Chapter 6

Summary and Conclusion

6.1. Summary

- The Box-Behnken design was used to optimize the CE, MAE and UAE process for pectin extraction from Assam lemon peel and evaluate the effect of independent variables on the pectin, yield. In CE, the optimal conditions were extraction temperature at 90°C for 22.69 min and 1:30 (g/mL) solid liquid ratio. In MAE, optimal conditions were exposed power of 600 W for 7 min and 1:30 (g/mL) solid-liquid ratio, while for UAE, optimal conditions were ultrasound amplitude of 99.87% for 42.65 min and 1:30 (g/mL) solid-liquid ratio. On comparing the different extraction techniques, the maximum pectin yield was 32.17% by UAE, while MAE and CE exhibited lower pectin yields of 19.61% and 16.56%, respectively.
- Independent variables such as solid-liquid ratio had a non-significant effect on the yield of pectin extracted from CE, MAE and UAE, while other independent variables had a significant effect on the yield of pectin from all extraction methods. The relative deviation between the experimentally observed pectin yield values and those predicted by RSM at optimal conditions for CE, MAE and UAE of pectin was below 10%, indicating a strong correlation between the experimental and predicted outcomes.
- The extracted pectin under optimal conditions was characterized, and the results showed that extracted pectin from CE, MAE and UAE had DE values of 90.29%, 84.29% and 81.89%, respectively, which is more than 50%, resulting in high methoxy pectin. The FTIR spectra of pectin-rich extract showed the presence of all the functional groups of pectin, and XRD analysis showed relatively higher crystallinity in UAE extracted samples than MAE and CE.
- The standardized pectin extract was used in synthesis of pectin iron complex (PIC) aimed to cread functional ingredient. Extracted pectin was incubated for 180 h with iron chloride hexahydrate (0.90–1.80 mM) to maximise the complexation conditions.
- Experiments were conducted in triplets at the optimum condition for validation and compared with the predicted value predicted by the response surface methodology (RSM). The experimental and predicted value for the optimum condition (1.36 mM) was found to be 2657±23.61 ppm and 2542 ppm, respectively, with a relative deviation of 1.06% showing good agreement between them.

- The DE values for the pectin (51.87%) and PIC (50.35%) exceed 50%, indicating that it falls into the category of HM pectin. The concentration of galacturonic acid was higher than 65% for pectin and PIC however, pectin had higher galacturonic acid content (84.33±1.44%) compared to PIC (67.39±4.92%). The methoxyl group content of pectin and PIC was found to be 2.02±0.01% and 13.52±0.04%, respectively.
- The XRD pattern indicates that the PIC is relatively more amorphous compared to pectin, despite having a higher crystallite grain size. SEM micrographs signify that PIC exhibited more disordered structures or less well-defined crystal lattices along with large grain sizes.
- The rheological investigation found that the material had shear-thinning properties, indicating non-Newtonian flow. The viscoelastic properties indicated a strong gel-like network, which was confirmed by the frequency sweep data. The flow behaviour of pectin/PIC solutions was well represented by the Herschel-Bulkley model. Also, PIC was found to be more thermally stable than pectin. This behaviour of pectin and PIC solutions is required in the food formulations, such as jam and jelly production, and the pharmaceutical industry, for controlled-release drug delivery systems.
- The thermal analysis (DSC and TGA graphs) revealed that PIC had greater thermal stability than pectin, suggesting that PIC can be preferable to pectin when thermal food processing is involved.
- The total iron content of the pectin and PIC is 23 ppm and 2657 ppm, respectively. The iron concentration in pectin is negligible after exposure to *in vitro* conditions. While, after exposure to simulated oral, gastric, and intestinal conditions, the Fe concentration in PIC is 143.978±4.20, 144.235±0.85, and 141.913±4.20 ppm, respectively.
- The average Fe absorption in PIC upon introduction to Caco-2 cells was 77.807±0.697 ppm, representing 2.93% of the total Fe present in PIC, while 54.82% of the overall Fe was introduced to the differentiated cells.
- For developing pineapple leather, the mixture design approach was used to optimize the concentration of independent variables such as pineapple pulp, PIC and sugar and evaluate the effect of independent variables on the DPPH free radical scavenging activity, TPC, browning index and overall sensory score. The independent variables selected for the pineapple leather from the developed PIC were pulp, PIC and sugar with ranges 78.05 – 98.92 g, 0 – 2 g and 0 – 20 g, respectively

- The optimal PL was made using a blend of pineapple pulp (90.59% w/w), PIC (1.83% w/w), sugar (7.58% w/w) and potassium metabisulphite (2000 ppm). The total phenolic content, DPPH, browning index, and overall sensory score of standardized PL is 88.36±2.80 mg GAE/g extract, 81.01±1.43 % RSA, 0.017±0.001, and 8.2±0.14, respectively.
- The optimal PL was packed in LDPE pouches and stored at three different temperatures (4°C, 15°C and 25°C) for 35 days to study its storage stability by analysing its moisture content, pH, water activity, browning index, TPC, DPPH antioxidant activity, texture and microbiological quality every 7th day. It was observed that with an increase in storage duration, the moisture content, water activity, microbial load, and browning index of PL were also increased. While the hardness, TPC, and DPPH activity of PL continuously decreased with an increase in storage time. The results suggest that PL showed adequate stability during 35 days.

