DEVELOPMENT AND EVALUATION OF AN ULTRAVIOLET RADIATION ASSISTED WITH OHMIC HEATING SYSTEM FOR PRESERVATION OF PINEAPPLE JUICE

by

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TAVANUR , MALAPPURAM -679573

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2015

DECLARATION

I, hereby declare that this thesis entitled **"DEVELOPMENT AND EVALUATION OF AN ULTRAVIOLET RADIATION ASSISTED WITH OHMIC HEATING SYSTEM 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.

Place: Tavanur **Y. Dileep Sean**

Date: **(2013-18-110)**

CERTIFICATE

Certified that this thesis entitled **"DEVELOPMENT AND EVALUATION OF AN ULTRAVIOLET RADIATION ASSISTED WITH OHMIC HEATING SYSTEM FOR PRESERVATION OF PINEAPPLE JUICE"** is a record of research work done independently by **Mr. Y. Dileep Sean (2013-18-110)** 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 him.

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Y. Dileep Sean

Dedicated to

My Parents
And
Beloved Guide

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LIST OF SYMBOLS AND ABBREVATIONS

^{o}B	:	Brix
^{o}C	:	Degree Celsius
%	:	per cent
&	:	And
/	:	Per
AC	:	Alternate current
Amp	:	Ampere
ANOVA	:	Analysis of variance
cfu/ml	:	Colony forming unit per milliliter
cm	:	Centimeter
CRD	:	Completely Randomized Design
DMRT	:	Duncan's multiple range test
et al.	:	and others
etc.	:	Etcetera
g	:	Gram
Hp	:	Horse power
Hz	:	Hertz
i.e.	:	that is
KAU	:	Kerala Agricultural University
K.C.A.E.T	:	Kelappaji College of Agricultural Engineering and Technology
l/min	:	Liter per minute
M	:	Meter
m^2	:	Square Meter
min	:	Minute(s)
mJ/cm^2	:	Milli Joules per square centimeter
ml	:	Milli litre
mm	:	Millimeter

NaOH	:	Sodium hydroxide
Nm	:	Nanometer
No.	:	Number
NTU	:	Nephelometric Turbidity Units
OH	:	Ohmic Heating
PET	:	PolyEthylene Terephthalate
S	:	Second(s)
s/m	:	Siemens/meter
Sl.	:	Serial
SPSS	:	Statistical Package for the Social Sciences
SS	:	Stainless steel
TSS	:	Total Soluble Solids
UV	:	Ultraviolet
V	:	Volt
V/cm	:	Volt per centimeter
W/m^2	:	Watt per square meter
μ	:	Micro
λ	:	Lamda

CHAPTER 1

INTRODUCTION

Fruits and vegetables are important supplements to the human diet as they provide essential minerals, nutrients including vitamin C, vitamin K, foliate, thiamin, carotene, dietary fibre and antioxidant phyto-compounds required for maintaining health. They are easily digested and exercise a cleansing effect on the blood and digestive tract. Fruits and vegetables have gained increasing interest among nutrition specialists, food scientists and consumers, since frequent consumption of fruits reduces the risk of certain cardiovascular diseases and cancer (Liu, 2003). In the recent years, demand for both fresh and processed fruits and vegetables have been substantial and this trend is likely to continue in future. The increasing demand can be regulated by either increasing their production or by adapting suitable processing techniques for its preservation. India is blessed with a variety of fruits and vegetables whose production during 2012-13 was 81.285 and 162.187 MT respectively, produced in an area of 6.982 and 9.205 ha. The growing demand increased the fruit production to the tune of 6.36% between 2012 and 2013 (National Horticulture Board, 2013). India's exports of fresh fruit and vegetable have increased by 17.82% during the period between 2012 and 2013 (APEDA, 2013). In spite of being a major producer of fruits, nearly 72% of fruits are wasted in India due to poor facility or absence of storage, logistics and processing support (Arivazhagan *et al.*, 2012). In addition to this, the highly perishable nature of fruits and vegetables due to their high water content, make them susceptible to desiccation, mechanical injury and pathological breakdown. This results in changes in texture, colour, flavour and nutritional value of the food. The biochemical and microbial changes render food unpalatable and potentially unsafe for human consumption.

Traditional food-processing technologies such as freezing, canning, and drying rely on heating or cooling operations. Although these technologies have helped to ensure a high level of food safety, the heating and cooling of foods

contribute to the degradation of various food quality attributes. The colour, flavor and texture of foods processed solely by heating may be irreversibly altered. Consumer demands for high quality foods that are fresh tasting and nutritious have created considerable interest in the development of new food processing techniques. To eliminate undesirable thermal effects on foods, considerable effort has been made to develop non-thermal technologies that rely on techniques other than uncontrolled heating or cooling operations.

Ultraviolet radiation treatment of pumpable fruit and vegetable juices is one of the non-thermal preservation methods. Ultraviolet processing involves the use of radiation from the ultraviolet region of the electromagnetic spectrum for the purpose of disinfection. Typically, the wavelength for UV processing ranges from 100 to 400 nm. This range may be further subdivided into UV-A (315 to 400 nm) normally responsible for changes in human skin that lead to tanning; UV-B (280 to 315 nm) that can cause skin burning and eventually lead to skin cancer; UV-C, (200 to 280 nm) called the germicidal range since it effectively inactivates bacteria and viruses, and the vacuum UV range (100 to 200 nm) that can be absorbed by almost all substances and transmitted only in a vacuum (Guerrero-Beltrán and Barbosa-Cánovas, 2004). When UV-C photons collide with oxygen atoms, the energy exchange causes the formation of ozone. UV-C is almost never observed in nature, since it is absorbed quickly.

Ultraviolet processing can potentially provide more ideal food products with fresh like characteristics. Although UV radiation has been investigated for inhibiting microbial growth, for removing microbial contaminants on the surface of fresh produce, and for disinfecting drinking, waste, and feed waters, practical applications of UV to disinfect water and liquid food products has limitations due to its low penetration depth, especially in liquid foods with turbidity, and suspended solids. This fact limits the application of UV radiation alone in effecting pasteurization of fruit juices.

Ohmic heating (OH), has been widely applied in food processes such as extraction, bleaching, and milk and juice pasteurization (Icier *et al*., 2008; Sun *et al*., 2008). Ohmic heating is the process of internal heat dissipation generated by applying alternating current through a food product with direct contact to two electrodes. Thus, heat is generated instantaneously in the food product, creating a rapid and uniform heating that reduces thermal abuse, as opposed to conventional thermal processing methods. Ohmic heating results in disruption of both the interiors and exteriors of cell walls by electroporation, which is the formation of pores in cell membranes in response to an electric field (Lima and Sastry, 1999). Therefore, ohmic heating has potential to process high-quality food products and retain their nutritional and sensory qualities while inactivating microorganisms.

Although number of studies on UV radiation and ohmic heating have been conducted separately, there is less information concerning UV radiation assisted with ohmic heating on the inactivation of pathogens in juice processing. But from what is stated, it may be hypothesized that the two stage process; first, the application of ohmic heating to a pumpable fruit juice could results in generation of electrophoretic force resulting in locking the open/close function of cell pores thus destroying cell membranes, and second, UV light could penetrate through the cell pores and disrupts the microbial DNA, preventing its replication and growth while retaining the overall quality of the juice.

There are almost 180 families of fruits that are grown all over the world. In that, citrus fruits constitute around 20% of world's total fruit production. The fruits are mainly processed into fruit juices and juice concentrates. Among the juices pineapple juice is gaining more importance at present. It was surveyed and reported that among pineapple, mango, and passion fruit juices, pineapple juice is most preferred by consumers (Sabbe *et al*., 2008).

Pineapple (*Ananas comosus*) is one of the commercially important fruit crops of India. Total annual world production is estimated at 14.6 million tonnes of fruits. India is the fifth largest producer of pineapple with an annual output of about 1.2

million tonnes. It is also a commercial fruit crop widely grown in Kerala with an annual production of 102.4 thousand tonnes covering an area of 12.5 thousand hectares having a productivity of 8.2 t/ha (National Horticulture Board, 2008-2009). The fruit is a good source of vitamin A, B and C and also calcium, magnesium, potassium and iron. It is also a good source of bromelin, a digestive enzyme. Pineapple is consumed fresh or in the form of juice, jam, squash and syrup. Among all forms, canned slices and juice are in much demand in India, constituting about 70% of the production. Fresh pineapple juice deteriorates with time due to growth of microorganisms and ferment during storage. Therefore, it has to be processed at the earliest in order to enhance its shelf life.

Considering the above facts a study was undertaken on **"Development and evaluation of an Ultraviolet radiation assisted with ohmic heating system for preservation of pineapple juice"** with the following objectives**:**

- To develop an ultraviolet radiation assisted with ohmic heating system for pineapple juice.
- To evaluate the developed system towards the preservation of pineapple juice leading to the standardization of process parameters.
- To evaluate the organoleptic characteristics of the treated juice.

CHAPTER 2
REVIEW OF LITERATURE

2.1 PINEAPPLE

Pineapple (*Ananas comosus*) is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavour and immense health benefits. Pineapple contains considerable calcium, potassium, fibre, and vitamin C and used as dietary fibre. Vitamin C is the body's primary water soluble antioxidant, against free radicals that attack and damage normal cells. Fresh pineapples are rich in bromelain used for tendering meat. Pineapple enzymes have been used with success to treat rheumatoid arthritis, diabetic ulcers, arteriosclerosis, anaemia. Pineapple reduces blood clotting and helps remove plaque from arterial walls. Pineapple fruits are primarily used in three segments, namely, fresh fruit, canning and juice concentrate with characteristic requirements of size, shape, colour, aroma and flavor.

2.1.1 Global Scenario

Pineapple exhibits increasing demand worldwide, over the years. The global trade is around 50% as fresh fruit, 30% as canned product and 20% as juice concentrate. World trade on fresh pineapple has shown 100 % increase during the last one decade. The main pineapple producers are Brazil, Thailand, Philippines, Costa Rica, China, India and Indonesia. The leading exporters are Costa Rica, Belgium, Philippines, Ghana, Netherlands, USA and France. Major importers are USA, Belgium, France, Italy, Germany, Japan and UK. MD2 or Dinar pineapple developed through hybridisation by Del Monte scientists in Costa Rica is the most popular variety in the international market because of its colour, flavour, shape, life span and ripeness being superior to other varieties.

2.1.2 National Scenario

India ranked sixth with a share of about 8 % of the world production of pineapples. The total area under pineapple cultivation in India is 84000 hectares with a production of about 1341000 t. India exports pineapple mainly to Nepal, Maldives, United Arab Emirates, Saudi Arabia, Kazakhstan, Oman, Bahrain, Bangladesh, Zambia, Pakistan and Qatar. ‘Kew’ and ‘Mauritius’ are the two

varieties of pineapple grown in India. It is grown in Karnataka, Meghalaya, West Bengal, Kerala, Assam, Manipur, Tripura, Arunachal Pradesh, Mizoram, and Nagaland. It is also cultivated on limited areas in the coastal belt of Tamil Nadu, Goa and Orissa. Though Assam has the largest area under pineapple West Bengal is the largest producer. Karnataka, West Bengal and Bihar are the three states reporting high productivity.

2.1.3 State Scenario

In Kerala, pineapple is cultivated in an area of 12500 ha with a production of 102400 t with a low productivity of 8.2 t/ha, consistently stable over the last few years. The congenial humid climate has favoured the cultivation of pineapple. The finest quality 'Mauritius Pineapple' comes from Kerala. Although pineapple cultivation is practised in almost all districts, the extent and trend of cultivation differs widely among Kerala's districts. The major pineapple producing district of Kerala, Ernakulam accounts for more than 60% of the area under pineapple cultivation**.** In Kerala, pineapple is grown mainly as an intercrop in rubber and coconut, and also as pure crop in garden land and in converted paddy fields. However, only less than two percent of the potential area in Kerala is cultivated with pineapple.

2.1.4 Benefits of Pineapple

2.1.4.1 Potential anti-inflammatory and digestive benefits

Bromelain is a complex mixture of substances that can be extracted from the stem and core fruit of the pineapple. Among dozens of components known to exist in this crude extract, the best studied components are a group of protein-digesting enzymes (called *cysteine proteinases*). In addition, researchers believed that excessive inflammation, excessive coagulation of the blood, and certain types of tumor growth may all be reduced by therapeutic doses of bromelain when taken as a dietary supplement.

2.1.4.2 Nutritional and Health Benefits

One of the juiciest fruits that is absolutely a delight to eat is the pineapple. It can be taken with whipped cream, custard and is one of the favorite drinks of many people during hot weather. The best part about pineapples is that

it is loaded with nutrients and beneficial enzymes, which ensures that you not only have a healthy body but also a glowing complexion. Pineapple is known to be very effective in curing constipation and irregular bowel movement. Pineapple is effective in getting rid of intestinal worms and also keeps the intestines and kidneys clean. It is effective in flushing out the toxins from the body, thus making the metabolism healthy. Pineapples are very rich in manganese and even a single cup of pineapple is supposed to contain a good amount of it. This mineral is required for the growth of healthy bones and tissues.

Table 2.1. Nutritional value of fresh fruit juice. (Holland *et al.*, 1991).

Constituents	**Nutritional value pineapple juice**
Protein, g	0.40
Fat, g	0.20
Carbohydrate, g	10.10
Fiber, g	1.20
Vitamins	
Vitamin-C, mg	12.000
B-group, mg	0.500
Carotene, mg	0.018
Minerals	
Potassium, mg	160.00
Calcium, mg	18.00
Iron, mg	0.20

2.1.4.3 Food Uses

In Puerto Rico and elsewhere in the Caribbean, Spaniards found the people soaking pineapple slices in salted water before eating, a practice seldom heard of today. Field ripe fruits are best for eating fresh, and it is only necessary to remove the crown, rind, eyes and core. In Panama, very small pineapples are cut

from the plant with a few inches of stem to serve as a handle, the rind is removed except at the base, and the flesh is eaten out-of-hand like corn on the cob. The flesh of larger fruits is cut up in various ways and eaten fresh, as dessert, in salads, compotes and otherwise, or cooked in pies, cakes, puddings, or as a garnish on ham, or made into sauces or preserves. Malayans utilize the pineapple in curries and various meat dishes. In the Philippines, the fermented pulp is made into a popular sweetmeat called *nata de pina.* The pineapple does not lend itself well to freezing, as it tends to develop off flavours.

Canned pineapple is consumed throughout the world. The highest grade is the skinned, cored fruit sliced crosswise and packed in syrup. All residual parts cores, skin and fruit ends are crushed and given a first pressing for juice to be canned as such or prepared as syrup used to fill the cans of fruit, or is utilized in confectionery and beverages, or converted into powdered pineapple extract which has various roles in the food industry. Chlorophyll from the skin and ends imparts a greenish hue that must be eliminated and the juice must be used within 20 hours as it deteriorates quickly. A second pressing yields "skin juice" which can be made into vinegar or mixed with molasses for fermentation and distillation of alcohol.

2.2 OHMIC HEATING

Ohmic heating is a thermal processing method in which an alternating electrical current is passed through food products to generate heat internally (Jha *et al*., 2011; Marra *et al*., 2009; Shirsat *et al*., 2004). Ohmic heating is an advanced thermal processing method wherein the food material, which serves as an electrical resistor, is heated by passing electricity through it. Electrical energy is dissipated into heat, which results in rapid and uniform heating of the food material. The technology is useful for the treatment of fruit juices which are generally characterized by high acidity conditions, which lead to the growth of yeast and mold, in addition to a few types of low-acid-tolerant bacteria. To avoid microbial spoilage, it is necessary to cause inactivation by applying heat by high temperature heating with very short exposition.which tend to denature when thermally processed. This holds

good for pineapple juice because pure pineapple juice can be ohmically heated in a fraction of a seconds.

Electrical fields, applied during ohmic heating of lipoxygenase and polyphenol oxidase, caused their faster inactivation than during conventional heating (Castro *et al.,* 2004).

Leizerson and Shimoni (2005) reported that ohmic heating is found to be more efficient for the required microbial and pectin esterase inactivation due to a shorter residence time while released flavor compounds are not degraded as quickly as during conventional pasteurization.

It was found that ohmic heating yields better products, clearly superior in quality than those processed by conventional heating (Allali *et al.,* 2010). Many reviewers reported the advantages of ohmic heating compared to conventional heating also include the more uniform and faster heating, cleaner and more environmentally friendly; higher yield and higher retention of nutritional value of food (Vikram *et al*., 2005; Nolsoe and Undeland, 2009; Sagar and Kumar, 2010; Ghnimi *et al*., 2008; Zareifard *et al*., 2003; Castro *et al*., 2004).

The ability to heat materials rapidly and uniformly through ohmic heating leads to a less aggressive thermal treatment. De Halleux *et al*. (2005) concluded that ohmic heating provided 82–97% of energy saving while reducing the heating times by 90–95% compared to conventional heating. They suggested that it could be possible to obtain efficiencies greater than 90% in an industrial process in which these losses were controlled by the wall insulation. Additionally, it is comparatively less difficult to clean an ohmic heater than traditional heat exchangers because of reduced product fouling on the heater's food-contact surface.

2.2.1 Principles and Techniques of Ohmic Heating

The basic relation for the energy generation rate in food under ohmic heating is given by Sastry (1992) as:

$$u = |\Delta V|^2 \sigma \qquad \ldots\ldots(2.1)$$

where,

u = energy generation per unit volume, W/m^3

ΔV = voltage gradient, V/cm

σ = electrical conductivity, S/m

It was reported that the electrical conductivity for most solid materials increased sharply with temperature at around 60°C due to breakdown of cell wall materials.

