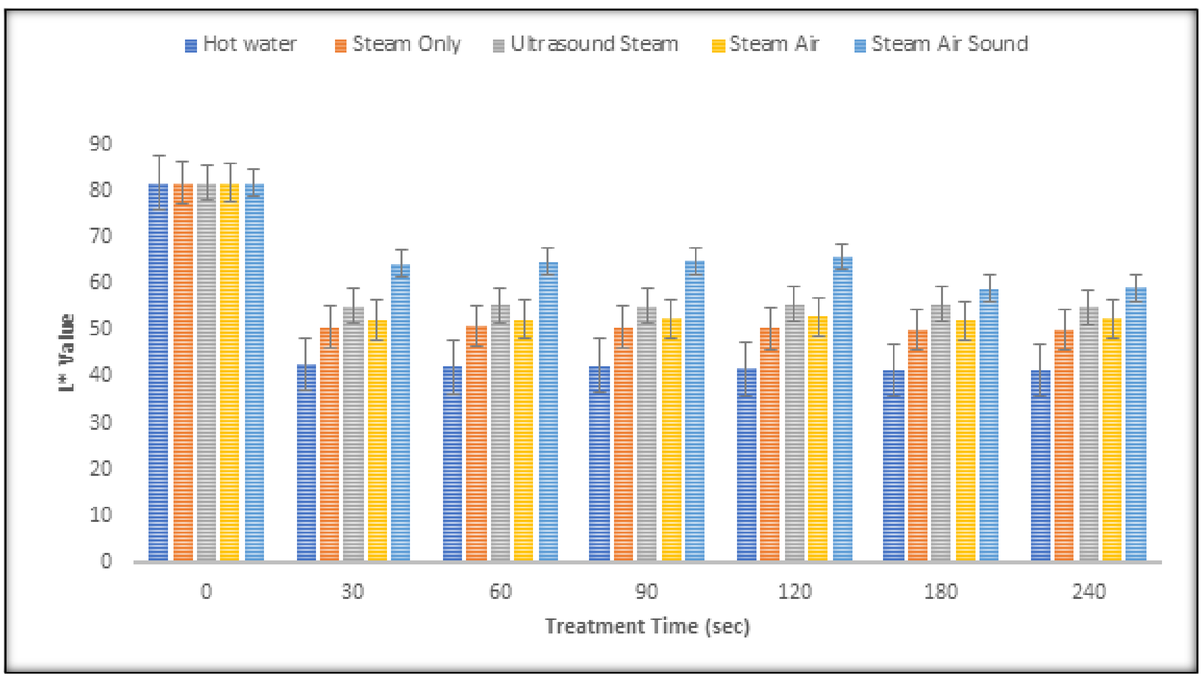


Figure 4.24. L* value of zucchini

4.4. Conclusions

This study showed that the combined ultrasound-steam blanching technique resulted in a more rapid inactivation of enzyme peroxidase (permitting the use of a shorter blanching time) and better product relative quality retention as compared to the conventional hot water blanching or steam blanching with or without added air (~25% air / 75% steam).

The textural qualities (hardness) of the treated vegetables - carrots, cauliflower, broccoli, zucchini, and green bell pepper was analysed in detail under different blanching techniques. Ultrasound steam blanching caused less textural damage in the blanched products.

The retention of ascorbic acid in different vegetables when subjected to different blanching treatments was also analysed. Less ascorbic acid loss was observed in combined ultrasound and steam as compared to conventional hot water or steam blanching alone.

The hue angle of carrots, broccoli and green bell pepper subjected to different blanching treatments was measured. Not much difference was observed in terms of color after different blanching techniques. However, the initial green color in broccoli was better retained in ultrasound-steam treated sample as compared to other treatments. The redness of carrots increased with an increase in blanching time and ultrasound steam showed better retention. The luminescence (L*) of cauliflower and zucchini was also better retained in ultrasound-steam treated sample.

All vegetables demonstrated superiority with added ultrasound for enhancing the rate of enzyme inactivation, better texture and color protection and limiting ascorbic acid loss.

The quality change kinetics evaluated in this chapter permit a relative assessment of change in parameters as influenced by a given treatment time. It should be noted that time required for blanching to result in ~95 % peroxidase enzyme inactivation of different for different treatment conditions. The magnitude differences in quality parameter as influence by different treatments gets compounded and wider when blanched according to the needs. This is because a treatment showing a slower quality change may be associated with a treatment that required a longer heating time for blanching.

4.5. References

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Connecting Statement to Chapter 5

In Chapter 4, through kinetic studies, the required blanching times for all five different vegetables under each different blanching techniques (hot water blanching, steam blanching and combined ultrasound steam blanching in presence and absence of air) were reported in addition to discussion on associated quality change kinetics. For quality change kinetics however, the treatment times were kept the same under different conditions and therefore they will not give quality output under equivalent blanching conditions which is necessary in order to optimize the treatments. In this chapter quality retention studies of frozen vegetables were conducted after blanching the vegetables for the specific times that are appropriate for the treatment condition resulting in 95% percent inactivation of peroxidase enzyme, followed by freezing in liquid nitrogen and storage at -20C for 56 days (8 weeks). The quality changes hardness, color (L value), ascorbic acid and drip loss were evaluated during subsequent frozen storage at different time intervals.

The results of this study were presented at the annual meeting of RITA, several students participated in this (Dr. Ramaswamy's Lab): Ali R Taherian helped in carrying out freezing and guided in texture analysis; Neelakanth Lamani – assisted in freezing and coordinated packaging; Maheshwor Bastakot assisted in color analysis; Husain Dhariwala assisted in enzyme inactivation and Ali Rampurwala helped in ascorbic acid evaluation.

This study was part of RITA CTAQ project and was funded by Consurtium RITA.

Results have been presented as poster presentations in conferences:

Basumatary R, and Ramaswamy HS. 2022. Ultrasound-Steam Treatment Process for Surface Microbial Decontamination and Enzyme Inactivation of vegetables. Oral Presentation in RITA-CTAQ precompetitive co-creation network, Technology Transfer Workshop (May 16th, 2022, Château Vaudreuil, Quebec.)

Basumatary R, Bastakoti M, Taherian A, Lamani N, Dhariwala H, Rampurwala A, Ramaswamy HS. 2022. Ultrasound-Steam Blanching for Enzyme Inactivation and Quality Retention during Frozen Storage of Vegetables. Poster Presentation in RITA-CTAQ precompetitive co-creation network, Technology Transfer Workshop (May 16th, 2022, Château Vaudreuil, Quebec).

Basumatary R, Bastakoti M, Taherian A, Lamani N, Dhariwala H, Rampurwala A, Ramaswamy HS. 2022. "Ultrasound-Steam Treatment Process for Quality change during Frozen storage of Vegetables." Poster presentation in 2022 Canadian Food Summit-CIFST National Conference (1st June 2022 -3rd June 2022, University of Guelph).

Chapter 5

Comparison of ultrasound-steam, steam/air and hot water blanching for quality characteristics of frozen vegetables

Abstract

This study evaluated the effectiveness of combined ultrasound combination blanching with steam, steam/air and hot water blanching prior to freezing and storage of frozen vegetables. Vegetables (broccoli, cauliflower, green bell pepper, carrots, and zucchini) were blanched for specific blanching times as determined in previous chapter for 95% inactivation of peroxidase enzyme under different blanching conditions (hot-water blanching, steam blanching and combined ultrasound steam blanching in presence and absence of air). Texture (hardness, stiffness, and chewiness), color (L*value) and ascorbic acid content of all vegetables were evaluated before freezing to study the effect of different blanching techniques on vegetables. Blanched vegetables were then frozen and evaluated on day 1, 14, 28 and 56 for texture, color, ascorbic acid content and drip loss. Ultrasound steam blanching showed better retention of texture (hardness), color (L* value) and ascorbic acid content compared to conventional steam or hot water blanching. Drip loss was seen highest in hot water blanched vegetables compared to other blanching techniques.

5.1. Introduction

Vegetables need to be preserved in some way to be available for later consumption because they are seasonal and perishable. Freezing is an effective method for preserving the nutritional content, texture, sensory quality, and shelf life of vegetables. According to literature freezing can help retain the vitamin C, carotenoids, and antioxidant capacity of vegetables (Xin et al.,2015; Ishevskiy et al., 2017; Muthukumarappan et al., 2018). According to James et al., (2015), the longer the storage life of food commodities, the lower the frozen storage temperature. Freezing can significantly extend the shelf life of vegetables. According to Alsailawi et al., (2014), frozen vegetables can be stored for up to 12 months without significant changes in quality. Although freezing slows down enzymatic reactions, senescence, and microbiological growth, it does not completely stop the process of food deterioration (Bahceci et al., 2005).

Li et al., (2017) found that freezing is an effective method for preserving the nutritional content of vegetables, particularly when combined with blanching. Blanching helps to inactivate enzymes that can cause deterioration of texture and flavor during storage as well as preserve the antioxidant capacity and nutrient content of the vegetables. Gonzalez et al., (2018), found that blanching before freezing helped to preserve the texture and color of broccoli, while also retaining its vitamin C content. Pervin, et al., (2015), investigated the effect of blanching and freezing on the quality and nutritional parameters of carrots and found that blanching and freezing helped to preserve the color and texture of the carrots, while also retaining their antioxidant capacity. Samsel, et al., (2021), found that blanching before freezing helped to preserve the color, texture, and nutritional content of the spinach leaves. Nilsson, et al., (2004), found that blanching before freezing helped to preserve the antioxidant capacity and vitamin C content of the green peas. Bamidele et al., (2017), found that blanching helped to preserve the antioxidant capacity and total phenolic content of the vegetables. It was found that blanching helped to preserve the vitamin C content and thiamine of frozen vegetables (Canet et al., 2005). Overall, these studies suggest that blanching is an important step in preserving the quality and nutritional content of vegetables during freezing.

Different methods of blanching have been practised in the food industry for blanching of fresh vegetables – hot water blanching and steam blanching being the most popular ones. Many novel blanching techniques have also been explored such as microwave blanching, pulsed electric fields, high pressure processing, pulsed light, and cold plasma blanching. But literature shows lack of combined ultrasound steam blanching of fresh vegetables.

In this study the effects of combined ultrasound and steam blanching, steam alone blanching in presence or absence of air and hot water blanching on fresh vegetables (carrots, cauliflower, broccoli, zucchini, and green bell pepper) were compared prior to freezing. The impact of frozen storage on the quality of vegetables that have undergone different blanching techniques was also evaluated. The quality of vegetables was assessed via inactivation of peroxidase enzyme, texture, color, and ascorbic acid retention and drip loss.

5.2. Materials and methods

5.2.1. Blanching treatment

The raw vegetables were peeled and sliced into appropriate dimensions (carrots 10mm thick & 25 mm dia; zucchini 10 mm thick & 30 mm dia; green bell pepper 8 mm thick & 15mm dia; broccoli 30 mm thick flower section and flattened on one side; cauliflower 20mm thick flower section and flattened on one side) in bulk (some shown in Figure.5.1) and known amount (50 g each batch) were blanched with different thermal processing techniques. The vegetables were subjected for specific predetermined blanching times under different blanching techniques (combined steam ultrasound and steam alone in presence and absence of air and hot water blanching) as determined in Chapter 4 based on 95% inactivation of peroxidase enzyme. Immediately after the treatment, they were dipped in ice cold water (4-5°C) to cool and stop the enzyme inactivation process.

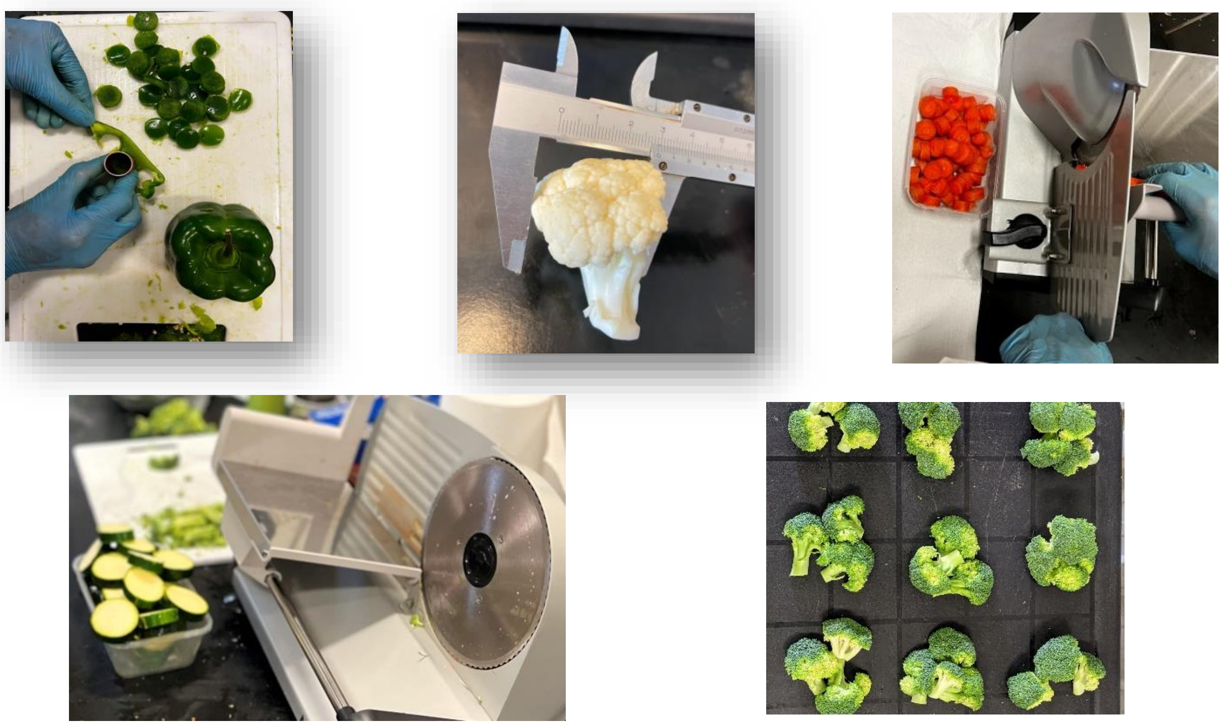

Figure 5.1. Cutting of vegetables into specific dimensions

5.2.2. Freezing and storage studies

The blanched vegetables were packed in ziplock bags (Figure 5.2) and frozen using liquid nitrogen (-196°C) and stored at -20ºC for storage studies and quality analysis. The vegetables were analyzed for quality parameters – (color, texture and ascorbic acid content) before freezing and after freezing at different time intervals (Day 1, 14, 28 and 56).

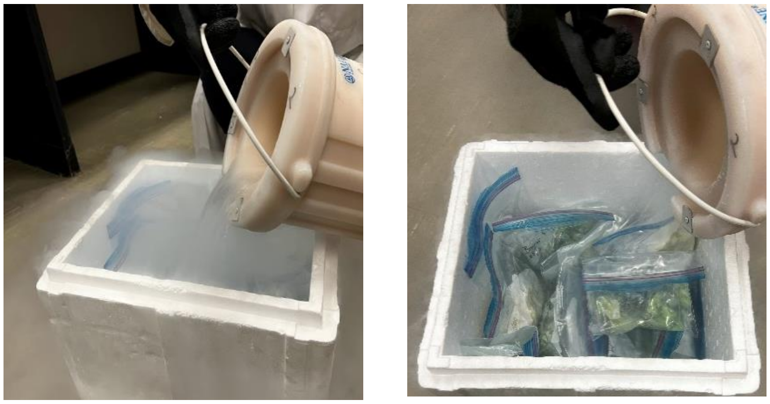

Figure 5.2. Freezing of vegetables using liquid nitrogen

For quality analysis the frozen vegetables were thawed by placing the packages in a refrigerator at 4-5°C 24 h before performing any analysis. Drip loss was evaluated in thawed vegetables at different time intervals of storage. The quality parameters were studied before freezing and after freezing and during storage for all the vegetables. The quality parameters such as hardness, color, drip loss and ascorbic acid content were studied before freezing and after frozen storage for different time intervals (1–56-day storage) for all five selected vegetables to compare the effect of different blanching techniques on change of quality parameters during frozen storage.

5.2.3. Quality analysis

The effect of different blanching techniques on the quality parameters (texture, color, and ascorbic acid content retention) of different vegetables were studied before freezing, after freezing and during storage for all the vegetables (most methods are detailed in Chapter 4) and briefly mentioned below and detailed where necessary.

5.2.3.1. Texture profile analysis (TPA)

The hardness, chewiness and stiffness of blanched carrots, broccoli, cauliflower, zucchini, and green bell pepper were obtained using the TA-XT Plus Texture Analyser (Texture technologies corp., Scarsdale, NY, USA). The software used to obtain the texture parameter values was Texture Exponent 32 software (Texture Technologies Corp., Scarsdale, NY/ Stable Micro Systems, Godalming, Surrey, UK). The machine was equipped with 50cm OD acrylic probe. For each experiment the processed products were placed under the probe.

The pre- and pro-test speeds were 1.0 mm/s and 0.50 mm/s, respectively, and the target mode of the test was set on compression distance where distance was 2mm for carrots, 3 mm for zucchini, 4 mm for green bell pepper, 15 mm for broccoli and 10 mm for cauliflower and time was set to 5 sec with a trigger force of 5 g. The parameters of probe height calibration were set to return distance 15 mm, return speed 1.7 mm/sec and contact force 1 g. For texture assessment, a minimum of 10 samples were tested for each commodity in triplicates. To obtain the TPA, a two-cycle compression test was used that mimicked two bites. The software represents these two bites as two peaks on a force vs distance graph. The maximum force required to compress the sample in the first compression was noted as its texture with hardness (Gram force to compress the samples by adjusted compression distance); Chewiness (work under the compression-decompression curve); and Stiffness (slope of upward compression curve).

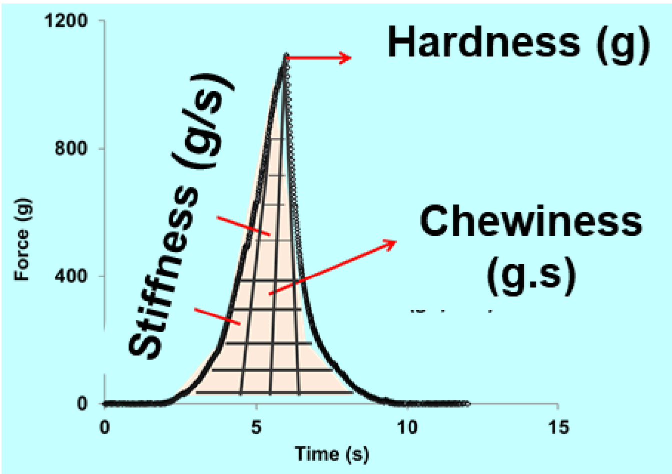

Figure 5.3. Example of TPA graph

5.2.3.2. Color analysis

The CIE L* (lightness), color attributes of the treated and frozen samples and raw control were determined using Minolta Tristimulus Chroma Meter (Minolta Corp., Ramsey, NJ, USA) and displayed by the software (SpectraMagic, Minolta Corp., Ramsey, NJ, USA).

5.2.3.2. Ascorbic acid analysis

Ascorbic acid content of all the vegetables was measured using AOAC Method 967.21 - 2, 6 Dichlorophenol Indophenol visual titration method (Nielsen, 2010)

5.2.3.3. Drip loss

The drip loss (equation 5.1) for each vegetable was determined after freezing relative to initial weight of fresh vegetables (Schudel et al., 2021) and expressed as loss percentage.

$$\text{Drip loss} = \left(\frac{Initial\ weight\ \ before\ freezing - Final\ weight\ after\ thawing}{Inital\ weight\ before\ freezing}\right) \text{ x } 100\ \% \qquad (5.1)$$

5.2.Results and discussion

5.3.1. Texture profile analysis

5.3.1.1. Effect of different blanching techniques on texture of vegetables before freezing

It is important to maintain optimal textural properties of vegetables after blanching to ensure their overall quality and acceptability (Neri, et al. 2011). Blanching has a significant effect due to changes in cell wall structure of vegetables such as softening or toughening on the texture of the vegetables which varies depending on the type of vegetable and blanching method used (Xiao, et al., 2017). Table 5.1. to 5.5. show the effect of different blanching techniques on the textural properties – hardness, chewiness, and stiffness of broccoli, cauliflower, carrots, green bell pepper and zucchini before freezing. The table also compares chewiness and stiffness parameters of the vegetables. Variability associated with the measured texture parameters were within a maximum 10 % of the mean values which is typical of texture values of biological materials. The tables show that the textural properties were highest in raw samples and least after hot water blanching and highest among the blanched in combined ultrasound steam blanching (among the blanched samples). Hot water blanching time was longer for enzyme inactivation compared to other blanching techniques. The longer treatment time likely has caused loss of textural properties in vegetables leading to softer texture in hot water blanched vegetables.

Figure 5.4. shows that the texture (hardness) of the vegetables were maximum when vegetables were subjected to combined steam and ultrasound blanching both in presence and absence of air compared to hot water blanching. The red line represents the texture values for the hot water blanching and all other techniques had values higher than this. The quality parameters shown in these figures are prior to freezing. Similarly, Figure 5.5 shows the graph as percentage loss in hardness relative to fresh and was seen maximum in all the vegetables subjected to hot water blanching and maximum retention of texture was observed in combined steam ultrasound blanching in presence of air. These results agree with the research findings of previous studies where longer blanching time could lead to loss of textural properties in the vegetables (Ravi et al., 2019).

Table 5.1. Effect of different blanching techniques on textural properties of broccoli

Treatments	Hardness	Chewiness	Stiffness
Raw	8126±275	72903±3497	396±10
HW	3332±150	26617±2420	126±7.2
Steam	5454±260	37391±2326	248±15
Steam-Air	5191±74	33120±1923	227±9.9
Steam-US	5748±224	43549±1952	292.±14
Steam-Air-US	5970±372	45335±3018	304.±10

Table 5.2. Effect of different blanching techniques on textural properties of cauliflower

Cauliflower	Hardness	Chewiness	Stiffness
Raw	23308±1747	77282±3745	1646±121
HW	1507±125	12622±1064	85±3.6
Steam	8770±391	59747±4017	541±41
Steam-Air	7952±404	57745±290	525±19
Steam-US	9463±464	67688±4095	657±52
Steam-Air-US	10397±997	68995±4153	676±23

Table 5.3. Effect of different blanching techniques on textural properties of carrots:

Treatments	Hardness	Chewiness	Stiffness
Raw	20049±594	20447±737	4908±258
HW	4399±395	4644±682	1394±98
Steam	6599±252	6998±282	1824±118
Steam-Air	6271±307	6723±314	1717±117
Steam-US	7156±341	7550±571	2090±131
Steam-Air-US	7114±311	7519±347	2055±85

Table 5.4. Effect of different blanching techniques on textural properties of green bell pepper

Treatments	Hardness	Chewiness	Stiffness
Raw	27969±1466	66088±3429	6561±293
HW	9266±470	23164±1276	1758 ±135
Steam	14471±660	31559±1876	2440±179
Steam-Air	12521±622	23358±1843	2220 ±120
Steam-US	19337±931	40051±1809	3238±253
Steam-Air-US	15943±756	32545±1758	2845±218

Table 5.5. Effect of different blanching techniques on textural properties of zucchini

Treatments	Hardness	Chewiness	Stiffness
Raw	28344±1306	65113±3755	6489±407
HW	10146±772	16956±1354	1731±156
Steam	15980±893	32459±2600	3211±231
Steam-Air	13362±872	24369±1755	2827±268
Steam-US	17698±1361	35749±2920	3484±280
Steam-Air-US	16383±1238	33229±2053	3325±233

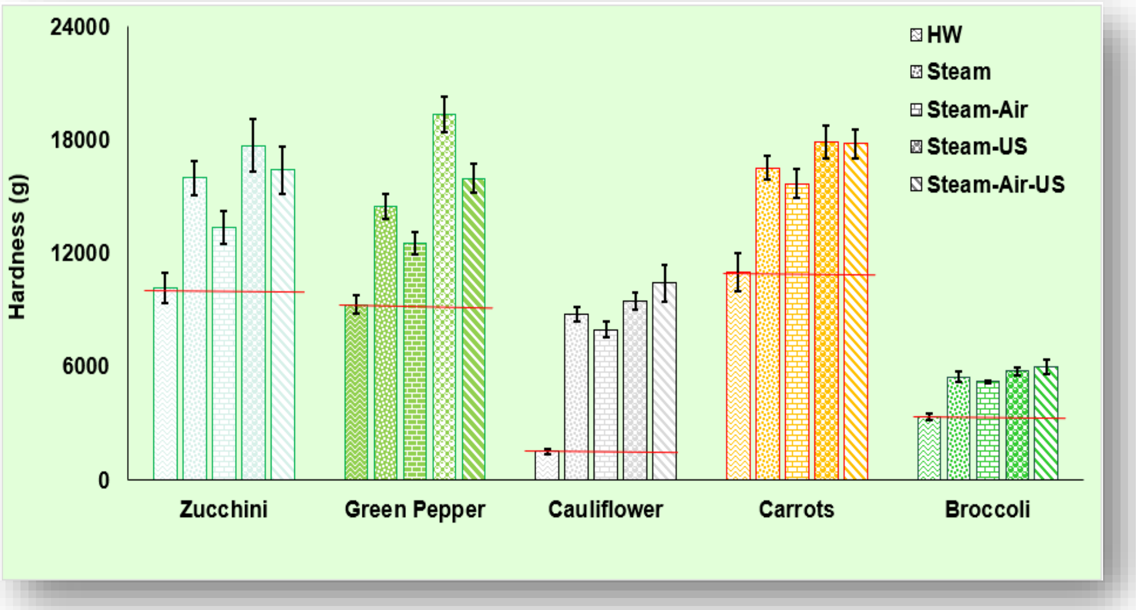

Figure 5.4. Effect of different blanching techniques on hardness of vegetables

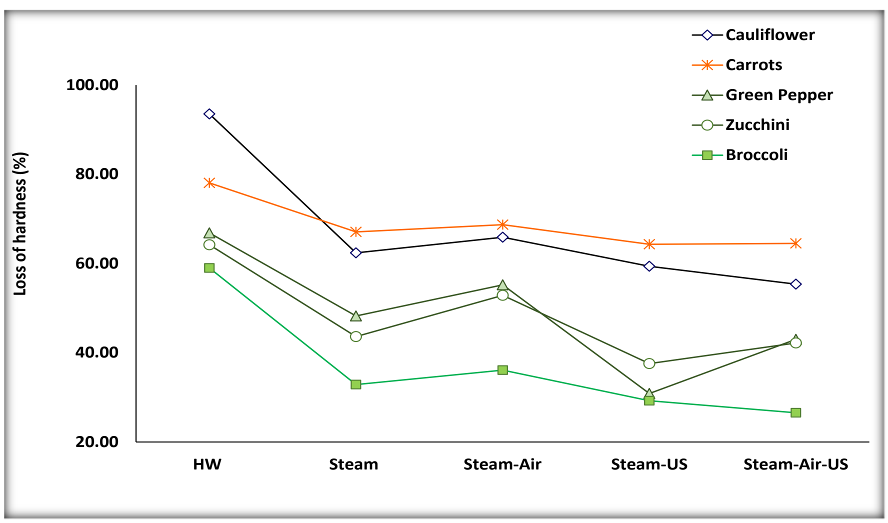

Figure 5.5. Effect of different types of blanching on loss of hardness of vegetables

5.3.1.2. Effect of frozen storage on textural properties of vegetables

Texture in frozen fruits and vegetables is an important component of product quality. Research shows that blanching caused significant changes in the texture and color which is further affected by frozen storage (Kidmose, et al.,1999). Figure 5.6. shows changes during frozen storage time on hardness of broccoli after different blanching treatments. All samples were thawed overnight in a refrigerator prior to analysis. Hot water showed the least retention of hardness compared to steam and combined steam-ultrasound treatments. Combined steam ultrasound showed slightly better overall retention of hardness both in presence and absence of air. The hardness of broccoli reduced after blanching and freezing. There were progressive changes in hardness values with storage time during frozen storage for different days; but the changes associated with ultrasound combination treatments were small.

