EFFECT OF COMBINED TREATMENTS OF ULTRASOUND AND ULTRAVIOLET RADIATION FOR PRESERVATION OF PINEAPPLE JUICE

by

ANJALY M. G.

(2018-18-021)

DEPARTMENT OF PROCESSING AND FOOD ENGINEERING

KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

TAVANUR, MALAPPURAM- 679573

KERALA, INDIA

2021

EFFECT OF COMBINED TREATMENTS OF ULTRASOUND AND ULTRAVIOLET RADIATION FOR PRESERVATION OF PINEAPPLE JUICE

by

ANJALY M.G.

(2018-18-021)

THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF TECHNOLOGY

IN

AGRICULTURAL ENGINEERING

(Agricultural Processing and Food Engineering)

Faculty of Agricultural Engineering and Technology

Kerala Agricultural University

DEPARTMENT OF PROCESSING AND FOOD ENGINEERING

KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

TAVANUR, MALAPPURAM - 679573

KERALA, INDIA

2021

DECLARATION

I, hereby declare that this thesis entitled **"Effect of combined treatments of ultrasound and ultraviolet radiation for preservation of pineapple juice"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Anjaly.

Place: Tavanur **ANJALY M. G.**

Date: 30/6/2021 **(2018-18-021)**

CERTIFICATE

Certified that this thesis entitled **"Effect of combined treatments of ultrasound and ultraviolet radiation for preservation of pineapple juice"** is a record of research work done independently by **Ms. ANJALY M. G. (2018-18-021)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Place: Tavanur

Date: 30/6/2021

Dr. Prince M. V
(Major Advisor, Advisory Committee)
Professor & Head
Dept.of Processing and Food Engineering
KCAET, Tavanur

CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Anjaly M. G (2018-18-021)**, a candidate for the degree of Master of Technology in Agricultural Engineering with major in Agricultural Processing and Food Engineering, agree that the thesis entitled **"Effect of combined treatments of ultrasound and ultraviolet radiation for preservation of pineapple juice"** may be submitted by Ms. Anjaly M. G, in partial fulfilment of the requirement for the degree.

Dr. Prince M.V
(Major Advisor,
Advisory Committee)
Professor and Head,
Department of Processing and Food Engineering
K.C.A.E.T., Tavanur.

Dr. Rajesh G. K.
(Member,
Advisory Committee)
Assistant Professor
Department of Processing and Food Engineering
K.C.A.E.T., Tavanur

Ms. Sreeja R
(Member,
Advisory Committee)
Assistant Professor (Biochemistry)
Department of Processing and Food Engineering
K.C.A.E.T., Tavanur.

(Shivaji K.P.)

Er. Shivaji K. P.
(Member,
Advisory Committee)
Assistant Professor (FMPE)
RARS, Ambalavayal
Kerala Agricultural University
Wayanad - 673 593

ACKNOWLEDGEMENT

While bringing out this thesis to its final form, I came across a number of people whose contributions in various ways helped my field of research and they deserve special thanks. It is a pleasure to express my deep sense of gratitude towards all those who have made it possible for me to complete this project with success.

First and foremost, I bow my heads before God Almighty for the blessings bestowed upon me to materialize this endeavour. I am deeply indebted to the Kerala Agricultural University for providing this opportunity to do the project work.

I would like to express my deep sense of gratitude and indebtedness to my guide, **Dr. Prince M.V.**, Professor and Head, Department of Processing and Food Engineering, K.C.A.E.T, Tavanur for his valuable encouragement, suggestions and support from an early stage of this research and providing me extraordinary experiences throughout the work. Above all, his priceless and meticulous supervision at each and every phase of the work inspired me in innumerable ways. I am proud to record that I had the opportunity to work with an experienced Professor like him.

With extreme pleasure I express my whole hearted gratitude to **Dr. Sathian K. K.**, Dean, Faculty of Agricultural Engineering and Technology for his interest and kind advices given to me at all stages of my study. .

I extend my sincere thanks to **Dr. Rajesh G.K**., Assistant Professor, Department of Processing and Food Engineering, K.C.A.E.T, Tavanur, a member of advisory committee for his guidance. I also owe my sincere thanks to all my Advisory Committee members for their valuable suggestions and guidance during my thesis work. I would also like to express my sincere gratitude to **Ms. Sreeja R.,** Assistant Professor (Bio-chemistry), Department of Processing and Food Engineering K.C.A.E.T, Tavanur and **Er. Shivaji K.P.,** Assistant Professor (FMPE), RARS Ambalavayal, for rendering timely advices and support during the entire period of my research work.

It is my pleasure to offer whole hearted thanks to **Mrs. Jojitha**, Technical assistant of Department of Food and Agricultural Process Engineering, K.C.A.E.T, Tavanur and **Mr. Radhakrishnan, M.V**., **Mrs. Geetha**., Lab Assistants for their

immense help and constant backing at all stages of this research work. I express my heartfelt gratitude to **Mr. Vipin P**., and **Mr. Lenin**., Technicians for their immense help and support throughout the project.

I would also like to thank my classmates and my senior **Er. Ashitha G.N** for their valuable suggestions and helpful discussions. I also express my sincere thanks to all staff members of library, KCAET, Tavanur for their ever willing help and cooperation.

Last but not the least, I must express my gratitude to my parents and family members for being with me at every moment and providing continuous moral boosting and affection during thesis work. This accomplishment would not have been possible without them. I must add that it has been a great experience studying at KCAET campus and all the time spent in this campus will remain in my memory for years to come. I once again express my heartfelt thanks to all those who helped me in completing this venture.

Anjaly M. G.

Dedicated to
My family and teachers

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS USED

°B	:	Brix
et al.	:	and others
Fig.	:	Figure
ha	:	Hectare
Int.	:	International
J.	:	Journal
m^3	:	Cubic metre
mm	:	Millimetre
cm	:	Centimetre
°C	:	Degree Celsius
AC	:	Alternate current
%	:	per cent
&	:	And
ANOVA	:	Anaysis of variance
Cfu/ml	:	Colony forming unit per millilitre
CRD	:	Completely Randomized Design
DMRT	:	Duncan's multiple range test
etc.	:	Etcetera
g	:	Gram
PET	:	Poly Ethylene Terephthate
Hz	:	Hertz
K.C.A.E.T	:	Kelappaji College of Agricultural Engineering and Technology
l/min	:	Litre per minute
M	:	Metre
min	:	Minute(s)
mJ/cm^2	:	Milli Joule per square centimetre

ml	:	Milli litre
NaOH	:	Sodium hydroxide
Nm	:	Nanometre
No.	:	Number
s	:	Second
Sl.	:	Serial
Sci.	:	Science
SPSS	:	Statistical Package for Social Sciences
SS	:	Stainless steel
TSS	:	Total Soluble Solids
UV	:	Ultraviolet
US	:	Ultrasound
V	:	Volt
t/ha	:	tonnes per hectare
Viz.	:	Namely
W/m^2	:	Watts per square metre

Introduction

CHAPTER I

INTRODUCTION

Fruits and vegetables are inevitable part of human diet. They provide essential nutrients like vitamins, minerals, dietary fibre, antioxidants and other phyto nutrients which are essential for maintaining health and metabolism. Frequent consumption of fruits reduces the risk of cardiovascular diseases and cancer. Fruits are easily digestible and have a cleansing effect on blood and digestive system. In recent years considerable attention has been focused on fruits and fruit juices due to these nutritional aspects. Fruit juices play an important role in daily nutrition because of the presence of essential nutrients and ease of consumption.

Pineapple (*Ananas comosus*) is a prime fruit in India. India is the fifth largest producer of pineapple and contributes 9% of global production. The total annual production is estimated to be 1.86 million tons. It is also an important commercial crop in Kerala with production of 3.10 lakh tonnes with productivity 17.6 t/ha (Indian Agristat 2017-18). The fruit is a rich source of vitamin A, vitamin B, vitamin C, and other nutrients.

Pineapple juice is gaining popularity all around the world due to its unique flavour, taste and nutrients. It also provides antioxidants, ascorbic acid, carotenoids, phenolic compounds and flavanoids. Besides, it is a rich source of minerals such as magnesium, calcium, potassium and iron. It also reduces the risk of cardiovascular and other degenerative diseases. The juice contains the digestive enzyme called 'bromelain', which exhibits anti-inflammatory and pharmacological effects and also helps in digestion (Lagnika *et al.*, 2017).

Fresh pineapple juice spoils gradually over time due to contamination of microorganisms and fermentation during storage. Therefore, it has to be processed at the earliest in order to enhance its shelf life. Generally, thermal treatments are used

for preservation of pineapple juice to extend its shelf life. Due to exposure to higher temperature, thermal treatment cause undesirable changes in its organoleptic qualities like flavour, taste, colour and odour. Prolonged heating also adversely effects the nutritional qualities. Present day, consumers are highly conscious about the nutritional qualities of processed foods. The demand for minimally processed food with fresh like nutritional and organoleptic qualities is increasing day by day. Therefore, suitable non thermal processes are to be deviced, developed and adopted in pineapple juice preservation.

Ultraviolet (UV) radiation is one of such alternative non-thermal tool for inactivation of harmful microorganisms. UV radiation can be classified into three regions based on spectral wavelength: UV- A (315 to 400 nm), UV-B (280-315 nm) and UV- C (200 -280 nm). UV – C radiation is found to possess significant germicidal effect at 254 nm. The UV rays at this germicidal wavelength induce changes in the genetic material of microorganisms such as bacteria, viruses, molds, and eventually leads to cell inactivation (Shah *et al.,* 2016). Although UV radiation has considerable potential in disinfection of microbial contamination on surfaces of fresh produce, drinking water and liquid foods, the limitation is its low penetration depth due to shadow effect. UV- C process does not produce chemical residues and it is a low cost operation (Bintsis *et al.,* 2000).

UV – C radiation is a promising technique used for inactivation of bacteria and spores in different fruit juices. Also UV – C radiation is capable to suppress oxidative enzymes such as polyphenol oxidase and peroxidase and thereby prevent browning reaction.

Ultrasound (US) treatment, another non-thermal technology widely applied in food processing for preservation, extraction, homogenization, filtration, degassing, non-destructive testing, cutting, emulsification, and crystallization. Ultrasound is defined as sound waves with frequencies higher than the upper audible range of

human hearing (>20 kHz). Higher-power ultrasound is commonly used in food processing to inactivate microorganisms at lower frequencies (20 to 100 kHz), which is referred to as 'power ultrasound'. The microbial inactivation in US is mainly due to cavitation. Cavitation is the phenomenon of generation, growing and eventual collapse of bubbles during the propagation of US through the product. Cavitation leads to destruction of cells, production of free radicals, formation of shock waves and denaturation of enzymes. US preserves the organoleptic and nutritional qualities of food products. It also offers greater homogeneity in processed product. The efficacy of ultrasound processing relies on the type of microorganism being treated. Gram positive bacteria and spores are comparatively more resistant to ultrasound treatments due to their cell wall structure. Therefore extended treatment time would be essential to render a safe product (Piyasena *et al*., 2003).

It has been reported that US is a suitable strategy to control the growth of spoiling yeast in fruit juices and the effect of treatment depends on duration of treatment and power (Bevilacqua *et al.,* 2014). The treatment reduces microbial population while retaining organoleptic and nutritional qualities of fruit juices.

Although both UV radiation and US treatment has their own capabilities in microbial inactivation, as a preservative method, application of any one method alone would not be efficient enough to kill all microorganisms. It could be hypothised from the above statements that development of an ultrasound assisted ultraviolet radiation system for pineapple juice could result in a hybrid effect in which the microbial destruction is achieved by a synergic mechanism of each technology with less energy, dosage and time. Such a process could produce pineapple juice with increased shelf life with minimum energy, retaining fresh like nutritional and organoleptic characteristics and ensuring safety which can attract remunerative price in the market. Therefore, this research entitled "Effect of combined treatments of Ultrasound and Ultraviolet radiation for preservation of Pineapple juice" was undertaken with the following objectives:

- To develop an ultrasound assisted ultraviolet radiation treatment system for preservation of pineapple juice
- To evaluate the developed system towards the preservation of pineapple juice leading to standardization of the process

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

2.1 PINEAPPLE

Pineapple (*Ananas comosus)* is a prime fruit grown in India. Pineapple is also known as “queen of fruits”. The name pineapple is originated due to its resemblance to pine cones. It belongs to the family Bromeliaceae. It is a wonderful tropical, drought – tolerant perennial plant. Pineapple is a compound fruit having high juice content, vibrant flavours and health benefits. Vitamin A, vitamin C, dietary fiber, potassium, calcium, iron and other micronutrients are abundant in pineapple. Fresh pineapple is a rich source of bromelin used for tenderization of meat. Pineapple can be mainly consumed in the form of fresh slices, juice, canned slices, candy, jam, jelly and squash etc.

2.1.1 Global scenario

Pineapple is considered as the third most important tropical fruit after bananas and mangoes. Pineapple demand has risen over the years. Philippines, Brazil, Thailand, China, India, Indonesia, Nigeria, Costa Rica, and Mexico are the major pineapple producing countries. The first five countries contribute nearly 50% of global production. Costa Rica, Belgium, Ghana, USA, Philippines and France are important exporters of pineapple in the global market. The principal importers are USA, Belgium, France, Italy, Germany, Japan and UK. Due to its exceptional flavour, colour, shape, life cycle and ripeness, MD2 or Dinar pineapple is a strain grown in Costa Rica that has high demand in the international market.

2.1.2 National scenario

India is the fifth largest producer of pineapples in the world. India contributes almost 9% of global production of fresh pineapple. Pineapple is grown in India in an area of 107,000 hectares with a production capacity of 1.86 million tons (Indian

Agristat 2017-2018). India exports pineapples to United Arab, Saudi Arabia, USA, Nepal, Kuwait, Oman, and Maldives. Kew or Giant Kew, Queen and Mauritius are the major varieties cultivated in India. West Bengal, Assam, Karnataka, Tripura, Bihar, Manipur, Meghalaya, Kerala and Arunachal Pradesh are the major states cultivating pineapple. West Bengal is the key producer of pineapple in India contributing 21.5% of national production. , Karnataka, West Bengal, Bihar, Tripura, Assam and Nagaland are the major states having high productivity.

2.1.3 State scenario

In Kerala, pineapple is grown in an area of 17.25 thousand hectares with a production capacity of 3.10 lakh tonnes. The productivity of pineapple in Kerala is around 17.6 t/ha (Indian Agristat 2017-18). Kerala contribute 6% of national production. Climate of Kerala favours pineapple cultivation. Pineapple is grown in Kerala as an inter-crop between rubber and coconut. Pineapple is grown mainly in Ernakulam, Kottayam, Pathanamthitta and some places in the district of Idukki in Kerala. Ernakulam accounts for 60% of area of pineapple cultivation in Kerala. In Kerala, Vazhakulam serves as a pineapple trading centre. Vazhakulam pineapple is very much in demand throughout in India and foreign countries due its sweetness, vibrant flavour and good quality.

2.1.4 Nutritional benefits of pineapple

Pineapple is a highly potential fruit contain a lot of nutrients. It has a vibrant flavour and taste. It is an abundant source of vitamin A, vitamin C and organic acids (Bartolomew *et al*., 1995). It contain carbohydrates, water, crude fiber, protein, and other micronutrients like calcium, potassium, manganese which are essential for maintaining balanced nutrition. Pineapple even referred as a medical diet for person suffering with certain disorders.

In the pineapple, 81.2 to 86.2 % water and 13-19 % total solids are present. 85% of the total solids are carbohydrates, including sucrose, glucose and fructose. Whereas, fiber makes up for 2 -3%. 25 -30% of nitrogenous compounds of pineapple fruit pulps are true proteins. Pineapple is a rich source of bromelain and a lot of other protein digesting enzymes. Bromelain can be taken as a dietary supplement to prevent excessive inflammation, acute sinusitis, excessive blood coagulation, gout, arthritis and certain type of tumour growth. Pineapple is very helpful in treating digestive problems such as constipation and irregular bowel movements. It also helps to eliminate toxins from body.

Pineapple is an abundant source of vitamins. It contains 10 – 25 mg vitamins (Rasid and Hosain, 1987). Pineapple juice contain considerable amount of vitamin C which has antioxidant properties. Vitamin C or ascorbic acid reduces bacterial and viral infections and increase iron absorption. Studies show that half a cup of pineapple juice can provide 50% of the recommended amount of vitamin C per day. Fresh pineapples are rich in calcium, potassium, manganese, sodium, phosphorus, iron and sodium (Farid *et al*., 2015). Manganese is essential for formation of bone and activation of certain enzymes in human body. Copper helps iron absorption, controls blood pressure and regulates heart rate. (Debnath, 2012).

2.1.5 Food uses

Pineapple is a prime tropical fruit with abundant health benefits. Pineapple can be consumed as fresh slices, juice, canned slices and in a wide array of processed products like jam, jelly, candy, squash, vinegar and ice cream etc. Global trade accounts for 50 percent of fresh fruit, 30 percent of canned product and 20 percent of juice concentrate.

In some regions, green pineapple is utilized for preparation of pickles. In Panama, very small pineapples are cut from the plant with some part of stem. The stem is used as a handle. In Malaya, pineapple is also used for preparation of curries

and in some meat dishes. In Puerto Rico and in some other regions pineapple slices subjected to soaking in salt water before consumption. Field ripe fruits are consumed as fresh slices after removal of crown, rind, and eye.

Nata de pina is a famous sweet meat balls in Philippines. It is made by using fermented pineapples. Peoples also prefer pineapple as juice due to its vibrant flavour and taste. The residue is used as livestock feed after juice extraction. The demand of canned pineapple is also increasing gradually. The highest grade pineapple is selected for preparation of canned slices. The skinned and cored fruit slices are canned with sugar syrup. The left over part is used canned juice preparation or also used for preparation of confectionary, or converted into powder. The skin juice prepared from the skin and end portions used for production of vinegar or mixed with molasses for fermentation.

2.2 ULTRAVIOLET

Ultraviolet radiation is a well-established promising technique used for inactivation of microorganisms such as bacteria, fungi and viruses. It is also useful for disinfection of pathogens and reduces food spoilage. Currently thermal processing and preservatives are used to prevent food spoilage. But thermal processing adversely affects colour, flavour, organoleptic and nutritional qualities of food material (Montenegro *et al*., 2002). The FDA ensured that thermal processing of fresh food products such as fruits and vegetables could be replaced by UV treatment (US FDA, 2000).

UV can be classified into different regions in electromagnetic spectrum; UV-A, UV- B and UV-C. The UV-A range is between 400 and 315 nm, resulting in skin lesions and tanning. UV – B ranges from 315- 280 nm. They are responsible for skin burning and skin cancer. UV – C ranges from 280 -200 nm. From the studies it was found that UV – C radiation have germicidal properties. Maximum inactivation occurs at 254 to 264 nm (Choudhary and Bandla, 2012).

2.2.1 UV light sources

There are different types of UV lights used in food processing sector such as mercury lamps, pulsed UV lamps, excimer lamps and LED lamps. The UV light sources are selected based on the requirement of application and economical aspects. In general, traditional low-pressure mercury lamps at 254 nm have been primarily used in the food sector for disinfection and liquid food treatment.

Mercury lamps often considered as the most reliable UV light sources. Mainly three types of mercury lamps are available in market; namely Low pressure mercury lamps, medium pressure mercury lamps and low pressure high output lamps. Low pressure lamps are most commonly used in industries. Low pressure lamps are monochromatic in nature whereas medium pressure lamps are poly chromatic in nature.

In low pressure mercury lamps the nominal total gas pressure ranges from 10^2 to 10^3 Pa at temperature about 40 °C. The emission spectrum is mainly focused about 253.7 nm. This wavelength is incredibly effective in the aspects of germicidal action, since photons are absorbed in this specific wavelength range by the DNA of microorganisms. Low pressure mercury lamps are suitable for juice processing and water treatment according to US FDA regulations (FDA, 2000).

Mercury medium-pressure lamps run at 40 °C at a total gas pressure of 10^4 to 10^6 Pa. In sustainable operating environment, the temperature can increase up to 600 to 800 °C. The emission spectrum ranges from 250 nm to 600 nm, mainly for medium-pressure mercury lamps. Generally Medium pressure mercury lamps are not used for germicidal treatment but the strong UV radiations have high penetration depth. It can be used for oxidation and photo degradation applications by varying gas composition. Low pressure high output lamps are developed recently, for disinfection applications.

UV pulsed lights also used in food industry. Pulsed light have a lot of advantages than continuous sources: high intensity, broad spectrum and instantaneous start. They are highly packed without mercury. Jun *et al*. (2003) reported that in corn meal, pulsed light inactivated Aspergillus niger spores. However, studies also showed that the light produced by pulsed lamps had lower penetration power and produced excess heat in the treatment chamber. UV light efficiently inactivated yeast and E.coli in fresh lettuce. Artes and Allende. (2003) verified that without altering its organoleptic properties, UV light efficiently inactivated yeast and E.coli in fresh lettuce.

Warriner *et al*. (2002) performed Studies on implementations of excimer lamp. UV excimer lamps generate a monochromatic output that can be coupled with gases and tuned to the appropriate wavelength. The benefit of excimer lamps is the ability to operate at a lower surface temperature. The lower temperature requirement reduces fouling behaviour of liquid foods. Excimer lamps were highly efficient in sterilization of packaging carton surfaces.

Light emitting diodes have a lot of advantages like low cost, energy efficiency, prolonged life and easy control. LED does not produce mercury waste. The commercial UV LED lamps produce radiations within wavelength ranges from 240 to 400 nm. UV LEDs are currently accessible on the market in research level and in small amounts.

