CHAPTER 5
SUMMARY AND CONCLUSION

A lab-scale continuous ohmic heating (COH) set-up made of Teflon material was developed. The heating behaviour of different fresh fruit juices (pineapple, orange, tomato, cucumber, and lemon) was analysed at varied EFS (25 to 45 V/cm) and flow rates (80 to 120 mL/min). The COH system heated all the fruit juices efficiently. Come-up time (CUT) they were decreased significantly ($P < 0.05$) with an increase in electric field strength. The reason was that the flow of higher electric current at higher EFS brought greater electrical energy within the juice samples, thus heating fruit juice at a faster rate and reducing overall heating time. Also, the CUT period increased with an increase in flow rates at lower EFS of 25 and 30 V/cm, while the effect was non-significant at higher EFS of 40 and 45 V/cm. The results also showed that the heating rate (HR) increased significantly with an increase in EFS. The effect of flow rate on HR was significant ($p < 0.05$) at lower EFS of 25 V/cm, while the change was non-significant ($p > 0.05$) at higher EFS of 40 and 45 V/cm. This may be due to larger voltage gradients that allowed greater current to pass through the sample, causing faster heat generation. Comparing the heating time of different fruit juices to achieve the targeted temperature of 90 °C, the pineapple juice took the maximum while the tomato juice took the least. Thus, tomato was heated faster than other juices, while pineapple juice was slowest among the selected juices. Variations in the CUT period and, therefore, variations in heating rate among different fruit changes were observed because of the fruit juice's intrinsic properties like acid content, electrical conductivity at room temperature, sugar content, consistency/viscosity, and ionic concentrations. Apart from juice's intrinsic properties, the ohmic heating behaviour is also influenced by factors like EFS and the frequency of the electric current. The temperature rise of the fruit juice was higher at higher applied EFS, and the heating rate increased multiple folds when EFS increased from 25 to 45 V/cm at all three flow rates. Statistical analysis showed that HR increased significantly ($p < 0.05$) with an increase in EFS for all the samples and under all three flow rates. These results were also in agreement with the volumetric energy gene-squared which $Q = \sigma \times \nabla V^2$ which interpreted that the amount of generated heat was directly proportional to the current produced by the voltage gradient and the EC of the foodstuff. So, the inherent properties of the food material and electrical conductivity influenced the heating rate. The electrical conductivity of all five fruit juices increased significantly with an increase in temperature and EFS at each flow rate of 80 – 120 mL/min. The present study also showed a linear relationship of electrical conductivity with temperature during COH of cucumber, orange, pineapple, and lemon juice under different EFS and flow rates. On the other hand, the electrical conductivity and temperature relationship of tomato juice during COH was better explained by a second-order polynomial relationship. Since ohmic heating is pure volumetric and direct resistance heating, almost all the energy supplied to the system is used to heat the food samples. Therefore, the COH system was highly energy efficient, with more than 90% efficiency. It was also observed that with an increase in EFS value, the SPC was found to increase and reach a maximum value, and then a further increase in the EFS resulted in the reduction of the SPC. Therefore, the EFS where maximum SPC or energy efficiency was achieved was critical. The critical EFS for tomato and pineapple juice was 40 and 35 V/cm, respectively. On the other hand, the critical EFS for cucumber and lemon juice was 30 to 40 V/cm, depending on the flow rates of the juice during ohmic heating. The critical EFS for orange juice ranged from 35 to 45 V/cm, depending on the flow rate.

Further, the pineapple juice was standardized at varied °Brix/Acid of 18, 22, and 26 and the heating behaviour was analysed at varied EFS (25 to 45 V/cm) and flow rate (80 to 120 mL/min). The study showed that heating performance was significantly affected by changes in °Brix/Acid, juice flow rate, and EFS. At low °Brix/Acid, there was a quick temperature rise, and the increment followed a non-linear path. The degree of non-linearity increased with an increase in the °Brix/Acid ratio of juice. The results also showed that °Brix/Acid, EFS, and flow rate significantly affected the CUT period. The heating rate greatly depends on the acid content of the juice, and it reduced significantly ($p < 0.05$) with an increase in °Brix/Acid while increased significantly ($p < 0.05$) with an increase in EFS. It was observed that the effect of flow rate on the CUT period was high at lower EFS. In some cases, like low EFS, high °Brix/Acid, and flow rate, the temperature of the sample did not reach the desired level, so it is imperative to select optimum treatment conditions. The optimum conditions for 18 °Brix/Acid were observed at an EFS of 35 V/cm, where maximum SPC was observed, while for juice with 22 and 26 °Brix/Acid, the maximum SPC was observed at 45 V/cm. SPC of the COH was decreased significantly with an increase in °Brix/Acid. The EC of the juice decreased significantly ($p < 0.05$) with an increase in the °Brix/Acid. EC decreased when °Brix/Acid of the juice increased from 22 to 26 compared to 18 to 22 at all temperatures. This reduction in EC with an increase in °Brix/Acid was due to a lowering of acid content and a decline of ionic movement that resulted in low current flow across the juice samples. The results showed that the EC increased significantly ($p < 0.05$) with an increased temperature for all °Brix/Acid pineapple juice.