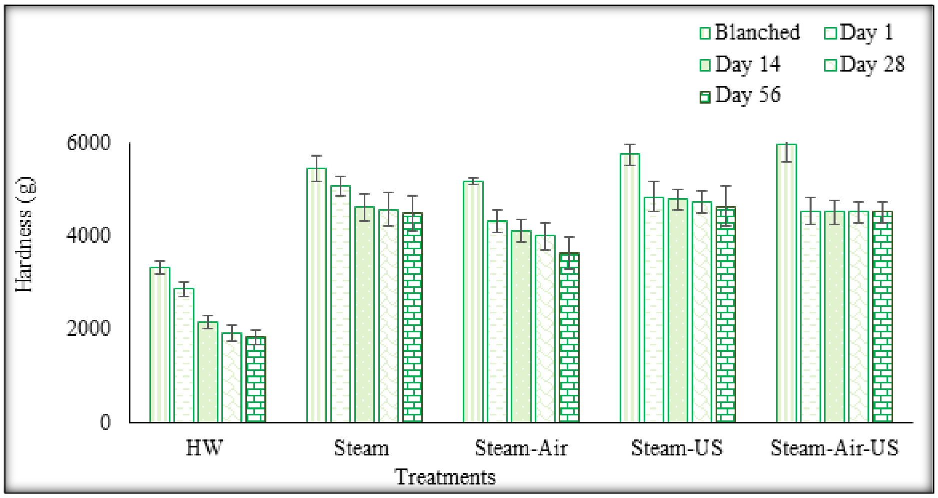

Figure 5.6. Effect of frozen storage on hardness of broccoli

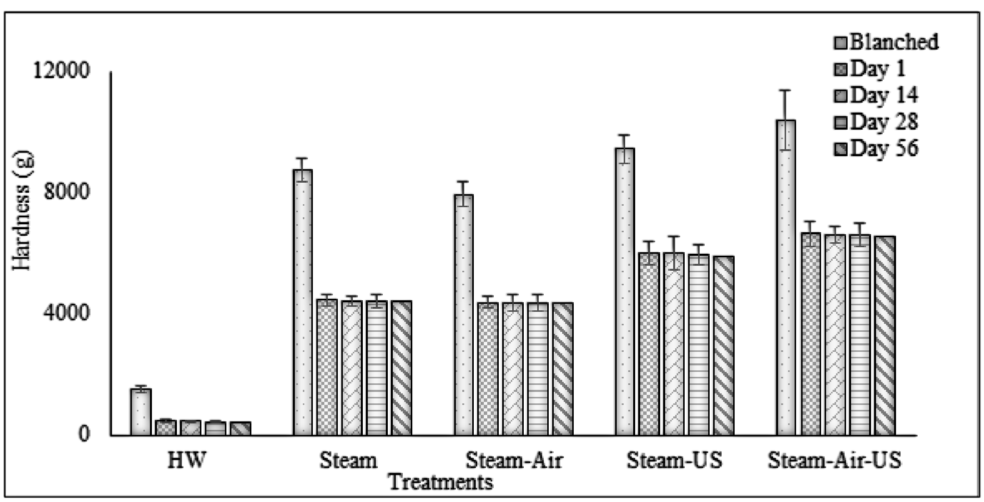

Figure 5.7 Effect of frozen storage on hardness of cauliflower

Figure 5.8 shows the changes during different blanching and freezing treatments for hardness in carrots. Again, the hot water resulted in the least retention of hardness compared to steam and combined steam-ultrasound treatment. Combined steam ultrasound showed better retention of hardness both in presence and absence of air. The hardness of carrots is reduced after freezing. Minimal changes in hardness were observed under frozen storage for different days. Unlike with cauliflowers, the texture values showed a progressive drop in hardness for carrots.

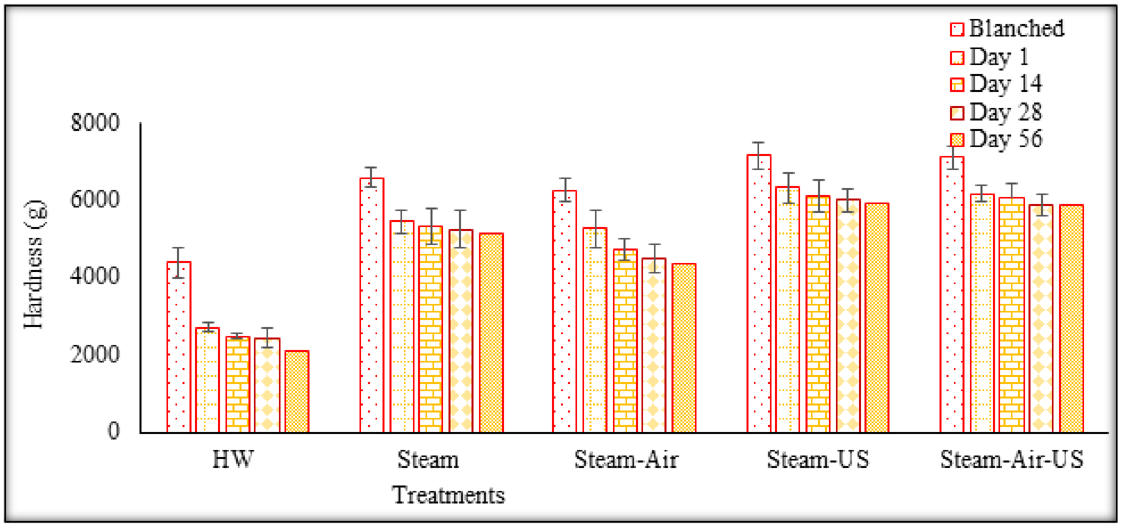

Figure 5.8 Effect of frozen storage on hardness of carrots

Figure 5.9 shows the typical changes during different blanching and freezing treatments for hardness in green bell pepper. Hot water appears to be the least favorable again with least retention of hardness compared to steam and combined steam-ultrasound treatment. Combined steam ultrasound showed better retention of hardness both in presence and absence of air. Though freezing reduced the hardness of green bell pepper the change was not drastic. With the increase in storage days the texture values dropped progressively.

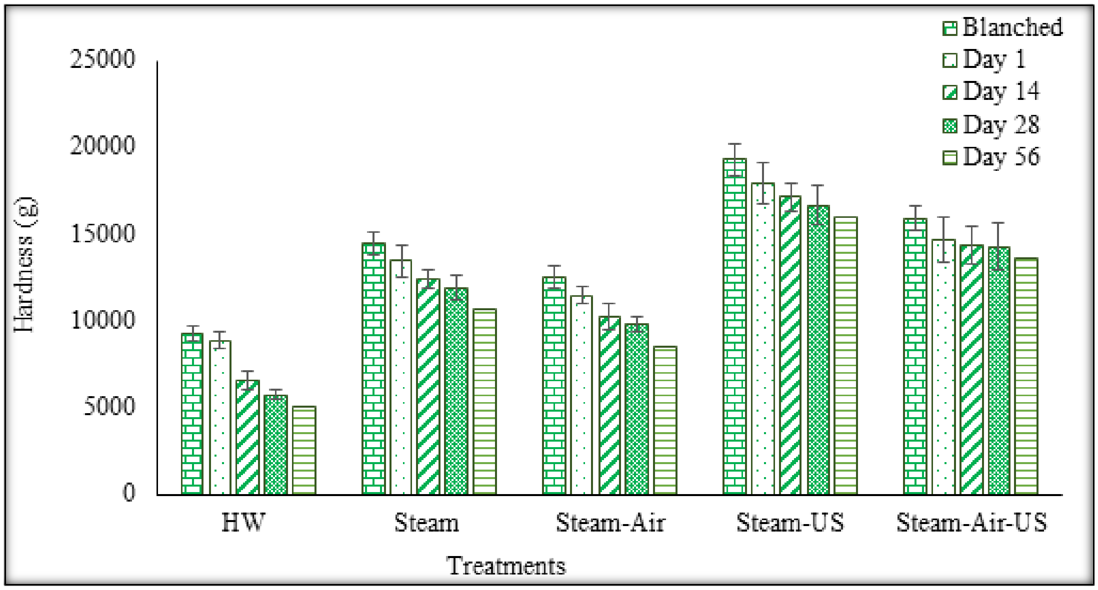

Figure 5.9 Effect of frozen storage on hardness of green bell pepper

Figure 5.10 shows the typical changes during different blanching and freezing treatments for hardness in zucchini. The hardness of zucchini showed a sudden drop after freezing and while during storage the changes were minimal. Hot water showed the least retention of hardness compared to steam and combined steam -ultrasound treatment. Combined steam ultrasound again showed better retention of hardness both in presence and absence of air.

In the case of most of the vegetables there was a significant drop in texture value on day 1 (blanching and freezing result), but a moderate further decrease during storage.

An overall representation of how frozen storage of vegetables blanched under different techniques is represented in Figure 5.11. All five vegetables showed highest loss of texture under hot water blanching followed by steam blanching in presence of air and then in absence of air. The treatment times were both longer in these 3 cases compared to combined ultrasound -steam blanching in presence and absence of air. Some differences and similarities could be noted as well. Carrots and green pepper showed the largest span of variations in hardness, ranging from 5000 to 15000 g. On the other hand, broccoli and cauliflower showed the least (under 5000 g) while had the middle range up to 10000 g. These differences were presumed to arise from the natural differences in their cell structure.

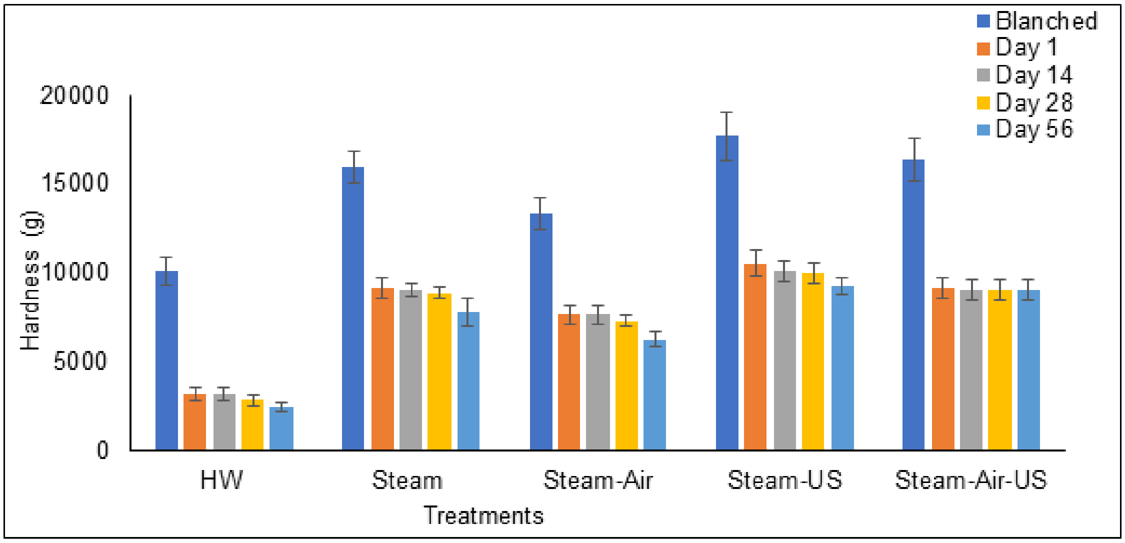

Figure 5.10. Effect of frozen storage on hardness of zucchini

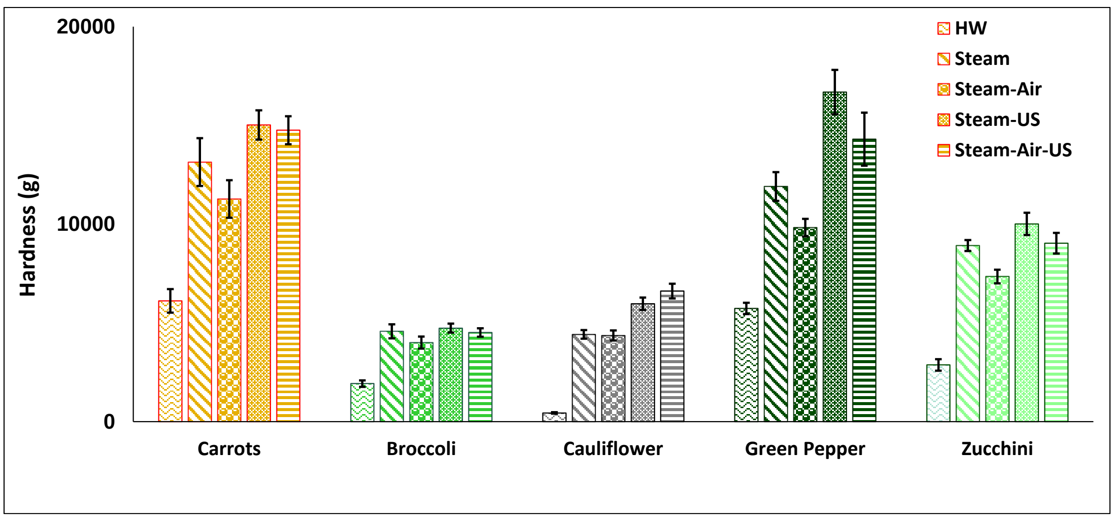

Figure 5.11. Effect of different blanching techniques and frozen storage on hardness of different vegetables

5.3.2. Ascorbic acid content

5.3.2.1. Effect of different blanching techniques on ascorbic acid retention in vegetables

Blanching can significantly reduce the ascorbic acid content of the vegetables depending on the type of vegetable and blanching time (Wen et al., 2010). Studies on vegetables such as cauliflower, broccoli, green beans, and brussels sprouts showed that blanching caused a significant reduction in the ascorbic acid content due to thermal degradation (Wen et al., 2010;

Ruiz-Ojeda et al., 2013). Steam blanching causes smaller reduction in ascorbic acid content compared to hot water boiling (Gupta et al., 2008) but the extent of reduction depends on the type of vegetables and blanching time (Wickramasinghe, et al., 2020).

In Figure 5.12, it can be observed that the ascorbic acid content retention of the vegetables was least when vegetables were subjected to hot water blanching, followed by different steam blanching techniques. Maximum retention was observed when vegetables were subjected to combined steam and ultrasound blanching both in presence and absence of air. This shows that adding ultrasound to steam blanching can reduce loss of ascorbic acid content in vegetables. The quality parameters shown in these figures are prior to freezing. Zucchini and carrots were not big contributor of vitamin C and are placed at the lowest end both with level and changes associated. Maximum changes were observed with green pepper with a similar pattern but at a lower level with broccoli the two green vegetables. Cauliflower showed minor variations with mean values between green pepper and broccoli.

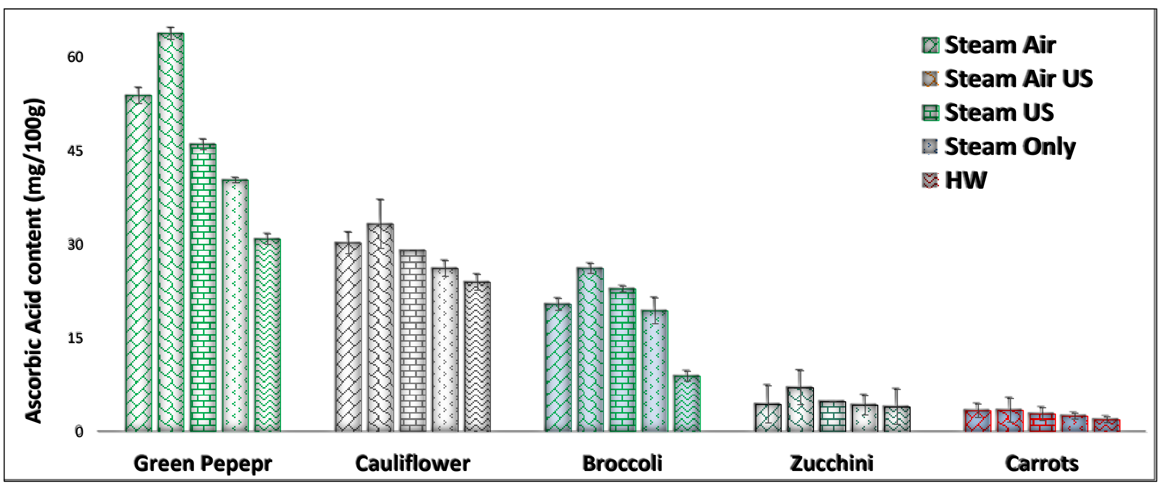

Figure 5.12. Effect of different blanching techniques on ascorbic acid content of vegetables

5.3.2.2. Effect of frozen storage on ascorbic acid retention in vegetables

Figures 5.13 to 5.17 show the changes in ascorbic acid content during frozen storage after different blanching treatments in broccoli, cauliflower, carrots, green bell pepper and zucchini respectively. It is clear that hot water showed least retention of ascorbic acid compared to steam and combined steam-ultrasound treatment. Here again the combined steam ultrasound showed better retention of ascorbic acid content in all the vegetables both in presence and absence of air. Day 1 after freezing showed higher content of ascorbic acid in few vegetables like broccoli, green bell pepper and zucchini in case of all the blanching techniques. This might be due to more extraction of ascorbic acid during analysis due to the blending of vegetables with extraction solvents. Later, the number of days stored in frozen conditions didn't show much significant difference, but the trend showed that with longer storage time the ascorbic acid content reduced. The storage time effect was very steady and similar for most treatments.

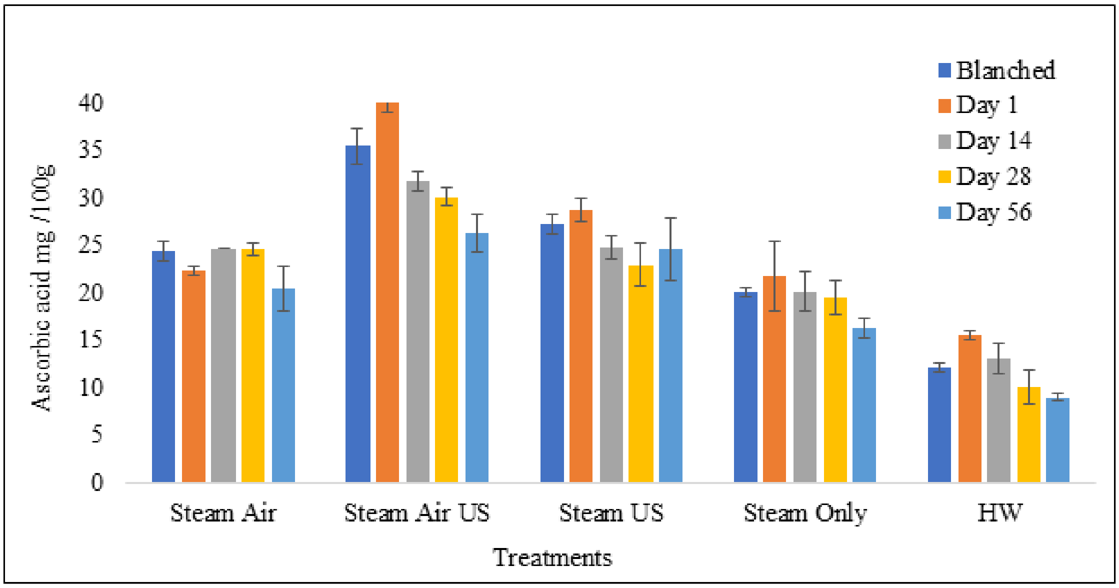

Figure 5.13. Effect of frozen storage on ascorbic acid content retention of broccoli

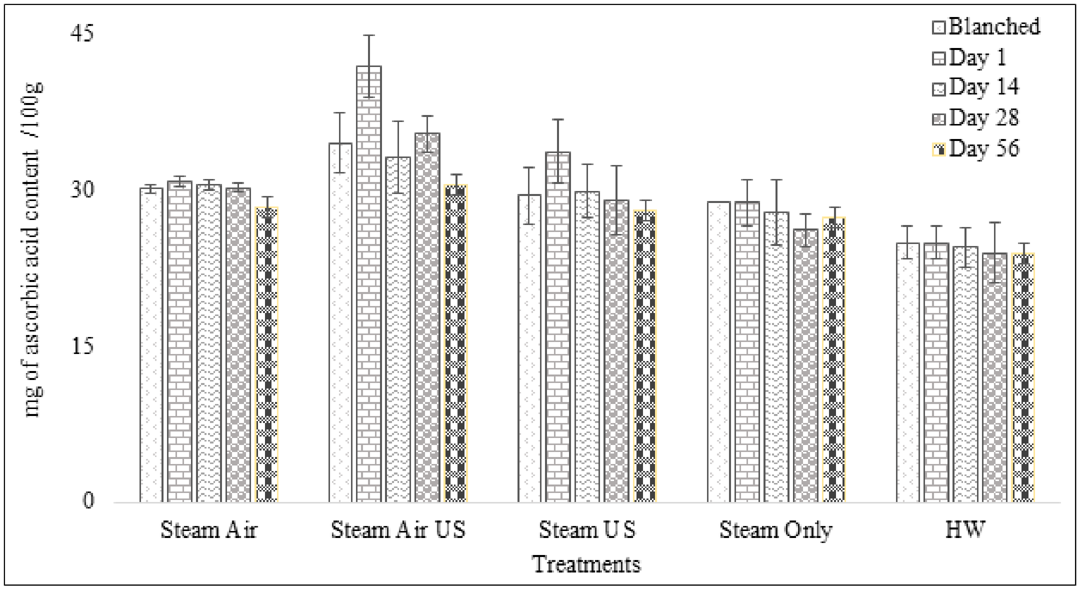

Figure 5.14. Effect of frozen storage on ascorbic acid content retention of cauliflower

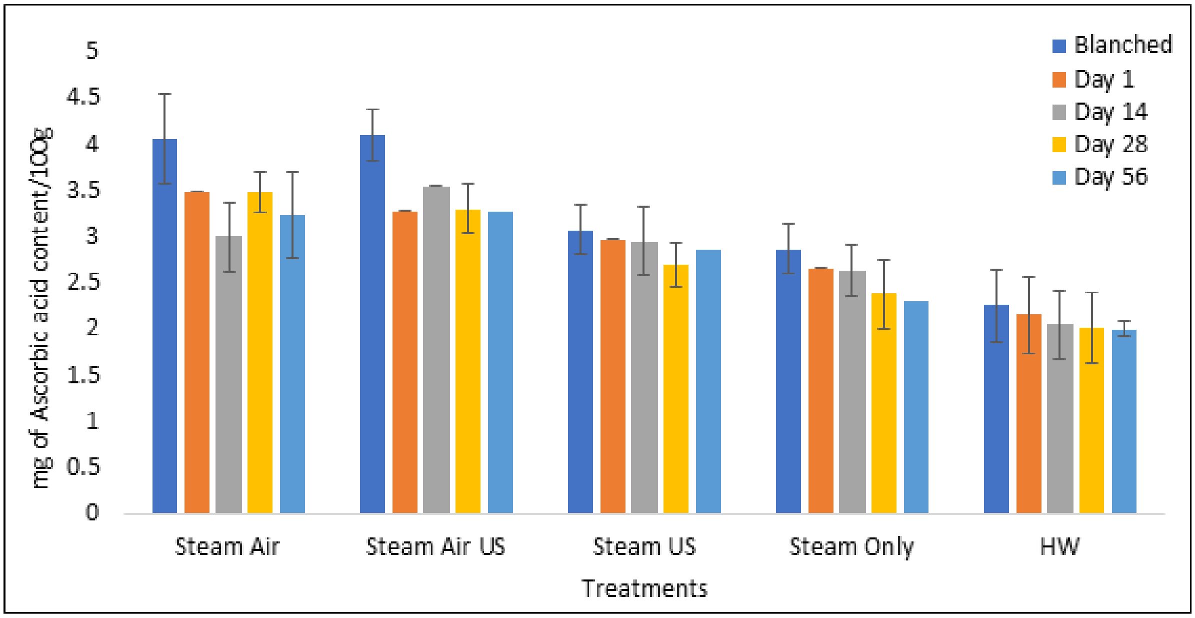

Figure 5.15. Effect of frozen storage on ascorbic acid content retention of carrots

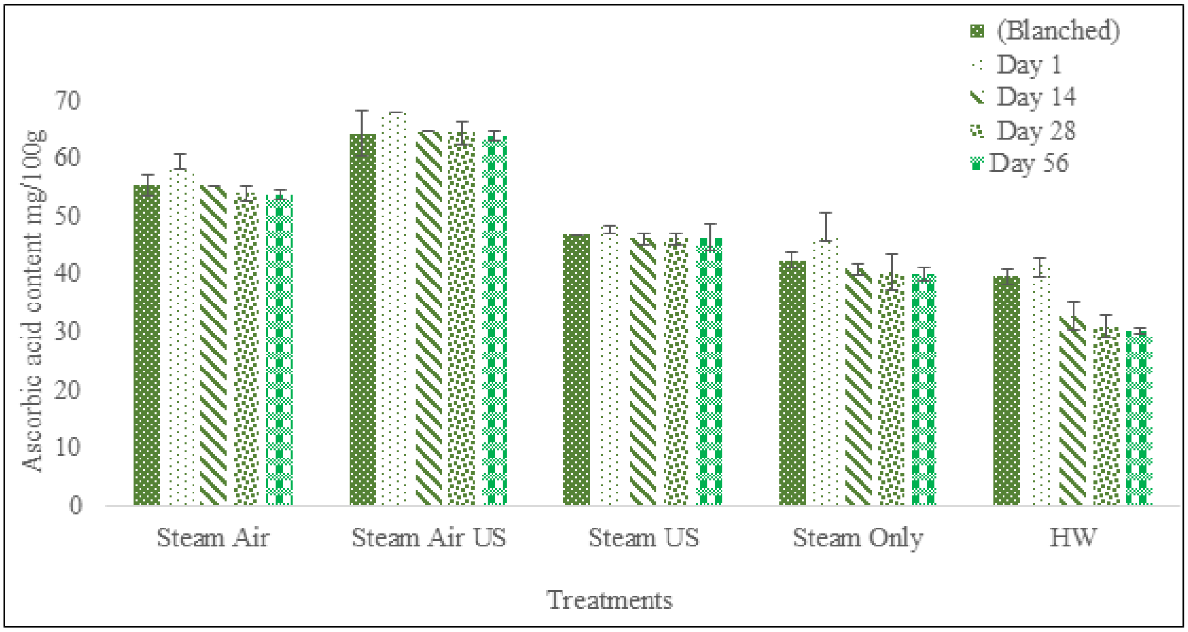

Figure 5.16. Effect of frozen storage on ascorbic acid content retention of green bell pepper

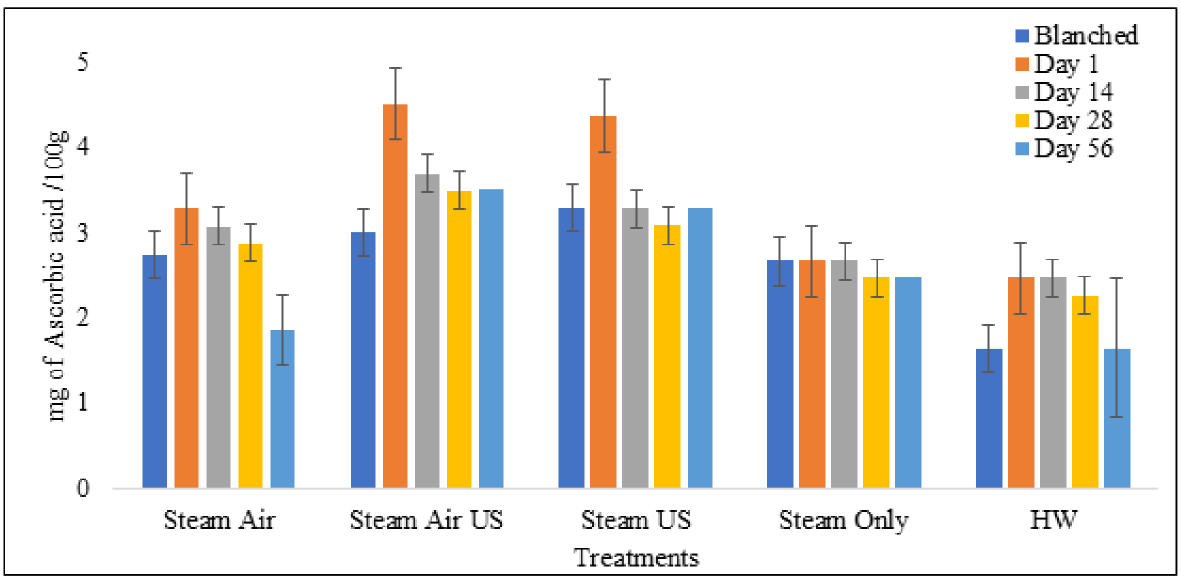

Figure 5.17. Effect of frozen storage on ascorbic acid content retention of zucchini

Thermal blanching can significantly reduce ascorbic acid content in vegetables, but frozen storage time does not have much effect on ascorbic acid content (Gupta et al.,2008). Figure 5.18 shows the effect of frozen storage comparing different blanching techniques on all the five vegetables. Hot water blanching causes least retention of ascorbic acid content followed by steam blanching. Combined ultrasound steam blanching showed the highest retention in all the vegetables.