2.2.2 Effect of UV treatments on microorganism

Ultraviolet processing technique uses non ionizing UV radiation of electromagnetic spectrum for inactivation of pathogens. The microbial inactivation is mainly due to DNA denaturation. DNA absorbs UV lights, leads to cross linking between adjacent pyramidine nucleoside bases such as thymine and cytosine in the same strand (Miller *et al*., 1999). Due to this mutation in nitrogen bases of DNA, replication and transcription processes did not takes place and eventually leading to

cell death. The amount of cross linking and mutation depends on exposure time. To obtain microbial inactivation the product should be subjected to UV irradiation at least 400 J/m^2.

The efficiency of the process mainly depends on the factors such as light source, presence of suspended particles, flow profile, geometric configuration of treatment chamber and type of microorganisms etc. Type of light source is an important factor determines efficiency of the process. The wavelengths of light sources are generally restricted to 254 nm. Low pressure mercury lamps are mainly used in industrial applications.

Similarly geometric configuration of treatment chamber and flow properties are two critical factors determines microbial stability of food products. Bintis *et al*. (2000) reported that UV light debts 30% intensity at 40 cm underneath the surface of distilled water whereas 10 cm below the surface of seawater. So various designs are employed to maintain turbulent flow and thin film cross sectional flow of product to overcome the limitations.

Presence of suspended solids also important factor affects efficiency of the process. Suspended solids cause absorption and scattering of UV lights. Suspended solids block UV light act as a site for aggregation of microorganisms (Christenen and Linden, 2000). Templeton *et al.* (2005) observed that the particles less than 2 µm in diameter are capable of protecting viruses from UV light.

The UV light sensitivity also depends upon the type of microorganisms. The variation in efficiency of UV treatment is mainly due to cell wall structure, thickness, composition, presence of UV absorbing proteins etc. studies shows that bacterial spores and viruses are more resistant to UV light (Koutchma,2009).

Several studies showed that there are repairing mechanisms exist to repair the damaged DNA such as photo reactivation, excision repair, recombinational repair and

inducible error – prone repair (Knudson, 1985). Photo reactivation is a light dependent enzymatic reaction. Photo reactivating enzymes are present in several bacteria. Carson and Peterson (1975) observed subsequent growth of microorganism due to photo reactivation in water after sterilization of water in commercial UV water sterilizer unit.

2.2.3 UV processing equipments

Tran and Farid (2004) developed UV treatment system for orange juice. The reactor consists of vertically fixed glass tube of 50 mm diameter. The orange juice flows down the inner wall of the tube as a thin layer due to the gravitational force, as shown in Figure 2.1.

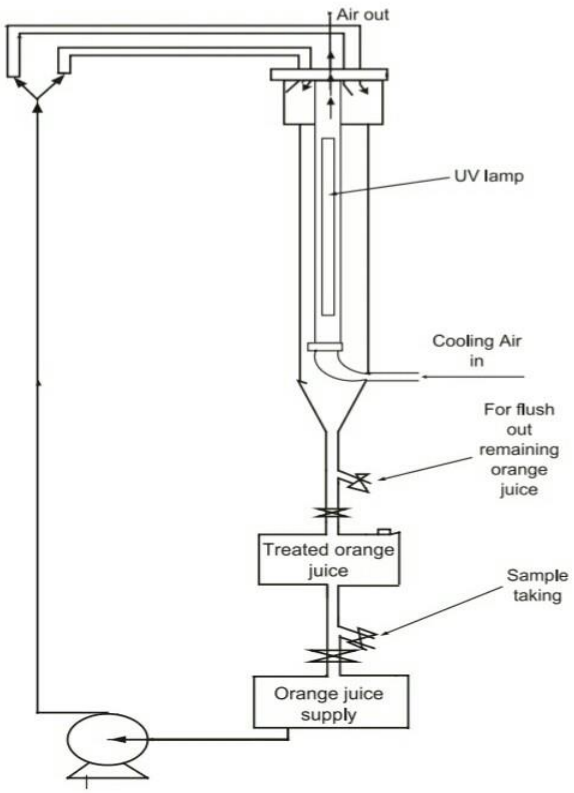


Figure 2.1 UV reactor system for orange juice.

A 30 W low pressure mercury lamp located along the axis of glass tube. The lamp was shielded by a quartz tube which prohibits the direct interaction of lamp with juice. Air was circulated into the annular space that enclosed the lamp. Air circulation facilitates chilling of lamp in order to maintain lamp efficiency. Juice was recirculated by using a peristaltic pump to study the effect of UV doses. The findings show that the physicochemical properties were not significantly altered by UV treatments and energy requirement of UV treatment was comparatively less than the conventional thermal treatment.

Bhullar *et al*. (2018) designed a continuous flow spiral reactor for coconut water (Figure 2.2) It consists of a coiled Teflon tube through which the coconut water was circulated and a middle low pressure mercury lamp of 40W. This system provides adequate mixing and consistent fluence to the feed.

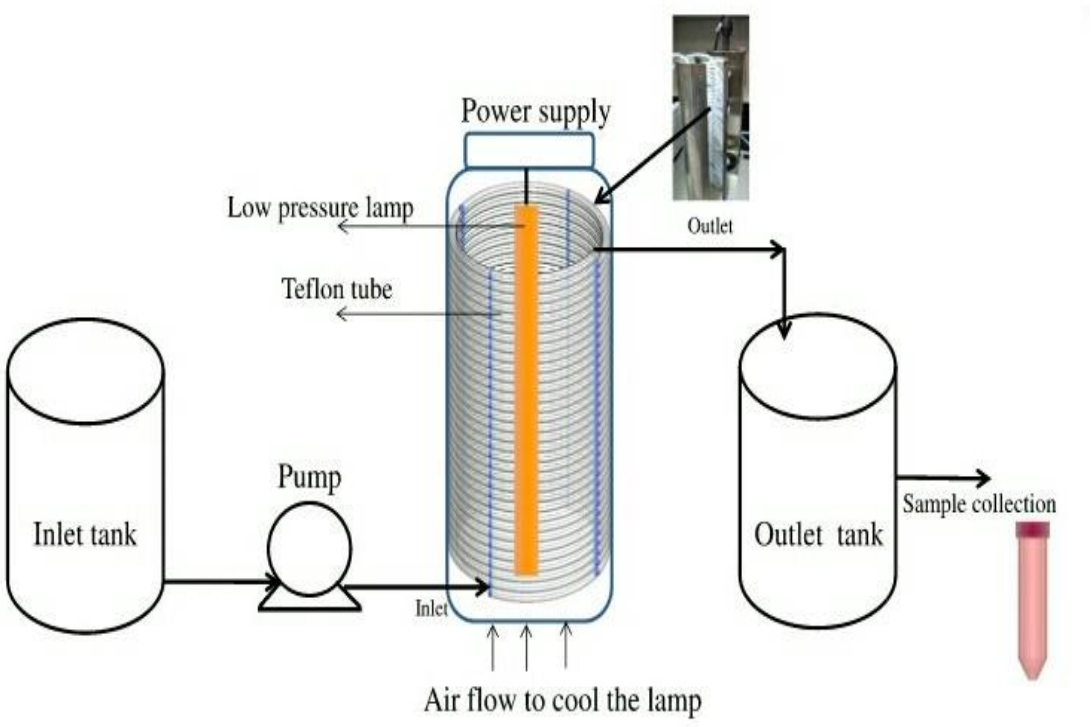


Figure 2.2 UV flow - through treatment system

Tanaka *et al*. (2016) developed a three dimensional UV-C irradiation model for strawberry based on the DO method and sliding mesh technique. The model consist of UV – C lamps installed in parallel with the direction of movement to provide more uniform incident dose distribution over the surface of strawberry without rotation. This model gave three log cycle reduction of *Pencillium digitatum* in 131 seconds without under or over exposure.

2.2.4 UV treatment of liquid foods

UV-C light treatment is a promising technique for inactivation of pathogens in liquid foods like fruit juices and milk. Generally penetration depth of UV in liquid food surfaces is less compared to water (Shama, 1999). UV light's penetration depth in to the juices is about 1 mm (Sizer and Balasubramaniam, 1999). UV-C light radiation's penetration capacity depends on the type of liquid to be treated, its absorptivity, the TSS of the liquid, and the suspended particles. The penetration depth will decrease with increase in solid content. The presences of large suspended particles reduce the incidence of light on the microorganisms (Bintsis *et al*., 2000). In order to overcome this limitation the UV processing equipments are designed carefully to ensure turbulent flow during processing or to maintain thin film of products.

Farid *et al.* (2001) found that due to UV irradiation, the shelf life of orange juice was doubled with a dosage of about 214.2/m2. Similarly Choi and Nielsen (2005) confirmed that, compared to thermally processed samples, UV processed apple cider retained its color and organoleptic qualities. Compared with thermally processed samples, the UV treated samples did not show any noticeable changes in their organoleptic properties during their storage.

Reinemann *et al.* (2006) observed a three-log cycle reduction in the natural flora of milk with a dosage of 1.5 J/ml after UV treatment. The treatment conducted by using two UV reactors. The reactors comprise UV low-pressure mercury lamps

mounted within the sleeves of quartz glass. A stainless steel chamber encircles the entire setup.

Pala *et al*. (2011) investigated the impact of UV- C light on quality attributes of pomegranate juice. The results of the research showed that UV- C treatment maintained anthocyanin content, anti-oxidant activity and other physico chemical properties of pomegranate juice compared to heat treatment. The UV- C treatment also enhanced its shelf life during refrigerated storage.

Experiments conducted by Geveke. (2008) shows that UV- C treatment can be used as an alternative tool for microbial inactivation of apple cider. UV- C treatment resulted 3.4 log reductions in *E. coli k -12* and 2.5 log reduction in *Listeria innocua* with treatment duration of 19 s and 58 s respectively. The process energy requirement was analogous to the heat treatment.

Experiments on the inactivation kinetics of *E. coli* 0157: H7 were carried out by Nagadi *et al.* (2003) in samples of liquid foods such as apple juice and egg white by UV-C treatment. The experiments were conducted with different UV dosage and depth of medium. The findings revealed that inactivation increases with decrease in depth and increase in dosage. More than 5 log cycle reduction was obtained when fluid depth was 1 mm and dosage was about 390 mJ/cm^2.

2.2.5 UV treatment of fresh produce

Surface of fresh produce contain pathogenic bacteria like *E. coli* and *Salmonella*. The presence of this pathogen may constitute a threat to human health. Several studies are reported that UV – C irradiation is also suitable for fresh fruits and vegetables to control post-harvest diseases and to delay ripening. Fresh fruits and vegetables exposed to UV-C treatment before storage to reduce initial microbial load on the surface of products. Park and Kim (2015) applied UV – C light to peeled garlic before storage. UV-C treated garlic remained firm than controls after 15 days of

storage. The treated samples maintained its colour, total flavanoids, antioxidants and other functional properties.

Sclerotinia sclerotirum is the major spoilage microorganism of carrots. Ojaghian *et al*. (2017) conducted experiments to study the efficacy of UV – C light radiation to prevent the spoilage of carrots due to *Sclerotinia sclerotirum.* They found that 5 minutes UV treatment reduced myceliogenic and carpogenic germination of *Sclerotinia sclerotirum.* UV radiations significantly increased the levels of proteins such as phenylalanine ammonia lyase, β-1,3-glucanase and 6- methoxymeliein in carrot roots.

Gogo *et al.* (2017) reported that UV light application at lower dosage (1.7 kJ/m^2) reduced fresh weight loss of African indigenous leafy vegetables during 14 days storage at 20 °C and 0 °C.

Collings *et al*. (2017) observed that UV treatment at lower dosage (<15k J/m^2) enhanced the biochemical profile of black pepper. The increase in biochemical load reduces the amount material required to produce equivalent flavour. The products with higher biochemical load can get higher prices in the market.

Obande *et al.* (2011) conducted experiments on shelf life of tomatoes. The tomatoes initially subjected to UV- C irradiation (8 kJ/m^2) and then inoculated with *Pencilium digitatum.* They observed that after 10 days of storage at 20 °C, the fruits processed with UV were firmer and the fungal lesion diameter was substantially smaller than the controls. The higher resistance to post-harvest diseases of UV treated products was due to the physiological effects induced by UV light. UV causes phytochemicals that have antifungal and antimicrobial effects to accumulate. They act as a strong barrier against invading pathogens. Similarly, Yaun *et al.* (2004) observed a 3.3 log cycle reduction of 240 w/m^2 in apples subjected to Ultra violet treatment.

2.3 ULTRASOUND

Ultrasound is a versatile, nonthermal, novel technology used in food industry Ultrasound is defined as sound waves with frequencies higher than the upper audible range of human hearing (>20 kHz) (Jayasooriya *et al.,* 2004). In nature bats and dolphins use ultrasound to locate their prey. It is possible for ultrasound to move through gas, liquids, and solid materials. Ultrasound propagated through the biological structures in the form of longitudinal waves. They induce compressions and rarefactions in the medium and a huge amount of energy is transmitted to the material. In the food sector, ultrasound is used for different purposes (Dolatoszwski *et al*., 2007).

Based on its frequency and energy intensity, ultrasound can be classified into two types; low power ultrasound and high power ultrasound (Knorr *et al*., 2004). The low power ultrasound (high frequency) applications use small power level (less than $1Wcm^{-2}$) and higher frequencies (greater than 100 kHz). Due to low energy level low power ultrasound do not cause any changes in physiochemical properties of food material hence they widely used as a nondestructive analytical tool in food industries. Low power ultrasound has been used for evaluate texture, composition and physical state of foods (Fellows, 2000). The high power ultrasound (low frequency) has high energy intensity (greater than $1Wcm^{-2}$) and low frequencies usually within the range 18 to 100 kHz (McClements, 1995). High-power ultrasound triggers physical, chemical and mechanical changes due to cavitation in the food products, so they are widely used for different applications such as degassing, crystallization, filtration, enhanced drying, microbial inactivation, enzyme inactivation, homogenization, emulsification, defoaming and meat tenderization (Cho and Irudayaraja, 2003).

2.3.1 Ultrasound generation

Ultrasound production system comprises a generator, transducer and the application system. Generator generates electrical or mechanical energy and

transducer transforms this energy into ultrasound of suitable frequencies. There are primarily three types of transducers: fluid-driven, magnetostrictive, and piezoelectric. The fluid driven transducers produce ultrasound energy by forcing liquid to a thin metallic blade. It is mainly used for mixing and homogenization of food products.

Magnetostrictive transducers are based on magnetostriction effect discovered by joule in 1874. These transducers were made of ferromagnetic material that changed dimensions when the magnetic field was applied, and these changes sought to generate mechanical vibrations after (Raichel, 2000). The efficiency of the system is quite poor, with a 60% conversion to acoustic energy. These setups are basically limited to 30 kHz.

Piezoelectric transducers work on the basis of the piezoelectric effect discovered in the 1800s by Pierre Curie. He reported that when mechanical pressure was applied to asymmetrical crystals like quartz and Rochelle salt electrical signals were produced. Conversely, mechanical vibrations can be produced by employing electrical oscillations to these salts. Paul Langevin performed the first practical examination in 1915 (Cruz *et al.,* 2014). The commonly used piezoelectric materials are lead zirconatetitanate, barium titanate, and lead metaniobate. The piezoelectric transducers are most widely employed equipments and more energy efficient (80%-90% transfer to acoustic energy).

2.3.2 Ultrasonic processing equipments

There are different types of ultrasound devices available in market based on size, geometry, materials of manufacturing, output power, frequencies and capacities. They are mainly divided into laboratory scale and large scale equipments. Large scale equipments are further divided into batch type and flow type devices.

Ultrasonic cleaning baths are the most commonly used laboratory scale equipment. They are economically feasible and mainly used for liquid samples

immersed in water (Manson, 1999). Ultrasonic baths are also used as batch type equipment in industries. Ultrasonic bath operate around 40 kHz or lower frequencies/power to avoid formation of cracks or damages to the tank walls due cavitation. Generally the bath consists of single or multiple transducers at the bottom. The area of transducers coming in contact with medium (liquid/water) should be kept maximum in order to get maximum distribution of energy. Ultrasonic baths also used for cleaning or decontamination in industries eg. For cleaning of chicken shackles in industries to avoid cross contamination (Watson, 1988).

The commonly used another batch type equipment is ultrasound probe device (horn). They are an immersion – type transducers. Generally used for more energy intensive applications and in order to get more homogenous products. The selection of probe is most important factor which determines the success of the application. The volume of sample to be treated, gap between probe apex and bottom of vessel, probe size, probe shape, and material of manufacturing are the major factors to be considered. Probes are generally manufactured using titanium, aluminum alloys or stainless steel. Probes are available in various shapes such as cylindrical, tapered, exponential, stepped, full wave and half wave (Cruz *et al.*, 2014).

Liquid whistle and resonating tube reactors are most common type of flow type reactors. Liquid whistle is the oldest type of device commonly used for emulsification. In liquid whistle the medium is forced under pressure through an orifice using a powerful pump. The medium emerges and expands into a mixing chamber. There are no any other moving parts in a liquid whistle other than a pump so it is a highly durable device.

In resonating tube reactor the liquid is conveyed through a pipe having ultrasonically vibrating walls. Ultrasound energy is readily transmitted to the moving liquid. Stainless steel is mainly used for commercial construction of resonating tube

reactors. They have different cross section like rectangular, pentagonal, hexagonal, circular etc.

2.3.3 Mechanisms of ultrasound processing

Ultrasound moves through the material in the form of longitudinal waves. It induces alternate compression and rarefaction cycles in the medium particles (Povey and Manson, 1998). When power is sufficiently high the rarefaction cycles exceeds the attractive forces of molecules of the medium and form cavitation bubbles. These bubbles grow over the cycles due to rectified diffusion. When the bubbles exceed critical size, they become unstable and leading to intense collapse. The process of bubble formation, growth and collapse is referred as cavitation. Cavitation produces mechanical, chemical and biochemical changes in the medium. It produces localized hot spots, which causes destruction of microorganisms and enzymes. The cavitation bubbles collide with each other and create shock waves which eventually cause inactivation of microorganisms by destruction of cell walls and cell membranes, and DNA denaturation through sonolysis of water. Cavitation also results microstreaming. Microstreaming is a phenomenon, in which the cavitation bubbles produce a vigorous circulatory motion and produce strong eddy currents in the medium. Microstreaming facilitate heat and mass transfer process (Zheng and Sun, 2006).

The effectiveness of cavitation depends on wave characteristics (frequency, intensity), product characteristics, treatment time and environment factors (temperature, pressure). The type of microorganism is also an important factor to be considered. The studies revealed that gram positive bacteria are comparatively resistant to ultrasound due to its cell wall composition and structure.

The microbial inactivation also depends on the environmental factors such as temperature and pressure. Generally ultrasound is combined with other techniques in

order to inactivate resistant microorganism. The different methods of application of ultrasound are given below;

2.3.3.1 Ultrasonication

In this method ultrasound is applied at low temperature.

2.3.3.2 *Thermosonication*

In this method ultrasound is combined with moderate heat in order to get more effective microbial inactivation than normal heat treatments (Manson *et al.*, 1996; Villamiel *et al.,* 1999).

2.3.3.3 *Manosonication*

This treatment is a combination of ultrasound and pressure. Generally use moderate pressure (100 to 300 kPa) at low temperature (Ercan and Soyal, 2013).

2.3.3.4 *Manothermosonication*

In this method heat, ultrasound and pressure applied simultaneously to the product. This method can be used to inactivate microorganisms having high thermal tolerance (Chemat *et al.*, 2011).

2.3.4 Applications of ultrasound in food processing

Ultrasound is widely employed in food processing sector for various unit operations such as degassing, crystallization, filtration, enhanced drying, microbial inactivation, enzyme inactivation, homogenization, emulsification, defoaming and meat tenderization.

2.3.4.1 *Food preservation*

Thermal processing like pasteurization and sterilizations serve as a preservation tool in food industry. In thermal treatments food products are exposed to

higher temperature which causes undesirable changes in food products. It will cause changes in texture, smell, colour and nutritional aspects of food products (Roobab *et al.,* 2018). Now a day's consumer demands for products with fresh like qualities. In order to meet consumer demands food processing sector now looking for non-thermal techniques which increase shelf life and retain nutritional profile of food products.

Ultrasound treatment can be used as promising technique for food processing. It has been proved that ultrasound and its combination techniques can be used for preservation of food products while keeping its nutritional and organoleptic properties with reduced energy usage. It is indeed an environmentally friendly, cost-effective approach that ensures a high degree of safety.

2.3.4.1.1 *Microbial inactivation*

Enzymes and microorganisms such as bacteria and fungus are the major cause of food spoilage. Food products, rich in water and nutrients are an appropriate medium for their growth and multiplication. The mechanism responsible for microbial inactivation is cavitation. Cavitation leads to breakdown of cell walls and membranes and denaturation of DNA. The microstreaming produced by cavitation bubbles also induces stress in microbial cells and leads to cell lysis. Free radicals formed by sonolysis of water also results microbial inactivation.

The major factors determine the extents of microbial destruction are amplitude of US waves, treatment duration, composition and volume of products (Ercan and Soyal, 2013). Nature of microorganism present in the food product also determines effectiveness of the process. Studies have shown that larger cells are much more susceptible than small cells to ultrasound due to the difference in their surface area. Drakopoulou *et al.* (2003) reported that due to the presence of thick cell wall, gram negative bacteria are much more resistant to ultrasound (thick layer of peptidoglycan is present in cell wall of gram positive bacteria). Similarly, spores are

very resistant to ultrasound especially in comparison to vegetative cells because of the difference in the composition and structure of the cell wall.

