ALTERNATIVE PROCESSING TECHNIQUES FOR PASTEURIZATION OF LIQUID FOODS: MICROWAVE, OHMIC HEATING AND ULTRAVIOLET LIGHT

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI’I AT MĀNOA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MOLECULAR BIOSCIENCES AND BIOENGINEERING

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Last but not least, I owe great thanks to my family; my amazing parents, brothers and sisters. Words alone cannot express the gratitude I owe to my wife, Kaniaw, for all her encouragement, love and support while I worked hard to fulfil our dream.
ABSTRACT

The objectives of this study were to reduce the microbial contamination of fruit juice, different treatments were applied in order to treat microorganisms efficiently without degradation of bioactive nutrient values and to reduce unnecessary waste and time.

This dissertation is divided in five chapters: Chapter one reviews numerous studies of the impacts of batch or continuous flow ohmic and microwave heat treatments as a novel technology on nutritional values, enzymatic inactivation, and microbial destruction. Profiles during processing were needed to predict the effect of using different temperatures. Additionally, UV-light is tested an alternative to traditional pasteurization for inactivation of microorganisms.

Chapter two includes continuous flow ohmic heating for pasteurization of raisin juice. This study attempted to establish kinetic models of raisin juice inoculated with E. coli K-12 that was treated using a continuous flow ohmic heater (50, 60, 70, and 80°C) with different residence times (23 to 208 s). The results showed that continuous flow ohmic heating was more efficient compared to conventional heating at the same conditions to reduce the microbial and PME activity, and it minimized the loss of total phenolic acids and antioxidant values in raisin juice.

Chapter three covers simulation to validate the percentage of antioxidants levels and heat distribution of grape juice when pasteurized by ohmic heating with different residence times by comparing experimental data with numerical simulation. The result showed the outlet temperature profiles of 60, 70 and 80°C were similar to simulation data within 2-3°C. However, the correlation coefficient between experimental and simulation data for antioxidant levels after ohmic treatments was above of 0.92.
Chapter four aimed to investigate the effect of continuous flow microwave heating on the reduction of microorganisms in kava juice to extend the shelf life. Chemical and microbial properties of treated juice were analyzed using key parameters such as microbial counts, kavalactones, and pectin methylesterase activities. The results obtained from this chapter showed that continuous flow microwave heating was confirmed to be more efficient than the conventional method for the inactivation of microorganisms and pectin methylesterase, and there was no indicator to show significant deterioration of kava juice quality.

Chapter five involves apple juice inoculated with *E. coli* K-12 and exposed with pulsed ultraviolet (PUV) and non-pulsed ultraviolet (NPUV) modes as continuous and batch light treatments for 11 to 51 ml/min and 5 to 15 mins respectively. The highest log reductions of *E. coli* K-12 in apple juice after PUV and NPUV as continuous system were 3.35 and 3.99, respectively, at 11 ml/min. Also, log reductions reached 0.85 and 1.35 in samples pasteurized by PUV and NPUV, respectively, as batch systems after 15 mins. In addition, there was no significant difference between PUV and NPUV as continuous and batch systems.
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CHAPTER I

Introduction and Literature Review

INTRODUCTION

Consumers expect premium food to be fresh, safe, natural, and also to have high quality and be convenient products to obtain (Soliva-Fortuny and Martin-Bellos, 2003; Song et al., 2007). In the past year, conventional pasteurization is a typical method for reducing the number of feasible microorganisms and inactivation of enzymes in foods, but it can reduce the essential nutrients and content of bioactive compounds of food products (Saguy et al., 1978; Chen, 1993; Rosset et al., 2007; De-Awuah et al., 2007; Pereira and Vicente, 2010; Morales-de la Pena et al., 2011). Commercial fruit juices are the result of changing the properties and components in the original fruit juice which allows them to be perceived as healthy and refreshing products (Mitic et al., 2011). Moreover, fruit juice properties such as color, taste and flavor are made undesirable via impacts of enzymes such as polyphenoloxidase, peroxidase and pectin methylesterase (De-Awuah, 2007). Phytochemicals such as carotenoids, flavonoids, phenolic compounds and vitamins in fruit juices play an important role in disease prevention (Gardner et al., 2000). The consumption of polyphenol-rich juice improves antioxidant status, decreases oxidation of DNA, and stimulates immune cell functions (Bub et al., 2003).

Preservation of food products, quality and safety are the main concerns of food processors. During the last few decades, with the goal to develop or alter conventional processing technologies, a number of innovative thermal technologies have been developed such as ohmic and dielectric heating which include radio frequency and microwave heating. These are considered volumetric forms of heating in which thermal
energy is generated directly inside the food. It has been seen that products treated with ohmic and microwave heating have higher quality than those processed by conventional technologies, and, as a result, are gaining more popularity (Castro et al., 2003; Pereira and Vicente, 2010). Non-thermal technologies which include high-voltage pulsed electric fields, high intensity light pulses, mano-thermosonication, high hydrostatic pressure, and ultraviolet light (UV) are also referred to as “emerging” or “novel” technologies have been proposed, and in some cases developed (Gould, 2001; Gomez-Lopez et al., 2005; Caminiti et al., 2011; Torkamani and Niakousari, 2011). This is mainly due to its ability to heat materials rapidly and uniformly, leading to a less aggressive thermal treatment, which, otherwise, often leads to over processed volumes (Castro et al., 2004; Sastry, 2005).

The aims of this investigation are to provide a detailed review of novel thermal technologies such as ohmic and microwave heating treatments of some foods and non-thermal technologies such as ultraviolet light, compared to conventional heating methods on the destruction of microorganisms, inactivation of enzymes and the subsequent changes in food qualities. Modeling and prediction are ways to make sure that the experiment gives good and reproducible results for any operating protocols as well as for design development and control to logics save time and money.
Literature Review

*Ohmic heating Process*

Ohmic heat treatment of food is not new; it was developed and used in the 19th century and during the first half of the 20th century (Getchell, 1935; Ball, 1937). Ohmic heating, or joule heating, is the electro-conductive heating when electrical currents (AC) are passed through the foods to achieve sterilization and the preferred extent of cooking. Heat is internally generated within the food due to electrical resistance. For processing liquids and solid-liquid mixtures, ohmic heating as a novel technology has been accepted by industries (Piette *et al.*, 2004). Ohmic heating can be considered a high temperature, short time aseptic process. Therefore design of effective ohmic heaters depends on the voltage gradient in the field and the electrical conductivity of the food material (De-Alwis and Fryer, 1990b; Sastry and Li, 1996). In general, fruits are less conductive than meat samples and lean meat is more conductive than fat (Ruan *et al.*, 2001; Sarang *et al.*, 2008). The heating rate in an electrical resistance system depends on many aspects such as specific heat, electrical properties, particle nature, concentration, size, shape and direction in the electrical field (Marcotte, 1999; Mckenna *et al*., 2006).

Ohmic heating is used in a broad range of applications such as preheating, blanching, pasteurization, sterilization and extraction of food products (Leizerson and Shimoni, 2005). Therefore the quality of foods, especially the volatile components can be better preserved compared to using the traditional method (Sastry, 1994). Moreover, the FDA (2000) reported that products containing particulates, resulted in less thermal breakdown of the product, and absence of a hot surface in ohmic heating reduces fouling problems in comparison to conventional heating because ohmic heating has the ability to heat
materials rapidly and uniformly (Sastry and Barach, 2000). Ohmic heating could be used as one of the alternative methods in manufacturing fruit juice processing; unlike conventional heating which is a fast process (Yildiz et al., 2009). Conventional thermal sterilization has been applied to most infant foods, but it can reduce the levels of essential nutrients and degrade the organoleptic qualities (Rooset et al., 2007).

**Impact of ohmic heating on nutritional values**

Ascorbic acid is used to prevent browning, discoloration, and also to prolong shelf life. Consequently, it is also a natural antioxidant. Castro et al. (2004b) showed that the decimal reduction time (D-value) for degradation of ascorbic acid in strawberry pulp after ohmic heating for 256 and 192 min. at temperatures of 60 and 70°C, were lower than conventional heating (294 and 196 min. while at the high temperatures (80 and 90°C) showed opposing results, and follows first order degradation kinetics for both methods. Their results were unlike the Vikram et al. (2005) study which presented high D-values for the effect of thermal degradation of vitamin C in orange juice by ohmic heating at 50, 60 and 90°C, and low D-values at 75°C dissimilar to conventional treatments (Table 1.1). According to Icer (2005) and Wang and Sastry (2002) ohmic heating is better than conventional heating in terms of involved retaining the nutritional value of foods, higher products, and short processing. It is therefore clear that the ohmic heating effect on flavor concentration in orange juice is higher than for conventionally heated orange juice (Leizerson and Shimoni, 2005).
Table 1.1 Nutrition values after ohmic and conventional heating treatments.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatments</th>
<th>Parameters</th>
<th>D-values (min)</th>
<th>References</th>
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<tr>
<td>Strawberry pulp</td>
<td>$D_{(60, 70, 80 and 90^\circ C)}$ Conventional heating</td>
<td>Ascorbic acid</td>
<td>294, 196, 152, 123</td>
<td>Castro et al., 2004b</td>
</tr>
<tr>
<td></td>
<td>$D_{(60, 70, 80 and 90^\circ C)}$ Ohmic heating</td>
<td></td>
<td>256, 192, 154 &amp; 130</td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td>$D_{(50, 60, 75 and 90^\circ C)}$ Conventional heating</td>
<td>Vitamin C</td>
<td>65.67, 49.81, 27.02 &amp; 12.91</td>
<td>Vikram et al., 2005</td>
</tr>
<tr>
<td></td>
<td>$D_{(50, 60, 75 and 90^\circ C)}$ Ohmic heating</td>
<td></td>
<td>95.96, 58.55, 23.72 &amp; 14.66</td>
<td></td>
</tr>
</tbody>
</table>

**Microbial and enzymatic inactivation by ohmic heating**

Sun *et al.* (2008) reported the decimal reduction time (D-value) of *S. thermophilus* in milk after ohmic heating at 75°C was lower than in conventional heating as shown in Table 1.2. Moreover, Handan (2010) found the D-values of *Alicyclobacillus acidoterrestris* spores in orange juice via ohmic heating at 70, 80 and 90°C were extremely small at 58.48, 12.24 and 5.97 min, respectively, than those achieved from conventional heating at 83.33, 15.11 and 7.84 min. under the same conditions. Pereira *et al.* (2007) showed the change in D-values after ohmic heating at 63 and 80°C for 1.90 and 11.1 min respectively, on *Escherichia coli* in goat milk and *Bacillus licheniformis* in cloudberry jam than those pasteurized conventionally under the same conditions 3.9±0.5, and 18.1±1.1min. Considering that mechanisms of microbial destruction by ohmic heating are related to the voltage gradient and thermal properties, a mild electroporation mechanism may take place during ohmic heating operating at low frequencies which
allows electrical charges to build up and form pores across the cell wall. Their results are in accordance with previous research (Yoon et al., 2002).

The results of both ohmic and conventional heating of Geobacillus stearothermophilus spores are summarized in Table 1.2. D-values obtained after ohmic heating at 10 KHz (125°C, 0.43 min and 60 KHz 125°C, 0.34 min. were extremely smaller than those calculated from conventional heating for 2.53 and 0.64 min, respectively (Somavat et al., 2011). Various studies have investigated ohmic heating treatments resulting in more inactivation of microorganisms when comparing D-values to those of conventional heating (Palaniappan and Sastry, 1992; Cho et al., 1999). Hence, Leizerson and Shimoni (2005) reported that microbial counts were reduced by at least 2-3 orders of magnitude, after exposure to ohmic heating of fresh orange juice compared to conventional heating at the same time there was no deference between both methods on the inactivation of microorganisms.

Alternative processing technologies causing more inactivation of enzymes in order to increase the quality foods have been investigated, because enzymes also have a negative influence on food products such as undesirable odours, tastes, and texture (Leizerson and Shimoni, 2005). Castro et al. (2004a) reported that the D-value for Lipoxygenases (LOX) in soyabean, during ohmic heating (75 and 85°C) was much lower (6.92 and 0.58) than conventional heating (117.8 and 0.99) under the same conditions. The authors also investigated D-values for polyphenoloxidase (PPO) in apples. The ohmic heating results (3.52-19.37 min) were extremely lower than the results obtained under conventional heating (10.30-61.61 min). The obtained data showed that ohmic heating was more
efficient than conventional heating because the electric field reduces the D-values for those enzymes.

Alternative processing technologies causing more inactivation of enzymes in order to increase the quality foods have been investigated, because enzymes also have a negative influence on food products such as undesirable odors, tastes, and texture (Leizerson and Shimoni, 2005)
Table 1.2. Microbial and enzymatic inactivation after ohmic and conventional heating treatments.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatments</th>
<th>Parameters</th>
<th>D-values (min)</th>
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</tr>
</thead>
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<tr>
<td>Milk</td>
<td>(D_{75^\circ C}) Conventional heating</td>
<td>S. thermophilus</td>
<td>3.3± 4.2, 3.09± 0.55</td>
<td>Sun et al., 2008</td>
</tr>
<tr>
<td>Orange juice</td>
<td>(D_{70, 80, and 90^\circ C}) for 0-30 min. Conventional heating</td>
<td>Alicyclobacillus acidoterrestris spores</td>
<td>83.33, 15.11 &amp; 7.84, 58.48, 12.24 &amp; 5.97</td>
<td>Handan, 2010</td>
</tr>
<tr>
<td>Milk</td>
<td>(D_{63^\circ C}) Conventional heating</td>
<td>Escherichia coli</td>
<td>3.9± 0.5, 1.9</td>
<td>Pereira et al., 2007</td>
</tr>
<tr>
<td>Orange juice</td>
<td>(D_{80^\circ C}) Conventional heating</td>
<td>Bacillus licheniformis</td>
<td>N/A, 18.1± 1.1, 11.1± 1.3</td>
<td></td>
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<tr>
<td>Orange juice</td>
<td>(D_{121, 125\ and\ 130^\circ C}) Conventional heating</td>
<td>Geobacillus stearothermophilus spores</td>
<td>N/A, 2.53ᵇ, 0.64ᵇ &amp; 0.06ᵃ, 0.88ᵃ, 0.43ᵃ &amp; 0.05ᵃ, 1.17ᵇ, 0.34ᵃ &amp; 0.05ᵃ</td>
<td>Somavat et al., 2011</td>
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<td>Orange juice</td>
<td>(D_{90^\circ C\ for\ 50\ s}) Conventional heating</td>
<td>Pectin Methylesterase (PME%)</td>
<td>5% 90-98%</td>
<td>Leizerson and Shimoni, 2005</td>
</tr>
<tr>
<td>Soybean</td>
<td>(D_{60 and\ 75^\circ C}) Conventional heating</td>
<td>Lipoxygenases (LOX)</td>
<td>117.8 and 0.99, 6.92 and 0.58</td>
<td>Castro et al., 2004a</td>
</tr>
<tr>
<td>Apple juice</td>
<td>(D_{75\ and\ 85^\circ C}) Conventional heating</td>
<td>Polyphenoloxidase (PPO)</td>
<td>61.61 and 10.30, 19.37 and 3.52</td>
<td></td>
</tr>
</tbody>
</table>
Computational modelling

Computer simulation can be used predict the temperature distribution within two or three-dimensions in either a static or continuous ohmic heating system. Shim et al. (2010) described the temperature profiles of solid-liquid food complexes under a static ohmic heating system. The simulation data were compared to experimental observations and it showed the predicted temperature values agreed with the experimental measurements with a maximum error of 6°C. Computer simulation is the key to realize the development of simulation as its representations of the experimental data. Three-dimensional (3D) finite differential computer modeling with arbitrary values of surface heat transfer coefficient and thermal conductivity were investigated to predict the temperatures distribution in package pasteurization of solid foods, using a numerical model for predictive log reduction of microorganism and then the relationship between simulation and experimental data was illustrated (Ghazala et al., 1995). Moreover, Jun and Sastry (2007) observed a three-dimensional model to envision the physical locations and temperature profiles using electrodes at the sealed end of pouches of tomato soup. In some cases, two-dimensional (2D) heated systems are used to calculate the heat distribution as a function of solid foods (De-alwis and Fryer, 1990b). In order to achieve results reliable with experimental values, it is essential to find accurate physical property values in the simulation (Fu and Hsieh, 1999). Moreover, electrical conductivity values of fruit juice impact the ohmic heating process.

