CHAPTER 6

CHARACTERIZATION OF BLENDED BEVERAGE INCORPORATED WITH CURCUMIN-ENRICHED PICKERING NANOEMULSION

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Characterization of blended beverage incorporated with curcumin-enriched Pickering nanoemulsion

This chapter covers the research work done and the results obtained with respect to Objective 4 of the study. The chapter has been divided into two sections (6A and 6B). First section reports the studies conducted on nanoemulsified beverage wherein specific concentration of curcumin-enriched Pickering nanoemulsion was added to the blended beverage of defatted coconut milk and pineapple juice, pasteurised, and stored at 25±2 ℃ and 4±2 ℃. In the second section, the blended beverage was employed as the aqueous phase together with Tween 80 and coconut waste nanocellulose to boost the curcumin concentration in the drink. This section presents the data on the characterization of the blended beverage that were generated during storage at 25±2 ℃ and 4±2 ℃ and interprets the findings. *In vitro* bioaccessibility of both kind of curcumin nanoemulsified beverages was studied.

Section 6A

6A.1. Introduction

Research has concentrated on creating food-grade colloidal systems that can encapsulate functional food constituents such as antimicrobials, flavours, food colourings, micronutrients, and nutraceuticals (Joung et al., 2016). The creation of functional foods that promote health can be achieved by incorporating bioactive components into food systems (Tapal and Tiku, 2012). Pickering emulsions are used for the encapsulation of bioactive compounds like curcumin and carotenoids, and essential oils like marjoram essential oil, cumin seed oil, etc. (Ngwabebhoh et al., 2018; Almasi et al., 2020; Chutia and Mahanta, 2021; Foo et al., 2022). A controlled release of soluble and insoluble bioactive molecules from a Pickering emulsion is desirable. Because of their biocompatibility, lack of cytotoxicity, and ability to degrade, solid particles from natural sources have been utilised as stabilisers in Pickering emulsion systems over time (Erdagi et al., 2020). Bioactive compounds can be more easily incorporated into food and beverage systems with the help of the micro- and nanoencapsulation, which also helps to disguise the colour and negative effects on sensory qualities (Kumar et al., 2016).

In India and other Asian countries, turmeric (*Curcuma longa*) is used as a food spice or colouring ingredient. Curcumin, also known as diferuloylmethane, is a naturally occurring hydrophobic yellow pigment that is derived from turmeric (Goel et al.,2008). Curcumin is well-known for its range of biological properties, including antioxidant, antimicrobial, and

antiproliferative properties (Tao et al., 2019). A stable curcumin nanoemulsion was prepared by Joung et al. (2016) using different ratios of oil, surfactant, and water; the impact of these ratios on stability was then investigated and authors reported that the nanoemulsion was physically stable for 30 days. Curcumin nanoemulsion was further added to the drinking milk system and was found to control lipid oxidation. Kumar et al. (2016) reported that maximum dosage of 8 g/day of curcumin used in clinical trials showed limited toxicity.

Whether a blended beverage of defatted coconut milk and pineapple juice can be used as a liquid medium to develop a stable nanoemulsified beverage enriched in curcumin has not been reported. It is also not known whether such a nanoemulsified beverage can be stable and deliver curcumin in the intestinal phase of in vitro digestibility is also not reported. Our aim of this work was to add the most stable Pickering nanoemulsion (PN2) (reported in chapter 5, containing curcumin, virgin coconut oil, Tween 80 and nanocellulose) into the blended beverage of defatted coconut milk and pineapple juice so that the beverage becomes a source of stable curcumin. This curcumin-enriched blended beverage was characterised for morphology, curcumin storage stability, microbial load, physicochemical properties, and *in vitro* bioaccessibility.

6A.2. Materials and methods

Fresh and mature coconuts (10–12 months) and ripe pineapples were purchased from the local market in Tezpur. Standards were purchased from Sigma-Aldrich, TCI, Merck, Himedia or SRL. Standard curcumin of ≥98% purity was used. Chemicals were purchased from Merck (Mumbai, India), Sisco Research Laboratory (Mumbai, India), and HiMedia (Mumbai, India). Solvents used for separation and identification were of HPLC grade.

6A.2.1 Pickering nanoemulsion preparation

The process for the synthesis of Pickering nanoemulsion (PN2) is described in section 5.2.5. of chapter 5.

6A.2.2 Development of nanoemulsified blended beverage

Pineapple was peeled, and juice was extracted using a mixer grinder. The pulp was pressed through a muslin cloth for complete extraction of juice using the method of Zheng and Lu (2011). Coconut milk was extracted by scraping the coconut with the help of a tabletop coconut scraper (Wise WCS001, India). The grated coconut was mixed with lukewarm water (2:1 ratio) and pressed through muslin cloth to extract and separate the milk using the method of Tangsuphoom and Coupland (2008). Coconut milk (CM) was defatted by centrifugation

(Eppendorf, model 5430 R, Germany) at 19630 × g for 15 min. Defatted coconut milk coded as C100 was blended with pineapple juice (P100) in equal ratios (C50:P50). Blended beverage was brought to 3.5 pH with citric acid and the TSS was increased to 13°Brix. The blended beverage of defatted coconut milk and pineapple juice was incorporated with PN2 at a concentration of 10% and shaken well. This curcumin-enriched blended beverage sample was pasteurized at 80 ℃ for 1.80 min (pasteurization temperature and time obtained from study reported in chapter 4) and coded as CP-Cur.

6A.2.3. Morphology of Pickering nanoemulsion and curcumin-enriched nanoemulsified blended beverage

Field emission scanning electron microscopy (FESEM) was performed at a voltage up to 10 kV and magnifications of 30,000x and 100,000x. The FESEM (JEOL, model- JSM 7200 F) was used to examine the architecture of freeze dried nanocellulose and Pickering nanoemulsion samples. Sample was placed on the holder using a double-sided cellophane tape and allowed to dry for 24 h. The sample was then exposed to iridium for 15 min.

6A.2.4. RP-HPLC analysis of curcumin-enriched nanoemulsified blended beverage

The method of Lu et al. (2018) was used to determine curcumin content in the blended beverage (CP-Cur). RP-HPLC unit (ThermoFisher Ultimate 3000), C18 column (5µm, 120A, 4.6X 250 mm) and UV-Vis detector were used for the separation of curcumin using mobile phases of 0.1% formic acid in water (A) and acetonitrile (B) with the following gradient conditions: 60% A and 40% B in the beginning decreased to 36% A at 7 min, maintained for 3 min, decreased to 10% A at 15 min, and returned to original ratio at 17 min with column temperature at 35 ℃, flow rate at 1 ml/min and wavelength at 425 nm. Curcumin content was identified by comparing retention time and absorption spectra and mass spectra of Pickering nanoemulsion peaks with reference standards. Six different concentrations (0.1, 0.5, 1, 5, 10, and 15 µg/ml) of curcumin standard were prepared in acetonitrile. The curcumin samples were then quantified from linear regression equations.

6A.2.5. Microbial characterization of curcumin-enriched nanoemulsified blended beverage

The aerobic plate count was determined through serial dilutions using pour plate method on nutrient agar following the method of Pala and Toklucu (2013) with modification.

The plates in duplicate were incubated for 48 h at 30 ℃. The total yeasts and moulds were also counted using the same dilutions and pour plate method on potato dextrose agar at 25 ℃ for five days. The results were expressed as CFU (colony forming units)/ml.

6A.2.6. Peroxidase residual activity in curcumin-enriched nanoemulsified blended beverage

The peroxidase (POD) activity was determined according to the method described by Kunitake et al. (2014). For POD activity, phosphate buffer (pH 5.0), hydrogen peroxide, and alcohol-based guaiacol solution were used and incubated for 15 min at 30°C. Sodium metabisulfite was then used to stop the reaction. In a spectrophotometer (Cary 60 UV-Vis, Agilent), absorbance was measured at 470 nm. Phosphate buffer solution added to the curcumin-enriched blended beverage (which served as a substitute for the reagents) was taken as the blank. The measurement for enzyme activity was in U/ml, where one unit was equal to a variation of 0.001 absorbance per ml per min of sample. The enzyme activity was determined using Eq. 6A.1:

$$\text{Activity (U/ml)} = \frac{\text{Ab (sample)} - \text{Ab (blank)}}{0.001 \times t} \quad \text{(Eq. 6A.1)}$$

where Ab (blank) is the blank absorbance; Ab (sample) is the sample absorbance; and t the sample's reagent incubation period (min).

6A.2.7. DPPH radical scavenging activity of curcumin-enriched nanoemulsified blended beverage

The Saikia et al. (2016) method was utilised to assess the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the sample extracts. In particular, 100 ml of a curcumin-enriched blended beverage was mixed with 1.4 ml of a methanolic solution of DPPH radical ((10^{-4} M). After 30 minutes, the absorbance at 517 nm was measured using a UV-vis Spectrophotometer (Cary 60 UV-Vis, Agilent) against a blank (100 ml methanol in 1.4 ml of DPPH radical solution). Results were reported in terms of the radical scavenging activity of the beverage (Eq.6A.2).

$$\text{Radical scavenging activity (\%)} = \frac{Ac - As}{Ac} \times 100 \quad \text{(Eq. 6A.2)}$$

Where, As is absorbance of sample and Ac is absorbance of control blank.

6A.2.8. Determination of soluble solids

The total soluble solid was determined by method described in section 3.2.2.1 of chapter 3.

6A.2.9. Determination of pH

The pH was measured by method reported in section 3.2.2.2 of chapter 3.

6A.2.10. *In vitro* bioaccessibility of curcumin from curcumin-enriched nanoemulsified blended beverage

The bioaccessibility of ingested curcumin from CP-Cur in gastro-intestinal fluids was determined after *in vitro* digestion process using the method described previously by Zhou et al. (2021) with modifications.

Oral phase: Briefly, 5 ml of the sample were placed into a test tube, and 5 ml of simulated saliva fluid which included 15 mg of mucin and different salts were then added. In order to replicate the oral phase, the material was kept in it for 2 min at 37 °C while being continuously swirled at 100 rpm.

Stomach phase: Following the completion of the oral phase digestion, 10 ml of the oral fluid was collected, and 10 ml of double-distilled water and simulated gastric fluid containing HCl and pepsin (activity 2000 U/ml) were added. After adjusting the pH to 3, the sample was kept in the stomach phase for two hours at 37 °C while being continuously swirled to simulate the gastric phase.

Intestinal phase: After gastric phase digestion, the resulting fluid (20 ml) was collected and then transferred into 100 ml beaker. An equivalent volume of small intestinal fluid containing bile extract (10mM), calcium chloride, and pancreatin (trypsin activity 100 U/ml and lipase activity 2000 U/ml) was added. The pH was adjusted to 7 and kept at 37 °C for 2 h. After completion of the entire process, the aliquot of digested sample of curcumin-enriched beverage was centrifuged at 18000 rpm for 50 min at 4 °C. After centrifugation, the supernatant containing the micelle fraction in which the curcumin had solubilized was collected and mixed with acetonitrile in the ratio of 1:9, vortexed and centrifuged at 4000 rpm for 10 min at 25 °C. The top acetonitrile layer with the solubilized curcumin was collected and analysed using the RP-HPLC (detailed method given in section 6A.2.4).

The bioaccessibility of curcumin was calculated using Eq. 6A.3 below:

$$\text{Bioaccessibility } \% = \frac{\text{Cmicelle}}{\text{Cintial}} \text{X100} \qquad \text{(Eq. 6A.3)}$$

Here, Cmicelle and Cinitial are the curcumin concentrations in the micelle phase (supernatant) after digestion and the initial curcumin concentration in blended beverage before digestion, respectively.

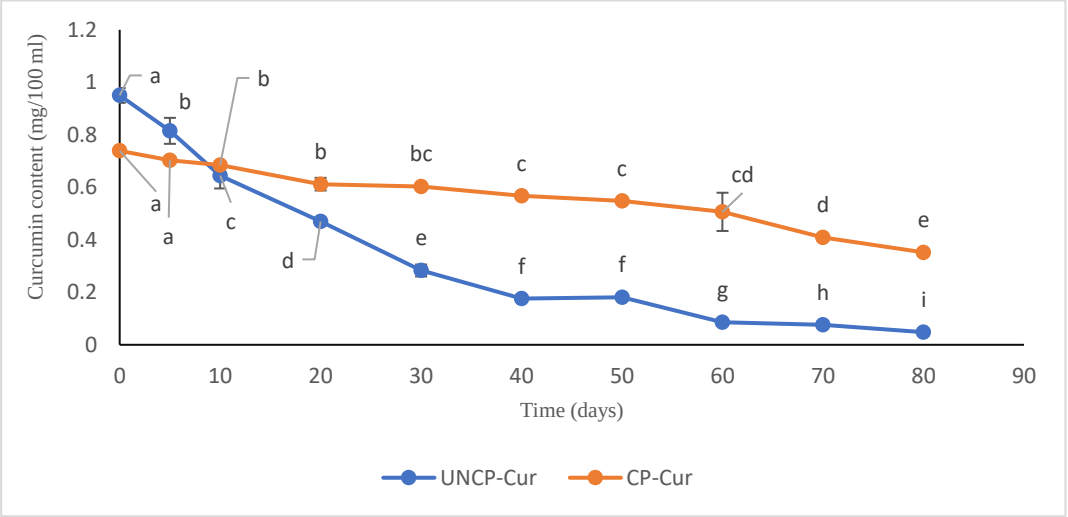
6A.2.11. Statistical analysis

Every component was analysed in triplicates, and the results are given as the mean ± standard deviation of all separate studies. The data analysis was performed using IBM SPSS 20.0 software. The analysis of variance (ANOVA) and the Duncan's multiple range tests were performed to determine if there were significant differences between the values ($p < 0.05$).

6A.3. Results and discussion

6A.3.1. Effect of pasteurization on curcumin stability in curcumin-enriched nanoemulsified blended beverage during storage

As reported in chapter 5, 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 was pasteurised. This nanoemulsified beverage was coded as CP-Cur. As the stability of curcumin in food systems is very low (Aditya et al., 2015), a storage study for 80 days at 25±2 ℃ and 4±2 ℃ was conducted to assess the stability of curcumin in CP-Cur. The changes were compared with non-pasteurized curcumin-enriched nanoemulsified blended beverages (coded as UNCP-Cur). As seen in Fig. 6A.1 (a-b), there were significant changes in curcumin content during the storage period at 25±2 ℃ and 4±2 ℃. The degradation of curcumin in non-pasteurized beverages was greater at room and refrigeration temperatures than CP-Cur. The curcumin content in UNCP-Cur and CP-Cur stored at 25±2 ℃ for 80 days (Fig. 6A.1. a) reduced from 0.95±0.02 mg/100 ml to 0.05±0.01 mg/100 ml and from 0.73±0.004 mg/100 ml to 0.35±0.04 mg/100 ml, respectively. Furthermore, curcumin content at 4±2 ℃ (Fig. 6A.1. b) ranged from 0.94±0.007 mg/100 ml to 0.11±0.01 mg/100 ml in UNCP-Cur and from 0.73±0.04 mg/100 ml to 0.40±0.07 mg/100 ml in CP-Cur. In UNCP-Cur, curcumin concentration decreased by 88.2% at 4±2 ℃ and 94.9% at 25±2 ℃ on day 80 of storage. On the other hand, reduction of curcumin concentration in CP-Cur was 45.2% at 4±2 ° and 51.7% at 25±2 ℃. Thus, curcumin degradation was observed to be less at 4±2 ℃ as than at 25±2 ℃. Aditya et al. (2015) reported that curcumin-loaded emulsions showed 91% and 87% curcumin stability after 15 days at 4 ℃ and 23 ℃, respectively. The number of variables, including temperature, co-excipients, and formulation stability, may have a direct or indirect impact on stability. Baek et al. (2020) reported that the stability of oil-soluble antioxidant compounds like β-carotene in nanoemulsions depends on the composition of the stabilizer used and the surrounding environmental factors, such as exposure to UV radiation and high temperatures.