Total solid content (TDS), viscosity, acidity and acidity of the heating sample have effect on the ohmic heating rate. Ghnimi *et al*. (2008) have evaluated the ohmic heating performance for highly viscous liquids, they reported that, higher viscous fluids tend to result in faster ohmic heating than lower viscosity fluids. Icier *et al*. (2012) reported vice versa. The extraordinary results may be due to different reactions occurring during ohmic heating depending on their composition. Assiry *et al.* (2011) have mentioned that pure water is not a good conductor of electricity and it has a conductivity of 0.055 mS/cm. that because ions in solution is helping electric current to transports.

Samaranayake and Sastry (2005) studied the effect of pH on electrochemical behaviour of an electrode material using a 60 Hz sinusoidal alternating current. The experimental results showed that, all the electrode materials exhibited intense electrode corrosion at pH 3.5 compared to that of the other pH values, although the titanium electrodes showed a relatively high corrosion resistance.

Darvishi *et al*. (2013) investigated the behaviour of pomegranate juice under ohmic heating by applying voltage gradients in the range of 30– 55 V/cm. The results indicated that, as the voltage gradient increased, time and pH decreased.

Imai *et al.* (1995) examined the effect of various frequencies (50 Hz – 10 kHz) in ohmic heating of Japanese white radish. At 50 Hz, there was a sharp initial rise of temperature and short time to raise the temperature at the mid-part of radish to 80ºC. From the results, it is suggested that ohmic heating at low frequency is very effective for the rapid heating of agricultural products to improve texture.

Khalaf and Sastry (1996) investigated the effect of fluid viscosity on the ohmic heating rates of fluid-particle mixtures. Fluids of identical electrical conductivity but different viscosity were used. Under static ohmic heating conditions, the heating rates of fluid and particle were not significantly affected by

fluid viscosities, whereas in a vibrating batch or continuous flow ohmic heater, the rate of heating increased with fluid viscosity.

Meatballs containing spores of *B.Stearothermophilus* and precursors of chemical makers were thermally processed in a starch solution with 30-40% solids content using a 5 kW ohmic system (Kim *et al.,* 1996). Different temperatures, flow rates, holding tube lengths and fluid electrical conductivities were used. Higher lethality was observed, microbiologically and chemically, at the center of the meatball rather than near the surface.

Mizrahi (1996) reported that blanching by ohmic heating considerably reduced the extent of solute leaching as compared to a hot water process. This was possible by eliminating the dicing and having a relatively small surface to volume ratio and a short blanching time.

Thawing of frozen shrimp blocks requires large amount of fresh water that become wastewater. An ohmic unit was designed (Roberts *et al.,* 1998) to thaw two shrimp blocks. It was observed that the ohmic thawing did not use any water, or generate much wastewater and was more energy efficient. Thus the major obstacle of ohmic thawing had been solved and the method could be used to thaw shrimp blocks.

Eliot Godereaux *et al.* (2001) investigated the feasibility of processing cauliflower by ohmic heating. Experiments performed in a 10kW APV ohmic heating pilot plant resulted in a final product with interesting firmness properties and stabilized at 25ºC and 37ºC.

Sweet potato cubes were ohmically heated to three endpoint temperatures (45, 60 and 80ºC) using three electrical field strengths (50,70 and 90 V/cm) and both ohmic heated and non-heated samples were placed in freeze dryer for drying. The freeze drying vacuum pressure was maintained at $50x10^{-3}$ bar and the condenser temperature was -50ºC. Results showed that the vacuum drying rates of ohmically heated samples were faster than raw samples for most treatment combinations, and that the maximum reduction of drying time was 24 per cent at electrical field strength of 50V/cm, endpoint temperature of 45º C (Zhong and Lima, 2003).

Ayadi *et al.* (2004) have optimized the ohmic heater design, in particular inlet cells, to reduce the fouling ability in the laminar flow of dairy products. An evaluation study of a continuous ohmic heating apparatus was carried out using a whey protein solution. It was found that the whey protein solution generated fouling layers on the electrode surfaces and the deposit on the electrode surfaces acted as an additional electrical resistance. But the deposit was removed when the temperature reached boiling point.

Halleux *et al.* (2005) observed that the ohmic cooking of processed meats effectively reduced the cooking time to 90 to 95 per cent with energy savings of 82 to 97 per cent than conventional smoking methods.

Icier *et al.* (2006) performed the ohmic blanching for pea puree samples by applying four different voltage gradients in the range of 20-50 V/cm and compared with conventional blanching. Results showed that the ohmic blanching at 30 V/cm and above voltage gradient inactivated peroxidase enzyme at lesser time than the water blanching. The ohmic blanching at 50 V/cm gave the shortest critical inactivation time of 54s with the best color quality.

Praporscic *et al.* (2006) studied the effect of ohmic heating on juice yield from potato and apple tissues. The best juice yield was obtained when the plant tissues were treated electrically at a moderate temperature of 50ºC.

2.2.2 Electrical Conductvity

de Alwis and Fryer (1991) conducted a series of simulations, using a finite element model to examine the range of solid and liquid electrical conductivities for uniform heating in the ohmic heating process. It was observed that there was a considerable variation occured in heating rate if liquid and solid conductivities differed significantly. It was reported that electrical conductivity of food material critically determined the local rate of heat generation in ohmic heating. The rate of heat generation was given by the equation

$$Q = kE^2 \qquad \ldots\ldots (2.2)$$

where,

Q = rate of heat generation, W

k = electrical conductivity of product, S/m

E = electric field strength, V/m

The main critical factor in thermal processes is the thermal history and location of the "cold spot", locating cold zones during OH requires special consideration as the current knowledge of conventional heating cannot be extrapolated to OH technology (Knirsch *et al*., 2010).

The main critical parameter in ohmic heating is the electric conductivity (σ). In a non-homogeneous material, such as soups containing slices of solid foods, the electric conductivity of the particle and its relation to fluid conductivity is pointed as a crucial factor in ohmic heating, critical parameter to the understanding of particles' heating rate under ohmic heating (Darvishi *et al.*, 2013).

Assiry *et al*. (2010) and Nguyen *et al.* (2013) reported electric conductivity for different materials including fresh fruits under ohmic heating such as apple, pineapple, pear, strawberry and peach, in which their electric thermal conductivity in the range from (0.05 to 1.2) S/m pure water has poor electric conductivity, and it is around 0.055 μS/cm.

Halden *et al.* (1990) investigated the changes in electrical conductivities of pork meat and potato during ohmic heating. Electrical conductivity of pork was slightly lowered at higher temperatures due to fat melting. But for potato, electrical conductivity increased at around 80º C, due to starch gelatinization.

Non homogeneous material, such as soups containing slices of solid foods, the electrical conductivity(s) of the particles and its relation to the fluid conductivity is pointed as critical parameter to the understanding of the particles heating rate under OH (Palaniappan & Sastry, 1991b).

Kusnadi *et al*. (2012); Samprovalaki *et al*. (2012); Sarkis *et al*. (2013); Somavat *et al*. (2012) and Somavat *et al*. (2013) concluded that variation of electric conductivity with temperature of food products during ohmic heating. It was found that electrical conductivity increased with temperature mainly due to increase of ionic mobility and this phenomenon should be factored in to the design of continues ohmic heaters.

Low conductivity solid particles, comparatively to the fluid conductivity, tend to lag behind the fluid at low concentrations related to the volume of the fluid.

However, in conditions where the concentration of the particles is high, those same low conductivity particles may heat faster than the surrounding fluid. So, the phenomenon of particle-lagging or particle-leading depends on the significance of particle resistance to the overall circuit resistance. With the increase of the particles concentration, the electric current path through the fluid becomes more tortuous, forcing a greater percentage of the current to flow through the particles. This can result in higher energy generation rates within the particles and consequently in greater relative particle heating rate (Sarang *et al*., 2007; Sastry and Palaniappan, 1992).

Palaniappan and Sastry (1991a) conducted studies on electrical conductivities of vegetable and meat samples in a static ohmic-heating device. It was reported that conductivity increased linearly with temperature whereas the conductivity-temperature curve gradually became non-linear when field strength was decreased.

A device was developed by Palaniappan and Sastry (1991b) to determine the electrical conductivities of foods under ohmic or conventional heating conditions. Orange and tomato juices were tested in the device. They have reported that the electrical conductivity of juices increased with temperature and decreased with solid content.

Ohmic heating behavior and electrical conductivity of two-phase food systems were studied by Zareifard *et al.* (2003) for carrot puree and cubes of different sizes. It was found that the electrical conductivities increased linearly with temperature and decreased as particle size and concentration increased.

Icier and Ilicali (2005) carried out studies on apricot and peach purees in a laboratory scale ohmic heater by applying voltage gradient in the range of 20-70 V/cm. The study showed that there existed a linear temperature dependent electrical conductivity relationship. This fact indicates that it may be possible to adjust the heating pattern of solid fluid systems by adjusting the overall influence of particle resistance in the system through setting the particles concentration in the fluid. The electric conductivity of some systems may also be altered to achieve the ideal OH situation, when the conductivity of the particles is equal to the surrounding fluid (Wang & Sastry, 1993a).

The electric conductivity is a function of the structure of the material and often changes by heating (cooking); however in some foods, the overall heating effect might be too little to alter the pattern of electrical conductivity (Pongviratchai and Park, 2007). There are also critical σ values below 0.01 S/m and above 10 S/m where OH is not applicable. This is because very large voltages or very large amperage values would be needed to generate the amount of heat required raising temperature substantially by the Joule effect, in case of very low or very large σ values, respectively (Piette *et al*., 2004; Piette, *et al*., 2001b). Palaniappan and Sastry (1991a) reported that the infiltration of a salt solution might increase the conductance of vegetables while, in contrast, the leaching out of ions from vegetable tissue during immersion in water may decrease conductance.

Halden *et al*. (1990) monitored conductance during the blanching of vegetables and suggested that the destruction of cell walls released cytoplasm contents thus changing the conductance value. Wang and Sastry (1993a) suggested that the infiltration of salt solution to improve the conductance of vegetable tissue. Increasing the electrolytic content within foods to increase electrical conductivity may be accomplished by salt infusion via soaking or blanching of solids in salt solution.

Amongst the principal causes of electrical conductivity changes in foods during OH, the destabilization of cellular membranes is pointed to be the main responsible effect for the reduction of the system's impedance (Lebovka, *et al*., 2005; Pongviratchai and Park, 2007). It was pointed out that electrical conductivity changes in foods during OH, is also affected by cell rupture, cell electroporation, tissue shrinkage, phase change, dehydration, starch gelatinization, salt concentration and mobility, moist mobility, pH value and the presence of fat or other non-conducting substances, among other factors.

2.2.3 Electrode Material

Electrode design problem such as electrode polarization and fouling was the major hurdle in development of suitable ohmic heating system though it enables to heat the food at extremely rapid rate (Singh *et al*., 2014). Previous designs attempted to use different conductive electrode materials such as titanium, stainless

steel, platinized-titanium, aluminium and graphite, electrodes etc. based on price and resistance which may affect the efficiency of the ohmic heater. When the product quality is not essential such as waste treatment, low carbon electrodes are often employed. For high product quality applications, metals such as stainless steel are preferred. At the same time the frequency of the power supply must be increased significantly to prevent corrosion and apparent metal dissolution (Stancl *et al*., 2010)

Yadav *et al.* (1986) investigated the electrical deacidification of milk. Stainless steel, silver and aluminium were tried as electrode material. Aluminium was found to be the most acceptable, as stainless steel electrodes were conducive to pitting and silver electrodes lost their weight rapidly and hence were uneconomical.

Wang and Sastry (1997) employed ohmic heating for starch gelatinization using 30 cm long, 2.54 cm diameter glass tube provided with titanium disc electrode. It was observed that heating rate increased significantly once the gelatinization was over.

Ohmic heating of foods inside a flexible package was modeled and optimized by Jun and Sastry, (2005). A package configuration with V-shaped electrodes with dimensionless width of 0.147 was validated to be most appropriate for uniform heating with a minimum cold zone of 2 per cent of total area.

Arrhenius equation was used by Assiry *et al.* (2006) to study the degradation kinetics of ascorbic acid in a batch type ohmic heater using stainless steel electrodes. It was observed that at high temperature, the reaction rate decreased due to decreased dissolved oxygen concentration.

2.2.4 Electroporation

The applied electric field under OH causes electroporation of cell membranes. The cell electro- poration is defined as the formation of pores in cell membranes due to the presence of an electric field and as consequence, the permeability of the membrane is enhanced and material diffusion throughout the membrane is achieved by electro-osmosis (An and King, 2007; Lima and Sastry, 1999). An and King, (2007); Sensoy and Sastry, (2004) reported that the electric breakdown or

electroporation mechanism is dominant for the non-thermal effects of OH. Yoon *et al.* (2002) observed that under OH the electric field appeared to have both direct and indirect effect on the cell wall, and intracellular materials were exuded to the culture medium. The exudates seemed to be composed of amino acids, protein, nucleic acids, coenzymes, and related material (Yoon *et al.*, 2002).

Yoon *et al*. (2002) hypothesized and concluded that at temperatures above 50C, the concentration of exuded materials from the ohmic heated groups were higher than those from conventional groups ($p < 0.01$) and that the rate of protein exuded per unit temperature increase was found to be significantly higher ($p < 0.01$) with OH than with conventional heating. The influence of the electrical field within OH might have increased the rate of electroporation, thereby leading to excess exudation and cell death. It was also observed that the amount of exuded protein increased significantly as the electric field increased from 10 to 20 V/cm. Spectroscopic analysis has shown that for OH at 20 V/cm the absorbance at 260 nm typically attributed to nucleic acids was 2-fold ($p < 0.01$) and the total protein content was 3-fold higher ($p < 0.01$) when compared with that at 15 V/cm.

2.3 ULTRAVIOLET LIGHT PROCESSING

Ultraviolet light can be used to inactivate many types of organisms, including viruses. A monochromatic UV light (254nm) is obtained by using low-pressure mercury vapour germicidal lamps. The UV light acts as a physical method for microbial disinfection. Due to the wide variety of organisms, including strains, the dose levels required for disinfection can vary according to the final effect required for each food product (Guerrero and Barbosa, 2004).

The effect of UV light on microorganisms may vary from species to species and in the same species, may depend on strains, growth media, and stage of culture (Chang *et al.*, 1985; Wright *et al.*, 2000), density of microorganisms and other characteristics such as type and composition of the food. Fungi and yeasts are more resistant during disinfection. Hijnen *et al.*, (2006) showed that UV radiation affects the DNA of bacteria, viruses, fungi and other microorganisms exposed to it in such a way that it prevents them from reproducing.

It was reported that UV radiation has a significant germicidal effect at wavelength of 254 nm with doses between 0.12 and 9.0 kJ m^{-2} in the UV-C range because of radiation hormesis, which causes inactivation of microorganisms as a consequence of DNA damage (Liu *et al*., 1993; Stevens *et al*., 1997; Nigro *et al*., 2000)

Sizer and Balasubramaniam (1999) concluded that the UV penetration depth into fruit and vegetable juices as approximately 1 mm with a maximum absorption of 90% of the light. In order to meet the required 5 log microbial reduction in liquid foods, a thin layer form for non-thermal UV processing should be required (Geveke and Torres, 2012).

A very important concern during UV processing is the application of appropriate dose to ensure delivery of safe food products and to avoid the possibility of spoilage due to photo reactivation. To avoid this disadvantage, the product should be maintained under refrigeration or dark packages.

2.3.1 Application of UV light in Food Preservation

UV-C radiation is a non-ionising radiation and has the advantage that it does not produce chemical residues, by-products or radiation. Also, it is a simple dry and cold process (Bachmann, 1975; Morgan, 1989) requiring very low maintenance. It is a low cost technology, as it does not need energy as a treatment medium. (Sastry *et al.,* 2000).

In the food industry UV-C irradiation has been mainly applied in various processes and products such as air disinfection in meat or vegetable processing, on the water that will be used in some stages of the process, on surfaces of fresh products, chicken, fish, eggs, and various liquid food: milk, fruit juice or cider as reported by Basaran *et al.*, (2004); Quintero-Ramos *et al.*, (2004); Matak *et al*., (2005); and Hadjock *et al.*, (2008).

2.3.1.1. Liquid foods

UV-C light only penetrates a very short depth into the surface of liquids other than clear water (Shama, 1999). The penetration of UV light into juices is about 1 mm for absorption of 90 per cent of the light (Sizer and Balasubramaniam, 1999).

This is the main reason for using a turbulent flow during liquid food processing. More recently, it has been reported that the Food and Drug Administration (FDA, USA) is considering allowing UV-C to be used to eliminate pathogens from fruit juices (Bintsis *et al.,* 2000).

Hoyer (1998) pointed out that photoreactivation of cells may occur when cells are exposed to visible light in the blue spectral range. These photoreactivated cells can be more resistant to UV-C light when a second UV treatment is applied. In this study it was observed that greater UV-C doses are required to obtain a 4 log reduction of photoreactivated cells previously UV-C treated in water.

The penetration effect of UV-C radiation depends on the type of liquid, its UV-C absorptivity, soluble solutes in the liquid and suspended matter. Increasing the amount of solids will diminish the intensity of penetration of the UV-C radiation; large suspended particles may also block the incidence of light on the microbial load Bintsis *et al.,* (2000).

Wright *et al.* (2000) developed a thin film UV-C disinfection unit (ten individual chambers in series) to treat inoculated unpasteurised apple cider with a mixture of five strains of acid resistant *E. coli* 0157:H7. Using various flow rates, ranging from 0.999 to 6.48 l/min, corresponding to a dose range of 610 to 94 J/m^2 and found a 3.81 log cfu/ml reduction of *E. coli* 0157:H7 in apple cider. However, this reduction is not enough to achieve the recommended 5 log microbial reduction in liquid foods.