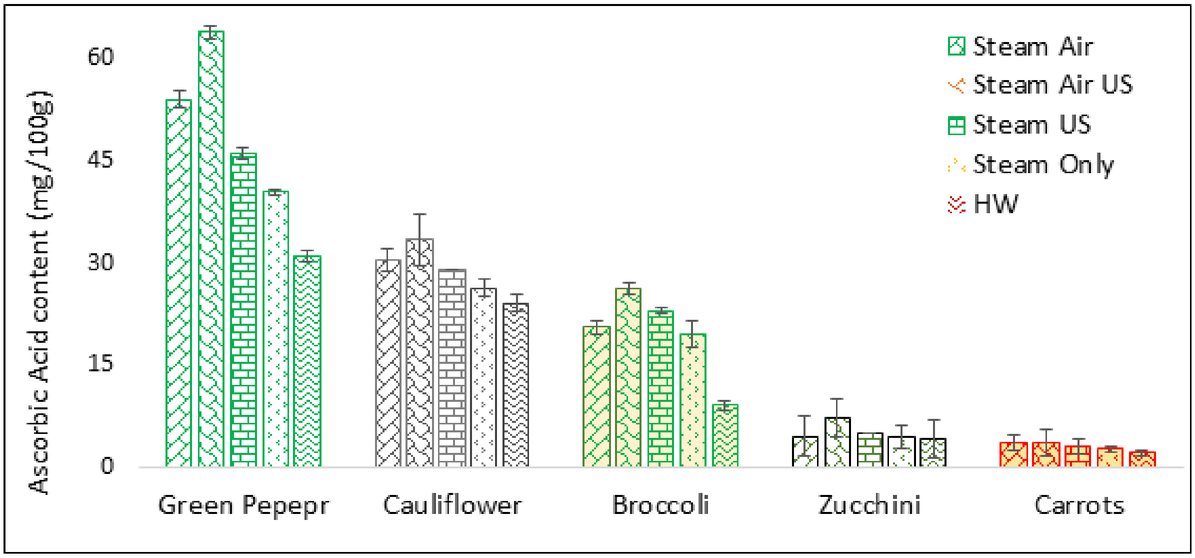

Figure 5.18 Effect of frozen storage on ascorbic acid content of different vegetables

5.3.3. Color analysis

5.3.3.1. Effect of different blanching techniques on color of vegetables

The color (L* values representing brightness) of the vegetables were maximum when vegetables were subjected to combined steam and ultrasound blanching both in presence and absence of air compared to hot water blanching. The color quality parameter shown in figure 5.19 are prior to freezing of all the vegetables treated under different blanching methods.

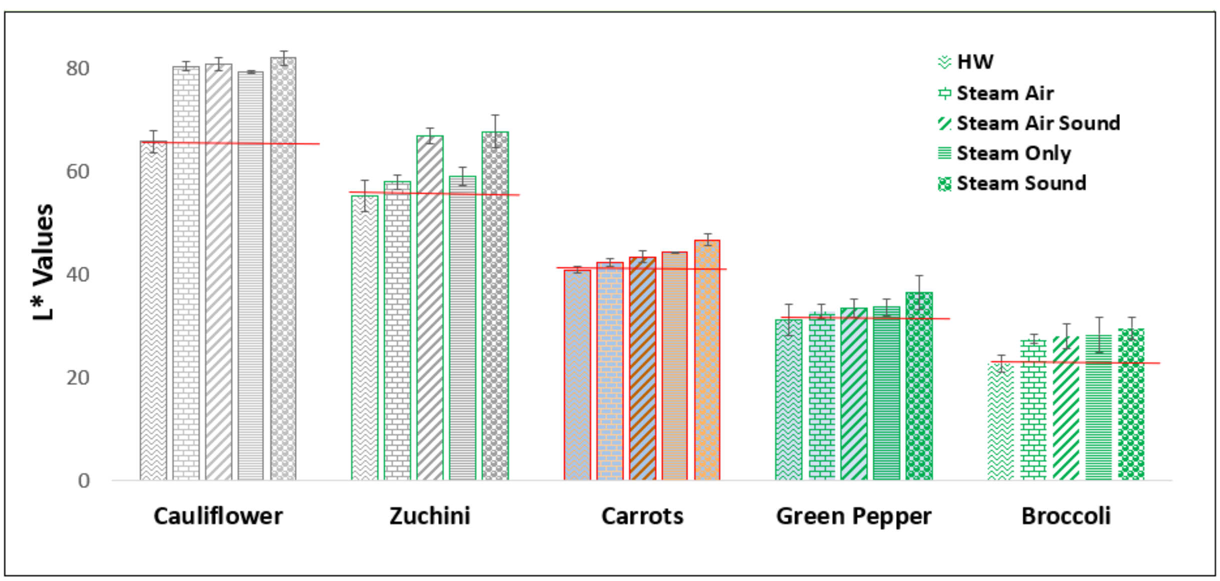

Figure 5.19. Effect of different blanching techniques on L* values of vegetables

5.3.3.2. Effect of frozen storage on color of vegetables

Figures 5.20 to 5.24 show the typical changes in L* values during different blanching and freezing treatments in broccoli, cauliflower, carrots, green bell pepper and zucchini respectively. Except for green bell pepper it is shown that the L* values of all the vegetables decrease gradually with the storage time indicating loss in retention of brightness. It is clear that hot water showed least retention of color compared to steam and combined steam ultrasound treatment in all the cases. Like other quality parameters the combined steam ultrasound treatment showed better retention of color in the vegetables both in presence and absence of air. For green bell pepper, the color changes were somewhat more comparable as influenced by the different treatments. The steam ultrasound treatment in presence of air appeared to show much better retention of color in green pepper compared to the one in absence of air, but still it was better than hot water blanching. The storage time effect was very steady and similar for most treatments.

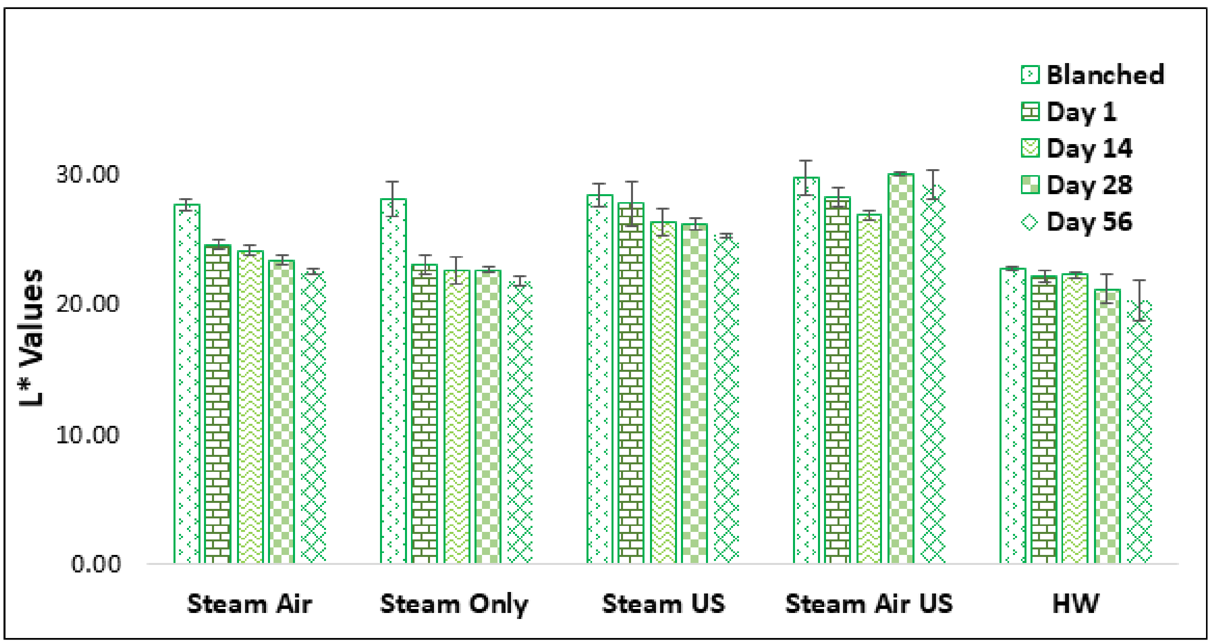

Figure 5.20. Effect of frozen storage on L* values of broccoli

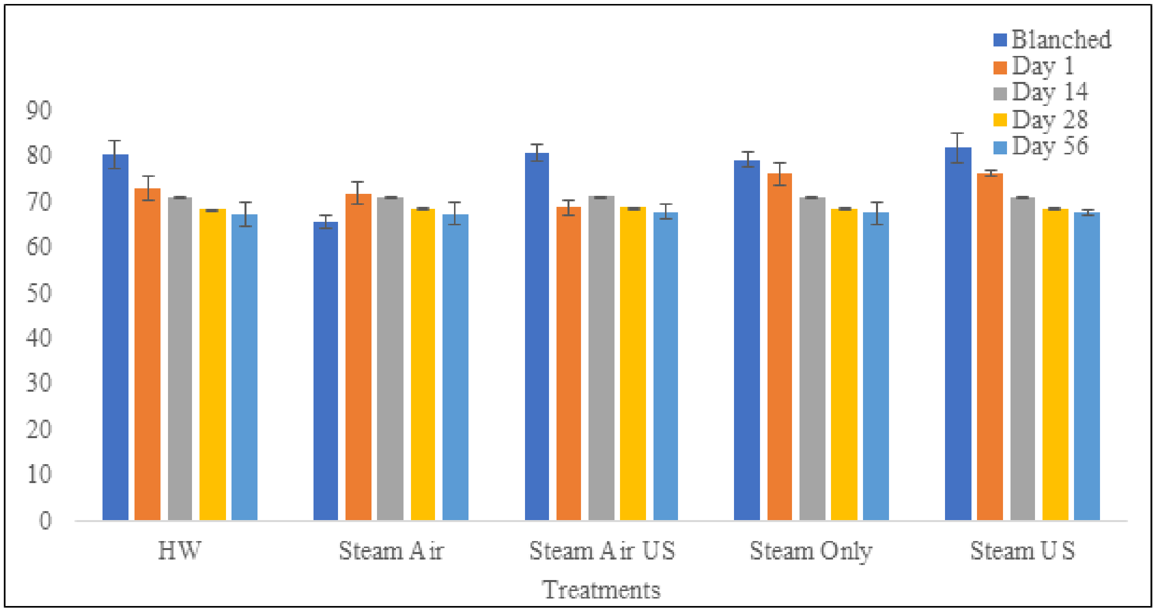

Figure 5.21. Effect of frozen storage on L* values of cauliflower

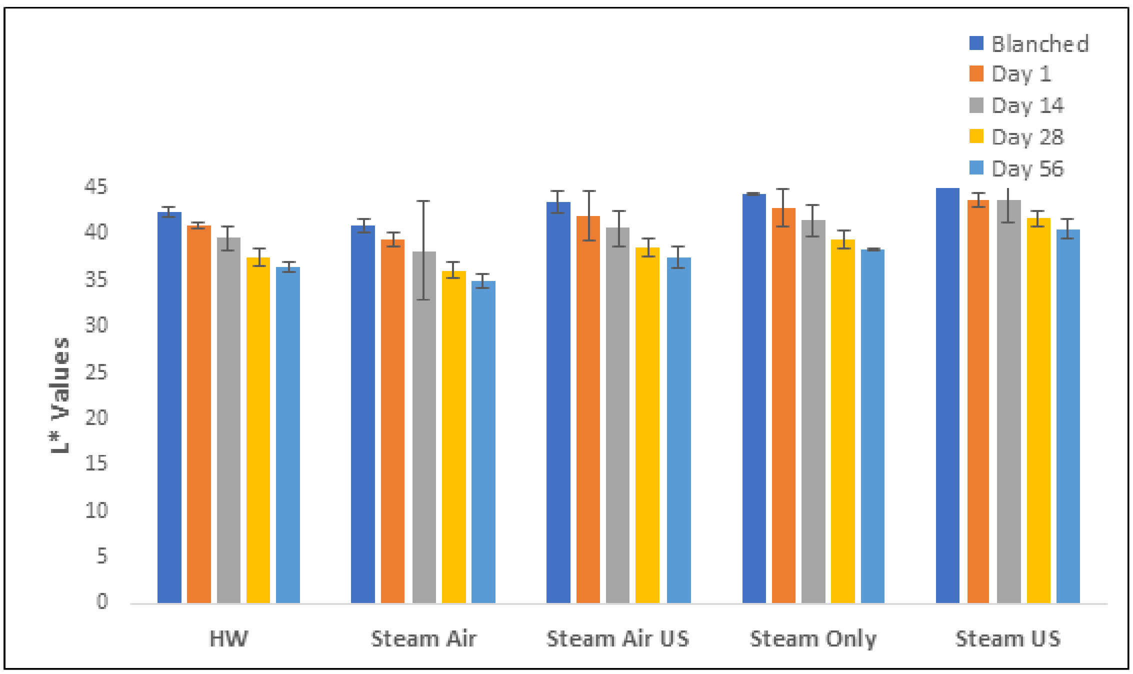

Figure 5.22. Effect of frozen storage on L* values of carrots

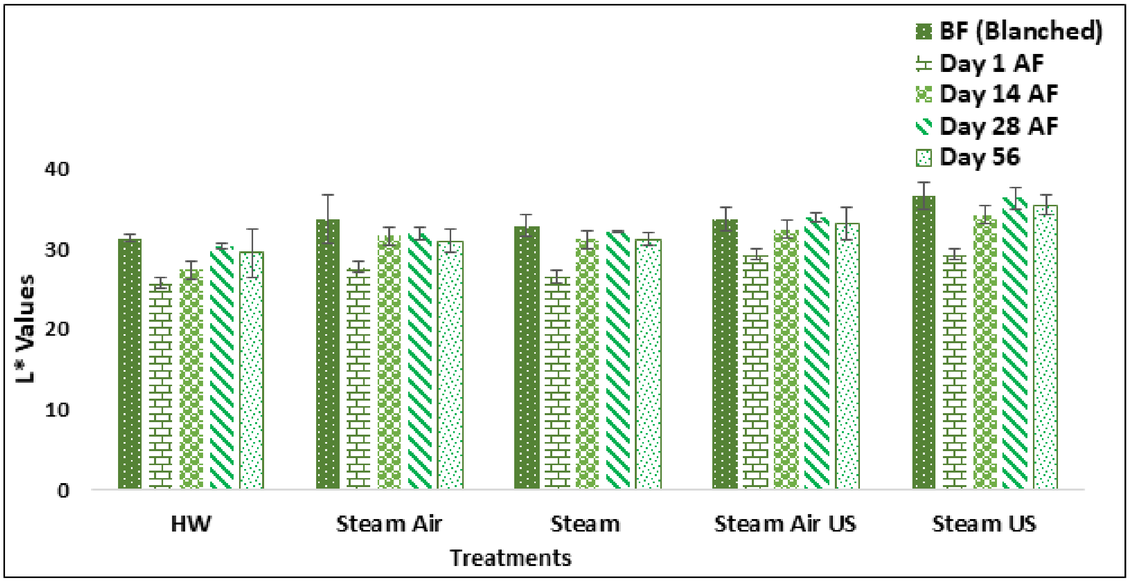

Figure 5.23. Effect of frozen storage on L* values of green bell pepper

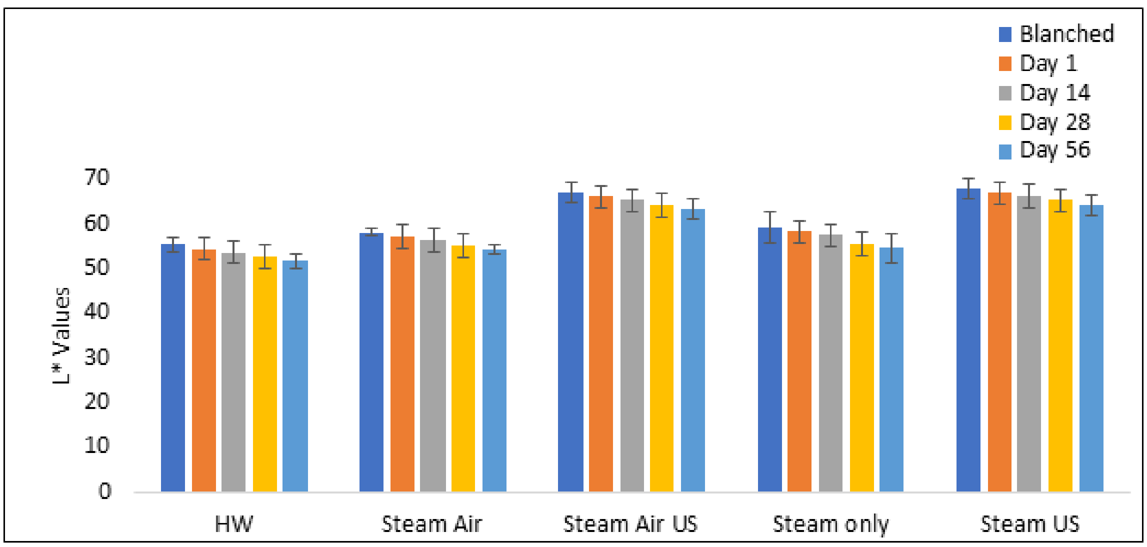

Figure 5.24 Effect of frozen storage on L* values of zucchini

Figure 5.25 shows the effect of frozen storage on color comparing different blanching techniques on all the five vegetables. In case of all the vegetables it is shown that hot water blanching has the lowest retention compared to all the other blanching treatments. Overall, when ultrasound is added to the blanching treatment, the brightness (L* value) of all the vegetables are retained compared to treatment without ultrasound.

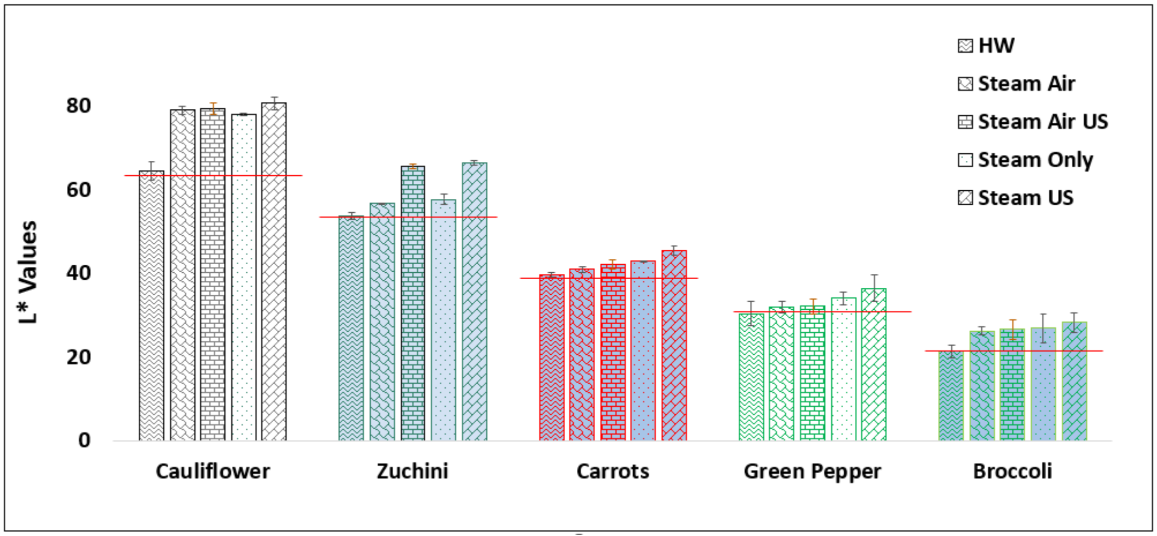

Figure 5.25 Effect of different blanching techniques and frozen storage on L* values of different vegetables

5.3.4. Drip Loss

Figure 5.26 shows the typical changes in drip loss of broccoli when subjected to different blanching techniques during frozen storage. Hot water blanching showed highest drip loss % and steam air ultrasound treatment showed least drip loss up to day 28th. On day 56th the drip loss% is highest for hot water treatment followed by steam air ultrasound. The drip loss increased in all the types of blanched samples during the frozen storage except for steam only treated sample.

Figure 5.27 shows the typical changes in drip loss of cauliflower when subjected to different blanching techniques during frozen storage. Hot water blanching once again showed highest drip loss and steam air ultrasound treatment showed least drip loss. Drip loss increased with frozen storage time. It almost reached the maximum value with just a day's frozen storage with hot water blanching, while other treatments showed a progressive increase in drip loss.

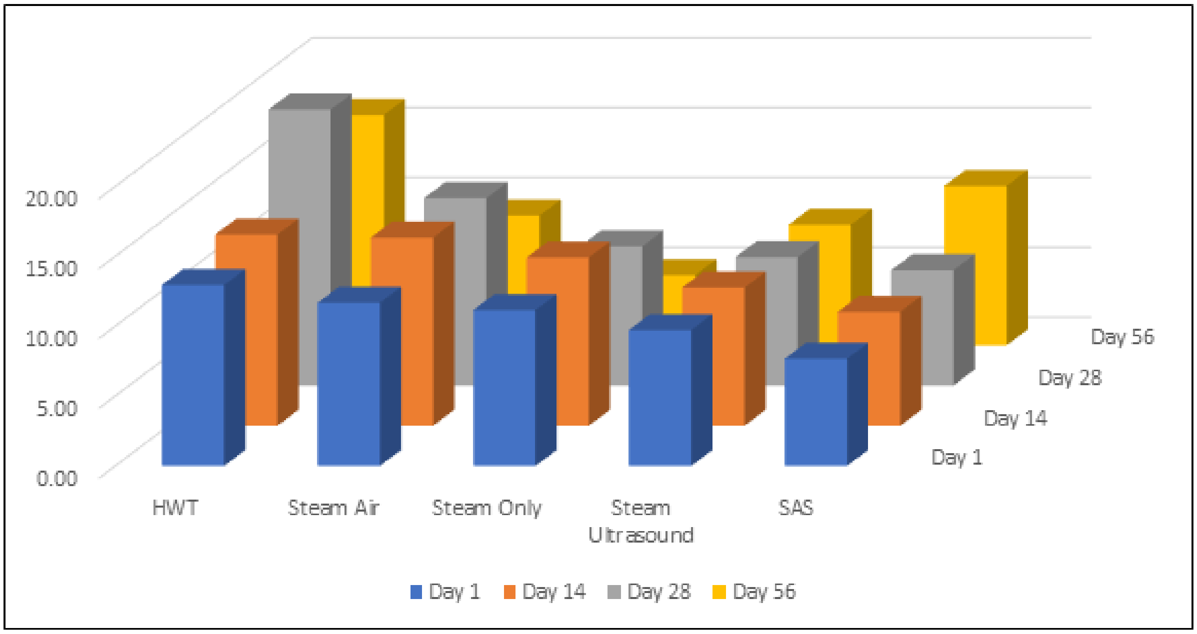

Figure 5.26. Changes in drip loss % of broccoli during frozen storage

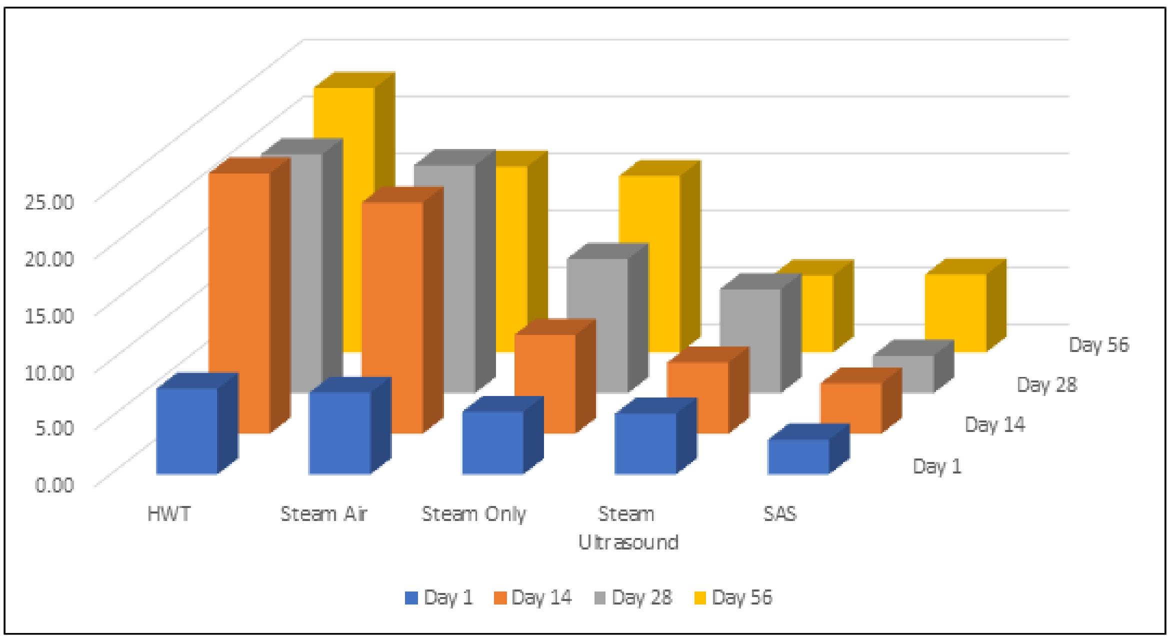

Figure 5.27. Changes in drip loss % of cauliflower during frozen storage

Figure 5.28 shows the typical changes in drip loss of carrots when subjected to different blanching techniques during frozen storage. Hot water blanching showed highest drip loss % and steam air ultrasound treatment showed least drip loss %. Drip loss increased with frozen storage time, but the changes associated with carrots were more moderate with all treatments.

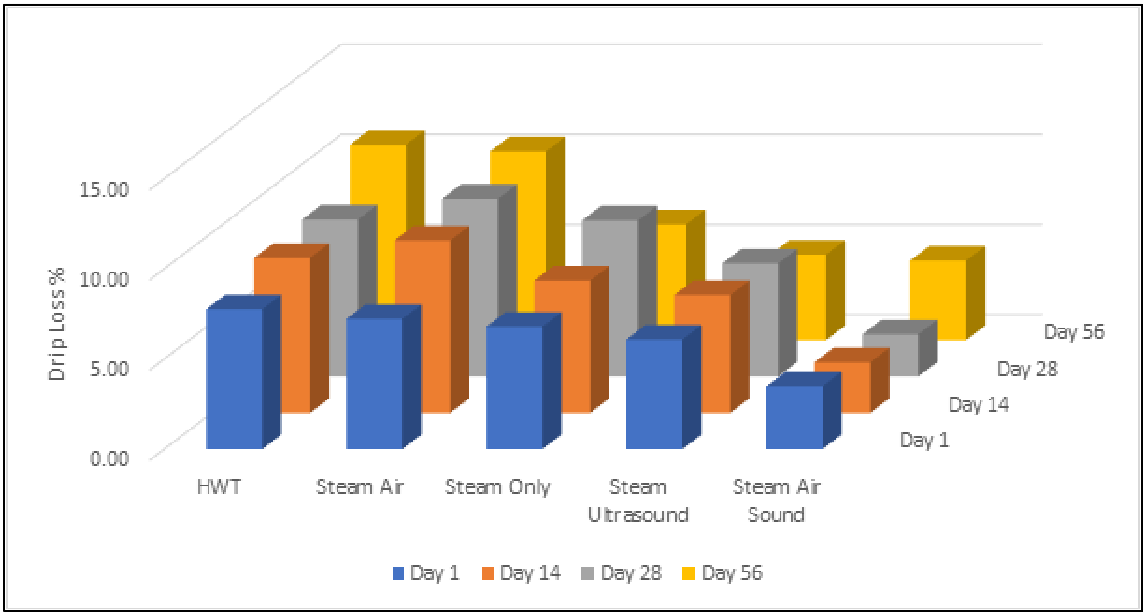

Figure 5.28. Changes in drip loss % of carrots during frozen storage

Figure 5.29 shows the typical changes in drip loss of green bell pepper when subjected to different blanching techniques during frozen storage. Hot water blanching again showed highest drip loss % and steam air ultrasound treatment showed least drip loss. Again, there was a big jump in drip loss with water blanched samples with just a day of frozen storage, while they were steadier with other samples. There appeared to be also a spike at 56-day storage for almost all the treatments except for steam only treatment.

Figure 5.30 shows the typical changes in drip loss of zucchini when subjected to different blanching techniques during frozen storage. Hot water blanching showed highest drip loss and steam air - ultrasound treatment showed least drip loss. Steam-air sound was the best up to 28-day storage, but by 56 days, the drip loss increased. But still when compared to hot water or steam treatment in presence of air, it showed less drip loss.