Experiments performed by Cameron *et al*. (2009) have shown that ultrasound treatment can replace thermal pasteurization of milk. They found that there was a considerable decrease in *E.coli* and *Listeria monocytogenes* after 10 min sonication process and 6 min exposure shows significant decrease in *Pseudomonas fluorescens*in inoculated milk. Odonnel *et al.* (2010) stated that treatments with ultrasound showed limited negative impacts on the quality characteristics of fruit juices such as orange, guava and strawberry. Similarly Aadil *et al.* (2013) stated that overall quality of grape juices was maintained and improved due to sonication treatment. Bevilacqua *et al.* (2014) also observed that sonication is a promising technique to control the growth of spoiling yeast in fruit juices and the treatment effectiveness depends on treatment duration and applied power.

According to studies performed by Valero *et al.* (2007), ultrasound treatment of orange juice (500 kHz, 240w for 15 min) reveals a small degree of microbial inactivation (about 1.08 log cfu/ml) without showing any adverse impact on the quality characteristics of juice such as limonine content, browning and color change. The microbial growth observed during storage studies indicate that ultrasound should combine with other potential methods to get more shelf life.

To increase the efficiency of microbial inactivation, ultrasound can be easily combined with other techniques. Fitriyanti and Narasimhan, (2017) observed that when ultrasound treatment combined with Anti-microbial peptide (AMP) Cecropin $P_{1,}$ there was a considerable reduction in *E. coli* count than individual treatments. They also found that the combination treatment was more economical and efficient than the separate individual treatments. Likewise Lilliard.(1993) conducted experiments to check the effectiveness of sonication treatments on *Salmonella* present in poultry skin using a ultrasound bath. He observed that sonication treatment reduce

the count by 1 -1.5 log cycle. But when the experiment repeated with chilled chlorinated water the count was reduced by 2.5 – 4 log cycles.

Similarly Ferrante *et al.* (2007) found that a combined technique of ultrasound, mild heat and antimicrobial agent decrease the growth and multiplication of *L. monocytogens*in orange juice. Researchers also found out that combined treatment of ultrasound and $Ca(OH)_2$ can replace chlorine treatment to prevent contamination of alfalfa seeds from *Salmonella* and *E.coli* (Scouten and Beuchat, 2002).

Cabeza *et al.* (2005) demonstrated that thermosonication (54 °C for 15 min) can be used for pasteurize surface of intact eggs to eliminate *S.enteritidis.* The process did not affect its nutritional and functional properties such as shelf life, emulsifying and foaming capacities, stability, textural properties of egg white gel, breakage resistance of shell and sensory properties of cooked eggs.

*Brettanomyces bruxellensis*is an important spoilage organism in wine processing. They produce volatile phenols which results in production of off-odours. Gracin *et al*. (2017) found that high power ultrasound treatment (20 kHz with a diameter probe of 12.7 mm) triggered maximum inactivation of *B. bruxellensis* at 43 °C for 3 minutes.

Spores such as Bacillus and Clostridium are generally more resistant to environmental factors, so it is difficult to inactivate spores compared to vegetative cells. Ultrasound is usually combined with pressure and heat to inactivate spores. Raso *et al.* (1998) recorded that 99% of *B. subtils* was inactivated by manosonication (117 μm amplitude) about 500 kPa over 12 min. They also confirmed that amplitude of sonication was an important factor to be considered. They performed Manosonication (20 kHz, 300 kPa) for 42 min at 90 μm destroyed 75% of *B. subtils.* The very same experiment was performed at 150 μm, resulting in 99.9 percent destruction. When temperature increased from 70 to 90 °C in the same experimental

conditions (20 kHz, 300 kPa, 117 µm amplitude) for 6min resulted more efficient microbial destruction.

Pagan *et al*. (1999) examined the influence of pH on sonication treatment. They found that ultrasound processing (20 kHz) at 200 kPa gave more reduction of *Listeria monocytogenes* at pH 4 than pH 7.

2.3.4.1.2 *Enzyme inactivation*

Enzymatic reactions cause food spoilage. Generally heat treatments are used for enzyme inactivation, but thermal treatments reduce the organoleptic and sensory qualities of food products which reduces consumer satisfaction. As a preservation method, non-thermal techniques are being used for this purpose.

Enzyme inactivation takes place due to cavitation in ultrasonic treatments. The first enzyme inactivated by sonication was pepsin around 60 years ago. Since then sonication is used as a promising technique for enzyme inactivation. For the inactivation of enzymes such as glucose oxidase, peroxidase, pectin methyl esterase, protease and lipase, sonification alone or in conjunction with other techniques is used.

All enzymes are generally proteins in nature. Ultrasound creates cavitation bubbles in the medium or products. The violent collapse of these bubbles creates localized hotspots. Cavitation also creates shock waves and microstreaming of liquid. All this effect induces structural changes in the protein's secondary and tertiary structures. Because of this, there will be enzyme denaturation. The free radicals formed due to sonolysis of water will react with amino acids of protein and destroy the biological activity of enzymes (Feng *et al., 2011)*.

Manas *et al.* (2006) reported that US treatment combined with temperature and pressure increased efficiency of inactivation of egg white lysozyme. Similarly Villamiel and Dejong. (2000) observed that normal sonication treatment was not efficient for inactivation of endogenous milk enzymes such as alkaline phosphatase,

G-glutamyl trans peptidase and lactoperoxidase at normal room temperature, but when combined with heat (60 – 70 °C) sonication effectively denatured the proteins. Likewise Lopez *et al.* (1998) observed that inactivation rate of endo polygalacturonases using Manosonication is higher than the heat treatment at 62.5 °C.

Mechanism and effectiveness of enzyme inactivation using ultrasound is different for different enzymes due to the difference in amino acid composition and structural changes (Ozbek and Ulgen, 2000). Lopez and Burgos. (1995) stated that the inactivation of peroxidase by manothermosonication was due to the splitting of the enzyme portion of the prosthetic heme, while lipoxygenase was inactivated by the free radical mechanism by denaturation of protein. Research studies also show that some enzymes are resistant to ultrasound (Sala *et al.*, 1998).

Thermosonication, manosonication, manothermosonication and other combination techniques using ultrasound can replace thermal treatments. Vercet *et al.* (2002) observed 100% inactivation of pectin methyl esterase in tomato puree takes place during sonication treatment at 200 kPa and 70 °C. Lopez *et al.* (1998) reported that application of heat to sonication treatment decrease the D value of enzyme inactivation. They conducted sonification at two separate temperatures of 52 °C and 86 °C and noticed that the D value of pectin methyl esterase decreased from 45 min to 0.85 min and also the D value of poly galactrounase decreased from 20.6 min to 0.24 min. Similar results were obtained from the study of Ercan and Soyal. (2013).They found that rate of inactivation of tomato pectin methyl esterase was increased drastically when temperature was combined with ultrasound.

Researchers also found that manothermosonication resulted enzyme inactivation at lower temperature or in a small treatment time. Vercet *et al*. (2001) observed that D value for heat treatment of pectin methyl esterase present in orange juice at 72 °C was 500 min. It was reduced to 1.2 min by manothermosonication.

The inactivation of enzymes by ultrasound depends on frequency, power, type and concentration of enzyme, pH and temperature. Potapovich *et al.* (2003) investigated the effect of factors such as power, pH, frequency etc. and conducted experiments at four different conditions;

1) 20 kHz frequency and 48 w/cm^2 power
2) 20 kHz frequency and 62 w/cm^2 power
3) 264 kHz frequency and 0.05 w/cm^2 power
4) 264 kHz frequency and 1 w/cm^2 power

In a buffer medium, catalase inactivation was reported to improve with increased power and decline with enzyme concentration. They also reported that inactivation rate increased with increase in frequency due to increased production of free radicals. Ultrasound and combined techniques can be used as a preservation tool to inactivate enzyme as a substitution to heat treatment.

2.3.4.2 *Filtration*

Filtration is a major unit operation in food industry. The conventional methods require replacement of filters or cleaning of filters on certain intervals due to clogging. The application of ultrasound enables the filtration system to operate more efficiently and extend processing time without maintenance. Ultrasound energy increases the flux by reducing concentration polarization. This helps to preserve a frictionless filter without altering filter membrane permeability. It helps to maintain a frictionless surface without affecting permeability of filter membrane. Ultrasonically assisted filtration (usually referred to as acoustic filtration) enhances the life of filter. It is widely employed for waste water treatments and filtration of fruit juices and drinks. It is also broadly used in dairy industry for processing of milk and whey products and separation of milk components (Zisu and Chandrapala, 2015). Acoustic filtration reduces energy requirements for processing of whey solutions with high solid content (Koh *et al*., 2012).

2.3.4.3 *Crystallization*

Crystallization is an important process in food industry used to produce chocolate, butter, margarine, whipped cream and ice cream. Crystallization can be aided in many ways by ultrasound with frequency of approximately of 20-100 kHz; it helps nucleation, monitor crystal growth rate, optimize tiny and even sized crystal production and also avoid surface fouling of newly created crystals (Virone *et al*., 2006). Bund and Pandit (2007) reported that ultrasound can be used to control crystallization process by applying sound energy at nucleation phase. Sonocrystallization reduces induction times and rate of nucleation of fats (Ueno *et al*., 2003).

2.3.4.4 *Freezing*

Freezing is an important preservation tool to increase shelf life of food products. Freezing is a unit operation which converts water molecules into ice crystals. Several studies have shown that ultrasound could be used to increase the freezing rate and also decrease the damage to cells during freezing.

Sun and Li (2003) reported that high power ultrasound treated frozen potatoes shows better cell structure than normal frozen potatoes. During ultrasound assisted freezing cavitation bubbles serve as nuclei for crystal growth which results an increasing freezing rate. Under such freezing conditions, a stable microstructure was attained due to the high filtration rate. The size of ice crystals is lesser than the ice crystals made by conventional method, which reduce in damage of product microstructure. Dette and Janseen (2010) reported that ultra sound assisted freezing preserve the aroma, colour and flavor in concentrated juice. Owing to its shorter freezing time and high product quality, ultrasound assisted freezing was found to be advantageous.

2.3.4.5 *Extraction*

High power ultrasound can be used as a promising technique for improving extraction of bioactive components. The penetration power of solvent into cellular materials increases due to the mechanical effect produced by the cavitation. Sonication also enhances the process of mass transfer and product release due to cell wall destruction.

Hromadkova and Ebringerova (2003) reported that ultrasound assisted extraction increases efficiency and maintain structural and molecular properties of hemicellulose, cellulose, xyloglucanand water soluble xylansectracted from buckwheat hulls.

Wu *et al. (*2001) observed that ultrasound assisted extraction can be conducted at lower temperature and it can be useful for increasing extraction of thermally unstable compounds. Halzhou *et al.* (2008) and Zhang *et al.* (2008) observed that application of power ultrasound in the process of extraction enhanced the yield of soybean and flax seed edible oil.

2.3.4.6 *Drying*

High-power ultrasound is a way to dehydrate food without affecting the important attributes and quality of the product. In ultrasound assisted drying products are dried more rapidly and required lower temperature compared to conventional methods which preserve quality characteristics of the product (Garcia *et al.,* 2009). Ultrasonic energy may be used alone or in conjunction with the other forms of energy, including hot air. The use of high-power ultrasound to dehydrate porous materials can be very useful in the process of handling heat-sensitive materials such as foodstuffs. High power ultrasound waves are capable of influencing mass transfer processes as a result of rising material drying rate. Ultrasonically assisted hot air

drying processes may thus allow lower temperatures or short processing times to be used. Application of ultrasound also increases the heat transfer rate between a solid heated surface and liquid by 30-60% (Ensminger, 1988).

The ultrasound-assisted drying process mechanism involves a series of intense and consecutive compressions and rarefactions in the ultra sound-induced food materials. A very small quantity is released through the surface of the substance with each contraction and then this water is allowed to evaporate by the hot gas stream. Fernandes and Rodrigues (2007) reported that ultrasound pretreatment of banana before drying produces microscopic channels in fruit structure which results an increase in water diffusion rate and reduction in drying time. Studies also shows that the rehydration properties of ultrasound pretreated samples such as banana and pineapples are higher when compared to the untreated samples (Fernandes *et al*., 2008). As a result, this method can be effective for the dehydration of vegetables and fruits without altering their key quality characteristics and nutritional properties.

2.3.4.7 *Emulsification*

Emulsification is a unit operation in which two or more immiscible liquids are dispersed from a mixture together. The energy required to disperse a liquid phase in small droplets in a continuous phase is provided by ultrasound. Collapsing cavitation bubbles induce shock waves with in liquid in the dispersing zone which result in high liquid velocity liquid jets forming. Emulsifiers (surface active substances, surfactants) and stabilizers are used in the emulsion to stabilize the newly formed droplets against coalescence.

The emulsification assisted by ultrasound requires less surfactant to form more stable emulsions. These methods are generally economically feasible, simple to use and integrate with existing manufacturing systems to increase the quality of the final product (Krasulya *et al.,* 2016). Riener *et al*. (2009) reported that the introduction of ultrasound reduced process temperature and time in homogenization.

Similarly chemat *et al*. (2011) found that ultrasonic homogenization at lower frequency ranges from 16 to 100 kHz produced more small, evenly distributed and stable emulsions.

2.3.4.8 *Degassing*

Degassing is an important application in food processing. In degassing operation ultrasound forces suspended gas bubbles from liquids to the surface and release the gas. It reduces the level of dissolved gas (Juan and Juarez, 2010). When sonicating liquids, small cavitation bubbles are created by the sound waves that spread from the radiating surface into the liquid media. A high total surface area is created by the series of tiny bubbles. Even, the bubbles are dispersed well throughout the liquid. The dissolved gases diffuse into vacuum bubbles and enhance the size of the bubbles. The acoustic waves encourage neighboring bubble agglomeration, leading to the rapid growth of bubbles. The ultrasound waves can also assist to shake bubbles off container surfaces and cause smaller bubbles to arise through and expel the trapped gas into the atmosphere just below liquid surface. This technology could be used in the food processing industry to degass carbonated drinks such as beer before bottling. The ultrasound assisted degassing process limits the number of broken bottles and overspill of the drink compared with conventional mechanical agitation.

2.3.4.9 *Cutting*

Ultrasound-assisted cutting improves the quality of the cut surface, decreases the energy needed for cutting and increases the precision of the cut (Schneider *et al.*, 2009). In general, an ultrasonic cutting device comprises of a generator, a transducer, an amplifier and a sonotrode (blade) that is capable of operating frequencies in the low ultrasonic frequency range of 20-100 kHz. Compared to other traditional technologies such as laser cutters and water jet cutters, the induced oscillation at the cutting edge of the sonotrode with specified amplitude resulting quicker and more

accurate cutting due to less mechanical cutting force required (Rawsoff,1998). The cutting blade is made of titanium that is very inert to food and materials that are robust. The vibration actually decreases the resistance to friction on the cutting board. Where the products contain particles that vary in hardness and elasticity from the surrounding mass or when they consist of layers that show substantially different mechanical properties, cutting with ultrasound-excited devices is a potential replacement to traditional cutting.

There are many benefits using ultrasound in food processing operations. The ultrasonic cutting characteristics depend on the food type and condition, e.g. frozen or thawed (Brown *et al.,* 2005). The ultrasound assisted cutting is mainly employed for fragile, fatty, sticky and heterogneous foodstuffs such as cakes, pastry and bakery products and cheeses. Another feature of this method is hygiene improvements, as the vibration avoids the substance from adhering to the blade and thus prevents the growth of micro-organisms on the blade, i.e. ultrasonic vibrations enable 'auto-cleaning' of the blade (Anita Ranai *et al*., 2017).

2.3.4.10 *Meat tenderization*

Ultrasonic meat tenderizing is a fast and simple mechanical process. In kitchens and industrial production lines, ultrasonic meat tenderization is employed effectively. The meat quality relies on the aroma, flavor, appearance, tenderness and juiciness of the meat. Consumer buying behaviour has also revealed that tenderness is the key factor in deciding the quality of meat. Meat tenderness is affected by the configuration of the skeletal muscle and its composition (Jayasooriya *et al*., 2004). Mechanically (e.g. pounding, piercing), thermally (by cooking, grilling, braising) or enzymatically, tenderization could be achieved.

High power ultrasound is an innovative mechanical approach for tenderize meat, such as beef, lamb, pork, and poultry. Ultrasound induces disruption of cell

membrane which results tenderness directly. It also causes release of cathepsins or Ca^{2+} ions from intercellular stores which results tenderness indirectly.

Effectiveness of meat tenderization depends on frequency, intensity, treatment time and temperature. Dickens *et al.* (1991) reported that meat tenderness increased with sonication treatment at frequency around 22 – 40 kHz. Ozuna *et al.* (2013) also stated that diffusion of NaCl and moisture increased due to sonication treatment. The treatment also reduced salting time and improved flavour and shelf life of products.

Studies also resulted that application of ultrasound (25 kHz, 2 w/cm^2) during rigormortis period improved tenderness and water holding capacity of meat samples (Dolatowski, 1999; Dolatowski and Tward, 2004). Research indicates that ultrasound can be used to modify meat properties as a potential tool that will increase consumer satisfaction.

2.3.4.11 *Application of low power ultrasound*

Low power ultrasound (LPU) is widely used as a nondestructive tool for examining physicochemical and structural properties of food materials. It is widely used as a reasonable method to study characteristics of impervious fluids and to identify extraneous matters present in the food without contact (Coupland, 2004).

Ultrasounds propagate through the material in the form of longitudinal waves. Ultrasound waves have their own characteristic properties like velocity, frequency, amplitude, pressure and period. When the US waves interact with materials velocity will change due to absorption and or scattering mechanism (Mc Clements, 2005). The change in velocity measurement can be calculated by Newton – Laplace equation (Blitz, 1963). These velocity measurements are used to determine composition, structure, physical state and phase transitions (Buckin *et al.*, 2002). It is also useful to detect presents of foreign matters and defects in processed food products (Haggstrom and Luukkala, 2001; Leemans and destain, 2009).

Attenuation coefficient and acoustic impedance are other main two factors which can correlate with physical properties of food product. Attenuation coefficient is a property which can correlate with properties like bulk viscosity, compressibility, rheology, microstructure and composition (Dukhin and Goetz, 2001). Acoustic impedance will vary for different products having different densities. The two methods used to track the properties of food materials are pulse-echo techniques and continuous wave techniques.

Low power ultrasound (LPU) is used as a fast and reliable technique in food industry. Low power ultrasound was used for estimating fat, muscle and chemical composition of carcass (Faulkner *et al*., 1990). It also used to study moisture and fat content of cod fillets. Researchers also developed a relationship between temperature dependence of ultrasound and fish composition using pulse echo method (McClements *et al.,* 1987). Benedito *et al.* (2001) conducted experiments to study the composition of raw meat mixtures by measuring ultrasound velocity. They studied the behaviour of ultrasound velocity at different temperature for different components like meat, fat, moisture and protein.

Low power ultrasound also used to check quality of fruits and vegetables. Mizrach *et al*, (1991) conducted experiments to study the ripeness of fruits using attenuation properties of ultrasound. They found that amplitude of ultrasound wave was increased when the peel colour changes from green to yellow. It can be used as an index to check ripeness of fruits.

Physical properties of batters are one of the important factor determine final product quality. Low power ultrasound techniques are employed to check the physical properties (density, viscosity, and rheology) of batter and final products such as cake, doughnut and cupcake Salzar *et al.* (2004) used low power ultrasound to measure batter consistency. They used the correlation between acoustic impedance and batter consistency to measure consistency of different samples.

Low power ultrasound is often used to verify the crystallization of emulsions, which is critical for emulsion consistency determination, such as ice cream, butter, margarine, etc. the phase transition of emulsion cause changes in internal structure, morphological properties and molecular packing. As they move through solids and liquids, the speed of ultrasound can vary. These ultrasound velocity measurement can be used to detect extend of crystallization and phase changes (Awad, 2004; Awad *et al*., 2001; Awad and Sato, 2001). Low power ultrasound can be as nondestructive and effective technique used for analysis of food products.

2.4 COMBINATION TREATMENTS

Novel technologies like pulsed electric field (PEF), ultrasound (US), ultraviolet (UV), high pressure processing (HPP), high intensity light pulses (HILP) and combinations of these treatments are widely used recently for efficient preservation of food products (Irene *et al*., 2011). Effectiveness of combination treatments also depends on product characteristics, technical compatibility and suitability of selected parameters besides microbial inactivation. Product characteristics are a critical factor, as PEF treatment is generally limited to liquid foods, whereas UV treatment is limited to food products and packaging surface disinfection (Barbosa *et al*., 1998; Sizer and Balasubrama- niam, 1999).

Sale *et al*. (1970) reported that mild dose application of gamma radiations before HPP treatment increased the pressure sensitivity of *B.coagulans* spores which resulted increased rate of inactivation spores. Similarly, Craw *et al.* (1996) found that, relative to individual procedures, combined irradiation treatment and HPP obtained a higher rate of inactivation of *Clostridium sporogenes* spores.

Noci *et al.* (2008) observed that the maximum microbial inactivation comparable to high temperatures processing (94 °C) resulted in combined PEF and UV treatment with fresh apple juice. But the quality characteristic of treated juice was superior than

severe heat treated samples. The quality characteristics of combination treatment samples were similar to mild heat treated samples at 72 °C.

Lee *et al*. (2013) investigated the efficiency of combined UV and ohmic heating on microbial inactivation of apple juice. They found that the fabricated combined UV and mild ohmic heating system resulted 6.39 ± 1.30 log cycle reductions in population of *E. coli* K12 in apple juice. The rate of microbial inactivation of combined treatment was significantly higher than individual treatments.