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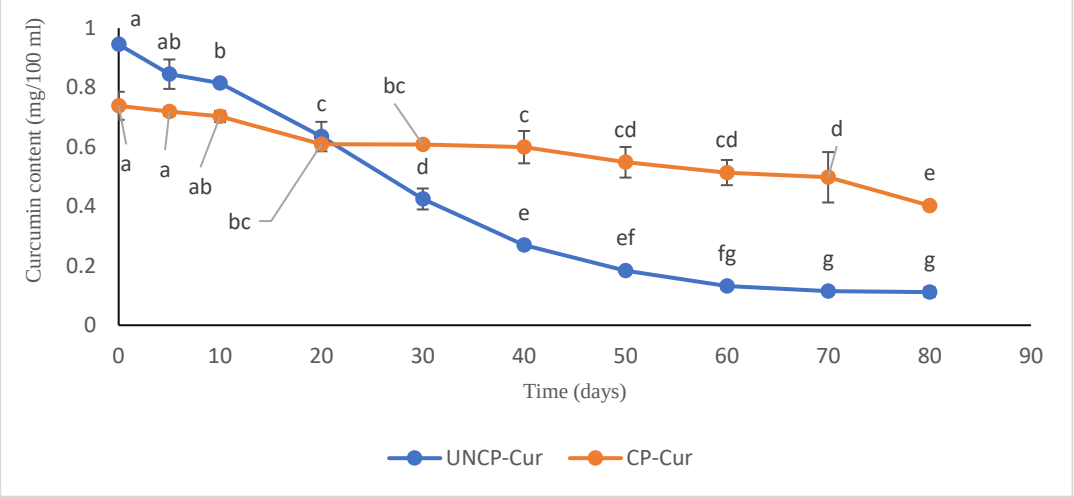

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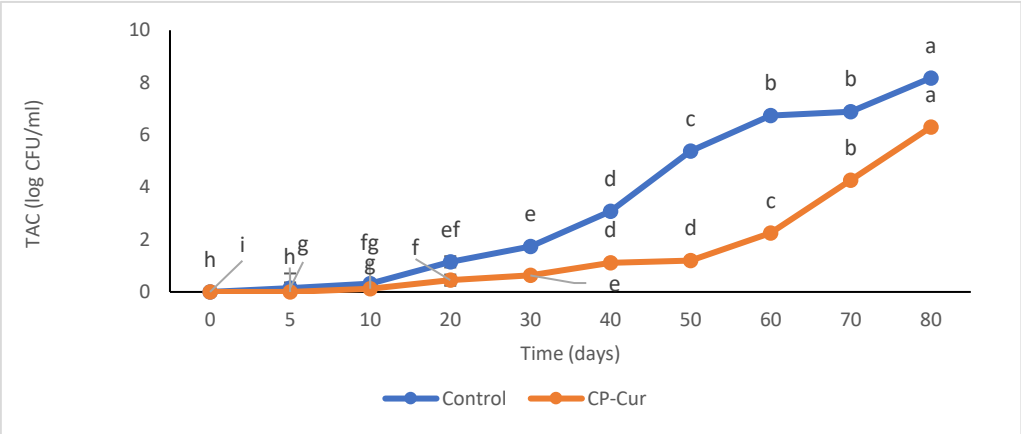
Fig 6A.1: Effect of pasteurization on curcumin content of UNCP-Cur (curcumin-enriched nanoemulsified blended beverage without thermal pasteurization) and CP-Cur (curcumin-enriched nanoemulsified blended beverage with thermal pasteurization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

6A.3.2. Effect of addition of curcumin-enriched Pickering nanoemulsion in blended beverage on microbial load during storage

In this study, CP-Cur and pasteurised blended beverage without curcumin (Control) were stored at 25±2 ℃ and 4±2 ℃. The total aerobic count (TAC) and yeast and mould count (YMC) were determined at 10-day intervals. It was observed that the control and CP-Cur samples showed no growth after pasteurization; however, on day 5 of storage, the TAC of the control sample showed growth of 0.15±0.20 log CFU/ml at 25±2 ℃, while CP-Cur did not show any

growth. Hussain et al. (2022) reported that curcumin has antimicrobial properties, which might be the reason in our study for no microbial growth on day 5 of storage at 25±2 ℃ (Fig. 6A.2. a). On day 80 of storage at 25±2 ℃ and 4±2 ℃, the TAC of Control was 8.17±0.04 log CFU/ml and 5.95±0.01 log CFU/ml, respectively (Fig. 6A.2. a-b). In comparison, CP-Cur had 6.29±0.01 log CFU/ml and 4.86±0.1 log CFU/ml, respectively on storage at 25±2 ℃ and 4±2 ℃. Thus, a significant increase in microbial growth was observed in the Control sample compared to CP-Cur.

YMC count on day 80 of storage at 25±2 ℃ and 4±2 ℃ for Control was 5.23±0.5 log CFU/ml and 5.88±0.1 log CFU/ml, respectively, while CP-Cur showed 3.27±0.01 log CFU/ml and 2.67±0.47 log CFU/ml, respectively, as shown in Fig. 6A.3. a-b. Pasteurisation decreased the microbial load during storage and extended the beverage shelf life. Citric acid serves as a preservative by making prepared beverages more acidic, which creates an environment that is unfavourable for the development of microorganisms (Mane et al., 2019). The aerobic plate count as per the FSSAI microbial standard for pasteurized juice should not be more than $1x10^4$ /ml, and the yeast and mold counts should not exceed $1x10^3$/ml. At 4±2 °C and 25±2 °C, the CP-Cur blended beverages did not exceed the maximum limit set by the FSSAI for total aerobic count of pasteurized juice until day 70 and day 60 of storage, respectively, with a count of 3.74±0.20 log CFU/ml and 2.25±0.01 log CFU/ml. With respect to the maximum limit set by the FSSAI for YMC, pasteurised CP-Cur blended beverage at 25±2 ℃ and 4±2 ℃ did not exceed the maximum limit until day 50 and day 80 of storage (1.59±0.2 log CFU/ml and 2.67±0.4 log CFU/ml), respectively. Joung et al. (2016) reported that the addition of curcumin nanoemulsion to milk systems increased the storage stability of milk. Mwangi et al. (2019) observed that unpasteurized carrot juice increased in bacteria counts by 3 logs after being kept at 20°C for a day. The bacteria count in the juice surged tenfold even when refrigerated. At 20°C, the addition of curcumin (0.4 mM) in the form of curcumin-methyl-β-cyclodextrin inclusion complex stopped the growth of microorganisms in the juice. It was observed that the CP-Cur samples showed no growth of TAC and YMC at 4±2 ℃ up to day 20 and 30, respectively, and at 25±2 ℃ up to day 10 and 20, respectively, which was attributed to the antimicrobial property of curcumin present.



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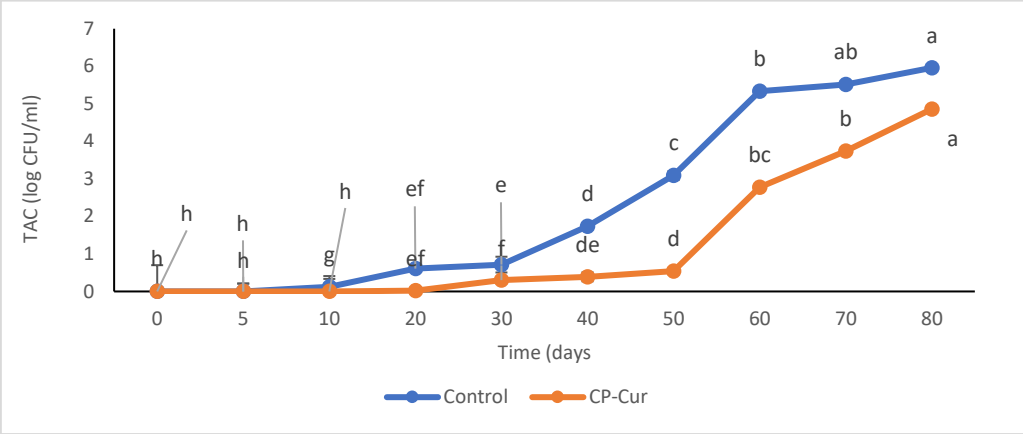

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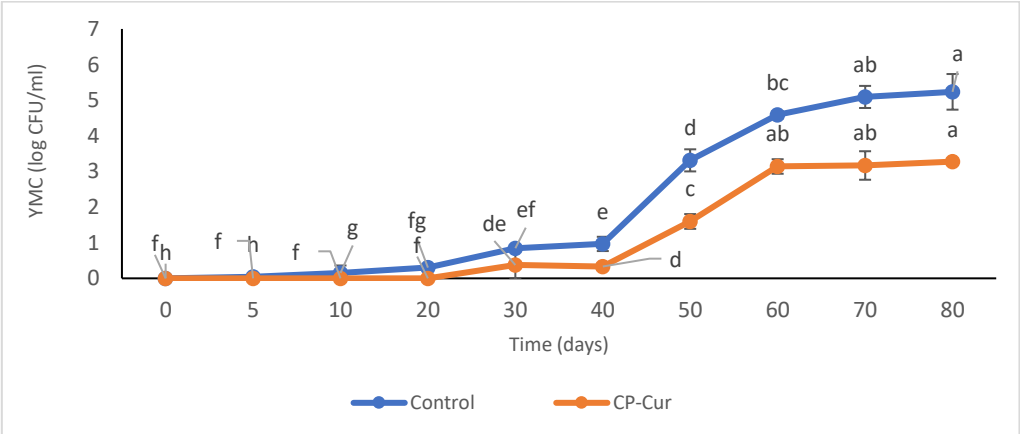
Fig. 6A.2: Effect of curcumin addition on total aerobic count of Control (pasteurized blended beverage without curcumin) and CP-Cur (pasteurized curcumin-enriched nanoemulsified blended beverage) on storage at (a) 25±2 °C and (b) 4±2 °C temperature.



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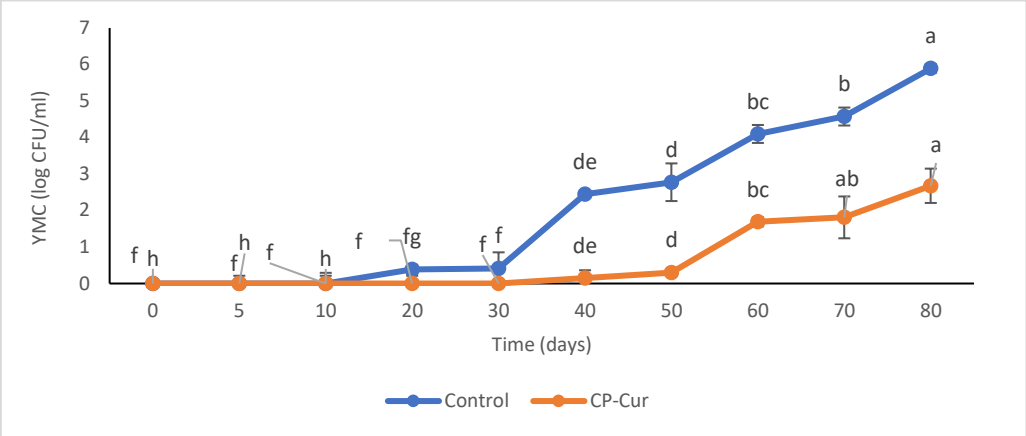


(b)

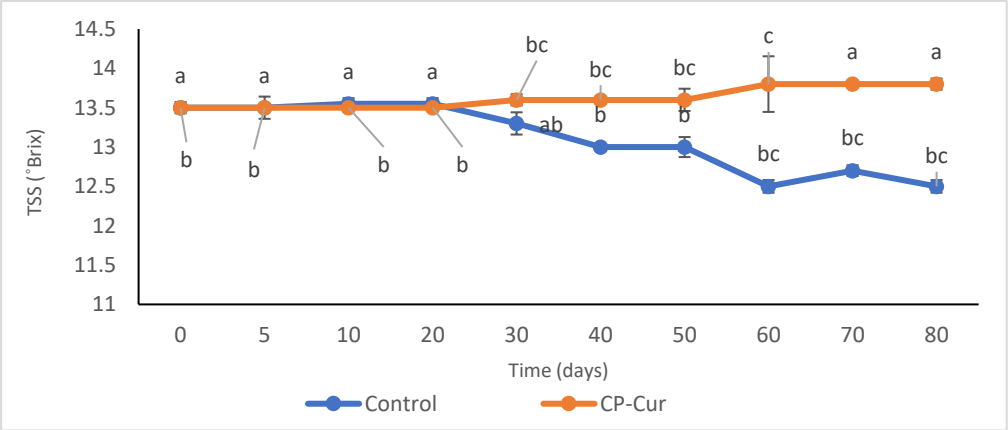
Fig. 6A.3: Effect of curcumin addition on yeast and mould count of Control (pasteurized blended beverage without curcumin) and CP-Cur (pasteurized curcumin-enriched nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

6A.3.3. Effect of addition of curcumin-enriched Pickering nanoemulsion in blended beverage on physicochemical properties during storage

To evaluate the impact of adding curcumin-enriched Pickering nanoemulsion to blended beverage (CP-Cur), total soluble solids (TSS) and pH were measured at 25±2 °C and 4±2 °C during storage. The alterations were contrasted with the blended beverage that had Pickering nanoemulsion but was not curcumin-encapsulated.

TSS content in blended beverages with and without curcumin after pasteurization are shown in Fig. 6A.4. (a) and (b), respectively. It was observed that no significant difference of TSS on day 30 of storage occurred (changed from 13.5±0.01 ºBrix to 13.3±0.14 ºBrix) but TSS significantly decreased to 12.5±0.07 ºBrix on day 80 of storage at 25±2 ℃ in Control sample. In CP-Cur, no significant difference of TSS was observed until day 20 at 25±2 ℃, but after that the TSS increased significantly up to day 60 of storage and thereafter decreased to the value at the start (Fig. 6A.4. a). The CP-Cur samples did not show any significant differences during the 80 days of storage at 4±2 ℃. In comparison, on day 80 of storage at 4±2 ℃, as seen in Fig. 6A.4 (b), TSS value of the Control sample significantly decreased from 13.5±0.01 ºBrix to 12.3±0.14 ºBrix. Similar results were expressed by Shukla et al. (2017) in their study, wherein the TSS of pasteurized mango-based dairy beverage, stored for 10 days at 5 ℃ reduced from 15.91 ºBrix to 14.84 ºBrix. According to Rivas et al. (2006), the bacteria present cause the fruit juice to deteriorate because of sugar fermentation through biochemical pathway, which is responsible for the change in total soluble solids. The CP-Cur samples did not show any

significant differences during the 80 days of storage at 4±2 ℃. Perhaps because of the addition of curcumin, which has antioxidant and antibacterial properties (Inal et al., 2022).