After studying the performance of the heating chamber of the COH set-up, an isothermal holding chamber was developed to study the temperature–time effect on the various quality parameters of the standardized pineapple juice (22 °Brix/Acid). The flow rates were determined for attaining a steady temperature for all the combinations of EFS (30, 35, and 40 V/cm) and temperature (70, 80, and 90 °C). The isothermal holding chamber was successfully developed for each combination of EFS – temperatures for holding juice isothermally for 0, 15, 30, 45, and 60 s.

After the development of the complete set-up of the COH system, standardized pineapple juice was ohmically treated at different EFS (30, 35, and 40 V/cm), temperature (70, 80, and 90 °C), and time (0, 15, 30, 45, and 60 s). The effect of COH treatment on quality parameters (colour and vitamin C), enzyme inactivation (PPO, POD, and bromelain), and microbial inactivation in stnadardized pineapple juice was studied. Significant ($p < 0.05$) reduction in PPO, POD, bromelain, microbial load, and vitamin C content was observed during the CUT period and isothermal treatment at different EFS and temperatures. Time and temperature had a significant ($p < 0.05$) effect on enzyme and microbial inactivation. A minimum residual activity of 31.8 ± 0.8, 17.8 ± 0.4, and 1.2 ± 0.4% was observed for PPO, POD, and bromelain, respectively, at 90 °C, 60 s, and 40 V/cm. It was also observed that the inactivation of the POD enzyme was higher than PPO under each treatment condition, suggesting that the PPO was thermally more resistant than the POD enzyme in the pineapple juice. A maximum microbial load reduction of 4.32 ± 0.03 log CFU/mL was observed at 90 °C when treated for 60 s at 35 V/cm. More than 50% of bromelain was inactivated during the CUT period and almost completely inactivated during isothermal treatment, suggesting the high thermal sensitivity of the enzyme.

The mechanism of enzyme inactivation may be due to the conformational change of the secondary structures in the enzymes from an α-helix to β-sheet, aggregation between the molecules, and distortion of the tertiary structures during thermal or non-thermal treatment. Also, the biochemical reactions might have influenced the presence of an electric field by altering molecular spacing and increasing inter-chain reactions that resulted in the additional inactivation of the enzymes during ohmic treatment. The other reason may be removing the metallic prosthetic groups of the PPO in the presence of an electric field, causing higher enzyme inactivation. The presence of an electric field might have altered the enzyme's surface charge and environment by ionizing solution components, and distributing these ions might have resulted in additional enzyme inactivation during ohmic heating. The mechanism for microbial inactivation was mainly the thermal effect leading to cell membrane destruction during ohmic heating. However, the non-thermal effect due to the electric field also influenced microbial growth inhibition through chemical and mechanical effects. The mechanical effects mainly result in the rupture or disruption of cell membranes through electroporation, which releases inner cellular contents and microbial inhibition. In contrast, the chemical effects form free radicals and ions, resulting in microbial inactivation.

A significant ($p < 0.01$) effect of treatment parameters were also observed on the overall colour change and vitamin C content. The L^* of the COH-treated pineapple juice was significantly ($p < 0.05$) affected by temperature, time, and EFS. The L^* values were slightly increased, indicating that the COH-treated pineapple juice was comparatively lighter than the untreated juice. A maximum of one-fourth of the vitamin C degradation occurred during the CUT period at 90 °C, while 38.2% of vitamin C content was retained after isothermal treatment of 60 s at 90 °C and 40 V/cm. The main reason for vitamin C degradation during ohmic heating was chemical decomposition due to heating temperature and processing time. Also, temperature, oxygen, metal catalysts, pH, enzymes, and light affect its degradation. In contrast, during ohmic heating, the strength of the electric field and frequency below 100 Hz greatly affect the degradation of Vitamin C.