Riberio *et al.* (2009) observed that combined thermosonication and pulsed electric field treatment increased shelf life of orange juice up to 168 days at 25 °C. The samples didn't show any significant changes in its physical properties. Although consistently maintained the microbial counts within the safe level during 168 days of storage.

Gachovska *et al*. (2008) investigated the efficiency of combined UV and PEF treatments on inactivation of *E.coli* in apple juice. They observed that the combined treatment regardless the order of treatment resulted 5.35 and 5.30 log cycle reduction of *E. coli* with PEF treatment (field strength 60 kV/cm) followed by UV treatment (treatment time1.8 s) and UV treatment (treatment time1.8 s) followed by PEF treatment (field strength 60 kV/cm) respectively.

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

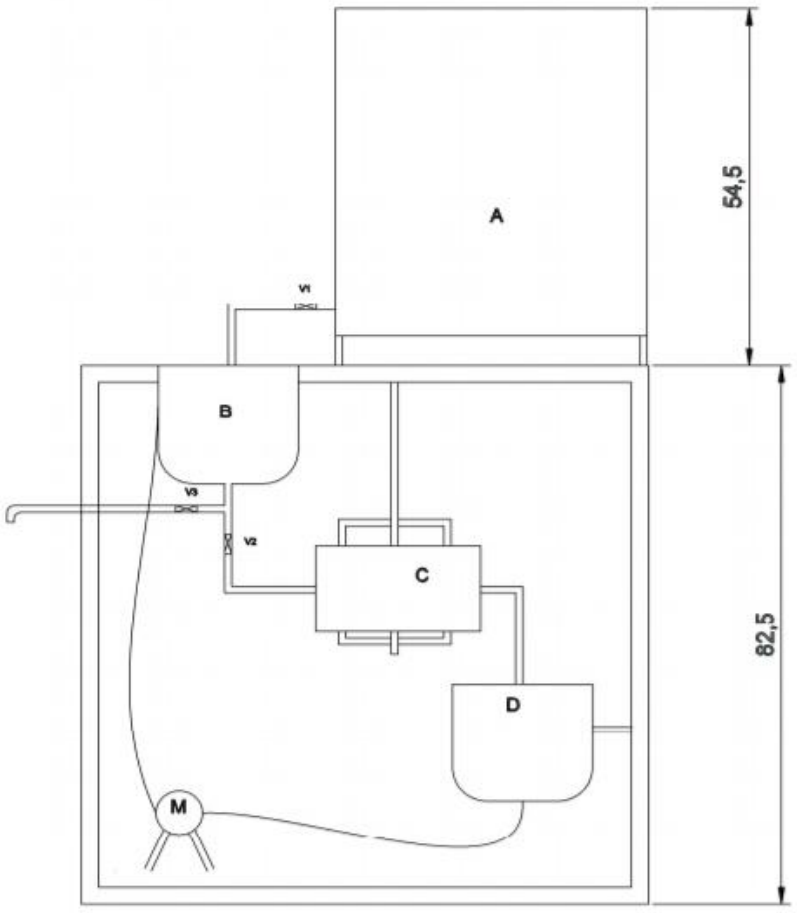
This chapter describes the details of development of an ultrasound assisted ultraviolet radiation treatment system for pineapple juice. The materials used for development, instrumentation and controls for setting various process parameters are also explained. The process of evaluation of the developed system towards preservation of pineapple juice and standardization of process parameters, methodologies for determination of quality of treated samples are also detailed.

3.1 DEVELOPMENT OF A COMBINED ULTRASOUND AND ULTRAVIOLET RADIATION TREATMENT SYSTEM

In this study, a combined US and UV treatment system was conceptualized and developed for preservation of pineapple juice. Although both UV and US have their own potential as a preservation method, the combined application could result in a synergic effect of microbial destruction. Such a process could lead to production of safe, nutritionally and organoleptically superior juice with minimum energy for processing and can attract consumers leading to reduction in post harvest losses.

A small scale combined ultrasound and ultraviolet treatment system was conceptualized and fabricated based on a thorough review of literature on ultrasound and UV radiation system for pumpable fluids. The developed system is shown in Figure 3.1, Figure 3.2 and Figure 3.3. It consists of the following components:

1. Ultrasonic bath with chiller (Sonicator)
2. Intermediate storage tank
3. UV treatment system
4. Circulation system



Part	Name
A	Ultrasonic bath with chiller
B	Intermediate storage tank
C	Ultraviolet treatment system
D	Recirculation tank
M	Motor
V_1, V_2,V_3	Valves

Figure 3.1 Schematic diagram of the Ultrasound assisted UV treatment system

1. US bath with chiller
2. Intermediate storage tank
3. UV treatment system
4. Recirculation tank
5. Motor

Figure 3.2 CAD diagram of developed Ultrasound assisted UV treatment system

Figure 3.3 Developed Ultrasound assisted UV treatment system

3.11 Ultrasonic bath with chiller

Ultrasound is an oscillating sound wave which has a frequency greater than the human hearing threshold (> 20 kHz). It is considered to be a promising technology in food industry. High power US at lower frequencies (20 to 100 kHz) has an ability to produce cavitation, which can be utilized for disruption of microbial cell membranes, leading to microbial inactivation. US preserve nutritional and organoleptic qualities of food products. It also offers greater homogeneity and significant energy savings. To improve inactivation efficacy, US is combined with other treatments such as pressure, heat and antimicrobials.

Ultrasound production system comprises a generator, transducer and the application system. Generator generates electrical or mechanical energy and transducer transforms this energy into ultrasound of suitable frequencies.

Ultrasound treatment was carried out in an ultrasound bath with chiller (Athena technology, Mumbai, model ATSC – 10) operating at a frequency of 33 kHz and output power 250W. The ultrasound bath (Figure 3.4) is made up of stainless steel (SS 304). The length, width and height of bath are 445 mm, 420mm and 545 mm respectively with a capacity of 10 L.

The sonicator consists of advanced MOSFET based SMPS generator and five PZT transducer. High frequency electrical energy is converted into ultrasound waves of required frequency by using piezoelectric sandwich type transducers attached to the base of stainless steel tank. These high-frequency sound waves will produce numerous microscopic vacuum bubbles that grow and collapse briskly. This process is called cavitation. Intense cavitation would result in microbial inactivation. Digital tuning of transducers with generator was applied to avoid frequency shifting during operation.

The compact in-build cooling system in the sonicator helps to maintain desired temperature from 10 to 30 °C. The copper cooling coils around the outer surface of tank, used for refrigerant circulation are properly insulated and connected to a condenser, and compressor assembled in the system, which controls the bath temperature as per the requirement. A PT – 100 simplicon sensor was employed for measurement of temperature. A digital temperature controller with setting 10 to 30 °C and timer with setting of 0 to 99 min is also provided to set the time and temperature of sonication.

The display panel comprised of switches for setting time and temperature, turning ON the ultrasound treatment and chilling. Also two more switches were provided for degassing and pulse sweep power process. The process of auto

degassing was carried out for 5 min, which helps to remove the dissolved gases, which in turn increases effective ultrasonic action. Pulse sweep power process was performed to ensure uniform distribution of ultrasonic energy. An LCD screen display temperature and time. The display also shows the balance time for completion of set cycle. An SET/ACT button is also provided for displaying the actual or set value according to the treatment.

The outlet valve of the US bath (V_1) is connected to a flexible nylon pipe of 12.7 mm diameter so that the juice after US treatment would be supplied to the intermediate storage tank for further supply to UV treatment chamber.

Figure 3.4 Ultrasonic bath with chiller

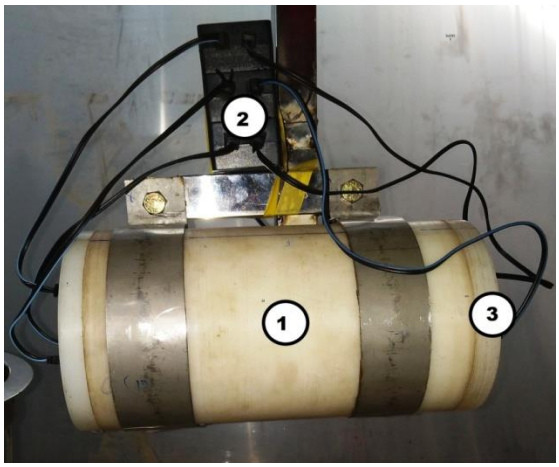
3.1.2 Intermediate storage tank

An intermediate storage tank made up of food grade stainless steel (SS 304) was fixed as shown in Figure 3.1. This tank serves to store the pineapple juice after US treatment and supply the treated juice for further subjecting to UV radiation. The capacity of tank is 5 liters with inner diameter of 200 mm and wall thickness 0.6 mm. The tank is connected to UV treatment chamber via 12.7 mm stainless steel pipe

through valve V_2. Arrangements were also provided to bypass the juice supply so that it can be collected without UV treatment through valve V_3.

3.1.3 Ultra violet treatment system

The ultraviolet treatment system (Figure 3.5) consists of three low pressure mercury lamps of 3 W output power and wavelength 254 nm, a quartz tube and nylon treatment chamber. The treatment chamber was drilled out from a cylindrical nylon block so that it has 130 mm and 100 mm outer and inner diameter respectively. The length of the chamber is 230 mm and its wall thickness 15 mm. The cylindrical chamber accommodates the quartz tube and UV lamps. Both the ends of cylindrical treatment chamber were closed with nylon end caps of 40 mm thickness and 130 mm diameter. At the centre of both end caps, 14 mm holes were drilled which in turn supports the quartz tube.



1. UV treatment chamber
2. UV choke
3. Nylon end cap

Figure 3.5 UV treatment system

A quartz tube of 14 mm and 10 mm outer and inner diameter respectively was installed centrally through the treatment chamber parallel to its longitudinal axis (Figure 3.7). Quartz glass is highly permeable to wavelength of less than 350 nm (Mansor *et al.,* 2015). Therefore, the UV-C radiation would be fully transmitted via the quartz tube. Besides, quartz tube also minimizes direct heat contact from UV lamp to the juice. One end of quartz glass tube forms the inlet and the other end outlet of the treatment chamber.

(a) Low pressure mercury lamp **(b) UV choke**

Figure 3.6 UV treatment chamber accessories

In order for the fruit juice be subjected to UV-C radiation uniformly, three low pressure mercury lamps (Figure 3.6(a)) of 3 W, each with a length of 240 mm were installed 120º apart axially to the chamber at the cylinder inside wall (Figure 3.7). These UV lamps were connected to the 230 V, 50 Hz, AC supply through UV chokes (Poker RO, Aquafresh UV choke). The chokes (Figure 3.6 (b)) are wired in series with the lamps. They endow with a high voltage charge to ionize the mercury. Besides, they reduce the voltage and amperage to an optimum level in order to keep the mercury ionized and to emit a stable stream of UV light. The flow rate of pineapple juice into the UV –C treatment chamber can be controlled by the valve V_3,

The treatment chamber was suitably attached to the main frame on a vertical stainless steel square section (175 mm × 175 mm) through o-clamps (Figure 3.5).

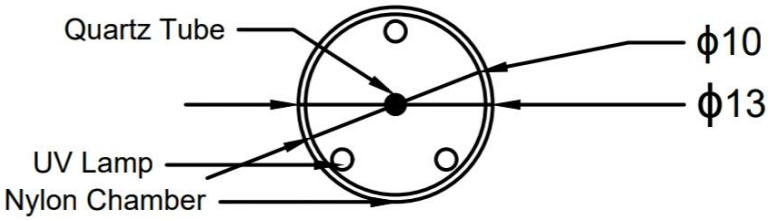


Figure 3.7 Side view showing details inside UV treatment chamber

The Bunsen-Roscoe reciprocity law for photochemical processes stated that the intensity of a photochemical reaction, such as the impact of UV-C radiation on nucleic acid of micro-organism is directly proportional to the total radiant energy dose that reaches the object. The total dosage is the product of the light intensity and treatment time. The overall impact of treatments depends on combination of applied flow rate and exposure time. The dosage was calculated by using the formula suggested by Stevens *et al*. (1999). The intensity of UV irradiation was measured by using UV – C radiometer (model UV C – 254) (Figure 3.8).

$$D = I \times t \qquad 3.1$$

Where,

I = intensity of UV radiation on the surface of quartz tube (W/cm^2)

t = treatment time in seconds

Figure 3.8 UV- C Radiometer

3.1.4 Circulation system

Circulation system consists of a 5 liter stainless steel recirculation tank with diameter and depth of 200 mm and 180 mm respectively, a 24 V DC pump and associated connecting pipes and valves. This system circulates the pineapple juice through the UV- treatment chamber until it subjected to the required dosage for the required time as per the experimental design.

3.2 PREPARATION OF PINEAPPLE JUICE

The developed ultrasound assisted ultraviolet treatment system was evaluated towards the preservation of pineapple juice. Fresh sound pineapples of '*kew*' variety were collected from local market (Tavanur, Malappuram) after visual inspection. Fruits were washed in running tap water. The crown and outer skin of fruits were removed by using sterilized knife. After peeling, fruit slices were cored and then cut in to small pieces. The juice was extracted in a mixer and the juice was collected in sterile stainless steel vessel. The extracted juice was filtered using muslin cloth, collected in sterile PET bottles and stored in refrigerated condition (4±2°C) for conducting experiments.

3.3 EXPERIMENTAL PROCEDURE

The experiments of ultrasound, ultraviolet and combined ultrasound and ultraviolet treatments of pineapple juice samples were conducted at Food Engineering lab, KCAET, Tavanur. The filtered fresh pineapple juice was fed to ultrasound bath with chiller. The electric supply of sonicator was switched on. The temperature was adjusted to 20 °C to maintain the quality of the juice upon US treatment. The required treatment time was configured on configuring unit of display panel. The pineapple juice samples were then subjected to US treatment for required treatment time. The experiments were carried out at three different time intervals *viz.* 10 min, 20 min and 30 min. After ultrasound processing, juice samples were collected in the intermediate storage tank by operating the valve $V_{1.}$ The juice samples were subsequently subjected to ultraviolet treatment. The processed samples were also collected in amber coloured PET bottles and stored in refrigerated condition (4±2°C) for further analysis.

The US treated juice was allowed to flow into UV treatment chamber by opening the valve V_2 (Figure 3.1). The valve would control the flow rate of juice into the UV- chamber. The valves were so opened that the flow rate was 1200 ml/min. The experiments were conducted at three different dosages as per the experimental design obtained by switching on three UV lamps one by one. The lamps were switched on prior to treatment to avoid intensity fluctuations. The juice was UV treated for 10 minutes by recirculation. The UV treated juice was collected in the recirculation tank and was pumped to the intermediate storage tank for further subjecting to UV treatment. For experiments, the juice could be individually and combined treated. The treated samples were collected through valve V_3 and bottled in amber coloured sterile PET bottles and sealed for further analysis. The process flow chart is shown in Figure 3.9.

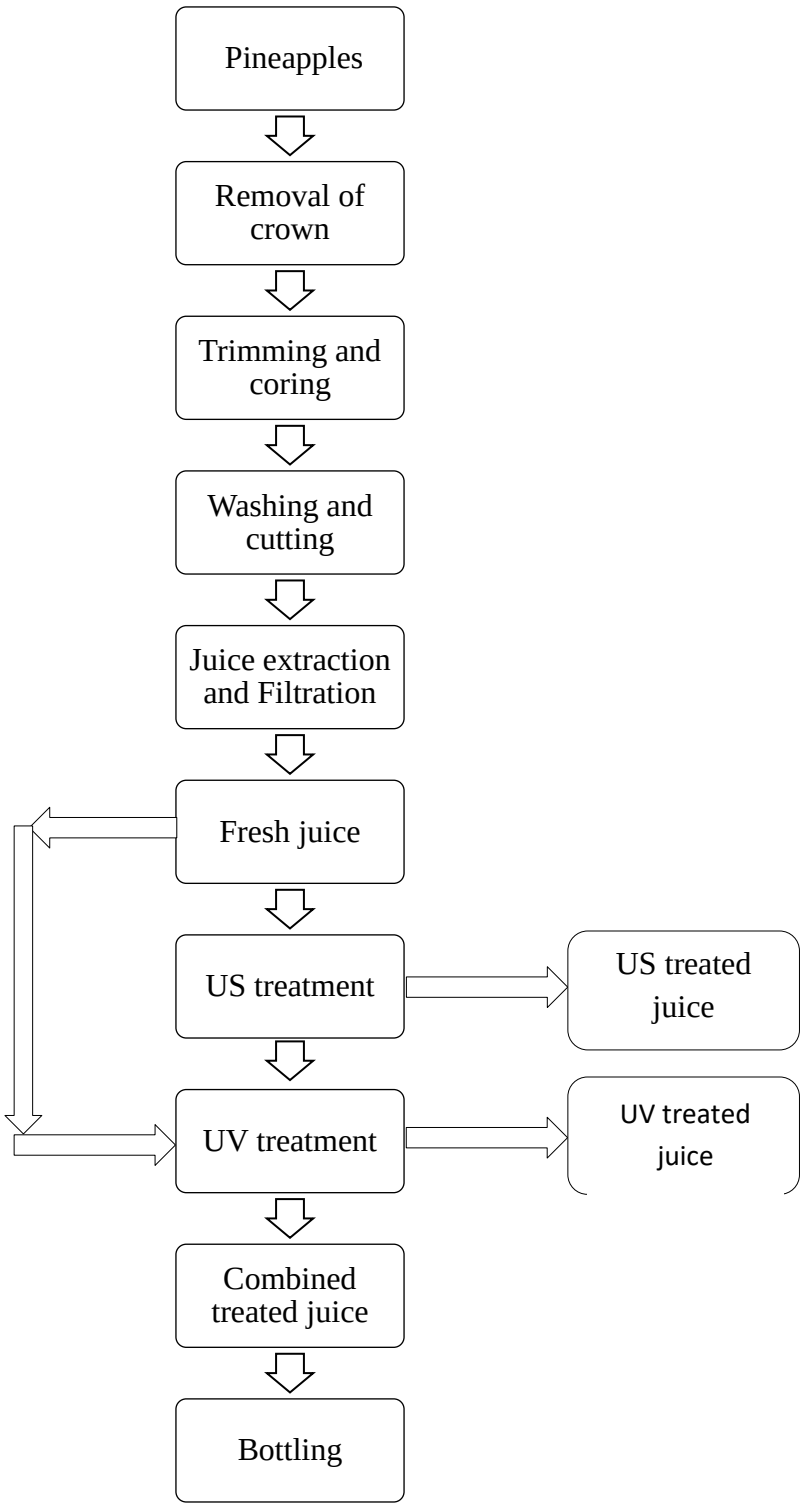


Figure 3.9 Process flow chart for production of treated pineapple juice

3.4 EXPERIMENTAL DESIGN

The entire study was divided into three experiments such as development of ultrasound assisted ultraviolet treatment system, evaluation of combined system leading to standardization of process parameters and organoleptic evaluation of treated juice samples. The effectiveness of developed system towards preservation was evaluated using pineapple juice as raw material. The process parameters of developed system need to be standardized for pineapple juice preservation. The standardized parameters would provide optimum operating conditions for microbial reduction while maintaining its organoleptic and nutritional qualities.

3.4.1 Parameters of the experiment

The process operating parameters which influence the effectiveness of the system such as US exposure time and UV dosage were selected as independent variables based on the review of literature and preliminary studies conducted. The quality characteristics such as total colour difference, pH, TSS, titrable acidity, ascorbic acid content, bacterial reduction and mold reduction were taken as dependent variables.

All the experiments were conducted in triplicate and mean values were taken. The data were statistically analysed by using Analysis of variance (ANOVA) technique. ANOVA is a statistical tool to test the homogeneity of several means. Completely Randomized Design (CRD) method is employed to find out the effect of independent variables on dependent variables. Analysis of variance using Duncan multiple range test (DMRT) was performed to determine the effect of independent variables on dependent variables. DMRT test reduce the possibilities of type 2 error. The treatments and their interactions were compared at $p< 0.05$ level using SPSS software version 20.0.

Table 3.1 Experimental design of US, UV and combined US & UV treatment of pineapple juice

Sl No	Independent variables		Dependent variables
I.	**Fruit juice**	**1 level**	***Physico chemical characteristics***
(a)	Pineapple juice	J_1	(a) Total colour difference
II.	**US exposure time**	**-3 levels**	(b) pH
(a)	10 min	T_1	(c)TSS
(b)	20 min	T_2	(d)Ascorbic acid (vitamin C)
(c)	30 min	T_3	**(**e) Titrable acidity
III.	**UV dosage**	**-3 levels**	***Microbial characteristics***
(a)	1000 mJ/cm^2	T_4	(a) Total plate count (Bacterial count)
(b)	1300 mJ/cm^2	T_5	(b) Total yeast count
(c)	1600 mJ/cm^2	T_6	
IV.	**Combined treatments**	**-9 levels**	
(a)	10 min & 1000 mJ/cm^2	T_7	
(b)	10 min & 1300 mJ/cm^2	T_8	
(c)	10 min & 1600 mJ/cm^2	T_9	
(d)	20 min & 1000 mJ/cm^2	T_{10}	
(e)	20 min &1300 mJ/cm^2	T_{11}	
(f)	20 min & 1600 mJ/cm^2	T_{12}	
(g)	30 min & 1000 mJ/cm^2	T_{13}	
(h)	30 min & 1300 mJ/cm^2	T_{14}	
(i)	30 min & 1600 mJ/cm^2	T_{15}	
	Replication	**3**	
Total number of experiments = (3+3+9)×3 = 45			

3.5 ANALYSIS OF QUALITY CHARACTERISTICS OF PINEAPPLE JUICE

The physicochemical, biochemical and microbiological quality characteristics of fresh, US treated, UV treated and combined US and UV treated pineapple samples were analysed. All experiments were conducted in triplicate and average values were reported.