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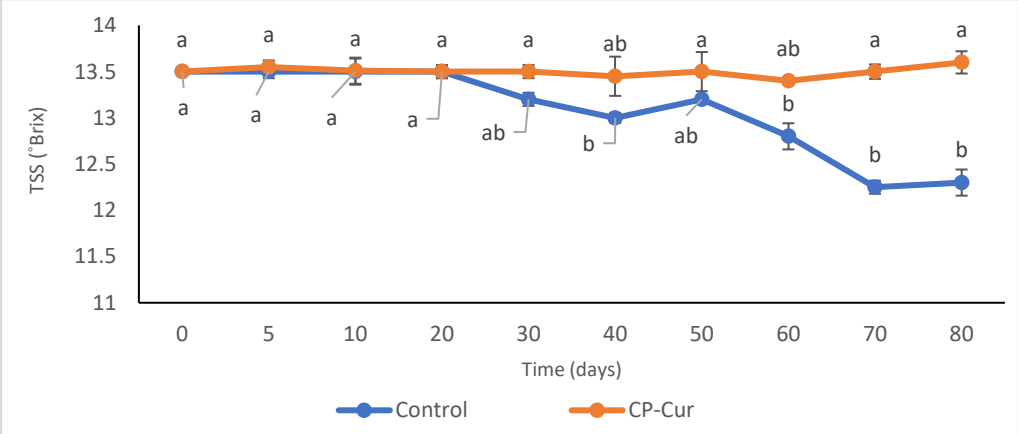
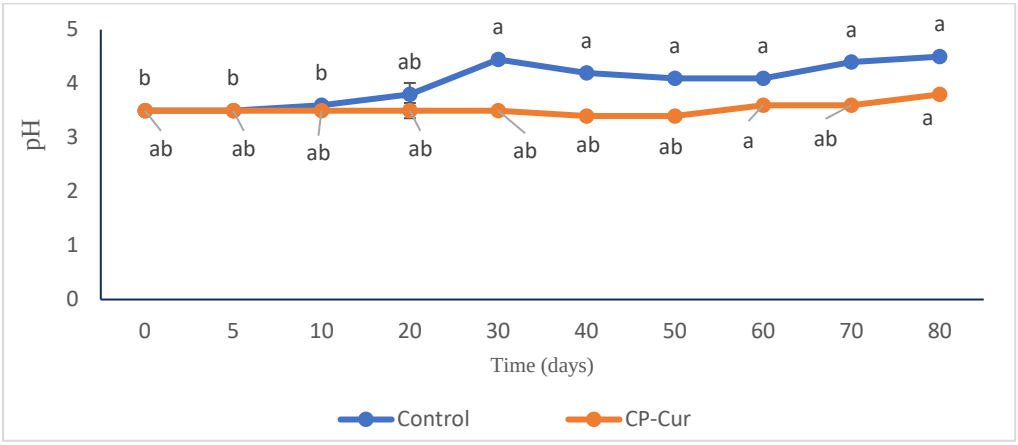


(b)

Fig 6A.4: Effect of curcumin addition on TSS of Control (pasteurized blended beverage without curcumin) and CP-Cur (pasteurized curcumin-enriched nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

The changes in the pH of the blended beverage were obtained by monitoring the pH at 25±2 ℃ and 4±2 ℃ during 80 days of storage, and the changes are presented in Fig. 6A.5. The initial pH of the blended beverage was 3.5 and on day 80 of storage at 25±2 ℃, no significant difference was observed in CP-Cur, while pH in control sample significantly increased to 4.1±0.07 on day 80 of storage at 25±2 ℃. At 4±2 ℃, the pH on day 80 of storage was 3.0±0.2 in CP-Cur and for 4.5±0.01 in the control sample (Fig. 6A.5. a-b). Shukla et al. (2017) also reported that the pH of pasteurized mango-based dairy beverage decreased from 6.57 to 5.99 within 10 days of storage at 5 ℃. The sugar present in beverage, may be a source of microbial spores. During storage, these spores and the bacteria converted the reducing sugars into acid. However, the curcumin present in the beverage, because of its antimicrobial property, was able

to check the growth of microbes and the resulting pH change. Igual et al. (2010) had reported that in pasteurized grape juice, the °Brix and pH remained stable during storage for 60 days at 4 ℃, indicating that the juice was not affected by storage time.



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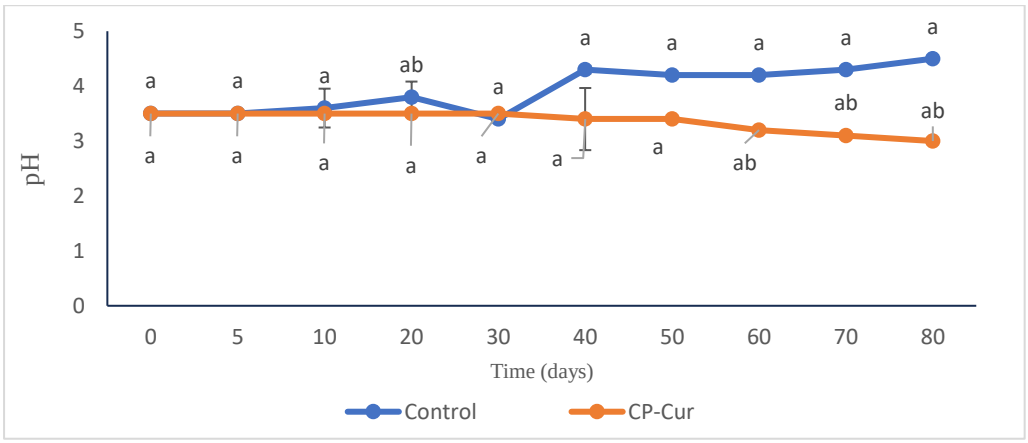


(b)

Fig 6A.5: Effect of curcumin addition on pH of Control (pasteurized blended beverage without curcumin) and CP-Cur (pasteurized curcumin-enriched nanoemulsified blended beverage) on storage at (c) 25±2 ℃ and (d) 4±2 ℃ temperature.

6A.3.4. Effect of addition of curcumin-enriched Pickering nanoemulsion in blended beverage on POD activity during storage

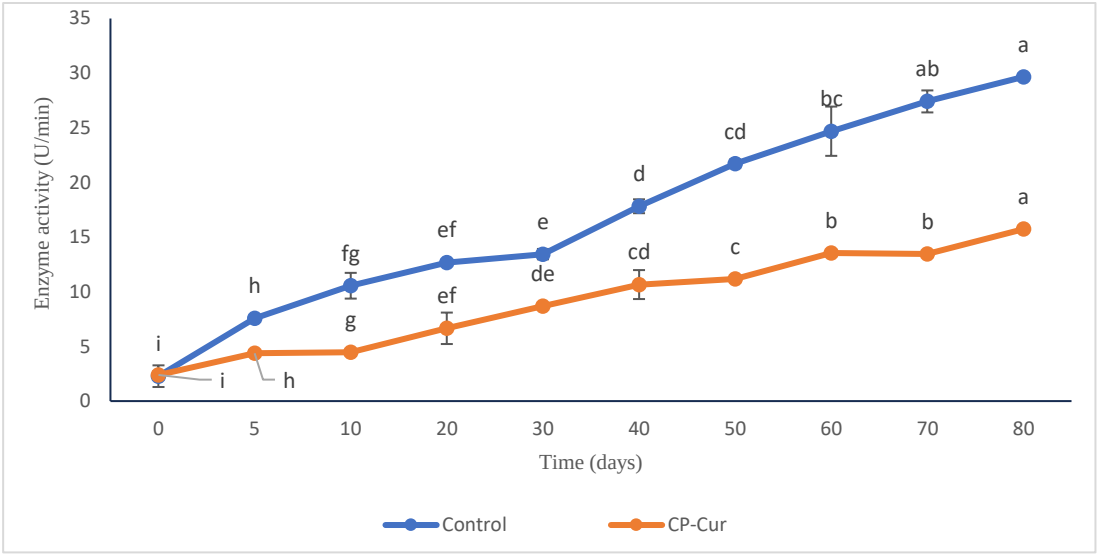
Peroxidase (POD) activity of CP-Cur (curcumin-enriched blended beverage), and Control (pasteurized blended beverage without curcumin) ranged from 2.3±0.32 U/min to 15.7±0.07 U/min and 2.2±0.98 U/min to 29.6±0.14 U/min, respectively at 25±2 ℃ on 80 days of storage, as shown in Fig. 6A.6 (a). CP-Cur registered lower peroxidase activity as compared to the Control. Furthermore, at 4±2 ℃, Control sample showed higher peroxidase activity range (2.4±0.32 U/min to 28.4±0.13 U/min) as compared to CP-Cur (2.3±0.32 U/min to 12.8±0.20 U/min) during 80 days of storage (Fig. 6A.6. b). The study results revealed that the POD of

CP-Cur at 25±2 ℃ was higher as compared to 4±2 ℃ during the entire period of storage. Addition of curcumin-enriched Pickering nanoemulsion in blended beverages and thermal pasteurization have positive effects on reducing the POD activity of blended beverages. Heat treatment, such as pasteurization, is an essential component of fruit juice processing, and is undertaken to inactivate enzymes like peroxidase that might cause unfavourable sensory and nutritional alterations (Petruzzi et al., 2017). POD is a significant food quality-related enzyme that has been linked to alterations in the nutritional value, color, and flavour of food (Tao et al., 2019). POD requires at least five minutes of thermal treatment at 80 ℃ of mango juice to achieve an 80% reduction in activity since it is more thermoresistant than PPO (Mandha et al., 2023). The POD activity of CP-Cur and Control on day 80 at 25±2 ℃ showed an increase by 5.5 and 12.0-fold, respectively. On the other hand, peroxidase residual activity increase at 4±2 ℃ of CP-Cur and Control on day 80 of storage was 4.3 and 10.6-fold, respectively. The POD residual activity increased with storage time in both the samples. Shaik and Chakraborty (2023) reported that the untreated lime juice samples had 68.3% residual POD activity on 6 days of storage at 25℃. POD gets revive with time and causes browning and oxidation of natural compounds present in juice. The authors observed that the thermally treated lime juice residual POD activity increased to 6.5% and 8% at 15 ℃ and 25 ℃, respectively. Deng et al. (2023) reported that curcumin is a promising therapeutic agent due to its natural and strong antimicrobial, anti-inflammatory functions and antioxidant properties. The authors opined that a possible explanation for curcumin ability to suppress enzymatic browning is because it increases antioxidant capacity. Accordingly, the addition of curcumin-enriched Pickering nanoemulsion provided antioxidant property to the beverage.

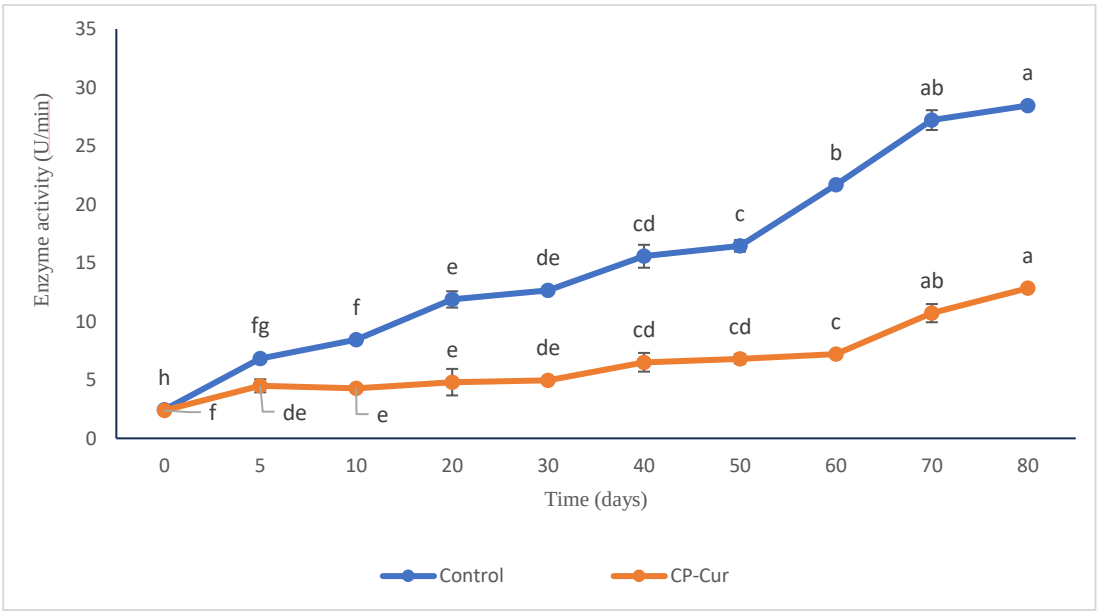
6A.3.5. Effect of addition of curcumin-enriched Pickering nanoemulsion in blended beverage on antioxidant property during storage

The DPPH radical scavenging activity ranged from 78.6±0.37% to 20.3±0.38% in CP-Cur stored at 4±2 ℃ during 80 days of storage and for control sample it ranged from 69.1±0.79% to 10.1±0.38% (Fig. 6A.7. b). At 25±2 ℃, DPPH activity significantly reduced in CP-Cur and Control sample. The DPPH activity reduced from 78.4±0.31% to 10.0±0.88% and from 69.3±0.75% to 8.4±0.49%, respectively (Fig. 6A.7. a). Khaksar et al. (2019) reported that on day 6 of storage, the DPPH values of the juices of guava, carrot, white dragon, and pineapple drastically decreased at RT (~28 ℃). However, no significant difference in DPPH activity was observed during the refrigeration storage (4±2 ℃) on day 6 of storage by the authors. DPPH activity in CP-Cur at 4±2 ℃ showed no significant difference up to day 10 of storage. But with further storage, DPPH activity of CP-Cur significantly decreased. The DPPH activity of CP-

Cur at 25±2 °C decreased significantly with increase in storage time. Curcumin addition in blended beverage CP-Cur showed positive impact as curcumin is reported to have strong antioxidant activity (Inal et al., 2022).