Farid *et al*. (2001) treated a thin film of orange juice falling over the wall of a UV system at 214.2 W/m^2 and found that the shelf life of UV-treated orange juice doubled without changes in colour and taste.

Ngadi *et al*. (2003) studied the inactivation kinetics of *Escherichia coli* O157:H7 in liquid foods such as apple juice and egg white by ultraviolet light. The applied UV dose ranged from 0 to 6.5 mW min/cm^2, while the depths of the medium were 1.0, 3.5, 5.0 and 10.0 mm. It was found that pH of the medium did not affect the inactivation of *E coli* O157:H7 and decreasing the depth of the medium increased the inactivation of *E coli* O157:H7. More than 5 log reduction was obtained when the fluid depth and UV dose were 1.0 mm and 390 mJ/cm^2.

However, less than a 1-log reduction was obtained when the fluid depth was 10.0 mm.

2.3.1.2 Effect of UV light in fruits and vegetables

UV-C light is also applied to fresh fruits, vegetables and roots before being stored to reduce the initial count of microorganisms on the surface of the product, which could induce host resistance to the microorganisms. The beneficial effect of UV-C light on fresh food products is called 'hormesis' and the agent (UV light) is called 'hormetin' or 'hormetic effect' (Stevens *et al.*, 1990).

Stevens *et al.* (1997) applied low UV-C light doses as a hormetic agent to reduce the brown rot caused by *Monilinia fructicola* on peaches, green mould caused by *Penicillium digitatum* on tangerines, and Rhizopus soft rot caused by *Rhizopus stolonifer* on tomatoes and sweet potatoes during storage. It is indicated that an integration of UV– C with the yeast treatment can be effective as commercial post-harvest fungicide treatment in reducing storage rots.

Stevens *et al.* (1998) applied UV-C light to peaches and found an augmented Phenylalanine Ammonia-Lyase (PAL) concentration and a diminishing of the ethylene synthesis that improved the shelf life of the fruit by delaying ripening. Stevens *et al.* (1999) studied induced resistance of sweet potato to fusarium root rot by UV-C hormesis. It was found that hormetic effect may stimulate the production of PAL that induces the formation of phytoalexins (phenolic compounds), which may, in turn, improve the resistance of fruits and vegetables to microorganisms.

Brown *et al.* (2001) investigated the effect of low dose ultraviolet light-C seed treatment on induced resistance in cabbage to black rot. The optimum UV-C dose of 3.6 kJ/m^2 was effective in reducing black rot and the population density of *Xanthomonas campestris pv. campestris* in infected cabbage leaves. Seeds treated with UV-C produced plants with the most desirable color, highest weight, largest head diameter and delayed maturity.

2.3.1.3 Application of UV light in surface sterilization

Allende and Artés (2003) studied the effect of UV-C radiation on minimally processed Lollo Rosso lettuce. Fresh processed lettuce was exposed to different

doses (0.40, 0.81, 2.44, 4.07 and 8.14 kJ m^{-2}) of UV-C radiation, and stored up to 9 and 10 days at 5°C. All UV-C radiation doses decreased growth of psychrotrophic bacteria, coliform, and yeast, but only significant differences were found when the highest level was applied. After seven days of storage the highest dose resulted in increased tissue brightness, and browning was reduced when dose of 2.44, 4.07, and 8.14 kJ m^{-2} were applied. UV-C radiation at an appropriate dose could reduce microbial loads without adversely affecting sensory quality of Lollo Rosso lettuce.

Yaun *et al.* (2004) used ultraviolet energy (UV- C) to investigate the bactericidal effects on the surface of red delicious apples, leaf lettuce and tomatoes inoculated with cultures of *Salmonella* spp. and *E. coli* O157:H7. Inoculated samples were subjected to different doses ranging from 1.5 – 24 mW/cm^2 of UV-C to determine effective log reductions of microbial populations. UV- C applied to apples inoculated with *E. coli* O157:H7 resulted in the highest log reduction of approximately 3.3 logs at 24 mW/cm^2. Lower log reductions were seen on tomatoes inoculated with *Salmonella* spp. (2.19 log) and green leaf lettuce inoculated with both *Salmonella* spp. and *E. coli* O157:H7 resulted 2.65 and 2.79 log reduction, respectively.

2.3.1.4 Water treatment

Sommer *et al.* (1996) used a standardized biodosimetric method to quantify the influence of reflection on the Reduction Equivalent UV Doses (RED) in two water disinfection systems. One device, possessing a reflector made of aluminium, showed a decrease of RED of about 40 per cent after removing the reflector. In the second device, the inner surface of the irradiation made of stainless steel chamber served as a reflector by itself. After eliminating the reflectance by black lacquering in the inner surface of the irradiation chamber a distinctive decrease of RED was measured. This effect was dependent on the transmittance of the water.

Shaban *et al.* (1997) studied the effect of UV radiation on inactivation of a range of microorganisms combined with factors affecting the radiation. It was reported that 1min contact time (0.5 l/min^1 flow rate) was effective against vegetative cells levels almost reaching zero (except with *Staphylococcus aureus*). On the other hand, spore-forming bacteria, *Candida albicans* and coliphage were more resistant to UV. In reactivation experiments, it was clear that

photoreactivation, and not dark repair, takes place with bacterial cells and irradiated algae regained their normal shape after 3 days in suitable media and enough light.

Sommer *et al.* (1998) found that efficiency of UV water disinfection device depends on flow, lamp intensity and water transmittance. An investigation was carried out to investigate the influence of transmittance against intensity on disinfection of water at the same sensor readings in a specially designed laboratory flow through UV irradiation system with one single UV lamp. Lamp intensity was decreased by diminishing the supply voltage. UV transmittance was reduced by pumping aqueous sodium thiosulphate solution into water inflow. The disinfection capacity was determined by measuring the Reduction Equivalent UV Doses (RED) using a standardised biodosimetric method. It was found that equal sensor readings, either achieved by reducing the lamp intensity or by lowering the UV transmittance of the water, resulted in different reduction equivalent UV doses in one-lamp systems.

2.3.2 UV Processing and Equipments

The most common approach to disinfecting liquids by UV-C light is by running the liquid through an annulus, as in those used for drinking water disinfection. However, the disinfection may not be effective if the thickness of the layer of liquid is not optimum, since UV penetration depends on the absorptivity of the liquid. Thin films of liquid are recommended to increase the effectiveness of UV-C penetration into liquids to ensure a lethal dose against bacteria (Shama, 1992).

Shama *et al.* (1996) developed a thin film photoreactor that had a nozzle with a special design that could spray the liquid by forming a liquid bell. The equipment had a UV-C lamp positioned axially inside the falling liquid bell and four UV-C lamps circumferentially located outside the liquid bell to improve the UV germicidal effect. Each lamp was held at a distance of 10 cm from the liquid bell. *E. coli* (1.2×10^7 cfu/ml) suspended water or humic acid was recirculated at a rate of 13.5 l/min. It was reported that the initial load reduced to survival fractions of 1.88×10^{-5} and 1.84×10^{-4} for absorptivities of 0.18 cm^{-1} (water) and 4.0 cm^{-1} (humic

acid), respectively, after 30 min of treatment. The dosage delivered was between 20.3 and 48.4 J/m^2 for one and five sources, respectively.

Sastry *et al.* (2000) used mixing devices before and after the UV-C treatment unit to ensure appropriate mixing of microorganisms in the system and to obtain a representative sample to assess residual microorganisms after processing.

Koutchma *et al.* (2004) examined the effectiveness of UV inactivation of pathogens in juice / cider and liquid model systems with a thin film UV reactor and a turbulent flow UV reactor. The thin film flow through laboratory UV unit incorporates three individual chambers connected in tandem with tubing. Eight low-pressure mercury lambs are mounted within a quartz sleeve running centrally through all three chambers. Apple juice / cider are pumped from a reservoir through a 0.08 cm annular gap between inner surface of each chamber and outer surface of the quartz sleeve. In turbulent flow UV reactor the treatment is achieved by passing liquid through a stainless steel chamber containing 12 UV emitting low-pressure arc tubes. The arc tube is mounted in a quartz sleeve and fitted within the chamber allowing liquid to pass the sleeve on all sides.

Tran and Farid (2004) designed a thin film UV reactor for treating the fruit juices. The UV reactor was made up of glass tube, which is fixed vertically with orange juice flowing by gravity as a thin uniform film along its inner surface. A low pressure UV lamp was fixed at the axis of the glass tube and enclosed by a quartz tube to prevent direct contact of the lamp with the juice. Air was pumped through the annular space surrounding the lamp for cooling as lamp efficiency drops at high temperature. The effect of UV doses was studied by circulating the juice more than once through the reactor rather than changing the lamp intensity. The energy required for UV treatment of orange juice (2.0 kW h/m^3) was much smaller than that required in thermal treatment (82 kW h/m^3). The colour and pH of the juice were not significantly influenced by the treatment.

2.3.3 Dose Measurement and Calculation

Three methods used to measure the UV dose delivered by a low pressure UV disinfection system are bioassays, chemical actinometry and mathematical modeling. The bioassay and chemical actinometry approaches are direct but time

consuming (Giese and Darby, 2000). The UV-C dose emitted from a lamp is usually measured using UV sensors in W/m^2 units. Radiometers (thermal or photonic) are instruments used to measure UV irradiance. Actinometers are used for measuring concentrations of products that come from photochemical reactions; these concentrations are directly related to the amount of UV light absorbed by the treated product (Shama, 1999).

Qualls and Johnson (1983) developed a bioassay method to measure the average intensity in flow through reactors as well as to verify a method of intensity calculation. The survival of spores of *Bacillus subtilis* ATCC 6633 was determined as a function of the UV dose to prepare a standard curve. Spores were added to unknown systems and survival rate was used to determine the average intensity.

Chang *et al.* (1985) used a radiometer to measure the incident intensity (I_o) at the liquid surface at 254 nm. Stirred suspensions of 0.5 cm depth were irradiated in small petri dishes. The average intensity in the stirred suspension (I_{avg}) was calculated using the equation:

$$I_{avg} = \frac{I_o\left(1 - e^{-A_e L}\right)}{A_e L} \qquad \ldots(2.3)$$

Where,

A_e = absorbance per centimeter

L = path length in mm

Giese and Darby (2000) used a calibrated radiometer to measure the irradiance delivered by a single UV lamp. Three narrow band pass filters with maximum outputs at 254, 280 and 301 nm were used to give monochromatic irradiation treatments. The radiometer measures a 30 s average intensity. The radiometer was positioned at elevation of water surface of irradiated samples. The measured intensity was corrected for absorbance of the liquid sample according to Beer's Law. Dose was calculated as the product of intensity and exposure time. Mathematical model was used to predict the dose delivered by a system based on both measured and assumed characteristics of the system. The germicidal intensity at a point in a reactor system delivered by a single medium pressure lamp is

$$I_g = \int_{220\,nm}^{300\,nm} I_\lambda G_\lambda d\lambda \quad \ldots (2.4)$$

Where,

I_g = germicidal intensity at a point in the reactor system

I_λ = intensity of light at wavelength λ (nm) at that point.

G_λ = germicidal efficiency

$$G_\lambda = \frac{\text{dose at } 254\,nm \text{ to achieve a specified inactivation level}}{\text{dose at wavelength } \lambda \text{ to achieve a specified inactivation level}} \quad \ldots (2.5)$$

Bolten and Linden (2003) developed a protocol for determination of the fluence (UV dose) in a bench scale apparatus containing UV lamps emitting either monochromatic or broadband UV light. This protocol includes specifications for construction of a bench scale apparatus, methods for determination of the average irradiance in water, details on UV radiometry and considerations for microbiological testing.

Adhikari *et al.* (2005) evaluated 4, 4′, 4″-tris-di-B-hydroxyethyl aminotriphenyl-acetonitrile (HHEVC) dye as a chemical actinometer in model buffers for UV treatment of apple juice and cider. The sensitivity of HHEVC dye to UV light was greater than that of the other standard chemical actinometers such as ferrioxalate and potassium iodide, in the dose range of 10 mJ/cm^2.

UV intensity flux or irradiance is usually expressed in W/m^2 and the dose or radiant exposure is expressed as J/m^2 (Bintsis *et al*., 2000). The UV-C dose (D) (Morgan, 1989; Stevens *et al.*, 1999) is defined as:

$$D = I_{254} \times t \quad \ldots\ldots (2.6)$$

Where,

D = dose in J/m^2

I_{254} = intensity or dosage rate in W/m^2

t = retention time in s

In a flow system, the retention time is obtained as:

$$t = \frac{\text{Volume of chamber}}{\text{Flow rate}} \quad \ldots\ldots (2.7)$$

2.3.4 Parameters in UV Light Treatment

The effectiveness of UV penetration on liquid foods is affected by factors such as light source, flow profile, geometric configuration of the UV treatment chamber, repairing mechanisms of the microorganisms, transmittance and absorbance of the liquid food.

2.3.4.1 Light source

The light source is restricted to UV-C light or, more specifically, to 254 nm because of its germicidal effect on microorganisms. As the UV-C radiation passes through the liquid, its intensity is reduced (Shama, 1999). It was reported that UV radiation loses 30 per cent of its intensity at 40 and 10 cm below the surface of distilled water and seawater, respectively (Bintsis *et al*., 2000). For this reason, exposure time, dosage and flow profile are critical in achieving the required effect on microbial load in liquids to deliver microbiologically safe food products.

2.3.4.2 Geometric configuration and flow profile

Geometric configuration is critical to ensure required disinfection in the food system. Various configurations reported were A thin film throughout the pipes (Wright *et al*., 2000), A liquid bell formed by spraying the liquid with nozzles (Shama,1992; Shama *et al.*, 1996), Laminar and turbulent flow reactors (Koutchma *et al.*,2004), Thin film UV reactor (Tran and Farid, 2004). It is essential that the UV system should be arranged to produce a flow profile that best suits the desired germicidal effect.

2.3.4.3 UV Reparing Mechanisms

It is reported that Irradiation of cells with UV light (220 to 320 nm) would result in the formation of intrastrand cyclobutyl – pyrimidine dimmers in the DNA, leading to mutagenic changes or cell death. Several repair pathways exist for the repair of UV induced DNA damage including photoreactivation, excision repair, recombinational repair and inducible error-prone repair (Knudson, 1985).

Photoreactivation is the error-free, light dependent (300 to 600 nm) enzymatic monomerization of UV induced pyrimidine dymers. The phooreactivating enzyme has been found in many species of bacteria. Carson and Petersen (1975) have reported that commercial UV water sterilizer unit sterilize water in dark, containing levels of 10^5

to 10^6 cells of *Pseudomonas cepacia* per ml. However, if the water was exposed to average room light conditions after UV exposure there was significant photoreactivation and subsequent growth of the organism.

Knudson (1985) studied the sensitivity of *Legionella* species to low does of UV. He reported that *L.pneumophila* and six other phenotypically related but genetically distinct *Legionella* species are sensitive to low doses of short wave UV light. In some trials the germicidal effect of the UV light was countered by as much as 4 log increase in survival when the UV irradiated cells were exposed to photoreactivating light.

Tosa and Hirata (1999) determined the susceptibility of enterohemorrhagic *Escherichia coli* O157:H7 and O26 to UV radiation at 254 nm and photoreactivation. The study showed that the dose of UV light required for 90 and 99% inactivation of EHEC O157:H7 was 1.5 and 3.0 mJ/cm^2, respectively. The dose of UV light required for 90 and 99% inactivation of EHEC O26 without photoreactivation was 5.4 and 8.1 mJ/cm^2, respectively. Apparent photoreactivation with visible light from a fluorescent lamp was observed in EHEC O26 but not in EHEC O157:H7. The dose of UV light required for 90 per cent inactivation of EHEC O26 after photoreactivation was 12.0 mJ/cm^2.

2.3.4.4 Presence of particulate materials

Particles can protect bacterial cells from UV radiation by shielding, absorbing, scattering and blocking not necessarily through bacterial attachment to particles as in chemical disinfection. Microorganisms can be present in the shadow cast by attaching to particles, thus allowing escape from full exposure to the UV radiation. The productive effect against UV inactivation of attached bacteria depends on particle size and turbidity levels (Christensen and Linden, 2003).

2.3.4.5 Suspended particles

Templeton *et al.* (2005) studied the inactivation of particle associated viral surrogates by ultraviolet light. The results of the study suggest that particles < 2 μm in diameter are large enough to protect viruses from UV light and that particulate chemical composition (UV- absorbing organic content) may be a critical factor in survival of particle associated viruses during UV disinfection.

Wu *et al.* (2005) examined the impacts of goethite particles on UV disinfection of drinking water. A unique association between bacterial cells and small goethite particles (~ 0.2 to 2 μm) protected *Escherichia coli* and *Pseudomonas putida* from UV inactivation was observed in this study. The protection increased with the particle concentration in the turbidity range of 1 to 50 Nephelometric Turbidity Unit (NTU) and with the bacterium – particle attachment time prior to UV irradiation. The lower degree of bacterial inactivation at longer attachment time was mostly attributed to the particle aggregation surrounding bacteria that provided shielding from UV radiation.

Adhikari *et al.* (2005) added suspended apple solids (1 to 4g / 100 ml) to malate buffer solution (0.13 or 0.4 g per 100 g caramel) to stimulate apple cider with turbidities of 100 – 1200 NTU. The effect of suspended solids in malate buffer solution on the delivery of UV dose to the juice or cider in a static system was examined. The decrease in delivered UV dose was much greater between 0 and 0.4 g caramel per 100 ml than it was between 0 and 4 g suspended solids per 100 ml and can cause a negative impact on the effectiveness of UV light for microbial destruction.