Huang (2006) attempted to measure the temperature profiles within the packages of frankfurters during pasteurization by hot water immersion and compared the number of L. monocytogenes found experimentally the numerical simulation results after
pasteurization. The temperature profiles were similar to simulation data, and there was a 1-2 log variation between experimental inspections and simulated data for the reduction of *L. monocytogenes* after pasteurization. Castro *et al.* (2004c) investigated the fluid residence time distribution of strawberry pulp, which was pumped through the ohmic heater at various flow rates, and performed computational fluid dynamics to find the best heat treatment because the optimization of the heating process was changed when the residence time distribution changed, otherwise it would be over-processing or un-sterilized.

**Microwave heating process**

Over the past few decades, thermal processing of materials, using microwaves as a source of energy is a well-known and viable alternative for high temperature short-time processing of thermo-sensitive materials. In contrast to conventional heating, where heat is transferred from the surface to the interior of the product, microwave penetrates the sample and causes heating throughout its volume. This volumetric heating leads to faster heat transfer rates and shorter processing times than conventional processes, because volumetric heating means that materials can absorb microwave energy directly within then can convert it to heat (Mullin, 1995; Tong, 2002; Salvi *et al.*, 2011).

Microwaves have electromagnetic waves in the range of infrared and radio waves between 300 MHz to 3 GHz (Thostenson and Chou, 1999; Meredith, 1998; Thostenson and Chou, 1999). Moreover, Zhao *et al.* (2000) and Tewari (2007) proposed that dielectric heating has involved microwave and radio frequencies, but microwave heating has a higher frequency (915 and 2,450 MHz) and a smaller wavelength unlike radio-
frequency heating, which has a low frequency (13.56, 27.12 and 40.68 MHz) and a larger wavelength compared to the samples being heated.

Microwave heating entails two distinct mechanisms: specifically, dipolar and ionic interactions. Due to their dipolar nature, water is the most common polar molecule and also a major component of foods. The water molecules form a “dipole” with a positive stimulated end and negative one. Similar to the action of a magnet, these “dipoles” will familiarize themselves when they are subject to electromagnetic radiation as it oscillates at the very high frequencies listed, and such oscillations create heat. (Buffler, 1993; Knorzer et al., 2011). The rotation of water molecules would generate heat for cooking whereas the mechanism of microwave heating is that the ions’ vibratory migration generates heat under the impact of an oscillating electric field. Microwave processing is versatile if properly designed and has been used extensively in the past to successfully inactivate enzymes and bacterial cells present in foods (Heddleson and Doores, 1994; Terigar et al., 2010). Theoretically, selective heating, electroporation, cell membrane bursting and magnetic field coupling were four major processes proposed for the investigation of non-thermal inactivation of microorganisms by microwaves (Kozempel et al., 1998).

Microwave heating has numerous advantages over conventional heating methods. These advantages include lower cost of microwaves, faster switch-on and shut-down time and smaller scale of operation (Giese, 1992). Studies on the non-uniform temperature distribution have been investigated by several authors (Lee et al., 2002; Gunasekaran and Yang, 2007). Non-uniform temperature distribution is one of the main problems in microwave heating systems. Hence, Lin et al. (1995); and Vilayannur et al. (1998); Yang
and Gunasekaran (2004) developed a simulation of microwave heating to predict temperature distribution in the microwave heated food.

Microwave heating has been confirmed to be more efficient than conventional methods for the extraction of isoflavones (>80%) at the same conditions (Terigar et al., 2010). Continuous flow microwave heating has been of interest to some researchers because all the parameters (i.e. colour, texture, flavour and nutrient value) of the food products by continuous flow microwave heating are improved (Giese, 1992; Tajchakavit et al., 1998; Sumnu, 2001). Specific heat, density, shape, surface to volume ratio, evaporative cooling, and thermal conductivity are physical factors of food that determine microwave penetration, overall heating rate, and conventional heat transfer (Gentry and Roberts, 2005).

*Impact of microwave heating on nutritional values*

Fratianni et al. (2010) has reported the concentration of total carotenoids in fresh orange juice was 25.60±2.69 µg/100 ml. Table 1.3 shows the total concentration of carotenoid pigments in orange juice detected after microwave pasteurization was decreased by 10%, 40%, and 50% at 70, 75 and 85˚C, respectively, at 1 minute integrals. However, Igual et al. (2010) found no significant difference between the impacts of microwave and conventional treatments on the total phenolic acids and DPPH% in grapefruit juice, while the concentration of ascorbic acid before and after microwave heating was similar, unlike conventional heating, ascorbic acid in grapefruit juice presented the lowest statistically significant difference (P ≤ 0.05). Villamiel et al., (1998) found the concentration of ascorbic acids had no significant difference between continuous flow microwave and conventional treatments with fresh orange juices. Abed-El-Al et al. (1994) found that the
amount of ascorbic acid decreased more in orange juice heated in a batch microwave system than in a hot water-bath. However based on Valero et al. (1999), the assessment of 12 components of milk product treated by continuous flow microwave heating had a higher level of 9.15 µg/l at 70°C, while individually the constituents were within 10 and 23%. The results were similar among the treatments due to the uniformity of heating in the microwave system, and, perhaps, the low thermal productions are used.

Sierra et al. (1999) described the influence of microwave and conventional heating (plate heat exchanger) on the concentration of vitamin B1 in milk. Table 1.3 shows no significant losses of vitamin B1 concentration, including the concentration after continuous microwave heating of raw milk at 85°C, 16.5 s come-up-time (CUT) and 0 s holding time (P ≤ 5%). However, raw milk pasteurized by conventional heating at 80°C, 44.37 s come-up-time (CUT) and 0 s holding time, showed significant degradation of this vitamin. Vitamin B1 retention is superior in the microwave heating of milk with time resided in the heating section (85°C for holding time 40 s) than in the plate heat exchanger (80°C for holding time 40 s).

Sierra and Vidal-Valverde, (2000) studied the effect of microwave treatment on the quality of milk, recovering a higher vitamin B1 retention at 90°C without a holding time. However, using the same pasteurization treatment but applying retention times of 30, and 60 s lead to decrease vitamin B1, which increased with increased holding time. The same author found no difference between the effects of tubular heat exchanger and microwave heat treatment on the concentration of vitamin B2 in milk, which was similar to the report of Van Zante and Johnson (1970).
Table 1.3 Nutrition values after microwave and conventional heating treatments.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatments</th>
<th>Parameters</th>
<th>Quantities</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice</td>
<td>Fresh Conventional heating</td>
<td>Total carotenoids (µg/100ml)</td>
<td>25.60 ± 2.69 a, not 22.49± 1.92 a, 15.55± 1.24 b, 12.76± 1.84 b</td>
<td>Fratianni et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Microwave heating (70˚C for 1 min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grape fruit juice</td>
<td>Fresh Conventional pasteurization 80˚C ±2.5 , 11 s</td>
<td>DPPH (%)</td>
<td>44.4</td>
<td>Igual et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Microwave pasteurization 900 W 30 s</td>
<td></td>
<td>26.1</td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td>Fresh Conventional pasteurization (89.8 and 96.˚C)</td>
<td>Ascorbic acid (mg/100g)</td>
<td>36</td>
<td>Villamiel et al., 1998</td>
</tr>
<tr>
<td></td>
<td>Microwave pasteurization (89.7 and 96.4˚C)</td>
<td></td>
<td>36</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>Fresh Conventional pasteurization (70, 80, and 90˚C)</td>
<td>Total volatile content (µg/l)</td>
<td>4.55, 4.84 and 5.89, 9.15, 4.29 and 4.93</td>
<td>Valero et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Microwave pasteurization (70, 80, and 90˚C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>Raw Conventional heating (90˚C (0, 30 and 60 s)</td>
<td>Vitamin B1 (mg/l)</td>
<td>0.276 ±0.003 a, 0.270 ±0.000 b and 0.265 ±0.001 c</td>
<td>Sierra and Vidas-Valverde, 2000</td>
</tr>
<tr>
<td></td>
<td>Microwave heating (90˚C for 0, 30 and 60 s))</td>
<td></td>
<td>0.277 ±0.001 b, 0.271 ±0.001 b  and 0.266 ±0.002 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Raw Conventional heating (90˚C for 0, 30 and 60 s)</td>
<td>Vitamin B2 (mg/l)</td>
<td>1.678 ±0.011 a, 1.672 ±0.021 a and 1.655 ±0.008 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microwave heating (90˚C for 0, 30 and 60 s))</td>
<td></td>
<td>1.682 ±0.061 a, 1.675 ±0.017 a and 1.688 ±0.009 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Raw Plate heat xchanger heating (80˚C for 0 and 40 s)</td>
<td>Vitamin B1 (mg/l)</td>
<td>0.395 ±0.006 a, z</td>
<td>Sierra et al., 1999</td>
</tr>
<tr>
<td></td>
<td>Microwave heating (85˚C for 0 and 40 s))</td>
<td></td>
<td>0.380 ±0.003 b, y and 0.360 ±0.003 c, y</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.393 ±0.006 a, z and 0.378 ±0.004 b, z</td>
<td></td>
</tr>
</tbody>
</table>
Microbial and enzymatic inactivation by microwave heating

In Table 1.4, Nikdel and Mackeller (1992) reported that the number of microorganisms in orange juice before treatment was $2 \times 10^4$ CFU/ml, and inactivation of microorganisms was less than 20 CFU/ml by continuous flow microwave pasteurizer at 88-91°C for 15 s and $5 \times 10^2$ CFU/ml by steam heat treatments at 91°C. Moreover, Nikdel et al. (1993) showed the initial of L. plantarum $2 \times 10^4$ CFU/ml in citrus juice, but the result after microwave heating (90°C for 30 s) produced higher inactivation of L. plantarum (< 10 CFU/ml) compared to that expected from traditional hot water pasteurization (91°C for 30 s, 550 CFU/ml). Consequently, researchers Tajchakavit et al. (1998) showed thermal kinetic considerations of D-values for S. cerevisiae, and L. plantarum in apple juice during continuous flow microwave heating at 55 and 60°C were lower than conventional heating.

On the other hand, Giuliani et al. (2010) illustrated the reduction of spore number of Alicyclobacillus acidoterrestris in cream of asparagus samples (1.90, 1.81, and 2.08 CFU/g) by microwave processing powers of 80%, 90%, and 100%, respectively. According to Canumir et al. (2002), the impact of microwave pasteurization at power levels of 900 and 720 W for 60 and 90 s on the survival of E. coli in apple juice showed no significant differences between both methods. Several studies, including that conducted by Yeo et al. (1999) showed that heat waves generated kill microorganisms.

Generally, juices contain different amounts of pectin methylesterase. Clarity and cloudiness juices and wines for the reduction of viscosity relate to pectin methylesterase activity. Regarding the impact of the continuous flow microwave heating at 75 °C with
10-15 s holding times, the inactivation of pectin methylesterase was 98.5 and 99%, while PME inactivation was 99% by conventional heating at 90.5 °C for 15s. While inactivation of pectin methylesterase has been more resistant than microorganism to heating, Igual et al. (2010) described that the percentage of residual pectin methylesterase under the microwave pasteurization at 900 W for 30 s was 10.07 lower than under conventional pasteurization 12.04 at 80 °C for 11 s.

In addition, the same authors found a difference between the impacts of microwave and conventional pasteurization on the percentage of inactivation of peroxidase (88.1 ±0.3 and 94.3 ± 0.7) respectively, under the same condition as above. Moreover, Tajchakavit and Ramaswamy (1997) found the D-value for the inactivation of PME in orange juice after conventional pasteurization was higher than after microwave pasteurization.
Table 1.4 Microbial and enzymatic inactivation after microwave and conventional treatments.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatments</th>
<th>Parameters</th>
<th>Quantities &amp; D-values</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange juice</td>
<td>Fresh Conventional pasteurization (90˚C)</td>
<td>Bacteria (CFU/ml)</td>
<td>2 × 10⁴</td>
<td>Nikdel, and Mackeller, 1992</td>
</tr>
<tr>
<td></td>
<td>Microwave heating (88-91˚C for 30 s)</td>
<td></td>
<td>5 × 10²</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; 20</td>
<td></td>
</tr>
<tr>
<td>Citrus juice</td>
<td>Fresh Conventional pasteurization (91˚C for 30 s)</td>
<td>Lactobacillus plantarum (CFU/ml)</td>
<td>2 × 10⁴</td>
<td>Nikdal et al., 1993</td>
</tr>
<tr>
<td></td>
<td>Microwave heating (90˚C for 30 s)</td>
<td></td>
<td>550</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt; 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fresh Conventional pasteurization (90.5˚C for 15 s)</td>
<td>Pectin methyl esterase (PME %)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microwave heating (75˚C with 10-15 s)</td>
<td></td>
<td>99</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>98.5-99.5</td>
<td></td>
</tr>
<tr>
<td>Apple juice</td>
<td>D(55 &amp; 60˚C) Conventional heating</td>
<td>L. plantarum</td>
<td>25.1 &amp; 21.9 min.</td>
<td>Tajchakavit et al., 1998</td>
</tr>
<tr>
<td></td>
<td>D(55 &amp; 60˚C) Continuous flow microwave heating</td>
<td>S. cerevisiae</td>
<td>2.08 &amp; 3.83 min.</td>
<td></td>
</tr>
<tr>
<td>Apple juice</td>
<td>Fresh Conventional pasteurization (83˚C for 30 s)</td>
<td>Escherichia coli (CFU/ml)</td>
<td>N/A</td>
<td>Canumir et al., 2002</td>
</tr>
<tr>
<td></td>
<td>Microwave heating (900 and 720 W for 40 to 90 s)</td>
<td></td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 - 3.4 × 10⁵</td>
<td></td>
</tr>
<tr>
<td>Grapefruit juice</td>
<td>Fresh Conventional pasteurization (80˚C ±2.5 for 11 s)</td>
<td>Residual Pectin methyl esterase (PME %)</td>
<td>N/A</td>
<td>Igual et al., 2010</td>
</tr>
<tr>
<td></td>
<td>Microwave pasteurization (900 W for 30 s)</td>
<td></td>
<td>12.04 ± 3.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10.07 ± 0.63</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fresh Conventional pasteurization (80˚C ±2.5 for 11 s)</td>
<td>Peroxidase (POD %)</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Microwave pasteurization (900 W for 30 s)</td>
<td></td>
<td>94.3 ± 0.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>88.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Orange juice</td>
<td>D(60˚C) Conventional pasteurization</td>
<td>Pectin methyl esterase (PME)</td>
<td>154 s</td>
<td>Tajchakavit and Ramawamy, 1997</td>
</tr>
<tr>
<td></td>
<td>D(60˚C) Microwave pasteurization</td>
<td></td>
<td>7.37 s</td>
<td></td>
</tr>
</tbody>
</table>
**Ultraviolet light**

Ultraviolet treatment is a promising bactericidal alternative to conventional methods because it does not undesirably affect the texture, color, flavour and bioactive components of food products since it can be accomplished at an ambient temperature (Yousef and Marth, 1988; Hollingsworth, 2001). Ultraviolet light has been used for the inactivation of microorganisms for a very long time and it is one of the many non-thermal technologies for food preservation. It is part of the electromagnetic spectrum, wavelengths in the visible light range between 400 and 700 nm. The germicidal portion is between the ranges of 200 to 390 nm, but 254 nm is the peak effective wavelength. The main mechanism of germicidal and microbial inactivation effect appears to be due to the UV part of the spectrum and its photochemical, thermochemical and physical properties, which involves structural changes in DNA of bacteria, and other pathogens, consequently, preventing cells from replicating (Anderson et al., 2000; Takishita et al., 2003; Elmnasser et al., 2007).