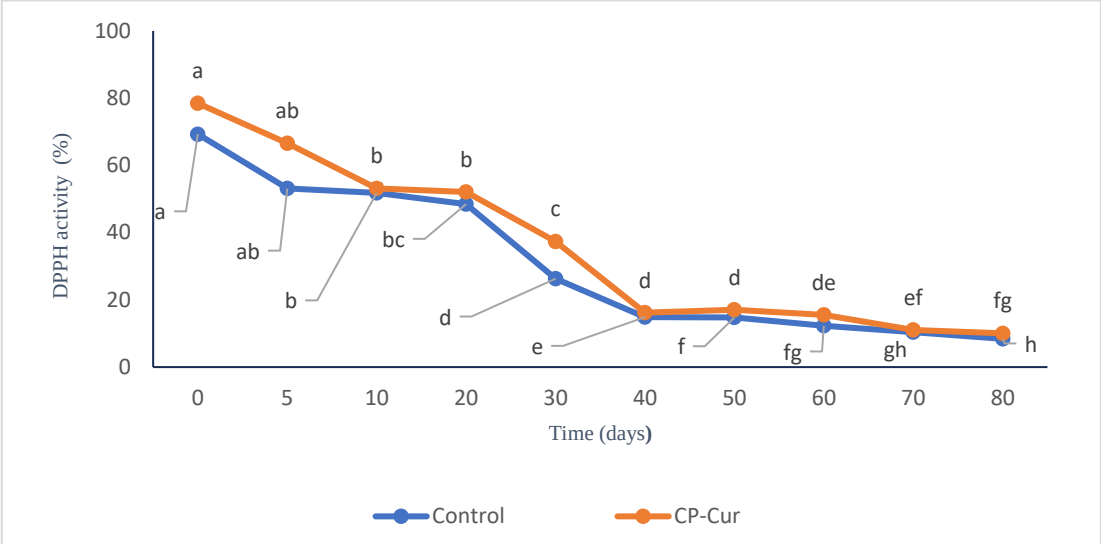


(a)

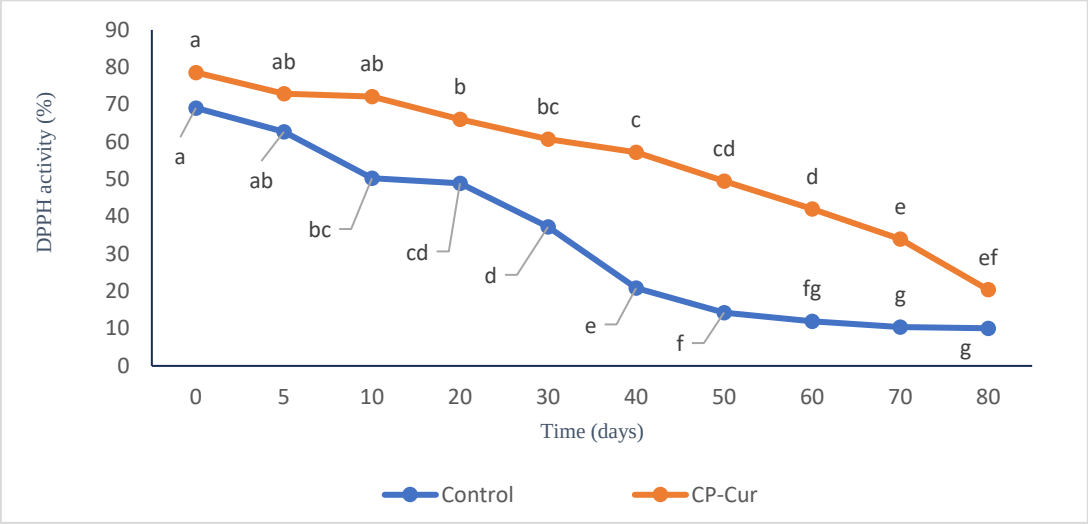


(b)

Fig 6A.6: Effect of curcumin addition on POD of Control (pasteurized blended beverage C50:P50 without curcumin) and CP-Cur (pasteurized curcumin-enriched nanoemulsified blended beverage) on storage at (a) 25±2 °C and (b) 4±2 °C temperature.



(a)



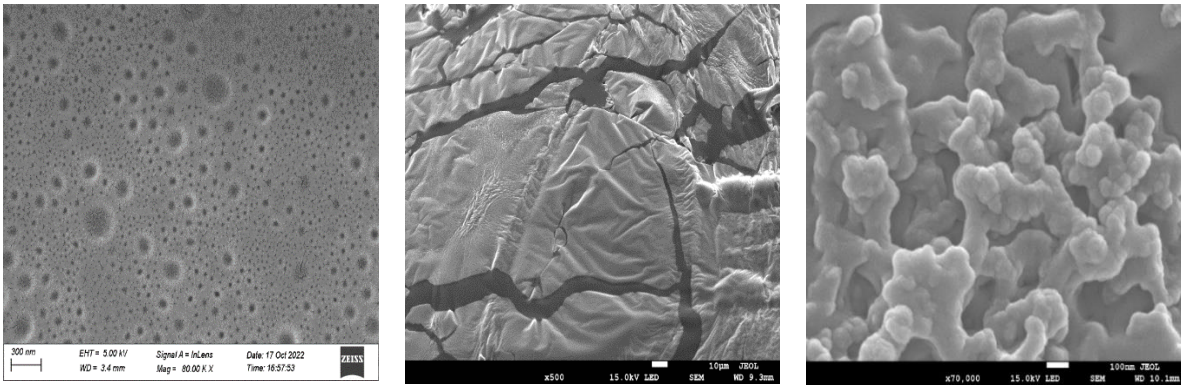
(b)

Fig 6A.7: Effect of curcumin addition on DPPH free radical scavenging activity of Control (pasteurized blended beverage C50:P50 without curcumin) and CP-Cur (pasteurized curcumin-enriched nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

6A.3.6. Morphology of curcumin-enriched nanoemulsified blended beverage

The morphology of Pickering nanoemulsion (PN2) as given in Fig 6A.8 (a) was spherical and the measured diameter was nearly similar (≤259.6 nm) to the average particle size measured by DLS (reported in Chapter 5, section 5.3.10 and Table 5.9). Ngwabebhoh et al. (2018) also reported the spherical morphology of Pickering emulsion with a mean particle size of <200 nm by DLS, whereas TEM study revealed diameter of 151 nm. The morphology of Pickering nanoemulsified blended beverage of defatted coconut milk and pineapple juice showed the

presence of agglomerated nanoparticles (Fig. 6A.8. c) which were not visible in blended beverage of coconut milk and pineapple juice without Pickering nanoemulsion (Fig. 6A.8. b).



(a) (b) (c)

Fig 6A.8: FESEM micrographs of (a) PN2 (curcumin-enriched Pickering nanoemulsion), (b) C50:P50 (blended beverage), (c) CP-Cur (curcumin-enriched nanoemulsified blended beverage).

6A.3.7. *In vitro* bioaccessibility of curcumin-enriched nanoemulsified blended beverage

Curcumin concentration in the micelle phase and total digesta were measured to investigate the impact of the addition of PN2 to the blended beverage of defatted coconut milk and pineapple juice (CP-Cur). PN2 had curcumin concentration of 0.95 mg/100 ml. The results demonstrated that the bioaccessibility of curcumin from CP-Cur after digestion was 51.1%. Zhou et al. (2021) reported the bioaccessibility of lipophilic polyphenol compounds from nanoemulsions encapsulated with coconut oil to be 51.8%, which supports our results. The results showed that the incorporation of Pickering nanoemulsion in blended beverages acted as a good carrier for maintaining curcumin stability and curcumin delivery. The *in vitro* release percentages of curcumin from nanocellulose-stabilized Pickering emulsion in stomach medium (pH-2.5) and small intestinal fluid (pH-7) was 38.2% and 51.6% (Fig. 6A.9), respectively. These results suggested that the simulated gastric phase did not affect curcumin release from the Pickering emulsion, whereas higher release in the simulated intestinal phase might be attributed to the destabilization of emulsion droplets (Tikekar et al., 2013). According to Ribeiro et al. (2022), Pickering emulsion in the stomach phase face harsh conditions, namely they contact with gastric fluids in stomach phase characterized by a highly acidic pH and high enzymes content. Due to the better stability of PN2 in different pH condition (as reported in chapter 5 section 5.3.11), release of curcumin in stomach phase was observed to be lower as compared to intestinal phase. Inal et al. (2022) reported that enzymatic lipid digestion occurs only in intestinal phase with bile salts and pancreatin. The fatty acid release during invitro digestion of

curcumin added echium oil nanoemulsion and echium oil nanoemulsion was 40.9%, and 33.9% respectively. The amount of curcumin released in the intestinal phase of the CP-Cur was greater than in the stomach phase, may be because of virgin coconut oil used for encapsulation of curcumin. The increase in curcumin release from CP-Cur could be explained by lipase dispersion between the aqueous phase and emulsion interface as lipase gets activated in intestinal phase by enzymatic reaction. Ahmed et al. (2012) reported that the release of fatty acid was higher for medium chain fatty acid (113%) as compared to long chain fatty acids (68%). The relatively lower amount of fatty acid digestion of long chain fatty acid is due to its property of accumulation at droplet surface, which inhibits lipase digestion of emulsion. Medium chain fatty acid is known to dissolve easily in aqueous phase than long chain fatty acid, and therefore is less likely to accumulate at droplet surfaces and inhibit the digestion of lipids. The VCO used in curcumin encapsulation is rich in medium chain fatty acids and contains nearly 49.3% lauric acid, 0.6% caproic acid, 7.4% caprylic acid, 5.9% capric acid, 18.5% myristic acid, 2.8% palmitic acid, and 6.1% stearic acid (chapter 5, section 5.3.7). The nanoparticle concentration is an important parameter for coalescence stability. Stability is typically assessed by analysing the physical changes in the emulsion over a practical period (Jiménez and Capron, 2018). The resulting nanocellulose-stabilized, curcumin-enriched Pickering nanoemulsion exhibited excellent stability in the gastric as well as intestinal phase. Integrating curcumin into a blended beverage containing defatted coconut milk and pineapple juice enhanced the bioavailability of curcumin.

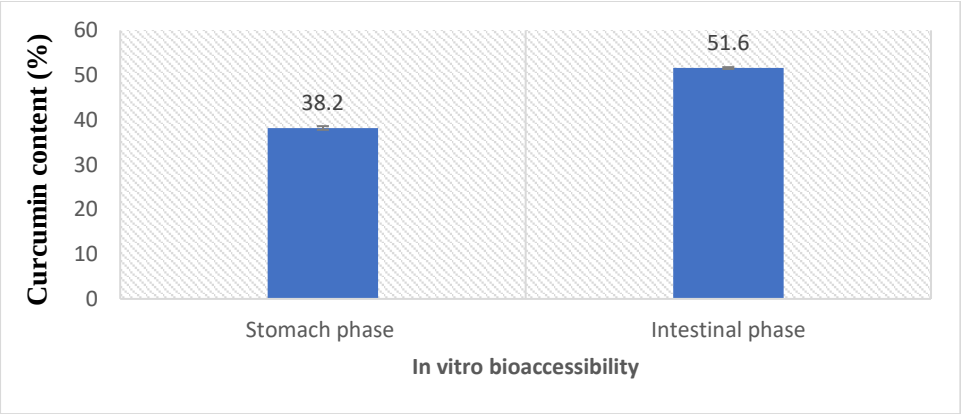


Fig 6A.9: *In vitro* bioaccessibility of curcumin-enriched blended beverage of defatted coconut milk and pineapple juice (CP-Cur).

6A.4. Conclusion

In this study 10% of PN2 was added to the blended beverage in order to control the amount of water (as aqueous phase) and to keep the concentration of Tween 80 within permissible limit. The stability of curcumin in CP-Cur after pasteurization was better than that of the UNCP-Cur up to 30 days of storage at 25±2 ℃ and 4±2 ℃. The morphology of Pickering nanoemulsion (PN2) assessed by FESEM was spherical and the measured diameter was nearly similar to the average particle size (≤259.6 nm) measured by DLS. Dispersion of nanoparticles was observed in CP-Cur after addition of PN2 to the blended beverage, which were not visible in blended beverage of coconut milk and pineapple juice without PN2. CP-Cur did not exceed the maximum threshold established by the FSSAI for the total aerobic count of pasteurized juice until days 70 and 60 of storage, at 4±2 °C and 25±2 °C, respectively. YMC of CP-Cur at 25±2 °C and 4±2 °C was within the maximum limit of FSSAI until days 50 and 80 of storage. As the storage duration increased, there was a substantial decrease in the DPPH activity of CP-Cur at 25±2 °C. On day 10 of storage, there was no significant difference in DPPH activity in CP-Cur at 4±2 °C, but on day 80 of storage, there was a substantial drop. CP-Cur's TSS and pH, however, did not significantly change over the course of storage at 25±2 °C and 4±2 °C. Blended beverage added with PN2 had curcumin concentration of ~0.95 mg/100 ml. The curcumin-enriched nanoemulsified blended beverage increased the bioaccessibility of curcumin in the intestinal phase *in vitro*. Curcumin bioaccessibility, which was 38.2% in the gastric phase, increased to 51.6% during *in vitro* intestinal phase digestion. The results revealed that Pickering nanoemulsions in blended beverages could successfully carry curcumin into the intestinal phase in a stable form. It can be concluded that nanocellulose from coconut milk waste residue has desirable characteristics that help stabilize Pickering nanoemulsions against coalescence when incorporated into blended beverages and act as a carrier for stable curcumin.

Section 6B

6B.1. Introduction

Emulsions are delivery systems that can be used to incorporate bioactive ingredients into food or pharmaceutical products. These systems can shield ingredients from the adverse effects of processing and allow for their controlled release at specific GI tract locations, such as the mouth, intestine, and stomach. Emulsifiers and surfactants are frequently used to stabilize emulsion systems (Saffarionpour, 2020). Emulsifiers prevent flocculation, creaming, coalescence, and sedimentation. Emulsification helps encapsulate, oxidatively stabilize, and release bioactive molecules. Natural or artificial solid particles are used in Pickering emulsions to stabilize the oil–water interface. These solids, like surfactants, reduce the free energy that must be overcome to maintain system stability (Souza et al., 2022). The effectiveness of Pickering emulsions in enhancing the oxidative stability of oils and the delivery of bioactive ingredients was assessed (Cahyana et al., 2022). Nanoparticles improve the stability of Pickering emulsions and bioavailability of drugs/functional components for delivery (Niu et al., 2017). To provide nutraceuticals with therapeutic benefits and regulate their release in the gastrointestinal tract, nanoemulsion systems stabilized with nanocellulose (NC) are created (Saffarionpour, 2020). The anionic polysaccharides, nanocrystalline cellulose (NCC) and the three-dimensional structure of nanofibrillated cellulose (NFC) stimulate electrostatic repulsive forces that lead to more stable emulsions with less phase separation.