Studies have shown that blanching caused an increase in drip loss, but the effect of freezing on drip loss may vary depending on the type of vegetable (Thakur et al., 2022). Figure 5.31 shows drip loss of different vegetables under different blanching techniques. A drip loss was observed to be highest under hot water blanching in case of all the vegetables. But depending on the type of vegetables the drip loss varied. Carrots had least drip loss followed by cauliflower, broccoli, zucchini, and then green pepper. Green pepper showed highest drip loss may be due to its higher water content and the way the vegetables were cut before blanching.

Van et al. (2020), reviewed about the effect of blanching on the water holding capacity and drip loss of selected vegetables including green beans, broccoli and carrots and found that blanching caused a significant increase in drip loss, which was attributed to the loss of water-soluble components.

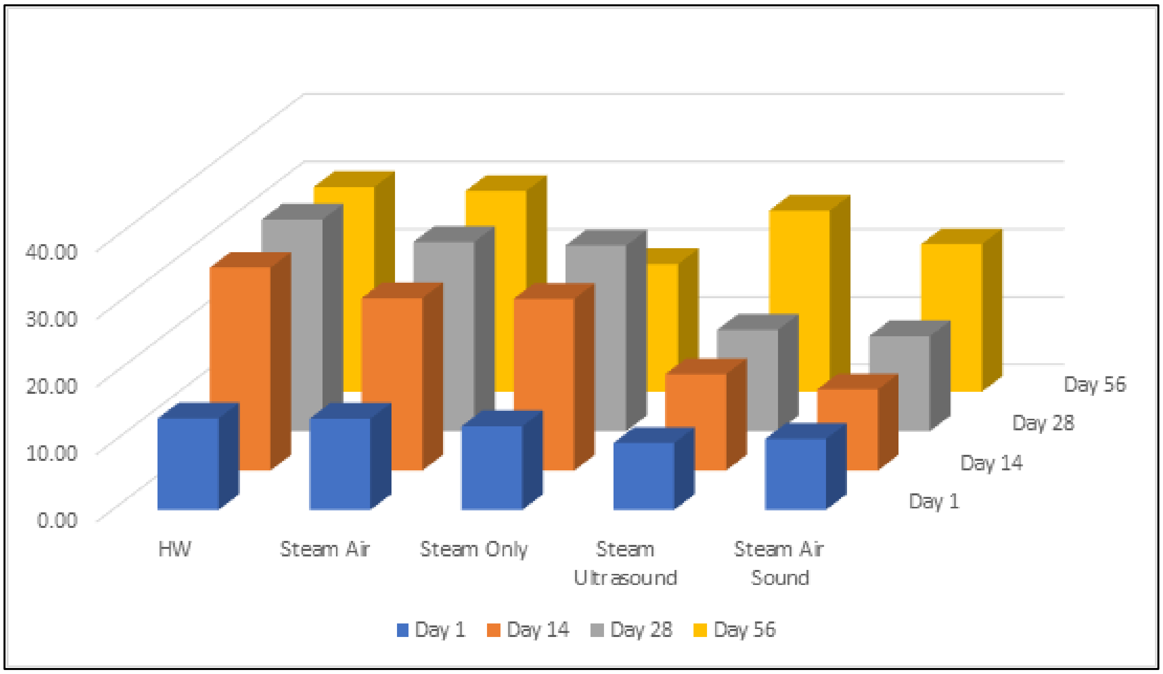

Figure 5.29. Changes in drip loss % of green bell pepper during frozen storage

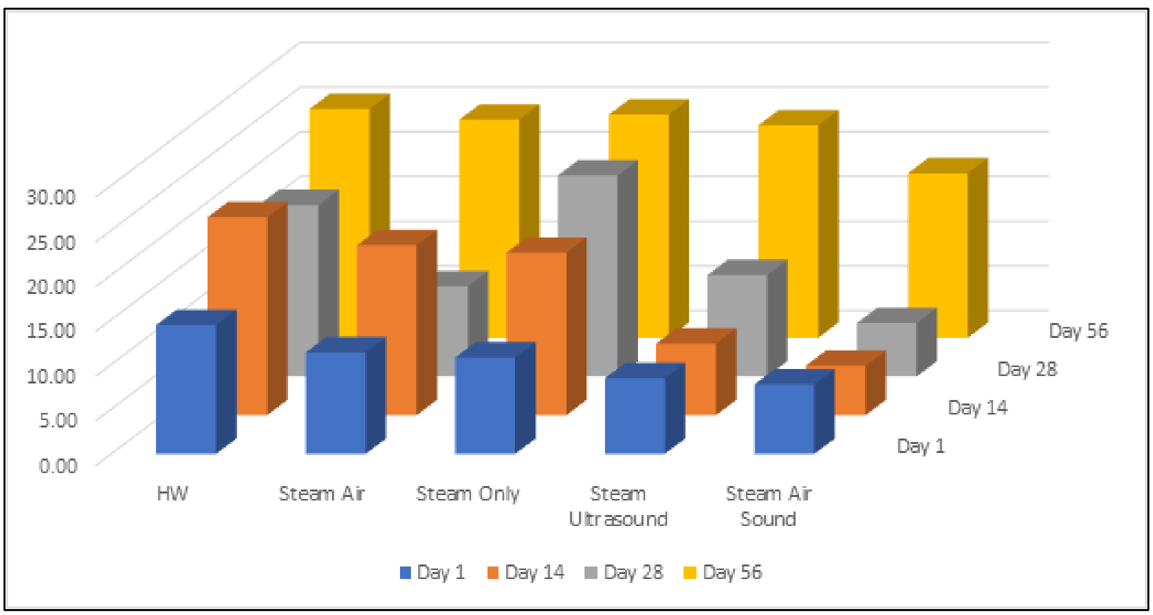

Figure 5.30. Changes in drip loss % of zucchini during frozen storage

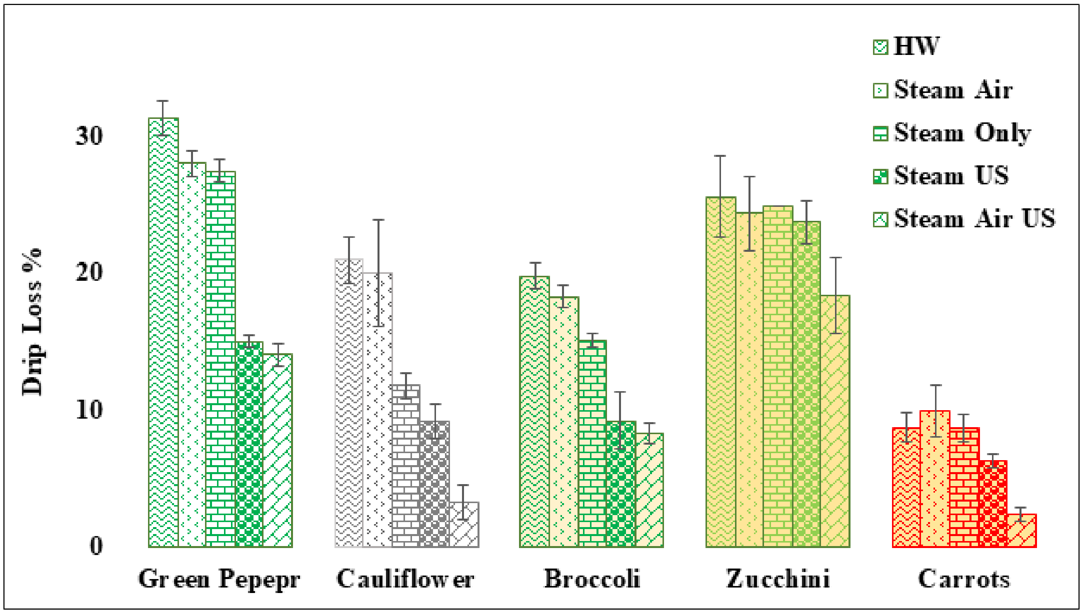

Figure 5.31. Changes in drip loss % of vegetables under different blanching techniques during frozen storage

5.4. Conclusions

Based on the research findings, it can be concluded that ultrasound steam blanching of vegetables can be a viable alternative to conventional blanching methods. It was observed that ultrasound steam combination blanching resulted in better color, texture, and ascorbic acid retention compared to conventional hot water and steam blanching methods. In addition, the ultrasound steam blanching method resulted in a short blanching time, which could be beneficial for industrial processing.

Hot water treatment shows the least retention of quality compared to steam and combined ultrasound steam blanching techniques both in presence and absence of air. Freezing of vegetables also showed variable effects on the texture, color, ascorbic acid content and drip loss of vegetables. Highest effect of frozen storage was observed on textural quality of the vegetables.

5.5. References

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Connecting Statement to Chapter 6

As discussed previously, steam is the most effective heating medium used in thermal processing applications and ultrasound can be used to enhance heat transfer. Heat transfer degradation from steam with the addition of air has been well documented in literature and has been discussed and implicated in the last three chapters. Use of ultrasound with steam, steam/air and hot water has been demonstrated to improve surface microbial decontamination and enzyme inactivation. Other than the detailed early study of Dr. Ramaswamy (PhD thesis, University of British Columbia) on heat transfer from steam/air mixtures, little basic work has been reported in the context of use of steam/air media for food processing applications. Almost no work has been reported in the literature on basic heat transfer from steam and steam/air mixtures in combination with ultrasound for heat transfer enhancement. In this chapter, therefore, some concept testing evaluation is reported to demonstrate the effectiveness of ultrasound-steam technology for heat transfer enhancement.

Part of this study has been included in a publication and several presentations:

Basumatary, R**,** Vatankhah, H**, Dwivedi**, M**,** John,D**,** Ramaswamy,HS. Ultrasound-steam combination process for microbial decontamination and heat transfer enhancement. *J Food Process Eng*. *2020*; 43:e13367. https://doi.org/10.1111/jfpe.13367

Basumatary, R., Vantankhah, H., and Ramaswamy, H.S. 2019. Evaluation of heating characteristics of porous materials in the presence of steam and sound combinations. Northeast Agricultural and Biological Engineering Conference (NABEC) 2019. June 16-19, 2019 Laval University, Quebec City, QC, Canada.

Basumatary, R., Vantankhah, H., Dwivedi, M., John, D., and Ramaswamy, H.S. 2020. Ultrasound-steam combination process for microbial decontamination and heat transfer enhancement: Overview and Concept Testing. Poster presentation in IFTPS Annual Meeting in San Antonio (March 3-5, 2020). Won 3rd prize in 2020 IFTPS Charles R. Stumbo Student Paper Competition.

Bastakoti, M *, Basumatary, R, Ali R. Taherian, Ramaswamy, H.S 2022. "Evaluation of heat transfer from steam/air and water under the influence of ultrasound waves." Poster presentation in ASABE NABEC Section Meeting July 31st -August 3rd, Edgewood Maryland, USA

Contribution of authors:

Basumatary is the PhD candidate who carried out bulk of the work under the supervision of Prof. Ramaswamy who conceived the idea, provided the technical advice and facility. Authors Vatankhah, Dwivedi, John, Taherian, Bastakoti provided technical assistance as needed at various phases of this research and assisted in dissemination of the research at conferences.

Chapter 6

Evaluation of ultrasound enhancement of heat transfer in steam and steam/air heating media

Abstract

In commercial thermal processing applications steam is the most effective heating medium used. However, for rapid short time (~30 s) direct heating of food products it poses serious difficulties because of the presence of air along the heat transfer surface, which interferes with the condensation heat transfer mechanism and slows down the effective heating of the surface. Addition of an ultrasound environment to steam can assist in removing this surface barrier and promote better heat transfer. For medium and long-time heating (several minutes), the benefit derived may be more from creating turbulence/vibrations in the heat transfer medium in or out of the container. This study was aimed at evaluating the combined influence of ultrasound in steam, steam/air, and water media on the associated heat transfer. Heat transfer from steam, steam/air, and water as influenced by ultrasound on container surface was compared using high thermal conductivity materials (aluminum) of regularly shaped metal transducers (cylinders and plates) and using product filled into retort pouches for heat transfer enhancement inside the container. Heating rate index has been traditionally used in thermal processing as a measure of the rate of heat transfer to foods packaged in different containers. It was demonstrated for both short time heating at the surface and longer time internal heating in the pouch, the combination heat treatment was more efficient than steam alone for enhancing heat transfer rates contributing to achieve a lower heating rate index, higher temperatures, and higher process lethality values. The steam-sound combination seems to be a promising approach in surface decontamination of fruits and vegetables as well as for industrial processing of foods without affecting their physico-chemical characteristics significantly like thermal processing. The focus of this study was to demonstrate the success of ultrasound-steam combination process**.**

6.1. Introduction

Thermal processing is one of the most common methods for achieving safe foods with an extended shelf life but, in general, it also leads to some loss in nutritional and sensory quality of foods (Ball and Olson, 1957). By far, steam with the highest surface heat transfer coefficient has been employed the most for the processing of foods either in rigid containers or in pouches

(Ramaswamy, 1983). However, in the presence of air along the container surface heat transfer rates are degraded as air interferes with the condensation mechanism and as a result diminishes the effective heating of the food surface. In addition, a short steam exposure for decontamination of fruits and vegetables has been used without change in the physico-chemical attributes of food quality (Basumatary et al., 2020).

The air which may be present naturally or intentionally added, can result in undesirable consequences with respect to heat transfer, container integrity and storage stability/quality of the product. For instance, the air can occur naturally in the treatment chamber as well as inside or along the food surface which delays heat transfer during short time steam treatment and in reducing the microbial load to a safe level as air interferes with the steam condensation mechanism (Pokhrel et al., 2017). The surface air acts as an insulator and decreases heat transfer enhancement along the surface of food resulting in higher processing time which destroys the nutritional and sensory properties of food. Using pure steam only during the sterilization of flexible pouches, deep concerns exist both with respect to deterioration of heat transfer and package integrity (Tung et al., 1984). The mixture of steam and air or water and air is used to maintain the retort pressure more than the pressure within the pouch during the processing. During heating, the introduced air pressure prevents the inflation of pouches due to the expansion of gases that can result in decreased heat transfer rates in food. Similarly, air prevents the bursting of pouches during cooling by lowering the internal vapor pressure to exceed the retort pressure (Tung et al., 1984). Though air can be removed from the food products using a vacuum system or during blanching, the eradication of air in the treatment system during food processing or compensation using a turbofan during short time heating as in conventional processing remains unfeasible for short time direct steam heating of food products (Basumatary et al., 2020).

Adding an ultrasound environment in combination with steam can help to remove this surface resistance barrier contributed by air and promote a better heat transfer. The usage of ultrasound is interesting to the food industry as it minimizes processing time, removes surface air barriers, and minimizes the deterioration of foods with higher energy savings (Ercan and Soysal, 2013a). The ultrasound can be applied to food products in various ways such as applying directly to the product, coupling with the product, or submerging in an ultrasonic water bath (Ercan and

Soysal, 2013b). Generally, sound waves with frequencies higher than 16 kHz are regarded as ultrasound and have shown promising results in microbial inactivation in fruits and vegetables (Pokhrel et al., 2017). Ultrasound is one of the widely used technologies for many commercial applications. The introduction of ultrasound waves, mainly in between the range of 20 kHz and 100 MHz, works by generating acoustic cavitation, growing, and subsequent collapse of bubbles near the food surface (Yusaf and Al-Juboori, 2014). This finally results in the removal of the surface resistance barrier contributed by air on the surface of the package or food product to produce enhanced heat transfer (Yusaf and Al-Juboori, 2014). The basic mechanism postulated for microbial inactivation is the acoustic cavitation. Cavitation is the formation of low-pressure voids in the liquid which grow, briefly oscillate, and then asymmetrically implode with great intensity. This generates extremely high local temperature zones, heating/cooling rates and pressure waves, giving rise to many chemical reactions (sono-chemical). Many studies show the successful application of ultrasound for enhancement of food processing efficacy and preservation methods with minimal or no damage to the product quality (Ercan and Soysal, 2013; Tao and Sun, 2013; Kentish and Feng, 2014; Turantaş et al., 2015; Musielak et al., 2016; Gallo et al., 2018; Rodríguez et al., 2018).

Ultrasound-steam and ultrasound-water based technologies can also be used to induce agitation within flexible thin profile packages which enhances the mixing and heat transfer within the package. Thermal processing of foods in flexible packages has been well researched. For agitation, mostly external mechanisms involving container movement like end-over-end, axial and reciprocal agitation have been researched. However, there are no studies on the induced agitation in flexible packages under the influence of sound in steam and water.

The surface decontamination of food surface can be achieved via the combination of ultrasound and short-time steam treatment where steam reduces the microbial load in food and ultrasound enhances the heat transfer by the removal of surface air present in cavities to reduce the treatment time (Basumatary et al., 2020). However, in order to optimize the application of ultrasound for thermal processing, it is critical to understand the heat transfer characteristics i.e. heating rate index (f_h) or surface heat transfer coefficient (h) of the treatment media (Tung et al., 1984). The determination of surface heat transfer coefficient using real food products or low thermal conductivity materials such as bentonite suspensions which closely mimic the real food products in pouches could be underestimated because of higher internal resistance as compared to the surface resistance (Ramaswamy et al., 1983). Therefore, high thermal conductivity materials are applicable for the evaluation of heat transfer characteristics. The experimental determination of the heating rate index, f_h can be correlated to the surface heat transfer coefficient as done in the study by (Ramaswamy et al., 1983). The objective of this study was to evaluate the influence of different treatment media such as steam-sound, steam-sound/air, steam/air, steam only, water only, and water-sound mixture on thermal process parameter, f_h using highly thermal conductive material (aluminum) and to study the effect of size and shape of highly thermally conductive materials on the heat transfer value, f_h under different treatment conditions.

6.2. Materials and methods

6.2.1. Test materials

Aluminum with centrally located copper/constantan thermocouple (diameter 0.762 mm, Omega Engineering Co., Stamford, CT) Teflon insulated were used for the study. The thermo-physical properties of the test material used, aluminum as used in earlier studies, were obtained from Tung et al. (1984): specific heat = 938 J/kgC, thermal conductivity = 239 W/mC, density = 2700 kg/m^3, and thermal diffusivity = 944 x 10^{-7} m^2/s.

The cylinders of two diameters, 1.9 and 2.6 cm respectively of various heights were taken. And two plates of various lengths with the same thickness (0.7 cm) were taken. Overall dimensions (cm) were as follows: 1.9 X 1.9, 1.9 X 3.9, 1.9 X 5.8, 2.6 X 2.6, 2.6 X 5.2, 2.6 X 7.7, 0.7 X 10.4, and 0.7 X 20.1 cm (Figure 6.1) Different liquid model systems including -*honey*, *glycerin*, *sugar syrup,* and *water* were filled into flexible retort pouches with thermocouples inserted into Nylon Sphere at the centre locations (Figure 6.2) for heat transfer enhancement studies. For preliminary heat transfer enhancement studies with food materials, mango slices were also considered instead of nylon sphere with honey inside the flexible pouches.

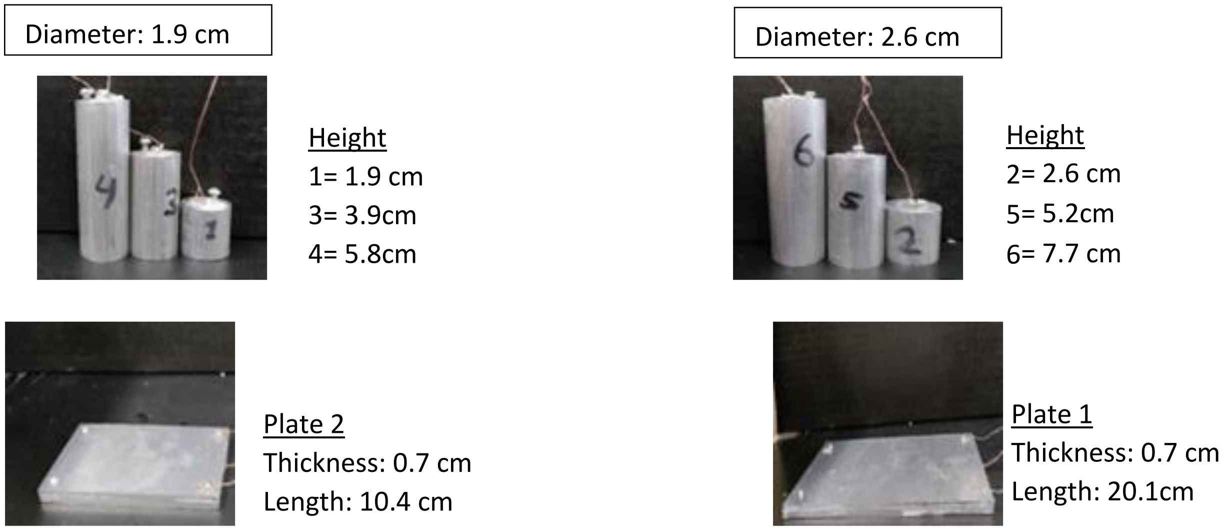


Figure 6.1- Overall dimension of cylinder and plate

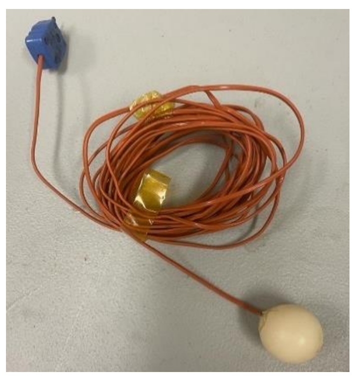


Figure 6.2. Nylon sphere with thermocouple

6.2.2. Experimental setup for heat transfer studies

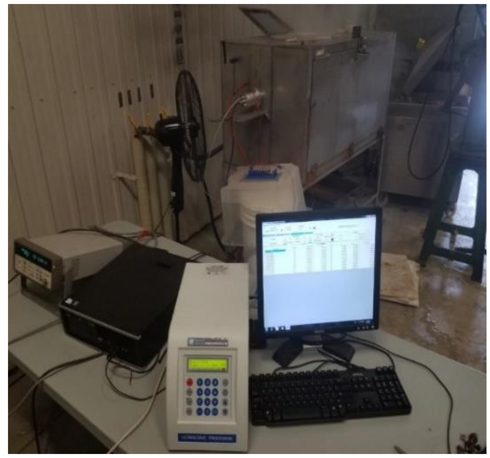
The equipment set used for this study is the same as what has been described in chapter 3. Some details are given below for providing continuity.

Steam chamber: The experimental set up for ultrasound-steam treatment consisted of a steam chamber with a manually operated steam inlet at atmospheric pressure at ~100^{o}C from bottom with a vent at the top for continuous flow of steam through a 15-psig heat pressure at a nominal low flow rate. After 10 min of purging saturated steam heating conditions with temperature close to 100°C could be established in the chamber.

Ultrasound system: The ultrasound equipment (LSP 500, Industrial Sonomechanics, LLC, Miami, Florida) consisted of a 500W ultrasonic generator (500 W LSP-500 generator) which transformed the 50Hz AC line power to a 20 kHz signal that drove the piezoelectric transducer (ACT-19-LSP). The amplitude of oscillation (power) could be varied between 20 to 100 %. The transducer was coupled to a horn (CH-type horn with 12.7 mm tip**)** which amplified and transmitted the vibration down its length to the steam in the treatment chamber. The vibrating surface of the horn promotes acoustic cavitation in the steam/liquid medium. This phenomenon can typically be seen as a cloud of bubbles forming in the vicinity of the tip and heard as an intense hissing noise. The system was operated using the software driven instrument controller and temperature data were gathered using an Agilent data acquisition system. The set-up is illustrated as shown in Figure 6.3.

Ultrasound water bath treatment: A digital ultrasound cleaner (Model – TH-SPQXJ-40A, made in China) of 10-liter capacity with ultrasonic frequency of 28/40 Hz, ultrasonic power of 240W and heating power of 500W was used for ultrasonic water bath processing. The chamber used to hold the sample had dimensions of 53cm x 32cm x 22cm. A heating coil was used along with the heating mechanism of the cleaner to reach the required processing temperatures and maintain them. The ultrasonic waves were employed in full wave mode when required.

Conventional hot water processing: During the HW processing the settings of the ultrasound water bath and temperature were the same only the ultrasound was turned off.



a. Steam Chamber with a sample **b. Ultrasound generator/controller**

c. Ultrasound Transducer **d. Ultrasound Horn**

Figure 6.3. Experimental set-up for heat transfer studies

6.2.3. Methodology for heat transfer studies

6.2.3.1. Aluminum materials

Before carrying out each ultrasound and/or steam treatment run, the environment temperature in the steam chamber was checked using a Teflon-insulated copper/ constantan thermocouple attached to the Agilent data logger. Next, the thermally conductive aluminum transducers with thermocouples at their geometric center were attached using thin wire and exposed to the steam chamber in front of the short distance of the horn. The temperature at the center of the test material was recorded after the instantaneous drop at a one-second interval using the Agilent data acquisition system (HP34970A, Hewlett Packard, Loveland, CO). After each treatment, the test material was cooled down using ice water.

Similarly, heat transfer study in water was studied by placing thermocouples in water bath, and thermally conductive materials were exposed to water only and water + ultrasound treatment media.

The experiments were carried out to characterize the effect of treatment conditions such as ultrasound-steam, ultrasound-steam/air, water-sound, steam /air, steam-only, and water-only. The steam/air mixture (75%/25%) was used to evaluate the effect of air on surface heat transfer. The experiments were designed to study the relationship between the shape & size of materials with heat transfer.

6.2.3.2. Nylon sphere & mango slices in flexible pouches

Different liquid model systems including -*honey*, *glycerin*, *sugar syrup*, and *water* filled into flexible retort pouches with thermocouples inserted into Nylon sphere at the centre locations were used (Figure 6.2). The heat penetration curves, collected using Agilent data acquisition system (HP34970A, Hewlett Packard, Loveland, CO) with and without ultrasound (amplitude = 100%) were compared to see if there was any influence of ultrasound in terms of enhancing heat transfer.

Test runs were carried out with mango slices filled into flexible pouches with thermocouples inserted into pieces positioned at the center locations. Medium temperatures were simultaneously recorded. The heat penetration curves with and without ultrasound were compared to see if there was any influence of ultrasound in terms of enhancing heat transfer. Mango pieces (15 x 8 x 8 mm) were packaged in retort pouches (10 x 15 cm) 350 g each with thin wire thermocouples inserted to their geometric centre, and time temperature data were gathered using a data logger. The tip of thin thermocouple wire (diameter 0.762 mm, Omega Engineering Co., Stamford, CT) was positioned at centre of the particle; for liquid temperature measurement, the tip of rigid thermocouple wire (24-gauge Teflon coated copper-constant) was in the center of the pouch. A third thermocouple was also used for gathering steam temperature. The thermocouple outputs were recorded using an Agillent data acquisition system (HP34970A, Hewlett Packard, Loveland, CO) at 5-s intervals. These were used to determine the associated f_h

values as the negative reciprocal slope of the semi-logarithmic curve of log Tr-T vs t where Tr is the steam temperature, T is the temperature of the honey or mango cube at time t.

6.2.3.3. Calculation of heat penetration parameters

A heating curve was plotted between the log of the difference of the heating medium and sample temperature (Tr-T) against time where Tr represents the heating medium temperature, T is the product temperature. The negative reciprocal of the slope of the straight-line portion gives the heating rate index (f_h). Mathematically,

$$f_h = -\frac{1}{slope} \tag{6.1}$$

$$Slope = \frac{Log(Tref - Ti) - \log(Tref - Tf)}{ti - tf} \tag{6.2}$$

where,

f_h= heating rate index (min)

Tref= reference temperature

Ti = initial temperature

Tf = final temperature

ti= time at (Ti); tf = time at (Tf)

These equations (6.1 and 6.2) were used to determine the associated f_h values and back calculation of surface heat transfer coefficients.

6.3. Results & discussion

6.3.1. Heat transfer studies in cylinders

Figure 6.4 shows that hot water and steam with included air showed the highest heating rate index, f_h as compared to other treatment media for three different heights of cylinders i.e., 1.9 cm, 3.9 cm, and 5.8 cm. These are finite cylinders represented by the intersection of an infinite cylinder and an infinite plate. The heat transfer is associated with a combination of infinite plate and an infinite cylinder. Higher the value of f_h, slower is the rate of heating. The air introduced acted as a barrier to the heat transfer on the surface of test materials, therefore the heating rate index was found to be higher when air was associated. The amount of air introduced was 25% which mimics the maximum industrial situation for the presence of air during food processing. For instance, the steam/air mixture represents 75% steam and 25% air composition. It was found that with an increase in the height of the cylinder by 2 cm for the same diameter of 1.9 cm, the heating rate index was found to be increased in a similar fashion by 2 s. Heating rate index is also proportional to the size and dimension of the material. The bigger the size the slower is its heating rate and hence higher is the heating rate index.