6.2. Conclusion

This work aimed to valorise lemon peels by developing a pectin complex and using it in the food system. The goal was to make use of food and agricultural waste and byproducts. Initially, the CE, MAE, and UAE processes for extracting pectin from Assam lemon peel were standardised using the Box-Behnken design, which also assessed the impact of independent factors on the pectin yield. When the various extraction methods were compared, the UAE showed the maximum pectin yield, followed by MAE and CE. The yield of pectin extracted from CE, MAE, and UAE was not significantly impacted by independent variables like the solid-liquid ratio, but it was significantly impacted by time and temperature/power/amplitude for all respective extraction techniques. The standardized pectin from the respective extraction techniques was further characterized and compared using chemical studies and instrumentation methods such as FTIR, XRD and SEM.

After standardizing and characterizing pectin from Assam lemon peels, the pectin complex was synthesized by supplementing iron with the pectin iron complex (PIC) aimed to treat iron deficiency, a global health concern. Extracted pectin underwent incubation for a duration of 180 h with iron chloride hexahydrate at concentrations ranging from 0.90 to 1.80 mM to optimize the conditions for complexation. The optimized PIC was characterized for its chemical (degree of esterification, methoxyl group content and galacturonic acid concentration), rheological, thermal (DSC and TGA), structural (FTIR and XRD) and surface morphological properties. Moreover, Caco-2 cell monolayers and *in-vitro* simulation digestion were used to evaluate the iron bioavailability and absorption of iron in the PIC. Following exposure to *in vitro* conditions, the iron content of pectin was insignificant. Additionally, it was observed that PIC's bio-accessibility was significantly higher than that of extracted pectin. The bioaccessibility of iron released from the selected PIC was determined to be 5.34±0.16%, indicating effective absorption potential. The findings showed that PIC could improve fibre consumption and decrease iron deficiency, which would have a number of positive health effects. Additionally, PIC supernatants and pectin were introduced to Caco-2 cells. However, there was no possibility of iron uptake by the differentiated Caco-2 cells because the intestinal fluid's pectin had a very small amount of iron.

Further, the developed PIC was studied for its application by formulating pineapple fruit leather (PL). To formulate PL, the mixture design was utilized to standardize the ratio of pineapple pulp, PIC, and sugar. Additionally, the impacts of independent variables on the DPPH free radical scavenging activity, TPC, browning index, and overall sensory score were assessed. The optimal PL was prepared with the combination of pineapple pulp (90.59% w/w), PIC (1.83% w/w) and sugar (7.58% w/w). Additionally, the ideal PL was packed and kept at three distinct temperatures (4°C, 15°C, and 25°C) for 35 days to investigate its shelf life. Every seven days, the PL was subjected to microbial studies (standard plate count, total yeast and mold count), water activity, moisture content, browning index, TPC, DPPH antioxidant activity, texture, and moisture content. According to the current study, the PL's moisture content, water activity, microbiological load, and browning index increased as storage duration increased. However, PL's hardness, TPC, and DPPH activity steadily declined as storage time increased. According to the findings, PL demonstrated sufficient stability during 35 days.

6.3. Future scope of work

- To study the form of iron (Fe(III) or Fe(II)) in the PIC reduced by Caco-2 cells.
- *In vivo* study of pectin complex-based fruit leather.

- The effect of packaging materials on the quality attributes of pectin complex-based fruit leather during storage should be further explored to improve product stability under various storage conditions.
- The developed green extraction methods for pectin (MAE and UAE) can be scaled up to pilot-scale studies to assess feasibility under industrial processing conditions in terms of yield, energy input, and process efficiency.
- Certain food applications apart from fruit leather, such as jams, baked goods, or low-calorie formulations, can be further linked to the structural and functional properties (such as gelling ability, emulsifying property, and degree of esterification) of the developed pectin-iron complex.
- Scale up the optimised pectin complex-based pineapple leather formulation from lab to pilot scale to assess consistency, batch reproducibility, and yield.
- The pectin–iron complex can be commercialised as a functional powder supplement, similar to commercial pectin.