CHAPTER 7

Summary and Conclusion

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This chapter summarizes the present study with key findings and conclusions from the investigation.

7.1 Summary

Oranges are one of the most important citrus fruits cultivated across the world. Orange juice is a rich source of polyphenols, flavonoids, carotenoids, vitamins, sugars, minerals, amino acids, and antioxidants. Many varieties or cultivars of oranges are available in India, including Malta, Khasi, Wakro, and Kinow, respectively. However, different varieties of oranges possess distinct bioactive compounds, nutritional compositions, aromas, and flavours. Wakro orange has a distinctive mandarin orange cultivar with an excellent source of vital bioactive components, good taste, and flavour. It is cultivated in Arunachal Pradesh, Northeast (NE) India. Another cultivar, Malta, is also grown mainly in Punjab, Uttarakhand, and Haryana, India.

Atmospheric cold plasma (ACP) is an emerging non-thermal technology that is an alternative non-thermal approach and is gaining significant interest day by day. Therefore, the feasibility of ACP treatment in orange juice was investigated. The first objective was to study the effect of ACP treatment on both cultivars' orange (Wakro and Malta) juice and optimize the treatment process conditions. Further investigation was on the kinetic modelling of *Escherichia coli* (*E. coli)* and pectin methylesterase (PME) inactivation in orange (cv. Wakro) juice during ACP treatment. Based on optimized process conditions obtained from objective 1, a storage study was conducted on orange (cv. Wakro) juice at different temperatures. In addition, a lab-scale continuous-type ACP setup was developed, and performance was evaluated using orange (cv. Wakro) juice.

The major findings of the present study are summarized chapter-wise as follows:

Chapter III To standardize the process parameters for atmospheric cold plasma (ACP)-assisted processing of orange juice from different cultivars

The changes in quality parameters and PME inactivation in orange juices from both cultivars (Wakro and Malta) during ACP processing have been investigated. The response surface methodology (RSM) optimized the processing conditions. The ACP treatment on orange (cv. Wakro) juice with voltage (16–24 kV), juice depth (3–5 mm), and treatment time (1–3 min) resulted in predicted optimum conditions of 19 kV voltage, 4.6 mm juice depth, and 2.6 min treatment time. The treatment conditions gave predicted and experimental values of 28.08 mg/100 mL ascorbic acid (AA), 43.80% residual activity (RA) of PME, 77.25% BI of AA, 53.92% DPPH radical scavenging activity, and 50.80 mg GAE/100 mL total phenolic content (TPC), respectively. On the other hand, ACP treatment on orange juice (cv. Malta) with voltage (16–20 kV), juice depth (2–4 mm), and treatment time (2–10 min) resulted in the predicted optimum conditions of 20 kV voltage, 3.3 mm juice depth, and 2 min treatment time, with the predicted values of AA, RA of PME, and BI being 31.54 mg/100 mL, 61.33%, and 79.10%, respectively. ACP treatment on the orange juice of the Wakro cultivar showed maximum PME inactivation of 56.20% at optimized conditions, which was higher than the Malta cultivar (PME inactivation: 28.67%). The AA content and DPPH radical scavenging activity were reduced during ACP treatment.

Chapter-IV To model the inactivation kinetics of enzyme (pectin methylesterase) and microbe (*Escherichia coli*) in orange juice during atmospheric cold plasma processing.

In this study, various mathematical models (first-order, Weibull, n^{th}-order, fractional conversion, and Membre) were applied for kinetics of PME, and *E. coli* inactivation in orange (cv. Wakro) juice and those were investigated. The n^{th}-order model was a best-fit model for PME inactivation ($R^2 > 0.98$; RMSE < 0.012; $\Delta_i = 2$). On the other hand, the Weibull model was best for explaining the characteristics of *E. coli* inactivation kinetics ($R^2 > 0.85$; RMSE < 0.2841; $\Delta_i = 0$ (for 16 and 24 kV) and $\Delta_i = 8.67$ (for 20 kV)).