3.5.1 Physicochemical characteristics

The physicochemical characteristics such as total colour difference, pH, TSS, titrable acidity and ascorbic acid (Vitamin C) content of samples were evaluated.

3.5.1.1 Total colour difference

The total colour difference (ΔE) of fresh as well as treated samples were measured by using Hunter Lab colorimeter (Hunter Association laboratory, Inc., Reston, Virginia, USA; model: Hunter-Lab's Colour Flex EZ) (Figure 3.10). The colorimeter consists of an opaque cover, sample port and display unit. Hunter Lab colorimeter works based on the principle of focusing light to the sample and measuring the energy reflected from it cross the visible spectrum. The mathematical model employed is called Hunter model. The colour of samples were measured based on three colour coordinates namely L^*, a^* and b^* where, L indicates whiteness to darkness, a (+) redness, a (-) greenness, b (+) yellowness and b (-) blueness. The colorimeter is calibrated by using black and white tiles. Δa, Δb and ΔL values indicates the deviation of individual values of treated samples compared to fresh juice sample. The total colour difference (ΔE) was calculated by the equation (Adekunte *et al.,* 2010).

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \qquad 3.2$$

3.5.1.2 pH measurement

The pH is a measure of negative log of the hydrogen ion concentration. It is a measure of free acidity. The pH value of fruit juices effect characteristic flavour and its consumer acceptance. The pH of samples were measured by digital pH meter (m/s SYSTRONICS Ahemedabad, model MK VI) (Figure 3.11). The digital pH meter was standardized with three buffer solutions of pH 4.0, 7.0 and 9.2. The probe was wiped with soft tissue paper before taking readings. The calibrated probe dipped in samples to measure the pH values.

Figure 3.10 Hunter Lab Colorimeter

Figure 3.11 Digital pH meter

3.5.1.3 *Total soluble solids*

A total soluble solid of a juice sample indicates the presence of various soluble chemical substances in it in soluble form. TSS is an indication of sugars present in the juice samples. TSS of treated juice samples were measured using a digital refractometer (Erma, Italy) (Figure 3.12). Calibration of refractometer was carried out employing distilled water. After wiping distilled water with blotting

paper, one drop of sample was placed on measuring port of refractometer and readings were taken.

Figure 3.12 Digital refractometer

3.5.1.4 *Titrable acidity*

Acids are important constituent in fruit juices. Titrable acidity was calculated by using standard titration method (AOAC, 2000). Five milliliters of juice sample were made up to 100 ml with distilled water in a standard flask. From this, 10 ml of sample was pipetted out into a conical flask. Few drops of phenolphthalein was added as an indicator. The sample was titrated against 0.1N NaOH until end point (colour turns into pink) was reached. The percentage of Titrable acidity was calculated using equation 3.3.

Percentage acidity

$$= \frac{\text{Titre value (ml)} \times \text{Normality of alkali} \times \text{Meq weight of citric acid}}{\text{weight of sample taken}} \times 100 \qquad 3.3$$

3.5.1.5 *Ascorbic acid (Vitamin C) content*

Ascorbic acid content was calculated by using titrimetric method using 2, 6 – dichlorophenol indophenol dye as described by Sadasivam and Manickam (1992).

The dye solution was prepared by dissolving 52 mg of 2, 6-dichlorophenol indophenols, and 42 mg of sodium bicarbonate in 200 ml distilled. Standard solution was made by dissolving 100 mg of ascorbic acid into 100 ml of 4% oxalic acid. 10 ml of standard solution was pipetted into a standard flask and made up to 100 ml with 4% oxalic acid so as to prepare a working standard solution. The 5 ml fruit juice samples were made up to 50 ml using 4% oxalic acid. The dye was standardized by titrating against standard ascorbic acid till pale pink colour persists for few minutes. The amount of dye consumed was equal to the amount of ascorbic acid present in the working standard solution (v_1). 10 ml of filtered juice was makeup to 100 ml with 4% oxalic acid. 10 ml of sample from the makeup solution was pipetted out into conical flask. The sample was titrated against dye solution till pale pink colour persists for few minutes. The titration was repeated till concordant values were obtained (V_2). The dye factor and ascorbic acid contents were calculated by using the formula.

$$\text{Dye factor} = \frac{0.5}{\text{Titre value (v1)}} \qquad 3.4$$

$$\text{mg of ascorbic acid}/100 \text{ of sample} = \frac{0.5\text{mg}}{V_1\text{ml}} \text{x} \frac{V_2}{5\text{ ml}} \text{x} \frac{100\ ml}{\text{Wt. of the sample}} \text{x}100 \qquad 3.5$$

3.5.2 Microbiological analysis

The microbial analyses of fresh as well as treated samples were carried out by standard plate count method.

3.5.2.1 *Enumeration of bacterial population*

The bacterial population in juice was analysed by spread plate method (Pollack *et al.*, 2002). The selective media used for bacterial enumeration is nutrient agar. 1ml of fruit juice was pipetted out into sterile test tube containing 9 ml distilled

water which gave 10^{-1} dilution. For a consistent dispersion of microbial cells in water blanks, test tubes were shaken well. 1 ml from 10^{-1}dilution pipetted to 9 ml of distilled water in another test tube to obtain 10^{-2} dilution. The procedure was repeated till 10^{-6} dilutions. For enumeration 0.1 ml of 10^{-4} and 10^{-5} dilutions of fresh juice sample and treated samples were pipetted out into nutrient agar plates and it was spread uniformly using sterilized glass L rod. The dilutions were chosen based on the preliminary studies conducted and a thorough review of literature. The plates were kept in upright position for few minutes. The plates were inverted and incubated at 35 °C for 24 to 48 hours. The colonies were counted after incubation. The average number of bacteria per ml of juice was estimated by using equation 3.6.

$$\text{Plate count } (\text{cfu/ml}) = \frac{\text{Average number of colonies from duplicate plate}}{\text{Dilution factor} \times \text{volume plated}} \qquad 3.6$$

3.5.2.2 ***Enumeration of yeast population***

Yeast is an important spoilage microorganism responsible for spoilage of fruit juices. The selective media used for yeast enumeration is Rosebengal agar. Serial dilutions of samples were prepared as described in 3.5.2.1. For enumeration 0.1 ml of 10^{-3} and 10^{-6} dilutions of fresh juice sample and treated samples were pipetted out into selective media (Rosebengal agar) plates and it was spread uniformly using sterilized glass L rod. The dilutions were chosen based on the preliminary studies conducted. The plates were kept in upright position for few minutes. The plates were inverted and incubated at 22 to 25 °C for 4 to 5 days. The results were expressed in terms of cfu/ml using formula 3.6.

3.6 ORGANOLEPTIC EVALUATION

Organoleptic evaluation is a research methodology used by the senses such as sight, smell, taste, touch and hearing to induce, quantify, analyze and comprehend responses to certain characteristics of food materials. It provides valid and reliable

information of the product. After standardization of parameters, the selected samples of combination treatments and control (fresh juice) were organoleptically evaluated by a panel of untrained judges. Colour, flavour, taste and overall acceptance of samples were evaluated. The assessment was conducted by using hedonic scale of 9 points as per IS 6272: 1991. The sensory parameters of treated juice samples were then compared with fresh juice samples. The score card model is given in appendix B.

3.7 COST ECONOMICS

The total cost of production of combined treated pineapple juice was calculated using standard procedure with suitable assumptions (Appendix C).

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSION

The chapter describes on the development of ultrasound assisted ultraviolet radiation treatment system and results of evaluation of the developed system towards preservation of pineapple juice. The results of the experiments conducted in order to standardize the operating parameters are discussed in detail.

4.1 DEVELOPMENT OF ULTRASOUND ASSISTED ULTRAVIOLET TREATMENT SYSTEM

In order to verify the hypothesis that an ultrasound assisted ultraviolet radiation treatment of pineapple juice would result in a combined effect towards microbial destruction as explained earlier, a system was conceptualised and developed at the college lab.

The ultrasound cum ultraviolet system suitable for fruit juices was fabricated as detailed in section 3.1. The main components of system were ultrasonic bath with chiller (sonicator), intermediate storage tank, ultraviolet treatment system and a circulation system. The US treatment was carried out in an ultrasonic bath with chiller operating with a frequency of 33 kHz. It consists of a generator and five piezoelectric sandwich type transducers which helps to produce intense cavitation. The compact in-build cooling system helps sustain desired temperature from 10 to 30 °C. A digital temperature controller and timer is also employed to set the required temperature and time of treatment. The outlet of sonicator is connected to the intermediate storage tank through a flexible nylon pipe. A stainless steel intermediate storage tank is used to store the pineapple juice after US treatment and supply the treated juice for further subjecting to UV radiation. A separate valve arrangement was also provided to bypass the juice supply without UV treatment. The ultraviolet treatment system consists of three low pressure mercury lamps of 3 W

output power and wavelength 254 nm, a quartz tube and nylon treatment chamber. A quartz tube was installed centrally through the cylindrical nylon chamber parallel to its longitudinal axis. In order to achieve uniform UV treatment, three low pressure mercury lamps of 3 W were installed 120º apart axially to the chamber at the inside cylinder inside wall. The ends of chamber were perfectly sealed by nylon end caps. The UV treatment system was connected to 230 V, 50 Hz input AC supply through UV chokes wired in series with lamps. Circulation system consists of a stainless steel recirculation tank, a 24 V DC pump and associated connecting pipes and valves. This system helps to attain the required dosage by continuous circulation of the pineapple juice through the UV- treatment chamber. The flow of fluids in the system was controlled by means of valves. The developed system is shown in Figure 4.1.

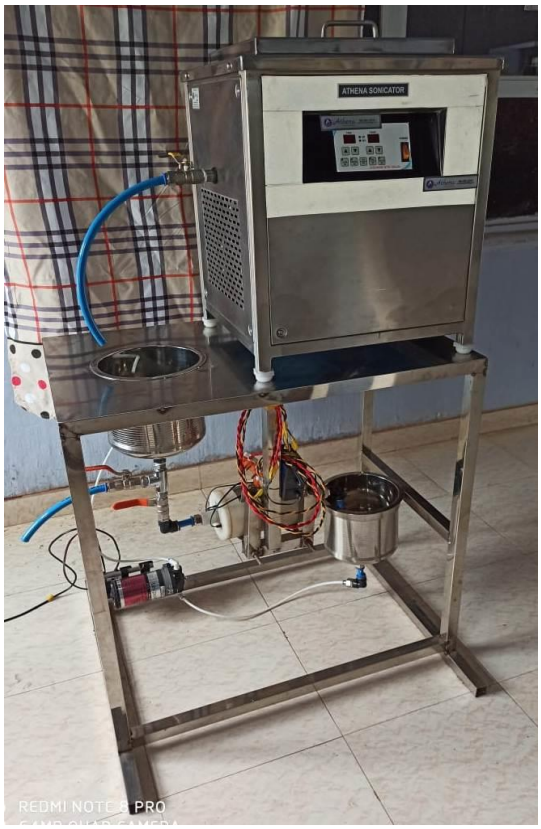


Figure 4.1 Developed Ultrasound assisted UV treatment system

The effectiveness of fabricated system was evaluated using pineapple juice as raw material. The system was designed in such a way that the juice could be subjected to individual ultrasound and ultraviolet and combined treatments through flow control valves.

4.2 STANDARDIZATION OF PROCESS PARAMETERS OF ULTRASOUND ASSISTED ULTRAVIOLET TREATMNT SYSTEM FOR PINEAPPLE JUICE

The standardization of process parameters such as ultrasound treatment time and UV dosages for individual as well as combined treatments for pineapple juice were carried out as detailed in section 3.3. The US exposure time and UV dosage levels was selected for individual and combined treatments based on the preliminary studies conducted in the lab. The data were analysed using SPSS software version 20.0. The Completely Randomized Design (CRD) method was used to determine the interdependency of the variables.

4.2.1 Effects of UV and US treatments on physicochemical properties

The effects of individual and combined treatments on physico - chemical properties of pineapple juice were analysed as per the procedure explained under section 3.5.1. The individual effects of ultrasound and ultraviolet treatments are discussed as follows.

4.2.1.1 *Effects of treatments on colour*

Colour is an important visual indicator to determine quality of juice and also determines the consumer acceptance of fruit juices. The total colour difference in various samples were analysed and presented in Table 4.1.

The experimental results show that individual as well as combined treatments did not show any significant colour change. During individual ultrasound treatments, the total colour difference varied between 0.24 and 0.34, implying that Ultrasound

treatment could not cause any noticeable colour changes in pineapple juice. Similar results were also reported by Aadil *et al.* (2013) during sonication treatment of grape juice. US treatments did not induce any significant effects on colour scoring. It did not promote any non- enzymatic browning reaction which would result brown pigment production and degradation of pigments (Lopez *et al*., 2010).

Table 4.1 Total colour difference in treated juice samples

Sample	Total colour difference (ΔE)
T_1	0.24
T_2	0.26
T_3	0.34
T_4	0.25
T_5	0.30
T_6	0.48
T_7	0.30
T_8	0.42
T_9	0.35
T_{10}	0.45
T_{11}	0.50
T_{12}	0.42
T_{13}	0.54
T_{14}	0.76
T_{15}	0.78

Similarly, as could be observed from Table 4.1 individual UV treatments also could not induce any changes in colour values. Choi and Nielsen (2005) observed that the UV pasteurization maintained superior colour of apple cider than thermal treatment. It could be hypothesized that UV treatment up to dosage 1600 mJ/cm^2 could not have any harmful effects on conjugate bonds and disulphide bonds and could also avoid pigment photo degradation. Therefore individual UV treatments maintained the colour of juice samples. Prolonged UV treatment could accelerate pigment degradation and leads to discolouration of fruit juices (Pala *et al*., 2011)

The total colour difference of combined treatments varied from 0.3 to 0.78. The maximum colour change was observed at a US treatment time 10 min and UV dosage of 1600 mJ/cm^2. It was observed that, combined treatment also could not produce any significant colour change in pineapple juice. The combined effect of ultrasound and ultraviolet radiation resulted in an increased colour change when compared to individual treatment through the colour change was negligible and insignificant statistically. It could be correlated that, the combined treatment resulted in an increased chemical reactions in the juice system but could not contribute to any appreciable pigment degradation and therefore, the natural colour in the juice is maintained.

4.2.1.2 *Effects of treatments on pH of pineapple juice*

pH is an important property of fruit juices. The pH of various samples were analysed and presented in Figure 4.1. The fresh pineapple juice had a pH value about 3.86 ± 0.0055. The pH values of treated juices varied between 3.86 and 3.81. It was found that the individual US and UV and combined treatments could not induce any significant changes in pH value ($p < 0.05$). The pH values were found to be stable even after different treatments. Similar observations were reported by Adekunte *et al.* (2010). They reported that during ultrasound treatment of tomato juice ultrasound could not induce any significant changes in pH of juice irrespective of amplitude and

time of US treatment. Similarly, Bhat *et al.* (2011) stated that ultraviolet treatment also could not produce any significant changes in pH of starfruit juice compared to fresh samples.

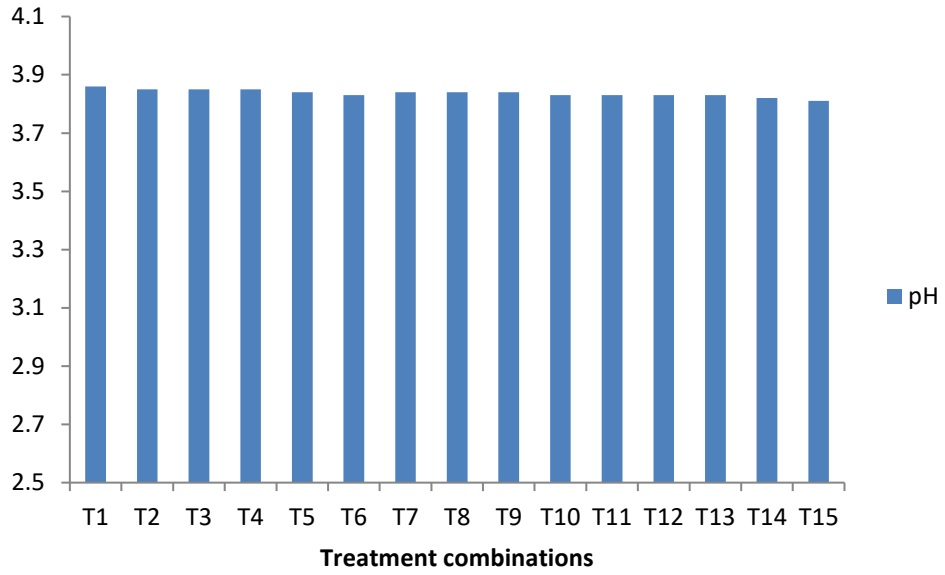


Figure 4.2 Effect of US and UV treatments on pH of pineapple juice

4.2.1.3 *Effects on treatments on TSS of juice*

The effects of treatments on TSS juice were represented in Figure 4.2. The fresh pineapple juice had a TSS 11.6 °Brix. No significant changes were observed in individual and combined treatments ($p < 0.05$). Individual sonication changed TSS values from 11.6 to 11.63 °Brix. The results are in agreement with the observations reported by Tiwari *et al.* (2008) during sonication of orange juice using a 19 mm probe at frequency of 20 kHz. It should be concluded that, sonication was unable to cause the breakdown of organic acids, chemical bonds and cell wall components for up to 30 minutes. Therefore, after treatment, the TSS of the samples remains stable (Yikmis, 2019).

UV treatment increased TSS values from 11.67 to 11.8 °Brix with increase in UV dosages. In combined treatments also TSS values were found to increase from 11.63 to 11.87 °Brix with increase in US treatment time and UV dosage though the changes were insignificant ($p < 0.05$). Aadil *et al.* (2014) also reported similar findings during combined ultrasound and pulsed electric field treatment of fresh grape juice.

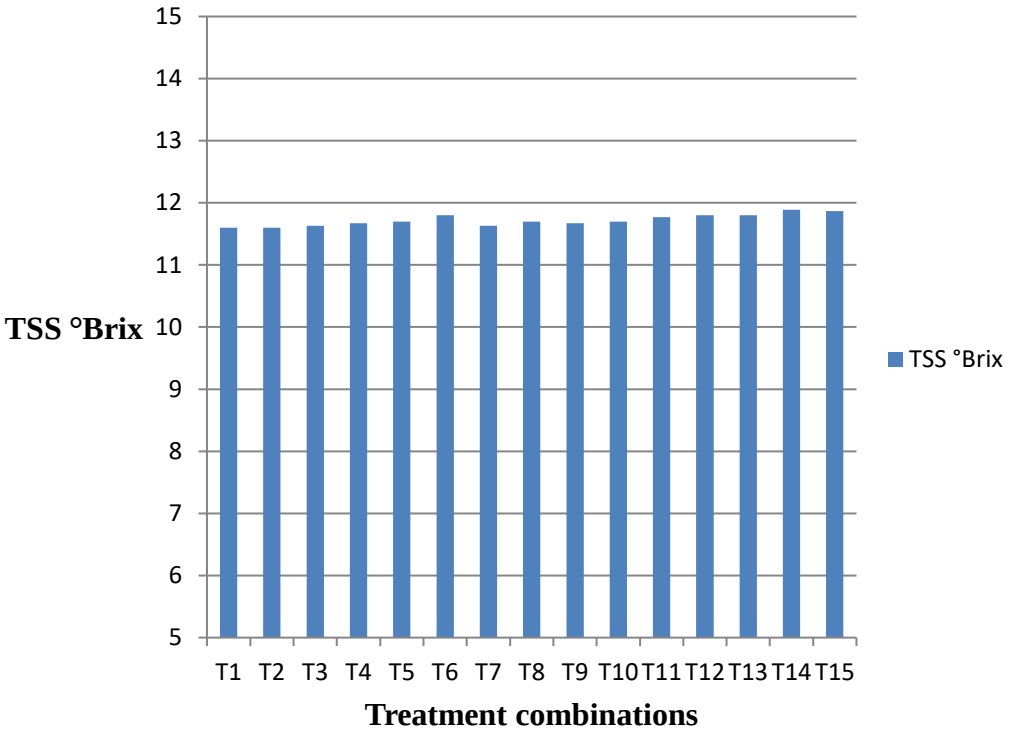


Figure 4.3 Effect of US and UV treatments on TSS of pineapple juice

4.2.1.4 *Effects of treatments on Titrable acidity of pineapple juice*

The Titrable acidity of different samples was given in the Figure.4.3. The fresh juice had titrable acidity around 0.330 ± 0.0003 % citric acid. The titrable

acidity varied from 0.333 to 0.336 % citric acid during individual US treatment and 0.332 to 0.334 %citric acid during individual UV treatment. Whereas on combination treatments as per the experimental design, the variation was between 0.332 to 0.338 % citric acid. Statistically the variations were found to be insignificant and values were close to that of fresh pineapple juice. Similar observations were reported by Noci *et al.* (2008) during combined pulsed electric field and UV treatment of fresh apple juice which recorded no significant changes in titrable acidity and pH.