However, NFC produces larger number of oil droplets and a stronger negative charge than NCC. Many reasons are assigned to emulsion stability, but two main ones are the bridging flocculation phenomenon and the high viscosity that inhibits droplet movement (Fitri et al., 2022). These days, a vast array of nutritious, extremely stable, and delicious foods are being created globally using natural but complex mechanisms. Milk-based beverages tend to develop serum separation due to the acidic fruit juice. Iranian natural gums were used in a study on the serum separation of whey-based pina colada beverages, which analyzed the combination of pineapple and coconut juice to improve stability (Dehghan et al., 2022). The particles in fruit juice sediment easily because they do not contain substances that can slow down particle motion. Juice sedimentation may be viewed as a technological challenge for the food sector. Methods to prevent sedimentation include reducing particle size via homogenization or grinding and adding thickening agents to enhance viscosity (Silva et al., 2019). Preservatives continue to be one of the key methods for extending food shelf life and preserving food quality.

The lingering risks that synthetic preservatives pose to the environment and human health have come to light as knowledge about them has grown (Lan et al., 2023). The food industry is searching for natural ingredients that are safe and non-toxic to replace preservatives as a result of this trend. As a natural food preservative with effective broad-spectrum antibacterial and antioxidant capabilities, curcumin is playing a crucial role in the preservation of food (Lan et al., 2023). Numerous efforts have been made to find a way to overcome the low oral bioavailability of curcumin and a great number of novel formulations have been developed by researchers to achieve this goal (Liu et al., 2018). Several systems have been explored for their capability to encapsulate and carry curcumin, including microemulsions, nanoemulsions, solid lipid nanoparticles, liposomes, biopolymer nanoparticles, and microgels. On the other hand, Pickering particle-stabilized emulsions have been reported to encapsulate more amount of curcumin and do not show alteration in droplet size after curcumin incorporation and storage (Neves et al., 2020).

Section 6A evaluated a blended beverage with a very low curcumin concentration (~0.95 mg/100 ml), as only 10% of curcumin-enriched Pickering emulsion was added to the coconut milk and pineapple juice blend in order to control the amount of water (as aqueous phase) and to keep the concentration of Tween 80 within permissible limit. The study reported in Section B was carried out to develop a curcumin-enriched Pickering nanoemulsion using a blend of defatted coconut milk and pineapple juice as the aqueous phase and virgin coconut oil as the oil phase to increase the concentration of curcumin in blended beverage and decrease serum separation. For this, a higher concentration of curcumin was added. Nanoemulsified blended beverages were studied for their emulsion stability, morphology, creaming index stability, and shelf-life after storage. Mechanism of curcumin release and bioaccessibility from Pickering nanoemulsion.

6B.2. Materials and methods

Fresh and mature coconuts (10–12 months) and ripe pineapples were purchased from the local market in Tezpur. Chemicals were purchased from Sisco Research Laboratory (Mumbai, India), Merck (Mumbai, India), and HiMedia (Mumbai, India). Solvents used for the separation and identification of sample were of HPLC grade. Standards were purchased from Sigma-Aldrich (USA), TCI (Japan), Merck, Himedia or SRL make. Standard curcumin (purity ≥98%) was used for HPLC study.

6B.2.1. Beverage preparation of defatted coconut milk and pineapple juice

Extraction of pineapple juice and defatting of coconut milk (C50:P50) were done using the methods described in chapter 3 (section 3.2.1).

6B.2.2. Preparation of nanocellulose

The nanocellulose ACU-10 was extracted from coconut milk waste residue using the method described in section 5.2.2 and chapter 5.

6B.2.3. Curcumin-enriched Pickering nanoemulsion blended beverage

The Pickering nanoemulsion was prepared as described by Ngwabebhoh et al. (2018) with some modifications. The Pickering nanoemulsion was developed using virgin coconut oil (7%), Tween 80 (0.1 to 0.2%), and curcumin (0.2% in virgin coconut oil w/v, %) in blended beverage. With the help of a high-speed homogenizer (IKA, Model T25, Digital Ultra Turrax), the mixture was first coarsely homogenized for 5 min at 11000 rpm. Next, it was homogenized under pressure (260 bar) for 5 passes by combining the organic phase, which consisted of VCO Tween 80 and blended beverage, with nanocellulose in concentrations of 0.05, 0.1, and 0.2 wt%. The produced Pickering nanoemulsions were transferred to glass vials and kept at RT (25±2 °C) for further investigations. The emulsions PNCP-Cur1, PNCP-Cur2, and PNCP-Cur3 containing 0.1 wt% Tween 80 were added with 0.05, 0.1, and 0.2 wt% nanocellulose, respectively. PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6 containing nanocellulose concentrations of 0.05, 0.1, 0.2 wt%, respectively were treated with 0.2 wt% Tween 80.

6B.2.4. Particle size measurement by dynamic light scattering (DLS)

Particle size of the Pickering nanoemulsion samples (PNCP-Cur1, PNCP-Cur2, PNCP-Cur3, PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6) was measured following the method described in section 5.2.6 of chapter 5.

6B.2.5. Morphology of curcumin-enriched blended beverage

The field emission scanning electron microscopy (FESEM) was used to determine the morphology of curcumin-enriched blended beverage, as described in section 6A.2.3.

6B.2.6. RP-HPLC analysis of curcumin-enriched blended beverage

Curcumin content in Pickering nanoemulsions (PNCP-Cur1, PNCP-Cur2, PNCP-Cur3, PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6) was determined by method described in section 6A.2.4.

6B.2.7. Thermal stability of curcumin-enriched blended beverage

The Pickering nanoemulsion samples were stored at room temperature for 1 h before analysis. Effect of heating conditions (63 ℃ for 30 min and 95 ℃ for 10 min) on curcumin content of Pickering nanoemulsions (PNCP-Cur1, PNCP-Cur2, PNCP-Cur3, PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6) was determined by the method described in section 5.2.14 of chapter 5.

6B.2.8. Creaming index of curcumin-enriched blended beverage

The physical stability of curcumin-enriched blended beverage was evaluated by determining the percentage creaming index. For this, 50 ml of the samples was taken in bottles that were firmly sealed and kept at 25 °C for 30 days. When the layers of water and oil were clearly separated and were not recombining, nanoemulsion cracking was considered to have begun (Pengon et al., 2018).

Using Equation 1, the creaming index (CMI%) was calculated.

$$\text{CMI\%} = \frac{\text{CC}}{\text{CT}} \text{X} 100 \qquad \text{(Eq. 6B.1)}$$

where CT is the total height of the emulsion layer and CC is the total height of the cream layer.

6B.2.9. *In vitro* bioaccessibility of curcumin-enriched blended beverage

The bioaccessibility of ingested curcumin from PNCP-Cur1, PNCP-Cur2, PNCP-Cur3, PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6 in gastro-intestinal fluids was determined by the method described in section 6A.2.5 of subsection 6A.

6B.2.10. Storage study of curcumin-enriched blended beverage

The curcumin-enriched blended beverage having maximum curcumin content and stability was pasteurized at 80 ℃ for 1.80 min and coded as PNCP-Cur2. The selection of pasteurization conditions has been reported in chapter 4.

6B.2.10.1. Microbial characterization of curcumin-enriched blended beverage

The total aerobic plate count and yeast and mould count were determined by the method described in section 6A.2.6 of subsection 6A.

6B.2.10.2. Peroxidase residual activity of curcumin-enriched blended beverage

The peroxidase activity (POD) was determined by the method described in section 6A.2.7 of subsection 6A.

6B.2.10.3. DPPH radical scavenging activity of curcumin-enriched blended beverage

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the curcumin-enriched blended beverage was determined by the method described in section 6A.2.8 of subsection 6A.

6B.2.10.4. Determination of soluble solids

The total soluble solids was determined by the method described in 3.2.2.1 of chapter 3.

6B.2.10.5. Determination of pH

The pH was measured by the method described in section 3.2.2.2 of chapter 3.

6B.2.11. Antimicrobial activity of curcumin-enriched blended beverage

The defatted coconut milk (C100), pineapple juice (P100), blended beverage (C50:P50), curcumin-enriched Pickering nanoemulsion added blended beverage (CP-Cur), and curcumin-enriched blended beverages (PNCP-Cur2) were analysed for antimicrobial activity. The agar well diffusion assay was used to evaluate the antibacterial efficacy against various test organisms using the method of Singh et al. (2016) with some modifications. In order to assess antimicrobial activity, 15–20 ml nutrient agar plates were prepared and left to solidify. The agar plates were maintained at 4 ℃ for 1 h before wells were punctured out of the plates using sterilized glass. Microbial inocula of *E. coli* and *S. aureus* were spread onto the agar plates by sterile glass rods under completely aseptic conditions. Then, aliquots (0.1 ml) of all the samples were pipetted into the wells, after that wells were sealed using one drop of soft agar. The plates were again kept at 4 °C for 2–4 h to allow the supernatant to diffuse and then incubated for 24–48 h at 37 °C. A clear zone of 1 mm or more (apart from the 6 mm well diameter) was deemed positive inhibition. The inhibition zone surrounding the well was measured laterally.

6B.2.12. Statistical analysis

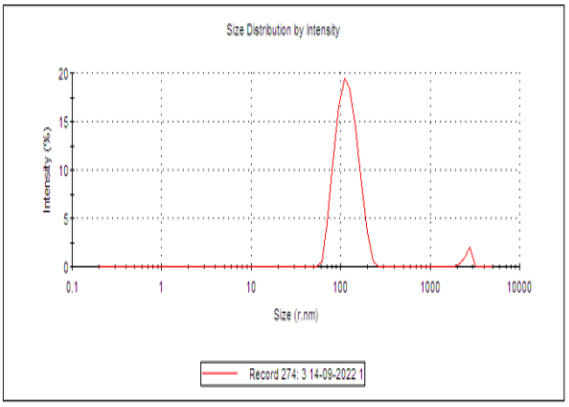
Every component was analysed in triplicate, and the results are presented as the mean ± standard deviation of all separate studies. All experiments on treated blended beverages are shown as significant differences ($p < 0.05$) based on the findings of analysis of variance (ANOVA) using IBM SPSS 20.0, and Duncan's multiple range tests were performed.

6B.3. Results and discussion

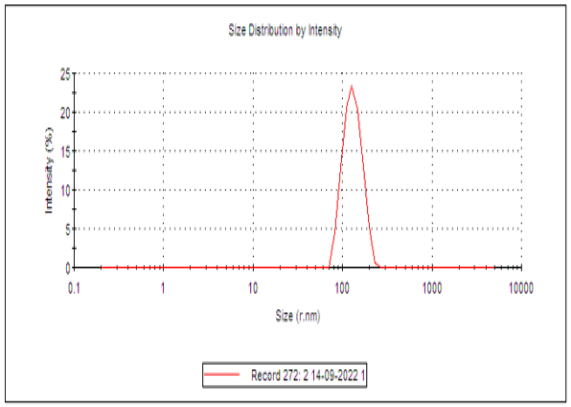
6B.3.1. Particle size of curcumin-enriched blended beverage

The particle size distribution for each curcumin-enriched Pickering nanoemulsified blended beverage that was stabilized with coconut milk waste nanocellulose and developed via high-pressure homogenization are shown in Fig. 6B.1 and Table 6B.1. For curcumin-enriched beverage production, the aqueous phase of the nanoemulsified beverage was blended with nanocellulose, which was selected as a stabilizer along with Tween 80 for better and longer-term stability. In drug-delivery systems, particle size greatly influences in *vitro* release of biomolecules. The PDI is the index of size distribution representing the similarity between particles, and a large PDI value indicates that the particle sizes are substantially different. By applying high-pressure homogenization to the oil, water, and surfactant mixture, nanoscale emulsions, hereafter referred to as nanoemulsions, can be created with small droplet sizes (50–200 nm) (Baek et al., 2020). An unequal particle size can result in irregular pharmacokinetic parameters and affect the therapeutic efficiency of a drug formulation. The average particle size and PDI value of PNCP-Cur1, PNCP-Cur2, PNCP-Cur3, PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6 was 120.5, 131.7, 145.3, 137.1, 141.1, and 172.5 nm, respectively, and 0.241, 0.119, 0.245, 0.207, 0.238, and 0.105, respectively, as shown in Fig. 6B.1 (a-f). The results indicated that the size and PDI of the nanoemulsified beverages were low. PDI measures the size distribution, and broadness indicates the deviation from the mean particle size (Tadros et al., 2004). The relatively lower PDI values of the nanoemulsions can be correlated with greater stability during storage. Jusril et al. (2022) reported that PDI value <0.25 indicated a narrow and concentrated particle size that gave better stability. Lei et al. (2022) reported that emulsions exhibited larger droplets and even demulsification when the content of bamboo shoot nanocellulose (BSNC) was increased. It is interesting to note that twice-shearing, which essentially decreases droplet size, greatly enhances the physicochemical characteristics of the emulsions. For the Pickering emulsions stabilized with a BSNC concentration of 0.5%–1.1%, the surface coverage was more than 100%. The droplet size of curcumin nanoemulsified beverages was significantly dependent on the oil, surfactant, and blended beverage ratios of defatted coconut milk and pineapple juice. The particle size of curcumin-enriched nanoemulsified beverages increased with increasing concentrations of the stabilizer (nanocellulose) and surfactant (Tween 80) Aw et al. (2022) reported that a nanocellulose-stabilized Pickering emulsion did not require a CNC concentration of more than 1.5 g/100 ml because it formed a very thick, gel-like Pickering emulsion. In contrast, when the stabiliser levels increase, the average particle sizes of the nanoemulsion stabilized with carboxymethyl

cellulose (CMC) shifted towards larger average particle size. This agrees with the work of Ntazinda et al. (2014), who found that increasing the CMC concentration above a certain level (to 6 g/L) caused a broadening of the particle size peak toward larger sizes. Addition of nanocellulose at a concentration of 0.05%, 0.1% and 0.2% with 0.1% Tween 80 increased the particle size of PNCP-Cur1, PNCP-Cur2, and PNCP-Cur3 to 120.5 nm, 131.7 nm, 145.3 nm, respectively (Table 6B.1). However, nanocellulose at 0.05%, 0.1% and 0.2% concentration with 0.2% Tween 80 concentration increased the particle size in samples PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6 to 137.1 nm, 141.1 nm, and 172.5 nm, respectively. A higher particle size was observed in curcumin-enriched blended beverage with 0.2% Tween 80 (PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6) than with 0.1% Tween 80 (PNCP-Cur1, PNCP-Cur2, and PNCP-Cur3).