UV-light includes two approaches, pulsed UV-light and continuous wave treatments. MacGregor et al. (1998) reported the destruction levels of conventional continuous UV light emission are four to six times slower than pulsed-light. There are several applications that can use UV as an alternative to thermal treatment for the rapid inactivation of pathogenic and spoilage microorganisms in foods, moulds, yeasts, bacteria and viruses (Oms-oliu et al., 2010).
Microbial destruction by Ultraviolet light

Table 1.5 summarizes results obtained during studies of microbial destruction with ultraviolet light in various foods. Sharifi-Yazdi and Darghahi (2006) investigated the inactivation of the bacteria *Pseudomonas aeruginosa* with an 8 log CFU/ml and *Bacillus megaterium* with a 4 log CFU/ml in water after 11 and 50 pulses of ultraviolet light, respectively. Results indicated that the gram-positive bacteria had higher resistance than the gram-negative bacteria tested. Shama (1999) and Sastry et al. (2000) have explained the same results because the latter have only a single or a few layers of peptidoglycan. In contrast, the gram-positive bacteria have many layers of peptidoglycan. Thus, Munoz et al. (2012) showed significant differences between microbial counts in apple juice before and after pulsed ultraviolet treatment by 3.1 and 4.9 log CFU/ml set at low and high energy, respectively. The microbial destruction increased with higher energy dose (Omis-Oliu et al., 2010).

Moreover, Geveke (2005) reported approximately 3.4±0.3 and 4.7 log reductions of *E. coli* in apple cider after exposure to pulsed ultraviolet light for 19 and 30 seconds, respectively at ambient temperature, while 2.5±0.1 log of *L. innocua* was reduced after a treatment time of 58 seconds. The inactivation of microorganisms depends on the kind of microorganisms and treatment time. Franz et al. (2009) found the number of *E. coli* in cloudy apple juice after ultraviolet treatment at higher flow rates were reduced by 4-5 logs, but did not find *L. brevis* in the same juice after the same treatment.
Table 1.5: Microbial inactivation after ultraviolet-light treatment.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatments</th>
<th>Parameters</th>
<th>Log counts</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>Pulsed Ultra violet light</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>8 log_{10} CFU/ml</td>
<td>Sharif-yazdi and Darghahi, 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Bacillus megaterium</em></td>
<td>4 log_{10} CFU/ml</td>
<td></td>
</tr>
<tr>
<td>Apple juice</td>
<td>Pulsed light (Low energy)</td>
<td><em>E. coli</em></td>
<td>3.1 log CFU/ml</td>
<td>Munoz <em>et al.</em>, 2012</td>
</tr>
<tr>
<td></td>
<td>Pulsed light (High energy)</td>
<td></td>
<td>4.9 log CFU/ml</td>
<td></td>
</tr>
<tr>
<td>Apple cider</td>
<td>Ultra violet</td>
<td><em>E. coli</em></td>
<td>3.4±0.3 log CFU/ml</td>
<td>Geveke, 2005</td>
</tr>
<tr>
<td></td>
<td>Ultra violet-light</td>
<td><em>L. innocua</em></td>
<td>2.5±0.1 log CFU/ml</td>
<td></td>
</tr>
<tr>
<td>Opaque apple juice</td>
<td>Ultra violet-light</td>
<td><em>E. coli</em></td>
<td>4-5 log CFU/ml</td>
<td>Franz <em>et al.</em>, 2009</td>
</tr>
<tr>
<td>Apple juice</td>
<td>Ultra violet-light</td>
<td><em>APC</em></td>
<td>3.5 log CFU/ml</td>
<td>Keyser <em>et al.</em>, 2008</td>
</tr>
<tr>
<td></td>
<td>Ultra violet-light</td>
<td><em>YM</em></td>
<td>3.0 log CFU/ml</td>
<td></td>
</tr>
<tr>
<td>Apple juice</td>
<td>High Intensity Light Pulses</td>
<td><em>E. coli</em></td>
<td>2.65, 4.5 and 4.7 log CFU/ml</td>
<td>Palgan <em>et al.</em>, 2011</td>
</tr>
<tr>
<td></td>
<td>Ultra violet-light</td>
<td><em>L. innocua</em></td>
<td>1.1, 1.4 and 1.93 log CFU/ml</td>
<td></td>
</tr>
</tbody>
</table>

On the other hand, Keyser *et al.* (2008) found that reduction of aerobic plate count (APC) bacteria was 3.5 log and yeast and moulds (YM) were 3.0 log after UV dosage (230 J/l) in apple juice, while the destruction of inoculated apple juice with *E. coli* K-12 reached to 7.42 log after treatments by UV dosages of 1377 J/L. Palgan *et al.* (2011) observed the destruction of *E. coli* and *L. innocua* showed in apple juice dissimilarity, however, *E. coli* appeared to be more sensitive than *L. innocua*, to high intensity light pulses for 2, 4 and 8 seconds.
CONCLUSIONS

In general, and concerning the parameters reviewed, the results indicated that there were significant differences between ohmic heating and conventional heating in death rates of most microorganisms, the inactivation of enzymes, and the degradation of nutrient values. These results suggest microwave and ohmic heating compares favourably against conventional heating at the same conditions. Because microwave and ohmic heating are volumetric heating processes and are more efficient than conventional heating. At the same time, the decimal reduction time for most parameters during microwave and ohmic heating were lower than conventional heating.

It was also concluded that numerical simulation procedures are able to successfully predict the temperature profiles and confirmed the impact of thermal or non-thermal processing on food quality and safety, which are strongly related to the boundary conditions and physical property values for consistent simulation.

Finally, the non thermal techniques such as UV- light can be used as alternative to conventional heating for the inactivation of microorganisms in fruit juice, milk, or on the surface of solid food products.
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CHAPTER II

Continuous Flow Ohmic Pasteurization of Raisin Juice and Kinetic Model Development: Antioxidant, Phenolic Acids, Pectin Methylesterase and Microbial Activity

ABSTRACT

Raisin juice is made primarily by sun drying and grinding of sweet black grapes along with the seeds. Because raisins are dehydrated, raisin juice is in turn a highly concentrated substance, with less water than grape juice. Thus, raisin juice is intensely sweet and gains a lot of interest from consumers. Pasteurization of raisin juice is essential to prevent spoilage due to microorganisms and certain enzymes. The continuous flow ohmic heating process is a type of volumetric heating that is very rapid and uniform, and it improves quality retention for liquid foods as opposed to conventional pasteurization. This study attempted to establish kinetic models of raisin juice inoculated with *E. coli* K-12 treated using a continuous flow ohmic heater (50, 60, 70, and 80°C) with different residence times (23 to 208 s). The proposed work involved quantification of product qualities, such as pectin methyl-esterase (PME), antioxidants, total phenolic acids and microbial properties of raisin juice when ohmic heating was applied. The degradation of phenolic acids, antioxidants, inactivation of *E. coli* K-12, and residual pectin methylesterase followed first-order reaction and half-life (t½) kinetic models were used. Results illustrated the amounts of phenolic acids (317.71 mg/100 g) and antioxidants (79.85%) that remained after ohmic heating, which were higher than the respective control data, (311.14 mg/100 g and 75.17%). The residual PME activities after ohmic and conventional heating methods were 53.66% and 58.28%, respectively. The survivals of *E.
coli K-12 in raisin juice after ohmic pasteurization were $0.63 \times 10^3$ CFU/ml, while conventional heating showed $0.97 \times 10^3$ CFU/ml.

Keywords: Raisin juice, Kinetics modelling, Continuous flow ohmic heating, parameters
INTRODUCTION

Fruit juice processing and development are two of the world’s main agro-based businesses. Presently, the market for fresh fruit juice in the USA and other countries has rapidly expanded (Tajchakavit and Ramaswamy, 1997). Concentrated raisin juice is made primarily by sun drying and grinding sweet black grapes along with their seeds. Traditionally, grapes have been dehydrated via open-air or by using convection ovens to make raisins. The drying process to produce raisins concentrates the amount of sugar, making them taste much sweeter. Raisins are naturally stable and resist spoilage due to their low moisture and low acidity.

Consumers typically prefer the sweet taste of raisin juice, making it more marketable than grape juice. The most universal phenolic acids in grapes consist of cinammic acids (coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, and neochlorogenic acid) and benzoic acids (p-hydroxybenzoic acids, protocatechuic acid, vanillic acid, and gallic acid) (Shi et al., 2003). Then most plentiful component of phenols represented in grape juice and grapes that are made into raisins may result in the loss of phenolic compounds and antioxidants (Braksa et al., 2010).

In general, cloudiness of pectin in concentrated juice has been correlated with pectin methyl-esterase activity, which is an undesirable quality in juice (Elez-Martinez et al., 2007). Raisin juice has a short life of a few days in the refrigerator, and shelf life may be reduced due to natural and artificial contamination. Consumer demands for safe and slightly processed foods with high quality properties have inspired better food production (Riahi and Ramaswamy, 2004). However, conventional pasteurization needs longer
heating times, which leads to degradation of the nutritional and organoleptic qualities of the end product (Vicram et al., 2005).

Ohmic heating obtained its name from Ohm’s law, which is another rapid heating technique for food products (Assawarachan, 2010). Ohmic heating or electric resistance heating is based on the channel of electrical current through a food product, which has electrical resistance that eventually converts energy to heat. Immediate heating takes place depending on the current passing through the substance using a variety of voltage and current combinations (Castro et al., 2004). Ohmic heating has many advantages, compared to conventional heating in terms of heating, high energy conversion efficiency, uniformity, and volumetric heating. Furthermore, the non-appearance of a hot surface in ohmic heating reduces fouling problems and thermal damage to the product (Wang and Sastry, 2002; Leizerson and Shimoni, 2005). Therefore, it has gained wide usage for many applications of food processing which involve thermal processes, aseptic processing, thawing, blanching, pasteurization, and sterilization (Assawarachan, 2010). It has been proposed that a continuous flow ohmic heating process not only improves quality retention for raisin juice, but is also a speedy process as opposed to conventional pasteurization. One of the significant issues in food thermal processing is the degradation of nutrients. For that reason, kinetic studies for nutrient degradation are essential to minimize undesired changes and to enhance the quality of food (Patras et al., 2010). Kinetic models have been extensively advanced to estimate vitamin destruction in juice during pasteurization. Hence, the kinetics of enzyme inactivation, the reaction order, the reaction constant and the energy of activation are essential to predict quality losses during thermal processes (Ganjloo et al., 2009).
Dhuique-Mayer et al. (2007) reported that to predict nutrient damage during thermal treatments, the knowledge of the kinetics behavior of these compounds, including the reaction rate as a function of temperature is required. Therefore, several mathematical models have been developed to describe the influence of heat treatment on enzyme activity by either a single deactivation curve at one temperature or the concurrent deactivation of enzymes achieved at different temperatures (Bryjak et al., 2004; Cruz et al., 2006). No data are available for the degradation kinetics modeling of E. coli K-12, antioxidants, PME, and total phenolics during the heat treatment of raisin juice at different temperatures.

This study was intended to explore the potential of a continuous flow ohmic heating technique for raisin juice pasteurization and build up the component kinetic models for degradation of key parameters such as microorganisms, pectin methylesterase, phenolic acids and antioxidants. Microbial and enzymatic inactivation during ohmic treatment have been investigated and compared to the control method.

**MATERIALS AND METHODS**

*Sample preparation*

The raisins with seeds used for this study were purchased from a local market. Raisins (1 kg) were washed in tap water, soaked in water for 15 minutes, mixed with 1L of water and ground before being squeezed with the cheese-cloth. The extraction process was repeated in a triplicate and each kilogram of raisins could produce approximately 2-3 L, of concentrated juice with the total solid mass of 18%. Finally, the juice was then
transferred to a clean feeding tank and kept at 4°C until the pasteurization experiments were performed.

*E. coli* K-12 from frozen stock cultures were obtained from the Food Microbiology Laboratory (University of Hawaii, Honolulu, HI, USA). The strains were incubated at 37°C for 24 h, and refreshed into Tryptic Soy Broth (TSB) to activate it. The initial concentration of *E. coli* K-12 was $10^6$ CFU/ml and was saved for subsequent experiments.

**Experimental set-up**

*Continuous flow ohmic heating*

Continuous flow ohmic heating was performed in a custom made horizontal cylindrical T glass apparatus 2 inches in length and 1 inch in diameter with the inlet and outlet on both sides of the cylinder. Fresh raisin juice was pumped through the insulator tube for ohmic heating until steady state was reached for the preferred condition. Samples were collected from the outlet tube, (Figure 2.1). According to Tulsiyan *et al.* (2009) the product residence time ($R_t$) was calculated using the formula:

$$R_t = \frac{\rho V}{\dot{m}}$$  \hspace{1cm} (2.1)

where $R_t$ is the residence time, $V$ is the volume of juice product and the density of the product ($\rho$) was calculated by measuring the mass per volume of product, and $\dot{m}$ is the average flow rate of the product measured during the run found by collecting and weighing the product and using a stop watch to time the flow.
Inlet and outlet temperatures were uninterruptedly collected using K-type wire thermocouples centrally located within the tubes and attached to the data logger. Finally, the product was refrigerated at 4˚C after cooling and made ready for experiments. The stainless-steel coil (L = 2.5 m and ID = 0.5 m) (Figure 2.2) was used for the conventional bath thermal treatments, and the power pump was adjusted to obtain the same residence time as in the respective continuous flow ohmic heating process.
**Property measurements**

Performed according to Gerard and Roberts (2004). The pH value of raisin juice was determined by using a pH meter (Mettler- T.led, model number 8603, Schwerzenbanch, Switzerland) with a penetration electrode, before and after treatments.