2.3.5 Effect of UV Radiation on Microorganisms

Begum *et al.*, (2009) reported that the UV-C light (254nm) is easy to use technology for disinfection of liquid foods. It has lethal effects on microorganisms such as bacteria, viruses, protozoa, yeasts, and molds. The germicidal effect of UV-C light on micro organismsis is at the DNA level. The absorption of UV-C light generates electronic changes that may cause breaking of the DNA bonds; therefore, microbial cells could be compromised. The photoproducts (pyrimidine nucleotide bases), enerated by the application of UV-C light, block the DNA transcription and replication; even more, inhibits cell functions that may cause the cell death (Guerrero-Beltran and Barbosa-Canovas, 2004).

Chang *et al.* (1985) studied the UV inactivation of pathogenic and indicator microorganisms. It was reported that vegetative bacteria and total coliforms exhibit resistance to UV light. However viruses, bacterial spores and amoebic cysts were 3 to 15 times more resistant to UV light when compared to *E.coli*.

Giese and Darby (2000) studied the sensitivity of microorganisms to different wavelengths of UV light. The responses of three species of coliform bacteria and bacteriophage to three wavelengths of UV light (254, 280 and 301 nm) were measured. The values of germicidal efficiency at 280 nm determined for each of the microorganisms were non significant. At 301 nm, the values of germicidal efficiency were significantly different, but all values were too small. From this study it is known that values of germicidal efficiency determined for one species of bacteria or virus may be used to represent the relative responses of all bacteria and viruses to medium pressure UV irradiation.

Sommer *et al.* (2000) examined effect of UV inactivation, dark repair and photoreactivaion of *Escherichia coli* O157:H7 and other pathogenic *Escherichia coli* strains in water. A wide divergence in the UV susceptibility was found within the strains tested. A 6-log reduction of bacteria that fulfils the requirement for safe water disinfection was varied from 12 J/m^2 (most susceptible strain) to 125 J/m^2 (most resistant strain). Dark repair did not played an important role in all the tested strains but in contrast all strains demonstrated photo repair ability. For 6 log reduction of these strains UV fluence up to 300 $J/m^2$is required. The results reveal that the minimum fluence of 400 J/m^2 is sufficient to inactivate pathogenic *E. coli* for water disinfection.

Green *et al.* (2004) studied the efficacy of ultraviolet germicidal irradiation to inactivate culturable fungal spores. The result indicated that Ultraviolet Germicidal Irradiation (UVGI) dose necessary to inactivate 90 per cent of the *Aspergillus flavus* and *Aspergillus fumigatus* was 35 and 54 mJ/cm^2, respectively.

Basaran *et al.* (2004) examined the influence of apple cultivars on inactivation of different strains of *E.coli* O157:H7 in apple cider by UV irradiation. Comparison of log reductions among the *E.coli* strains to the cider parameters of °Brix, pH and malic acid content failed to show any statistically significant relationships. The result of this study indicated that regardless of the apple cultivar used, a minimum 5 log reduction is achieved for all strains of *E.coli* O157:H7 tested with a 14 mJ/cm UV dose.

Wang *et al.* (2005) examined the inactivation of *E.coli* using UV radiation from a pulsed xenon flash lamp. Using 8 nm wide pulse of UV radiation, the most efficient inactivation is found to occur at around 270 nm and no inactivation is observed above 300 nm. A peak value of 0.43 log reduction per mJ/cm^2 of UV dose was observed in *E.coli*.

2.4 COMBINATION TECHNOLOGIES

Non-thermal processing technologies include the application of high-voltage pulsed electric fields (PEF), high hydrostatic pressure, ultraviolet light (UV), high intensity light pulses (HILP) and manothermosonication (MTS) Combinations of these have been recently exploited for complete and efficient microbial and enzymatic inactivation in fruit juices and milk (Irene *et al.,* 2011).

Successful combinations of nonthermal processes depend not only on increased microbial inactivation but also on the technical compatibility and suitability of the selected processes. Product characteristics are an important consideration, as PEF and ultrasonication are mainly restricted to treatment of liquid products, while ultraviolet radiation and pulsed light are limited to application on food or packaging surfaces (Barbosa *et al.,* 1998; Sizer and Balasubrama- niam, 1999; Butz and Tauscher, 2002).

Su *et al.* (1996) observed that applications ultrasound increased the inactivation of B. subtilis spores under PEF; however, no details reported were in their published abstract as to the intensity of the ultra sonication treatment, whether it was performed before, during or after PEF, and what were the actual levels of inactivation.

Halim *et al.* (2012) reported that Ultraviolet irradiation alone was found effective in reducing microbial counts of to 2 log reduction. Although the combined treatment did not achieve a 5 log microbial reduction, two-fold microbial reduction could be achieved compared to individual treatments.

Pressurization may reduce the effectiveness of a PEF treatment, as Knorr (2001) found that electric field pulse treatment under high pressure (200 MPa) exerted a protective effect against permeabilization of bacterial cell membranes although each treatment alone cause major damage at this site.

Pagan *et al*. (1998) studied the possibility of germinating Bacillus spores using HHP, then inacti- vating the germinated cells with a PEF treatment. They found that germination of more than 5-log cycles of spores was initiated by pressurization, and while the germinated cells did become sensitive to a subsequent heat treatment, they were not sensitized to PEF application below 40 ^{0}C. It was suggested that spore inactivation by these combined processes could be improved by adding an intermediate holding step to allow germinated spores to outgrow into vegetative cells.

Sale *et al.* (1970) investigated the use of gamma irradiation before to HHP treatment of B. coagulans spores and found that mildly lethal doses of radiation increased the pressure sensitivity of survivors. Craw *et al*. (1996) found that irradiation sensitized *Clostridium sporogenes* spores to pressure and that mild doses of irradiation and high pressure combined was more effective for inactivating *Clostridium* spores than application of either process alone.

Gould and Jones (1989) reported the effects of simultaneously applying pressure and ionizing radiation to spores. The two treatments were additive in their sporicidal effect; thus, the intensity of one or both treatments could be lowered while retaining a degree of inactivation. The mechanisms of spore destruction were postulated as either the germination of spores by HHP and consequent sensitization to radiation, or the disruptive effect of irradiation on peptidoglycan in the spore cortex, allowing partial rehydration of the core and increased sensitivity to both radiation and pressure

Noci *et al*. (2008) the application of PEF or a combination of UV irradiation and PEF to freshly squeezed apple juice resulted in a similar total microbial reduction compared to the severe heat treatment. However, the quality attributes measured in juice processed by PEF or by the combined approach were similar to those observed in juice treated by the milder heat process (72 °C) and consistently superior when compared to the severe heat treatment (94 °C), with the exception of enzyme inactivation.

Irene *et al.* (2011) reported that a blend of apple and cranberry juice was processed by a combination of a light-based technology (ultravioletlight (UV) (5.3

J/cm^2) or high intensity light pulses (HILP) (3.3 J/cm^2) in combination with pulsed electric fields (PEF) (34 kV/cm, 18 Hz, 93.l s) or manothermosonication (MTS) (5 bar, 43°C, 750 W, 20 kHz). Selected physical and chemical attributes were evaluated pre- and post-processing, and the sensory attributes of non-thermally treated samples were compared to conventional pasteurisation (26 s, 72°C). No significant changes were found in non-enzymatic browning, total phenolics and antioxidant activity of the juices. UV + PEF and HILP + PEF treatments did not affect the colour of the product and HILP + PEF processing retained more monomeric anthocyanins than any other combined treatment. Sensory analysis showed that UV + PEF and HILP + PEF combinations did not impact on odour and flavour of the juice, while combinations that included MTS adversely affected those attributes.

CHAPTER 3

MATERIALS AND METHODS

This chapter describes the conceptual design and development of an ultraviolet radiation assisted with ohmic heating system for fruit juices. The materials and methods used for fabrication of the various components and the instrumentation employed for measurement of parameters were explained. The process of evaluation and standardization of process parameters towards the preservation of pineapple juice such as the experimental plan and procedures for determination of quality characteristics of the treated juice in terms of biochemical, microbial and organoleptic qualities were narrated.

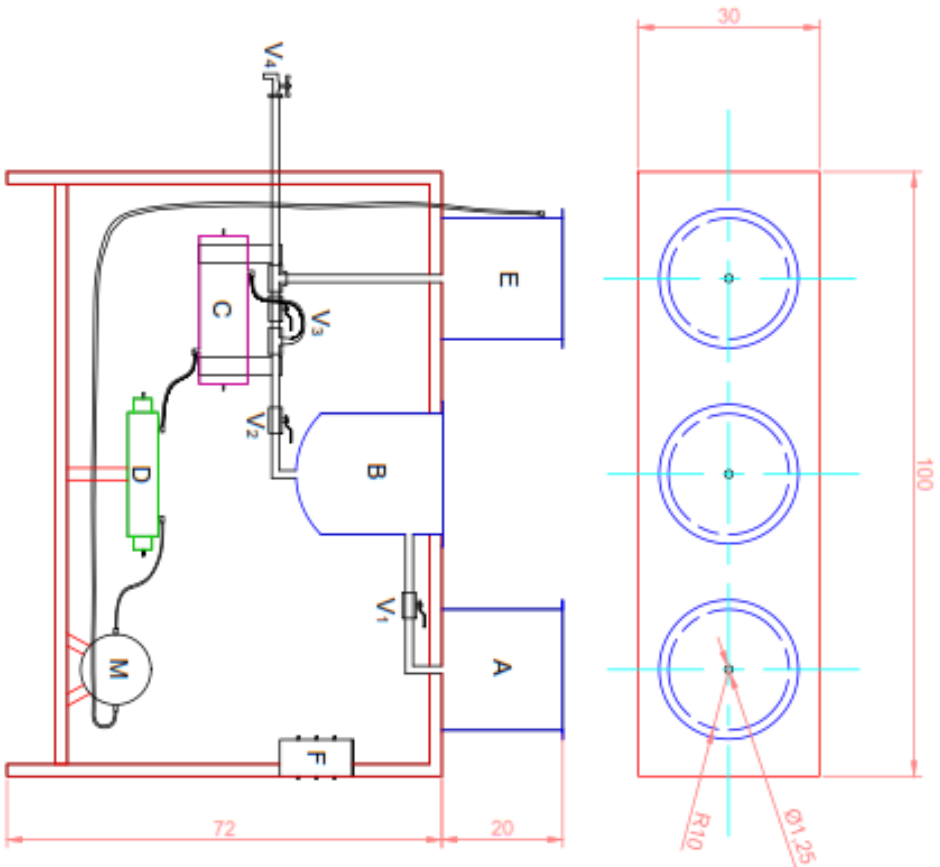
3.1 DEVELOPMENT OF A COMBINED OHMIC HEATING AND UV TREATMENT SYSTEM

Based on a thorough review of the works carried out in UV and ohmic heating separately and combined treatments, the design of a small capacity ohmic heating cum UV treatment reactor was conceptualized, further refined and then fabricated. The developed experimental system as shown in Figure 3.1 Plate 3.1 consists of the following main components.

1. Feed tank
2. Filtering unit
3. Ohmic heating chamber
4. UV treatment chamber
5. Recirculation system

3.1.1 Feed Tank

Feed tank is made up of 0.6 mm thick food grade SS 304 stainless steel. Diameter and height of the tank are 200 mm and 200 mm respectively with a capacity of six liters.



PART	NAME
A	Feed tank
B	Filtering Unit
C	Ohmic heating chamber
D	UV treatment chamber
E	Recirculating
V_1, V_2, V_3	Valves
V_4	Discharge valve
M	Motor
F	Control panel

Figure 3.1 Schematic diagram of the combined Ohmic heating and UV treatment apparatus

Plate 3.1 Developed combined Ohmic heating and UV treatment apparatus

3.1.2 Filtering Unit

Filtering unit consists of a SS tank with SS filter having 100 openings per square centimeter. The dimension and capacity of the tank is same as that of the feed tank. Filtering unit filters the pineapple juice and also acts as a baffer tank so as to ensure full continuous flow through the lines.

3.1.3 Ohmic Heating Chamber

The ohmic heating system primarily consists of an ohmic heating chamber, volt-amp meter for measuring the power and thermocouple probe for measuring inside temperature of the system.

The essential criteria for the construction of the ohmic heating chamber are that it should be electrically nonconductive, withstand process temperature and should not impart off flavor to the product. Accordingly, a cylindrical Teflon block of 80 mm diameter was used for construction of the chamber. A bore of 70 mm diameter was drilled so that the wall thickness of the chamber would be 10 mm. The ends of the cylinder was closed with the Teflon end caps (Plate 3.2) which

perfectly seals the hollow Teflon cylinder on either side. Holes of diameter 1.5 mm was drilled on both end caps for power connection to the electrodes installed on the either side chamber.

Plate 3.2 Ohmic heating chamber

In order to heat the juice ohmically, two electrodes are installed inside the chamber. The electrodes are made of food grade, non-corrosive SS 304 of 2 mm thick material. The electrodes are cut into circular shape with outer diameter of 70 mm so that they fit perfectly inside the cylinder (Plate 3.3). The ohmic heating rate depends on the spacing between the electrodes or the voltage gradient applied. Based on the preliminary studies conducted, the spacing between the electrodes were fixed as 6.5 cm. The ends of 1.5 mm aluminum rods connecting the electrode assembly will protrude through drilled holes on the end caps which will act as power terminals which is connected to power source. The terminals are connected to a 220v, 50Hz AC power supply. Nylon inlet and outlet couplings are provided at 13 cm from the center on either side for inserting nylon inlet and outlet tubes. The chamber is secured to the mainframe of the system. The accuracy and efficiency of the developed ohmic heating chamber was roughly tested by determination of the electrical conductivity of sodium chloride solution at narrow concentration and comparing the values with calculated values as described by Palaniappan and Sastry (1991a).

The electrical conductivities of samples were calculated from voltage and current data, using the equation,

$$\sigma = \frac{L}{AR} \qquad \ldots\ldots(3.1)$$

Where,

σ = specific electrical conductivity, (S/m)

A = area of cross section of sample, (m^2)

L = length of sample, (m)

R = resistance of sample, (ohm)

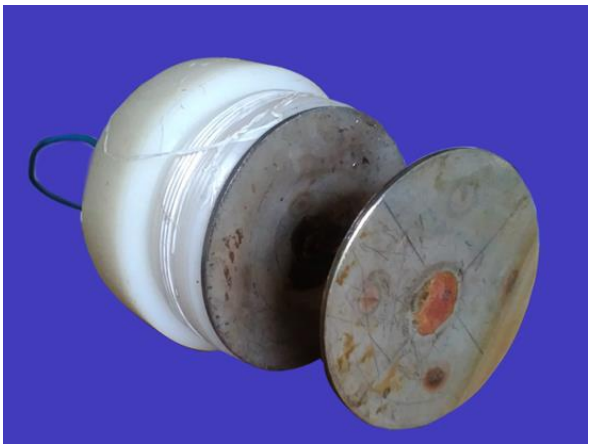


Plate 3.3 SS Electrodes

A multimeter with voltage rating up to 440 V connected in parallel to the circuit and an ammeter with rating up to 20 A connected in series with the circuit were used for the recording voltage and current respectively. A thermocouple probe inserted perpendicularly into the chamber record the temperature of the juice inside the chamber.

3.1.4 UV Treatment Chamber

The UV treatment chamber essentially consists of a 45 mm thick cylindrical aluminum alloy pipe with a length of 250 mm, inner diameter of 40 mm (Plate 3.4 a) the cylinder is so fabricated that it can hold a quartz tube 19.7 mm thick, 16.5 cm length, inner diameter of 17.8 mm at the axial center of the aluminum pipe so that the flow of juice will be through the annular space between the tube and the cylinder (Plate 3.4b). The quartz tube will act as a cover for the UV lamp protecting it from liquid flow. A UV lamp with 3W output power with a UV wavelength of 254 nm was housed at the center of the quartz tube axially. Nylon inlet and outlet couplings are provided at 130 mm from the center on either side for inserting nylon inlet and

outlet tubes. The fabricated UV treatment reactor is secured to the mainframe of the system.

(A) Front view (B) Side view

Plate 3.4 UV treatment chamber

The intensity of the UV radiation was measured using a UV-C radiometer (m/s LT Lutron, model UVC-254) (Plate 3.5) consisting of a narrow band solar bind photodiode and UV colour filter sensor with peak sensitivity at 254 nm wavelength.

Magnitude of a photochemical reaction, such as the effect of UV-C radiation on nucleic acid is directly related to the total dose of radiant energy that hits the target. It is the product of light intensity and exposure time (Bunsen-Roscoe reciprocity law for photochemical processes). The overall effect of the radiation is depended on combination of applied flow rate and exposure time used.

Plate 3.5 UV-C radiometer

In order to find out UV dose that results in maximum log reduction of microbial population in fruit juice, preliminary experiments were carried out at different dosage

levels. The applied dosage was calculated using the following formula as suggested Morgan (1989) and Stevens *et al.*, (1999).

$$D = I \times t \qquad \ldots\ldots(3.2)$$

Where,

I = intensity of UV-C light at the external surface of the irradiation tube in W/m^2

t = exposure time in s

In a continuous flow system, the retention time is obtained as

$$t = \frac{\text{volume of chamber}}{\text{flowrate}} \qquad \ldots\ldots(3.3)$$

3.1.5. Recirculation System

Recirculation system consists of a 6 liter capacity SS tank, with dimensions same as that of the feed tank two flow control valves, a-V-A, 0.25 hp pump and associated connecting pipes and nylon tubes (Plate 3.1, Figure 3.1).