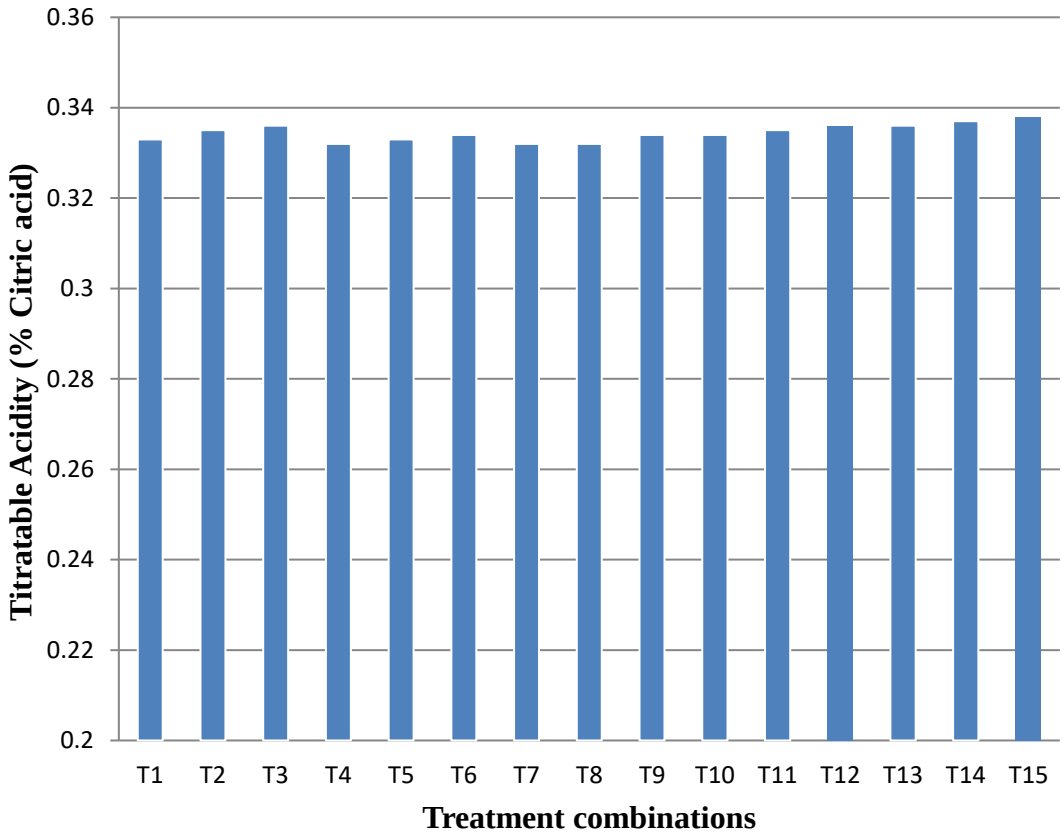


Figure 4.4 Effect of US and UV treatments on titrable acidity of pineapple juice

4.2.1.5 *Effects of treatments on ascorbic acid (Vitamin C) content*

Ascorbic acid (Vitamin C) is a water soluble vitamin present in fruit juices. It is an important nutritional quality indicator to fruit juices. Consumption of ascorbic

acid prevents cardio vascular and cancer diseases. Vitamin C is very sensitive to heat and O_2 presence.

The results of the experiments conducted to estimate ascorbic acid content during US and UV treatments are depicted in Figure 4.4. It may be observed form the results that, during sonication initially ascorbic acid content was found to increase to 46.3 mg of ascorbic acid /100 g of sample from an initial value of 46.1 mg of ascorbic acid /100 g of sample when treatment time was increased form 10 minutes to 2o minutes Followed by a decrease in ascorbic acid content to 45.9 mg of ascorbic acid /100 g of sample when the treatment time was further increased to 30 minutes. Bhat *et al*. (2011) also reported similar observations in sonication of kasturi lime juice. Ultrasound propagation in juice media results in production of compression and rarefaction leading to bubble growth and collapse which ultimately cause cavitation phenomena. During this process entrapped oxygen in the juice gets expelled. The reduced oxygen content minimizes degradation reaction rate which would be the reason for the increased ascorbic acid content per 100 g of juice sample. Sonication for more than 20 min could initiate ascorbic acid content degradation reaction mainly attributed to the formation of hydrogen ions and free radicals due to sonolysis of water (Riesz and Kondo, 1992). Though there is a decrease in ascorbic content after 20 min of sonication, it was found that the ascorbic acid content after 30 min sonication showed almost similar result as that of fresh juice.

Individual UV treatments and combination treatments also were found to have insignificant effect on ascorbic acid content at all treatment combination of experimental design ($p<0.05$). It may also be observed that all the treatment combination preserve vitamin C content when compared to conventional thermal pasteurization wherein a temperature increases to about 90 ℃ would results in about 94% reduction in vitamin C (Akinyele *et al*., 1990).

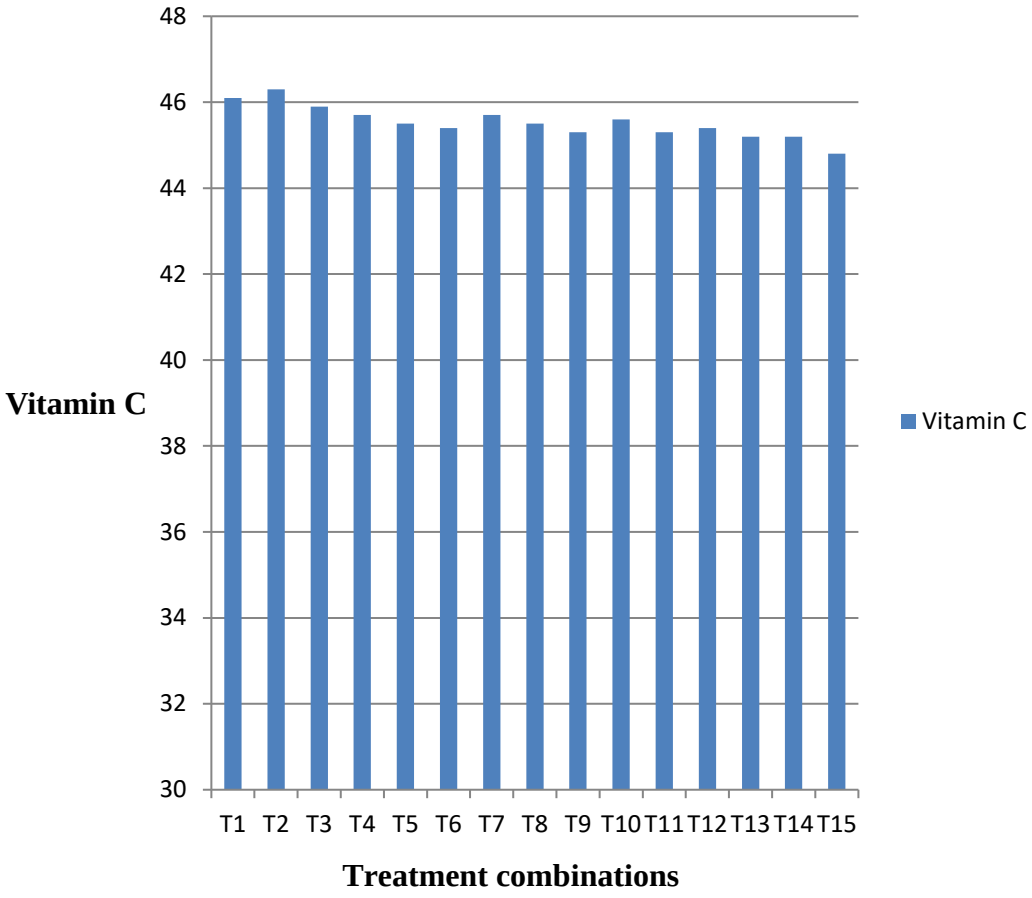


Figure 4.5 Effect of US and UV treatments on Vitamin C of pineapple juice

From the above results, it could be inferred that both individual and combination treatment could not cause any appreciable reduction in dominant quality characteristics of pineapple juice and the treated juice maintained fresh like characteristics even at highest US treatment time and UV dosage either at individual as well as at combined treatment stages. .

4.2.2 Microbial characteristics

In order to assess the microbial spoilage and safety, the treated pineapple juice samples were subjected to microbiological analysis such as bacteria and yeast population.

4.2.2.1 *Bacterial population*

The evaluation of bacterial load in individual and combined US and UV treated samples as per the experimental design were carried out as outlined in section 3.5.2.1 and the results are tabulated in Table 4.2. The bacterial population in the fresh juice samples were also assessed for analysing the bacterial log reduction.

Table 4.2 Bacterial population in treated samples

Treatment	**Time/ dosage (min & mJ cm^{-2})**	**Bacterial log reduction**
Ultrasound treatment	T_1 (10 min)	2.27 ± 0.018
	T_2 (20 min)	2.58 ± 0.019
	T_3 (30 min)	3.60 ± 0.051
Ultraviolet treatment	T_4 (1000 mJ cm^{-2})	3.76 ± 0.021
	T_5 (1300 mJ cm^{-2})	4.02 ± 0.055
	T_6 (1600 mJ cm^{-2})	4.86 ± 0.016
Combination treatment	T_7 (10 min & 1000 mJ/cm^2)	4.52 ± 0.041
	T_8 (10 min & 1300 mJ/cm^2)	4.97 ± 0.029
	T_9 (10 min & 1600 mJ/cm^2)	5.06 ± 0.021
	T_{10} (20 min & 1000 mJ/cm^2)	5.09 ± 0.055
	T_{11} (20 min & 1300 mJ/cm^2)	5.13 ± 0.027
	T_{12} (20 min & 1600 mJ/cm^2)	5.31 ± 0.036
	T_{13} (30 min & 1000 mJ/cm^2)	5.31 ± 0.036
	T_{14} (30 min & 1300 mJ/cm^2)	5.31 ± 0.036
	T_{15} (30 min & 1600 mJ/cm^2)	5.31 ± 0.036

When the pineapple juice subjected to US alone, a bacterial log reduction of up to 3.6 ± 0.051was observed for 30 min treatment time. Though the bacterial reduction increased with increase in treatment time, the treatment could not achieve safe bacterial limit for human consumption.

In the form of longitudinal waves, ultrasound passes through the material and induces cavitation. The implosion of bubbles creates shock waves which eventually cause inactivation of microorganisms by destruction of cell walls and cell membranes, and DNA denaturation through sonolysis of water. Zupanc et al. (2019) reported that gram-positive bacteria are more US-resistant than gram-negative bacteria. This is presumed to be due to cell wall thickness and peptidoglycan presence in the cell wall.

When pineapple juice samples subjected to UV treatment alone, with increase in UV dosage, the bacterial load reduction was found to be increased and reached a value of 4.86 ± 0.016 at UV dosage of 1600 mJ/cm^2. But in these treatments also, the system could not reach safe bacterial population. The microbial inactivation is mainly due to DNA denaturation. DNA absorbs UV lights; leads to cross linking between adjacent pyramidine nucleoside bases such as thymine and cytosine in the same strand. This dimmer formation inhibits the further replication and transcription process and inevitably leads to the death of cells.

Bintsis et al. (2000) reported that even though UV has bactericidal capabilities in liquid food media, it has low penetrating power, especially in turbid pumpable liquids. In order to counteract this reduction in microbial power, either the UV wattage has to be increased or the cross sectional thickness of the flow has to be lowered to obtain a log reduction of acceptable safety. This would lead to increased power consumption or decreased throughput capacity apart from compromising on juice quality. Therefore, in order to achieve a safe microbial limit, a combination of US and UV was taken up for study which could lead to reduction in bacterial load to

safe level simultaneously reducing the intensity of treatment parameters preserving its quality at low energy and increased throughput.

When pineapple juice was subjected to combination treatment, it was revealed that a 5 log reduction could be achieved at a treatment combination of T_9 (10 min & 1600 mJ/cm^2) itself. The decreasing trend continued until T_{12} (20 min & 1600 mJ/cm^2) (Table 4.2) thereafter bacterial load reduction was almost nil. It may be point out that the initial bacterial load was found to be 62×10^6 cfu/ml which got reduced to 3×10^2 cfu/ml at T_{15} combination. According to FDA specification a 5 log reduction in bacterial population in fruit juice could be considered as safe for human consumption (FDA, 1997). Lee *et al*. (2013) reported that when apple juice was subjected to UV assisted ohmic heating at a wavelength of 254 nm and 65 ℃ produced a high reduction of *E. coli* (6.39 ± 1.30) compared to individual UV and ohmic heating treatments.

It may be postulated that synergic effect of combined US and UV treatment resulted high level of inactivation than individual treatments. Two level of inactivation takes place; first ultrasound cavitation leading to destruction of cell membranes and structures and UV exposure resulting in denaturation of DNA leading to cell death.

4.2.3.2 *Yeast population*

The results of the experiments conducted to enumerate the yeast population and thus to found out the yeast log reduction in individual and combined US and UV treated pineapple juice samples are presented in Table 4.3. To have rigorous comparison, the yeast population in fresh pineapple juice were also found.

Table 4.3 Yeast population in treated samples

Treatment	**Time/ dosage (min & mJ cm^{-2})**	**Yeast log reduction**
Ultrasound treatment	T_1 (10 min)	1.87 ± 0.017
	T_2 (20 min)	2.39 ± 0.029
	T_3 (30 min)	2.42 ± 0.036
Ultraviolet treatment	T_4 (1000 mJ cm^{-2})	3.52 ± 0.021
	T_5 (1300 mJ cm^{-2})	3.91 ± 0.018
	T_6 (1600 mJ cm^{-2})	4.46 ± 0.036
Combination treatment	T_7 (10 min & 1000 mJ/cm^2)	4.52 ± 0.041
	T_8 (10 min & 1300 mJ/cm^2)	4.75 ± 0.029
	T_9 (10 min & 1600 mJ/cm^2)	4.81 ± 0.024
	T_{10} (20 min & 1000 mJ/cm^2)	4.89 ± 0.051
	T_{11} (20 min & 1300 mJ/cm^2)	4.94 ± 0.036
	T_{12} (20 min & 1600 mJ/cm^2)	5.08 ± 0.029
	T_{13} (30 min & 1000 mJ/cm^2)	5.08 ± 0.029
	T_{14} (30 min & 1300 mJ/cm^2)	5.08 ± 0.029
	T_{15} (30 min & 1600 mJ/cm^2)	5.08 ± 0.029

It may be derived from the results that US alone treatment could only result in an insignificant reduction in yeast population when subjected to ultrasonication from

10 to 30 min at US frequency of 33 kHz. Yeast showed more resistance to ultrasound treatment than bacteria due to their higher cell wall thickness and composition (Yikmis, 2019). It could be observed that the US alone treatment could only produce a yeast log reduction of 2.42 ± 0.036 which is much less than the safe limit. UV treatment alone resulted in a significant reduction of yeast population ($p< 0.05$) but at the same time could not achieve the safe yeast limit even at the highest experimental dosage of 1600 mJ/cm^2 (4.46 ± 0.036). The population reduced from 49×10^6 cfu/ml to 17×10^2 cfu/ml.

As may be seen from the results the combined treatments of pineapple juice, the yeast population showed a 5.08 ± 0.029 log reduction at treatment T_{12} (US treatment of 20 min & UV dosage of 1600 mJ/cm^2). Further increase in US time and UV dosage combination could not yield any reduction in yeast population. According to FDA specification, a 5 log reduction in yeast population in liquid food could be considered safe for human consumption (FDA, 1997). It may also be noted that, the treatment T_{12} could reduce the yeast population from an initial level of 49×10^6 cfu/ml to 3×10^2 cfu/ml.

4.3 OPTIMIZATION OF PARAMETERS

In order to analyse the effect of the chosen process variables of US and UV treatments of individual and combined treatments in the physico – chemical and microbial characteristics and thus to standardise the operating parameters, statistical optimization was carried out using SPSS software version 20.0. The results are presented in Table 4.4.

Table 4.4 Process parameters for obtaining best quality pineapple juice

Quality characteristics	Best treatment
Colour	The changes are insignificant. The maximum variation with respect to control was shown by $T_{15.}$
pH	All treatments shows minimum variations
TSS	The changes are insignificant. The maximum variation with respect to control shown by T_{14} and $T_{15.}$
Titrable acidity	All treatment combinations are on par into fresh juice.
Vitamin C	All treatments are insignificant. The maximum variation with respect to control shown by $T_{15.}$
Bacterial population	All US and UV combination treatments except T_7 and T_8 resulted 5 log cycle reductions in bacterial load.
Yeast population	The US and UV combination treatments T_{12} to T_{15} resulted 5 log cycle reductions in yeast count.

It may be deduced from the table that the individual and combined US and UV treatments could not induce any appreciable changes in physico chemical characteristics of pineapple juice. However, treatment T_{15} showed highest variation in colour, TSS and vitamin C and treatment T_{14} in TSS, though the intensity of the variations with respect to the control was minimum. On the other hand the individual US and UV treatments could not achieve the desired minimum bacterial and yeast reduction of 5 log cycle with in the selected process parameter limits and therefore could be eliminated.

In combination treatments (T_7 -T_{15}), treatments T_7 and T_8 and treatments T_7 – T_{11} could not achieve 5 log cycle reduction in bacterial and yeast population respectively. T_{12} -T_{15} could be treated as bacteriologically safe treatments. But as stated above, treatment T_{14} and T_{15} showed highest variation in terms of colour, TSS

and vitamin C content with respect to control though the variations are minimum. Also T_{14} and T_{15} consumed highest US treatment times and UV dosages compared to T_{12} and T_{13} which would result in high energy consumption and low throughput and would not be cost effective. Therefore US and UV treatment combinations T_{12} (20 min & 1600 mJ/cm^2) and T_{13} (30 min & 1000 mJ/cm^2) could be taken up for final organoleptic evaluation.

4.4 ORGANOLEPTIC EVALUATION

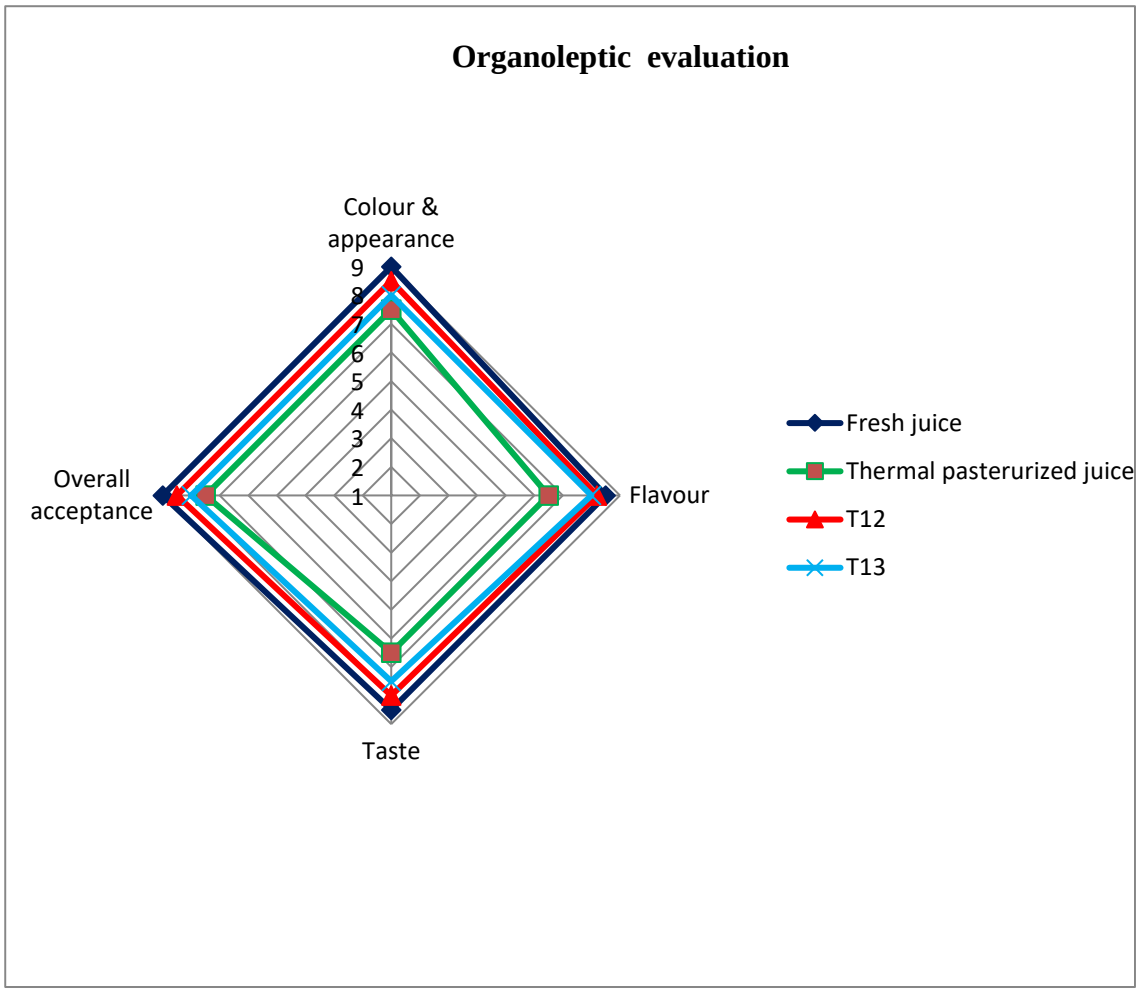
The combined US and UV treated samples under optimized operating conditions such as T_{12} and T_{13} were analysed for its sensory acceptance through organoleptic analysis as per the procedure explained in section 3.6. The treated compared with fresh and thermally pasteurized pineapple juice. For thermal pasteurization, pineapple juice was heated at 80 ℃ for 15 min and further cooled to ambient temperature (Lagnika *et al*.,2017). The mean sensory scores of important characteristics like colour and appearance, flavour, taste and overall acceptance given by a panel of judges are represented in Table 4.5. The radar chart showing variations in scores are given in Figure 4.5.