Similarly, in Figure 6.5, steam + sound and hot water + sound mixture had similar heating rate indexes i.e., 14, 20, 22 seconds for heights of 2.6 cm, 5.2 cm, and 7.7 cm of cylinders, respectively. The decreased heating index rate index was due to the creation of turbulence created by the ultrasound environment in combination with steam near the test material surface to remove the surface resistance air barrier. Specifically, the vibrating surface of the horn promotes acoustic cavitation in the steam/liquid medium which can be seen as a cloud of bubbles forming in the vicinity of the tip and heard as an intense hissing noise. With the increase in height of the cylinder, similar results were observed as seen in Figure 6.5.

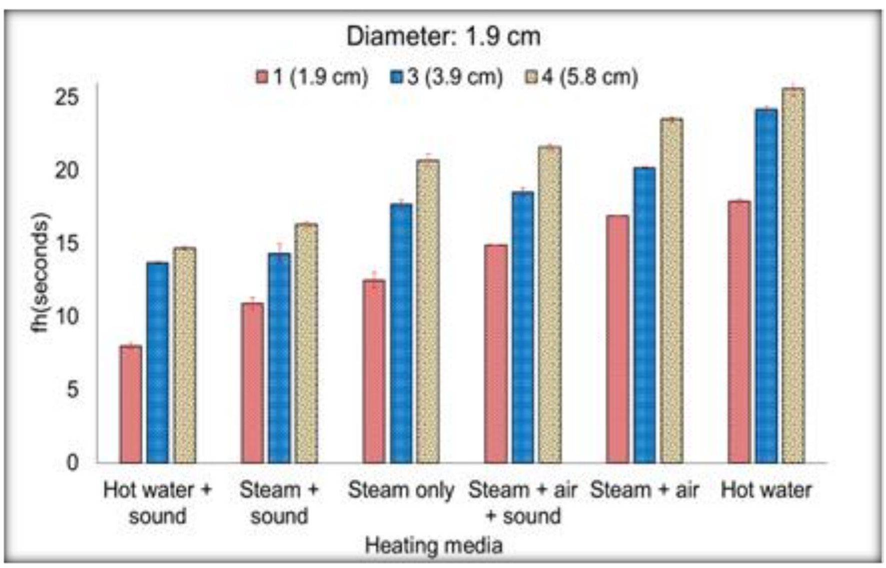


Figure 6.4. f_h value with and without sonication in steam and water for cylinders (diameter = 1.9 cm).

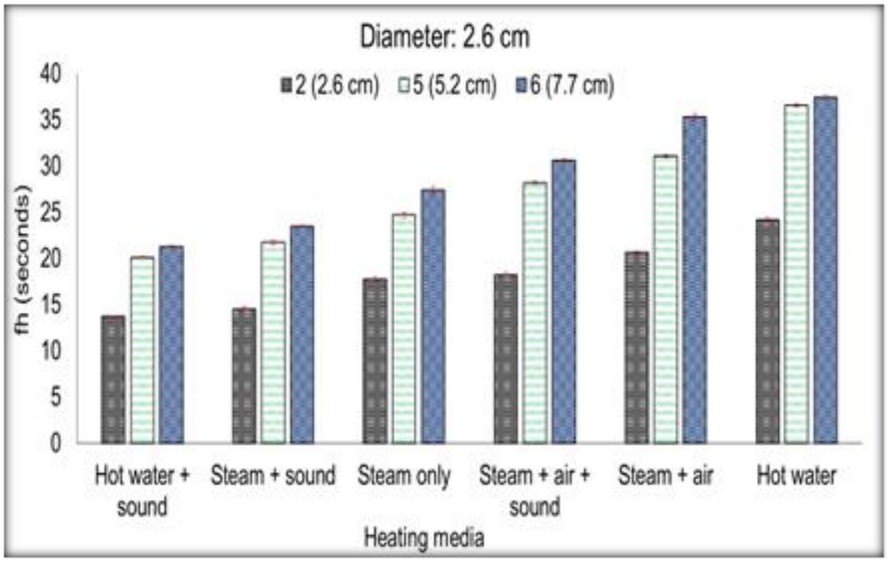


Figure 6.5. f_h value with and without sonication in steam and water for cylinders (diameter = 2.6 cm)

6.3.2. Heat transfer studies in plates

The plate represents a finite geometry intersected in x, y and z directions by three infinite plates. The heat transfer situation is a solution to heat transfer associated with three infinite plates (Figure 6.6). The length and width are significantly higher as compared with the thickness. Hence the heat transfer is primarily related to the thickness but moderated by the mass changes associated with the combined dimensions. As the plate length was increased, the time taken to heat when any of the heating media used was longer due to an increase in the mass dimension. However, sound combination with steam or water still resulted in a lower heating index as observed in the figures for cylinders. Therefore, the smaller the size of the test material or food product, the faster will it be heated, and when sound is used in combination with the heating media, the heating time will be decreased, thereby retaining the integrity and physico-chemical properties of original food products.

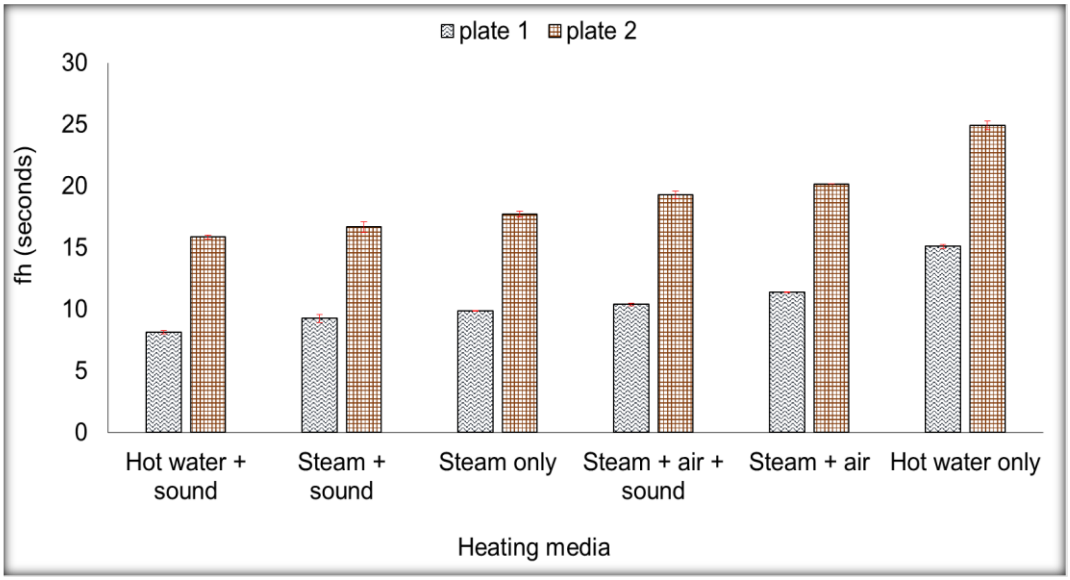


Figure 6.6. fh value with and without sonication in steam and water for plates of thickness 0.7 cm (Plate 1: length= 10.4 cm, width=5.25 cm, and thickness= 0.7 cm; plate 2: length=20.1 cm, width = 10.2 cm, and thickness=0.7 cm)

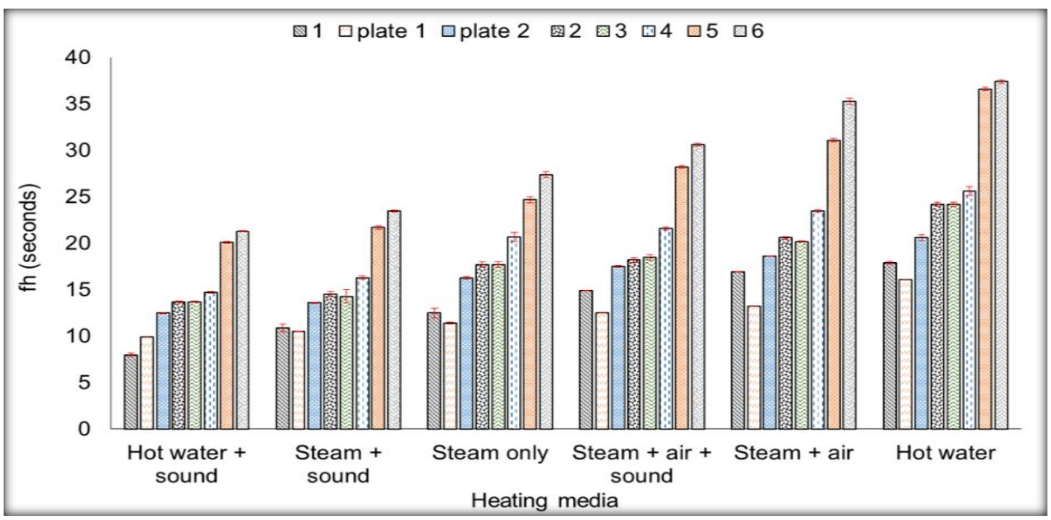


Figure 6.7. Overall comparison of different sized- aluminum plates f_h value influenced by different heating media

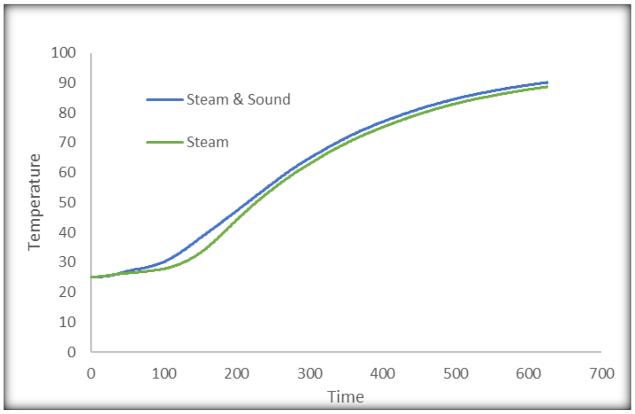
(Plates (L=Length, and W= Width):1: L= 1.9 cm, & W= 1.9 cm;2: L= 2.6 cm, & W= 2.6 cm;3: L= 1.9 cm, & W= 3.9 cm;4: L= 1.9 cm, & W= 5.8 cm;5: L= 2.6 cm, & W= 5.2 cm;6: L= 2.6 cm, & W= 7.7 cm; Plate 1: length= 10.4 cm, width=5.25 cm, and thickness= 0.7 cm; Plate 2: Length=20.1 cm, width = 10.2 cm, and thickness=0.7 cm)

Overall, f_h value for the ultrasound-steam combination was found to be lower with all size cylinders and plates as compared to other heating conditions (steam only, steam + air & and hot water only) as illustrated in figure 6.7. The ultrasound-water combination showed slightly better or similar effectiveness than the ultrasound-steam combination presumably because ultrasound waves create better turbulence and oscillated faster in water than steam media. However, steam offers better advantages as a heating medium and results in less affluent production (Ramaswamy et. al 1983), therefore the usage of steam is preferred over water. An ultrasound when combined with steam or water accelerates convective heat transfer by creating cavitation as well as turbulence which disrupts the air layer from the surface, known as the laminar sub-layer.

The usage of high thermal conductivity materials for the determination of surface heat transfer coefficient was shown in the studies done by (Tung et al., 1984). However, the problems associated with the determination of surface heat transfer coefficient using Aluminum included the accuracy with which f_h value can be estimated from the time-temperature data. As per the study, at least 7-10 data pairs in the linear region were necessary for the determination of the heating rate index value accurately. The center temperature of highly thermally conductive material increases rapidly with exposure to the heating media; therefore, the experiment must be carried out meticulously to accurately estimate the f_h. When the experiment was carried out the cylinders and plates with increasing size for the same diameter/thickness, the heating rate index increased such that a reliable estimate of f_h could be done. In addition, the linear region obtained from the plot of the logarithmic temperature difference between the treatment chamber and test material versus time had an R-squared (R^2) value of 0.99.

6.3.3. Heat transfer studies in flexible pouches with Nylon sphere and mango slices

The time–temperature data obtained from different liquid media is shown in Figures 6.8-6.11. The temperature at the center of the Nylon sphere was increased in the presence of ultrasound treatment. The ultrasound-steam combination improved the heat transfer in all media. However, the degree of the heat transfer improvement was dependent on the type of liquid used primarily due to the differences in the thermo-physical characteristics of different liquids. The highest effect was seen in glycerine while honey showed a smaller change in the heat transfer rate while using ultrasound. A temperature difference was observed in all samples and liquid medium used as filler when using the ultrasound assisted steam and the lag phase in the T-t data of all samples was moderated. Thus, in the presence of air especially at the stages of the heating, ultrasound assisted steam showed better heat transfer rates than steam alone.



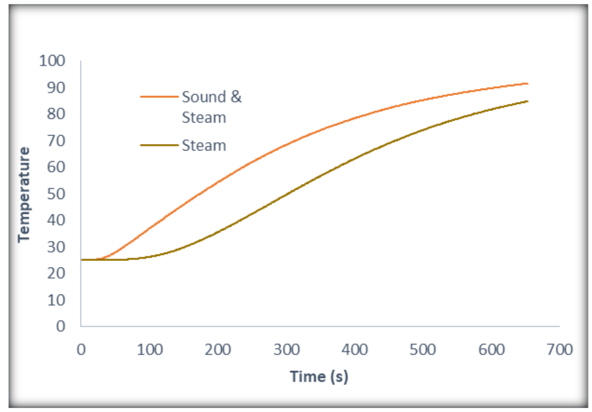
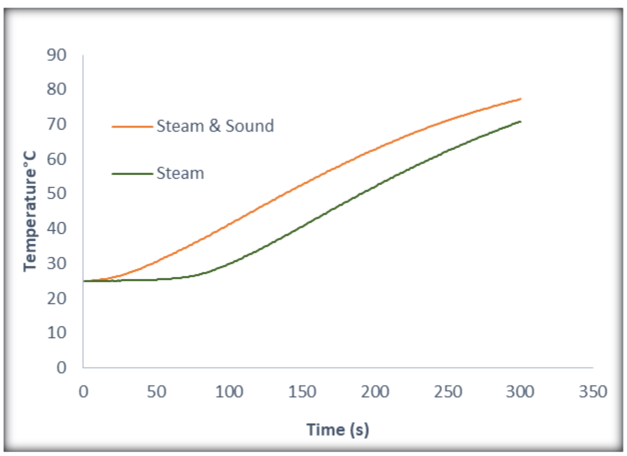


Figure 6.8 Nylon sphere in honey

Figure 6.9. Nylon sphere in glycerin



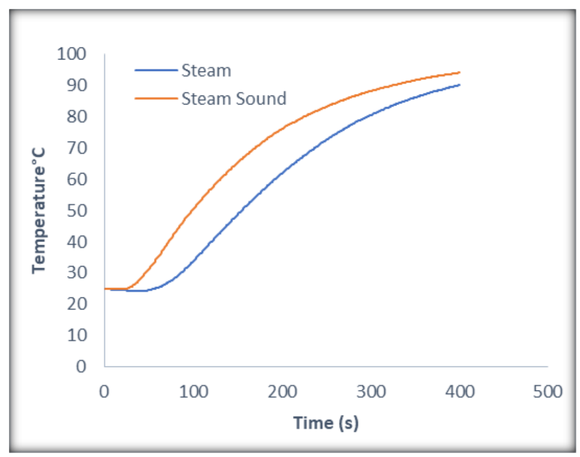


Figure 6.10. Nylon sphere in sugar solution

Figure 6.11. Nylon sphere in water

Figure 6.12 shows the typical heat penetration curves for mango pieces packaged in a retort pouch and heated under various conditions of ultrasound-steam treatment in steam only situation (without the ultrasound). Five curves are shown, the top one being that of steam chamber which reached 100°C in about 5 min of heating. There was no difference in temperature of steam with and without ultrasound. Next to follow was the honey in pouch with ultrasound-steam combination heating showing a considerable lag from the steam chamber heating, converging to 100°C after 10-12 min. Next to this was the honey in steam only heating which lagged the one with ultrasound combination especially in the middle regions. Convergence to 100°C almost followed the one without ultrasound taking 10-12 min. Fruit pieces showed further lag and the ones without ultrasound demonstrated the least effective heating showing a large deviation from the one with ultrasound combination heating. For reaching pasteurization temperatures of around 80°C, the time taken were about 70, 170, 220, 250, 320 s for steam chamber, honey ultrasound-steam, honey steam, mango piece ultrasound-steam and finally mango piece steam only. The trend clearly demonstrates the heat transfer enhancement with the ultrasound-steam combination treatments. However, to reach the processing temperature of 100°C, up to 700 s was required.

Figure 6.13. shows similar results under the ultrasound combination heating conditions providing some additional details in terms of accumulated lethality at 100°C expressed as equivalent heating in seconds at 100°C integrated over the heating period using a s value of 10°C as $\sum 10^{((T-100)/10)}$ dt. The heating time required to achieve a lethality of 4s was 420 s with ultrasound-steam combination heating of mango pieces as compared to 550 s for the same in steam only heating.

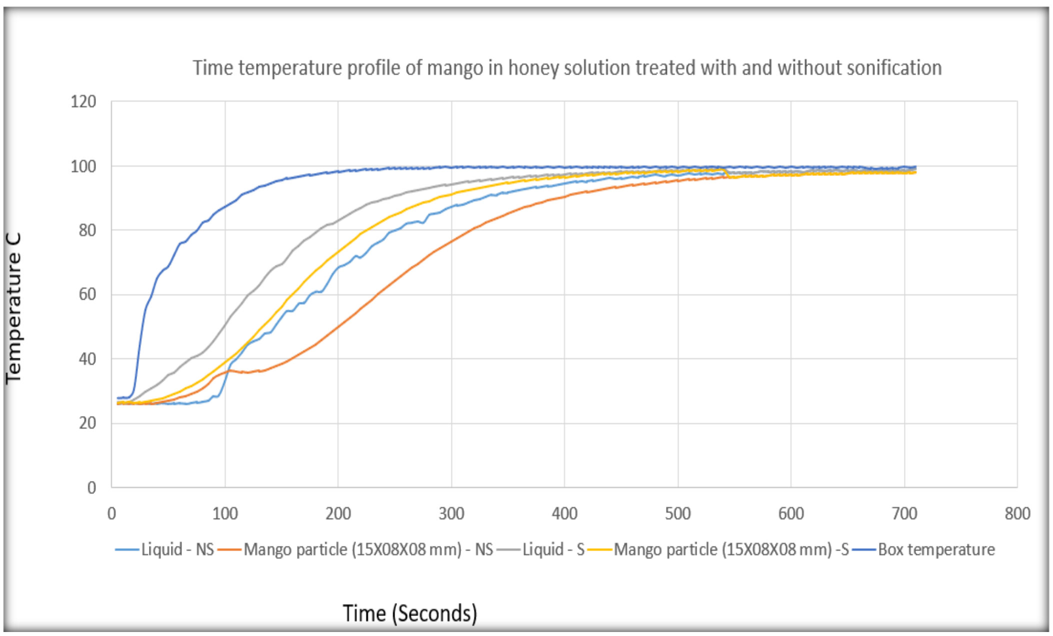


Figure 6.12. Ultrasonic improvement in steam heating of mango pieces in honey

Another way to look at the heat penetration curve from a heat transfer point of view is to compute the associated heating rate index defined as the negative reciprocal slope of the heat penetration curve: log (Tr-T) vs t. The data presented in Figure 6.13 were used to compute the associated f_h values, the associated average values of f_h are tabulated in Table 6.1. As shown, the f_h associated with steam only heating was about 30% longer than with the combination ultrasound-steam heating. Longer values of f_h indicate slower rate of heating. These values can be converted to heat transfer coefficient values if the thermo-physical properties of mango pieces and honey were available. Future studies will focus on gathering data on the associated heat transfer coefficients as influenced by process and product variables.

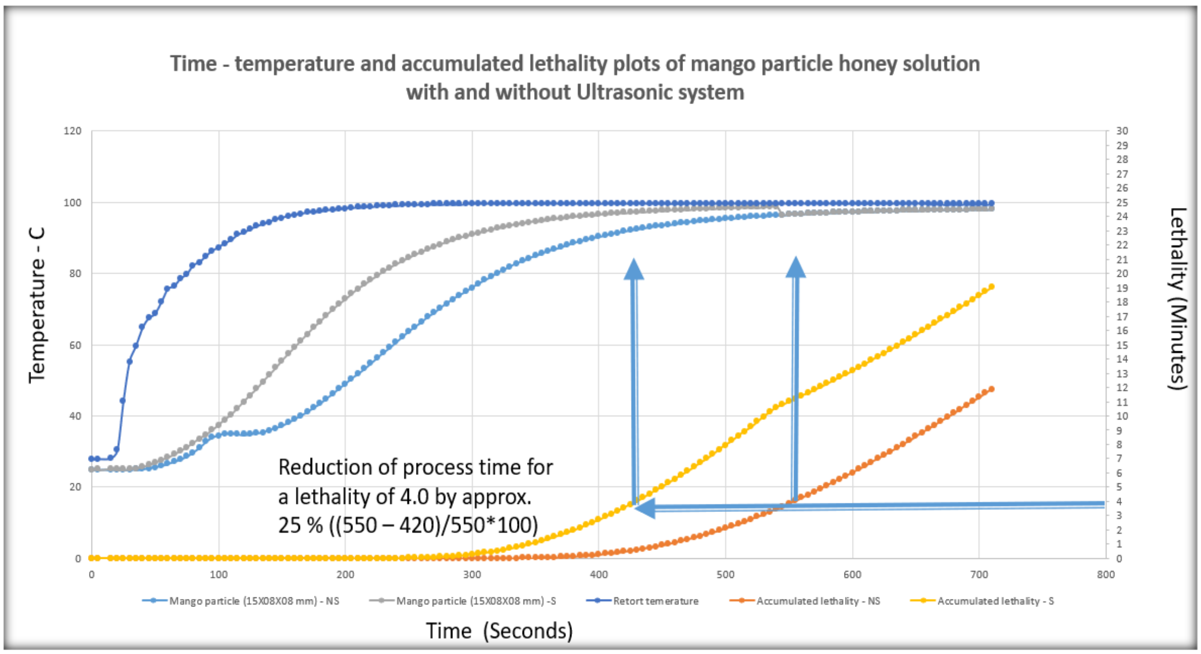


Figure 6.13. Time - temperature and accumulated lethality plots of mango particles in honey under steam heating with and without ultrasound

Table 6.1. f_h value of mango and syrup with and without sonication:

Medium	f_h value with ultrasound	f_h value without ultrasound
Mango particles	3.6 min	4.7 min (31% higher)
Syrup	min	4.5 min (33% higher)

6.4. Conclusions

The study shows some preliminary results for use of ultrasound-steam technology for effective heat transfer enhancement. The ultrasound-assisted steam/water heating enhances heat transfer by disrupting the surface air layer so that it shows a promising application in reducing the processing time to achieve safe food as well as to retain the quality of food. This combination treatment i.e., thermal, and non-thermal processing has been demonstrated to offer superior results as compared to thermal processing only in surface decontamination as well as in enzyme inactivation studies. Therefore, the outcome could contribute to a better understanding of the concept of ultrasound-assisted steam and steam air application to food processing.

This is the first study on the effect of ultrasound-steam heating on the heat transfer characteristics of mango slices packed in honey in flexible packages providing another way to agitate the contents (in addition to the existing commercial methods of end-over-end and axial agitation processing).

Different liquid food models with different viscosity were used for this study. Heat transfer coefficients was evaluated as a function of steam variables (flow rate, entrapped air) and sound variables (time, amplitude). To create steam air environments, air and steam was introduced at different flow rates through a flow regulator as used in steam/air retorts (Ramaswamy et al., 1983; Tung et al., 1984). For initial studies, nylon sphere was introduced in the packaged liquid food models of different viscosity which was subjected to different heating conditions to evaluate the heat transfer coefficients at different heating conditions. These studies will be extended to different packaged food products.

The enhancement of heat transfer into packaged mango slices as part of the preliminary studies of heat transfer studies was found to be effective. This research will be expanded to thermal processing of foods which can be accomplished in steam heating conditions at atmospheric pressures. Current setup cannot be used for sterilization of low acid foods, which requires higher steam pressures. This concept, however, should be applicable for pasteurization of acid and acidified foods.

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Connecting Statement to Chapter 7

This chapter is dedicated to the development of innovative process for pasteurizing acid and acidified foods in flexible pouches using ultrasound-steam and ultrasound-water techniques. In the following pages, the exciting realm of food preservation is detailed, exploring novel approaches that hold immense potential for enhancing food safety. In chapter 6 it was shown how addition of ultrasound to steam chamber or hot water can enhance the heat transfer in the medium by reducing the treatment time. Taking this concept further, an ultrasound assisted pasteurization process of acid /acidified foods in flexible pouches was developed. The agitation induced by ultrasound in the pouches enhances the heat transfer from the medium to the contents inside the pouch thereby reducing the treatment time, which results in better quality retention of the products.

The primary objective was to develop alternative pasteurization methods that address the unique requirements and characteristics of acid and acidified foods packaged in flexible pouches. In doing so, we sought to minimize the risk of spoilage, optimize the preservation process, and ultimately safeguard consumer health.

This chapter provides detailed experimental results, allowing for a deeper comprehension of the practical implementation and outcomes of this innovative technique. By embracing ultrasound -steam and ultrasound-water pasteurization process we aim to provide a viable and effective solution that not only ensures food safety but also promotes sustainability and resource efficiency.

This is the first time that this approach (ultrasound steam and hot water bath heating) has been used for agitation thermal processing of acid and acidified foods in thermal pasteurization applications. With enthusiasm and anticipation, we present this chapter, eager to share our findings and ignite the imagination of researchers, practitioners, and enthusiasts alike. Together, it is hoped that this will pave the way toward safer and more sustainable food processing methods, ensuring the availability of high-quality, nourishing products for generations to come.

Part of this study has been submitted for publication:

Basumatary R, and Ramaswamy H.S., 2023. Ultrasound enhanced steam and hot water pasteurization of pineapple and apple slices in flexible pouches. Paper submitted to Journal of Food Measurement and Characterization. (Paper under review)

Part of these results were presented as poster in conferences:

Basumatary R, and Ramaswamy HS. 2023. “Combined ultrasound-steam pasteurisation of acidified carrots.” Poster presentation in CSBE/SCGAB AGM and Technical Meeting July 23rd -26th July 2023 Lethbridge Alberta, Canada.

Basumatary R, and Ramaswamy HS. 2023. “Developing ultrasound-steam and ultrasound - water processes for pasteurizing acid and acidified foods in flexible pouches.” Poster Presentation in IFT FIRST 2023 July 16th -July 19th, 2023, Chicago, Illinois at McCormick Place Convention Center.

Contribution of authors

Basumatary is the PhD candidate and carried out all the experimental work under the direction of Prof. Ramaswamy.

Chapter 7

Developing ultrasound- steam and ultrasound - hot water processes for pasteurization of acid and acidified foods in flexible pouches

Abstract

This research investigated the ultrasound (U) enhancement of steam (S, SU), steam/air (SA, SAU) and hot water (W, WU) processes for the pasteurization of high acid foods (pineapples and apples) and acidified foods (carrots) in flexible pouches. A specially designed ultrasound setup was developed to process acidified carrots in brine solution, cut pineapples and apples in sugar solution that were pre-packaged in flexible retort pouches with steam and ultrasound combination treatments. An ultrasonic hot water bath was used to process these pre-packaged products under WU. All pasteurization techniques were designed to deliver a target lethality (F90°C-10min) through heat penetration testing and were compared for reduction in process time and improvement in product quality. Compared S, SA and W, the ultrasound assisted processes, SU, SAU, and WU, resulted in significantly reduced processing times presumably due to the induced ultrasound agitation and helped to improve the product quality. The resulting impact of these processes on quality of carrots, apple and pineapple slices depended on the specific process and quality indices, but the quality parameters were considerably better for the ultrasound assisted processes. Hence, ultrasound pasteurization was recommended as another viable technique for agitation processing of foods in flexible containers.