3.2 PREPARATION OF PINEAPPLE JUICE

In order to evaluate the developed UV assisted ohmic heating system, pineapple juice, rich in vitamins, minerals and other essential nutrients widely grown in kerala was selected. Pineapple juice was prepared in an aseptic environment with all precautions to minimize microbial ingression as described in Figure 3.2 Fresh ripe pineapple procured from local market were used for study.

3.3 EXPERIMENTAL PROCEDURE

Fresh pineapple juice was fed to the feed tank and then passed through the filtering unit. The valve (V_1) which is connected in between feed tank and the filtering is shutoff once the full unit of juice had passed through. Sequentially it passes from filtering unit to the ohmic heating chamber to UV treatment chamber. The pump was used to pump the juice from UV treatment chamber to recirculation tank. The valve (V_2) situated between filtering unit and ohmic heating chamber is shutoff after pumping entire juice to the recirculation tank. The juice is recirculated sequentially from recirculating tank to Ohmic heating chamber and then to the UV treatment chamber. The desired treatments such as ohmic heating alone, UV treatment alone and combined OH and UV are given by recirculating the juice for required number of cycles.

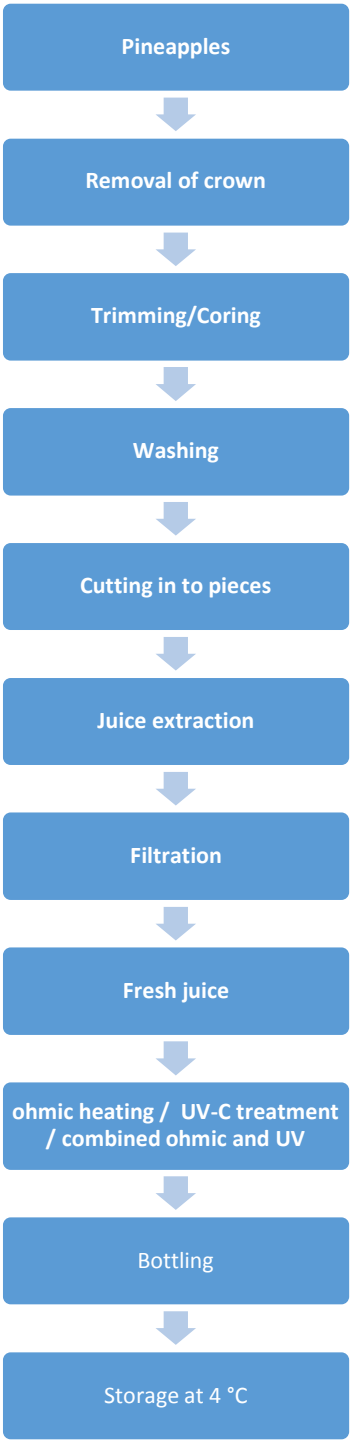


Figure 3.2 Process flow chart for production of treated pineapple juice

Ohmic heating alone involves preparation of the juice and recirculating the juice to the ohmic heating chamber. A potential difference of 220 V was applied to the terminals. A voltage gradient of 36 V/cm was achieved. The flow rate was 1 l/min. From the preliminary studies the target temperatures were set to 50 °C, 55 °C, and 60 °C respectively. The time required to reach the target temperatures were 360s, 420s, and 460s respectively. The change in temperature was monitored by using a thermocouple probe which was inserted perpendicularly into the ohmic heating chamber. The UV treatment was switched off during the treatment.

The UV treatment alone involves the circulation of juice through the UV chamber. The UV lamp was switched on prior to UV treatment of juice in order to minimize fluctuations in intensity. The flow rate was adjusted to 600 ml/min. The juice was recirculated through the UV treatment chamber to obtain higher levels of dosage. The applied dosage per cycle was 100 mJ/cm^2. Exposure time of pineapple juice to UV light during each cycle was 120s. Based on the preliminary studies, the dosage levels were fixed at 800 mJ/cm^2, 1200 mJ/cm^2 and 1600 mJ/cm^2. The time taken for achieving the dosage levels are 16, 24 and 32 min respectively. The ohmic heating treatment was switched off during the treatment

Combined Ohmic heating and UV treatment was also given to the juice as per the experimental design. During this, the UV and ohmic heating section were in operational and the ohmic heating and UV dosage is applied as per the required temperature and dosage combination. Once the ohmic heating temperature was reached the treatment was switched off and on to maintain the temperature. The treated juice was collected through the collection tap (V_4).

All the treated samples were stored at 4°C for 25 days in amber colour PET bottles as shown in Plate 3.6 for further analysis.

3.4 EXPERIMENTAL DESIGN

This study was broadly divided into three experiments such as development of UV radiation assisted into ohmic heating system, evaluation of developed system leading to the standardization of the process parameters and organoleptic evaluation of the treated juice. The development of such a system for a pumpable fruit juices has been dealt in previous sections. Evaluation of the system for its effectiveness in fruit juices

preservation pineapple juice has been taken up as pineapple is a potential fruit crop adaptable and highly grown in Kerala, rich in nutrients and needs novel technological inputs for value addition. The process parameters of the developed system needs to be standardized for pineapple which would ensure microbial destruction causing minimal changes in the quality characteristics. The levels of individual variables were fixed based on preliminary studies conducted on treatment of pineapple juice.

Plate 3.6 PET Bottles

3.4.1 Parameters of the Experiment

The following independent and dependent variables were selected for the experiments on Ohmic heating, UV treatment and combined UV and Ohmic heating treatment processing of fruit juice.

Sl No	***Independent Variables***		***Dependent variables***	
(i)	**Fruit juice**	**1 level**	**Biochemical characteristics**	
(a)	Pineapple juice	J_1	(a)	pH
(ii)	**Ohmic heating**	**- 3 levels**	(b)	Total soluble solids
(a)	50 °C	T_1	(c)	Titratable acidity
(b)	55 °C	T_2	(d)	Ascorbic acid (Vitamin 'C')
(c)	60°C	T_3	(e)	Total sugar
(iii)	**UV Dosage**	**-3 levels**	**Microbial load**	
(a)	800 mJ cm^{-2}	T_4	(a)	Bacterial population
(b)	1200 mJ cm^{-2}	T_5	(b)	Yeast population
(c)	1600 mJ cm^{-2}	T_6		
(iv)	**Combined ohmic/UV**	**9levels**		
(a)	50 °C 800 mJ cm^{-2}	T_7		
(b)	50 °C 1200 mJ cm^{-2}	T_8		
(c)	50 °C 1600 mJ cm^{-2}	T_9		
(d)	55 °C 800 mJ cm^{-2}	T_{10}		
(e)	55 °C 1200 mJ cm^{-2}	T_{11}		
(f)	55 °C 1600 mJ cm^{-2}	T_{12}		
(g)	60 °C 800 mJ cm^{-2}	T_{13}		
(h)	60 °C 1200 mJ cm^{-2}	T_{14}		
(i)	60 °C 1600 mJ cm^{-2}	T_{15}		
(v)	**Replication**	- 3		
Total number of experiments = (3+ 3 + 9) × 3 = 45				

All the experiments in the study were conducted in triplicate and mean values reported. Completely Randomized Design (CRD) was followed to study the effect of the predictor variables on the response variables. Analysis of variance (ANOVA) using Duncan test was performed to determine the significant effect of independent variables on response variables. The treatments and their interactions were compared at $p<0.05$ level using the SPSS software version 16.0.

3.5 MEASUREMENTS OF OPTICAL PROPERTIES OF JUICE

3.5.1 Absorption of UV light

The efficiency of UV light inactivation of microorganism in juice depends on absorbance of UV light (Koutchma *et al.*, 2004). Hence it is necessary to determine the absorbance of UV light by the fruit juice when it is passed through the quartz tube.

To classify a given material quantitatively as to its ability to absorb or transmit radiation of wavelength λ, it is necessary to define an absorption length or optical path length L_λ in the material. This length is the distance of penetration into the material at which the incident radiation has been attenuated a given amount i.e. the intensity to which the radiation has been reduced to a given fraction out of the intensity of the incident beam (McCabe *et al.*, 2001). It is known that the absorption length is reciprocal of the absorption coefficient μ

$$\text{i.e. } L_\lambda = \frac{1}{\mu_\lambda} \qquad \ldots. (3.4)$$

Where,

L_λ = absorption length in mm

μ_λ = absorption coefficient in mm^{-1}

Therefore,
$$\therefore \mu_\lambda = \frac{1}{L_\lambda} \qquad .... (3.5)$$

Absorbance of UV by fruit juice was measured using a UV 1800- VIS spectrophotometer (m/s Shimadzu ltd, Japan) (Plate 3.7). The absorbance of 254 nm wavelength, monochromatic UV light obtained from low pressure mercury vapour lamps was measured for the treatment parameters as per the experimental design.

Plate 3.7 Spectrophoto meter

3.6 ANALYSIS OF QUALITY CHARACTERISTICS OF FRUIT JUICE

The fresh and treated pineapple juice were analysed for biochemical, microbial and organoleptic characteristics.

3.6.1 Biochemical Analysis

Biochemical analysis of fresh and treated fruit juice were carried out as per procedures laid out by Ranganna (1986).

3.6.1.1 pH measurement

The pH is the logarithm of the reciprocal of hydrogen ion concentration is a measure of active acidity which influence the flavor of a product and also affects its processing requirements. The pH of the treated pineapple juice was measured by using digital pH meter (m/s SYSTRONICS Ahmedabad, model MK VI) (Plate 3.8). The pH meter was standardized with buffer solutions of different pH of 4.0, 7.0, and 9.2. The probe was then wiped dry and dipped in the juice samples to record the pH values.

3.6.1.2. Total soluble solids

The soluble solids are primarily sugars such as sucrose, fructose, and glucose. Citric acid and minerals in the juice also contribute to the soluble solids. The total soluble solids present in the fruit juice were determined by using a hand refrectometer with a range of 0-32°B. (ERMA Hand refrectometer, Tokyo, japan) (Plate 3.9). Several drops of juice are placed on the prism surface. The liquid on the prism plate should be free from bubbles or floating particles of pulp or other matter and then the prism lid is closed. The

instrument should be turned towards the light to get proper readings. If necessary the eye piece is focused until a clear image appears. The position at which the demarcation line between the light and dark regions crosses the vertical scale gives the percentage soluble solids reading.

Plate 3.8 pH meter

Plate 3.9 Hand refractometer

3.6.1.3. Titrable Acidity

Five milliliter of juice sample was dissolved in 100 ml of water. From the makeup, 10 ml of sample was pipetted out into a 100 ml conical flask and few drops of phenolphthalein indicator was added. The solution was titrated against 0.1 N NaOH till the colour turned to pale pink colour. This procedure was repeated to get concordant values. The percent acidity was calculated as follows.

$$\text{Percent acidity} = \frac{\text{Titre value} \times \text{Normality of alkali} \times \text{Meq weight of citricacid}}{\text{Weight of the sample taken}} \times 100 \quad ..(3.6)$$

3.6.1.4. Ascorbic acid (Vitamin 'C')

Standardization of dye was carried out by taking 5 ml of standard ascorbic acid solution and titrated against the 2,6 dichlorophenol indophenol dye till the pink colour persists for 10 s. Ten milliliter of juice sample was taken and made in to 100 ml with 4% oxalic acid solution and filtered. Ten milliliter of filtrate was taken and titrated against the dye solution. The dye factor and ascorbic acid content were estimated using the formula.

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}} \quad \ldots(3.7)$$

$$\text{mg of ascorbic acid}/100\text{g of sample} = \frac{\text{Titre value} \times \text{Dye factor} \times \text{volume made up} \times 100}{\text{Volume of filtratetaken} \times \text{Weight of sample}} \quad .(3.8)$$

3.6.1.5. Total sugar

Total sugar was extracted from the fruit juice by adding 80% (v/v) aqueous ethanol. Working standard solution of 0.2, 0.4, 0.6, 0.8 and 1.0 ml was pipetted out and made up the volume to 1.0 ml by adding distilled water. Juice sample, was made into colourless solution by adding distilled water. From this diluted sample 0.5 ml was taken in a separate test tube and made up to 1 ml by adding 0.5 ml of distilled water. Four milliliter of anthrone reagent was added to all the test tubes. Blank was prepared by taking 1 ml of distilled water and 4 ml of anthrone reagent. All the test tubes were heated in a water bath for 8 min and cooled rapidly. The absorbance at 620 nm wavelength was recorded using spectrophotometer.

3.6.2. Microbial Analysis

UV treated pineapple juice were stored at 4°C for recording the shelf life. The microbiological observation of the samples were carried out as per the procedure below.

3.6.2.1 Enumeration of bacterial population

The number of living organism per milliliter of juice sample is measured by determining the viable cell number through spread Plate method (Pollack *et al.*, 2002). Serial dilutions of juice sample were prepared by transferring 1 ml of sample into 9 ml sterile water (10^{-1}). For enumeration, 0.1 ml of 10^{-4} and 10^{-6} dilutions of control and treated pineapple juice samples were applied into two Plates of nutrient agar medium and it was spread out with the use of a curved glass rod. The Plates were then kept in inverted position and incubated at 37°C for 24 h. The average number of bacteria per ml of juice was calculated using the formula.

$$\text{Plate count} = \frac{\text{Average number of colonies from duplicate plate}}{\text{Dilution factor} \times \text{Volume plated}} \text{ cfu/ml} \qquad \ldots(3.9)$$

3.6.2.2 Enumeration of Yeasts population

Yeasts are aerobic microorganisms and for their enumeration surface plating is preferred (Yousef and Carlstron, 2003). Serial dilutions of fruit sample were prepared as described in section 3.6.2.1. For yeast suspensions from 10^{-3} and 10^{-6} dilutions were taken and applied in rosebengal chloramphenical agar Plates, which is used as a selective media for yeasts and molds. The suspension was then spread

out employing a curved glass rod. The incubation for total yeast and mould counts was done at 22 to 25°C for 5 days. Each test was replicated three times and results were expressed as colony forming units (cfu) per ml using the formula (3.9).

3.8 ORGANOLEPTIC EVALUATION

After conducting optimization of the process parameters, the selected samples which retained quality characteristic studies in each category such as ohmic heating alone, UV treatment alone, combined UV and ohmic heated, controls (fresh and stored) and conventionally heat pasteurized samples were organoleptically evaluated by a panel of 10 untrained judges for the colour, flavor, taste and overall acceptance. The evaluation was carried out using a 9 point hedonic scale as per IS 6272: 1991.

3.9 COST ECONOMICS

The cost economics of equipment in commercial level was estimated by considering the costs *viz,* raw material, production, labour, electricity and other related costs. The fixed cost and variable cost was used for determining the cost of operation. The fixed cost was calculated by using the following relationship as described by Palanisami *et al*. (1997). The variable costs, which are incurred on electricity charges, repairs and maintenance, raw materials etc., were calculated by collecting data during the production of equipment and assuming certain data reasonably wherever necessary.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter outlines the results of the evaluation of developed ultraviolet assisted ohmic heating system towards preservation of pineapple juice. The outcomes of the procedures laid out for the evaluation leading to the standardization of the main process parameters in terms of maintaining the quality characteristics of the juice are discussed in detail.

4.1 PHYSICO- CHEMICAL CHARACTERISTICS OF FRESH PINEAPPLE JUICE

In order to study the effect of various treatments, the physical and chemical properties that define the quality parameters of fresh pineapple juice relevant to the present study were determined and is presented in Table 4.1.

Table 4.1 Physico - Chemical and microbial characteristics of fresh pineapple juice.

Characteristics	**Mean ± SD***
pH	3.86 ± 0.0057
TSS (°Brix)	18.50 ± 0.0573
Titratable acidiy(%citric acid)	0.3223 ± 0.0003
Vitamin – C (mg)	46.49 ± 0.2423
Parameters	**Pineapple juice**
Total sugars (g)	10.25 ± 0.2406
Absorbance (cm^{-1})	0.573
Transmittance	42.70%
Turbidity	372 NTU
Microbial population	
Bacteria (cfu/ml)	65×10^6
Yeast (cfu/ml)	43×10^6

*SD – Standard deviation

The results of the chemical characteristics indicated that pineapple juice is basically a medium acid juice with a pH value of 3.86 ± 0.0057. A low turbitity value of 372 NTU revealed that the juice contained less suspended particles which resulted in slightly higher transmittance value of 42.70%. Initial microbial population in fresh juice along with particulates and organic matter were associated with the low transmissivity of UV light (Shama *et al.*, 1996). The initial bacterial

and yeast population in fresh pineapple juice were 65×10^6 cfu/ml and 43×10^6 cfu/ml respectively.

4.2 DEVELOPMENT OF A UV RADIATION ASSISTED OHMIC HEATING SYSTEM

In order to test the hypothesis that the electrophoretic force of an ohmic heating system would enhance the lethal capacity of low penetration depth of UV radiation for inactivation of microorganisms, an ohmic heating cum UV combination setup suitable for pumpable fruit juice was fabricated. As detailed in section 3.1 the main components of the system were Feed tank, Filtering unit, Ohmic heating chamber, UV treatment chamber and Recirculation system. The system is attached into instrumentations for control of the fluids such as motor, valves, regulators and switches and instrumentation for measurement of parameters such as temperature, UV dosage, Voltage, Current, Power etc. It was then tested for its effectiveness with pineapple juice as a raw material. The system is designed in such a way that pineapple juice could be subjected to UV treatment alone, ohmic heating alone and combined UV and Ohmic heating by diverting the flow through appropriate valves and pipe system as per the experimental procedure explained in section 3.3

4.3 STANDARDISATION OF PROCESS PARAMETERS OF THE UV ASSISTED OHMIC HEATING SYSTEM

The process parameters such as ohmic heating temperatures, UV dosage for individual treatments and combined treatments needs to be standardized for the efficient functioning of the system.

