

## Abstract

The aim of this research was to develop a curcumin-enriched blended beverage from defatted coconut milk and pineapple juice, utilizing defatted coconut milk, a by-product of virgin coconut oil extraction, in a novel way. Coconut milk was blended with pineapple juice to enhance sensory properties and diversify its food applications. To address curcumin instability and poor bioavailability in liquid food systems, a Pickering nanoemulsion stabilized by nanocellulose derived from coconut milk waste was developed. This oil-in-water emulsion, with virgin coconut oil as the oil phase, improved curcumin encapsulation, stability, and bioaccessibility. The resulting nanoemulsion was used to fortify the blended beverage, yielding a functional drink with enhanced curcumin content.

The study followed a systematic approach, identifying research gaps and organizing the work into four objectives, each contributing distinct insights into product development and characterization. **Chapter 3** deals with the study carried out on blending of defatted coconut milk and pineapple juice in different ratios and analysing the blends for biochemical, phytochemical, and sensory properties. Potassium and magnesium were the major minerals present. Total phenolic content and total flavonoid content of blended beverages were 32.4-38.4 mg GAE/100 ml and 23.9-20.5 mg QE/100 ml, respectively. The beverages exhibited high DPPH scavenging activity. RP-HPLC analysis showed the presence of gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid and catechin. The comparison of sensory properties of the blended beverages showed significant difference for color, taste, aroma, and overall acceptability, and the beverage with equal amounts of defatted coconut milk and pineapple juice scored higher than only pineapple juice. **Chapter 4** focuses on optimizing pasteurization, high-pressure homogenization, and guar gum stabilization to improve microbial stability, enzyme inactivation, and physical stability of a blended beverage (C50:P50). Blended beverage pasteurized at 80°C for 1.8 min was found effective in reducing peroxidase (POD) activity while retaining DPPH antioxidant activity. High-pressure homogenization (490 bar, 6 passes) achieved microbial lethality (*E. coli*) and lowered POD activity. To minimize serum separation (SS%), guar gum was added at varying concentrations (0.1–0.3%), with 0.3% (3GGCP) providing the lowest SS% (12.6±0.05%) over 15 days without processing treatment. Pasteurized beverages with guar gum (3GGPAS) outperformed those treated with high-pressure

homogenization (3GGHPH) in terms of serum separation, microbial stability, residual POD activity, and antioxidant properties over 30 days at both room ( $25\pm 2^{\circ}\text{C}$ ) and refrigerated ( $4\pm 2^{\circ}\text{C}$ ) conditions. Control samples without stabilizers showed rapid serum separation ( $23.63\pm 1.4\%$  after 1 hour) and lower storage stability. Pasteurization combined with guar gum stabilization is superior to high-pressure homogenization with guar gum, for enhancing the physical and microbial stability of the blended beverage during storage. **Chapter 5** deals with preparation and stabilization of a curcumin-enriched Pickering nanoemulsion using nanocellulose derived from coconut milk waste residue (CMR). Nanocellulose was processed via acid hydrolysis (38%, 40%, and 42% sulfuric acid; AC1, AC2, AC3), ultrasound treatments (5 and 10 min; UL-5, UL-10), and combined methods (ACU-5, ACU-10). The smallest particle size with superior PDI value (0.166) was achieved using ACU-10. Nanoemulsions (PN1, PN2, PN3, PN4) were stabilized with varying nanocellulose concentrations (0.05–0.3%), with PN2 (0.1% nanocellulose) exhibiting the best stability and functionality. PN2 demonstrated superior curcumin retention across pH levels (2–8), temperatures ( $63^{\circ}\text{C}$ ,  $95^{\circ}\text{C}$ ), and during a 30 days storage period without creaming. Its stability was attributed to its low PDI (0.284), which prevented coalescence despite larger particle size compared to PN1. The optimal stabilization of Pickering nanoemulsions relies on precise nanocellulose concentration, enhancing curcumin stability and minimizing physical separation over time. **Chapter 6** is divided into two sections (6A and 6B). In the study reported in **section 6A**, the curcumin-enriched Pickering nanoemulsion (PN2) at 10% level (containing a total curcumin concentration of  $\sim 0.95$  mg/100 ml) was added to the blended beverage of defatted coconut milk and pineapple juice and then pasteurized ( $80^{\circ}\text{C}$  for 1.80 min). Pasteurization reduced the curcumin content in the curcumin-enriched blended beverage to 0.73 mg/100 ml. The *in vitro* release of PN2 added blended beverage (CP-Cur) in the intestinal phase was 51.5%, which was higher than the release in the stomach phase (38.1%). CP-Cur after pasteurization showed better stability compared to unpasteurized CP-Cur. The microbial count in pasteurized CP-Cur did not exceed the maximum threshold established by the FSSAI for the total aerobic count of pasteurized juice until day 70 and 60 of storage at  $4\pm 2^{\circ}\text{C}$  and  $25\pm 2^{\circ}\text{C}$ , respectively. Yeast and mould count of CP-Cur at  $25\pm 2^{\circ}\text{C}$  and  $4\pm 2^{\circ}\text{C}$  was within the maximum limit of FSSAI until day 50 and 80 of storage. The findings clearly demonstrated that Pickering nanoemulsion is a promising carrier for the encapsulation of curcumin in beverage. **Section 6B** of **Chapter 6** reported the use of a blended beverage of defatted coconut milk and pineapple juice as the aqueous phase for