Table 4.5 Mean sensory scores of samples

Sample	Colour and appearance	Flavour	Taste	Overall acceptance
Fresh juice	9	8.5	8.5	9
Thermal pasteurized juice	7.5	6.5	6.5	7.5
T_{12} (US 20min& UV 1600 mJcm^{-2})	8.5	8.2	8	8.5
T_{13} (US 30min& UV 1000 mJcm^{-2})	8	8	7.5	8

It could be concluded from the results that combined US and UV treatment with a US exposure time of 20 min and UV dosage 1600 mJcm^{-2} retained all

organoleptic qualities close to that of fresh juice. The other optimized sample (T_{13}) showed less preference in terms of sensory characteristics with respect to fresh juice. The sample (T_{13}) which gave almost similar results in terms of physicochemical and microbial characteristics could be eliminated based on sensory characteristics. It could thus be derived that a combined US and UV treatment with a US treatment time of 20 min and UV dosage of 1600 $mJcm^{-2}$ (T_{12}) may be adjudged as the best treatment combination in terms of nutritional, microbiological and sensory qualities



.

Figure 4.6 Variation of organoleptic scores of samples

4.5 COST ECONOMICS

The cost of production of pineapple juice employing the combined US and UV treatment under optimized treatment parameters of 20 min of US treatment time and a UV dosage of 1600 mJ/cm^2 were calculated based on the standard procedure detailed in Appendix C. It was found that the cost of production of one litre pineapple juice was estimated to be Rs. 119.95. The cost of thermally processed pineapple juice available in the market is Rs. 175/litre.

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

Fruit juices are an important part of human diet. They provide essential vitamins, minerals and other nutrients which are essential for maintaining balanced health. Generally thermal treatments used as a common method of preservation for fruit juices. Due to exposure to higher temperature it adversely affects nutritional as well as sensory qualities of juices. Therefore, demand for alternative method which preserves the nutritional and sensory qualities of fruit juices increasing day by day. Non thermal techniques can play a major role in fruit juice preservation in future.

Ultraviolet (UV) is alternative tool for preservation. UV - C radiation at 254 nm has a significant germicidal effect. UV radiation inactivates microorganisms by DNA denaturation. During processing genetic materials absorb radiations, leading to cross linking between adjacent pyramidine nucleoside bases such as thymine and cytosine in the same strand. This mutation prevents DNA replication and transcription process, leading to cell death. UV irradiation is used for disinfection of water and liquid food products. The application of UV radiation is limited due to its low penetration depth.

Ultrasound (US) is another promising technique which can be employed for preservation of fruit food products. US is a form of energy produced by sound waves having frequency more than 20 kHz. US when passed through the material in the form of longitudinal waves, form series of compression and rarefaction waves. At sufficient high - power bubbles develop in the medium. The bubbles grow over the period of few cycles to critical size and violently collapse. This phenomenon is called cavitation. The shock waves produced by cavitation inactivate microorganisms by breaking down of cell walls and thinning of cell membranes. Free radicals formed

due to sonolysis of water and micro streaming are also relevant in microbial inactivation.

Even though UV radiation and US treatment has their own potential as a preservative method, but application of any single treatment would not be competent enough to kill all microorganism. This study was intended to develop an ultrasound assisted ultraviolet system for pineapple juice preservation and evaluation of developed system to produce a good quality microbiologically safe product.

An ultrasound assisted ultraviolet treatment system was conceptualized, further refined and then developed. The system consists of ultrasonic bath with chiller (Sonicator), storage tank, ultraviolet treatment system and recirculation system.

Ultrasound treatment was carried out by using ultrasound bath with chiller (Athena technology, model ATSC – 10) operating at a frequency 33 kHz and power 250 W. The ultrasound bath is made up of stainless steel (SS 304). Piezoelectric transducer was placed at the bottom of the tank that produced ultrasound waves. Digital time and temperature controller were also provided. The treatments were carried out with three different time intervals 10 min, 20 min and 30 min.

The UV treatment chamber was made up of cylindrical nylon tube having 100mm inner diameter, 130 mm outer diameter and 230 mm length. The ends of tube were perfectly sealed by nylon caps. A quartz tube of 10 mm inner diameter and 14 mm outer diameter passed through the centre of chamber. During ultraviolet processing, juice flow through the quartz tube. Three low pressure mercury lamps of 11 W output power and 254 nm wavelength placed at an angle 120^{o} from the centre. The flow through the system was regulated by means of valves. The experiments were carried out with three different dosages 1000 mJ/cm^2, 1300 mJ/cm^2, and 1600 mJ/cm^2. The dosages were varied by changing the no of light sources. The dosage of treatment would increases with increase in number of light sources.

Fresh juice was fed to ultrasound bath and temperature was adjusted to 20 °C. After ultrasound processing juice samples were transferred to storage tank and sequentially subjected to ultraviolet treatment. Pineapple juice was exposed to both individual and combined treatments as per the fixed US treatment time and UV dosages. The developed system was then evaluated based on selected parameters.

The physicochemical properties such as colour, pH, TSS, titrable acidity and ascorbic acid content were evaluated. The results showed that the individual as well as combined treatments did not induce any significant impact on the physicochemical properties of pineapple juice samples. The results showed that individual and combined treatments retained the quality characteristics of samples compared to fresh juice.

From the microbial analysis, it was concluded that combined treatment resulted 5 log cycle reduction in bacterial and yeast population whereas individual treatment failed to produce 5 log cycle reductions. From the study it was found that combined US and UV treatments with US exposure 20 min and UV dosage 1600 $mJcm^{-2}$ and US exposure 30 min and UV dosage 1000 $mJcm^{-2}$ ensured microbiologically safe product while retaining fresh like qualities.

Organoleptic evaluation of optimized samples, fresh juice and thermally treated samples revealed that combined US and UV treatments with US exposure 20 min and UV dosage 1600 $mJcm^{-2}$ retained all organoleptic qualities close to that of fresh juice. Therefore, the combined treatment with US exposure 20 min and UV dosage 1600 $mJcm^{-2}$ selected as the best treatment and therefore these process variables were selected as optimum parameters for the developed system.

Suggestions for future work

In order to enhance the effectiveness and commercialization of developed ultrasound assisted ultraviolet treatment system, the following suggestion for future research were recommended.

1. Combine US and UV in single treatment chamber
2. Provide electrical valves for varying UV dosage in single pass
3. Provide provision for varying US frequency
4. Incorporation of automatic controls
5. Improve quality standards by application of HACCP

References

REFERENCES

Aadil, R., Zeng, X.A., Sun, D.W., and Wang, M.S. 2014. Combined effects of sonication and pulsed electric field on selected quality parameters of grapefruit juice. *Food Sci. and Technol*. 62: 1-4.

Aadil, R.M., Zeng, X.A., Han, Z., and Sun, D.W. 2013. Effect of ultrasound treatments on quality of grapefruit juice. *Food Chem.* 141: 3201-3206.

Adekunte, A.O., Tiwari, B.K., Cullen, P.J., Scanell, A.G.M., and Odonnell, C.P. 2010. Effect of asonication on colour, ascorbic acid and yeast inactivation in tomato juice. *Food Chem.* 122: 500-507.

Akinyele, I.O., Keshinro, O.O., and Akinnawo, O.O. 1990. Nutrient losses during and after processing of pineapples and oranges. *Food Chem.* 37(3): 181-188.

Allendre, A. and Artes, F. 2003. Combined ultraviolet-c and modified atmosphere packaging treatments for reducing microbial growth of fresh processed lettuce. *Food Sci. Technol*. 36: 779-786.

Anita Rana., Meena., and Shweta. 2017. Ultrasonic processing and its use in food industry: A review. *Int. J. Chem. Stud*. 5(6): 1961-1968.

AOAC (Association of Analytical Chemists), 2000. Official methods of analysis.

Awad, T. and Sato, K. 2001. Effects of hydrophobic emulsifier additives on crystallization behavior of palm mid fraction in oil-in-water emulsion. *Journal of the American Oil Chemists Society*. 78(8): 837–842.

Awad, T. S. 2004. Ultrasonic studies of the crystallization behavior of two palm fats oil and water emulsions and its modification. *Food Res. Int*. 37(6): 579–586.

Awad, T., Hamada, Y., and Sato, K. 2001. Effects of addition of diacylglycerols on fat crystallization in oil-in-water emulsion. *Eur. J. of Lipid Sci. and Technol*. 103(11): 735–741.

Barbosa-Canovas, G.V., Pothakamury, U.R., Palou, E., and Swanson, B.G. 1998. *Nonthermal preservation of foods.* Marcel Dekker, New York.

Bartolomew, A.P., Pilar, R., and Foster, C. 1995. Pineapple fruit: morphological characteristics, chemical composition and sensory analysis of red Spanish and smooth cayenne cultivars. *Food Chem.* 53: 75-79.

Benedito, J., Carcel, J. A., Rossello, C., and Mulet, A. 2001. Composition assessment of raw meat mixtures using ultrasonics. *Meat Sci*. 57(4): 365–370.

Benedito, J., Carcel, J.A., Sanjuan, N., and Mulet, A. 2000. Use of ultrasound to assess Cheddar cheese characteristics. *Ultrasonics*. 38 ; 727–730.

Bevllacqua, A., Spernza, B., Campaniello, D., Sinigaglia, M., and Corbo, M.R. 2014. Inactivation of spoiling yeasts of fruit juices by pulsed Ultrasound. *Food Bioprocess Technol.* 7: 2189-2197.

Bhat, R., Ameran, S.B., Voon, H.C., Karim, A.A., and Tze, L.M. 2011. Quality attribute f starfruit (*Averrhoa carambola* L.) juice treated with ultraviolet radiation. *Food Chem.* 127(2): 641- 644.

Bhat, R., Kamaruddin, N.S., Tze, L.M., and Karim, A.A. 2011. Sonication improves kasturi lime (Citrus microcarpa) juice quality. *Ultrasound Sonochemistry*. 18(6):1295-300.

Bhullar, M.S., Patras, A., Nthengea, A.K., Pokharela, B., Yannama, S.K., Rakariyatham, K., Pan, C., Xiao, H., and Sasge, M. 2018. Microbial inactivation and cytotoxicity evaluation of UV irradiated coconut water in a novel continuous flow spiral reactor. *Int Food Res. J*. 103: 59-67.

Bintsis, T., Tzanetaki, E.L., and Robinson, R. 2000. Existing and potential applications of the ultraviolet light in the food industry- A critical review. *J. Sci. Food Agric*. 80: 637–645.

Blitz, J. 1963. Fundamentals of ultrasonics. London: Butterworths & Co. 214pp.

Brown, T., James, S.J., and Purnell, G.L. 2005. Cutting forces in foods: experimental measurements. *J. Food Eng*g. 70:165-170.

Buckin, W., Kudryushov, E., and ODriscoll, B. 2002. High-resolution ultrasonic spectroscopy for material analysis. *Am. Lab.* 28–31.

Bund, R.K. and Pandit, A.B. 2007. Sonocrystallization: effect on lactose recovery and crystal habit. *Ultrasonics Sonochemistry.* 14(2): 143-152.

Cabezae, M.G., Garcia, M.L., De La Hoz, L., Cambero, I., and Ordenzo, J.A. 2005. Thermoultrasonication Eliminates Salmonellae from Intact Eggshells without Changing the Functional Properties of their Components. *J. Food Sci.* 70: 292-295.

Cameron, M., McMaster, L.D., and Britz, T.J. 2009. Impact of ultrasound on dairy spoilage microbes and milk components. *Dairy Sci. and Technol*. 89: 83-98.

Carson, I.A. and Petersen, N.J. 1975. Photoreactivation of *Pseudomonas cepacia* after ultraviolet exposure: A potential source of contamination in ultraviolet treated waters. *J. Clin. Microbiol.* 1: 462-464.

Chemat, F., Huma, Z. and Khan, M.K. 2011. Applications of ultrasound in food technology: processing, preservation and extraction. *Ultrasonics sonochemistry.* 18(4): 813-835.

Cho, B.K and Irudayaraj, J.M.K. 2003. Foreign object and internal disorder detection in food materials using non- contact ultrasound imaging. *J. Food Sci.* 68: 967-974.

Choi, L.H. and Nielsen, S.S. 2005. The effects of thermal and nonthermal processing methods on apple cider quality and consumer acceptability. *J. Food quality* 28: 13-29.

Choudhary, R. and Bandla, S. 2012. Ultraviolet Pasteurization for Food Industry. *Int. J. of Food Sci. and Nutr. Engg*. 2(1): 12-15.

Christensen, J. and Linden, K.G. 2003. How particles affect UV light in the UV disinfection of unfiltered drinking water. *J. Am. Water Works Assoc.* 95: 179-189.

Collings, E.R., Gavidiaa, M.C.A., Cools, K., Redfern, S., and Terry, L.A. 2018. Effect of UV-C on the physiology and biochemical profile of fresh Piper nigrum berries. *Postharvest Biol. and Technol*. 136: 161-165.

Coupland, J. N. 2004. Low intensity ultrasound. *Food Res. Int.* 37 (6): 537–543.

Crawford, Y.J., Murano, E.A., Olson, D.G., and Shenoy, K. 1996. Use of high hydrostatic pressure and irradiation to eliminate Clostridium sporogenes spores in chicken breast. J. Food Prot. 59:711-715.

Cruz, R.M.S., Khmelinskii, I., and Vieira1, M.C. Ultrasound Applications in Food Technology: Equipment, Combined Processes and Effects on Safety and Quality Parameters. *Adv. in Food Sci. and Nutr.*13: 413-444.

Debnat, P., Dey, P., Chanda, A., and Bhakta, T. 2012. A survey on pineapple and its medicinal value. *Scholars Academic J. Pharm.* 1(1).

Dette, S.S. and Jansen, H. 2010. Freeze concentration of black currant juice. *Chemical Engg. And Technol.* 33(5): 762-766.

Dickens, J.A., Lyon, C.E., and Wilson R.L. 1991. Effect of ultrasonic radiation on some physical characteristics of broiler breast muscle and cooked meat. *Poultry Sci.* 70(2): 389-396.

Dolatowski, Z.J. 1999. The influence of low-frequency ultrasound processing on the structure and qualitative traits of meat. Rozpr. Nauk, AR Lubl, pp. 221.

Dolatowski, Z.J. and Twarda J. 2004. Einfluss von Ultraschall auf das Wasserbindungsvermögen von Rindfleisch. *Fleischwirtschaft* .12: 95-99.

Dolatowski, Z.J., Stadnik, J., and Stasiak D. 2007. Applications of ultrasound in food technology. *Acta Sci. Pol. Technol. Aliment.* 6(3): 89-99.

Drakopoulou, S., Terzakis, S., Fountoulakis, M.S., Mantzavinos, D. and Manios, T. 2009. Ultrasound-induced inactivation of gram-negative and gram-positive bacteria in secondary treated municipal wastewater. *Ultrasonics Sonochemistry.* 16: 629–634.

Dukhin, A. S. and Goetz, P. J. 2009. Bulk viscosity and compressibility measurement using acoustic spectroscopy. *J. Chem. Phys*. 130 (12).

Ensminger, D. 1988. Acoustic and electroacoustic methods of dewatering and drying. *Drying Technol*. 6(3): 473 - 499.

Ercan, S.S. and Soyal, C. 2013. Use of ultrasound in food preservation. *Nat. sci*. 5(08): 5-13.

Farid, M.H. and Shaheen, A. 2015. Nutritional value and medicinal benefits of pineapple. *Int. J. Nutr. and Food Sci.* 4(1): 84-88.

Farid, M.M., Chen, X.C., and Dost, Z. 2001. Ultraviolet sterilization of orange juice. In: J. *Proceedings of the Eight Int. Congress on Engg. and Food*, Lancaster: Technomic publishers, 1567-1572 pp.

Faulkner, D.B., Parrett, D.F., Mckeith, F.K., and Berger, L.L. 1990. Prediction of fat cover and carcass composition from live and carcass measurements. *J. Animal Sci.* 68(3): 604–610.

FDA, 1997. Fruit and vegetable juice beverages: Notice of intent to develop a HACCP program., interim warning statement, and educational program. Washington, D.C.: U.S. Food and Drug Administration. *Federal Register* 62: 45593 – 45596.

Fellows, P.J. 2000. *Food processing technology technology: Principles and practice.* Woodhead Publishing, Boca Raton.

Feng, H., Barbosa, C,G., and Weiss, J. (Ed.). 2011. *Ultrasound technologies for food and bioprocessing*. Springer, New York.

Fernandes, F.A.N. and Rodrigues, S. 2007. Ultrasound as a pretreatment for drying of fruits.: dehydration of banana. *J. Food Engg.* 82: 261-267.

Fernandes, F.A.N. and Rodrigues, S. 2008. Application of ultrasound and ultrasound assisted osmotic dehydration in drying of fruits. *Drying Technol.* 26(12): 1509 -1516.

Fernandes, F.A.N., Linhares, F.E., and Rodrigues, S. 2008. Ultrasound as a pretreatment for drying of pineapples. *Ultrasonics Sonochemistry.* 15(6): 1049-1054.

Ferrante, S., Guerrero, S., and Alzamora, S. M. 2007. Combined use of ultrasound and natural antimicrobials to inactivate Listeria monocytogenes in orange juice. *J. Food Prot.* 70: 1850–1856.

Fitriyanti, M. and Narasimhan, G. 2018. Synergistic effect of low power ultrasonication on antimicrobial activity of cecropin p1 against e.coli in food systems. *Food Sci. and Technol.* 96:175–181.

Gachovska, T.K., Kumar, S., Thippareddi, H., Subbiah, J., and Williams, F. 2008. Ultraviolet and pulsed electric field treatments have additive effect on Inactivation of E. Coli in apple Juice. *J. Food Sci.* 78 (9): 412-417.

Garcia-Perez, J.V., Carcel, J.A., Riera, E., and Mulet, A. 2009. Influence of the applied acoustic energy on the drying of carrots and lemon peel. *Drying Technol.* 27: 281-287.

Geveke, D.J. and Torres, D. 2012. Pasteurization of grapefruit juice using a centrifugal ultraviolet light irradiator. *J. Food Engg.* 111(2): 241-246.

Gogo, E.O., Opiyo, A.M., Hassenberg, K., Ulrichsa, C., and Keila, S.H. 2017. Postharvest UV-C treatment for extending shelf life and improving nutritional quality of African indigenous leafy vegetables. *Postharvest Biol. and Technol*. 109: 107-117.

Gracin, L., Krizanovic, S., Jambrak, A.R., Tomasevic, M., Kelsin, K., Lukic, K., and Ganic, K.K. 2017. Monitoring the influence of high power ultrasound treatment and thermosonication on inactivation of Brettanomyces bruxellensis in red wine. *Croatian J. of Food Technol. Biotech. and Nutr*. 12: 107-112.

Haeggstrom, E. and Luukkala, M. 2001. Ultrasound detection and identification of foreign bodies in food products. *Food Control*. 12(1): 37–45.

Halzhou, L., Pordesimo, L., and Weiss, J. 2004. High intensity ultrasound assisted extraction of oil from soyabean. *Food Res. Int.* 37: 731- 738.

Hromadkova, Z. and Ebringerova, A. 2003. Ultrasonic extraction of plant materials : investigation of hemicelluloses release from buckwheat hulls. *Ultrasonic Sonochemistry*. 10: 127-133.

Jayasooriya, S.D., Bhandari, B.R., Torley, P., and Arcy, B.R. 2004. Effect of high power ultrasound waves on properties of meat: a review. *Int. J. Food Prop*. 7, 2, 301-319.

Juan, A. and Juarez, G. 2010. High-power ultrasonic processing: recent developments and prospective advances. *Phys. Procedia.* 3: 35–47.

Jun, S., Iruddayyaaraj, J., Demirci, A., and Geiser, D. 2003. Pulsed UV-light treatment of corn meal for inactivation of Aspergillus niger spores. *International J. Food Sci. Technol.* 38: 883-888.

Knorr, D., Zenker, M., Heinz, V., and Lee, D.U. 2004. Applications and potential of ultrasonics in food processing. *Trends Food Sci. Technol*. 15: 261-266.

Knudson, G.B. 1985. Photoreactivation of UV – irradiated *Legionella pneumophilla* and other *Legionella* species. *Appl. Environ. Microbiol.* 49(4): 975-980.

Koutchma, T. 2009. Advances in ultraviolet light technology for non-thermal processing of liquid foods. *Food Bioprocess Technol*. 2: 138-155.

Krasulya, O., Bogush, V., Trishina, V., Potoroko, I., Khmelev, S., Sivashanmugam, P., and Anandan, S. 2016. Impact of acoustic cavitation on food emulsions. *Ultrasonics Sonochemistry*. 30: 98-102.

Lagnika, C., Adjovt, Y.C.S., Lagnika, L., Gogohounga, F.O., Sacramento, O.D., Koulony, R.K., and Sanni, A. 2017. Effect of combining ultrasound mild heat treatment on physicochemical, nutritional quality and microbiolgical properties of pineapple juice. *Food and Nutr. Sci.* 8: 227-241.

Lee, S.H., Yamada, K., and Jun, S. 2013. Ultraviolet radiation assisted ohmic current for microbial inactivation in apple juice. *Trans. of ASABE*. 56(2): 1085-1091.

Leemans, V. and Destain, M.F. 2009. Ultrasonic internal defect detection in cheese. *J. Food Engg*. 90(3): 333–340.

Lillard, H.S. 1993. Bactericidal effect of chlorine on attached Sallmonellae with and without sonification. *J. Food Protect*. 56: 716-717.