4.3.1 Biochemical characteristics

The effect of process variables on the biochemical quality characteristics such as pH, TSS, titratable acidity, ascorbic acid and total sugars were analysed and discussed below.

4.3.1.1 Effect of various treatments on the pH of pineapple juice

pH is an important characteristic determining the quality which describes the stability of the bioactive compounds in fruit juice. The values of pH of treated juices were analyzed and are presented in Figure 4.1. Fresh pineapple juice had a pH value of 3.86 ± 0.0057. It was found that significant difference of pH values were

observed for samples treated with ohmic heating alone while for the rest of the treatments, the pH values were on par with the fresh pineapple juice. Incidentally the decrease in pH values were minimum for the combined treatments indicating the fresh like characteristics. Similar results were reported by wright *et al.* (2000), for the apple cider when the same was subjected to UV treatment alone. Also, it was reported that high electric field and UV treatments did not substantially influence the pH of juice samples as compared with the untreated samples (Bhat *et al.*, 2011; Timmermans *et al.*, 2011).

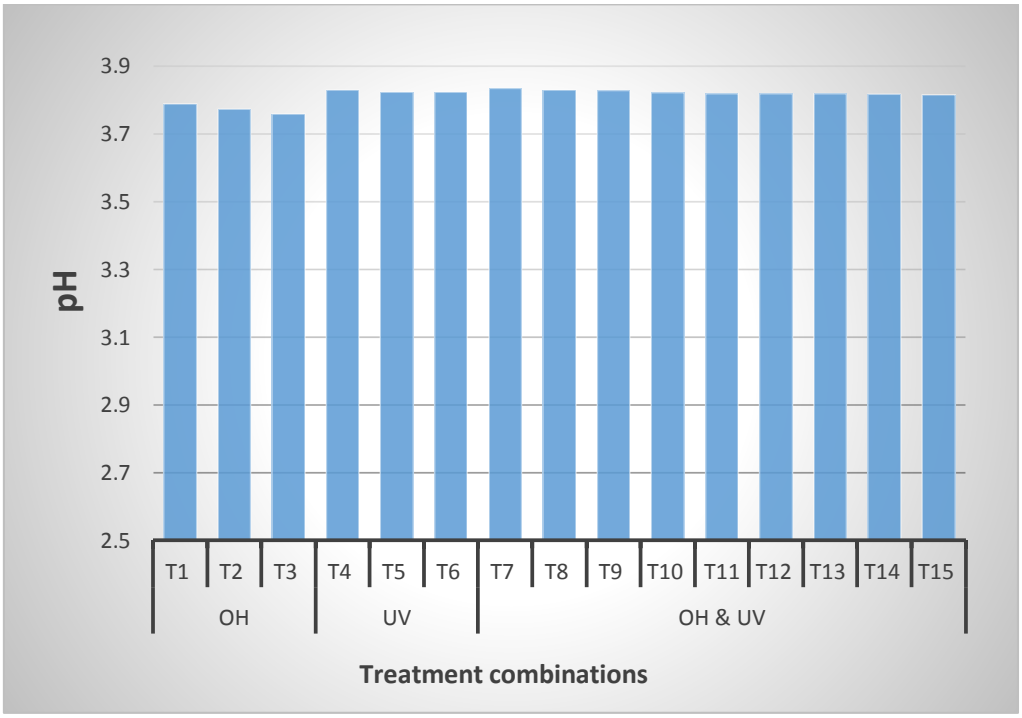


Figure 4.1 Effect of OH and UV treatments on pH of pineapple juice

4.3.1.2 Effect of treatments on TSS of pineapple juice

The effect of treatments on TSS of the pineapple juice are shown in Figure 4.2. It may be observed from the Figure that TSS values increased from 19.06 ± 0.057 to 19.13 ± 0.057 °Brix during the ohmic heating alone when the temperature increased from 50 °C to 60 °C. For all other treatment conditions, the TSS was found to decrease significantly from the ohmic heating alone and the values were more or less in the same range as that of the fresh juice which was 18.5 ± 0.00573 °Brix. During the UV treatment alone, the TSS was found to increase from a value of 18.566 ± 0.057 to 18.76 ± 0.057 °Brix with increase in UV dosage level. For the

combination treatments TSS values were found to increase with increase in ohmic heating temperature though the variations were not significant among the treatments. Similar results were also reported in combination treatments of hurdle strategy for preservation of fresh apple juice by Noci *et al.*, (2008). According to Castro *et al.* (2003) the structural changes caused due to the heating would lead to an increase in electrical conductivity. This is counterbalanced by water evaporation during heating, reducing the fluid movement and thus electrical conductivity as evidenced by increase in TSS values.

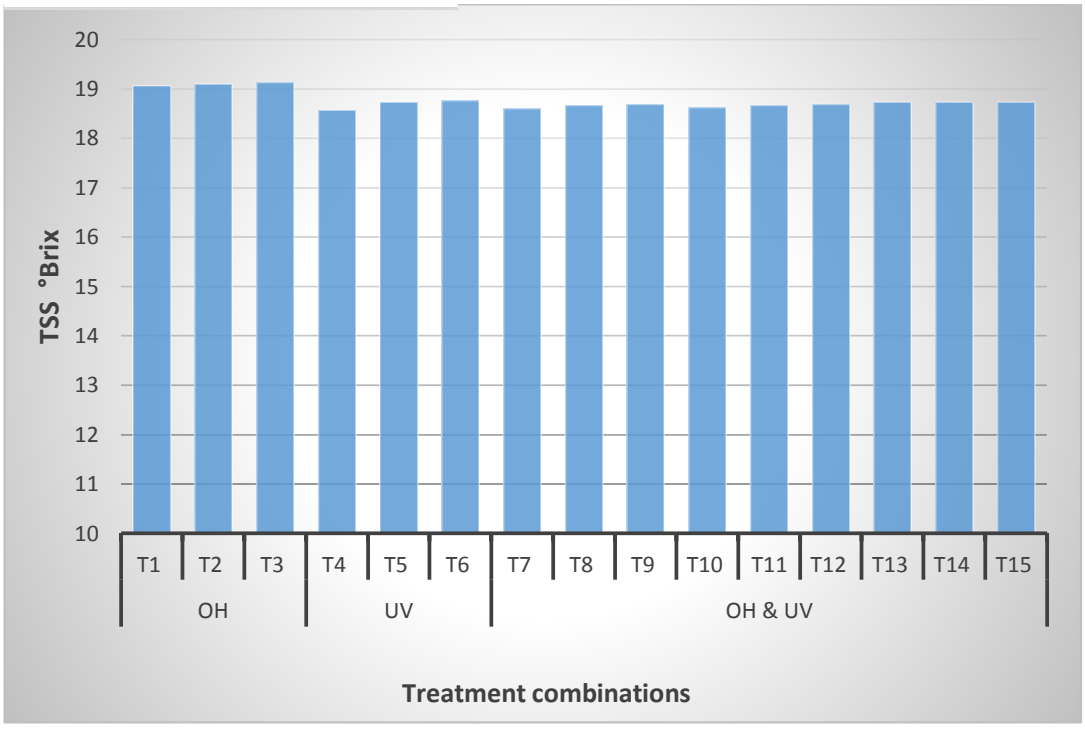


Figure 4.2 Effect of OH and UV treatments on TSS of pineapple juice

The maximum flow rate of the food product through the ohmic system will determine the power requirement to affect the appropriate temperature increase. In particular, ohmic heating systems heat at a very fast rate, and even small delays in the flow of some of the food product could lead to large differences in temperature. During ohmic heating alone, the flow rate of the juice was high resulting in reduced retention time. Therefore, the time to reach target temperature was high. The juice is subjected to heat for long time resulting in increased TSS content. On the other hand during combination treatment, the flow rate was lowered in order to provide required UV exposure dosage. The time required for juice to reach the target

temperature was less compared to ohmic heating alone. The juice is subjected to heat for less time resulting in reduced TSS value compared with ohmic heating alone though the temperature of both treatments remain same. Similar results were also observed in the case of titratable acidity, ascorbic acid and total sugars.

4.3.1.3 Effect of treatments variables on titratable acidity of pineapple juice

The titratable acidity of fresh juice was found to be 0.3223 ± 0.0003 (% citric acid). The results of the titratable acidity determination during various treatment conditions as per technical programme using the developed system is presented in Figure 4.3. In general titratable acidity increased with increase in ohmic heating temperature and UV dosages but the variation among each treatments sets were insignificant at 0.01% significant levels. The titratable acidity was found to decrease significantly between OH and UV alone treatments. Among the various treatments, combined treatments with lower ohmic heating temperatures showed titratable acidity values close to fresh pineapple juice.

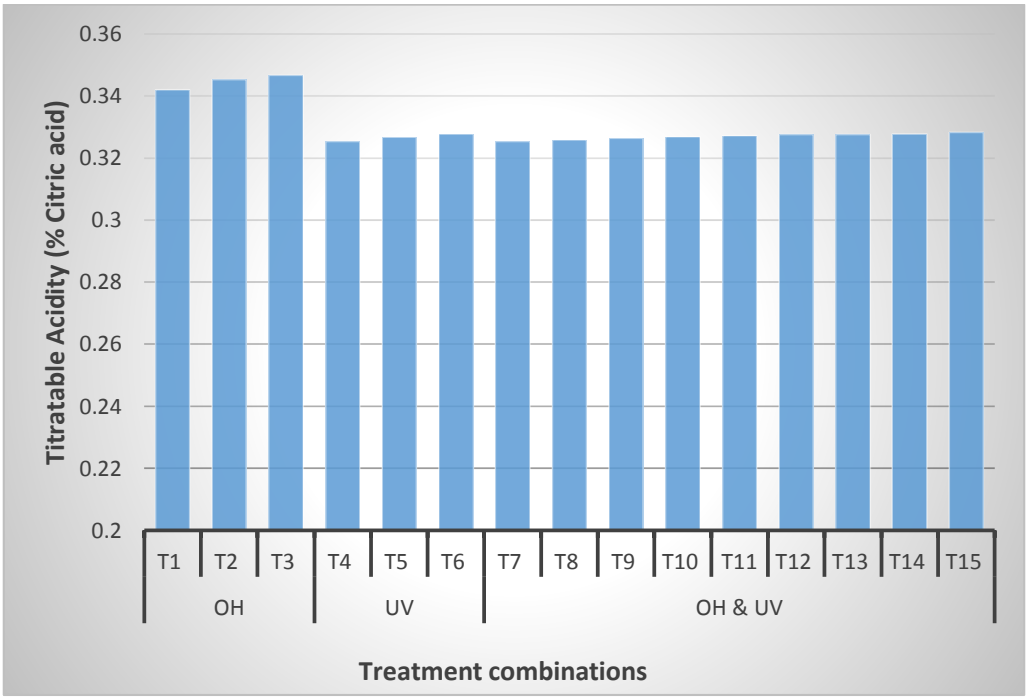


Figure 4.3 Effect of OH and UV treatments on titratable acidity of pineapple juice

4.3.1.4 Effect of treatments conditions on ascorbic acid of pineapple juice

Ascorbic acid (Vitamin-C) in fruit juices is a very important nutritional quality indicator. In food industry, Vitamin-C is used as a food additive and it is

frequently added to fruit juices to preserve and protect them from any colour changes. Vitamin-C content of fresh pineapple juice was found to be 46.49 ± 0.2423 mg. The ascorbic acid contents of treated pineapple juices after different treatments with the developed system are presented in Figure 4.4

It could be revealed that significant increase was observed between Vitamin C values of ohmic heating alone and other treatment combinations. Among ohmic heating alone, significant decrease in vitamin C values from 43.5 ± 0.196 to 42.3 ± 0.257 mg were observed, whereas the variation of vitamin C values of UV alone and combined treatments were insignificant from that of fresh juice. During ohmic heating alone, the heating effects due to the increase in temperature when UV dosage was absent might be the reason for loss of vitamin C through the aerobic pathway as vitamin C is heat sensitive bioactive compound (Odriozola-serrano *et al.*, 2008). The reduction in vitamin C could also be attributed due to the oxidation effects at high temperatures. Similar results were reported for UV- treatment alone by Tran and Farid (2004).

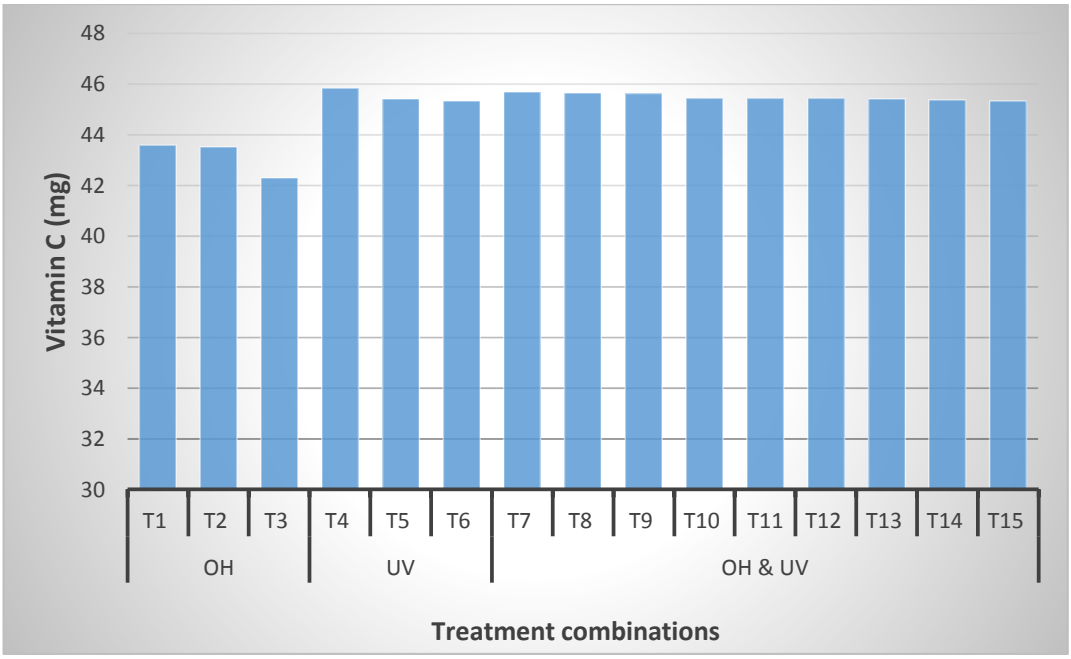


Figure 4.4 Effect of OH and UV treatments on Vitamin C of pineapple juice.

4.3.1.5 Effect of treatments variables on total sugar content of pineapple juice

The taste of fruit juice is compounded mainly by its sugar content, acids and of numerous volatile aromatic components which are present in meager quantities.

The observations on total sugar content of treated pineapple juice are presented in Figure 4.5.

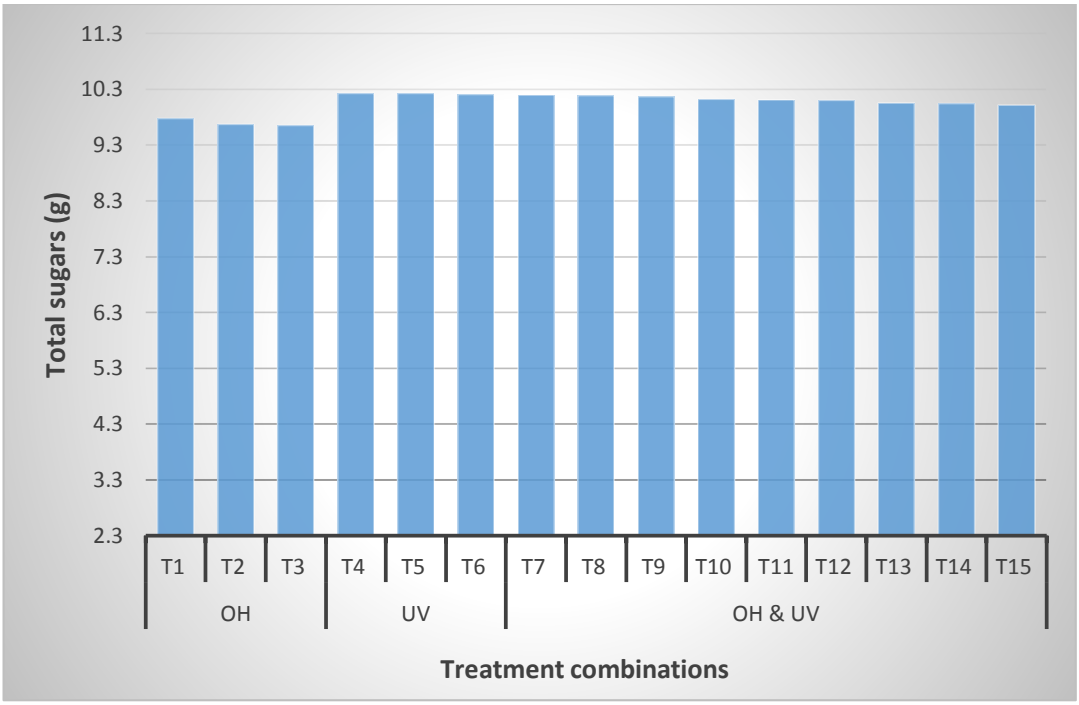


Figure 4.5 Effect of OH and UV treatments on Total sugars of pineapple juice

Total sugar content of fruit juices decreased with increase in temperature during the ohmic heating alone. Further a significant increase in total sugars were observed between ohmic heating and UV alone treatments. Among the various combined treatment combinations, no significant variations in total sugars were observed. Combined treatments show values of total sugars close to that of fresh pineapple juice.

