CHAPTER 7

CONCLUSION AND FUTURE WORK

Conclusion and future work

7.1. Conclusion

The present research work was carried out to develop and characterize curcumin-enriched blended beverage of defatted coconut milk and pineapple juice. Coconut milk was processed to yield nanocellulose and virgin coconut oil, both of which were useful in the development of curcumin-enriched nanoemulsified beverage. This work aimed to utilize the by-products in a novel way in the development of a functional blended beverage. To select the best blended beverage for curcumin enrichment in terms of nutrient composition, physicochemical, antioxidant and sensory properties, different parameters of blended beverages were analysed. To increase the shelf life of blended beverage, standardization of process conditions for thermal pasteurization and high-pressure homogenization of defatted coconut milk and pineapple juice was done. For incorporating curcumin in the blended beverage, a Pickering nanoemulsion was prepared using nanocellulose as a stabilizer obtained from coconut milk waste residue and the Pickering nanoemulsion was used to encapsulate curcumin. The nanocellulose was synthesized form coconut milk waste residue by acid hydrolysis, ultrasonication, and combined acid hydrolysis and ultrasonication and the process that gave desirable characteristics was ascertained. The ability of the nanocellulose to stabilise the Pickering nanoemulsion and retain the stability of curcumin was assessed. The release properties and bioaccessibility of curcumin from nanoemulsified blended beverage in intestinal phase *in vitro* was investigated. Another study was performed to increase the curcumin content in blended beverage, for which the curcumin-enriched blended beverage was taken as the aqueous phase in emulsion development.

The experimental works have been detailed in Chapters 3-6. The major conclusions drawn from the current thesis work are stated chapter-wise here.

7.1.1. To develop and evaluate defatted coconut milk and pineapple juice-based blended beverage (Chapter 3)

Defatted coconut milk and pineapple juice were blended in different ratios (C20:P80, C30:P70, C40:P60, C50:P50, C60:P40, C30:P70, and C80:P20) and evaluated for different properties.

- Blends of defatted coconut milk and pineapple juice demonstrated strong antioxidant activities and contained good levels of TFC (total flavonoid content) and TPC (total phenolic content). The TPC and TFC of the blended beverages ranged from 28.4 - 36.4 mg GAE/100 ml and 18.3-23.9 mg QE/100 ml, respectively. The blends possessed strong antioxidant properties, with 22.9-52.1% MCC (metal chelation capacity) and 14.9-16.4 mg/100 ml FRAP (ferric reducing antioxidant power assay) and 66.8-88.6±0.03% DPPH (2,2-diphenylpicrylhydrazyl) radical scavenging activity.

- The blended beverages possessed acceptable physicochemical properties.
- The addition of defatted coconut milk and pineapple juice contributed to acceptable juice blends in terms of colour, taste, and aroma by the sensory panellists.
- The blended beverages namely, C60:P40, C50:P50, C40:P60 scored higher for their sensorial properties than the other combinations. The blend C50:P50 scored the highest for overall acceptability.
- Blended beverages were rich in potassium and magnesium. Sodium, calcium, zinc, iron, and manganese were also present.
- The phenolic acids that were identified in blended beverage were gallic acid, chlorogenic acid, caffeic acid, coumaric acid, and catechin.
- Gallic acid was the major phenolic acid in C100 (154.5 µg/ml**)** and chlorogenic acid was the major one in P100 (147.5 µg/ml). The level of gallic acid and chlorogenic acid in C50:P50 was 114.2±0.01 µg/ml and 141.1±0.10 µg/ml, respectively.

7.1.2. To study the effect of pasteurization and high-pressure homogenization on microbial stability during storage of blended beverage (Chapter 4)

The D_{10} values were calculated for different microbes, viz. *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes*, and *Escherichia coli* to achieve 5-log reduction. Peroxidase (POD) residual activity and DPPH activity were determined in samples thermally treated for 60, 70, 80 and 90 °C) for different times (5, 20, 40, 60, and 120 s). The thermally pasteurized blended beverage with temperature 80 °C and time 1.80 min was used for further study based on POD residual activity and DPPH activity. The FCCD design was used to optimize the impact of homogenization pressure and number of passes on microbial count and POD residual activity, based on maximum desirability level of 0.99. The key conclusions are as follows:

- The microbial count of *E. coli* decreased with increasing homogenization pressure and passes. The ANOVA results show that homogenizing pressure and the number of homogenizing passes were found to be significant for reduction of microbial load ($p< 0.0001$) and POD residual activity ($p< 0.0001$). A predicted model for reduction of *E. coli* and POD residual activity was established as a function of the pressure and number of passes. The optimum process parameters for inactivation of *E. coli* and POD were 490 bar pressure and 6 number of passes.