Total soluble solids are estimated as °Brix with a refractometer (American optical corp. Keene NH, Catalog 10419). 1 ml of the sample after vortexing was put on the slide of the refractometer and the concentration was measured. The total soluble solids were then adjusted to 18°Brix by adding water to the original concentration.

**Pectin methylesterase (PME) activity**

The PME activity in raisin juice was measured by the methods given in Igual et al., (2010). 5ml of raisin juice was added to 20 ml of 1% peel citrus pectin dissolution with 0.02 M NaCl that was previously tempered in a 30°C water bath and then mixed together. The solution was adjusted to PH 7.7 (Mettler-Toledo AG 8603 Schwerzenbanch (Switzerland) by using NaOH. Then, 100 µL of 0.05 N NaOH was instantly added. The time required to bring the pH back to 7.7 by the enzyme reaction was recorded. The following equation explains the enzyme activity (A):

\[ A = \frac{V_{NaOH} \times N_{NaOH}}{t_R \times m} \]

where \( V_{NaOH} \) is the volume used in the titration (ml), \( N_{NaOH} \) is the normality of NaOH solution used (m\text{Eq.mL}^{-1}), \( t_R \) is the reaction time (min), and \( m \) is the mass of the sample (g). The following equation is used to find the percentage of residual enzyme activity (\( R_A \)):

\[ R_A = \frac{A_t}{A_o} \times 100 \]
Where \( A_t \) is the enzyme activity of treated samples and \( A_o \) is the enzyme activity of untreated samples.

**Antioxidant capacity**

The antioxidant capacity of raisin juice was assessed by loosing at the free radical-scavenging impact on 1,1-diphenyl-2-picrylhydrazyl (DPPH). Radical scavenging activity was estimated according to the method described by Orizola-Serrano et al. (2007a) with some modifications. 3.9 ml of DPPH (0.030 g/L) in methanol was added to 0.01 ml of raisin juice samples before and after pasteurization. The absorbance was measured with a Shimadzu spectrometer at 515 nm at 0.25 min intervals until the reaction reached steady state. Percentage of DPPH (%DPPH) was calculated by this equation:

\[
\% \text{DPPH} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

**Total phenolic acids**

The amount of total phenolic contents of raisin juice before and after pasteurization was determined using the Folin-Ciocalteu reagent assay described by Velioglu et al. (1998) with some modification. 100 µL of raisin juice was mixed with 0.75 ml of Folin-Ciocalteu reagent and was allowed to let stand at 22°C for 5-8 minutes. Then, 0.75ml of Sodium Carbonate (60 g/L) solution was added to the mixture. Following 2 hours incubation at the same temperature, absorbance was measured at 765 nm using a Shimadzu spectrometer (Japan). Total phenolics content of samples were expressed as mg/100g of gallic acid equivalent (GAE).
Degradation kinetics modeling

For the kinetic study, the rate constant (K-value) for *E. coli* K-12, pectin methylesterase activity, total phenolic acids, and antioxidants, a first order reaction and half-lives (t½) were used. Harbourne *et al.* (2008), and Wang and Xu (2007) reported the thermal degradation of anthocyanins followed a first-order reaction:

\[
\ln(C_t/C_0) = -Kt \tag{2.5}
\]

Where \(C_0\) is the initial concentration, \(C_t\) is the concentration at the t time, and k is the reaction rate constant.

The half-lives (t½) of the total phenolic acids, and antioxidants, were calculated using this equation:

\[
t_{1/2} = \ln[2]/K \tag{2.6}
\]

Statistical Analysis

Data were analyzed using statistical analysis system (SAS) software (version 9.0). Significant effects and interactions of heat treatments, times and temperatures at the confidence level 0.05 on all parameters listed were determined by Duncan’s and Least Squares Means.

RESULTS AND DISCUSSION

Total soluble solid (TSS) of raisin juice product adjusted to 18 °Brix was obtained with a pH of 3.68 according to the procedures described in the methodology. The raisin juice, initially at 18°C was pumped from liquid container to the pasteurizer system with a (Monstat Carter Casette Pump, model number 74-000-12131, USA). Sample was
collected from the outlet at 50 to 80°C in another container (temperature was measured with thermocouples) and the residence time as summarized in Figure 2.3.

Figure 2.3 Temperature –time profile of continuous flow ohmic and conventional heating for raisin juice.
Stumbo (1973) and Tajchakavit et al. (1998) showed that assessment of thermal resistance of microorganisms is typically achieved by exposing the microorganisms to assorted pasteurization treatments and then determining the survivors. The numbers of survivors of *E. coli* K-12 in raisin juice were determined after inoculation (1×10^6 CFU/ml) and after treatments by continuous flow ohmic and conventional heating as investigated in Table 2.1. The survival of *E. coli* K-12 in raisin juice after ohmic heating was lower (0.63×10^3 CFU/ml) than conventional heating at the same conditions (0.97×10^3 CFU/ml), the differences of ohmic heating from conventional heating may be the result of the influence of the electric current, and these results are in accordance with a previous study by Cho et al. (1999) that showed it may contribute to cell inactivation, bringing a non-thermal impact to inactivation.
Table 2.1 Survivals of *E. coli* K-12 in raisin juice after conventional and ohmic heating treatments

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Residence time (s)</th>
<th>Flow rate (ml/s)</th>
<th>Ohmic heating</th>
<th>Conventional heating</th>
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<td>0.78±0.071</td>
<td>1.77±0.071</td>
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<td>0.72±0.028</td>
<td>1.73±0.057</td>
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<td>1.15</td>
<td>0.64±0.042</td>
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<td>0.88</td>
<td>0.43±0.11</td>
<td>0.735±0.021</td>
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<td>0.03±0.042</td>
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<td>0.029±0.009</td>
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<td>0.021±0.001</td>
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<td>0.61</td>
<td>0.001±0.0</td>
<td>0.002±0.0</td>
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</table>
The electrical conductivity of raisin juice is 0.22 S/m at 20°C and increases with increasing temperature. Present results were similar to results found by Handan (2010) where conventional heating was less effective for reducing microorganisms. The impact of treatments by ohmic heating at 50°C on the CFU of *E. coli* K-12 in raisin juice product did not significantly increase as the time increased from 78 to 208 s (0.76, 0.48, 0.08 and 0.051), while there were significant differences of microorganisms from 78 to with 208 s at the same temperature for conventional heating.

**PME, antioxidant capacity, and total phenolic acids**

Table 2.2 presents the residual activity of PME in raisin juice. The reason for studying pectin methyl esterase enzyme inactivation behavior is important because it is responsible for the hydrolysis of the pectin in raisin juice that causes loss of freshness in juice. Concerning the impact of the pasteurization treatments, the residual activity of PME increased (P<0.05) by providing lower temperature or time in continuous flow ohmic heating as opposed to conventional heating. The activation of PME investigated retentions of 53.66% and 58.28% after ohmic and conventional heating treatments, respectively. The retention of PME activity in raisin juice with shorter ohmic and conventional heating exposures times, where residual activity increased by 75.63% and 79.25%, respectively, were close to the results found by Collet *et al.* (2005), where the enzyme inactivation was influenced by holding time.
Table 2.2 Residual pectin methylesterase activities after treatments

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Temperature (°C)</th>
<th>Residence time(s)</th>
<th>Flow rate (ml/s)</th>
<th>Pectin methylesterase (%)</th>
<th>Pectin methylesterase (%)</th>
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<td>70.36 ±1.76</td>
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<td>1.15</td>
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<td>62.14 ±2.49</td>
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<td>59.07 ±3.84</td>
<td>67 ±1.70</td>
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<td>53 ±4.53</td>
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<td>44.66 ±4.34</td>
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<td>52.91 ±0.83</td>
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<td>41.37 ±1.05</td>
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<td>31.92 ±1.03</td>
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<td>53.69 ±1.05</td>
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<td>36.89 ±1.22</td>
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<td>26.21 ±1.04</td>
<td>28.11 ±0.94</td>
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<td>0.61</td>
<td>8.74 ±0.41</td>
<td>15.09 ±0.31</td>
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</table>

Although Villamiel et al., (1998) reported that the percent activation of PME in orange juice by continuous flow microwave and conventional heating was 2.5% and 1.20% at 90°C and 96°C respectively, Leizerson and Shimony (2005) showed that the residual PME activation was 2.0% after ohmic heating treatment at 90°C for 50s. This is
because ohmic heating has more influence on the PME activity in raisin juice unlike conventional heating, where there is high energy conversion efficiency, uniform and volumetric heating.

Table 2.3 shows that the antioxidant capacities of raisin juice were considered as free radical-scavenging capacities in DPPH, where the color changed from purple to yellow. This color change is taken as a signal of the hydrogen donating ability of the tested compounds (Brand-Williams et al., 1995). Antioxidant capacity retention in continuous flow ohmic heating of raisin juice had higher levels (79.85%) compared to continuous flow with conventional heating (75.17%). The Elez-Martinez and Bellos-Martin (2007) reported that 35.4% inhibition of DPPH in orange juice and 39.6% inhibition of DPPH in gazpacho were observed when products were thermally processed (90°C, 60 s), and Igual et al. (2010) illustrated the %DPPH loss was 40% in orange juice after treatments by conventional and microwave heating.
Table 2.3 Retention of antioxidant capacity in raisin juice after conventional and ohmic pasteurization

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Residence time(s)</th>
<th>Flow rate (ml/s)</th>
<th>Retention of antioxidant capacity (%)</th>
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</thead>
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<td>Ohmic heating</td>
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<td>93.45 ±1.50</td>
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<td>90.07 ±1.80</td>
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<td>1.4</td>
<td>88.92 ±0.88</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>1.15</td>
<td>83.47 ±1.02</td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>0.88</td>
<td>82.91 ±1.59</td>
</tr>
<tr>
<td></td>
<td>208</td>
<td>0.61</td>
<td>77.22 ±0.99</td>
</tr>
<tr>
<td>60</td>
<td>23</td>
<td>2.21</td>
<td>89.51 ±2.21</td>
</tr>
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<td>49</td>
<td>2.07</td>
<td>86.94 ±4.07</td>
</tr>
<tr>
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<td>78</td>
<td>1.85</td>
<td>83.13 ±0.54</td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>1.4</td>
<td>80.04 ±1.63</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>1.15</td>
<td>76.95 ±0.12</td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>0.88</td>
<td>75.47 ±0.70</td>
</tr>
<tr>
<td></td>
<td>208</td>
<td>0.61</td>
<td>72.63 ±2.12</td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>2.21</td>
<td>88.32 ±4.80</td>
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<td>83.99 ±1.55</td>
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<td>78</td>
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<td>78 ±3.73</td>
</tr>
<tr>
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<td>107</td>
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<td>77.55 ±0.75</td>
</tr>
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<td>138</td>
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<td>73.45 ±2.38</td>
</tr>
<tr>
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<td>0.88</td>
<td>71.09 ±0.91</td>
</tr>
<tr>
<td></td>
<td>208</td>
<td>0.61</td>
<td>69.71 ±2.36</td>
</tr>
<tr>
<td>80</td>
<td>23</td>
<td>2.21</td>
<td>82.11 ±3.58</td>
</tr>
<tr>
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<td>49</td>
<td>2.07</td>
<td>78.63 ±1.71</td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>1.85</td>
<td>75.56 ±0.78</td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>1.4</td>
<td>73.17 ±1.46</td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>1.15</td>
<td>70.98 ±2.10</td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>0.88</td>
<td>69.45 ±1.35</td>
</tr>
<tr>
<td></td>
<td>208</td>
<td>0.61</td>
<td>64.44 ±2.45</td>
</tr>
</tbody>
</table>

The highest level of antioxidant capacity residence DPPH (95.18%) in raisin juice pasteurized by ohmic heating was found at 50°C after 23 s, therefore there was significant difference in %DPPH after treatments at different temperatures. The influence of
temperature and time caused dramatic effects on the concentration of DPPH, the same results found by Igual et al. (2010) and Deng et al. (2011).

Total phenolic acids of raisin juice were determined by the Folin-Ciocalteu assay, and then the standard curve was made regarding gallic acids. The contents of phenolic acids found after continuous flow ohmic and conventional heating treatments (Table 2.4), were significantly different (317.71 and 311.14 mg/100g) between both methods, as were the combined impact of treatment time and temperature on raisin juice.

The total phenolic acids concentration after 23 s at 50°C was at a maximum concentration of 331.0 mg/100 g after ohmic heating unlike conventional heating at the same conditions, which was found to be 330.62 mg/100 g. This is in good agreement with work done by Ruangsri et al. (2008), where the concentration of total phenolic acids decreased after pasteurization.
Table 2.4 Concentration of total phenolic acids after pasteurizations

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Residence time(s)</th>
<th>Flow rate (ml/s)</th>
<th>Total phenolic acids(mg/100gm)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ohmic heating</td>
<td>Conventional heating</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>23</td>
<td>2.21</td>
<td>331 ±2.29</td>
<td>330.62 ±0.94</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>2.07</td>
<td>330.43 ±0.36</td>
<td>329.76 ±0.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>1.85</td>
<td>330 ±0.87</td>
<td>329.38 ±1.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>1.4</td>
<td>328.94 ±0.99</td>
<td>328.86 ±0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>1.15</td>
<td>327.02 ±3.56</td>
<td>327.69 ±1.97</td>
<td></td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>0.88</td>
<td>326.64 ±4.54</td>
<td>324.08 ±4.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>208</td>
<td>0.61</td>
<td>324 ±22.61</td>
<td>322.31 ±2.71</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>23</td>
<td>2.21</td>
<td>330.05 ±10.04</td>
<td>326.08 ±1.01</td>
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</tr>
<tr>
<td></td>
<td>49</td>
<td>2.07</td>
<td>328.62 ±8.78</td>
<td>323.65 ±2.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>1.85</td>
<td>327 ±3.58</td>
<td>319.56 ±1.15</td>
<td></td>
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<td>107</td>
<td>1.4</td>
<td>322.5 ±3.59</td>
<td>319 ±1.47</td>
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<td></td>
<td>138</td>
<td>1.15</td>
<td>318.67 ±6.65</td>
<td>315 ±2.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>0.88</td>
<td>315.5 ±4.10</td>
<td>314.02 ±0.48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>208</td>
<td>0.61</td>
<td>313.72 ±4.03</td>
<td>311.43 ±2.12</td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>23</td>
<td>2.21</td>
<td>322.06 ±1.27</td>
<td>319.51 ±1.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>49</td>
<td>2.07</td>
<td>318.55 ±0.97</td>
<td>315.22 ±0.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>1.85</td>
<td>315.61 ±1.20</td>
<td>307.09 ±2.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>1.4</td>
<td>314.07 ±4.13</td>
<td>303.97 ±1.94</td>
<td></td>
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<td>138</td>
<td>1.15</td>
<td>308.46 ±4.30</td>
<td>295.03 ±2.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>0.88</td>
<td>305 ±2.54</td>
<td>292 ±3.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>208</td>
<td>0.61</td>
<td>300.09 ±3.26</td>
<td>287.5 ±2.43</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>23</td>
<td>2.21</td>
<td>321.16 ±0.85</td>
<td>317 ±2.89</td>
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</tr>
<tr>
<td></td>
<td>49</td>
<td>2.07</td>
<td>318.43 ±0.67</td>
<td>310.47 ±2.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>78</td>
<td>1.85</td>
<td>314.18 ±1.45</td>
<td>297.9 ±3.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>107</td>
<td>1.4</td>
<td>307.62 ±2.43</td>
<td>294 ±3.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>138</td>
<td>1.15</td>
<td>304.06 ±3.47</td>
<td>286.17 ±1.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>171</td>
<td>0.88</td>
<td>297.01 ±1.54</td>
<td>284.71 ±5.42</td>
<td></td>
</tr>
<tr>
<td></td>
<td>208</td>
<td>0.61</td>
<td>295.5 ±3.48</td>
<td>280 ±2.201</td>
<td></td>
</tr>
</tbody>
</table>
**Kinetics analysis**

The initial microbial concentration, and the electrical conductive have a cooperative role in the inactivation of microorganisms (Machado et al., 2010). Figure 2.4 shows the logarithmic survivals of *E. coli* K-12 via both methods plotted against time. A linear line under a first order reaction resulted for *E. coli* K-12 survivals.