4.3.2. Microbiological Characteristics

4.3.2.1 Bacterial Population

The experiments were carried out to determine the reduction in bacterial population during various treatment conditions such as Ohmic heating, UV treatment and combined OH and UV treatment of pineapple juice as explained in section 3.5.1 The results of the experiments conducted to analyze the effect of OH, UV treatment and combined OH and UV treatment variables on the bacterial disinfection of pineapple juice are presented in Table 4.2.

The pineapple juice treated with ohmic heating alone resulted in slight reduction in bacterial population with respect to fresh juice but the reduction was insignificant. It may also be seen that with increase in temperature during ohmic heating, the bacterial population was found to decrease insignificantly.

Table 4.2 Bacterial Population in treated juice.

Treatment	Temperature/dosage (°C & mJ cm^{-2})	Bacterial log Reduction [$\log(N/N_0)$]
Ohmic heating	T1 (50 °C)	2.57 ± 0.017
	T2 (55 °C)	2.59 ± 0.018
	T3 (60 °C)	3.73 ± 0.051
UV Treatment	T4 (800 mJ cm^{-2})	3.88 ± 0.036
	T5 (1200 mJ cm^{-2})	4.07 ± 0.055
	T6 (1600 mJ cm^{-2})	4.91 ± 0.077
Combination treatment	T7 (50 °C & 800 mJ cm^{-2})	4.93 ± 0.041
	T8 (50°C & 1200 mJ cm^{-2})	5.09 ± 0.029
	T9 (50 °C & 1600 mJ cm^{-2})	5.09 ± 0.029
	T10 (55 °C & 800 mJ cm^{-2})	5.09 ± 0.029
	T11 (55 °C & 1200 mJ cm^{-2})	5.09 ± 0.029
	T12 (55 °C & 1600 mJ cm^{-2})	5.18 ± 0.036
	T13 (60 °C & 800 mJ cm^{-2})	5.18 ± 0.036
	T14 (60 °C & 1200 mJ cm^{-2})	5.18 ±0.036
	T15 (60 °C & 1600 mJ cm^{-2})	5.18 ± 0.036

A similar trend was also observed for UV treatment alone. During ohmic heating at 60 °C, the bacterial population was found to be 12×10^3 cfu/ml, a 99.98% reduction compared to fresh juice whereas the reduction was 99.99% for UV treatment at highest dosage of 1600 mJ/cm^2. This indicates that only a 3.73 and 4.91 Log reduction could only be achieved during ohmic heating and UV alone treatments respectively which may not be considered safe for storage and human consumption.

When pineapple juice was subjected to combined ohmic heating and UV treatment in the developed system, marked reduction in bacterial population was noticed. The most severe treatment i.e. treatment at an ohmic heating temperature of 60 °C with UV dosage of 1600 mJ/cm^2 could result in bacterial population of 4×10^2

cfu/ml. But it may be seen that all the combined treatments except T_7 (50 °C, 800 mJ/cm^2) ensured a 5 log reduction in bacterial population and therefore considered safe for storage and human consumption as per (FDA 1997). The increased inactivation of the bacteria with increase in dosage level could be attributed not only to the dosage level but also due to the mixing of the fruit juice during the higher number of circulations which would have facilitated more uniform exposure of all the particles of juice for UV light. These results are in conformity with that of Li *et al.,* (2005) who reported that mixing of elements significantly enhanced the log reduction of microorganisms. It may be postulated that the synergistic effect of combined ohmic heating and UV treatment could have resulted in achieving a high level of inactivation. Although the electroporation generated by a strong electric field could contribute to the enhanced lethality rate, thermal impacts and UV exposure might also account for the irreversible collapse of cellular structures and consequent bacterial inactivation. Thus, the hypothesis of the two step bacterial inactivation process; first the electrophoretic force from the strong electric field could lock the open/close function of the cell pores and destroy the cell membranes, and second, UV light could penetrate deeply through the cell pores and disrupts the microbial DNA, killing the same stands validated. Similar justifications were also pointed out by Sun *et al.,* (2008) and Lee and Jun, (2011).

4.3.2.2 Yeast Population

The experiments were carried out to determine the reduction in yeast population during various treatment conditions such as Ohmic heating, UV treatment and combined OH and UV treatment of pineapple juice as explained in section 3.5.1 The results of the experiments conducted to analyse the effect of OH, UV and combined OH and UV treatment on the yeast disinfection of pineapple juice are presented in Table 4.3.

The pineapple juice treated with ohmic heating alone resulted in slight reduction in yeast population with respect to fresh juice but the reduction was insignificant. It may also be seen that with increase in temperature during ohmic heating, the yeast population was found to decrease insignificantly. A similar trend was also observed for UV treatment alone. During ohmic heating at 60 °C, the yeast

population was found to be 13×10^3 cfu/ml, a 99.9 % reduction compared to fresh juice whereas the reduction was 99.99% for UV treatment at highest dosage of 1600 mJ/cm^2.

Table 4.3 Yeast Population in treated juice.

Treatment	Temperature/dosage (°C & mJ cm^{-2})	Yeast log Reduction log (N/N_0)
Ohmic heating	T1 (50 °C)	2.3906 ± 0.017
	T2 (55 °C)	2.4162 ± 0.018
	T3 (60 °C)	3.5558 ± 0.051
UV Treatment	T4 (800 mJ cm^{-2})	3.7048 ± 0.036
	T5 (1200 mJ cm^{-2})	3.8949 ± 0.055
	T6 (1600 mJ cm^{-2})	4.7338 ± 0.077
Combination treatment	T7 (50 °C & 800 mJ cm^{-2})	4.7594 ± 0.041
	T8 (50°C & 1200 mJ cm^{-2})	5.0314 ± 0.041
	T9 (50 °C & 1600 mJ cm^{-2})	5.0314 ± 0.029
	T10 (55 °C & 800 mJ cm^{-2})	5.0314 ± 0.029
	T11 (55 °C & 1200 mJ cm^{-2})	5.0314 ± 0.029
	T12 (55 °C & 1600 mJ cm^{-2})	5.0314 ± 0.029
	T13 (60 °C & 800 mJ cm^{-2})	5.0314 ± 0.036
	T14 (60 °C & 1200 mJ cm^{-2})	5.0314 ± 0.036
	T15 (60 °C & 1600 mJ cm^{-2})	5.0314 ± 0.036

When pineapple juice was subjected to combined ohmic and UV treatment in the developed system, marked reduction in yeast population was noticed. The most severe treatment, i.e treatment at an ohmic heating temperature of 60°C with UV dosage of 1600 mJ/cm^2 could result in yeast population of 4×10^2 cfu/ml. The yeast showed more resistant to all treatments compared to bacteria as the chemical composition of the cell wall and thickness of yeast cells are from bacteria. This result is in agreement with the findings of Tran and Farid (2002) for UV treatment of carrot juice.

4.4 OPTIMISIATON OF THE PROCESS PARAMETERS

In order to find the process variables which could give the best quality characteristics based on the response variables chosen for the study on bio-chemical, microbial and sensory attributes, the obtained data were analysed statistically as explained in section 3.4.

Table 4.4 Process parameters responsible for obtaining the best quality attributes in the developed ohmic heating assisted UV radiation system.

Sl. No.	Quality characteristics	Best treatments
1	pH	All treatments except ohmic heating alone presented minimum variation from the pH of fresh pineapple juice. (T_4 to T_{15}).
2	TSS (° Brix)	Treatments T_4 (UV), T_7, T_8, T_{10} and T_{11} (OH and UV) shows minimum variation from fresh pineapple juice.
3	Titratable acidity (% Citric acid)	Treatments T_4 and T_5 (UV) and T_7 to T_{14} (OH and UV) represented minimum variation from fresh juice.
4	Vitamin C (mg)	Treatments T_4 and T_5 (UV) and T_7 to T_{10} (OH and UV) represented minimum variation from fresh juice.
5	Total Sugars (g)	All treatments combinations are on par into fresh juice.
6	Bacterial population (cfu/ml)	All combined UV and OH treatments except T_7 resulted in 5log reduction in bacterial count.
7	Yeast population (cfu/ml)	All combined treatements UV and OH treatments except T_7 resulted in maximum reduction in yeast count.

The process optimization was carried out by varying the predictor variables and corresponding results were discussed in previous sections. The data were tabulated in Appendix I. The process parameters responsible for yielding the most acceptable

quality attributes for the ultraviolet radiation assisted with ohmic heating system for preservation of pineapple juice are presented in Table 4.4

It may be concluded from the Table 4.4 that treatment T_8 and T_{10} representing combined ohmic heating and UV treatments with an ohmic temperature and UV dosage of 50 °C and 1200 mJ/cm^2 and 55 °C and 800 mJ/cm^2 respectively were found to be superior in preservation of pineapple juice through the mild pasteurization process in ohmic heating assisted UV radiation system developed. The system ensured the microbial inactivation yet retained the fresh like quality characteristics of pineapple juice.

4.4 ORGANOLEPTIC EVALUATION

In order to assess the sensory attributes of the treated juice the organoleptic evaluation of the pineapple juice with the optimized combinations of ohmic heating and UV treatment were carried out in comparison with fresh and conventionally heat pasteurized pineapple juice as explained in section 3.8. The mean sensory scores of the most important organoleptic characteristics that define the acceptance of the juice such as taste, colour, flavor, and overall acceptance provided by the judges are presented in Table 4.5. The spider chart showing the variation of mean scores are shown in Figure 4.6

Table 4.5 Mean scores of the Sensory evaluation

Sample	**Taste**	**Colour**	**Flavor**	**Overall acceptance**
Fresh	8.4	9	9	9
Thermal	7	6.5	6.5	6.6
OH and UV 50 °C,1200 mJ/cm^2	8.3	8.5	8.5	8.5
OH and UV 55 °C , 800 mJ/cm^2	8.1	8.1	8.2	8

It may be revealed from the table that combined UV and ohmic heating treatment with ohmic heating temperature 50°C and UV dosage of 1200 mJ/cm^2 showed the best results in terms of taste, colour, flavor and overall acceptance and close to that of the fresh juice. The other optmised treatments i.e 55 °C and 800 mJ/cm^2 reflected less preference in terms of all sensory attributes studied when compared to fresh as

well as the other combination treatment studied. Therefore, though this treatment showed similar results in terms of bio-chemical and microbial characteristics could be eliminated in terms of sensory quality. Thus it was concluded that the treatment T_8 could be adjudged best with ohmic heating temperature of 50 °C and UV dosage of 1200 mJ/cm^2 and therefore these process variables were selected as the best operating parameters for the developed ultraviolet assisted with ohmic heating system which retains the nutritional and sensory quality while inactivating the microorganisms.

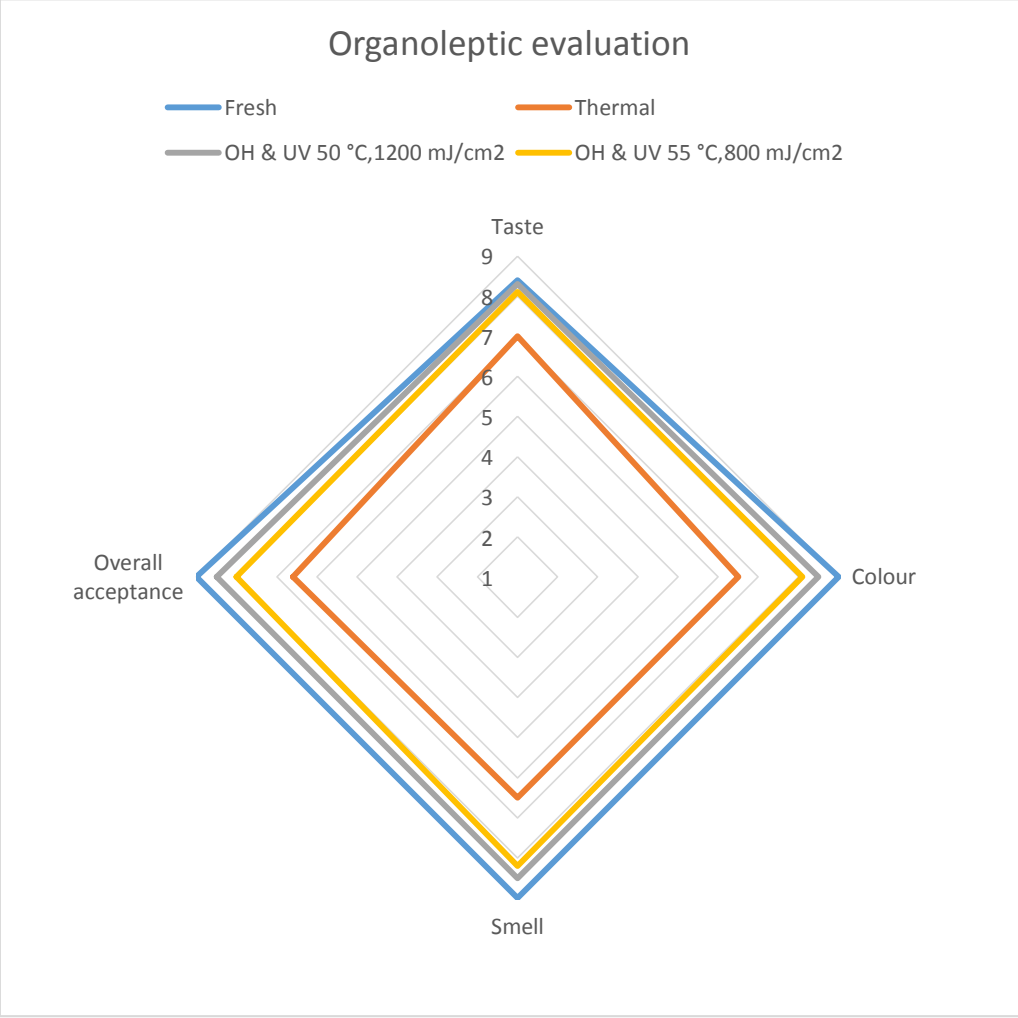


Figure 4.6 Variation of organoleptic scores of the fresh and treated pineapple juice

4.5 STORAGE STUDIES

In order to assess the effectiveness of the developed system towards preservation during the storage of pineapple juice, the juice treated at optimized process parameters

such as ohmic heating temperature of 50 °C and UV dosage of 1200 mJ/cm^2 and fresh juice (control) were stored at refrigerated condition at 4 °C. The results of biochemical and microbial characteristics of the juice during the storage period at 5days interval is presented in Table 4.6.

It may be observed from the table that the pH of the fresh juice increased significantly from 3.7 to 4.4, a 18.9 % increase at the end of the 25 days of storage whereas the pH of treated juice remains more or less same throughout the storage period studied. The increase in pH could be an indication of growth of microorganisms which could also be revealed through the decrease in TSS content of the untreated juice. Cortes *et al.* (2008) reported that an increase the pH values in the juices could be due to the microorganisms that resulted in juice spoilage in their studies on high pressurized pasteurization of orange juice. It was also revealed from the table that a significant reduction of TSS from a value of 18.5 °Brix to 15.2 °Brix was noticed for the fresh juice at the end of 25 days of storage and the rate of decrease of TSS increased from the 5th day of storage clearly indicating spoilage of the juice. The UV and OH treated juice showed no appreciable decrease in TSS values till the end of 25 days of storage. As stated earlier the decrease in TSS of the untreated juice could be due to the development of microbes on the substrates resulting in fermentation leading to an increase in pH of the same.

The titratable acidity values showed a reducing trend with the progress of storage days for the untreated juice whereas it remained same for the treated juice throughout. The titratable acidity decreased from 0.33 % to 0.17 % at the end of the 25th day marking a 48 % reduction for untreated juice whereas the treated juice could maintain the initial value of 0.33 % till the end. The reduction in titratable acidity could be attributed to the fermentation of organic acid by microorganisms leading to the spoilage of juice (sodeko *et al.*, 1987).

The variation in vitamin C content of control and treated sample during storage is presented in Table 4.6. Ascorbic acid (Vitamin C) is an important nutrient that possess antioxidant ability and provides protection against free radicals. It is also considered as an indicator of nutritional quality of juices. In the case of treated juice only 9 mg reduction in vitamin C was observed whereas 35 mg reduction was

observed for untreated juice at the end of the 25 days of storage which indicated a 75.04% reduction from its initial value. The reduction in vitamin C during storage could be due to the oxidation mechanism of not only oxygen but also exposure of samples to light (Odriozola-Serrano *et al.,* 2008). Though the treated sample showed a reduction in vitamin C content during storage, the rate of reduction was much faster in untreated sample.

Total sugars of the treated juice showed no significant change till the end of the storage period whereas the value varied between 10.39 g and 8.15 g for untreated juice. For treated juice, there occurred only a slight decrease from 10.2 g to 10.03 g which was insignificant. In case of untreated juice the sugars would broken down to other fractions and consequent fermentation which might have caused the spoilage and consequently a reduction in total sugars.

The comparison of bacterial count during the storage days summarizes the effectiveness the developed system. In case of untreated juice, marked increase in microbial count was noted and at the end of 25 days of storage and it has reached the level of too many to count. It is clear from the table that at the end of 25 days of storage the bacterial count was only 18×10^3 cfu/ml in case of treated sample. The limit of microbial shelf life for juice is 6 log cfu/ml (Mirrazavi, 2011). Therefore microbially, the treated juice is safe till the end of 25 days of storage.

Studies were continued on storage aspects of treated juice and it was found that the biochemical parameters and sensory characteristics of the treated juice slowly started to change after 25 days of storage under refrigeration and so is not reported.