The kinetic modelling of enzymes (PPO, POD, and bromelain), microbial inactivation, and vitamin C degradation of the COH-assisted isothermal treatment of standardized pineapple juice were carried out. PPO and POD inactivation kinetics were examined using first-order, distinct isozymes, Weibull distribution, sigmoidal logistic, and fractional conversion models. Bromelain inactivation and vitamin C degradation kinetics were examined using first-order, Weibull, and logistic models. On the other hand, the microbial inactivation kinetics were examined using first-order, Weibull, and modified Gompertz models. The Weibull distribution model showed the highest fitting accuracy for PPO inactivation ($R^2 > 0.990$; RMSE < 0.0101; $\Delta_i \leq 2$), POD inactivation ($R^2 > 0.980$; RMSE < 0.042; $\Delta_i \leq 2$), bromelain inactivation ($R^2 > 0.990$; RMSE < 0.0293; $\Delta_i \leq 2$), and microbial inactivation ($R^2 > 0.980$; RMSE < 0.0085; $\Delta_i \leq 2$) on the given dataset. On the other hand, the first-order model was best suited for vitamin C degradation kinetic ($R^2 > 0.840$; RMSE < 0.057; $\Delta_i = 0$). The accuracy factor (A_f) and bias factor (B_f) for respective models of PPO, POD, bromelain, microbial inactivation, and vitamin

C degradation were closer to the simulation line (closer to 1), suggesting the accuracy of these models in predicting. The Weibull model's scale factor (δ-values) also suggested higher thermal stability of the PPO enzyme than the POD enzyme because of the complex nature of these enzymes. Also, the shape factor ($n < 1$) suggested the concave nature of the fitted model.

Also, a second-order polynomial model fits well at a significance level of 5.0% with a full factorial design of experiments. ANOVA showed that the model for all the response parameters viz., PPO, POD, bromelain, microbial, and vitamin C was highly significant ($p < 0.001$) with R^2 greater than 0.985 for PPO, POD, bromelain, and microbial inactivation, while for vitamin C, it was 0.956 with low RMSE and residual value. COH-treated standardized pineapple juice's process parameters were optimized using food spoilage enzymes PPO and POD and total microbial load. The best solution was obtained at EFS 40 V/cm with a treatment temperature of 88.86 °C and a time of 59.94 s. The optimized parameters were validated under similar treatment conditions with a less than 5.0% deviation.

Further, the standardized pineapple juice was treated with optimized conditions and a hot water bath under similar conditions. The treated juice samples were stored for two months (60 days) at two different temperatures (4 °C and 25 °C), and a comparative storage study was done. Physico-chemical parameters, enzyme activities, and microbial growth were examined. The physicochemical parameters like pH, TSS, titratable acidity, and electrical conductivity of untreated pineapple juice stored at 25 °C were significantly affected. At the same time, the change was minor in COH and water bath-treated juice samples at both storage temperatures. The total colour changes of untreated pineapple juice attained a maximum value of 5.32 ± 0.15 and 5.42 ± 0.34, respectively, at 4 °C and 25 °C storage temperatures. On the other hand, the COH-treated pineapple juice observed a total colour change of 2.84 ± 0.08 and 3.69 ± 0.08 at 4 °C and 25 °C storage temperatures, respectively, while the water bath-treated juice obtained comparatively higher total colour of 3.30 ± 0.42 and 3.85 ± 0.63, respectively at 4 °C and 25 °C. Slow degradation of enzyme activity (PPO, POD, and bromelain) and vitamin C content was observed during the storage period at both temperatures; however, the degradation was faster at a higher storage temperature of 25 °C than at 4 °C for all the samples. The initial microbial load of untreated, COH-treated, and water bath-treated pineapple juice was 5.71 ± 0.08, 1.56 ± 0.07, and 1.67 ± 0.06 log CFU/mL of juice, respectively. The microbial load increased significantly with the storage period. The microbial load of COH-treated pineapple juice was comparatively lower than the water bath-treated juice samples. However, the increase in the microbial load of COH and HWB-treated pineapple juice was within the acceptable limit for human consumption.

In summary, the COH process is an energy-efficient, quick, and uniform heating of the fruit juice with a negligible thermal gradient within the food samples and a convenient method for processing pineapple juice. The ohmic heating methods would also help to deactivate spoilage enzymes and microorganisms and retain quality parameters. The continuous mode of operation of ohmic heating can be easily aligned with other unit operations like regeneration units and aseptic packaging, which is very much necessary from an industrial point of view.

The result obtained from the present thesis can form the basis for future studies on the design and development of a pilot-scale continuous ohmic heating system for various fruit juice processing and thermal treatment of other pumpable food products. A complete set-up of the COH system with regeneration and aseptic filling units can be designed for continuous aseptic processing of fruits and vegetable juice processing. Further, the different frequencies and waveforms of the electric current can be explored to examine its effect on heating performance and various nutritional parameters of ohmically treated pineapple juice. The electrochemical effect on specific nutrients and toxicological studies of ohmically treated pineapple juice can be further examined to ensure its safety for human consumption.