- The addition of guar gum to blended beverages decreased serum separation, according to our study. The blended beverage containing 0.3% guar gum that was pasteurised (3GGCP) demonstrated to have better properties among the beverage samples.
- On the other hand, guar gum and high-pressure homogenization have a detrimental impact on serum separation. When compared to 3GGHPH, the blended beverage 3GGPAS demonstrated lesser serum separation.
- The 3GGPAS sample showed improved results in terms of sensory properties, physicochemical properties, and bioactivity than 3GGHPH.
- The total plate count on day 30 of storage of blended beverages (3GGPAS and 3GGHPH) increased up to 3.9±0.38 log CFU/ml and 2.7±0.22 log CFU/ml, respectively at 25±2 °C and 6.8±0.08 log CFU/ml and 3.8±0.14 log CFU/ml, respectively at 4±2 °C.
- This study revealed that during storage, 3GGPAS had better properties than 3GGHPH in terms of microbial growth, peroxidase activity and antioxidant property during the storage period of 30 days at 25±2 °C and 4±2 °C.

7.1.3. To characterize curcumin-enriched Pickering nanoemulsion stabilized with nanocellulose synthesized from coconut milk waste residue (Chapter 5)

Curcumin was encapsulated in a Pickering nanoemulsion stabilized with nanocellulose synthesized from coconut milk waste residue and the emulsion was incorporated in a blended beverage of defatted coconut milk and pineapple juice to improve the encapsulation of curcumin and to increase the bioaccessibility and stability of curcumin. The virgin coconut oil obtained as a by-product in the processing of coconut milk was used as the oil phase in nanoemulsion development.

- The coconut milk waste residue (CMR) had 58.5% cellulose content, 23.5% hemicelluloses, and 1.3% lignin.
- The nanocellulose (coded as ACU-10) that was synthesized from coconut milk waste residue using low sulphuric acid concentration (42%) and ultrasonication time of 10 min had the smallest particle size of 99.6 nm and PDI value and 0.166. Nanocellulose (ACU-10) was able to form a stable Pickering nanoemulsion because of its small particle size and low PDI.
- Combined treatments of acid and ultrasonication developed higher crystallinity and thermal stability in the nanocelluloses, particularly in ACU-10. HPLC results suggested that nanoemulsion stabilized with ACU-10 was able to retain maximum amount of curcumin.
- The morphology of CMR in the FE-SEM images clearly illustrated the presence of non-fibrous components on its surface. Among all the nanocellulose samples that were obtained

by ultrasonication, acid hydrolysis, and combined ultrasonication and acid hydrolysis, the nanocellulose sample obtained by combined treatments (ACU-10) exhibited uniform distribution of cellulose nanofibers

- Among the various Pickering nanoemulsions that were prepared (PN1, PN2, PN3 and PN4) using ACU-10 nanocellulose the particle size of PN2 with nanocellulose concentration of 0.1% was 259.6 nm and PDI value was 0.284, which was the smallest.
- Curcumin degradation was lowest in the Pickering emulsion stabilized with 0.1% nanocellulose (sample PN2) as compared to other emulsions stabilized with 0.05%, 0.2% and 0.3% nanocellulose (PN1, PN3, PN4) after exposure to different pH (2-8) and temperature (63 °C and 95 °C) levels due to its better stability and lower PDI value. Emulsion stability in PN2 was maintained during storage at room temperature for 30 days.
- The results disclosed that with an increase in the concentration of nanocellulose in the Pickering emulsion the creaming index (CMI%) also increased. PN2 that had 0.1% nanocellulose had 0 CMI% up to day 30 of storage as compared to other Pickering nanoemulsions.

7.1.4. To characterize the blended beverage incorporated with curcumin-enriched Pickering nanoemulsion (Chapter 6A and 6B)

Chapter 6A: The curcumin-enriched Pickering nanoemulsion (PN2) at 10% level containing total curcumin concentration of 0.95 mg/100 ml was added to the blended beverage of defatted coconut milk and pineapple juice and pasteurised. The stability and release property of curcumin were assessed. The key conclusions are as follows:

- Addition of PN2 to blended beverage increased the bioaccessibility of curcumin. Curcumin bioaccessibility that was 38.1% in the *in vitro* gastric phase increased to 51.5% during *in vitro* intestinal phase digestion.
- The stability of curcumin in the nanoemulsion added blended beverage after pasteurization (CP-Cur) was better than the unpasteurized (UNCP-Cur) beverage.
- The morphology of Pickering nanoemulsion incorporated blended beverage showed the presence of agglomerated nanoparticles.
- No significant difference of TSS in the nanoemulsified beverages till day 30 of storage at 25±2 °C occurred,
- The initial pH of the nanoemulsified blended beverage was 3.5 and no significant change occurred on day 80 of storage at 25±2 °C. On the other hand, the pH in control sample significantly increased to 4.1±0.07 on day 80 of storage at 25±2 °C.