In this study an ultraviolet radiation assisted ohmic heating system for preservation of fruit juices was designed and successfully fabricated. The fabricated instrumentation was tested for its effectiveness in pasteurization of pineapple juice. It was found that the synergistic effect of UV radiation assisted by ohmic heating could improve the microbial lethality in pineapple juice, and it was presumed that the UV light could effectively penetrate and damage the cellular structure through the cell pores opened by electroporation of ohmic process. The process parameters of the developed system was optimized by considering the important process

variables. It was established that a combined UV assisted ohmic heating process with ohmic heating temperature of 50 °C and a UV radiation dosage of 1200 mJ/cm^2 could bring out a 5log reduction in bacteria causing minimum changes in the nutritional and sensory quality and thus the overall acceptability of the pineapple juice. In order to further validate the developed system, a storage study was also carried out with pineapple juice treated in the fabricated system with the optimized process parameters and compared the same with that of fresh untreated juice. It was revealed that system could effectively treat the juice which could give a shelf life of 25 days under a refrigeration temperature at 4 °C retaining its biochemical characteristics while keeping the microbial level safe. The developed system is simple in design and easy to operate and continuous in operation.

Table 4.6 Storage period at 5days interval

Days	Quality characteristics											
	pH		TSS (°Brix)		Titratable acidity (% citric acid)		Vitamin C (mg)		Total Sugars (g)		Bacterial population (cfu/ml)	
	Control	OH/UV	Control	OH/UV	Control	OH/UV	Control	OH/UV	Control	OH/UV	Control	OH/UV
0	3.7	3.8	18.5	18.6	0.32	0.33	46.49	45.6	10.39	10.2	68×106	6×102
5	3.9	3.8	18.1	18.5	0.32	0.33	39.35	44.29	9.81	10.1894	72×106	10×102
10	4.1	3.8	17.6	18.5	0.30	0.33	32.88	42.96	9.53	10.1788	8×107	17×102
15	4.2	3.8	16.9	18.5	0.26	0.33	19.94	40.31	9.11	10.1575	25×107	5×103
20	4.3	3.8	16.1	18.4	0.22	0.33	12.61	38.00	8.54	10.115	TMTC	13×103
25	4.4	3.9	15.2	18.2	0.172	0.33	11.60	35.39	8.15	10.03	TMTC	18×103

4.6 COST ECONOMICS

The cost of production of the developed combined ohmic heating and UV treatment system was estimated to be Rs. 15000/-. The cost of the production could be further scaled down once the production is taken up on a industrial scale.

CHAPTER 5

SUMMARY AND CONCLUSION

Thermal heat processing serves as a common preservation method for beverages. However, due to its adverse effects on nutritional and sensory qualities, such as degradation of fresh juice flavor and reduction in nutrients, there is an increasing demand for an alternative non-thermal processing method as consumer demands for fresh, high quality and safe foods with fresh like characteristics is increasing. Non-thermal methods allow the processing of foods lower than the temperatures used during thermal processing. So flavours, essential nutrients and vitamins undergo minimal or no change.

Ultraviolet (UV) radiation is one such non-thermal processing alternative that has been shown to be effective against many types of foodborne pathogens. UV radiation has a significant germicidal effect at wavelength of 254 nm with doses between 0.12 and 9.0 kJ/ m^2 in the UV-C range because of radiation hormesis, which causes inactivation of microorganisms as a consequence of DNA damage. Although UV radiation has been investigated for inhibiting microbial growth, practical application of UV to disinfect water and liquid food products are limited due to low penetration depth, turbidity, and suspended solids which is not sufficient to meet the required 5 log microbial reduction in liquid foods, as regulated by the Food and Drug and Administration (FDA).

Ohmic heating (OH), a promising technology, the mechanism of which the internal heat dissipation generated by applying alternating current through a food product with direct contact to two electrodes so that, heat is generated immediately in the food product, creating a rapid and uniform heating that reduces thermal abuse, as opposed to conventional thermal processing methods. This study envisages development of a UV radiation assisted with ohmic heating system for pineapple juice and evaluation of the developed system in retaining the quality characteristics and microbial safety. It was hypothesized that a two-step process could occur: first, the electrophoretic force from a strong electric field could lock the open/close functions of cell pores and/or destroy cell membranes, and second, UV light could

penetrate deeply through the cell pores and disrupt the microbial DNA, preventing replication of microorganisms while retaining overall qualities of the juice.

A small capacity ohmic heating cum UV treatment reactor was conceptualized, further refined and then fabricated. The system consists of a feed tank, filtering unit, ohmic heating chamber, UV treatment chamber and a recirculation system.

The ohmic heating chamber was constructed using a 80 mm diameter Teflon cylinder with 10 mm thick wall. Two stainless steel electrodes of 70 mm diameter with 6.5 cm electrode spacing was provided inside chamber. The power supply to the ohmic heating chamber consisted of a 220 V, AC which resulted a voltage gradient of 34V/cm. OH temperature of juice was fixed at three levels 50°C, 55°C and 60°C.

The UV treatment chamber consists of a 45 mm thick cylindrical aluminum alloy pipe with a length of 250 mm, inner diameter of 40 mm. The cylinder is so fabricated that it can hold a quartz tube 19.7 mm thick, 16.5 cm length, inner diameter of 17.8 mm at the axial center of the aluminum pipe so that the flow of juice will be through the annular space between the tube and the cylinder The quartz tube will act as a cover for the UV lamp protecting it from liquid flow. A UV lamp with 3W output power with a UV wavelength of 254 nm was housed at the center of the quartz tube axially. The UV treatment involves the circulation of juice through the UV chamber. The flow rate of juice was set to 300 l/min. The juice was recirculated through the UV treatment chamber to obtain higher levels of dosage. The applied dosage per cycle was 100 mJ/cm^2. Exposure time of pineapple juice to UV light during each cycle was 180s. Based on the preliminary studies, the dosage levels were fixed at 800 mJ/cm^2, 1200 mJ/cm^2 and 1600 mJ/cm^2.

Evaluation of the developed ohmic heating assisted UV radiation system was evaluated for its effectiveness of preservation on pineapple juice. Juice were treated with ohmic heating alone, UV treatment alone and combined UV and OH treatment as per the ohmic heating temperature and UV dosage levels studied.

It was found that fresh pineapple juice had an absorbance value of 0.573 cm^{-1}, turbidity value of 372 NTU, and transmittance of 42.70%. The bacterial and yeast

population in fresh pineapple juice were 65 × 10^6 cfu/ml and 43 × 10^6 cfu/ml respectively.

The biochemical characteristics such as pH, TSS, titratable acidity, ascorbic acid and total sugars were carried out and analyzed for the treatments studied. The pH showed a minimum variation from fresh juice except for ohmic heating treatment. TSS, titratable acidity, ascorbic acid and total sugars had shown a minimum variation in UV alone and combination treatments compared to ohmic heating. Bacterial and yeast population resulted 5 log reduction in combination treatments, whereas some of the UV and all ohmic heating treatments failed to achieve 5 log reduction.

From the biochemical and microbial analysis it was concluded that combined ohmic heating and UV treatments with ohmic temperature and UV dosage of 50 °C and 1200 mJ/cm^2 and 55 °C and 800 mJ/cm^2 respectively were found to be superior in preservation of pineapple juice through the mild pasteurization process in the developed ohmic heating assisted UV radiation system thus ensuring the microbial inactivation yet retaining the fresh like quality characteristics of pineapple juice.

Organoleptic evaluation of optimized treated juice, fresh juice and conventionally heat treated juice revealed that combined UV and ohmic heating treatment with ohmic heating temperature 50°C and UV dosage of 1200 mJ/cm^2 showed the best results in terms of taste, colour, flavor and overall acceptability close to that of the fresh juice. Therefore, combination treatment with ohmic heating temperature of 50 °C and UV dosage of 1200 mJ/cm^2 adjudged best and therefore these process variables were selected as the best operating parameters for the developed ultraviolet assisted with ohmic heating system which retains the nutritional and sensory quality while inactivating the microorganisms. The storage studies revealed that system could effectively treat the pineapple juice which could give a shelf life of 25 days under a refrigeration temperature at 4 °C retaining its biochemical characteristics while keeping the microbial level safe.

Suggesions for future work

In order to further improve the preservative effect of UV radiation assisted with ohmic current and scale up of process for commercialization, the following suggestions for future research are recommended.

1.) Combine the UV and ohmic heating effects in single reaction chamber.
2.) Provision for changing the UV dosage intensity in single pass itself.
3.) Application of ohmic current in pulsed wave modes.
4.) Use of better electrodes which are more efficient and that does not corrode such as platinum.
5.) Improving the bacteriological standards of the system through use of better material science such as stainless steel pipes, valves and controls and application of HACCP.
6.) Incorporation of better controls such as automatic cutoffs, flow rates etc.

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APPENDIX-I

Effect of Independent Variables on Dependent Variable

Sl No	Treatment	pH	TSS	Titratable acidity	Vitamin-C	Total sugars
1	T1	3.79 [b]	19.06[f]	0.342 [e]	43.58 [c]	9.77 [bc]
2	T2	3.77 [c]	19.1 [f]	0.345 [f]	43.52 [c]	9.66 [c]
3	T3	3.76 [c]	19.13 [f]	0.346 [f]	42.30 [d]	9.65 [c]
4	T4	3.83[a]	18.56[ab]	0.325 [a]	45.82 [a]	10.23[ab]
5	T5	3.82 [a]	18.73[de]	0.326[abcd]	45.41 [ab]	10.23[ab]
6	T6	3.82 [a]	18.76 [e]	0.327 [bcd]	45.32 [b]	10.2[abc]
7	T7	3.83 [a]	18.6 [abc]	0.325 [a]	45.68 [ab]	10.21[abc]
8	T8	3.83 [a]	18.67 [bcde]	0.325[ab]	45.63 [ab]	10.19[abc]
9	T9	3.82 [a]	18.69 [cde]	0.364 [abc]	45.62 [ab]	10.17[abc]
10	T10	3.82 [a]	18.62 [bcde]	0.326[abcd]	45.42 [ab]	10.10[abc]
11	T11	3.82 [a]	18.66[abcde]	0.327[bcd]	45.43 [ab]	10.11[abc]
12	T12	3.82 [a]	18.69 [cde]	0.327[bcd]	45.44 [ab]	10.12[abc]
13	T13	3.81 [a]	18.73 [de]	0.327[bcd]	45.40 [ab]	10.05[abc]
14	T14	3.81 [a]	18.73 [de]	0.327[cd]	45.38 [ab]	10.04[abc]
15	T15	3.81 [a]	18.73 [de]	0.328[d]	45.32 [b]	10.02[abc]

APPENDIX-II
Quality Characteristics of treated samples

Sl No	Treatment	pH	TSS	Titratable acidity	Vitamin-C	Total sugars
1	T1	3.79	19.06	0.342	43.58	9.77
2	T2	3.77	19.1	0.3453	43.52	9.66
3	T3	3.76	19.13	0.3467	42.30	9.65
4	T4	3.83	18.56	0.3253	45.82	10.23
5	T5	3.82	18.73	0.3267	45.41	10.23
6	T6	3.82	18.76	0.3277	45.32	10.21
7	T7	3.83	18.6	0.3253	45.68	10.2
8	T8	3.83	18.67	0.3259	45.63	10.19
9	T9	3.82	18.69	0.3264	45.62	10.17
10	T10	3.82	18.62	0.3269	45.42	10.10
11	T11	3.82	18.66	0.3273	45.43	10.11
12	T12	3.82	18.69	0.3276	45.44	10.12
13	T13	3.81	18.73	0.3276	45.40	10.05
14	T14	3.81	18.73	0.3278	45.38	10.04
15	T15	3.81	18.73	0.3283	45.32	10.02

DEVELOPMENT AND EVALUATION OF AN ULTRAVIOLET RADIATION ASSISTED WITH OHMIC HEATING SYSTEM FOR PRESERVATION OF PINEAPPLE JUICE

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ABSTRACT

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ABSTRACT

Ultraviolet (UV) radiation is one such non-thermal processing alternative that has been shown to be effective against many types of foodborne pathogens. But there is a limitation of practical application of UV to disinfect liquid food products due to low penetration depth. Ohmic heating (OH), another promising technology, has been widely applied in food processes. This generates heat immediately in the food product, creating a rapid and uniform heating that reduces thermal abuse, as opposed to conventional thermal processing methods. Often times, ohmic heating cause heat-sensitive nutrients within food to be deteriorated by excessive current flow. These advanced technologies could contribute to shorten processing times, energy savings, and highly balanced safe food; however, they alone still cannot guarantee food safety without damaging the food's quality. Therefore, a new concept to combine ultraviolet and ohmic heating has been extensively evaluated. This combination technology would optimize each of the individual technology's strengths and reduce each of their individual weaknesses. The present study envisages development of a UV radiation assisted with ohmic heating system for pineapple juice and evaluation of the developed system in retaining the quality characteristics and microbial safety. In this study, a dual cylindrical ohmic and ultraviolet treatment combination continuous flow chambers was designed and fabricated to pasteurize the pineapple juice. UV treatment 800, 1200 and 1600 mJ/cm^2. Ohmic treatment until the sample temperature reached 50°C, 55°C and 60°C; and ohmic heating combined with UV treatment as the temperature rose to 50 °C, 55 °C, and 60 °C along with 800, 1200 and 1600 mJ/cm^2 dosages. Combined ohmic heating at 50 °C and UV treatment of 1200 mJ/cm^2 were found to be superior based on biochemical, microbiological and organoleptical characteristics. Storage study of best sample revealed that could give a shelf life of 25 days under a refrigeration temperature at 4 °C retaining its biochemical characteristics while keeping the microbial level safe.

സംഗ്രഹം

അൾട്രാവയലറ്റ് രശ്മികൾ ഉപയോഗിച്ചുള്ള താപരഹിത ഭക്ഷ്യസംസ്കരണം ഭക്ഷ്യവസ്തുക്കളിലെ രോഗാണുക്കളെ ഫലപ്രദമായി നശിപ്പിക്കുന്നു. പക്ഷെ ഈ രശ്മികൾക്ക് വസ്തുക്കളിൽ ആഴത്തിൽ പ്രവേശിക്കാത്തതിനാൽ ദ്രാവകരൂപത്തിലുള്ള ഭക്ഷ്യവസ്തുക്കളിൽ ഈ രീതി ഫലപ്രദമല്ല. ഭക്ഷ്യവസ്തുക്കളിൽ വേഗതയിലും എല്ലായിടത്തും ഒരേപോലെ താപം സൃഷ്ടിക്കുകയും ചെയ്യുന്ന മറ്റൊരു സാങ്കേതികവിദ്യയാണ് ഒമിക് താപീകരണം, പക്ഷേ ഇതിന്റെ താപത്താൽ ഭക്ഷ്യവസ്തുക്കളിലെ ചില പോഷകങ്ങൾ നശിക്കുന്നു. എന്നാൽ ഭക്ഷ്യ സംസ്കരണസമയലാഭം, ഊർജ്ജസംരക്ഷണം, സുരക്ഷിതമായ ഭക്ഷണം എന്നിവയാണ് ഈ സാങ്കേതികവിദ്യകളുടെ ഗുണങ്ങൾ. അതിനാൽ ഈ രണ്ടു സാങ്കേതികവിദ്യകളേയും സംയുക്തമായി കൈതച്ചക്കയുടെ നീരിൽ ഉപയോഗിച്ച് ഒരു ഗവേഷണം നടത്തുകയും അതിന്റെ ഗുണമേന്മയും കീടാണുക്കളിൽ നിന്നുള്ള സംരക്ഷണവും വിലയിരുത്തുകയും ചെയ്തു. ഇതിനുവേണ്ടി കൈതച്ചക്കയുടെ നീരിനെ പാസ്ച്ചുറൈസ് ചെയ്യാനായി ഈ സാങ്കേതികവിദ്യകളുടെ സംയുക്തപ്രവർത്തനം സാധ്യമാക്കുന്ന ഒരു സംവിധാനം രൂപകൽപന ചെയ്ത് വികസിപ്പിച്ചെടുത്തു. 800, 1200, 1600 mJ/cm^2 തീവ്രതയുള്ള UV രശ്മികളും ഒമിക് താപീകരണത്തിനായ് 50°C, 55°C, 60°C താപനിലയുമാണ് ഈ ഗവേഷണത്തിനുപയോഗിച്ചിരിക്കുന്നത്. ഈ രീതിയിൽ സംകരിക്കപ്പെട്ട കൈതച്ചക്കയുടെ നീരിന്റെ ജീവരസതന്ത്രത്തിന്റെയും, സൂക്ഷ്മാണുശാസ്ത്രത്തിന്റെയും സ്വഭാവസവിശേഷതകളുടെയും അടിസ്ഥാനത്തിൽ, 50°C ഒമിക് താപീകരണത്തിന്റെയും 1200 mJ/cm^2 തീവ്രതയുള്ള UV രശ്മികളുടെയും സംയുക്ത ഉപയോഗത്തിലൂടെ സംസകരിക്കപ്പെട്ട കൈതച്ചക്കയുടെ നീരാണ് ഈ പഠനത്തില് മികച്ച ഗുണമേന്മയുള്ളതായി കണ്ടെത്തിയത്. ഈ രീതിയിൽ പാസ്ച്ചുറൈസ് ചെയ്ത കൈതച്ചക്കയുടെ നീര് സൂക്ഷ്മാണുക്കളിൽ നിന്നും സുരക്ഷിതമായി സംരക്ഷിച്ച് 25 ദിവസത്തേക്ക് സംഭരിക്കുകയും അതിന്റ ജീവരസതന്ത്രസ്വഭാവസവിശേഷതകളെ കുറിച്ച് പഠനം നടത്തുകയും ചെയ്തു.