Lopez, P. and Burgos, J. 1995. Peroxidase stability and reactivation after heat treatment and manothermosonication. *J. Food Sci*. 60(3): 451-455.

Lopez, P., Sala, F.J., Fuente, J.L.D., Condon, S., Raso, J. and Burgos, J. 1994. Inactivation of peroxidase, lipoxygenase and polyphenol oxidase by manothermosonication. *J. of Agric. and Food Chem.* 42: 252-256.

Lopez, P., Vercet, A., and Burgos, J. 1998. Inactivation of tomato pectic enzymes by manothermosonication. *Zeits chrift für Lebensmittel Untersuchung und Forschung* , 207:249-252.

Lopez, V.M.G., Orsolani, L., Yepez, A.M., and Tapia, M.S. 2010. Microbiological and sensory quality of sonicated calcium – added orange juice. *Food Sci. and Technol.* 43: 808- 813.

Manas, P., Munoz, B., Sanz, D., and Condon, S. 2006. Inactivation of lysozyme by ultrasonic waves under pressure at different temperatures. *Enzyme and Microbial Technol.* 39: 1177-1182.

Manson, T.J., Paniwnyk, L., and Lorimer, J.P. 1996. The use of ultrasound in food technology. *Ultrasonics sonochemistry*. 3(5): 253 -260.

Mansor, A., Shamsudin, R., Adzahan, N.M., and Hamidon, N.M. 2015. Performance of UV pasteurization with quartz glass sleeve on physicochemical properties and microbial activity of pineapple juice. *J. of Food Process Eng.* 30: 1-8.

Mason, T.J. 1999. *Power ultrasound in food processing- The way forward.* In: Povey, M.J.W. and Mason, T.J. (ed.). Blackie Academic and Professional, London, pp. 105-126.

McClements, D.J. 1995. Advances in the application of ultrasound in food analysis and processing. *Trends in Food Sci. and Technol*. 6(9): 293–299.

McClements, D.J. 1995. Advances in the application of ultrasound in food analysis and processing. *Trends Food Sci. Technol*. 6: 293-299.

McClements, D.J. 1997. Ultrasonic characterization of foods and drinks: Principles, methods, and applications. *Crit. Rev. in Food Sci. and Nutr*. 37 (1): 1–46.

McClements, D.J. and Povey, M.J.W. 1987. Solid fat content determination using ultrasonic velocity measurements. *Int. J. of Food Sci. and Technol.* 22(5): 491–499.

Miller, R., Jeffrey, W., Mitchell, D., and Elasri. M. 1999. Bacterial responses to ultraviolet light. *ASM News*. 65: 534–541.

Mizrach, A., Galili, N., Rosenhouse, G., and Teitel, D.C. 1991. Acoustical, mechanical and quality parameters of winter grown melon tissue. *Trans. of the ASABE*. 34(5): 2135–2138.

Montenegro, J., Ruan, R., Ma, H., and Chen, P. 2002. Inactivation of E. coli 0157:H7 using a pulsed nonthermal plasma system. *J. Food Sci*. 67: 646–48.

Nagadi, M., Smith, J.P., and Cayoutte, B. 2003. Kinetics of ultraviolet light inactivation of *Escherichia coli* 0157: H7 in liquid foods. *J. Sci. Food. Agric.* 83: 1551-1555.

Noci, F., Riener, J., Ribeiro, M.W., Cronin, D.A., Morgan, D.J. and Lyng, J. 2008. Ultraviolet irradiation and pulsed electric fields (PEF) in a hurdle strategy for the preservation of fresh apple Juice. *J. Food Engg*. 85: 141-146.

Obande, M.A., Tucker, G.A., and Shama, G. 2011. Effect of Preharvest UV-C Treatment of Tomatoes on Ripening and Pathogen Resistance. *Postharvest Biol. and Technol*. 62:188–192.

ODonnell, C.P., Tiwari, B.K., Bourke, P., and Cullen, P.J. 2010. Effect of ultrasonic processing on food enzymes of industrial importance. *Trends in Food Sci. and Technol.* 21: 358-367.

Ojaghiana, M.R., Zhanga, J.Z., Xiea, G.L., Wanga, Q., Lia, X.L., and Guob, D.P. 2017. Efficacy of UV-C radiation in inducing systemic acquired resistance against storage carrot rot caused by Sclerotinia sclerotiorum. *Postharvest Biol. and Technol*. 130: 94-102.

Ozbek, B. and Ulgen, K.O. 2000. The stability of enzymes after sonication. *Process Biochem*. 35:1037- 1043.

Ozuna, C., Puig, A., Garcia-Perez, J.V., Mulet, A., and Carcel, J.A. 2013. Influence of high intensity ultrasound application on mass transport, microstructure and textural properties of pork meat brined at different NaCl concentrations. *J. Food Engg.*119: 84-93.

Pagan, R., Manas, P., Alvarez, I., and Condon, S. 1999. Resistance of Listeria monocytogenes to ultrasonic waves under presure at sublethal (manosonication) and lethal (manother-mosonication) temperatures. *Food Microbiol.* 16: 139-148.

Pala, C.U. and Toklucu, A.K. 2011. Effect of UV-C light on anthocyanin content and other quality parameters of pomegranate juice. *J. Food composition and analysis*. 24: 790-795.

Park, M.H. and Kim, J.G. 2015. Low-dose UV-C irradiation reduces the microbial population and preserves antioxidant levels in peeled garlic during storage. *Postharvest Biol. and Technol*. 100: 109–112.

Piyasena, P., Mohareb, E., and McKellar, R.C. 2003. Inactivation of microbes using ultrasound: A review. *Int. J. of Food Microbiol*. 87(3): 207–216.

Pollack, R.A., Findlay, L., Mondschein, W., and Modesto, R.R. 2002. *Laboratory exercise in microbiology.* John Wiley and sons Inc., NewYork, 41p.

Potapovich, M.V., Eremin, A.N., and Metelitza, D.I. 2003. Kinetics of catalase inactivation induced by ultrasonic cavitation. *Appl. Biochem. and Microbiol.* 39:140-146.

Povey, M.J.W. and Mason, T.J. 1998. *Ultrasound in Food Processing.* SpringerBlackie A & P, London.

Raichel, D. R. 2000. The Science and Applications of Acoustics. Springer, New York, 178p.

Rasid, K. and Hosain. 1987. Fruits of Bangladesh. *Rashid publishing house.* 1: 232.

Raso, J. and Barbosa-Canovas, G.V. 2003. Nonthermal preservation of foods using combined processing techniques. *Crit. Rev. in Food Sci. and Nutr*. 43: 265-285.

Raso, J., Palop, A., and Condon, S. 1998. Inactivation of Bacillus subtilis spores by combining ultrasound waves under pressure and mild heat treatment. *J. Applied Microbiology.* 85: 849-854.

Rawson, F.F. 1998. An introduction to ultrasonic food cutting. ln: Povey, M.J.W., T.J. Mason (Eds.), Ultrasound in Food Processing, Blackie, Glasgow.

Reinemann, D.J., Gouws, P., Cilliers, T., Houck, K., and Bishop, J.R. 2006. New methods for UV treatment of milk for improved food safety and product quality. *ASABE presentation*. Paper No. 066088.

Ribeiro, M.W., Noci, F., Cronin, D.A., Lyng, J.G., and Morgan, D.J. 2009. Shelf life and sensory evaluation of orange juice after exposure to thermosonication and pulsed electric fields. *Food and Bioproducts processing.* 87: 102–107.

Riener, J., Noci, F., Cronin, D.A., Morgan, D.J., and Lyng, J.G. 2009. Characterisation of volatile compounds generated in milk by high intensity ultrasound. *Int. Dairy J.* 19: 269-272.

Riesz, P. and Kondo, T. 1992. Free radical formation induced by ultrasound and its biological implications. *Free Radic Biol Med*. 13(3): 247-70.

Roobab, U., Aadil, R.M., Madni, G.M., and Bekhit, A.E. 2018.The impact of nonthermal technologies on microbiological quality of juices: a review. *Comprehensive Rev. Food Sci and Technol.* 17: 437-457.

Sadasivam, S. and Manickam, C. 1992. *Biochemical methods for agricultural science* (1st ed.). Wiley Eastern Limited, New Delhi

Sala, F.J., Burgos, J., Condon, S., Lopez, P. and Raso, J. 1995. Effect of heat and ultrasound on microorganisms and enzymes. In: Gould, G.W. (ed), *New Methods of Food Preservation*, Blackie Academic and Professional, London, pp. 176-204.

Salazar, J., Turo, A., Chavez, J.A., and Garcia, M.J. 2004. Ultrasonic inspection of batters for on-line process monitoring. *Ultrasonics.* 42(1): 155–159.

Sale, A.J.H., Gould, G.W., and Hamilton, W.A., 1970. Inactivation of bacterial spores by hydrostatic pressure. *J. Food Sci. Technol.* 47 (1): 15-26.

Sastry, S. K., Datta, A. K., and Worobo, R. W. 2008. Ultraviolet light. *J. Food Sci. Supplement*. 65(12): 90-92.

Schneider, Y., Zahn, S., Schindler, C., and Rohm, H. 2009. Ultrasonic excitation affects friction interactions between food materials and cutting tools. *Ultrasonics*. 49: 588–593.

Scouten, A.J. and Beuchat, L.R. 2002. Combined effects of chemical, heat and ultrasound treatments to kill salmonella and e. colio157:H7 on alfalfa seeds. *J. Appl. Microbiol.* 92: 668- 674.

Shah, N.A.K., Shamsudin, R., Rahman, R.A., Adzahan, N.M. 2016. Fruit Juice Production Using Ultraviolet Pasteurization: A Review. *Beverages*. 2(22): 1-20.

Shama. G. 1999. Ultraviolet light. In: R.K.. Robinson, C. Batt and P. Patel (ed.), *Encyclopedia of Food Microbiology-3*, London, Academic Press, pp. 2208-22011.

Sizer, C.E. and Balasubramaniam, V.M. 1999. New invention process for minimally processed juices. *Food Technol.* 53(10): 64-67.

Stevens, C., Khan, V.A., Lu, J.Y., Wilson, C.L., Chalutz, E., Droby, S., Kabwe, M.K., Haung, Z., Adeyeye, O., Pusey, L.P., and Tang, A.Y.A. 1999. Induced resistance of sweet potato to Fusarium root rot by UV-C hormesis. *Crop prot*. 18 (7): 463-470.

Sun, D.W. and Li, B. 2003. Microstructure change of potato tissues frozen by ultrasound – assisted immersion freezing. *J. Food Engg.* 57 (4): 337-345.

Tanaka, F., Nashiro, K., Trivittayasil, V., and Uchino, T. 2016. Simulation of UV-C Dose Distribution and Inactivation of Mold Spore on Strawberries in a Conveyor System. *Food Sci. Technol. Res*. 22 (4): 461-466.

Templeton, M.R., Andrews, R.C., and Hofmann, R. 2005. Inactivation of particles associated viral surrogates by ultraviolet light. *Water Res.* 9: 47-50.

Tiwari, B.K., Muthukumarappan, K., Odonnell, C.P., and Cullen, P.J. 2008. Effects of sonication on kinetics of orange juice quality parameters. J. Agric. F00d chem. 56: 2423- 2428.

Tran, M.T.T. and Farid, M.M. 2004. Ultraviolet treatment of orange juice. *Innov. Food Sci. Emerg. Technol.* 5: 495-502.

U. S. Food and Drug Administration. 2000. 21 CFR Part 179. Irradiation in the production, processing and handling of food. *Fed. Regist*. 65: 71056-71058.

Ueno, S., Ristic, R.I., Higaki, K., and Sato, K. 2003. In situ studies of ultrasound – stimulated fat crystallization using synchrotron radiation. *The J. Physical Chemistry.* 107 (21): 4927-4935.

Valero, M., Recrosio, N., Saura, D., Munoz, N., Marti, N. and Lizama, V. 2007. Effects of ultrasonic treatments in orange juice processing. *J. Food Engg.* 80: 509-516.

Vercet, A., Burgos, J., Crelier, S., and Lopez-Buesa, P. 2001. Inactivation of protease and lipase by ultrasound. *Innovation Food Sci. and Technol*. 2: 139-150.

Vercet, A., Sanchez, C., Burgos, J., Montanes, L., and Lopez, B.P. 2002. The effects of manothermosonication on tomato pectic enzymes and tomato paste rheological properties. *J. Food Engg.* 53(3): 273-278.

Villamiel, M. and deJong, P. 2000. Influence of high intensity ultrasound and heat treatment in continuous flow on fat, proteins, and native enzymes of milk. *J. Agric. and Food Chem.* 48: 472-478.

Villamiel, M., Hamersveld, E.H., and DeJong, P. 1999. Effect of ultrasound processing on quality of dairy products. *Milchwissenschaft.* 54: 69-73.

Virone, C., Kramer, H.J.M., Rosmalen, G.M., Stoop, A.H., and Bakker, T.W. 2006. Primary nucleation induced by ultrasonic cavitation. *J. Crystal Growth.* 1: 9-15.

Warriner, K., Kolstad, J., Rumsby, J., and Waites, W. 2002. Carton sterilization by uv-c excimer laser light: recovery of bacillus subtilis spores on vegetable extracts and food simulation matrices. *J. Appl. Microbiol*. 92: 1051-1057.

Watson, T. 1998. The importance of power ultrasound in cleaning and disinfection in the poultry industry – a case study. In Ultrasound in Food Processing (Povey M, Mason TJ, eds), *London: Blackie Academic and Professional*, pp. 144–150.

Wright, J.R., Sumner, S.S., Hackney, C.R., Pierson, M.D., and Zoecklein, B.W. 2000. Efficacy of ultraviolet light for reducing Escherichia coli O157:H7 in unpasteurized apple cider. *J. Food Protection*. 63: 563–67.

Wu, H., Hulbert, G.J., and Mount, J.R. 2001. Effects of ultrasound on milk homogenization and fermentation with yogurt starter, *Innovative Food Sci. and Emerging Technol.*1: 211 – 218.

Yaun, B.R., Sumner, S.S., Eifert, J.D., and Marcy, J.E. 2004. Inhibition of lopathogens on fresh produce by ultraviolet energy. *Int. J. Food Microbiol*. 90(1): 1-8.

Yikmis. 2019. Effect of ultrasound on different quality parameters of functional sirkencubin syrup. *Food Sci. and Technol.* 40: 1-8.

Zhang, Z.S., Wang, L.J., Li, D., Jiao, S.S., Chen, X.D., and Mao, Z.H. 2008. Ultrasound assisted extraction of oil from flaxseed. *Sep. Puri. Technol.* 62: 192-198.

Zheng, L. and Sun, D.W. 2006. Innovative applications of power ultrasound during food freezing process – A review. *Trends Food Sci. Technol.* 17(1): 16-23.

Zisu, B. and Chandrapala, J. 2015. High power ultrasound processing in milk and dairy products. *Emerging dairy processing technol-opportunities for the dairy ind.* 149-179.

Appendices

Appendix A.1 Analysis of variance (ANOVA) for Total colour difference

ANOVA					
Total colour difference					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.426	14	.185	1.54	.421
Within Groups	.082	30	.012		
Total	.509	44			

Appendix A.2 Analysis of variance (ANOVA) for pH

ANOVA					
pH					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4580760.00	14	327197.143	1.000	0.478
Within Groups	9815864.57	30	327195.486		
Total	14396624.5	44			

Appendix A.3 Analysis of variance (ANOVA) for TSS

ANOVA					
TSS					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.057	14	.019	2.408	.134
Within Groups	.071	30	.008		
Total	.129	44			

Appendix A.4 Analysis of variance (ANOVA) for Titrable acidity

ANOVA					
Titrable acidity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.001	14	.016	2.351	.140
Within Groups	.001	30	.007		
Total	.003	44			

Appendix A.5 Analysis of variance (ANOVA) for Ascorbic acid content

ANOVA					
Ascorbic acid content					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.445	14	.148	.841	.505
Within Groups	1.588	30	.176		
Total	2.032	44			

Appendix A.6 Analysis of variance (ANOVA) for Bacterial log reduction

ANOVA					
Bacterial log reduction					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	42.812	14	3.058	9173914.28	<.001
Within Groups	.000	30	.000		
Total	42.812	44			

Appendix A.7 Analysis of variance (ANOVA) for yeast reduction

ANOVA					
Yeast reduction					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	52.149	14	3.725	11174871.4	<.001
Within Groups	.000	30	.000		
Total	52.149	44			

APPENDIX B
Sensory score card for organoleptic evaluation

SENSORY SCORE CARD

Department of processing and Food Engineering
KCAET, Tavanur

Name of judge: **Date:**

You are requested to assess the product in terms of general acceptability on a 9 point hedonic scale

Characteristics	**Sample code**						
	A	B	C	D	E	F	G
Colour & Appearanc							
Flavour							
Taste							
Overall acceptability							

Score system:

Dislike extremely: 1, Dislike very much: 2, Dislike moderately: 3, Dislike slightly: 4

Neither like nor dislike: 5, Like slightly: 6, Like moderately: 7, Like very much: 8

Like extremely: 9

Comments if any:

Signature

APPENDIX C

Cost Economics of developed ultrasound assisted ultraviolet radiation system

Total production cost - Rs.102500

Assumptions

Life span (L)	=	10 years
Annual working hours (H)	=	275 days (per day 8 hrs) = 2200 hours
Salvage value (S)	=	10% of initial cost
Interest on initial cost (i)	=	15% annually
Repair and maintenance	=	8% of initial cost
Insurance and taxes	=	2% of initial cost
Electricity charge	=	Rs.7/unit
Labour wages/person	=	Rs. 350/day

1. Total Fixed cost per day		
i. Depreciation	=	$\frac{C-S}{L\ x\ H} = \frac{102500-10250}{10x2200} =$ Rs. 4.19/h
ii. Interest	=	$\frac{C+S}{2}\ x\ \frac{i}{H} = \frac{102500+10250}{2}$ x $\frac{15}{100x2200}$ =Rs.3.83/h
iii. Insurance & taxes	=	2% of initial cost

		$=\frac{2}{100x2200}$x 102500 = Rs. 9.32/h
Total Fixed cost	=	i + ii + iii = Rs. 17.34/h = Rs. 138.72/day
2. Total variable cost per day		
i. Repair & maintenance	=	8% of initial cost $=\frac{8}{100\ x\ 2200}$ x 102500 = Rs 3.73/h
ii. Electricity cost a) Cost of energy consumed by US bath / day b) Cost of energy consumed by UV treatment system / day c) Cost of energy consumed by motor / day Total energy cost/day	 = = = =	 Rs.14/ day Rs. 0.50/ day Rs. 1.63/day Rs. 16.13/day
iii. Labour cost	=	Rs. 700/day (2 persons)

iv. Packaging cost	=	Rs. 100/day
v. Cost of raw material	=	Rs.2880/day
Total variable cost	=	i + ii + iii + iv + v =Rs. 3699.86/-
Therefore total cost for production of 32 litre of pineapple juice/ day	= = =	Fixed cost + Variable cost 138.72+ 3699.86 Rs. 3838.58/day
Processing cost of 1 litre of pineapple juice	=	Rs. 119.95/litre

Processing cost of 1 litre of pineapple juice in market = Rs. 175/litre

Benefit-cost ratio = $\frac{175}{119.95}$ = 1.46

Therefore the total processing cost of 1 litre of pineapple juice in ultrasound assisted ultraviolet radiation system was found to be Rs. 119.95 The benefit cost ratio was found to be 1.46 :1

EFFECT OF COMBINED TREATMENTS OF ULTRASOUND AND ULTRAVIOLET RADIATION FOR PRESERVATION OF PINEAPPLE JUICE

By

ANJALY M.G.

(2018-18-021)

ABSTRACT OF THESIS

Submitted in partial fulfillment of the requirement for the degree of

MASTER OF TECHNOLOGY

IN

AGRICULTURAL ENGINEERING

(Agricultural Processing and Food Engineering)

Faculty of Agricultural Engineering and Technology

Kerala Agricultural University

Department of Processing and Food Engineering

KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND TECHNOLOGY

TAVANUR, MALAPPURAM- 679573

KERALA, INDIA

2021

ABSTRACT

Ultraviolet (UV) treatment is an alternative tool for food preservation. UV radiation inactivates microorganisms by DNA denaturation and is widely used for disinfect water and liquid food products. The application of UV radiation is limited due to its low penetration depth. Ultrasound (US) is another promising technique which can be employed for preservation of fruit food products. The microbial inactivation in US is mainly due to cavitation. Cavitation leads to destruction of cells, production of free radicals, formation of shock waves and denaturation of enzymes.US preserve the organoleptic and nutritional qualities of food products. Even though UV radiation and US treatment has their own potential as a preservative method, but application of any single treatment would not be competent enough to kill all microorganism. Therefore, a new concept has been extensively evaluated to combine ultraviolet and ultrasound. This combined treatment would optimize the strength of each individual treatment and reduce each of their individual weaknesses. This present study envisages development of US assisted UV radiation treatment system for pineapple juice and evaluation of developed system in retaining the quality characteristics and microbial safety. The system consists of ultrasonic bath with chiller (Sonicator), storage tank, ultraviolet treatment system and recirculation system. The treatments were carried out with three different US time intervals 10 min, 20 min and 30 min and three different UV dosages 1000 mJ/cm^2, 1300 mJ/cm^2, and 1600 mJ/cm^2. Combined US and UV treatments with US exposure 20 min and UV dosage 1600 $mJcm^{-2}$ were found to be superior based on physicochemical, microbiological and organoleptical characteristics.