Figure 2.4 Survives of *E. coli* K-12 of samples after pasteurization methods.
This is in agreement with the previous study by Huang et al., (2007). Even when raisin juice was exposed to ohmic and conventional heating separately, there was no significant \textit{E. coli} K-12 inactivation with the change in time from 78 s to 138 s. On the other hand, temperature was also one of the significant factors in degradation kinetic modeling, the log reduction of \textit{E. coli} K-12 increased significantly from 50 to 80°C. Machado \textit{et al.} (2010) also found they could significantly reduce the counts of microorganisms by increasing the temperature. Table 2.5 showed the K-value increased with increasing temperature from 50 to 80°C after pasteurization of raisin juice with ohmic and conventional heating.
Table 2.5 Impact of temperatures on the K-values of survivals E. coli K-12 and residual pectin methyl esterase in the model raisin juice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameter</th>
<th>Temperature (°C)</th>
<th>K-value (s⁻¹)</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ohmic heating</td>
<td>PME</td>
<td>50</td>
<td>0.003</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>0.004</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>0.009</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>0.009</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>E. coli K-12</td>
<td>50</td>
<td>0.015</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>0.009</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>0.008</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>0.019</td>
<td>0.88</td>
</tr>
<tr>
<td>Conventional heating</td>
<td>PME</td>
<td>50</td>
<td>0.003</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>0.003</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>0.007</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>0.008</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>E. coli K-12</td>
<td>50</td>
<td>0.015</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>0.010</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>0.013</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>0.017</td>
<td>0.90</td>
</tr>
</tbody>
</table>

First order reaction kinetics modeling was fitted for pectin methylesterase degradation during both methods of pasteurization at different temperatures and at various times as indicated in Figure 2.5. They were roughly the same as those formerly reported in the study of Gunawan and Barringer (2000); Ahmed et al. (2002) and Tajchakavit and Ramaswamy (1997). The reaction rate constant further confirmed the impact of temperatures.
Figure 2.5 Residual PME activities of samples after pasteurization methods.

Residual PME activity in raisin juice detected by ohmic heating was extremely smaller (53.66%) than those obtained from conventional heating (58.28%). This
designates that ohmic heating is more effective than conventional heating in inactivation PME in raisin juice.

Figures 2.6 and 2.7 shows the results of the first order regression for the model functional to the values of the antioxidants activity and degradation of phenolic acids component of pasteurized in raisin juice.

![Graph showing Ohmic Heating](image)

![Graph showing Conventional Heating](image)

Figure 2.6. Retention of antioxidant capacity (%) of samples after pasteurization methods.
The $K$-values of the residual of phenolic acids and antioxidants in raisin juice were lower after ohmic heating than conventional heating, which designates that the concentration of phenolic acids and percentage of antioxidants after ohmic heating are more stable than those after conventional heating at most of temperatures. On the other hand, the half-live ($t_{1/2}$) values were opposite the $K$-values for the same parameters. Hence, both components are more damaged with time at all temperatures and those
antioxidants and phenolic acids are further rapidly decreased at higher temperature. There have been several researchers that have used the pseudo first-order reaction kinetics for ascorbic acid, and anthocyanins degradation in fruit juice (Cemeroğlu et al., 2003, and Matei et al., 2009). Moreover Table 2.6 shows the first-order reaction rate constant and half-lives of the model raisin juice with regression coefficients ($r^2$) values between ranges 0.90 to 0.99 in both methods. The K-value increased with increasing temperature, unlike the half-lives, ($t^{1/2}$), which decreased with increasing temperature in both parameters for most samples. This is in agreement with previous reports by Wang and Xu (2007) and Harbourne et al. (2008).
Table 2.6 Impact of temperatures on the K-values and half of times \( (t_{\frac{1}{2}}) \) of antioxidant capacity and phenolic acids in the model raisin juice.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameter</th>
<th>Temperature (°C)</th>
<th>( K \times 10^{-3} )</th>
<th>( t_{\frac{1}{2}} ) (sec.)</th>
<th>( r^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ohmic heating</strong></td>
<td>Total phenolic</td>
<td>50</td>
<td>5.322</td>
<td>130.25</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>acids</td>
<td>60</td>
<td>6.19</td>
<td>111.98</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>5.605</td>
<td>123.66</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>6.674</td>
<td>103.86</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>Anti-oxidant</td>
<td>50</td>
<td>5.669</td>
<td>122.27</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>7.121</td>
<td>97.33</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>9.484</td>
<td>73.09</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>8.088</td>
<td>85.71</td>
<td>0.90</td>
</tr>
<tr>
<td><strong>Conventional heating</strong></td>
<td>Total phenolic</td>
<td>50</td>
<td>4.646</td>
<td>149.2</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>acids</td>
<td>60</td>
<td>6.365</td>
<td>108.9</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>6.382</td>
<td>108.61</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>9.047</td>
<td>76.62</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Anti-oxidant</td>
<td>50</td>
<td>6.072</td>
<td>114.16</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>7.24</td>
<td>95.74</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>8.582</td>
<td>80.77</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>10.641</td>
<td>65.14</td>
<td>0.95</td>
</tr>
</tbody>
</table>
CONCLUSIONS

In this study, the influence of kinetic modelling of ohmic heating on the residual antioxidants, PME activity, degradation of phenolic acids, and survivors of *E. coli* K-12 in raisin juice were investigated. First-order kinetic modelling and half-lives were used to predict the safety and quality of raisin juice under the studied investigational conditions. This model gives a result of suitable processing parameters (e.g. treatment, temperature and time) to achieve microbial damage and inactivation of PME, with minimal loss of nutrients in raisin juice after treatments. The number of surviving *E. coli* K-12 decreased with increasing residence time, and correspondingly, there was reduced residual PME activity, antioxidant capacity retention (%DPPH), and phenolic acids. Therefore, continuous flow ohmic heating was more efficient compared to conventional heating at the same conditions to reduce the microorganisms and PME activity, and it minimized the loss of total phenolic acids and antioxidants unlike conventional heating.


Elez-Martinez, P., and Martin-Belloso, O. (2007). Effect of high intensity pulsed electric field processing condition on vitamin C and antioxidant capacity of orange juice and gazpacho, a cold vegetable soup. *Journal of Food Chemistry*, 102, 201-209.


Tajchakavit, S. (1997). Microwave heating of fruit juices: Kinetic of enzyme inactivation /Microbial destruction and evaluation of enhanced thermal effects. A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of philosophy.


CHAPTER III

Numerical Model for Predictive Retention of Antioxidant Activity of Grape Juice Pasteurized with Continuous Flow Ohmic Heating

ABSTRACT

Non-homogenous heat distribution of liquid foods in a continuous flow ohmic heater has a major impact on the degradation of nutrient values and bioactive compounds. Therefore, physical and thermal properties of grape juice were key not only for the designing of ohmic heating to achieve heating uniformity, but also for the prediction of changes in the nutrient values and bioactive compounds of grape juice during ohmic treatment. Simulation of ohmic heating was conducted via finite element analysis using the commercial multiphysics software, COMSOL. The objectives of the presented study were to simulate and validate the percentage of antioxidants levels and heat distribution of grape juice when pasteurized by ohmic heating with different residence times. The result showed the outlet temperature profiles that were experimentally observed were similar to simulation data. The electrical conductivity of the sample increased linearly with increasing temperature. In addition, the experimental observations and simulated data for antioxidant levels after ohmic treatments were consistently close with a correlation coefficient above 0.92. These results indicate that the developed model was reliable and suitable for prediction of nutritional values of grape juice pasteurized with ohmic heating.

Keywords: COMSOL Multiphysics, continuous flow ohmic heating, antioxidant capacity.
INTRODUCTION

Ohmic heating is a novel thermal technology that can be used as an alternative to conventional heating and has significant potential for the pasteurization of foods. Ohmic heating has many advantages such as volumetric heating, uniform and rapid heating for both homogenous and heterogeneous products and it has the ability to achieve desirable quality attributes in foods, unlike other heating methods. It also has a lower principal cost compared to other electric field heating such as microwave and radio frequency heating (Fryer and Li, 1993; Marra et al., 2009). The industry uses ohmic heating technology for liquid and solid-liquid mixture foods product (Piette et al., 2004). Bioactive compounds in foods are impacted by treatment temperature and residence time. It is possible to model and predict the retention of nutrient values of food products after ohmic treatments. In fact, modelling and prediction are the ways to make sure that the experiment gives good results for any methods as well as for design development and control to save time and money. Moreover, Ye et al. (2004) reported that the ability to control the heating rates is the crucial advantage of ohmic heating to ensure homogeneous heating in the product, thus minimally processing foods, improving nutrient retention and organoleptic attributes that are essential to consumers.

Recently, the COMSOL Multiphysics software has been used for various fields of science research. It is involved for all steps in the modeling procedure, defining geometry, specifying physics, meshing, solving, and then post processing the outcomes. (COMSOL AB, 2009). Shim et al. (2010) showed the temperature gradient values for each solid food and carrier medium that were simulated using computational fluid
dynamics (CFD) for ohmic heating were similar to experimental data with a maximum prediction error of 6°C. In addition, Ye et al. (2004) simulated the static ohmic heating process of liquid-particulate mixtures. In another study, in order to present the prediction of the temperature distribution in a static ohmic heating system, a two-dimensional (2D) simulation was made (Fu and Hsieh, 1999).

Many researchers including Tavman et al. (1997); De-Moura et al. (1998); Maroulis (2001); Muramatsu et al. (2010) and Assawarachan (2010) Saravacos and have reported that knowledge of the thermophysical characteristics of foods, such as thermal diffusivity, electrical conductivity and specific heat were needed to design, optimize and show how these characteristics react during processing as a function of temperature in an ohmic heating system. However, the thermophysical characteristics of foods are important for the design of processing devices as well as for the prediction and management of an assortment of modifications that take place in food during thermal processing (Muramatsu et al., 2010). Theoretically, rapid and uniform heating are the major advantages of ohmic processing (Marra et al., 2009; Peter et al., 2011).

Knowledge of three dimensional (3D) temperature distributions of heated products is essential when optimizing electrical heating processes (Knoerzer et al., 2006). The key problem of existing processing technologies during ohmic processing is non-uniformity, in spite of current advancements in volumetric heating to retain product quality by reducing over-processing nutrients and improving nutrient preservation and organoleptic aspects (Ye et al., 2004). However, no studies have reported using COMSOL Multiphysics three-dimensional simulation with continuous flow ohmic heating to predict the concentration of nutrient values.
Thus, the primary objectives of the recent investigation were to validate the percentage of antioxidants levels and heat distribution of grape juice when pasteurized by ohmic heating with different residence times by comparing experimental data with the numerical simulation.

**MATERIALS AND METHODS**

*Sample preparation*

The grapes used for this study were purchased from the local market in Hawaii. Grapes were washed in tap water then crushed using a blender after removing the clusters and the juice was extracted by squeezing it through a cheese-cloth then storing it at 4°C until the experiments were performed (not more than one day).

Total soluble solids are estimated as °Brix with a refractometer (American optical corp. Keene N.H, U.S.A. CATALOG 10419) at 20°C, and were adjusted to a concentration of 15.5°Brix.

*Determination of antioxidant activity*

Radical scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was estimated according to the method described by Orizola-Serrano *et al.* (2007a) with some modification. 3.9 ml of DPPH, (0.030 g/L) in methanol, was added to 0.01 ml of raisin juice samples before and after pasteurization. The absorbance was measured with a Shimadzu spectrometer at 515 nm at 0.25 min intermission until the reaction reached a steady state steady constant. Percentage of DPPH (%DPPH) was calculated by this equation:
%DPPH$ = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \quad (3.1)$

**Experimental set-up**

*Continuous flow ohmic heating-set up*

The continuous flow ohmic heating experiment was performed in a horizontal insulator tube made of Pyrex glass 152.4 mm in length, with a 25.1 mm internal length, 50 mm height, and 6.7 mm inner diameter. The space between inlet and outlet was 72.5 mm. Two titanium electrodes were situated, one at both ends of the cylinder, through a pair of spacers. The gap between electrodes was 92.5 mm (Figure 3.1). For the thermocouple inlets two threaded holes were incorporated to allow insertion of two threaded plastic thermocouple holders at the end of the cell branches. Thermocouples were prepared with K-type thermocouple wires.

![Figure 3.1 Schematic of continuous flow ohmic heating](image)

Fresh grape juice was pumped through the insulator tube for ohmic heating until steady state was reached for the preferred conditions. Samples were collected from the
outlet tube. According to Tulsiyan et al. (2009) the product residence time ($R_t$) was calculated using the formula:

$$R_t = \frac{\rho V}{\dot{m}}$$  \hspace{1cm} (3.2)

The density of the product ($\rho$) was considered by measuring the mass per volume of product, and $\dot{m}$ is the average flow rate of the product measured during the run by collecting and weighing the product in a vessel and using a stop watch to time the flow. Inlet and outlet temperatures were uninterruptedly obtained using K-type wire thermocouples centrally placed within the tubes and attached to the data logger. Finally the product was refrigerated at 4°C after cooling and made ready for experiments.

**Mathematical model for continuous flow ohmic heating system**

Simulation of continuous flow ohmic heating was conducted via finite element analysis using COMSOL Multiphysics Software (COMSOL 4.1, COMSOL, Inc., Palo Alto, CA).