Chapter-V To study the shelf life of atmospheric cold plasma (ACP) treated orange juice during storage.

This study found that the quality of orange (cv. Wakro) juice was significantly influenced by storage temperature (10 and 25 ºC) and duration (0–9 days for untreated juice and 0–30 days for ACP treated juice). Total soluble solids (TSS), pH, and titratable acidity (TA) in orange (cv. Wakro) juice were observed to be minor changes over 0–30 days. The total colour change (ΔE) in ACP-treated juice was significantly increased at the end of the 30^{th} day with values of 1.95 ± 0.05 and 3.21 ± 0.02 at 10 ºC and 25 ºC. On the other hand, the quality parameters, such as AA, DPPH, and TPC in juice, were decreased at both storage temperatures (10 ºC and 25 ºC) with increasing the storage period. An insignificant variation was observed for total viable counts

(TVC) in ACP-treated juice from 0 to 9 days at 10 ºC. The growth of yeast and mold counts (YMC) in ACP-treated juice significantly increased from 9 to 30 days with values of 3.08 ± 0.05 to 7.06 ± 0.03 log CFU/mL, respectively.

Chapter VI To develop a lab-scale continuous system of atmospheric cold plasma (ACP) for juice processing and its performance evaluation with orange juice.

The study revealed no significant effect on pH, TSS, and TA in orange juice (cv. Wakro) while treated with continuous ACP treatment. AA and DPPH radical scavenging activity decreases with increasing the voltages in continuous ACP treatment. The RA of PME was observed less (29.64 ± 1.47 %) at 28 kV voltage and 50 mL/min, indicating 70.36 % PME inactivation. Electrical conductivity (EC) increased at a low flow rate (50 mL/min) and high voltage (28 kV). The ΔE was slightly changed during continuous treatment. First-order kinetics was successfully fitted with the experimental data of AA, DPPH radical scavenging activity, and TPC.

7.2 Conclusion

- RSM showed good predictability and performance in assessing the effect of ACP treatment in orange (cv. Wakro and Malta) juice.
- ACP treatment significantly affected PME activity, AA, and BI of AA in orange juice. However, the TPC in orange (cv. Wakro) juice increased with a maximum of 51.93 ± 0.26 mg GAE/100 mL under the condition of juice depth: 5 mm, voltage: 20 kV, time: 2 min.
- The n^{th} order and Weibull model was successfully fitted for predicting inactivation kinetics of PME and *E. coli* inactivation during ACP treatment.
- In the kinetic study, the n^{th} order and Weibull model exhibited the highest fitting accuracy for both PME inactivation ($R^2 > 0.98$; RMSE < 0.012) and *E. coli* ($R^2 > 0.85$; RMSE < 0.284).
- The untreated and ACP-treated (optimized condition) orange juice (cv. Wakro) was studied for shelf life at two different temperatures (10 ºC and 25 ºC). The AA and DPPH radical scavenging activity was significantly reduced during storage. The ΔE increased with the increase in temperature and storage time, indicating both significantly affected the juice quality. The ACP-treated orange (cv. Wakro) juice demonstrated a potential benefit over the untreated juice by exhibiting reduced microbial (TVC and YMC) growth during storage and less degradation of TPC compared to the untreated samples.

- A continuous mode of ACP setup was developed to process orange (cv. Wakro) juice, which significantly impacted the quality characteristics of the juice. However, insignificant ($p > 0.05$) effect of cold plasma on the juice's TA, pH, and TSS ensures that the juice retains its natural quality.
- Lab-scale continuous ACP treatment significantly inactivated 70.36 ± 1.47% PME enzyme in orange (cv. Wakro) juice at 28 kV and 50 mL/min flow rate, which may enhance the juice quality by maintaining clarity, ensuring smoothness, and increasing the shelf-life.
- ACP-assisted processing could effectively be used as an alternative non-thermal technology for fruit juice processing.

7.3 Future scope of the present study

Some of the future perspectives of the present study are as follows:

- Toxicological study.
- Determination of plasma reactive species and its concentration.
- Continuous system for plasma treatment.