- At 4±2 ℃, the pH on day 80 of storage was 3.0±0.2 in CP-Cur and 4.5±0.01 in the Control sample.
- The presence of curcumin in CP-Cur exhibited lower peroxidase residual activity of 5.5-fold as compared to Control with residual activity of 12.0-fold on day 80 of storage at 25±2 ℃. On the other hand, peroxidase residual activity at 4±2 ℃ of CP-Cur and Control on day 80 of storage was 4.3 and 10.6-fold, respectively.
- The pasteurized CP-Cur beverage was microbially safe. The yeast and mould count (YMC) on day 50 and day 80 of storage at 25±2 ℃ and 4±2 ℃, respectively and the total aerobic count (TAC) on day 60 and day 70 at 25±2 ℃ and 4±2 ℃, respectively did not exceed the maximum limit recommended by FSSAI.

Chapter 6B: The blended beverage was employed as the aqueous phase together with Tween 80 and coconut waste nanocellulose to increase the curcumin concentration in the beverage. The blended beverages that were generated were analysed for several properties during storage at 25±2 °C and 4±2 °C. *In vitro* bioaccessibility of curcumin-enriched Pickering nanoemulsified blended beverage was studied.

- PNCP-Cur2 (curcumin-enriched blended beverages with 0.1% nanocellulose and Tween 80) and PNCP-Cur5 (curcumin-enriched blended beverages with 0.1% nanocellulose and 0.2% Tween 80) had particle size of 131.7 nm and 141.1 nm and lower PDI value 0.119 and 0.238, respectively. Their small size and low PDI enabled the nanoemulsified beverages to resist any creaming during 30 days of storage at 4±2 ℃.
- An increase in the concentration of nanocellulose increased particle size, which affected the stability of curcumin-enriched Pickering nanoemulsion blended beverages. The most stable formulation was obtained with 0.1% nanocellulose and 0.1% or 0.2% Tween 80.
- Significantly higher curcumin content was retained in PNCP-Cur2 and PNCP-Cur5 where the drop in curcumin level was 43.6% and 46.8% at 63 ℃, and 51.5% and 51.2% at 95℃ from initial curcumin content 12.6±0.68 mg/100 ml and 12.5±0.62 mg/100 ml, respectively.
- PNCP-Cur2 and PNCP-Cur5 accounted for the exceptional efficacy of curcumin during *in vitro* bioaccessibility of 62.2±0.33% and 61.4±0.67% with 0.1% nanocellulose and 0.1% and 0.2% Tween 80, respectively. The nanocellulose and Tween 80 stabilized curcumin-enriched blended beverage provided good barrier at the oil- in- water interface of the Pickering nanoemulsion.
- After exposure to the oral and stomach phases of *in vitro* digestibility, no change in shape of emulsion droplets in PNCP-Cur2 was observed, whereas the size of droplet size increased during the stomach phase due to flocculation. A change of shape of emulsion droplets in the

intestinal phase was observed in FE-SEM morphology which is desirable for the release of curcumin in intestinal phase.

- At 25±2 °C and 4±2 °C, relative POD activity of PNCP-Cur2 samples was 3.4 and 0.98-fold, respectively. Whereas, in comparison, CP-Cur showed higher residual activity of 5.5 and 4.3-fold on day 80 of storage at 25±2 °C and 4±2 °C, respectively.
- The results revealed that pasteurised beverage, namely, PNCP-Cur2 had higher reduction of TAC and YMC count during storage at 25±2℃ and 4±2℃ temperatures than the control sample that had no curcumin.
- The microbiological quality of curcumin-enriched Pickering nanoemulsified blended beverage was improved due to the increased concentration of curcumin. The observed TAC and YMC were below the maximum limit recommended by FSSAI. The PNCP-Cur2 beverage was microbially safe on day 80 for YMC and day 80 for TAC at 25±2℃ and 4±2℃.
- The antimicrobial activity of PNCP-Cur2 and CP-Cur was reflected in the greater zone of inhibition as compared to blended beverage without curcumin.
- Utilising a blended beverage as the aqueous phase increased the concentration of curcumin incorporation (∼12.63 mg/100ml). In section 6A, only 10% of the curcumin-enriched Pickering nanoemulsion could be added to a blended beverage with a ~0.95 mg/100 ml curcumin content.
- Due to increased concentration of curcumin, antimicrobial property, antioxidant, physicochemical and enzymatic properties were augmented in PNCP-Cur2 as compared to CP-Cur during storage.

7.1.5 Future scope of work

The findings of the research work and insights gained from the study provided with the following areas of research for further consideration:

- *In vivo* study of curcumin-enriched Pickering nanoemulsified blended beverage can be conducted.
- More in-depth studies regarding the bioavailability of curcumin from the nanocellulose are required.
- The curcumin-enriched Pickering nanoemulsion can be incorporated in other food products.
- The effect of coconut milk waste nanocellulose used as a stabilizer in other food products can be explored.
- Effect of storage on the quality of the oil phase in the nanoemulsified beverage can be undertaken.