(a)



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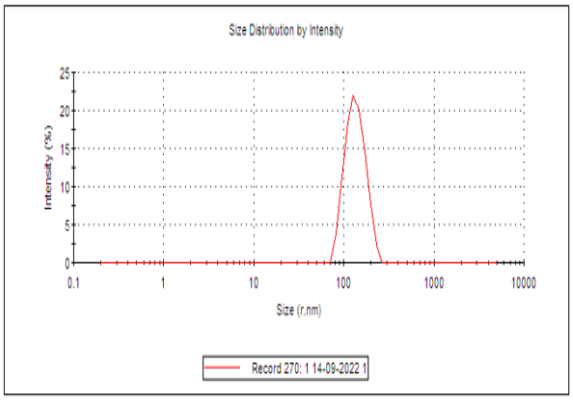

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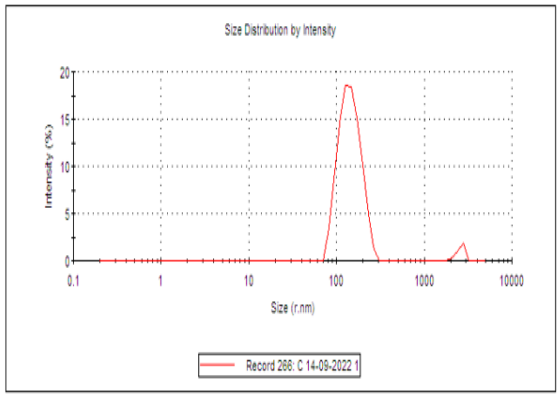

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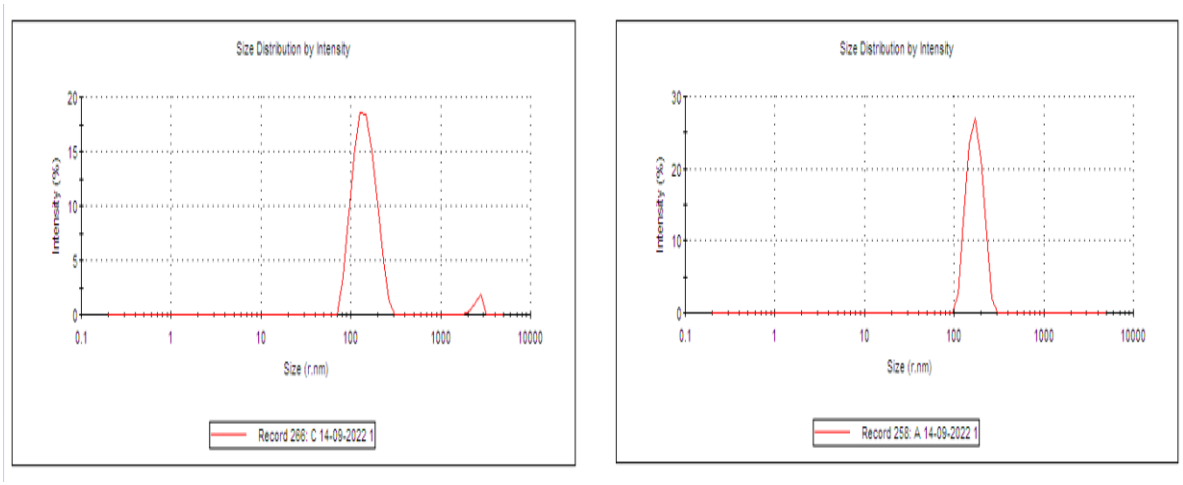


(e) (f)

Fig. 6B.1: Particle size and PDI values of curcumin-enriched Pickering nanoemulsified blended beverage (a) PNCP-Cur1, (b) PNCP-Cur2, (c) PNCP-Cur3, (d) PNCP-Cur4, (e) PNCP-Cur5, and (f) PNCP-Cur6.

Table 6B.1. Particle size and polydispersity index of curcumin-enriched blended beverages

Samples	Particle size (nm)	Polydispersity index
PNCP-Cur1	120.5	0.241
PNCP-Cur2	131.7	0.119
PNCP-Cur3	145.3	0.245
PNCP-Cur4	137.1	0.207
PNCP-Cur5	141.1	0.238
PNCP-Cur6	172.5	0.105

6B.3.2. Creaming index of curcumin-enriched blended beverage

Creaming index is a good indicator of the physical stability of nanoemulsified beverage. The volume of the separated layer was expressed as %CMI of the curcumin-enriched Pickering nanoemulsified blended beverage (Fig. 6B.2) on storage. %CMI of Control sample on day 5 was 27.3±0.94% and day 35 was 42.3±0.29%. Stability is commonly addressed by analysis of physical alterations of the nanoemulsion over a practical length of time. PNCP-Cur1, PNCP-Cur2, PNCP-Cur3, PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6 showed no separation until day 10. PNCP-Cur6 showed %CMI of 13.3±0.01% on day 15 of storage. PNCP-Cur1 exhibited

cream separation on day 20 with %CMI of 15.3±0.82%, PNCP-Cur3 and PNCP-Cur4 showed %CMI of 15.6±0.29% 21.6±0.35% respectively, on day 25. While %CMI of Control, PNCP-Cur1, PNCP-Cur3, PNCP-Cur4, and PNCP-Cur6 on day 35 of storage was 40.0±0.01%, 43.3±0.71%, 35.6±0.29%, 42.6±0.77%, 35.6±0.29%, respectively. PNCP-Cur2 and PNCP-Cur5 did not show any separation till day 35 of storage. PNCP-Cur2 with 0.1% nanocellulose and PNCP-Cur5 with 0.2% nanocellulose registered 0 %CMI on day 35 and were the most stable formulations (Fig. 6B.2). The stability of the nanoemulsified beverages is related to the particle size and PDI value of droplets. Sari et al. (2015) reported that the individual use of stabilizer like Tween 80 and whey protein concentrate-70 did not yield positive results in stability of creaming index. Moreover, in the blended beverage of coconut milk and pineapple juice, we used nanocellulose along with Tween 80 at a very low concentration. The absence of any creaming in PNCP-Cur2 and PNCP-Cur5 may be due to its smaller particle size and PDI value (Table 6B.1). Jusril et al. (2022) reported that PDI value <0.25 indicated a narrow and concentrated particle size that is required for better stability. Pengon et al. (2018) have reported that the type of surfactant used had a direct impact on the durability of emulsions. Aw et al. (2022) reported that a nanocellulose-stabilized Pickering emulsion did not require a CNC concentration of more than 1.5 g/100 ml because it formed a very thick, gel-like Pickering emulsion. In contrast, when the stabiliser levels increase, the average particle sizes of the nanoemulsion stabilized with carboxymethyl cellulose (CMC) shifted towards larger average particle size. This agrees with the work of Ntazinda et al. (2014), who found that increasing the CMC concentration above a certain level (to 6 g/L) caused a broadening of the particle size peak toward larger sizes. The stability of curcumin-enriched Pickering nanoemulsified blended beverage was affected by the concentration of nanocellulose used. PNCP-Cur1 and PNCP-Cur4 having 0.05% nanocellulose started separation on day 20 and day 25, respectively. On other hand, PNCP-Cur3 and PNCP-Cur6 with 0.2% nanocellulose showed separation on day 25 and day 15 of storage, respectively. Nanocellulose concentration has been reported to be an important parameter for preventing coalescence of the Pickering emulsion droplets (Ngwabebhoh et al., 2018).

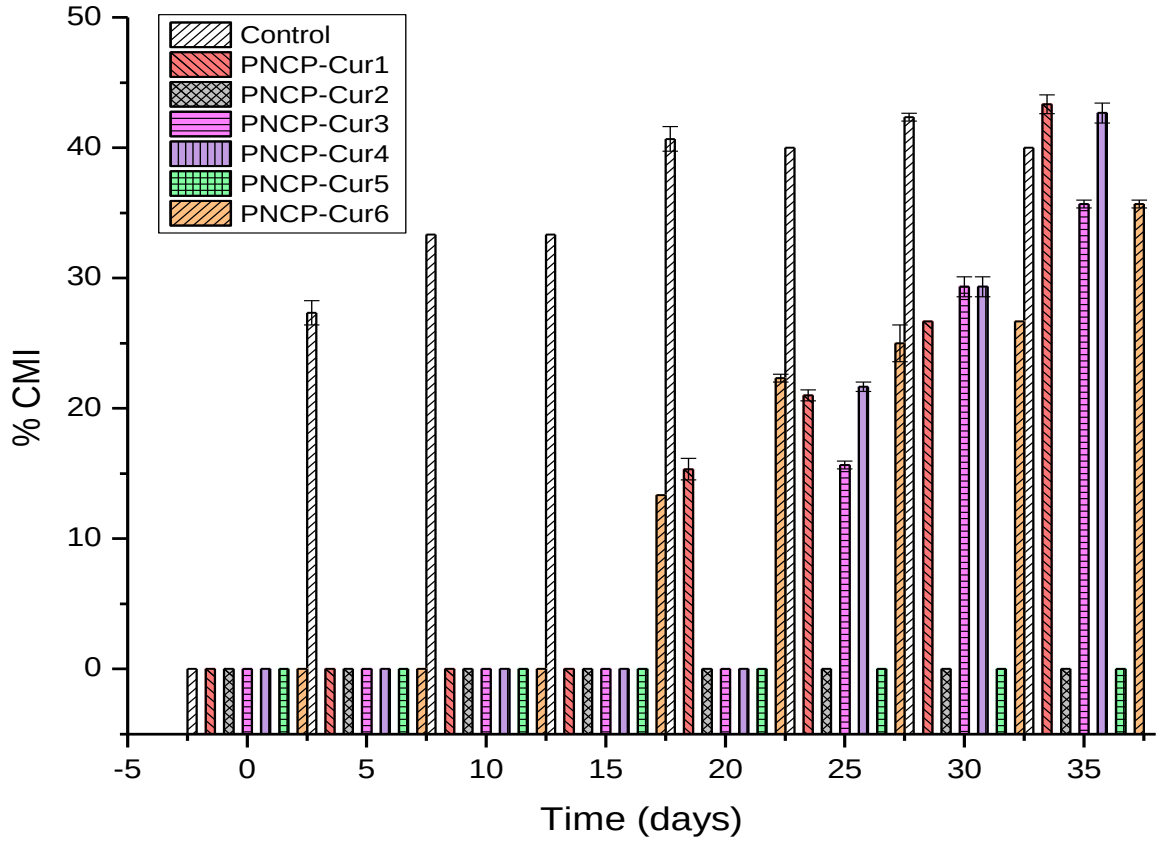


Fig 6B.2: Creaming index of curcumin-enriched Pickering nanoemulsion blended beverages (PNCP-Cur1, PNCP-Cur2, PNCP-Cur3, PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6).

6B.3.3. Curcumin stability in curcumin-enriched Pickering nanoemulsified blended beverages stored at different temperatures

The stability of curcumin in curcumin-enriched Pickering nanoemulsified blended beverages was determined at two different temperatures, and the results are shown in Table 6B.2. Curcumin content in PNCP-Cur3 and PNCP-Cur6 was low, i.e. 5.7±0.05 mg/100 ml and 5.3±0.04 mg/100 ml, respectively, at 63 ℃, and 5.7±0.14 mg/100 ml and 5.2±0.24 mg/100 ml, respectively at 95 ℃. The maximum curcumin content of 7.1±0.22 mg/100 ml was observed in PNCP-Cur2 at 63 ℃. An increase in the concentration of nanocellulose increased particle size, which affected the stability of curcumin-enriched Pickering nanoemulsion blended beverages PNCP-Cur 3 and PNCP-Cur6 having the highest concentration of nanocellulose i.e. 0.2% registered particle size of 145.3 nm and 172.5 nm, respectively. The significantly lower curcumin content was observed in samples PNCP-Cur6 and PNCP-Cur3 with 54% and 50.4% drop in curcumin content at 63 ℃ and with 55.1% and 50.4% drop at 95℃, from initial curcumin content of 11.6±0.26 mg/100 ml and 11.5±0.22 mg/100 ml, respectively. Significantly higher curcumin content was retained in PNCP-Cur2 and PNCP-Cur5 where drop of curcumin 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. Curcumin stability was observed to be related to creaming index, PDI, and particle size. The stability of PNCP-Cur2 and PNCP-Cur5 was justified in section 6B.3.2 taking into account the fact that there was no %CMI on day 35 of storage. The most stable formulation was obtained with 0.1% nanocellulose and 0.1% or 0.2% Tween 80. Moreover, PNCP-Cur2 and PNCP-Cur5 also had very small particle size of 131.7 nm and 141.1 nm, respectively and PDI values of 0.119 and 0.238, respectively. Jusril et al. (2022) reported that PDI value <0.25 correlated with more stability on storage. According to Li et al. (2016), curcumin may migrate into hot water because medium-chain triglyceryl oil and curcumin have improved water solubility at higher temperatures. After release of curcumin from nanoemulsion droplets, it gets exposed to oxidation and hydrolysis in the aqueous solution. However, the curcumin content in the nanocellulose-stabilized curcumin-enriched Pickering nanoemulsion blended beverage at 63 ℃ for 30 min and 95 ℃ for 1 min showed no significant difference after treatment. No significant difference in curcumin concentration was observed (6.1±0.15 mg/100 ml and 6.1±0.14 mg/100 ml) at 95 ℃ for PNCP-Cur2 and PNCP-Cur5 having 0.1% nanocellulose and 0.1% and 0.2% Tween 80 concentration. Tween 80 concentration did not show any effect on curcumin degradation of PNCP-Cur2 and PNCP-Cur5 at 95 ℃. The nanoemulsified blended beverage PNCP-Cur1 and PNCP-Cur4 having nanocellulose concentration of 0.05% and 0.1% and 0.2%Tween 80 showed significantly lower curcumin content as compared to PNCP-Cur2 and PNCP-Cur5 at 63 ℃ (Table 6B.2). At 95 ℃, the curcumin content of PNCP-Cur1 (6.2±0.23 mg/100 ml) had significantly higher curcumin content as compared to PNCP-Cur3 (5.7±0.14 mg/100 ml), PNCP-Cur4 (5.5±0.12 mg/100 ml), and PNCP-Cur6 (5.2±0.24 mg/100 ml) (Table 6B.2). No significant difference was observed in PNCP-Cur1, PNCP-Cur2 and PNCP-Cur5 at 95 ℃ (Table 6B.2). Winuprasith and Suphantharika (2015) reported that the droplet size of an emulsion increases with an increase in the concentration of micro-fibrillated cellulose due to its high viscosity and thus poorer mixing of the emulsion. In general, electrostatic interactions, van der Waals forces, hydrophobic interactions, and hydrogen bonding affect Brownian motion in solutions. The strong electrostatic repulsion between CNC particles due to their high surface charges delayed their adsorption at the oil/water interface, due to which nanocellulose alone cannot be used to produce Pickering nanoemulsion (Aw et al., 2022). Sari et al. (2015) also reported that the individual use of stabilizer like Tween 80 and whey protein concentrate-70 did not yield positive results in stability of creaming index. So, the use of nanocellulose along with Tween 80 extended the stability of curcumin-enriched blended beverage. As the storage duration extended, the downward migration of nanocellulose particles caused an increase in optical density. This occurred because intermolecular

interactions cannot keep larger particles balanced (Niu et al., 2017), which is why separation happens in the Pickering nanoemulsion. Further, Aw et al. (2022) reported that the surface coverage by cellulose nanocrystals (CNC) on the droplet interface is poor at low CNC concentrations (i.e., 0.1 to 0.5 g/100 ml), suggesting that the PE is not completely covered by solid particles. Curcumin is transferred more quickly from the oil phase into the aqueous phase when a portion of the oil phase is in contact with curcumin, this accelerates curcumin degradation. Aw et al. (2022) observed that the curcumin stability decreased at 50 ℃ when the CNC concentration increased from 0.1 g/100 ml to 1.5 g/100 ml, suggesting that the rate of curcumin degradation increases linearly with the increase in CNC concentration. For Pickering emulsion stability, the cellulose content should not be excessively high or low. Increase in the concentration of nanocellulose above 0.1% in curcumin-enriched Pickering nanoemulsion blended beverage increased the degradation of curcumin at the treatment temperatures of 63 ℃ and 95 ℃. Yuliarti et al. (2019) reported increased particle size in systems containing CMC and a small amount of pectin. The increase in particle size with increased CMC may be because of a limited depletion flocculation effect.