**General heat transfer equation**

The unsteady state heat equation by conduction plus generation expressions are used to explain the heat transfer during ohmic heating system.

$$pC_p \frac{\partial T}{\partial t} + pC_p v \cdot \nabla T = \nabla \cdot (k \nabla T) + q_{ohm}$$  \hspace{1cm} (3.3)

where $T$ is temperature (Kelvin), $t$ is time (s), $V$ is fluid velocity (m/s), $\rho$ is density (Kg/m$^3$), $C_p$ is specific heat (J/Kg. K) and $q$ is the heat (W/m$^3$) generated by ohmic heating.
$q_{ohm}$ is ohmic heating power source, and it is calculated from electrical conductivity ($\sigma$) and the voltage gradient ($\nabla V$) (Shim et al., 2010).

$$q_{ohm} = \sigma (T) |\nabla V|^2$$

(3.4)

Laplace’s equation which defines the electric field distribution in an ohmic conductor is given by (De-Alwis and Fryer, 1990):

$$\nabla [\sigma(T) \cdot \nabla V] = 0$$

(3.5)

**Turbulent flow equation**

Incompressible turbulent flow (k-$\varepsilon$ model) is governed by the Reynolds-Averaged Navier-Stockes (RANS) equations based on approximate time-averaged method.

$$\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} = -\frac{1}{\rho} \nabla p + \nabla \left( (\mathbf{v} + \mathbf{v}_T) [\nabla \mathbf{u} + \nabla \mathbf{u}^T] \right)$$

(3.6)

where, $\nabla \cdot \mathbf{u} = 0$, $\mathbf{u}$ is the velocity, $t$ is the time, $\rho$ is the density, $\mu$ is the dynamic viscosity, $p$ is the pressure and $\mu_T$ is the turbulent viscosity. Table 3.1 shows that the kinematic eddy viscosity is related with two dependents variables: the turbulent kinetic energy, $K$, and the distribution rate of turbulent energy, $\varepsilon$.

$$\gamma_T = C \mu \frac{K^2}{\varepsilon}$$

(3.7)

$C\mu$ is the constant model parameter and $k$ and $\varepsilon$ are calculated from two additional transport equations as follows:

The transport equation for $k$:

$$\frac{\partial k}{\partial t} + \nabla \cdot \left( k \mathbf{u} - \frac{\gamma_T}{\sigma_k} \nabla k \right) = P_k - \varepsilon$$

(3.8)

$$\frac{\partial \varepsilon}{\partial t} + \nabla \cdot \left( \varepsilon \mathbf{u} - \frac{\gamma_T}{\sigma_\varepsilon} \nabla \varepsilon \right) = \frac{\varepsilon}{k} \left( c_{\varepsilon 1} - c_{\varepsilon 2} \right)$$

(3.9)

$\sigma_k, \sigma_\varepsilon, c_{\varepsilon 1},$ and $c_{\varepsilon 2}$: constant model parameters
\[ P_K = \frac{\nu_T}{k} |\nabla u + \nabla u^T|^2 : \text{production term} \] (3.10)

Table 3.1 Model variables in the above equations

<table>
<thead>
<tr>
<th>Variables</th>
<th>Values</th>
<th>Parameters</th>
<th>Units</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_T )</td>
<td>Turbulent viscosity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \varepsilon = e_p )</td>
<td>Turbulent dissipation energy</td>
<td>( \text{m}^2/\text{s}^3 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K )</td>
<td>Turbulent kinetic energy</td>
<td>( \text{m}^2/\text{s}^2 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_k )</td>
<td>0.5</td>
<td>Tuning parameter</td>
<td>Crosswind diffusion of Navier-Stokes equations</td>
<td></td>
</tr>
<tr>
<td>( C_k )</td>
<td>1</td>
<td>Tuning parameter</td>
<td>Crosswind diffusion of Turbulence equations</td>
<td></td>
</tr>
<tr>
<td>( C_{el} )</td>
<td>1.44</td>
<td>Model constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_{e2} )</td>
<td>1.92</td>
<td>Model constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_{\mu} )</td>
<td>0.09</td>
<td>Model constant</td>
<td>Turbulent viscosity model</td>
<td></td>
</tr>
<tr>
<td>( \sigma_k )</td>
<td>1</td>
<td>Model constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \sigma_{\varepsilon} )</td>
<td>1.3</td>
<td>Model constant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_v )</td>
<td>0.42</td>
<td>Model constant</td>
<td>Boundary condition for wall</td>
<td></td>
</tr>
</tbody>
</table>

The k-ε turbulence model relies on several assumptions, the most important of which are that the Reynolds number is high enough and that the turbulence is in equilibrium in boundary layers, which means that production equals dissipation. The assumptions limit the accuracy of the model, because these assumptions are not always true. The spatial extension of recirculation zones, for example, is underestimated by the k-ε turbulence model. Furthermore, in the description of rotating flows, the model often shows poor agreement with experimental data. In most cases, the limited accuracy is a fair trade-off for the amount of computational resources saved compared to more complicated turbulence models. The flow pattern of juice in the inlet tube was governed by laminar flow before the inlet insulator, after that the flow was turbulent.
Transport equation (Mass balance equation)

\[ \frac{\partial C_i}{\partial t} + \nabla \cdot (-D_i \nabla C_i) + u \cdot \nabla C_i = R_i \]  \hspace{1cm} (3.11)

\( D_i \) is the diffusion coefficient and \( R_i \) is the reaction equation of species.

Boundary condition on the wall:

\[ -n \cdot N_i = 0, \quad N_i = -D_i \nabla C_i + u C_i \]  \hspace{1cm} (3.12)

Boundary condition of inlet: \( C_i = C_0 \)

Boundary condition of outlet: \( -n \cdot D_i \nabla C_i = 0 \)

Reaction equation of species

\[ \frac{\partial c}{\partial t} = -kc \]  \hspace{1cm} (3.13)

Reaction rate of the Arrhenius equation:

The temperature dependence of the rate constant and the reaction will occur with a faster rate if we increase temperature because there will be more collisions, thus a faster reaction. In this study the Arrhenius equation was used to predict total antioxidant activity after ohmic heating at 70°C. The Arrhenius equation is as follows:

\[ K = K_0 e^{\frac{-E_a}{RT}} \]  \hspace{1cm} (3.14)

where \( K \) is the thermal degradation rate constant (s\(^{-1}\)), \( K_0 \) is the pre-exponential factor (1/s), \( E_a \) is the activation energy (J/mol), \( R \) is the gas constant (J/mol.k), and \( T \) is the absolute temperature (K).

Thermal boundary

At the walls of the test cell, insulation boundary is given by:

\[ -n \cdot (-k \nabla T) = 0 \]  \hspace{1cm} (3.15)

Where \( n \) is the unit vector perpendicular the surface.
Ground electrode: \( V=0 \)

Electric boundary:

Electric insulation: \(-n \cdot J=0\) \( J\) \: current density \( (A/m^2) \)

Electric potential: \( V=V_0 \) \( (V) \)

Ground: \( V=0 \)

**Turbulent flow wall conditions**

Solution-wall interface: wall functions which are used to model the thin region near the wall with high gradients in the flow variables.

The boundary conditions for the velocities are no-penetration condition:

\[ u \cdot n = 0 \]

The turbulent kinetic energy is subject to a homogeneous Neumann condition:

\[ \nabla k \cdot n = 0 \]

\[ ([\mu + \mu T] (\nabla u + (\nabla u)^T) - \frac{2}{3} \rho kl] n = -\rho \frac{u_k}{\delta w} u_{tang} \quad (3.16) \]

\[ u_{tang} = u - (u \cdot n) n \]

Turbulent flow inlet conditions

\[ U = -U_0 \ n \quad U_0: \text{normal inflow velocity} \quad (3.17) \]

\[ K = \frac{3}{2} (U_0 l_T)^2 \quad l_T: \text{turbulent intensity} \quad (3.18) \]

\[ \epsilon = c_\mu \frac{3}{2} \frac{k^2}{l_T} \quad l_T: \text{turbulent length scale (m)} \quad (3.19) \]

\[ l_T: 0.07D \quad D: \text{pipe diameter} \]

Turbulent flow outlet conditions (pressure and Neumann boundary)
At the outlet, viscous stresses vanish because the normal gradients of all variables are set equal to zero

\[ P = P_o \quad \text{n. } [\nabla u + \nabla^T u] = 0 \] \hspace{1cm} (3.20)

\[ \nabla k \cdot n = 0, \nabla \epsilon \cdot n = 0 \]

**Thermal conductivity**

Thermal conductivity of fruit juice can be expressed as functions of temperature and total solid content (Muramatsu et al., 2003 and 2005). The following equation was utilized to predict the thermal conductivities of fruit juice (Riedel, 1949)

\[ K = (326.58 + 1.0412T - 0.0033T^2) \times (17.30 \times 10^{-4} - 9.34 \times 10^{-6}C) \] \hspace{1cm} (3.21)

where \( K \) is the thermal conductivity (W/m °C), \( T \) is the temperature and \( C \) is the total solid content.

**Thermal diffusivity**

Thermal diffusivity was accounted based on this equation (Martens, 1982)

\[ D = [0.057363 \times W_x + 0.000288 \times (T + 273.15)] \times 10^6 \] \hspace{1cm} (3.22)

where \( D \) is the thermal diffusivity and \( W_x \) is the sample moisture (in a decimal form).

**Electric conductivity**

Electric conductivity of grape juice at different temperatures is measured by using a micro conductivity meter (OMEGA, CDH-287).
RESULTS and DISCUSSION

Total soluble solid (TSS) of grape juice product was adjusted to 15.5˚Brix. For each temperature in continuous flow ohmic treatments, 1000 ml samples were used and their temperatures were measured with thermocouple. Temperatures measured at the outlet ranged from 50 to 80˚C and 20˚C at the inlet. Exposure times were altered by changing the pump flow rate between 8-40 rpm, and the residence time (25 to 215 s) was measured as summarized in Figure 3.2.

Figure 3.2 Temperature-time profile continuous flow ohmic heating for grape juice.

Accurate physical and thermal properties are crucial to achieve unfailing results with experimental values as well as for the food properties during thermal processing. In this study, references and equations were used to obtain physical and thermal properties of grape juice and used in the simulation Table 3.2.
Table 3.2 Physical and thermal properties of grape juice used in the simulation COMSOL

<table>
<thead>
<tr>
<th>Properties</th>
<th>Equations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic viscosity (pa.s)</td>
<td>$\mu = \text{fluid viscosity/density}$</td>
</tr>
<tr>
<td>Electric conductivity ($\sigma$) (S/m)</td>
<td>$\sigma = a.T + b$</td>
</tr>
<tr>
<td>Heat capacity ($C_p$) (J/kg. k)</td>
<td>$Q = mC_p \Delta T$</td>
</tr>
<tr>
<td>Density ($\rho$) (kg/m$^3$)</td>
<td>$\rho = m/v$</td>
</tr>
<tr>
<td>Electric potential (V)</td>
<td>135</td>
</tr>
<tr>
<td>Fluid velocity (m/s)</td>
<td>$(0.030 - 0.0113$m/s), from $V=\text{flow rate (m}^3$/s)/Area (m$^2$)</td>
</tr>
</tbody>
</table>

Using the recorded data from the experiment including values for the outlet temperatures, residence time, and percentage of antioxidants of grape juice, then residence time, and percentage of antioxidants at different temperatures (50, 60, 70 and 80°C), the reaction rates were calculated. Therefore, pre-exponential factor ($K_0$) was calculated by natural logarithm of reaction rates with one over those temperatures except at 70°C. Hence, the percentage of antioxidants of grape juice after pasteurization by continuous flow ohmic heating at different temperatures was determined experimentally and then 50, 60 and 80°C were used as a model to predict total antioxidant activity after ohmic heating at 70°C for validation models by the Arrhenius equation.

The geometry and mesh mode in a horizontal insulator tube was investigated in Figure 3.3. The model was designed in 3D to provide a better estimation of the treatment of the sample in the cell cavity. In this modelled system, the complete mesh consists of 179,346 elements.
Because the electric conductivity of food components is key in an ohmic heating system, it decreases for some components such as fat, lipid, and alcohols, unlike moisture content, which increases. In Figure 3.4, the electric conductivity of the sample increased linearly with increasing temperature by experimental methods, as was expected and consistent with literature data by Palaniappan and Sastry (1991); Shim et al. (2010), and Muramatsu et al. (2010) observed a linear relationship present between the thermal conductivity and temperature; and Assawarachan (2010) reported the quantity of electric conductivity at the highest voltage was slightly higher than those at lower voltages.
Material processing using ohmic heating is possible due to the electric field distribution within the chamber (Santos et al., 2011). Moreover, Figures 3.5 and 3.6 show the predicted variation of electrical field strength distributions in three and two dimensional simulations between two electrodes by providing an electric potential of 135 V. The Laplace equation is necessary to calculate of electrostatic fields because most of the electric field data is converted to an electrostatic field problem by using high voltage (Anderson, 1977). The maximum value of the electric field was 2,549.3 V/m for the above electrical potentials, and had the highest strength at boundary of the electrodes. However, it is weaker in a void of the electrodes, which was similar to the observation of Buckow et al., (2010).
Figure 3.5 Distribution of electric potential (V) in ohmic heater

Figure 3.6 Distribution of electric field (V/m) in the ohmic heater at 70°C in (a) 3D and (b) 2D
Figure 3.7 Simulated temperature distributions of grape juice at the residence time of 215 sec. and three exit temperatures: (a) 60, (b) 70 and (c) 80°C. * (The numbers in the above color bars are temperature values at Kelvin).

Figure 3.7 investigates the temperature distribution in the 3D-COMSOL multiphysics software. The area to the right closest to the electrode was the hottest location based on the condition that there was observable temperature slope in the tube. The temperature decreased from the right to left because, during the process, the temperature of grape juice is lowest before being pumped through the inlet to the system.
The results are similar Salvi et al. (2011). However, the exit temperature profiles at 70 and 80°C that were experimentally found were similar to simulation data when statistical T-test analysis was used as shown in Figure 3.8. This is consistent with the observation by Fu and Hsieh, (1999). The temperature was higher in the simulation than in the experimental data, partially because there was energy loss to the environment. Assawarachan (2010) previously showed that higher temperatures were obtained under higher voltage.

![Figure 3.8. Exit temperatures of processed grape juice](image)

Basically, retention of antioxidant levels (%) in grape juice is a crucial. In this study, 50, 60 and 80°C were used as a model to predict retention of total antioxidant activity after ohmic heating at 70°C via the Arrhenius equation (Figure 3.9).
Fig 3.9 Simulated antioxidant level (%) at the residence time of 215 sec and exit temperature of 70°C.

Figure 3.10 observes simulated values of antioxidants activity. Experimental observation and simulation data for antioxidant levels after ohmic treatments were consistently close with the correlation coefficient above 0.92 because there is an inverse relationship between temperature and concentration of antioxidants. As previously stated, the temperature distribution in the COMSOL multiphysics simulation was not significantly higher than in experimental data. These results indicate that the simulation was reliable and suitable for grape juice pasteurized with ohmic heating.
Fig 3.10 Correlation coefficients between measured and simulated antioxidant levels (%).
CONCLUSIONS

Based on this study, it can be included that the simulation software can be used to predict total antioxidant activity through Arrhenius equation after ohmic heating. It also allows design of more accurate ohmic heating pasteurization equipment to ensure minimization of the thermal degradation of food nutrient value and sensory properties as well as to save time and money. Comparing the results of the experimental and simulation data shows where heat loss takes place in the system design of the ohmic heating tube. The exit temperature profiles 60, 70 and 80°C that were experimentally found were similar to simulation data within 2-3°C. In addition, the experimental observations and simulated data for antioxidant levels in grape juice after ohmic treatments were consistently close with a correlation coefficient above 0.92.
REFERENCES


CHAPTER IV

Pasteurization of Kava Juice Using a Novel Continuous Flow Microwave Heater

ABSTRACT

Kava drink, a traditional Polynesian beverage, has a short life of three days in refrigeration. Thermal pasteurization is known to destroy smooth mouth-feel associated with have kava drink due to heat sensitive starch particles, kavalactones. This study was aimed to investigate the effect of continuous flow microwave heating on the reduction of microorganisms in kava juice to extend the shelf life. Chemical and microbial properties of treated juice were analyzed using key parameters such as microbial counts, kavalactones, and pectin methylesterase activities. The microbial population was reduced from $3.25 \times 10^6$ CFU/ml on average to $5 \times 10^5$, $8 \times 10^3$, and $4.5 \times 10^2$ CFU/ml at 41.4, 52.3, and 65.2°C respectively. The amounts of kavalactones such as Kavain (K), demethoxyyangonin (DMY), Yangonin (Y) were kept constant or increased after pasteurization at different treatment temperatures. The effect of microwave heating on the pectin methylesterase activities decreased significantly from 80% to 34% by increasing the temperature values from 41.4 to 65.2°C, enhancing juice cloud stability. The developed pasteurization is expected to deliver the adequate lethal effect to the kava juice without major deterioration of food quality.