7.1. Introduction

The recent emphasis in the consumer service food sector has been to deliver better-quality products without compromising safety. The necessity for pre-packed meals and the rising public awareness of a healthy diet have made the minimal processing concepts a potential alternative to conventional processing as an efficient way to supply high-quality, safe, extended shelf-life products with or without refrigeration. The traditional thermal processing (TP) technique established scientifically over a 100 years ago has undergone significant improvements through optimization procedures like high temperature short time process, agitation processing, thin profile processing, aseptic processing etc as well as through the use of non-conventional heating media like microwave, radiofrequency and ohmic heating techniques (Ramaswamy and Marcotte, 2005). A typical way of achieving minimal processing has been to pasteurize foods, instead of achieving commercial sterility, and combine it with refrigeration to have high quality and a short extension of high-quality shelf-life because the high temperature thermal processing could have an adverse effect on chemical composition and organoleptic properties (Qu et al., 2021).

Due to the prolonged thermal processing durations needed to inactivate spores of the key pathogen *Clostridium botulinum*, "low acid" foods (pH>4.6) processed conventionally suffer significantly reduced quality. By converting low-acid foods to "acid" foods, product acidification changes the process regulation from high-temperature sterilisation to low-temperature pasteurisation conditions. Foods with high acidity (pH < 4.6) can be processed under moderate processing conditions (< 100°C) with greater efficiency and lower energy consumption technology (Tola and Ramaswamy, 2015). While lowering pH can reduce energy and processing time, not all foods can benefit from this approach as the addition of acid can result in unacceptable sensory attributes (Tola and Ramaswamy 2018). These processing conditions are only aimed at inactivating the oxidative enzymes and vegetative bacteria which have low thermal resistance. Microbial spores are unable to grow under these pH conditions and the pasteurization process will yield shelf stability as well (Ramaswamy and Marcotte, 2005). However, these products are very delicate, and their sensory characteristics can be adversely influenced by even these minimal processing conditions. With growing consumer demand for higher quality products, efforts are constantly being made to improve such treatment methods with newer concepts and technologies. Many studies have compared traditional methods (hot water or steam pasteurization) of pasteurization of acidic foods with newer methods such as microwave, radio frequency (RF), ohmic heating, pressure-assisted thermal processing, and found to be more effective in maintaining quality in a shorter process time (Koskiniemi et al., 2011; Tola & Ramaswamy, 2014; Tola & Ramaswamy, 2018; Qu et al., 2021; Bao et al., 2022).

Ultrasound has been recently used as a process aid in many food processing applications (Villamiel et al, 2017). Abid et al. (2014), found that combination of ultrasound (US) with high hydrostatic pressure (HP) represented a possible barrier to the production of safe, high-quality apple juice with highest enzyme and microbial inactivation and enhanced nutritional value. The literature (Abid et al., 2014;Tola and Ramaswamy 2018;Yildiz et al.,2023) has shown that only MW, RF, ohmic heating, pressure assisted thermal processing and ultrasound combined with HP have been investigated as alternatives to conventional heat treatment of acidic foods and no literature is available on the use of ultrasound steam and ultrasound water treatment combinations for pasteurizing acidic foods. Heat treatment of food in flexible packaging has also been well studied (Dileep et al., 2007; Wu et al., 2012; Ravishankar et al., 2013; Maldonado et al., 2015; MacNaughton et al., 2018; Puthanangadi et al., 2021). In the case of agitation, mainly the external mechanisms related to the movement of the container have been studied, such as: end-over-end, axial and reciprocal agitation (Singh et al., 2017). Ultrasound pasteurization of fruit products or acidified products in flexible containers has never been studied.

Most ultrasound studies have been carried out with liquid media for a variety of applications including heat transfer enhancement. However, work in combination with steam is rather limited. Some studies have shown ultrasound steam processes can be successfully employed for surface inactivation of microorganisms. Musavian et al. (2022), found that steam-ultrasound decontamination treatment on naturally contaminated broilers significantly reduced the *Campylobacter, Enterobacteriaceae*, and total viable count (TVC) on three different broiler surface areas at a slaughter speed of 10,500 birds per h at temperatures more than 80ºC. Another study by Moazzami et al. (2021) showed use of combined ultrasound (30 to 40 kHz) and steam (84 to 88ºC) reduced *Campylobacter jejuni, Enterobacteriaceae, E. coli* and total aerobic bacteria significantly from the surface of broiler carcasses at slaughter with line speed of 18000 birds per hr. But no major work using combined ultrasound and steam on heat transfer or for food processing has been found. So far there has been no studies on enhancing heat transfer of foods in flexible packaging under the influence of ultrasound in steam or water. The agitation created by ultrasonic water and ultrasonic steam in a flexible, thin-profile package to promotes overall heat transfer to the contents was concept tested recently (Basumatary et al., 2020). Extension of this concept to pasteurize high acid foods is the basis for this work.

Apples and pineapples are nutritious fruits loaded with many vitamins and minerals and in addition many disease fighting phytochemicals, enzymes and antioxidants are found. An interesting comparison made between how both are good and one better than the other & vice versa is published in general literature source [https://foodstruct.com/compare/apples-vs-pineapple]. These fruits and fruit products consumed fruits both in fresh and different processed forms like canned or bottled, frozen, dehydrated etc. They both belong to the acid group (pH <4.6) classification of food based on pH (Ramaswamy and Marcotte, 2005) and can be processed under temperature below 100°C. Both are popular fruit and canning is a commonly used thermal processing technology. Quality enhancement in these thermally processed fruits is highly desirable as with any conventionally canned product. Carrots are also one of the most consumed vegetables in the United States, one-fourth of which are consumed in processed form, largely canned or frozen (Jing et al., 2017). Carrots are low acid foods and to be processed at lower temperature for microbial safety it needs to be acidified.

Therefore, the main objective of this study was to develop ultrasound-steam and ultrasound-water processes for pasteurizing acidified and high acid foods pre-packaged in flexible pouches with the aim of improving the quality attributes in relation to the conventional methods on an equivalent lethality basis to establish a traditional pasteurization process such as a target lethality of 10 min at 90°C (F90/10=10min). This study should help in providing useful information to develop a ultrasound assisted pasteurization process.

7.2. Materials and methods

7.2.1. Sample preparation

The same batches of Red Delicious Apple (Malus domestica), pineapples (*Ananas comosus)* and carrots (Daucus carota- Farmers market Canada) were purchased from a local supermarket (Maxi & Cie, QC, Canada) one or two days before the experiments were planned and stored at 4ºC. The samples were then pretreated as per their requirements the same day before their processing.

Apples were halved equatorially, and each half-segment was cored and equally cut into 12 pieces using an apple cutter. For the anti-browning treatments, the apple slices were immersed in ascorbic acid solution (40 g ascorbic acid in 1 L deionized water) for 5 min (Rux et al., 2019). Pineapples were washed and sliced in 1.5 cm thickness using a slicer. The core was removed from the slices, and it was cut into wedges of length 2 cm using a sharp knife. Carrots were peeled, washed, and then diced into 6 mm slice thickness. It was further dipped in 0.6% citric acid solution for 24 h for acidification process to bring down the sample's pH to < 4.5 (4.00 ± 0.2).

The pretreated samples were then separately filled into the pouches with some hot processing liquids. A total of 195± 0.5 g carrot slices and 130g ± 0.5g brine (0.2% NaCl in distilled water, w/w, total samples in each pouch); 250 ± 0.5 g of pineapples and 200 ± 0.5 g of sugar syrup (15% w/v) and 250 ± 0.5 g of apples and 200 ± 0.5 g of sugar syrup (15% w/v) were filled into each 8-mil resealable aluminium foil cooking bags (18 X 26 cm SumDirect, US). The salt (NaCl) was added to improve the taste and the ratio of carrots to brine and percentage of NaCl was determined from the commercial canned carrot products (Jing et al., 2017). The ratio of pineapple to sugar syrup and apples to sugar syrup was adjusted based on the pouch's capacity.

The end of a flexible type-T thermocouple was inserted into the apple, pineapple, and carrots center, keeping the piece with thermocouple in the geometric center of the pouch. All pouches were hot filled with brine or sugar solution respectively and were sealed using a pouch sealer. Prepared pouches were loaded to the Ultrasound steam and ultrasound water heating system for processing immediately. The color and texture of the processed samples were measured on the same day of processing. The sample pouches for total carotenoids assay, total phenolic content, ascorbic acid analysis and DPPH were stored at -30 º C until the day of analyses.

7.2.2. Ultrasound-steam treatment setup

The US-steam treatment setup used for the study is explained in detail in chapter 3 under materials and method section. The samples were introduced into the steam chamber from the top. A small opening was cut on top of the steam chamber for introducing the samples to be treated and then partially covered to allow purging of the medium (discussed in detail in chapter 3). The pouches with the samples (carrots, apples, and pineapples) to be treated were then introduced from the top to the ultrasound steam treatment setup.

7.2.3. Ultrasound water bath treatment setup

A digital ultrasound cleaner (Model – TH-SPQXJ-40A, made in China) of 10-liter capacity with ultrasonic frequency of 28/40 Hz, ultrasonic power of 240W and heating power of 500W was used for ultrasonic water bath processing. The chamber used to hold the sample had dimensions of 53cm * 32cm * 22cm. A heating coil was used along with the heating mechanism of the cleaner to reach the required processing temperatures and maintain them. The ultrasonic waves were employed in full wave mode when required.

7.2.4. Conventional hot water processing setup

Conventional HW heating was also conducted on the pouches with carrots, apples, and pineapples with the ultrasound water bath system. During the HW processing the settings of the ultrasound water bath and temperature were the same only the ultrasound was turned off. The holding time of food pouches were adjusted to achieve target process. After processing the samples were taken for color and texture analysis and were stored at the same conditions for the rest of the quality analysis as that of ultrasound steam and ultrasound water processed samples.

7.2.5. Time and temperature data gathering

The time–temperature data was collected using three thermocouples. The tip of thin thermocouple wire (diameter 0.762 mm, Omega Engineering Co., Stamford, CT) was positioned at the center of the particle; for liquid temperature measurement, the tip of rigid thermocouple wire (24-gauge Teflon coated copper-constant) was located at the center of the pouch. A third thermocouple was also used for gathering the ultrasound -steam chamber or ultrasound water bath temperature of the heating medium. The other ends of these thermocouples were connected to a data logger, which was set to read the temperatures at every 5 seconds.

These were used to determine the associated f_h values as the negative reciprocal slope of the semi-logarithmic curve of log Tr − T vs. t; where Tr is the temperature of the heating medium, T is the temperature of the liquid or food pieces at time t.

7.2.6. Thermal processing and processing time determination

Each pouch with different food samples (carrots, pineapple, and apple) were processed under different treatment conditions – steam alone and combined ultrasound -steam in presence and absence of added air, hot water, and ultrasound hot water. Three experimental runs were done for each test. The samples were processed for extended lengths of time (30 min) to provide time-temperature data, from which the processing time could be interpolated to achieve a targeted lethality. Time-temperature data were gathered every 5 seconds and the accumulated lethality was calculated by using the following equation at a reference temperature of 90℃ using a z value of 10℃:

$$F_o = \int_0^t 10^{\frac{T(t)-T_R}{z}}\, dt \tag{7. 1}$$

where *t* is time in minutes, *T(t)* is temperature as a function of time, *z* is the temperature required to reduce the F value by 90%, and T_R is the reference temperature 90˚C for thermal processing of acidic foods.

Equation 1 is traditionally used in thermal process calculations and is related to the destruction of *Clostridium botulinum* in low acid foods. The temperature sensitivity parameter z value of spore forming bacteria is generally taken as 10 °C, with a reference temperature of 121.1 °C. The minimal thermal processing for commercial sterility is generally accepted as a Fo value 3.0 min. For acid foods a similar concept is used for establishing a pasteurization process. Generally, a lower z value (5-8 °C) is often used corresponding to vegetative bacteria; but for process establishment in European standards, a pasteurization value calculated at the reference temperature of 90 °C with a z value of 10 °C to an accumulated value of 10 min is used (adequate for non-proteolitic strains of *C. botulinum*). This was used in this study and heating time was calculated to reach a target $F_{90°C}$ lethality of 10 min. Once the processing time was obtained, the next batch of samples were processed according to this time and cooled to 50 °C using cold water, then refrigerated for further cooling to room temperature. Triplicate runs were done for each treatment.

7.2.7. Calculation of heat penetration parameters

A heating curve was plotted between the log of the difference of the heating medium and sample temperature (T_r-T) against time. Where, T_r represents the constant heating medium temperature, T is the product temperature. These were used to determine the associated f_h values which was computed as the negative reciprocal of the slope of the straight-line portion which gives the heating rate index (f_h). Mathematically,

$$f_h = -\frac{1}{slope} \tag{7.2}$$

where,

f_h= heating rate index (min)

Equation (7.2) was used to determine the associated f_h values.

7.2.8. Quality evaluation

7.2.8.1. Texture profile analysis

The hardness of pineapple wedges, apple and carrot slices were obtained using the TA-XT Plus Texture Analyser (Texture technologies corp., Scarsdale, NY, USA). The software used to obtain the texture parameter values was Texture Exponent 32 software (Texture Technologies Corp., Scarsdale, NY/ Stable Micro Systems, Godalming, Surrey, UK). The machine was equipped with 50cm OD acrylic probe. For each experiment the processed products were placed under the probe. For carrots the pre- and pro-test speeds were 1mm/s and 0.50 mm/s, respectively and the target mode of the test was set on distance where distance was 2 mm and time was set to 5 s with a trigger force of 5 g. For apples the pre- and pro-test speeds were 1mm/s and 5 mm/s, respectively and the target mode of the test was set on strain where strain was 75% and time was set to 5 s with a trigger force of 5 g. For pineapples the pre- and pro-test speeds were 1mm/s and 5 mm/s, respectively and the target mode of the test was set on distance where distance was 2mm and time was set to 5 s with a trigger force of 5 g. The parameters of probe height calibration were set to the return distance 15mm, return speed 1.7 mm/sec and contact force 1 g. For texture assessment, a minimum of 10 samples were tested for each commodity in triplicates. To obtain the TPA, a two-cycle compression test was used that mimicked two bites. The software represents these two bites as two peaks on a force vs distance graph. The maximum force required to compress the sample in the first compression was noted as its hardness.

7.2.8.2. Color analysis

The CIE L* (lightness), a* (redness), and b* (yellowness) color attributes of the treated samples and raw control were determined using Minolta Tristimulus Chroma Meter (Minolta Corp., Ramsey, NJ, USA) and displayed by the software (SpectraMagic, Minolta Corp., Ramsey, NJ, USA). The total color differences (ΔE) were calculated by the following equation:

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

where the raw samples were used as the control in the calculation of ΔE.

7.2.8.3. Total carotenoid analysis

The extraction of carotene from the raw and processed carrots followed the methods described in Jing et al., (2017), and Fuji Chemical Industries with modifications. Briefly, 20g of carrots with 2g of anhydrous sodium carbonate homogenate was mixed in a mechanical blender with 30 ml extraction solvent hexane-acetone (1:1) and stirred for 2-3 minutes. After adding 4 ml of 1% BHT to the mixture and placing the mixture in 25ml centrifuge tubes, the tubes were subjected to ultrasound water bath (full wave) (Ultrasonic cleaner, Model: TH-SPQXJ-40A; Capacity:10L with 28/40 khz ultrasonic frequency, ultrasonic power:240W and heating power 500W) for 5 minutes at 50°C. The organic layer was collected and filtered through a Whatman filter paper. 25ml of acetone was added while filtration. The filtrate was then centrifuged at 17000 rpm for 15 minutes using Sorval SA 600 RC5C centrifuge. The supernatant was collected until clear solution. The clear supernatant was made up to 50ml volume with acetone in 50 ml standard flask. The absorbance value of the extract (supernatant) was measured at 474 nm against acetone blank using a spectrophotometer (Ultrospec 2100 pro UV/Visible Spectrophotometer).

The total carotenoid concentration % of the extract was calculated by the formula below:

$$\text{Total carotenoids content (\%)} = \frac{Absorbance\ X\ volume}{210}\ X\ \frac{W}{100} \qquad (7.5)$$

in which absorbance is the absorbance of the extract supernatant at 474nm, volume is the dilution volume for the extract supernatant preparation, 210 is absorbance of 1 (mg/mL) carotenoids solution in a 1 cm cuvette at 474nm (extinction coefficient), W is the weight, in mg, of the carrots taken to prepare the extract supernatant.

7.2.8.4. Ascorbic acid (vitamin C analysis)

Extraction of Vitamin C from raw and processed pineapples followed the method described by Hernández et.al, 2006, with modifications. Frozen pineapples were pulverized. 2.5g of pulverized pineapples were mixed with 25 ml of the extractant solution (3% MPA and 8% acetic acid for MPA-acetic acid extraction). The mixture was homogenised in a Politron PT 6000 (Kinematica AG, Switzerland) high speed blender at 18000g (in ice and darkness) for 1 min. It was then centrifuged at 9000g (refrigerated at 4°C) for 20 minutes using Sorval SA 600 RC5C centrifuge. This procedure was repeated twice, and the two resulting supernatants were mixed for each sample.

An Agilent 1260 Infinity HPLC system equipped with an Agilent quaternary pump module, refrigerated Agilent 1260 Infinity II Vialsampler and a diode array detector (DAD) WR, and an electrochemical detector (ECD) (945 Professional Detector Vario IC Amperometric Detector) operated by an OpenLAB Chemstation with Mass Profiler Pro software was used for sample analysis. Ascorbic acid in the samples was analyzed using a reverse phase HPLC Gemini-NX (5 µm, 100 mm×4.6 mm) column (Phenomenex, Torrance, CA,) and a 4.6 mm × 2.0 mm guard column based on the modified method of Hernandez et al., (2006). A constant gradient mobile phase consisting of 0.015% TEA (A) and 100% acetonitrile (B), with a flow rate of 1.2 mL/min was used for the analysis. Ascorbic acid was detected at 245 nm and confirmed by spectral scanning at 190-900nm. Quantification was by external standards calibration using ascorbic acid standard (Sigma Aldrich, ON).

7.2.8.5. Determination of total polyphenolic contents (TPC) analysis

Extraction of TPC from raw and processed pineapples and carrots followed the method described by Yahya et.al., (2019), with modifications. 10g of pineapple/carrots were mixed with 35 mL of ethanol: water solution (50:50 %, v/v) and homogenized at 2000 rpm for 2 minutes. The sample was then ultrasonicated using US-water bath at 100% amplitude for 6 minutes at 30°C. It was then centrifuged for 15 minutes at 17000 rpm at room temperature. The supernatant was collected and taken for spectrophotometric analysis.

0.30 mL of supernatant was mixed thoroughly with the Folin-Cioalteu's phenol reagent (0.30 mL) and distilled water (2.70 mL) and left for 5 min. The sample solution was then mixed with sodium carbonate, Na_2CO_3 solution (7 % (v/v), 3.00 mL) and distilled water (1.20 mL). The solution was left to stand at room temperature for 30 min before the measurements were made. The standard curve of gallic acid was prepared at 0.02 to 0.50 mg/ml concentrations individually in separate test tubes. For all samples, triplicate readings were taken at 765 nm on a UV/VIS Spectrophotometer (Ultrospec 2100 pro). TPC was presented as mean ± standard deviation, where the value was expressed as mg of gallic acid equivalent (GAE) per 100 g of fresh pineapple or carrots extract (mg GAE/100g of wet material).

7.2.8.6. Free radical scavenging activity (DPPH)/ antioxidant activity

For polyphenol extracts (PPE) from the samples (carrots and pineapple) the method of Sengkhamparn et al. (2014), was used with few modifications. Briefly, 10g of wet sample was blended with 20ml of Methanol (99%) for 2 minutes. The mixture was homogenized for 10 minutes at 2000rpm. It was then centrifuged at 10000 rpm for 10 minutes. The supernatant is collected as the extract and filtered using Whatman filter paper.

The DPPH scavenging capacities of PPE was measured according to the method of Hossain et al., (2011), with some modifications. An aliquot of 0.60 ul of PPE with 240 ul of DPPH solution (0.2mM solution 99% Methanol) was mixed. The reaction mixture was incubated for 30 min in the darkness at room temperature. The absorbance of resulting solution was measured at 517 nm with a plate reader. The control was prepared as above without any extracts and methanol was used for the baseline correction. The blank used was 300µl of 99% methanol. All samples were run in triplicate. The radical scavenging capacity of the test samples was measured as a decrease in the absorbance of DPPH and was calculated as the ratio of the difference between the absorbance of DPPH solution ($A_{control}$) and absorbance of sample and DPPH mixture (A_{sample}) to the absorbance of DPPH solution:

$$\%\text{Antioxidant Activity} = [(A_{control} - A_{sample})/A_{control}] \times 100 \qquad (7.6)$$

7.2.8.7. Statistical analysis:

Statistical analysis was conducted using IBM SPSS Statistics Version 26 software. One-way analysis of variance (ANOVA) was used for all data. Differences among treatments by means were determined by Tukey's method for comparison test with significant level at $p \leq 0.05$.

7.3. Results and discussions

7.3.1. Processing time

Processing conditions for both ultrasound assisted and conventional hot water or steam alone pasteurization was selected to result in equivalent microbial safety. The processing time for all the three commodities (apples, pineapples, and acidified carrots) under different treatment conditions (steam (S), steam ultrasound (SU), steam/ air sound (SAU), steam air(SA), hot water ultrasound (WU) and hot water (W) was calculated as the equivalent time required at a particular treatment condition to meet the target pasteurization lethality set at 90 °C for 10 min. Table 1 shows the processing times for all the three packaged commodities. Results show that the processing times for the apples, pineapples and carrots packaged in flexible containers were lower when used under ultrasound processing conditions. This was true for steam (S), steam/air (SA) and water bath (W) heating conditions. Hence it was clear that the addition of ultrasound helped to enhance the heat transfer from the medium to the product.

The longest time was associated with the steam/air medium in which there would be a significant resistance due to the presence of air. But it also provides better contact between the heating medium and the product due to the added external pressure of the air. The hot water bath came next with slightly better process times (5-10%) as compared to steam/air but again the ultrasound addition improved it further by about another 10% reduction process time. Generally, with better density than steam water tends to provide better contact and heat transfer than steam/air where the condensation of steam on the package surface could be impaired by the presence of air. The steam and ultrasound steam came out as the best heating media with 20-30% reduction in process times as compared with steam/air. Steam is the generally considered best heat transfer medium with contribution by both condensation and convection heat transfer and is proven from the reduced process times to achieve a target lethality. There were differences in process times between apple, pineapple and carrot slices arising from differences in the nature and composition of these commodities.

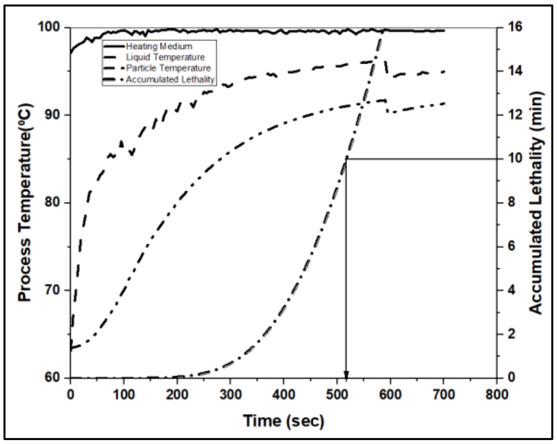
Figure 7.2 shows typical heat penetration curves along with the accumulated lethality for pineapple pieces packaged in flexible pouches with sugar solution processed under the different treatment conditions over a heating time of about 10-15 min. The smooth and transient nature of the curves demonstrate the expected temperature rise in the samples with temperature gradation. The bath temperature reached the target first. The liquid and the sample followed it not necessarily reaching the bath temperature before the target lethality was reached. Medium temperatures reached were somewhat different and depended on the treatment method. Steam heating was effective reaching 100 ºC, but steam/air medium reached only about 95C while the ultrasonic bath temperature reached only 90 ºC. In each case, however, experiments were carried out until the target lethality was reached in the test samples.

Also evident with the different curves for test samples are differences between the selected treatments conditions demonstrating better temperature rise in samples heated under ultrasound assisted conditions. Since these are from different experiments, variations in their temperature profiles are obvious depending on the respective initial temperatures and bath conditions, and direct comparisons cannot be made. However, they can be used to compute the accumulated lethality in the sample which were calculated as equivalent times at a reference temperature of 90 ºC. This parameter could be used for comparing the treatment methods.

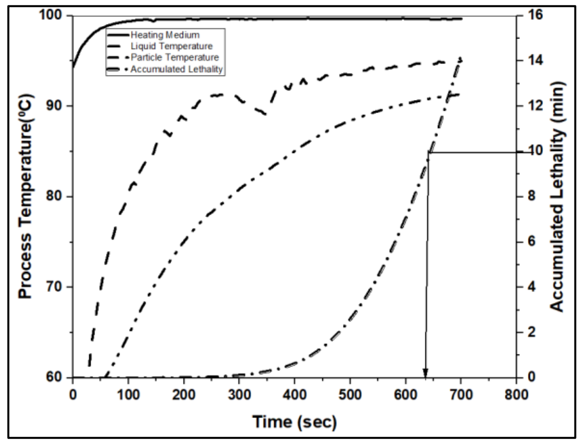
The six typical heat penetration curves are shown, the top two one being those heated under steam with and without ultrasound. Next to follow were the one treated in water with and without ultrasound and finally the last two were for steam/air with and without ultrasound. A clear comparison that could be made from these curves is that the time to reach the target lethality in ultrasound included treatments were clearly shorter than those without for each treatment type. The overall progression was also clear that the treatment time increased from steam heating to water bath heating to steam/air heating with or without ultrasound inclusion. Our previous concept testing preliminary studies (Basumatary et al., 2020) also showed the similar trends of heat transfer enhancement by addition of ultrasound to steam.

Figure 7. 3. showed similar trends of heat penetration and accumulated lethality curves for acidified carrots processed in flexible pouches under different treatment conditions. In this case the curves are much closer for each treatment compared to pineapple pieces treated under

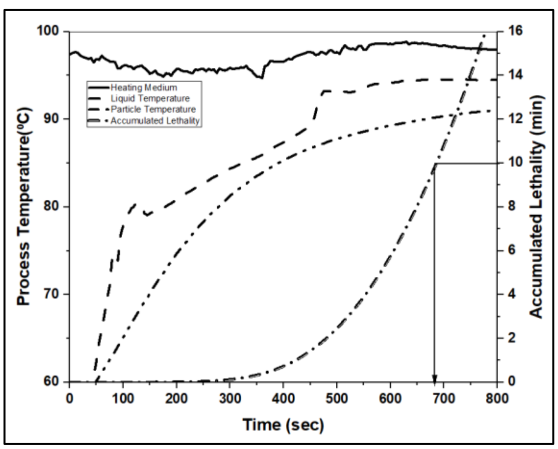
different conditions. But it shows similar trends of heat transfer enhancement and reduction of process time to achieve certain lethality under ultrasound assisted treatments compared to conventional treatments. Our previous preliminary studies (Basumatary et al., 2020) also showed the similar trends of heat transfer enhancement by addition of ultrasound to steam.



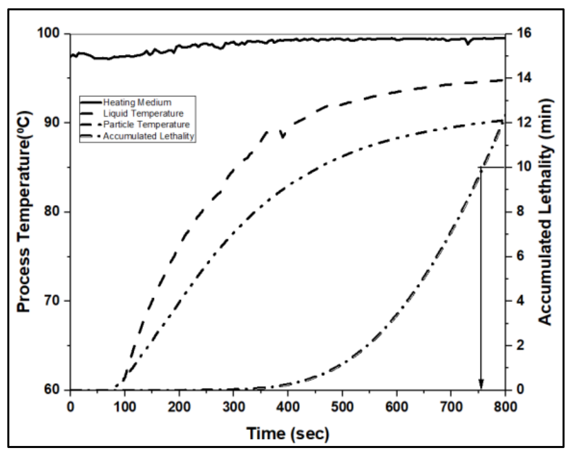
a. Steam Ultrasound (SU)



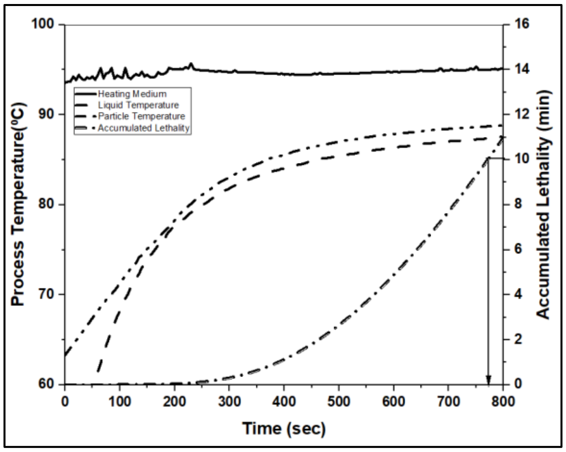
b. Steam (S)



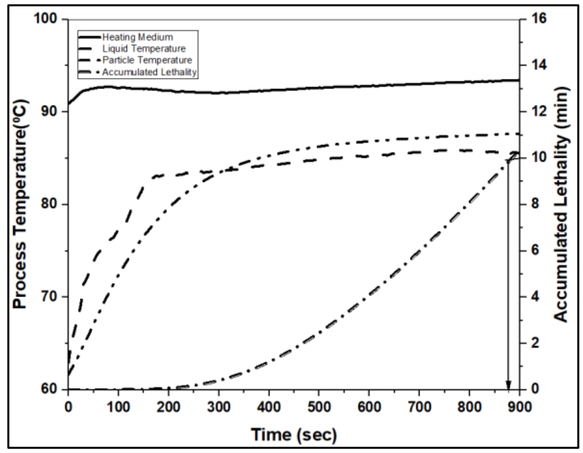
c. Water Ultrasound (WU)



d. Water (W)



e. Steam /air ultrasound (SAU)



f. Steam/air (SA)

Figure 7. 2. Process temperature and accumulated lethality evolution curve for pineapple pieces processed under different treatment conditions: a) Steam ultrasound (SU), b) Steam (S), c) Hot water ultrasound (WU), d) Hot water (W), e) Steam/air ultrasound (SAU), and f) Steam/air (SA).