Table 6B.2. Stability test of curcumin in curcumin-enriched Pickering nanoemulsified blended beverages at two different temperatures

Samples	Curcumin (mg/100 ml)		
	Control	**63 ℃**	**95 ℃**
PNCP-Cur1	10.6 ± 0.76^{bA}	6.4 ± 0.13^{bcB}	6.2 ± 0.23^{aB}
PNCP-Cur2	12.6 ± 0.68^{aA}	7.1 ± 0.22^{aB}	6.1 ± 0.15^{abB}
PNCP-Cur3	11.5 ± 0.22^{abA}	5.7 ± 0.05^{dB}	5.7 ± 0.14^{cB}
PNCP-Cur4	10.6 ± 0.34^{bA}	6.2 ± 0.10^{cB}	5.5 ± 0.12^{cdB}
PNCP-Cur5	12.5 ± 0.62^{aA}	6.6 ± 0.16^{bB}	6.1 ± 0.14^{abB}
PNCP-Cur6	11.6 ± 0.26^{abA}	5.3 ± 0.04^{eB}	5.2 ± 0.24^{dB}

$^{a-c}$ Values with different superscripts vary significantly within a column at $p<0.05$ and $^{A-B}$Values with different superscripts vary significantly within a row at $p<0.05$.

6B.3.4. *In vitro* bioaccessibility of curcumin-enriched Pickering nanoemulsified blended beverages

In order to gain further insight into the influence of the nanocellulose and surfactant concentration on PNCP-Cur1, PNCP-Cur2, PNCP-Cur3, PNCP-Cur4, PNCP-Cur5, and PNCP-Cur6, the *in vitro* lipid digestibility of all curcumin-enriched Pickering nanoemulsified blended beverages was examined using a simulated small intestine model. From Table 6B.3, it was observed that more the lipids in curcumin were digested, the more curcumin was released (). The curcumin content in curcumin-enriched Pickering nanoemulsified blended beverage was significantly ($P < 0.05$) decreased in intestinal phase with nanocellulose concentration of 0.05% and 0.2%. The samples PNCP-Cur2 and PNCP-Cur5 had the highest curcumin content in *in vitro* bioaccessibilty, with values of 62.2±0.33% and 61.4±0.67% respectively, that were not significantly different, as shown in Table 6B.3. This was possibly due to its higher stability as %CMI was not observed in PNCP-Cur2 and PNCP-Cur5 (Fig6B.2). A lower *in vitro* bioaccessibility of curcumin was observed in samples PNCP-Cur1 and PNCP-Cur4 having 0.05% nanocellulose with values of 36.3±0.49% and 48.4±0.38%, respectively. PNCP-Cur3, and PNCP-Cur6 with 0.2% nanocellulose registered *in vitro* bioaccessibility of 51.2±0.27% and 48.2±0.41%, respectively. Among all the samples PNCP-Cur1 had the lowest invitro bioaccessibility ($p<0.05$) with lowest nanocellulose (0.05%) and PNCP-Cur2 and PNCP-Cur5 having the highest *in vitro* bioaccessibility with 0.1% nanocellulose concentration. Winuprasith and Suphantharika (2015) reported that nanocellulose induces the development of porous, multilayered organizations that act as bridges between oil droplets. This process is known as the bridging flocculation phenomenon. The nanocellulose present on the surface of droplet of Pickering nanoemulsified blended beverage prevent the curcumin release in oral and stomach phase of PNCP-Cur2 and PNCP-Cur5. The lowest curcumin content was observed in oral phase of PNCP-Cur2 and PNCP-Cur5 with 0.1% nanocellulose and 0.1% and 0.2% Tween 80 with values of 12.1±0.81% and 12.4±0.52%, respectively. The significantly different and highest curcumin content after digestion in the oral and stomach phases was observed in PNCP-Cur1 with values of 24.06±1.49% and 46.51±0.53%, respectively. The results indicated that the encapsulated curcumin was highly stable in PNCP-Cur2 and PNCP-Cur5, and was not completely released from the delivery system during gastric phase of digestion. Resistance of PNCP-Cur2 and PNCP-Cur5 in oral and stomach phases may be attributed to the resistance of the nanocellulose to oral and gastric phases of digestion. The sample PNCP-Cur2 and PNCP-Cur5 with 0.1% nanocellulose showed significantly lower curcumin release in oral phase, i.e., 12.1±0.81% and 12.5±0.52%, respectively. PNCP-Cur2 and PNCP-Cur5 showed significantly

different and maximum curcumin content in the intestinal phase of digestion with values of 62.2±0.33% and 61.4±0.67%, respectively due to its better stability and no creaming, as specified in section 6B.3.2. According to Ribeiro et al. (2022), Pickering emulsion in the stomach phase faces harsh conditions, namely they contact with gastric fluids in stomach phase characterized by a highly acidic pH and high enzymes content. Due to the better stability of PNCP-Cur2 and PNCP-Cur5 in different pH condition (as reported in section 6B.3.3), release of curcumin in stomach phase was observed to be lower as compared to intestinal phase. Inal et al. (2022) reported that enzymatic lipid digestion occurs only in intestinal phase with bile salts and pancreatin. The fatty acid release during *in vitro* digestion of curcumin-added echium oil nanoemulsion and only echium oil nanoemulsion was 40.9%, and 33.9%, respectively. The amount of curcumin released in the intestinal phase of the PNCP-Cur2 and PNCP-Cur5 was greater than in the stomach phase, which may be because of virgin coconut oil that was used for the encapsulation of curcumin. The increase in curcumin release from PNCP-Cur2 and PNCP-Cur5 could be explained by lipase dispersion between the aqueous phase and emulsion interface as lipase gets activated in intestinal phase by enzymatic reaction. Ahmed et al. (2012) reported that the release of fatty acid was higher for medium chain fatty acid (113%) as compared to long chain fatty acids (68%). The relatively lower amount of fatty acid digestion of long chain fatty acid is due to its property of accumulation at droplet surface, which inhibits lipase digestion of emulsion. Medium chain fatty acid is known to dissolve easily in aqueous phase than long chain fatty acid, and therefore is less likely to accumulate at droplet surfaces and inhibit the digestion of lipids. The VCO used in curcumin encapsulation is rich in medium chain fatty acids and contains nearly 49.3% lauric acid, 0.6% caproic acid, 7.4% caprylic acid, 5.9% capric acid, 18.5% myristic acid, 2.8% palmitic acid, and 6.1% stearic acid (chapter 5, section 5.3.7).

This Pickering nanoemulsion with blended beverage as an aqueous phase effectively increased the stability of the blended beverage, ensuring the encapsulation of curcumin within the beverage system. The strong electrostatic repulsion between CNC particles due to their high surface charges delayed their adsorption at the oil/water interface. According to Aw et al. (2022) addition of salt (sodium chloride) in CNC formulation reduced the electrostatic repulsion between negatively charged nanoparticles of CNC, however the salts present in pepsin digestion used *in vitro* bioaccessibility study may affect the release of curcumin from curcumin-enriched Pickering nanoemulsion blended beverage. According to Ribeiro et al. (2022), in stomach phase the Pickering emulsion faces harsh conditions, namely they contact with gastric fluids in stomach phase characterized by a highly acidic pH and high enzymes content. Due to

which the release of curcumin in stomach phase was more as compared to oral phase in all samples of curcumin-enriched blended beverage. However, the release of curcumin in PNCP-Cur1 was higher in stomach phase (46.5±0.53%) as compared to intestinal phase (28.5±0.77%) with nanocellulose concentration of 0.05%, which may be due to its poor stability, as mentioned in section 6B.3.2. The separation of PNCP-Cur1 started on day 20 with %CMI of 15.3±0.82%. The sample PNCP-Cur2 with 0.1% nanocellulose showed significantly lower amount of curcumin release in the oral phase (12.1±0.81%) and maximum amount in intestinal phase (62.2±0.33%), due to its better stability as specified in section 6B.3.2, with no %CMI. Curcumin release in the beverage samples followed the order: PNCP-Cur1>PNCP-Cur3>PNCP-Cur4>PNCP-Cur6 during the oral phase with 0.05% and 0.2% nanocellulose and 0.1% and 0.2% Tween 80 concentration. Fitri et al. (2022) reported that fibers keep the cluster of oil droplets together when NCC and NFC concentrations increase from 0.05 to 0.20% (w/w). In the NFC-stabilized emulsion, this behaviour was more evident. The NFC formed three-dimensional networks in a continuous aqueous phase to provide functionality. Its diameter was in the nanometre range, whereas its length was several micrometres or longer (Fitri et al., 2022).

Table 6B.3. *In vitro* release of curcumin from curcumin-enriched Pickering nanoemulsified blended beverages

Samples	Oral phase (%)	Stomach phase (%)	Intestinal phase (%)	Bioaccessibility of curcumin (%)
PNCP-Cur1	24.1±1.49^{a}	46.5±0.53^{a}	28.5±0.77^{f}	36.3±0.49^{d}
PNCP-Cur2	12.1±0.81^{d}	34.5±0.45^{d}	63.0±0.20^{a}	62.2±0.33^{a}
PNCP-Cur3	18.7±0.39^{b}	40.3±0.21^{c}	53.4±0.58^{c}	51.2±0.27^{b}
PNCP-Cur4	18.5±0.66^{b}	43.1±0.17^{b}	49.2±0.09^{d}	48.4±0.38^{c}
PNCP-Cur5	12.5±0.52^{d}	29.4±0.46^{e}	61.9±0.17^{b}	61.4±0.67^{a}
PNCP-Cur6	15.1±0.11^{c}	42.1±0.83^{b}	45.3±0.47^{e}	48.2±0.41^{c}

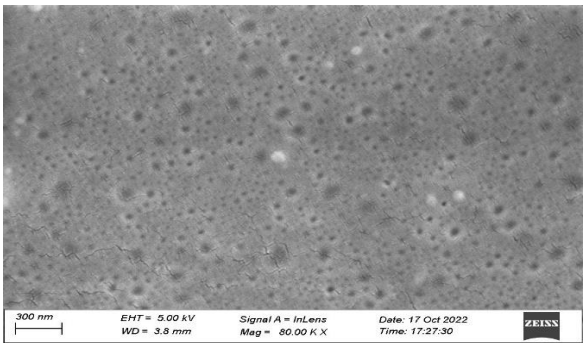
$^{a-f}$ Values with different superscripts vary significantly within a column at P <0.05

6B.3.5. Morphology of curcumin-enriched Pickering nanoemulsified blended beverage during *in vitro* bioaccessibility

Figure 6B.3 shows the field emission scanning electron microscopy (FE-SEM) images of PNCP-Cur2 during the different phases of *in vitro* digestibility. In the initial images of PNCP-Cur2, that are shown in (Fig. 6B.3. a) the droplets were oval or spherical with packed

nanocellulose and Tween 80 layer observed around the oil droplets, confirming the efficient stabilization role of the stabilizers. Liu et al. (2019) reported a similar spherical morphology of Pickering emulsion systems on FESEM images as for PNCP-Cur2 in Fig. 6B.3. a. Jiménez and Capron (2018) reported that distinct aspect ratios of nanocellulose exhibited diverse strategies for stabilising lipid emulsions. In particular, TEMPO-CNFs behaved between CNF and CNC, and cellulose nanocellulose fibre (CNF) with the largest aspect ratio was found to form an interconnected network around lipid droplets, while cellulose nanocellulose crystal (CNC) with the smallest aspect ratio could wrap outside each individual lipid droplet surface (Liu et al., 2019). After exposure to the oral phase of *in vitro* digestibility, no change in shapes of PNCP-Cur2 was observed, where no agglomerates were observed (Fig. 6B.3. b-c). Similar results were reported by Ribeiro et al. (2022) using CLSM. CLSM analysis images of *in vitro* bioaccessibility of vitamin E loaded nanohydroxyapatite Pickering emulsions show no changes in oral phase, no agglomerates and no evident changes in droplet size were observed. In the stomach phase, the PNCP-Cur2 faced stringent conditions, namely they came in contact with gastric fluids characterized by extremely acidic pH. The acquired FE-SEM images (Fig. 6B.3. d-e) revealed that stomach phase is where PNCP-Cur2 underwent some changes. Specifically, it is clear from Fig. 6B.3. (d-e) that PNCP-Cur2 droplet size increased during the stomach phase due to flocculation. Golding and Wooster (2010) explained the reasons for the increase in particle size and floc presence. Firstly, as the emulsions moved from the mouth stage to the stomach stage, the gastrointestinal environment became acidic instead of neutral, which affected the surface charge of the Tween 20-coated oil droplets. Secondly, electrostatic repulsion between droplets was reduced by counter-ions in the simulated gastrointestinal fluid. Thirdly, bridging flocculation with mucin from artificial saliva may result in flocs and a larger particle size. Nanocellulose stabilized PNCP-Cur2 did not exhibit any morphological changes in the oral phase of digestion. Nanocellulose fiber was less efficient as an emulsion stabiliser in the gastric phase than it was in the oral phase. Because nanocellulose fibrils or crystals have larger pore size than the molecules size of lipase or lipid droplet (Liu et al., 2019), nanocellulose did not impede the interaction between lipase and lipid droplets over time. However, nanocellulose along with Tween 80 reduced the diffusion or molecular collision of lipid droplets at a short time range. The change of shape of emulsion droplets in the present study was observed in the intestinal phase (Fig. 6B.3. f-g) due to the breakdown of the outer layer of surfactant and nanocellulose, which is desirable for the release of curcumin. FE-SEM enabled the imaging of PNCP-Cur2 during each digestion phase and allowed us to explore the emulsion stability of curcumin-enriched Pickering nanoemulsion blended beverage upon environmental changes. Fitri et al. (2022) reported that after exposure to the small intestine

phase, the mean particle diameters of all evaluated samples increased significantly ($p < 0.05$). Fat digestion started in the small intestine and some oil phase was removed from the emulsions, causing them to lose dimension. It was found that the biopolymer network's structure affects lipid digestion. Because lipid droplets can disperse in liquid or embed in big particles or gels, insight of the fiber's microstructure and that of the droplets during each stage of digestion is crucial to comprehending the transformation and possible interactions between the emulsions and the nanocellulose.