Keywords: Kava beverage, microwave pasteurization, heat sensitive, kavalactones, pectin methyl esterase.
INTRODUCTION

Kava (Piper methysticum G. Forester) belongs to the family piperaceae and is a tropical shrub that grows commonly throughout the islands of the South Pacific. The botanist Johann Georg Forester named the plant P. Methysticum or intoxicating pepper and gave the first detailed explanation of the plant. Thus, “methysticum” is the Latin dictation of the Greek “methustikos” and it is derived from “methu”, which means “intoxicating drinks” (Singh, 1992). Kava, which has been used as a folklore medicine, may have been the first foundation of income for farmers in Vanuatu less than 3,000 years ago (Bilia et al., 2004).

Kava juice is produced from the roots, rhizomes, and stems of the kava plant. Samples are chopped with tap water and extracted through a cheese-cloth. Kava root has been identified to consist of 43% starch, 20% fiber, 12% water, 3.2% sugars, 3.6% proteins, and 3.2% minerals and up to 20% kavalactones (Lopez-Avila and Yefchak, 2009). Eighteen kavalactones have been identified, and six of the major ingredients consist of a group of structurally associated lipophilic lactone derivatives with an arylethylene-α-pyrene core known as kavalactones (Amaral et al., 2008) such as: Kavain (1.8%), methysticin (1.2%), desmethoxyyaangoninn (1%), Yangonin (1%), dihydrokavain (0.6%), and dihydromethysticin (0.5) (Bilia et al., 2002 and Gautz et al., 2006). Kavalactones are measured to be the active ingredients of kava-kava accountable for the pharmacological activity in humans such as sedative, anxiolytic, anti-inflammatory and analgesic effects. (Martin et al., 2000; Simeoni and Lebot, 2002 and Singh and Singh, 2002). The total kavalactones presented in kava juice can be standard for quality. The relative concentration of each of the six major kavalactones in samples
indicates a chemical profile and is also commonly expressed in qualitative form called a chemotype.

In general, the precipitation of pectins leads to cloudiness in the juice (Laratta et al., 1995). Constant color in juice is an indicator of high quality, and the flavor, texture, and aroma of juice are all partially attributed to cloudiness (Krop and Pilnik, 1974). The complexity of kavalactones is a big concern when pasteurizing kava so that its taste and psychoactive properties are not altered. The kava beverage is made of temperature sensitive component; thus, kavalactones and starches begin to degrade when pasteurized at temperatures above 60°C. Traditional and unpasteurized kava drinks have a shelf life of less than three days with refrigeration at 4°C, which is not commercially accepted.

Pasteurization is a thermal technology used for killing pathogenic and spoilage microorganisms in fruit juices; however, it may destroy the organoleptic, nutritional and physiochemical characteristics of foods (Espachs-Barroso et al., 2003). Safe and minimally processed foods with high quality attributes are essential to consumers, and those traits encourage those in food and academic industries find innovative food techniques (Riahi and Ramaswamy, 2004).

Microwaves are defined as an electromagnetic spectrum in the mixture of infrared and radio frequencies, with a wavelength ranging from 1mm to 1m and functioning at frequencies of 300 MHz to 3GHz (Thostenson and chou, 1999). Continuous flow microwave pasteurization has a great potential as an alternative to the conventional heating process for viscous and pumpable liquid food products. It can contribute to enhancement color, flavor, texture, and nutrient preservation (Giese, 1992), furthermore, complete a very rapid short come-up-time (CUT) process, compared to conventional
pasteurization. Several studies have reported that microwave treatment is very effective against microorganisms and enzymes. (Heddleson and Doores, 1994; Villamiel et al., 1996; and Canumir et al., 2002).

In this study, fresh kava juice was pasteurized using the developed continuous flow microwave pasteurization unit. We hypothesized that microwave would be a great potential to deliver enhanced lethal wave doses and volumetric heat rate to pathogenic microorganisms while reducing heat damage, thus maintaining high quality of kava juice. Volumetric heating indicates that materials can absorb microwave energy directly and within them then convert it to heat. (Tong, 2002). To our knowledge, there have been no reports that describe the effects of microwave pasteurization on microbial reduction of juice products, such as kava juice, of which the components are so fragile in high heat.

The objectives of this study were to develop a pilot scale continuous flow microwave pasteurization system for kava juice and characterize the process parameters for best quality production, this study included exploration of the inactivation of pectin methylesterase, which was responsible for the hydrolysis of pectin and resulted in the loss of fresh juice cloudiness.

**MATERIALS AND METHODS**

*Sample preparation*

Fresh kava root and rhizome samples were obtained from local farm in the Big Island (HI, USA), and washed with tap water using a brush to remove crumbly soil from root surfaces and then chopped. Tap water was added to 250 g of sample and finely blended for 60 seconds. The blended mixture was squeezed using cheese-cloth to obtain juice. 4L
of Kava juice was used for further experiments. The pH value of kava juice was measured using a pH meter (METTLER-TOLEDO AG, model number 8603, Schwerzenbanch, Switzerland) prior to and after treatment.

**Continuous flow microwave pasteurization set-up**

A pilot scale system of continuous flow microwave pasteurization was developed as shown in Figure 4. 1.

![Figure 4.1 A schematic of continuous flow microwave pasteurizer. Ti: inlet, To: outlet, and M1 & M2: magnetrons](image)

A glass coil with a length 4.5 m and internal diameter of 0.7 cm was housed inside the cavity through two holes of (0.6 cm diameter) drilled into the center axis of the lower and upper (Inlet and Outlet) sides of the chamber, and two magnetrons each with 1 KW power were connected to the cavity via waveguide of the microwave system. The kava juice, initially at 20°C, was pumped from a fluid storage tank to the microwave pasteurizer system using a silicone tube (Manostat, model number 7.5-3000017 Tygon,
silicone, USA) and a peristaltic pump with 100 rpm (Manostat Carter Casette Pump, model number 74-000-12131, Thermo Fisher Scientific, USA). Samples were collected in another storage tank from the outlet tube and made ready for chemical and microorganisms analysis after cooling.

**Microbiological analysis**

To enumerate microorganisms in unpasteurized and pasteurized kava juice, samples were tested in triplicate for total plate counts with plate count agar. 1 ml of sample was serially diluted with 0.1% peptone water, and 0.1 ml of sample was spread on (PCA). Plates were then incubated at 37 °C for 48 hours. (Nguyen and Mittal, 2007).

**Pectin methylesterase (PME) activity measurement**

PME activity in kava juice was measured by titrating the liberated carboxyl group at pH 7.7 at 30°C, using method of (Igual et al., 2010). 10 ml of kava juice was added to solution which contains 40 ml of 1% peel citrus pectin dissolution and 0.02 M NaCl that were previously tempered to 30°C in a thermostat bath. The solution was adjusted to pH 7.7 by adding NaOH solution. Then, 100 μL of 0.05 N NaOH was instantly added. The accurate time required to lower the pH back to 7.7 by the enzyme reaction was recorded. The following equation explains the enzyme activity (A).

\[
A = \frac{(V_{NaOH} \times N_{NaOH})}{t_R \times m}
\]  

(4.1)

Where \(V_{NaOH}\) is the volume used in the titration, which \(N_{NaOH}\) is the normality of NaOH solution used (m\(_{Eq}\)m\(_L\)-1), \(t_R\) is the reaction time (min), and \(m\) is the mass of the sample
(g). To find the percentage of residual enzyme activity (RA), the following equation is used

\[
RA = \left( \frac{A_t}{A_o} \right) \times 100
\]

(4.2)

Where \( A_t \) is the enzyme activity of treated samples and \( A_o \) is the enzyme activity of untreated samples.

**Gas Chromatography (GC) conditions for detection of kavalactones**

Gas chromatography analysis was carried out using a Hewlett Packard 5890 gas chromatograph (flame ionization detector, FID) (Palo Alto, CA, USA). DB-5 capillary column (30 m x 0.25 mm, 0.25 µm; J & W Scientific) was prepared. Helium carrier gas was used at flow rate 1 ml/min. The column temperature was increases from 100 °C, raised to 260 °C at a rate of 30°C/min and then held at 260°C for 30 min. The juice sample (10 ml) was weighed and 10 ml methanol solution was added and filtered through a 0.45 µm filter prior to injection of GC to point out residual amounts of kavalactones before and after treatments preparation. All samples (4 µl) were injected in splitless mode using an HP auto-sampler. Fig. 4.2(A) shows gas chromatograms of typical four kavalactone standards (Kavain (K), demethoxyyangonin (DMY), and yangonin (Y), and dihydromethysticin (DHM) found in kava juice. However, DHM was not detected in kava juice (Fig. 4.2(B) because different pre-processing environments and processing conditions might influence the absence of DHM in kava juice (Whitton et al., 2003)
Statistical analysis was carried out using SAS software 9.0 (SAS Institute Inc., NC, USA). Significant effects of microwave treatments on all parameters listed were evaluated by Duncan’s test at level 0.05% and results were expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Effect of microwave power

Impedance matching of microwave cavity

Impedance matching of the microwave heating cavity was essential for improving microwave power conversion and for reducing microwave reflection, which could affect the energy efficiency and also damage the magnetrons. Preliminary impedance analysis was conducted using a custom-made calibration kit as shown in Figure. 4.3(A) by adjusting the stub position on the waveguide until the marker indicating S11 and S12
values were closer to the center of the smith chart, which implies minimum microwave reflection in Figure 4.3 (B).

Figure 4.3 Impedance matching: network analyzer and custom made calibration kit (A) and Smith chart (B).

After the impedance matching of microwave cavity, the energy efficiency of the microwave pasteurizer was calculated using the following equation, and water was used to estimate energy efficiency:

\[
\text{Energy efficiency} \quad (\%) = \frac{mC_p\Delta T}{\text{Measured wattage} (W)} \times 100 \quad (4.3)
\]

Where, \( m \) is flow rate (kg/s), \( C_p \) is the specific heat of water (4.186 kJ/kg°C), and \( \Delta T \) is the temperature difference between inlet and outlet temperatures (°C). It is commonly known that the maximum energy efficiency of microwave heating is close to about 65% experimentally; however, the calculated energy efficiency of fabricated microwave pasteurizer was about 43%. Efforts were made to minimize microwave reflection via impedance matching. However, energy was possibly lost due to the dissipated heat from the magnetrons and/or microwave power reflected or transmitted from the cavity to the
excitation ports. In addition, factors such as heat loss from the coil to the environment also contributed to the overall energy lost.

Outlet temperature values of kava juice treated by various microwave power levels (330W, 440W, and 550W) with adjusted flow rate (255ml/min) were measured as 41.4°C, 52.3°C, and 65.2°C, respectively. Figure 4.4 displays outlet temperatures of kava juice pasteurized by various microwave power levels were applied. The pH-values of kava juice samples at different temperatures were 6.34, 6.33 and 6.31 respectively and slightly lower than the pH-value (6.35) before treatments.

![Temperature vs Time graph](image)

Figure 4.4 Exit temperatures of kava juice pasteurized at various microwave power levels.

**Effect of continuous flow microwave pasteurizer on microorganisms**

The continuous flow microwave pasteurization was reported as an appropriate alternative to the conventional thermal processes because microwave is a volumetric and rapid heating method (Gerard and Roberts, 2004; Clare et al, 2005 and Giuliani et al.,
The number of microbes in unpasteurized kava juice was $3.25 \times 10^6$ CFU/ml on average. Although the surfaces of kava root and rhizome were thoroughly washed microorganisms originating from the soil such as *Pseudomonas fluorescens*, inside kava root could not be easily removed. In Figure 4.5, the number of microorganisms that survived after pasteurization decreased with increasing temperature, and significant differences were observed between microbial counts in kava juice before and after microwave treatments at 52.3, and 65.2°C. There were approximately 0.81, 2.61 and 3.86 log reduction in the microbial accounts when kava juices were treated at 41.4, 52.3, and 65.2°C, respectively.

![Figure 4.5 Log microbial counts in kava juice after microwave pasteurization at 41.4, 52.3, and 65.2°C. Means with different letters are significantly different (p ≤ 0.05).](image)

This finding is consistent with the study of Nikdal *et al.* (1993) reporting that the inactivation of microorganisms in unpasteurized, conventionally pasteurized and
microwave pasteurized orange juice were $2 \times 10^4$, 550, and less than 10 CFU/ml, respectively.

**Kavalactones concentration before and after pasteurization**

The concentrations of K, DMY, and Y among the six major kavalactones in kava juice were evaluated based on the GC calibration curves which were obtained using standard solution at different concentrations. Figure 4.6 shows that the initial concentrations of three kavalactones (K, DMY, and Y) in unpasteurized kava juice were 8.99±1.37, 29.05±1.36, and 39.13±1.71 μg/ml, respectively.

![Figure 4.6 Concentrations of kavalactones in unpasteurized and pasteurized kava juice at different temperatures. Means with different letters are significantly different (p ≤ 0.05).](image)

The concentrations of three kavalactones in kava juice treated at 41.4 and 52.3°C did not change significantly, while the concentrations of K and DMY increased significantly at 65.2°C. Amaral et al. (2008) indicated that conventional thermal pasteurization might
lead to severed composition of total pyrone moieties in foods due to prolonged exposure of heat sensitive compounds to external heat sources, whereas, microwave heating is instant and volumetric, thus, not permitting the reaction time needed for decomposition of key kavalactones.

Figure 4.7 shows that the PME activities detected in kava juice after pasteurization was 83% ± 4.2, 73% ± 1.4, and 34% ± 7.1 at the treatment temperatures of 41.4, 52.3 and 65.2°C, respectively.

Figure 4.7 Pectin methylesterase activities (PME%) in unpasteurized and pasteurized kava juice at different temperatures. Means with different letters are significantly different (p ≤ 0.05)

Nikdel and Mackeellar (1992) found that the range of PME in orange juice treated at temperatures of 70-90°C was 88 to 100%, while Nikdel et al. (1993) found that the range of PME activation was between 98.5-99.5% at 75-97°C when microwave and conventional heating were applied. Igual et al. (2010) reported that the PME inactivates remaining in grapefruit juice after conventional pasteurization was between 82 and 100%.
CONCLUSIONS

In conclusion, when kava juice was pasteurized with continuous flow microwave heating technique, the exit juice temperatures at different power levels at 330, 440, and 550 W were 41.4, 52.3 and 65.2°C, respectively. Max four log reduction of targeted microbes were obtained after microwave pasteurization with the time and temperature combination of 37 sec and 65.2°C. Also there was no indicator to show significant deterioration of juice quality, such as starch gel or clear separation of starch rich compounds after microwave treatments.