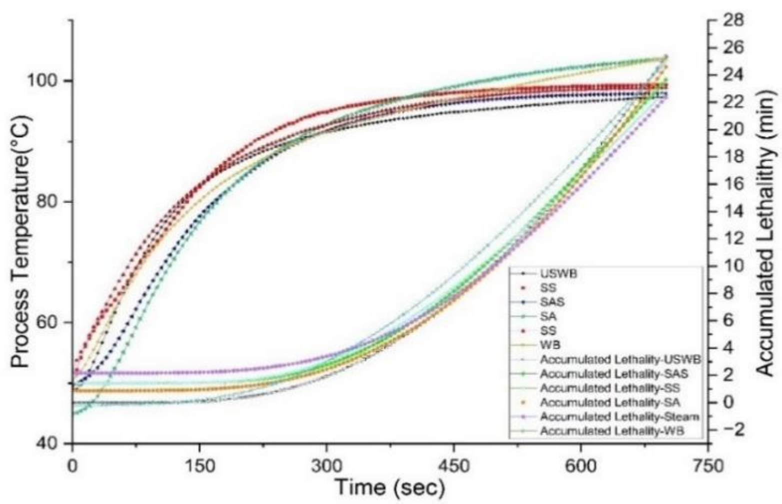


Figure.7.3. Process temperature and accumulated lethality evolution curve for carrots processed under different treatment conditions: Steam (S), Steam ultrasound (SS/SU), Steam/ air ultrasound (SAU/SAS), Steam/air (SA), Hot water ultrasound (WU/USWB) and Hot water (W/WB)

Table 7.1: Processing conditions for achieving an equivalent target lethality of 10 min at 90ºC under different steam, steam/air and water bath heating conditions with and without added ultrasound.

Sample	Treatments	Processing Time (min) ($F_{90°C}$ = 10 min)
Pineapple	Steam/air	14.7 ± 0.8
	Steam/air ultrasound	12.7 ± 0.5
	Hot water	12.6 ± 0.5
	Hot water ultrasound	11.3 ± 0.3
	Steam	10.2 ± 0.5
	Steam ultrasound	8.6 ± 0.1
Carrots	Steam/air	19.8 ± 0.5
	Steam/air ultrasound	17.8 ± 0.9
	Hot water	11.5 ± 0.6
	Hot water ultrasound	8.1 ± 0.7
	Steam	7.9 ± 0. 6
	Steam ultrasound	6.9 ± 0.3
Apple	Steam/air	12.0 ± 0.5
	Steam/air ultrasound	11.2 ± 0.6
	Hot water	11.0 ± 0.6
	Hot water ultrasound	10.1 ± 0.9
	Steam	9.00 ± 0.8
	Steam ultrasound	8.2 ± 0.6

7.3.2. Heating rate index (f_h)

Heating rate index is a measure of the rate index of the packaged foods during thermal processing. Since it is a parameter represented as the negative reciprocal slope of the semi-logarithmic plot of temperature difference between the heating medium and product vs time (typical curve shown in Figure 7.4), higher values of heating rate index represent slower hearing rate. Therefore, higher f_h means slower heating which would normally also result in longer process times as listed in Table 1. Figure 7.5 shows the f_h values of carrots, apples and pineapples under different treatment conditions (S, SU, SA, SAU, W and WU). The f_h values decreased when ultrasound was added to the steam or hot water treatments.

The results show that ultrasound assisted treatments increased the rate of heat transfer compared to conventional treatments. The highest f_h value of 13.08 minutes in carrots, 9.38

minutes in pineapples and 8.31 minutes in apples was observed under hot water treatments (W). Whereas the lowest f_h value 4.29 minutes in carrots, 5.16 minutes in pineapples and 4.50 minutes in apples was observed under combined ultrasound and steam treatment. Here there appear to be a bit of reversal because the longest process time was associated with SA rather than W. The reason for this is while f_h is extracted from well developed heating regime excluding the thermal lag (the curved portion the heating curve shown in Figure 7.4), the process time is obtained by the combination of time temperature including the lag period until the integrated lethality value reaches 10 min. The lowest f_h value 4.5 min in apples and 5.2 min in pineapples was observed under combined ultrasound and steam treatment (SU).

Figure 7.4 also illustrates that there was significant difference in the f_h values between hot water and combined ultrasound-hot water treatments. No significant difference was observed between steam treatment in presence of air and hot water treatment. Significant difference was observed between steam treatment in presence of air when combined with ultrasound and without ultrasound. However, the introduction of ultrasound in hot water treatment influenced acidified carrots much more than pineapples or apples– the heating rate index for acidified carrots decreased by 52% on average when ultrasound was added to hot water compared to 15% for pineapples and 22% for apples. Also, under steam treatment in presence of air, introduction of ultrasound decreased the heating rate index by 24% on average for acidified carrots compared to 14% for pineapples and 22% for apples. But in the absence of air, the introduction of ultrasound to steam it showed influenced pineapples much more than carrots and apples – heating rate index of pineapples decreased by 23% on average compared to 11% for acidified carrots and 13% for apples. These results overall indicate that addition of ultrasound to the hot water or steam treatment reduces the heating rate index thereby indicating heat transfer enhancement.

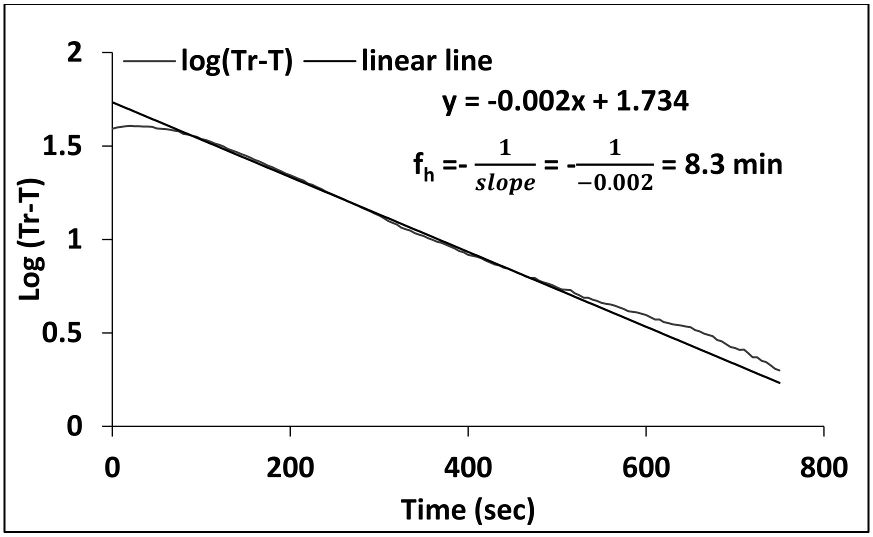


Figure 7.4. Example for calculating f_h values from log (Tr-T) vs time for a selected treatment condition: Hot water (W) heating of apple slices

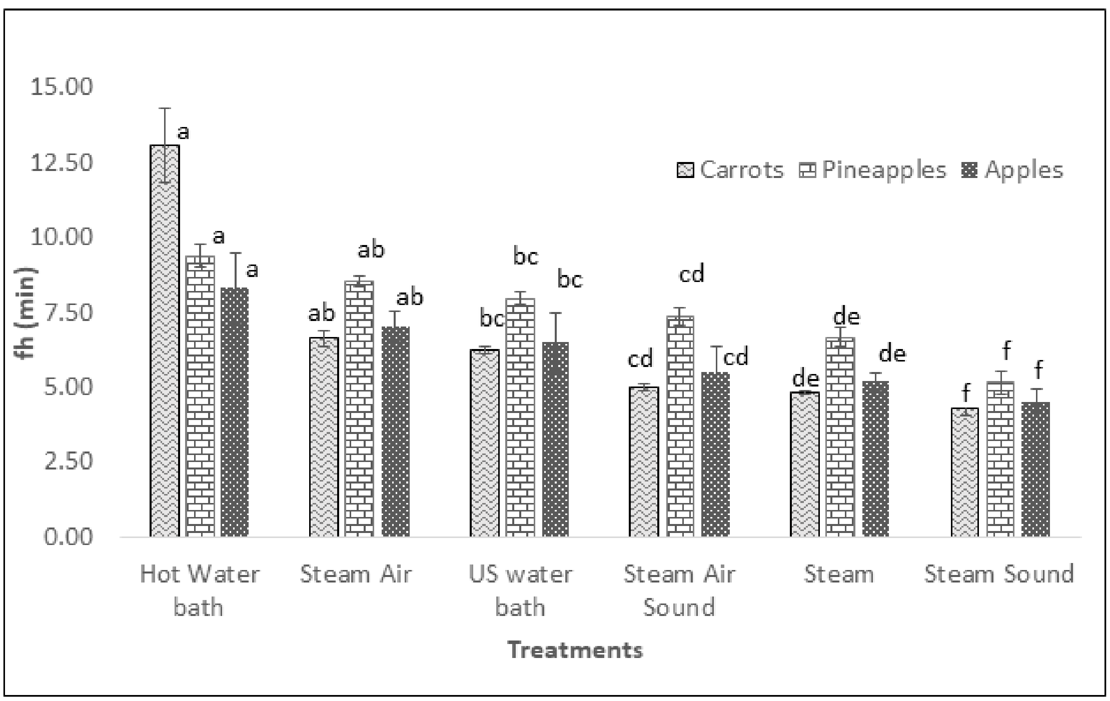


Figure 7.5: Heating rate index (fh) of carrots, pineapples and apples processed under different treatment conditions: Steam, Steam Sound, Steam Air Sound, Steam Air, Ultrasound water bath and Hot water bath.

*Significant differences ($p < 0.05$) are indicated with different letters, and values marked with a same letter for each vegetable are not significantly different.

7.3.3. Quality Evaluation

7.3.3.1. Color

The CIE LAB color values of untreated and processed carrots, apples and pineapples are given in Tables 7.2, 7.3 and 7.4 respectively. From Table 7.2 it was observed that both steam and hot water processes significantly reduced a* values in the carrot samples, from 35.05 of raw carrots to 27.38 – 32.95 of processed ones, 6-21% reduction compared with the initial value. All carrots processed by ultrasound assisted treatment had slightly higher a* values than those by HW or steam only heating and significant difference was found in samples treated under the influence of ultrasound both in presence and absence of air when compared without ultrasound and with ultrasound -hot water treatment when compared with hot water treatment. For b* values, the ones treated without ultrasound had higher values compared to the ones treated under the influence of ultrasound both in hot water and steam. The b* values were higher in treated carrots than the untreated carrots. The overall L* values of processed carrots had significant difference from untreated carrots but within the different treatments it remained relatively stable (with values around 50-55), though steam ultrasound treated showed significant higher values compared to other treatments indicating more lightness in the sample. The changes in a* and b* values indicate a deterioration in the initial deep orange color of the carrots, mainly due to carotenoids that may have suffered degradation and isomerization during heat treatment. The overall color differences (ΔE) between the treated samples and their controls (raw carrots) are also summarized in Table 2. In theory, a ΔE of 1 represents a color difference perceptible to the human eye under ideal viewing conditions; while ΔE values between 2 and 3 may be considered equivalent by some viewers under non ideal lighting conditions (Vervoort et al., 2012). It is clear from Table 7.2 that all ΔE values were greater than 3, indicating that color differences between all processed and unprocessed carrots are perceptible to the human eye under normal lighting conditions. Only carrots treated with hot water and steam had the highest ΔE value, indicating the lowest color retention of the carrots. The ΔE values of processed carrots ranged from 8.1 to 15.9, and all samples of carrots processed under the influence of ultrasound have lower ΔE values than treatments with only hot water or steam, indicating a better color stability. This could be due to the shorter heat-up time of ultrasonically assisted processing compared to hot water or pure steam processing.

Table 7.2: CIE L*, a*, b* values, and total color differences (ΔE) of diced carrots under different treatments. The color attributes of raw carrots were used as the control

.	Treatments	L*	a*	b*	Total color difference ΔE
Carrots	Untreated	61.4± 1.2^{a}	35.0± 0.9^{a}	55.4± 1.6^{de}	
	Steam	53.3± 0.05^{e}	30.0± 0.9^{de}	61.1± 1.5^{a}	11.1
	SU	55.2± 0.3^{b}	32.9± 0.9^{b}	60.2± 2.1^{ab}	8.1
	SA	51.3± 0.04^{def}	27.4± 1.3^{g}	58.5± 2.02^{bc}	15.9
	SAU	50.5± 0.3^{efg}	29.7± 0.3^{def}	50.3± 0.39^{g}	12.5
	WU	52.9± 0.2^{cde}	32.3± 3.9^{bc}	54.2± 0.8^{f}	10.5
	W	51.4± 0.2^{cde}	30.3± 0.4^{d}	56.3± 4.2^{d}	12.3

*Values in the same column, significant differences ($p < 0.05$) are indicated with different letters, and values marked with a same letter are not significantly different.

The L* values of apples treated under the influence of ultrasound had better retention of lightness when compared to the ones treated with steam or hot water only but were mostly lower than in the control (Table 7.3). There was an increase in a* value in treated samples compared to the raw apples. This might be due to the surface red pigments of apples dissolving in the liquid while processing. Within the treatments as well significant differences were observed for a* values between steam and ultrasound-steam in absence of air and hot water and ultrasound hot-water. The b* values showed that there no significant difference between raw and treated samples except for apples treated in presence of air. The ΔE values for treated apples were lowest for combined ultrasound steam treatment and highest for steam only treatment both in presence and absence of air indicating better color retention for apples treated under the influence of ultrasound compared to conventional heating.

There was a significant reduction of L* values in the pineapple samples processed under steam and W from 74.3 of raw pineapples to 43.5 – 51.3 of processed ones, with 31-42% reduction compared with the initial value (Table 7.4). When compared within the treatment's pineapples processed by ultrasound assisted treatment had slightly higher L* values than those by W or S heating. For a* values significant difference was seen between raw and processed pineapples, but no significant difference was seen within the different treatments. The b* values of processed pineapples had significant difference from untreated pineapples and as it shows the change in yellowness and greenness can be considered as an important factor to observe colour change in pineapples. The samples treated under influence of ultrasound had higher b* values indicating retention of yellowness. The changes in L* and b* values indicate a deterioration in the initial brightness and yellowness color of the pineapples. The overall color differences (ΔE) between the treated samples and their controls (raw pineapples) are also summarized in Table 3. It shows that all ΔE values are huge for all processed pineapples, but pineapples treated with hot water and steam only had the highest ΔE value, indicating the lowest color retention of the pineapples.

Table 7.3: CIE L*, a*, b* values, and total color differences (ΔE) of apple slices after thermal processing under different treatments. The color attributes of raw apples were used as the control.

	Treatments	L*	a*	b*	Total color difference ΔE
Apple slices	Untreated	78.7 ± 0.4^{a}	0.6 ± 0.2^{fg}	20.2 ± 1.1^{b}	
	Steam	66.7 ± 0.6^{e}	5.7 ± 1.8^{ab}	19.2 ± 0.8^{bc}	14.0
	SU	73.4 ± 1.2^{b}	3.4 ± 1.8^{e}	17.4 ± 1.5^{bcdef}	7.7
	SA	64.8 ± 0.8^{fg}	5.8 ± 1.6^{a}	22.3 ± 1.4^{a}	15.8
	SAU	70.9 ± 0.7^{c}	5.4 ± 0.7^{abcd}	17.3 ± 1.0^{efg}	10.6
	WU	69.4 ± 0.4^{cd}	2.0 ± 0.8^{ef}	18.7 ± 0.3^{bcde}	10.4
	W	65.4 ± 0.5^{ef}	6.4 ± 0.9^{a}	19.2 ± 1.6^{bcd}	15.5

*Values in the same column, significant differences ($p < 0.05$) are indicated with different letters, and values marked with a same letter are not significantly different.

Table 7.4: CIE L*, a*, b* values, and total color differences (ΔE) of pineapple wedges after thermal processing under different treatments. The color attributes of raw pineapples were used as the control.

	Treatments	L*	a*	b*	Total Color Difference ΔE
Pineapple wedges	Untreated	74.4± 0.1[a]	6.6± 0.1[a]	45.9± 0.1[a]	
	Steam	45.4± 1.0[g]	1.3± 0.4[bcde]	31.8± 0.6[f]	32.7
	SU	51.4± 0.2[b]	2.1± 0.6[b]	37.3± 4.1[b]	25.3
	SA	47.4± 0.8[de]	1.3± 0.1[bcd]	33.4± 1.1[d]	30.3
	SAU	50.8± 0.3[bc]	2.0± 0.3[bc]	36.0± 0.6[bc]	26.0
	WU	47.5± 0.2[d]	0.7± 0.1[bcdef]	29.9± 0.2[e]	31.9
	W	43.5± 0.2[f]	0.8± 0.1[bcdefg]	27.0± 0.3[g]	36.7

* For each parameter (Values in the same column), significant differences ($p < 0.05$) are indicated with different letters, and values marked with a same letter are not significantly different.

7.3.3.2. Texture profile analysis

Texture changes of acidified carrots and pineapples under different processing conditions are shown in Figure 7.6. Apparent texture loss was observed in all processed pineapple samples compared with the raw samples. The hardness parameter was used to measure the textural loss of the processed carrots and pineapples. There were significant differences in the hardness values between different treatments. The samples treated under the influence of ultrasound showed higher hardness values compared to the ones treated with steam or hot water only indicating better textural retention. The retention in hardness is due to the lower processing times associated with ultrasound assisted treatments. As with apples, ultrasound enhanced steam and steam/air pasteurization processes better preserved the texture characteristics.

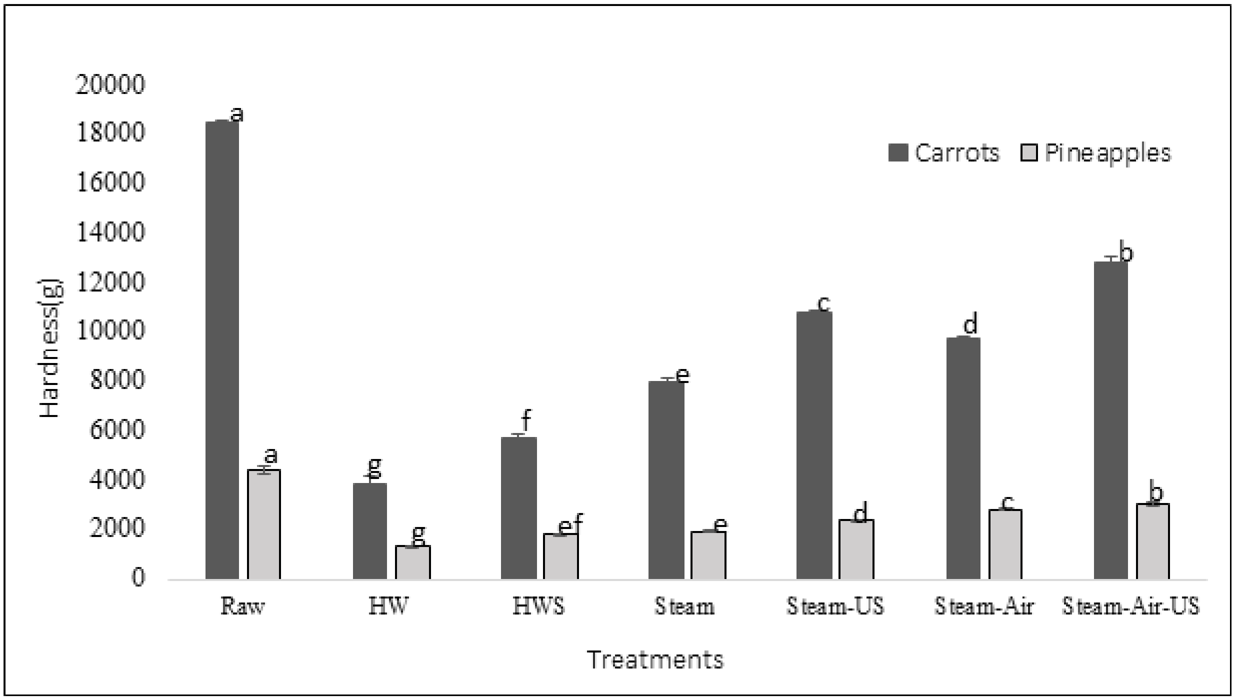


Figure 7.6: Texture of carrots and pineapples under different treatments. (HW/W (hot water); HWS/WU (hot water ultrasound); Steam /S; Steam-US/SU (steam ultrasound); Steam-Air/SA; Steam-Air US/SAU). Columns labeled with the same letters are not significantly different ($p < 0.05$).

For textural changes in apples the hardness and chewiness values were taken into consideration. Significant reduction in hardness and chewiness of processed apples were observed as shown in Figure 7.7 when compared to untreated apples. Within the different treatments as well the hardness and chewiness values showed difference with lowest values for hot water treated samples indicating most textural loss in hot water treated samples compared to steam treated samples. The results showed that ultrasound assisted treatments have better textural retention for apples processed in flexible pouches. The ultrasound enhances steam and steam/air pasteurization had better texture retention than others. Chewiness followed similar trends.

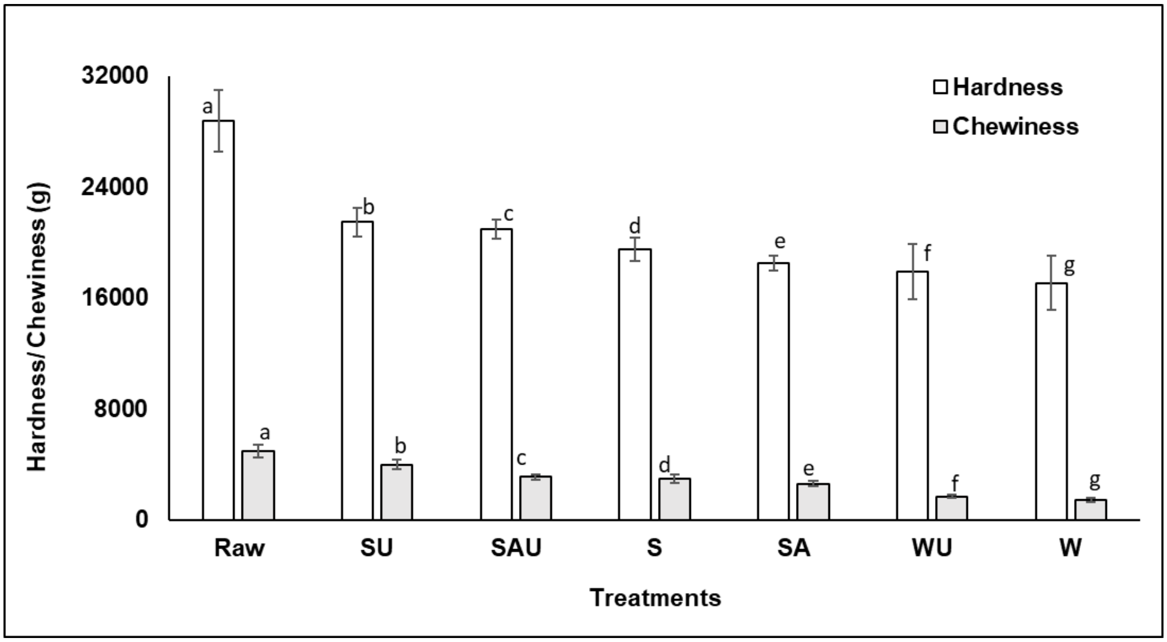


Figure 7.7: Texture (Hardness and chewiness) of apples under different treatments. Columns labeled with the same letters are not significantly different ($p < 0.05$).

7.3.3.3. Total phenolic content

Degradation of TPC for acidified carrots and pineapples under different pasteurization conditions are shown in Table 7.5. The highest retention was with ultrasound-steam process (SU) with about 25% loss as compared with fresh for pineapples and 22% loss as compared with fresh for carrots. Hot water ultrasound (WU) was next, but SAU and the ones without ultrasound produced similar low results. This work was carried out only with carrots and pineapples. So only pineapple wedges and carrots treated under the influence of ultrasound in steam and hot water had good retention results. This might have been due to the relatively longer exposure of the samples under the other treatment conditions.

Table 7.5: Total phenolic content of carrots and pineapples under different treatments.

Treatments	Total Phenolic Content (mg GAE /100g of fresh carrots)	Total Phenolic Content (mg GAE /100g of fresh Pineapples)
Raw	88.5 ± 0.9	280.0 ± 6.4[a]
Acidified Raw	75.2 ± 1.32[a]	NA
W	41.5 ± 0.7[fg]	178.4 ± 6.4[de]
WU	48.5 ± 1.3[c]	195.3 ± 6.3[bc]
S	42.7 ± 0.9[def]	185.8 ± 8.[cd]
SU	68.5 ± 3.3[b]	209.4 ± 4.9[b]
SAU	47.6 ± 2.3[cde]	172.1 ± 1.6[def]
SA	46.9 ± 1.3[cd]	165.8 ± 3.7[efg]

* The same letters in the tables indicate no significant difference between the treatments (p <0.05).

7.3.3.4. DPPH/antioxidant activity

The antioxidant activities of pineapples were studied under different treatment conditions are shown in Figure 7.8. Higher antioxidant activity in treated fruits is desirable. Changes associated with the antioxidant activity of pineapples during the pasteurization treatments were small with or without ultrasound assistance. The significance patterns were mixed with treated pineapples even as compared with fresh pineapples. It appears the antioxidants present in pineapple have relatively high thermal resistance.

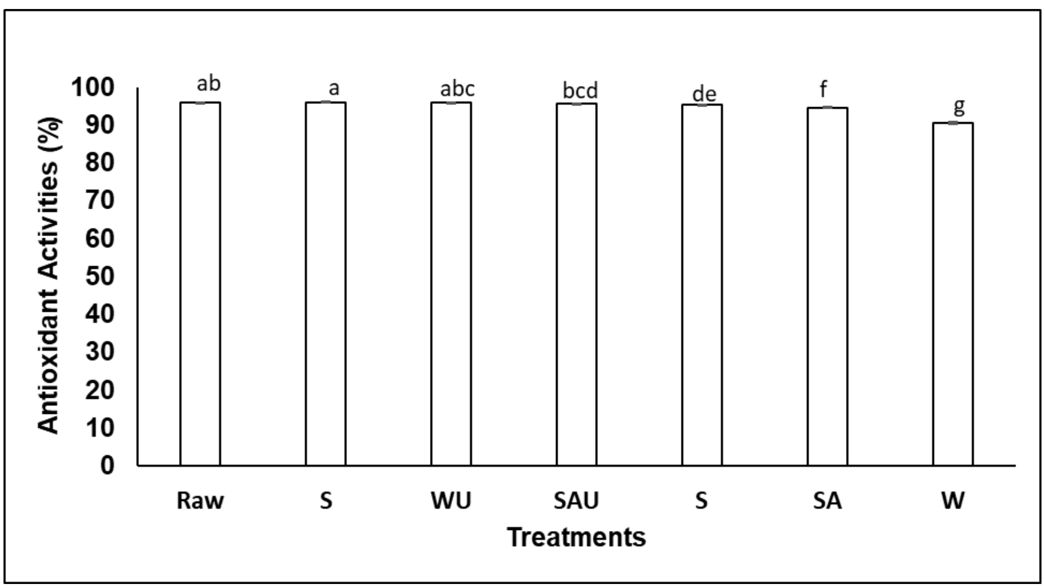


Figure 7.8: Antioxidant activity of pineapples under different treatments. Columns labeled with the same letters are not significantly different ($p < 0.05$).