preparing curcumin-enriched Pickering nanoemulsified beverages, focusing on the effects of nanocellulose concentration on curcumin stability, bioaccessibility, and storage properties. Curcumin-enriched Pickering nanoemulsions were stabilized with varying concentrations of nanocellulose and Tween 80. The formulations PNCP-Cur2 (0.1% nanocellulose and Tween 80) and PNCP-Cur5 (0.1% nanocellulose and 0.2% Tween 80) were evaluated for curcumin bioaccessibility, physicochemical stability, and microbial safety during 35 days of storage at  $25\pm 2^{\circ}\text{C}$  and  $4\pm 2^{\circ}\text{C}$ . Maximum curcumin bioaccessibility (~62%) was observed in PNCP-Cur2 and PNCP-Cur5, which also demonstrated strong stability with low PDI values (0.119 and 0.238) and narrow particle size distributions (131.7 nm and 141.1 nm). PNCP-Cur2 retained the highest curcumin content ( $7.1\pm 0.22$  mg/100 ml at  $63^{\circ}\text{C}$  and  $6.1\pm 0.15$  mg/100 ml at  $95^{\circ}\text{C}$ ) and showed no creaming index (%CMI) during storage. Both formulations resisted microbial growth, with total aerobic counts and yeast/mold counts remaining below FSSAI limits during storage. They were also resistance against *E. coli* and *S. aureus*. The nanoemulsions demonstrated robust stability in gastrointestinal conditions, showcasing the effectiveness of coconut milk waste-derived nanocellulose as a stabilizer and curcumin carrier. According to our findings, oil-in-blended beverage emulsions can be stabilised using coconut milk waste nanocellulose, which also works well as a carrier for curcumin in gastrointestinal tract.

This research work makes significant contributions to the area of blended beverage and curcumin-enriched nanoemulsions. In section 6A, only 10% of the curcumin-enriched Pickering nanoemulsion could be added to a blended beverage that could provide ~0.95 mg/100 ml curcumin content so as to keep Tween 80 concentration within the permissible level. Utilising a blended beverage as the aqueous phase helped to increase the concentration of curcumin incorporation up to ~12.6 mg/100 ml in the nanoemulsion while keeping Tween 80 level within permissible limit. The findings of our research may be useful for further designing of functional food using natural waste products, such as nanocellulose from coconut milk waste.

Keywords: Blended beverage, Pickering nanoemulsion, nanocellulose, bioaccessibility, antimicrobial activity