Microwave energy shows a great potential as an alternative process for kava juice since the results suggested that the microwave process based on all parameters tested in this study was superior to conventional heating in terms of thermal preservation of heat sensitive liquid foods. The continuous flow microwave pasteurization is expected to benefit not only kava juice industries, but also other dairy and food beverage industries by extension.
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CHAPTER V

Exploring the Use of Ultraviolet Light as an Alternative to Traditional Pasteurization of Apple Juice

ABSTRACT

Apple juice inoculated with *E. coli* K-12 was exposed to pulsed ultraviolet (PUV) and non-pulsed ultraviolet (NPUV) modes as a potential alternative to conventional pasteurization methods. The importance of the time and flow rate parameter can be seen on the impact of PUV and NPUV on microbial reduction in apple juice for both continuous and batch processing. The log reduction counts of microbes increased with increasing flow rate from 11 to 51 ml/min as a continuous system under PUV and NPUV modes, as well as increasing treatment times from 5 to 15 mins as a batch system under the same modes. The results of this study showed the bacterial population was significantly reduced after 11 ml/min and 15 min exposure to UV light in apple juice as continuous and batch systems, which were the highest log counts reduction reached at 3.35 and 0.85, respectively, of the *E. coli* K-12 in pasteurized samples. Additionally, there was no significant difference between pulsed ultraviolet and non-pulsed ultraviolet treatments under continuous and batch processing to reduce the microorganisms under two experimental modes.

Keywords: Ultraviolet light, apple juice
INTRODUCTION

Ensuring food safety, adequate nutrition content and bioavailability free from preservatives and additives are the curiosities of public health and consumer protection, which have been processed in a fashion to minimize the influence on the original food products (Reed and Grivetti, 2000). Thermal pasteurization is a typical method for reducing the number of viable microorganisms in foods, but it may extensively diminish sensory qualities and may cause some adverse effects on the nutritional and/or organoleptic attributes of foods (Elez-Martinez and Martin-Belloso, 2005). UV light irradiation is easy to use and lethal to most gram-positive and gram-negative bacteria at room temperature. It is one of the most promising treatment methods (Geveke, 2005; Gayan et al., 2011). In contrast, Shama (1999), Sastry et al. (2000) and Sharifi-Yazdi and Dargahi (2006) reported gram-negative bacteria are less resistant to UV light than gram-positive, yeast, bacteria spores, moulds and viruses. The application of ultraviolet light can be successfully utilized for the disinfection of spores on polystyrene surfaces and in water and liquid food products (McDonald et al., 2002 and Keyser et al., 2008).

The penetration of UV light into juices is about 1 mm for the absorption of 90% of the light (Guerrero-Beltran and Barbosa-Canovas, 2005). Key factors influencing the penetration of UV light include wavelength, type of fluid, concentration, and UV absorption capacity of the liquid foods (Franz et al., 2009). In addition, based on the wavelength in the electromagnetic spectrum, UV light is further subdivided into four regions: UV-A (315-400nm), UV-B (280-315), UV-C (200-280nm) and vacuum less than 200 nm. Among the four regions of UV that have been defined, vacuum UV is most effective for the destruction of microorganisms because the nucleotides of
microorganisms are able to absorb the photon energy at the wavelength of 254 nm by DNA of microorganisms is very strong (Harm, 1980; Bank et al., 1990; Diffey, 2002 and Keyser et al., 2008).

UV-light is a stream of photons and moves as a wave form. It is released as a gas universally containing xenon and argon or xenon and mercury. Low-pressure mercury, medium-pressure mercury and pulsed xenon are sources of UV. Blatchley and Peel (2001), Bolton (2005), and Lopez-Malo and Palou (2005) have reported the efficiency of ultraviolet light has been correlated of two universal laws of photochemistry. The first factor in creating a chemical reaction is that photons require an adequate amount of energy to break or form bonds. Merely the wavelength that is absorbed by atoms and molecules can be effective in producing photochemical change in the molecules. Chemical changes accrue when enough energy has been absorbed. The second factor, in order to motivate chemical reaction, the photon must absorb energy that is equal to or greater than the weakest bond in the molecules. Because microorganisms are so small, they are only absorbing less than one percent of photons.

Dosage is a crucial part of UV-light. It defines the amount of light energy absorbed by microbial population. Dosage was computed as the product of irradiance or intensity (I) multiplied by time (t). This is represented as (mJ/cm²), which is equal to (mW.sec/cm²) or (µW.sec/cm²) (Said et al., 2009) (Table 5.1). Scientific research confirmed that different microorganisms are inactivated by various intensities of ultraviolet radiation (Cristinel et al., 2011). Universally, it is impossible to select the minimum UV dose needed for disinfection of microorganisms because the UV dose can be changed depending on the initial microbial loads, specific properties of the
microorganisms, target of processes, type of foods and suspension solution (Regli et al., 1991 and Wright et al., 2000).

Table 5.1 Microorganisms inactivated by a certain intensity of the UV-light

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Intensity (mW.sec.cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus anthracis</em></td>
<td>13.7</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>21</td>
</tr>
<tr>
<td><em>Eschericia coli</em></td>
<td>9</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>10.5</td>
</tr>
<tr>
<td><em>Salmonella enteritidis</em></td>
<td>12</td>
</tr>
<tr>
<td><em>salmonella typhi</em></td>
<td>6.4</td>
</tr>
<tr>
<td><em>Shigella dysenteritidis</em></td>
<td>6.6</td>
</tr>
<tr>
<td><em>Tobacco mosaic</em></td>
<td>440</td>
</tr>
</tbody>
</table>

Based on literature by Keyser et al. (2008) a higher dose of ultraviolet radiation was effectively used to kill the microbial loads in orange juice. Since the dosages used did not change the organoleptic properties of the juice unlike clear juice such as apple juice, lower ultraviolet radiation has been used. Moreover, Maneerat et al. (2003) demonstrated that UV exposure does not negatively affect the quality of the product. Ultraviolet light disinfection is estimated because unlike the thermal decontamination process, the essential nutrients, odors and content of bioactive compounds are not significantly changed during treatment (Oms-Oliu et al., 2010; Palgan et al., 2011 and Bandla et al., 2012).
The results of several studies indicate that microorganisms are more resistant to non-pulsed ultraviolet than pulsed ultraviolet (PUV) because the energy is multiplied many fold (Dunn et al., 1995 and McDonald, 2000). The objective of this study was to determine the impact of the pulsed ultraviolet and non-pulsed ultraviolet action conditions as a non-thermal method on the reduction of microbial loads in apple juice under continuous and batch processing conditions.

**MATERIALS AND METHODS**

**Sample preparation**

Clear apple juice was obtained from a local market in Hawaii. *E. coli* K-12 was used in this study in order to achieve information on the response of this bacterial in apple juice to the UV light. The frozen stock culture was received from the Food Microbiology Laboratory (University of Hawaii, Honolulu, HI, USA). The strains were incubated at 37°C for 24h, and refreshed into Tryptic Soy Broth (TSB) to activate it. Then, the culture was inoculated into clear apple juice.

**Experimental design**

**Continuous system**

A double-Ultra-violet germicidal lamp with a pulsed and non-pulsed ultraviolet light with peak emission at 254nm, 120V and intensity of 30 mJ/cm² (Tank Master, Model number, TM16, Hg, lamp contains mercury, USA) was used for this study. The UV lamp had a quartz glass with a 2.0 cm inner diameter and the air gap of 0.5 cm between the UV lamp with quartz glass and a plastic tube (Figure 5.1). The juice was pumped from a fluid
feed tank to the plastic tube chamber 27 cm in length, containing an ultra-violet light. The length of the lamp was 22 cm house a silicone tube (Manostat, model number 7.5-300-017 Tygon, Silicone, USA) and a peristaltic pump with various flow rates, 11-51 ml/min (Manostat Carter Casette Pump, model number 74-000-12131, Thermo Fisher Scientific, USA).

![Schematic of continuous UV-light system](image)

**Figure 5.1 Schematic of continuous UV-light system.**

**Batch system**

2 ml of apple juice was pipetted onto a small plastic Petri dish in a chamber covered with aluminium foil to protect the emission of the UV-light. The air gap between the UV-light and samples was 3 cm. The apple juice was treated by pulsed ultraviolet (PUV) for 3 pulses and by non-pulsed ultraviolet for 5, 10 and 15 minutes, separately (Figure 5.2).
Statistical analysis

Data was analyzed by one-way variance using the ANOVA function of MINITAB. Tukey’s test was used to determine if there was a statistical difference in bacterial reductions among control, PUV and NPUV samples for both systems using a 95% confidence interval.

RESULTS AND DISCUSSION

In this study, UV-light pasteurization was used in two modes with each system: PUV and NPUV with a continuous and batch system for the inactivation of *E. coli* K-12 in apple juice after inoculation. Previous studies have used UV-light as alternative to thermal treatments for the destruction of harmful microbes in either liquid or solid foods, such as bacteria, yeasts, moulds, and viruses (Fine and Gervais, 2004; Torkamani and Niakousari, 2011 and Munoz *et al.*, 2012).

The highest log reduction counts reached were 3.35 and 3.57 after PUV and NPUV pasteurization, respectively, after 11ml/min, while reduction of 3.99 and 4.07 logs were achieved at 51ml/min. This suggests that a lower flow rate resulted in higher reduction. Inactivation of *E. coli* K-12 in all samples at different flow rates were statistically
significant. Otherwise, there were no significant differences between PUV and NPUV on the inactivation of *E. coli* K-12 in apple juice (Figure 5.3). The light intensity, treatment time, light components, the space between the light and sample, type of food, type of microorganism and wavelength are related to the efficiency of UV light on the inactivation of microorganisms.

![Bar graph](image)

**Figure 5.3** Log counts of *E. coli* K-12 at different flow rates under Pulsed ultraviolet (PUV) and non-pulsed ultraviolet (NPUV) modes as a continuous system. Means with different letters are significantly different (p \( \leq 0.05 \)).

The initial log count of *E. coli* K-12 in apple juice after inoculation was 5.99. The influence of time had dramatic effects on the survival of *E. coli* K-12. Figure 5.4 shows there was a significant log reduction of *E. coli* K-12 in apple juice with an increased number of treatment times for both PUV and NPUV, individually.
Figure 5.4 Log counts of Apple juice inoculated with *E. coli* K-12 after pulsed-ultraviolet (PUV) and non-pulsed ultraviolet (NPUV) pasteurization. Means with different letters are significantly different (*p* ≤ 0.05).

The highest log count reduction reached 0.85 and 1.35 from 5.99 of *E. coli* K-12 in samples pasteurized by PUV and NPUV, respectively after 15 min, the same results were found by Parikh (2007) where approximately 5 logs CFU/ml of *L. monocytogenes* population decreased in chilled brines after being exposed to UV light for 15 min. Moreover, Keyser *et al.* (2008) found that apple juice treated by UV-C light at high dosage killed 7.42 log of *E. coli* K-12. The main mechanism of microbial destruction is the photochemical ability of UV-light to breakdown microbial DNA during the structural changes when food absorbs the UV radiation (Takeshita *et al.*, 2003). Therefore, under the current experimental conditions, there were no significant differences between PUV and NPUV for the inactivation of *E. coli* K-12 in apple juice. It was known that because pulsed UV inactivation is capable of producing a great dose-rate light, it may possess adequate amounts to damage the DNA, and also sample temperature increased with an increase in the number of pulses of UV light (Upadhyaya *et al.*, 2004 and Krishnamurthy, 2006). Because the energy accumulated
using PUV is enough to produce strong pulsed and excite them at a short time, it might deliver more lethality than NPUV for decontamination of targeted microbes. The study by Bohrerova et al. (2008) showed that pulsed UV-light was more effective than non-pulsed ultraviolet or continuous wave treatment on bacterial inactivation. However, in a similar study, Upadhyaya et al. (2004) showed that water treated with a pulsed lamp and low-pressure Hg lamp separately showed no significant difference between both methods on the destruction of E. coli in water. On the other hand, McDonald et al. (2002) attempted to inactivate Bacillus subtilis spores on the surfaces of polystyrene with a flash lamp and medium pressure NPUV, but there was no significant difference between them.

Inactivation of E. coli K-12 in apple juice under PUV and NPUV with a batch system was higher than that with a continuous flow ultraviolet system; however, there was still no significant difference between PUV and NPUV treatments. It was presumed that it might be because the light power was not strong enough to distinguish the effect of pulsed from a continuous light, and perhaps the low penetration depth of UV-light compared to the sample volumes was a key limiting factor on the impact of the pulsed waves.
CONCLUSIONS

In this study, PUV and NPUV modes under continuous and batch system were individually applied as an effective technique to pasteurize apple juice that was contaminated with *E. coli* K-12. The results proposed to use UV to optimize the time and flow rates needed to destroy most of the microbes in juice. As expected, shorter treatment times and higher energy densities were necessary to inactivate the same number of microorganisms. In addition, there was no significant difference between the lethal capacities of both PUV and NPUV modes as continuous and batch systems individually.
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Efficacy of ultraviolet light for reducing Escherichia coli O157:H7 in
CHAPTER VI

CONCLUSIONS

Three different techniques were used as alternative to conventional heating for the reduction of microbial contamination of liquid foods. First, the technique of using continuous flow ohmic pasteurization and kinetics of pectin methylesterase for the inactivation of *E. coli* k-12 while maintaining essential nutrients and bioactive compounds of raisin juice was investigated. First-order and half-life models give suitable processing parameters to achieve raisin juice microorganisms’ damage and inactivation of PME with minimal loss of nutrients. Therefore, continuous flow ohmic heating was more efficient compared to conventional heating at the same conditions to reduce the microorganisms and PME activity, and it minimized the loss of total phenolic acids and antioxidants in raisin juice.

To determine heat distribution and the percentage of antioxidants in grape juice after pasteurization by continuous flow ohmic heating at different temperatures experimentally, temperatures of 50, 60 and 80°C were used as a model development to predict the retention of antioxidant values after ohmic heating at 70°C for validation models by the Arrhenius equation. Comparing the results of the experimental and simulation data shows where heat loss takes place in the system design of the ohmic heating tube. The exit temperature profiles at 60, 70 and 80°C that were experimentally found were similar to simulation data within 2-3°C. In addition, the experimental observations and simulated data for antioxidant levels in grape juice after ohmic treatments were consistently close with a correlation coefficient above 0.92.
The Second technique involved microwave heating as a continuous system to reduce microorganisms in kava juice. A maximum four-log reduction of targeted microbes was obtained after microwave pasteurization with the time and temperature combination of 37 s and 65.2°C. Also there was no indicator to show significant deterioration of juice quality, such as starch gel or clear separation of starch-rich compounds after microwave treatments. Microwave energy shows great potential as an alternative process for kava juice since the results suggested that the microwave process based on all parameters tested in this study was superior to conventional heating in terms of thermal preservation of heat sensitive liquid foods.

The third technique considered was pulsed- and non-pulsed ultraviolet light modes as batch and continuous systems used to reduce microorganisms in apple juice. The results proposed use UV to optimize the time and flow rates needed to destroy most of the microbes in apple juice. As expected, shorter treatment times and higher energy densities were necessary to inactivate the same number of microorganisms. In addition, there was no significant difference between the lethal capacities of both PUV and NPUV modes as continuous and batch systems individually.