7.3.3.5. Ascorbic acid content

The ascorbic acid content of pineapples (Figure 7. 8) reduced under all treatment conditions. All were statistically significant. The order of better retention from best to worst was SAU, SA, SU, S, WU and finally W. Hot water pasteurization was the worst case and SAU and SA were better than the others. It is not clear why. This was not dependent on the treatment time and all of them were given the same level of heat treatment to achieve the target pasteurization value. Nevertheless it was one of the ultrasound assisted process that provided better retention.

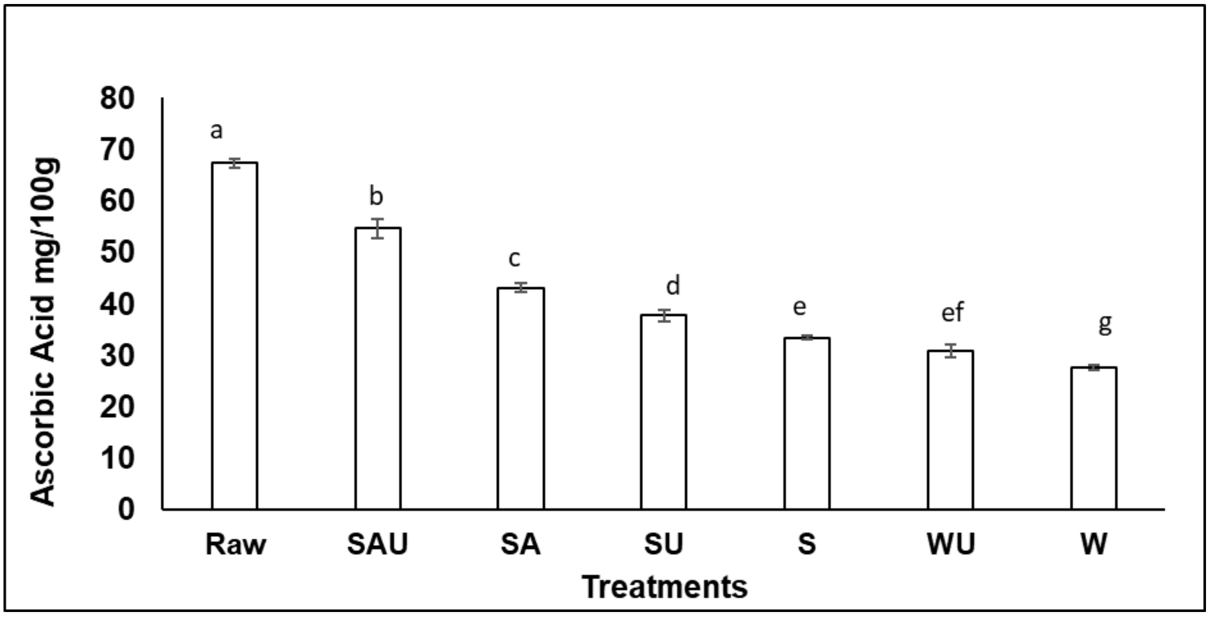


Figure 7.8.: Ascorbic acid content of pineapples under different treatments. Columns labeled with the same letters are not significantly different ($p < 0.05$).

7.3.3.6. Total carotenoids

Carotenoids are responsible for the bright orange color of carrots; the total carotenoids of carrots are shown in Figure 7.9. In raw acidified carrots, the total carotenoids analysed was 46.3± 0.10 mg/100 g. Both steam and hot water processing significantly reduced the contents of the total carotenoids. But combined steam and ultrasound treated carrots had no significant difference in total carotenoid contents from the raw carrots. Thermal processes ($F_{90°C}$ =10 min) for pure steam treatment caused a loss of around 25% loss in total carotenoids compared with the initial value; for combined steam ultrasound treatment it caused a loss of only 3%. In presence of air, it showed higher loss of total carotenoids – 46% loss for combined steam ultrasound and 53% for steam only treatment when compared with initial value. This might be due to less disintegration of carrot tissue due to lesser heating of the products compared to pure steam conditions or hot water treatment. For hot water processing as well, we see around 35% loss of total carotenoids in hot water treatment which is higher than ultrasound assisted hot water processing of carrots which showed 26% loss of total carotenoids.

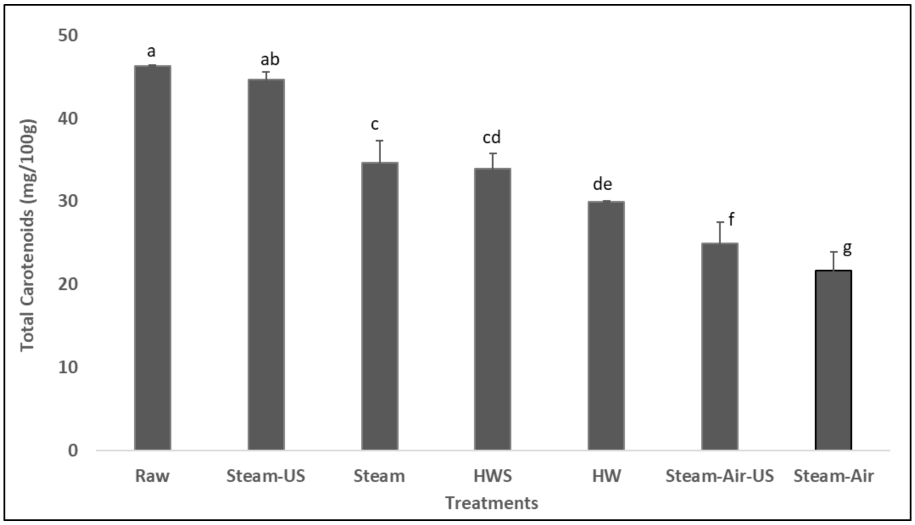


Figure 7.9: Total carotenoids of acidified carrots under different treatments. (HW/W (hot water); HWS/WU (hot water ultrasound); Steam /S; Steam-US/SU (steam ultrasound); Steam-Air/SA; Steam-Air US/SAU). Columns labeled with the same letters are not significantly different ($p < 0.05$).

7.4. Conclusions

This study demonstrated the applicability of ultrasound assisted pasteurization of pre-packaged acidified carrots in brine, pineapple wedges and apple slices in sugar solution. Steam in the presence and absence of air and hot water was used as the heating medium for processing the flexible pouches with all the three products. The thermocouples were placed in the food particles at the coldest spot in the pouch. Ultrasound steam and ultrasound -hot water pasteurization processes at $F_{90°C}$ = 10 min were established for carrots, pineapples and apples in brine and sugar solution in pre-packaged flexible retort pouches, along with conventional pure steam or hot water processes resulting in equivalent microbial safety. The total processing time of ultrasound assisted process was greatly reduced compared with an equivalent hot water process.

It also improved the quality uniformity of the processed products compared with an equivalent hot water or steam process, indicating less thermal deterioration of the overall quality in ultrasound assisted processed samples.

The results of this study demonstrated the impact of ultrasonic processing on each quality attribute of carrots, pineapple and apple products depending on the selected quality parameters as well as the products processed. For color, all carrot samples processed by ultrasound assisted treatments had lower ΔE values and slightly higher a* values than those by hot water or steam only treatment, denoting a better color retention in ultrasonic assisted processed samples. A significant difference was also observed in texture and total carotenoid retentions for acidified carrots treated under the influence of ultrasound compared with no ultrasound. No significant differences of TPC content retention were found in carrots processed by hot water or steam under the influence or absence of ultrasound.

For pineapples the quality attributes analysed were color, texture, TPC, antioxidant activity and ascorbic acid content. The results showed that ultrasound assisted treatments had better retention of all the quality attributes analysed compared to the ones without ultrasound. For apples only texture and color quality attributes were analysed and both attributes indicated better retention when treated under the influence of ultrasound.

Since all the quality attributes of pasteurized carrots, apples and pineapples were tested and compared immediately after processing only, further work should be carried out to investigate the quality changes during the shelf life of products. Further systematic research may also be needed to optimize ultrasound assisted pasteurization in steam and hot water treatments and process designs for improvement in the product quality. Future optimized steam and hot water - ultrasound assisted processes should take advantage of short heating time while considering different sensitivities of food quality attributes to thermal degradation.

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Chapter 8

8.0. Comprehensive Discussion

The use of ultrasound steam technology for microbial surface decontamination, enzyme inactivation of fresh vegetables, heat transfer enhancement and pasteurization of acid and acidified foods in flexible pouches represents a significant advancement in food processing and preservation. Throughout this exploration, numerous benefits and exciting outcomes that arise from the application of combined ultrasound-steam in the field of food processing have been demonstrated.

In Chapter 2 from the comprehensive literature review, the potential applications of ultrasound technology in food processing industry have been highlighted. It has been documented that steam is the most efficient and commonly used mode of thermal processing for microbial decontamination, enzyme inactivation and pasteurization. But when used for rapid surface heating, the presence of air can cause reduction in rate of heat transfer. An ultrasound environment can reduce the interference from this air surface barrier and increase the rate of heat transfer. To improve the traditional thermal processing method to achieve better quality products various improvements have been accomplished and several have been discussed in detail in Chapter 2 concerning thermal processing and ultrasound technology. Applications of ultrasound with liquid to enhance heat transfer has been widely explored but limited literature was found on combination of ultrasound and steam applications. A recent bibliometric analysis (July 2023) on combined ultrasound steam research showed only 30 documents on combination of steam and sound heating, most of these studies were conducted on poultry processing under high pressure steam conditions. These studies were also mostly propriety research conducted by an equipment manufacturing company. At the start of this PhD program in 2018 only 5 to 6 documents on combined ultrasound and steam were available. The increase in the documents currently, possibly some of them based on presentations of this thesis research at conferences, shows the increase in interest rate for research in this field. Since the potential of combined ultrasound steam technology was not well explored and very few scientific papers were available, the thesis objectives were set to explore the potentials of this novel technology.

In Chapter 3 the use of combined ultrasound steam technology for microbial surface decontamination in fresh vegetables was evaluated. The concept paper was published as cited.

Fresh vegetables are susceptible to microbial contamination after harvesting due to their high water and nutrient contents. The surface contamination of the vegetables presents a serious problem with respect to public health. Thermal processing is one of the most common methods used to provide safe foods with longer shelf life. Steam heating is one of the most common and cost-effective method of thermal processing as it has highest rate of heat transfer. But when used for rapid short time heating the presence of air on the surface and internal tissues of the products can cause barrier to the heat transfer. The presence of air can have significant effects on both heat transfer and surface decontamination processes for vegetables (Gomez et al., 2007). Air is relatively poor conductor of heat compared to other substances like metals or liquids. When heating vegetables, the presence of air can affect how efficiently heat is transferred to the food. This can lead to non-uniform heating and longer heating times, which might impact the quality and safety of the vegetables (Mafart et al., 2002). Air acts as an insulator, creating a barrier between the heat source and the vegetables. This can result in slower and less efficient heat transfer. In the presence of air, natural convection currents might form, causing uneven distribution of heat within the treatment environment. This can lead to hotspots and cooler areas in the treatment chamber, affecting the uniformity of heating. Surface decontamination of vegetables is crucial to reduce the risk of foodborne illnesses caused by pathogens present on the surface. The presence of air can impact the effectiveness of decontamination methods. One of the substantial advantages of ultrasound-steam technology is its ability to effectively eliminate microbial contaminants on the surface of fresh vegetables by removing the air surface barrier. The mechanism of elimination via combined steam-ultrasound wave lays on creation of a dynamic environment that disrupts the cell membranes of bacteria and other pathogens, leading to their inactivation. This process not only ensures food safety but also minimizes the reliance on chemical treatments, thus offering a more sustainable and environmentally friendly approach.

The first focus of this study (Chapter 3) was to enhance the surface bacteria decontamination via disruption of the air which is present along the surface and internal tissues of the food products. Our finding indicated that 3-6 log CFU reduction could be achieved within 30 s when carrots, cauliflower, broccoli, and zucchini inoculated with *E. coli K-12* were subjected to combined ultrasound-steam treatment compared to steam only treatment where 2-5 log CFU reduction was achieved in 30 s. In the presence of air (which is common during commercial application), it showed 1-4 log CFU reduction in 15s when subjected to combined ultrasound-steam treatment compared to steam only treatment where up to 1 log CFU reduction was achieved in 30s. To ensure the thermal destruction of pathogenic micro-organisms, thermal destruction kinetics study was conducted in the lab scale comparing the D values of pathogenic and non-pathogenic strains. In the pilot plant scale studies due to safety reasons, we used *E. coli K-12* a non-pathogenic strain. The D value of same non-pathogenic strain (*E. coli K 12*) was compared with pathogenic *E. coli 30800472* (gram –negative) and *Listeria monocytogenes* 1870 (gram-positive) bacteria. It was found that D-value of non-pathogenic strain was much higher at all the different temperatures (3.21 minutes at 60℃; 0.37 minutes at 65℃; 0.13 minutes at 70℃ at z value of 7.22℃) compared to the D-value of pathogenic strains (*Listeria monocytogenes*: 2.83 minutes at 60℃; 0.24 minutes at 65℃; 0.03 minutes at 70℃ at z value of 7.35℃ and E. coli 30800472: 2.88 minutes at 60℃; 0.32 minutes at 65℃; 0.12 minutes at 70℃ at z value of 5.18℃). This indicates that the inactivation of E. coli K- 12 ensures the inactivation of both pathogenic gram- positive and gram- negative bacteria. The z-values indicates the temperature sensitivity of the micro-organisms.

The study also showed that the presence of air can interfere with the rate/extent of heat transfer to the treated vegetable surfaces, thereby, reducing the microbial decontamination efficacy. It was concluded that ultrasound-steam combination heating is a clear winner in reducing heat treatment time to achieve target surface microbial lethality in vegetables. It is a promising technology for effective surface microbial decontamination of vegetables. Addition of ultrasound to the steam system reduced the surface barrier to heat transfer and enhanced the microbial decontamination efficiency.

As ultrasound-steam technology was successful in rapid short time heating in our first objective, it was further explored in Chapter 4 (second objective) to see if it would be efficient for medium treatment time to inactivate enzymes in fresh vegetables. Enzymes, while essential for physiological processes, can contribute to quality deterioration and spoilage of food products. By subjecting vegetables to ultrasound-steam, the enzymes can be deactivated, prolonging the shelf life, and maintaining the freshness of the produce. Our studies covered the effect of ultrasound on blanching (peroxidase enzyme inactivation) and resultant quality parameters such as texture, color, and ascorbic acid retentions in Chapter 4. Blanching is an important unit operation in which fruit and vegetable undergo a mild heat treatment to inactivate the enzymes prior to freezing, canning, or drying. It involves briefly immersing vegetables in boiling water or steam and then rapidly cooling them. The primary purposes of blanching include enzyme inactivation, microbial reduction, color preservation and texture retention. Using short blanching times often referred to as "minimal blanching", is a technique that aims to achieve these goals while minimizing the negative impact on the quality of fresh vegetables. The reason stands for development of off flavor and discoloration in fruits and vegetable caused by presence of enzymes prior to thermal processing. Enzymes in vegetables are responsible for various biochemical reactions that can lead to color and texture changes, as well as nutrient degradation during storage. Blanching inactivates theses enzymes, thereby preserving the quality of vegetables. However, blanching with hot water can, extensively, damage both texture and color of vegetables. Steam blanching is also long enough to soften the texture of fruits and vegetables in some extent. Earlier studies (Vu et al., 2004; Taherian and Ramaswamy 2009) related the softening of the tissue to the loss of turgor pressure, uptake, or adsorption of water during thermal processing, which can reduce the cohesiveness of the matrix and soften the cell wall and middle lamella in the plant foodstuffs. It was also found that the softening process follows two simultaneous pseudo first-order rate softening mechanisms, one more rapid than the other. The dominant factor which determines residual firmness in thermally treated samples is the slower mechanism since the substrate responsible for the rapid mechanism could be lost during a short exposure to 100°C. In this base if rapid mechanism could be reduced by introduction of ultrasound the main part of texture may be preserved. Our studies showed that the textural qualities (hardness) of the treated vegetables endured less textural damage when blanched with ultrasound assisted steam. However, further studies should be considered to understand the mechanism of ultrasound on softening kinetics of fruits and vegetables. Nutrient loss during blanching is a concern, especially for water-soluble vitamins. Shorter blanching times can help limit nutrient losses while achieving enzyme inactivation (Montero et al., 2019). Rapid cooling after blanching further prevents nutrient degradation. Ultrasound-assisted blanching also showed superior quality for all the tested vegetables in terms of ascorbic acid content retention. Better retention of texture and ascorbic acid content was found in carrots, cauliflower, broccoli, zucchini, and capsicum when treated with ultrasound steam compared to hot water or steam alone treatment and not much difference was observed in terms of color of the treated vegetables. The highest quality losses were associated with hot water blanching in all the vegetables.

The better-quality retention of ultrasound -assisted blanched vegetables is associated with shorter blanching times as vegetables are exposed to heat for shorter durations compared to the ones without ultrasound. Studies on carrots showed that enzyme inactivation of carrots was achieved within 3.11 min when blanched with combined ultrasound-steam in the absence of air and 3.78 min in presence of air. Whereas it took 3.43 min when blanched with steam alone and more than 5 minutes when blanched in the presence of air or with hot water. These values were as follows, respectively for different vegetables: Broccoli - 3.5. 4.3 and 5.0; cauliflower - 5.1, 6.4 and 10.0 min; zucchini - 2.7, 2.8 and 3.0 min; green bell pepper - 2.1, 2.6 and 2.2 min, respectively. These were detailed in Chapter 4.

The established new blanching technique opened a new potential for blanching for enhancing the quality and extending the storage for frozen vegetables as well. Our 3rd objective (Chapter 5) therefore was to study the effect of ultrasound assisted blanching on quality retention of frozen storage vegetables. Freezing is an effective method for preserving the nutritional content of vegetables, particularly when combined with blanching pre-treatment. Freezing food is a common method of food preservation that can dramatically increase the shelf life of vegetables while keeping their nutritional value and quality characteristics. However, the texture, color, and nutritional value of certain vegetables might be affected differently by freezing (Augustin et al., 2015). Vegetables texture can be affected by freezing because ice crystals can form, tear cell walls, and alter textures when thawed. Vegetables may get mushier or softer as a result.

The water content of the vegetable, the cell structure, and the freezing and thawing techniques used all affect how much the texture changes (Toivonen et al., 2008). Enzymatic and non-enzymatic processes are principally responsible for color changes in frozen veggies. Enzymatic reactions can still happen at low temperatures, but more slowly. Color-changing enzymes, such those involved in browning processes, may remain active during freezing and storage. Additionally, the oxygen included in packaging materials might lead to oxidation, which may worsen colour. The best method for preserving veggies' nutritious content is freezing, as opposed to other methods like canning or drying (Wani et al., 2019). Vitamins, minerals, and other nutrients are frequently kept in good condition when food is frozen. However, some nutrients may be lost during blanching (the process before freezing), as water-soluble vitamins may leak into the blanching water. Utilising shorter blanching durations and quick cooling can help to minimise nutrient losses. Even though freezing is typically effective in maintaining the nutritional value of vegetables, it's important to keep in mind that maintaining the quality and nutritional content of frozen produce necessitates proper blanching, rapid freezing, suitable packaging, and controlled storage conditions. Hence, our studies intended to evaluate the effectiveness of combined ultrasound-steam, steam/air and hot water blanching prior to freezing and storage of frozen vegetables. The obtained results indicated that ultrasound steam combination blanching yielded improved color, texture, and ascorbic acid retention compared to conventional hot water and steam blanching methods. The effect of frozen storage time on changes in textural properties of tested vegetables was found to be minimal.

Additionally, the use of ultrasound-steam has shown to enhance heat transfer during the processing of fresh vegetables. The rapid oscillations caused by ultrasound waves create microstreaming and cavitation effects, leading to improved heat distribution and penetration within the vegetable tissues. This phenomenon enhances heat transfer and allows more efficient and uniform heating, consequently, reducing processing times and ensuring consistent product quality. This finding agreed with Bargava et al., (2021) where the review paper mentioned about the enhancement of heat transfer and uniform heating achieved by addition of ultrasound to different food processing unit operations.

To make sure that heat transfer is affected by presence of ultrasound, a study in highly thermal conductive material (aluminum) was conducted in our 4th objective (Chapter 6). The objective was to evaluate the influence of different treatment media such as steam-sound, steam-sound/air, steam/air, steam only, water only, and water-sound mixture on thermal process parameter, f_h value and to study the effect of size and shape on the heat transfer value, under different treatment conditions (https://ifst.onlinelibrary.wiley.com/doi/toc/10.1111/(ISSN)1365-2621. Editor's Choice Transformational Research in Food Science and Technology). The f_h values were lowest for ultrasound assisted treatments compared to the ones without ultrasound. This showed a better rate of heat transfer in the presence of ultrasound. Nylon spheres were also used to simulate the food materials in flexible pouches with different liquids. From these experiments as well, we could confirm that ultrasound assisted treatments had better rate of heat transfer as it showed lower values. Preliminary studies were also conducted at this stage with mango cubes to understand the heat transfer rate when ultrasound is added. The results showed lower f_h values for ultrasound assisted treatment compared to steam alone. Taking these studies as a base of increased rate of heat transfer in ultrasound assisted treatment in pouches studies were further conducted as part of the 5th objective (Chapter 7) to evaluate if ultrasound-steam technology could be successfully used for pasteurization of acid or acidified foods in flexible pouches.

This study (Chapter 7) was conducted to investigate the effect of ultrasound assisted pasteurization on quality and safety aspects of acidic and acidified vegetable. The low acid vegetables such as carrots (pH range of 5.88 to 6.40) should undergo prolonged thermal processing durations to inactivate spores of the key pathogen Clostridium botulinum. Prolong heating could cause a great deal of damage to the quality of the low acid foods (pH>4.6). By converting low-acid foods to "acid" foods, the process regulation from high-temperature sterilization changes to low-temperature pasteurization conditions. Pineapple, with a pH range of 3.2 to 4.1, Red Delicious apple (pH of 3.9) and acidified carrots (pH of 4.0 to 4.2) were selected and pre-packaged in flexible pouches containing processing liquids (sugar solution). The study demonstrated the applicability of ultrasound assisted pasteurization of pre-packaged in flexible containers for carrots in brine solution, apple slices and pineapple wedges in sugar solution. Ultrasound was combined with steam, steam/air, and hot water in separate treatments for carrots, apples and pineapples. These high acid products only need a pasteurization process to make them safe and shelf stable. They are generally treated at temperatures <100oC and thereby receive much lower heat treatment as compared with low acid foods like vegetables, meat, and fish. Even these already low process times can further be reduced by the ultrasound addition to the traditional heat media like steam, steam/air and hot water. A target pasteurization lethality of F90°c = 10 min was employed for all treatment conditions so that they can be meaningfully compared between themselves irrespective of the treatment type and small differences in product heating profiles and temperatures during the treatment. The pasteurization treatment time was significantly reduced for the ultrasound assisted processes as compared to those without. This provided some energy reduction advantages even if there were no quality advantages.

However, the main goal was to develop ultrasound-steam and ultrasound – hot water pasteurization processes, evaluate and compare the quality attributes of the products with conventional methods on equivalent microbial safety basis. Our findings indicated less process time needed for both ultrasound- steam and ultrasound hot-water combination treatments due to the enhancement of heat transfer. Examination of f_h designated a significant difference between steam treatment in presence of air when combined with ultrasound and without ultrasound. The combination promotes rapid and uniform heat distribution through agitation, improving the overall efficiency of the pasteurization process. This ensures that the desired microbial reduction is achieved while preserving the sensory and nutritional qualities of the food products. fh values were lower in mango pieces, pineapple wedges, acidified carrots treated under ultrasound combination with steam and water due to the agitation caused across the package. This indicated better heat transfer rates with added ultrasound which promoted the overall heat transfer to the contents and helped reduce the process time. Better retention of color, texture, total phenolic content, antioxidant activities, ascorbic acid content and total carotenoids of the apple slices, pineapple wedges/acidified carrots were observed when the pouches were subjected to heat treatment in presence of ultrasound as compared to the ones without ultrasound.

It should be noted that only one processing temperature condition and one ultrasound frequency/power was employed in this study. In future, the process could be optimized taking in to account different temperatures and ultrasound intensities.

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Chapter 9

General Conclusions and Future Recommendations

9.1. General Conclusions and Summary

Ultrasound steam technology is a novel technology that can be used to improve the traditional thermal processing to enhance microbial surface decontamination, enzyme inactivation of fresh vegetables, heat transfer enhancement and pasteurization of acid and acidified foods in flexible pouches. Throughout this research we have explored the potential benefits of combining ultrasound with steam or water to provide a better quality and safe treated products. This adds to the advancement in food processing and preservation sector.

Several general conclusions from this research could be summarized as:

- Ultrasound–steam treatment is a clear winner in reducing heat treatment time to achieve target surface microbial lethality and enzyme inactivation in vegetables.
- The study showed that the presence of air can interfere with the rate/extent of heat transfer to the treated vegetable surfaces, thereby, reducing the microbial decontamination and enzyme inactivation efficacy.
- Addition of ultrasound to the steam system reduced the surface barrier to heat transfer and enhanced the microbial decontamination and enzyme inactivation efficiency.
- Studies on carrots, cauliflower, broccoli and zucchini showed that 3-6 log CFU reduction could be achieved within 15s when these vegetables were inoculated with *E.Coli K-12* and subjected to combined ultrasound-steam treatment compared to steam only treatment where 2- 4 log CFU reduction was achieved in 30s.
- In the presence of air (which is common during commercial application), it showed 1-4 log CFU reduction in 15s when subjected to combined ultrasound-steam treatment compared to steam only treatment where up to 1 log CFU reduction was achieved in 30s.
- Ultrasound-assisted blanching showed superior quality for all the tested vegetables in terms of color, texture, and ascorbic acid content retention.

- Studies on carrots showed that enzyme inactivation of carrots was achieved within 3.11 min when blanched with combined ultrasound-steam in the absence of air and 3.78 min in presence of air. Whereas it took 3.43 min when blanched with steam alone and more than 5 minutes when blanched in the presence of air or with hot water. These values were as follows, respectively for different vegetables: Broccoli - 3.5. 4.3 and 5.0; cauliflower - 5.1, 6.4 and 10.0 min; zucchini - 2.7, 2.8 and 3.0 min; green bell pepper - 2.1, 2.6 and 2.2 min, respectively
- Better retention of texture and ascorbic acid content was found in carrots, cauliflower, broccoli, zucchini, and capsicum when treated with ultrasound steam compared to hot water or steam alone treatment and not much difference was observed in terms of color of the treated product.
- The highest quality losses were associated with hot water blanching**.**
- The effect of frozen storage time on changes in textural properties of tested vegetables was minimal.
- f_h values were lower in mango pieces, pineapple wedges, acidified carrots treated under ultrasound combination with steam and water due to the agitation caused across the package. This indicated better heat transfer rates with added ultrasound which promoted the overall heat transfer to the contents and helped reduce the process time.
- Better retention of color, texture, total phenolic content, antioxidant activities, ascorbic acid content and total carotenoids of the pineapple wedges/acidified carrots were observed when the pouches were subjected to heat treatment in presence of ultrasound as compared to the ones without ultrasound.

Overall, the utilization of ultrasound steam technology offers a compelling solution for microbial surface decontamination, enzyme inactivation, and heat transfer enhancement in pasteurization process in flexible pouches. Its efficiency and sustainability make it a valuable tool for ensuring food safety, extending shelf life, and improving overall product quality. As research in this field continues to advance, we can anticipate further innovations and refinements that will revolutionize the way we process and preserve fresh fruits and vegetables, leading to safer, healthier, and more sustainable food systems.

9.2. Recommendations for future research

It is important to acknowledge that while ultrasound steam technology holds immense promise, there are still areas that require further research and development.

- Optimization of operating parameters, such as ultrasound frequency, power, and exposure time, is essential to maximize the efficiency and effectiveness of the technology. This is especially important to reduce blanching time and prevent further loss of texture and color in processed vegetables.
- Additionally, exploring its applicability to different types of vegetables and scaling up the process for commercial production are crucial for its widespread adoption in the industry.
- Future work should be carried out to investigate the quality changes during the frozen storage of the vegetables.
- Shelf-life studies of the blanched vegetables should be carried out for longer duration.
- Shelf-life studies for the products pasteurized in flexible pouches should be carried out.
- In future optimized steam and hot water-ultrasound assisted processes should take advantage of short heating time while considering different sensitivities of food quality attributes to thermal degradation.
- The same type of study can be extended to explore the potential of ultrasound-steam technology under atmospheric conditions for microbial surface decontamination of meat and poultry.

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