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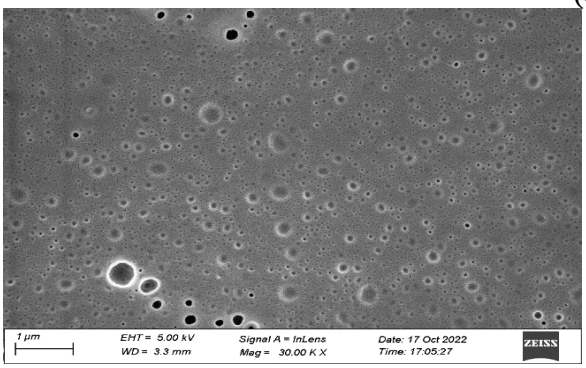

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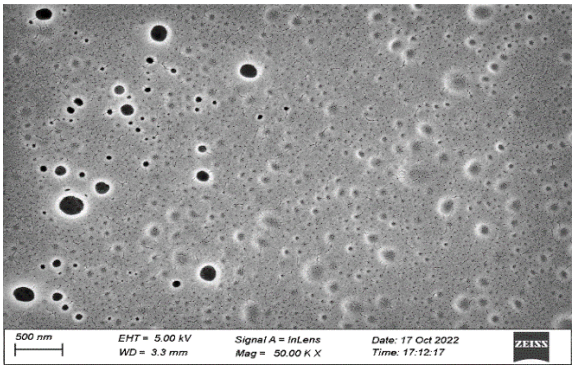

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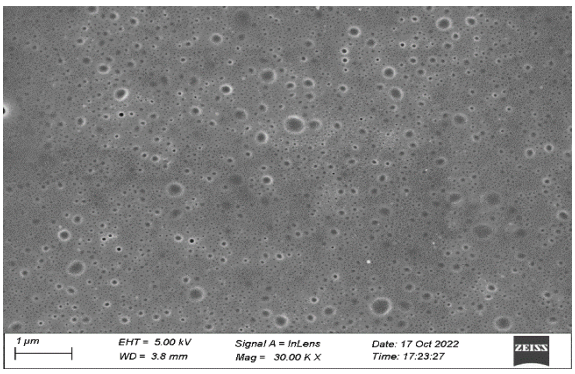

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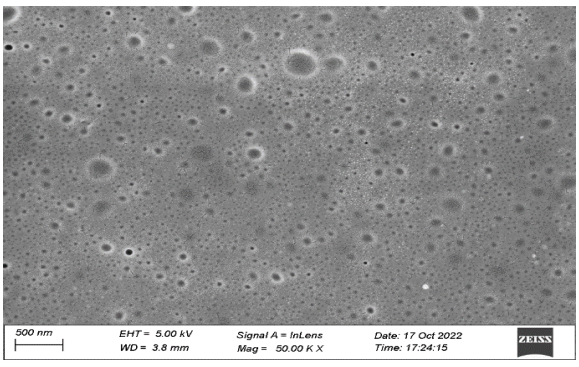

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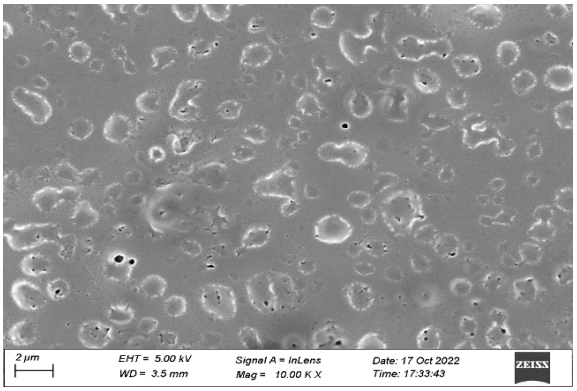
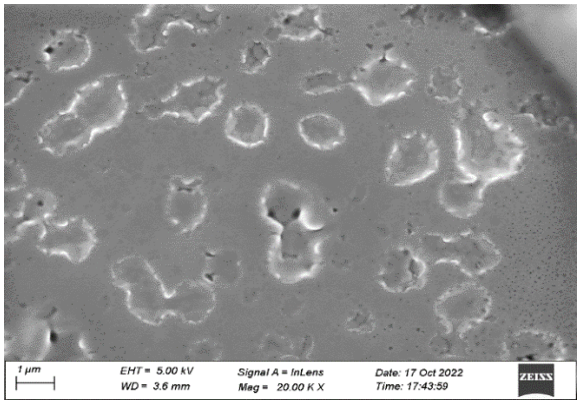


(f)



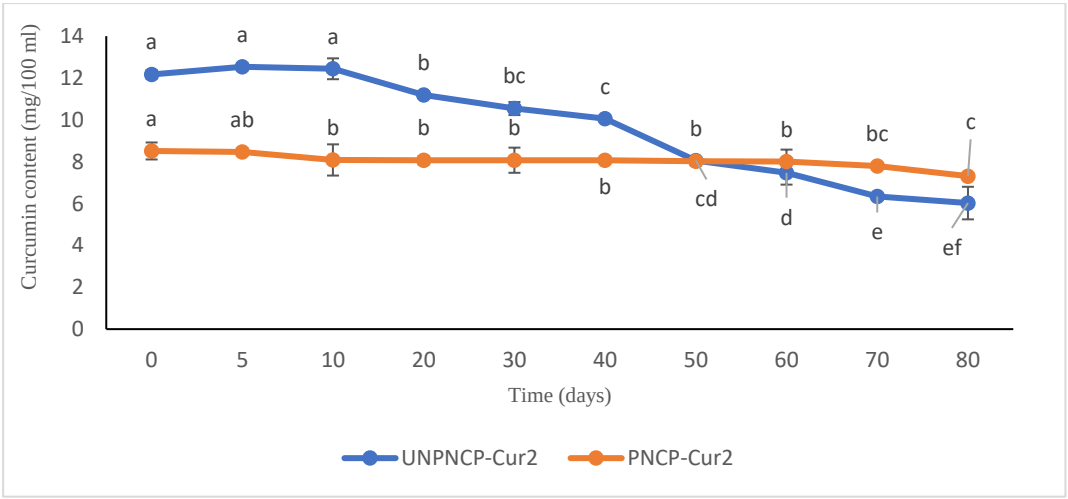
(g)

Fig 6B.3: FE-SEM micrographs of curcumin-enriched Pickering nanoemulsified blended beverage in nanometre range and micrometre range: (a) PNCP-Cur2; (b & c) PNCP-Cur2 in oral phase; (d & e) PNCP-Cur2 in gastric phase; (f & g) PNCP-Cur2 in intestinal phase.

6B.3.6. Effect of pasteurization on curcumin stability of curcumin-enriched Pickering nanoemulsified blended beverages

The stability of curcumin in food systems is very low and therefore, the degradation of curcumin during storage was evaluated. To ascertain the curcumin stability, the curcumin-enriched blended beverage that had the maximum concentration of curcumin throughout the *in vitro* bioaccessibility study, i.e., PNCP-Cur2 was stored for 80 days at 25±2 ℃ and 4±2 ℃. The effect of pasteurization was determined for curcumin stability in curcumin-enriched blended beverage with pasteurization (PNCP-Cur2) and without pasteurization (UNPNCP-Cur2). The initial curcumin content before pasteurization was 12.1±0.20 mg/100 ml and after pasteurization significantly reduced to 8.5±0.40 mg/100 ml. Li et al. (2016) reported that curcumin may migrate into hot water because medium-chain triglyceryl oil and curcumin improve water solubility at higher temperatures. Release of curcumin from nanoemulsion droplets exposes curcumin to oxidation and hydrolysis in the aqueous solution. As shown in Fig. 6B.4. (a-b), during storage, degradation of curcumin without pasteurization was more evident at room temperature and at refrigeration temperature. On day 80 of storage, the curcumin content in UNPNCP-Cur2 and PNCP-Cur2 was 6.0±0.77 mg/100 ml and 7.3±0.13 mg/100 ml, respectively at 25±2 ℃. The curcumin content at 4±2 ℃ of UNPNCP-Cur2 and PNCP-Cur2 was 8.4±0.08 mg/100 ml and 8.0±0.01 mg/100 ml, respectively, on day 80 of storage. Percentage drop of curcumin in UNPNCP-Cur2 and PNCP-Cur2 at 25±2 ℃ storage temperature was 44.3% and 14.1%, and 47.1% and 3.2% at 4±2 ℃ storage temperature, respectively. The curcumin degradation in UNPNCP-Cur2 was more as compared to PNCP-

Cur2 during storage, which may be attributed to microbial or physicochemical changes in UNCP-Cur2 during storage. However, curcumin is a naturally occurring polyphenol, its use in food preservation is restricted by photosensitization, pH instability, hydrophobicity, and heat sensitivity (Lan et al., 2023). Thermal pasteurization has proved to be a suitable method to reduce the microbial spoilage in food (Peng et al., 2017) Curcumin along with pasteurization reduced the microbial growth during storage of PNCP-Cur2 (section 6B.3.6). The storge stability of PNCP-Cur2 was better in terms of curcumin stability as compared to non-pasteurized curcumin enriched blended beverage. Aditya et al. (2015) reported that curcumin-loaded emulsions showed 91% and 87% curcumin stability after 15 days at 4 °C and 23 °C, respectively. When these emulsions were immediately placed in a model beverage system, a notable difference was observed. Approximately 40% of the curcumin remained stable at 4 °C and 23 °C, which was much less than the curcumin stability observed in the emulsion. The findings of Aditya et al. (2015) suggest that several variables, including temperature, co-excipients, and formulation stability, may have a direct or indirect impact on stability. Baek et al. (2020) reported that the stability of oil-soluble antioxidant compounds like β-carotene in nanoemulsions depends on the composition of the oil and surrounding environmental factors, such as exposure to UV radiation and high temperatures during storage. The curcumin degradation in unpasteurized curcumin-enriched blended beverage was more as compared to pasteurized curcumin-enriched blended beverage, may be the effect of microbial and physicochemical changes during storage.



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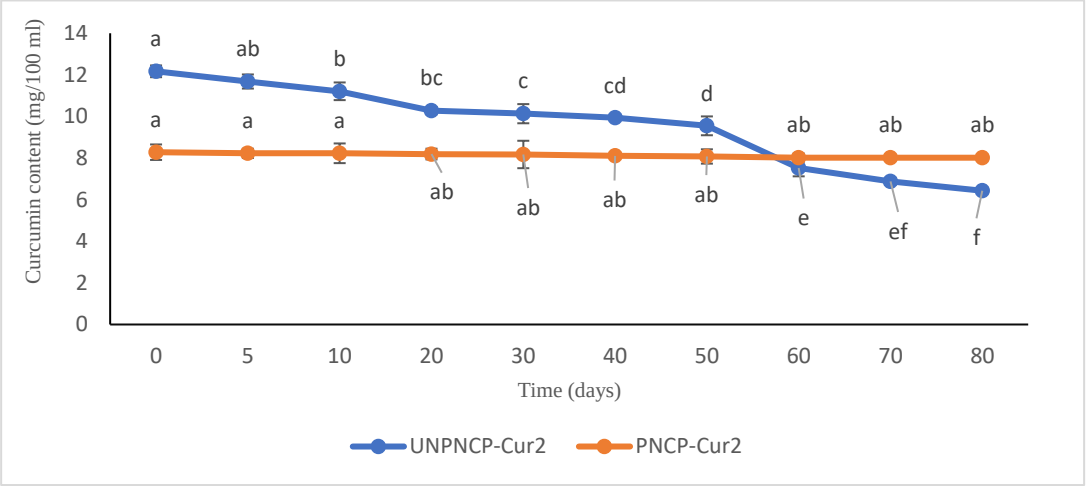


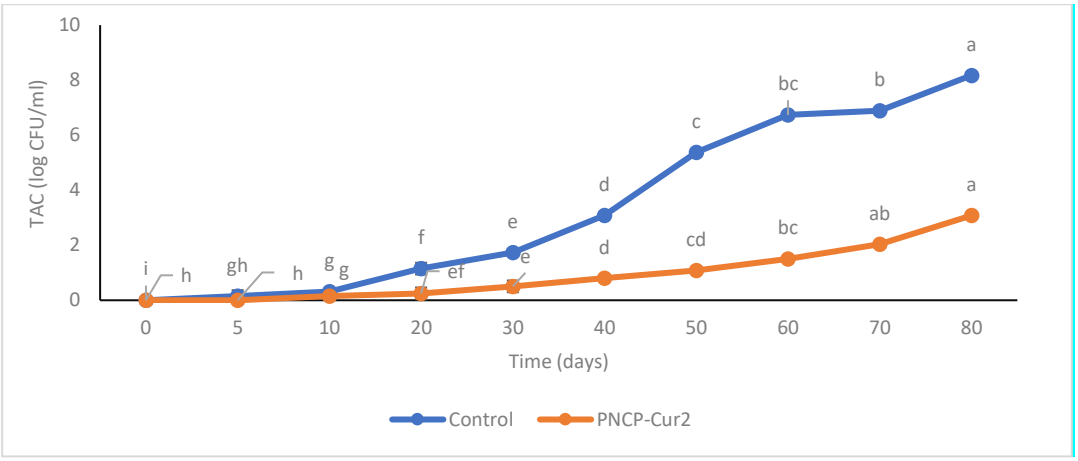
(b)

Fig 6B.4: Effect of pasteurization at (a) 25±2 ℃ and (b) 4±2 ℃ temperature on curcumin content in UNPNCP-Cur2 (curcumin-enriched Pickering nanoemulsified blended beverage without thermal pasteurization) and PNCP-Cur2 (curcumin enriched Pickering nanoemulsified blended beverage with thermal pasteurization).

6B.3.7. Effect of processing treatments on microbial load of curcumin-enriched Pickering nanoemulsified blended beverage during storage

The results of the bacteriological analysis of pasteurized blended beverage without curcumin (Control) and pasteurized blended beverage with curcumin and nanocellulose (PNCP-Cur2) are presented in Fig 6B.5. (a-b) and Fig. 6B.6 (a-b). After pasteurization, the Control sample had 0.15 log CFU/ml total aerobic count (TAC) and 0.03 log CFU/ml yeast and mould count (YMC) on day 5 of storage at 25±2 ℃. In comparison, the TAC load of the Control and PNCP-Cur2 registered no growth on day 5 of storage at 4±2 ℃. PNCP-Cur2 did not exhibit any YMC growth until day 60, while Control did not exhibit any YMC growth till day 10 of storage at 4±2 ℃. However, on day 80 of storage, the Control and PNCP-Cur2 sample showed TAC of 8.17±0.04 log CFU/ml and 3.08±0.02 log CFU/ml at 25 ℃, and 5.65±0.1 log CFU/ml and 0.77±0.1 log CFU/ml at 4 ℃, respectively. These results clearly indicated that bacterial growth was greater in Control than in PNCP-Cur2. The YMC of Control and PNCP-Cur2 on day 80 of storage was 5.23±0.5 log CFU/ml and 2.26±0.1 log CFU/ml at 25 ℃, 5.88±0.1 log CFU/ml and 0.50±0.6 log CFU/ml at 4±2 ℃ respectively. Mane et al. (2019) reported that after packaging, the produced turmeric-orange RTS beverage bottles were pasteurized, which decreased the microbial load during storage and extended the beverage shelf life. Citric acid also serves as a preservative by making prepared RTS beverages more acidic, which creates an environment that is unfavourable for the development of microorganisms. As per the FSSAI

microbial standard for pasteurized juice, aerobic plate count should not be more than $1x10^4$ /ml, and yeast and mould count not more than $1x10^3$/ml.



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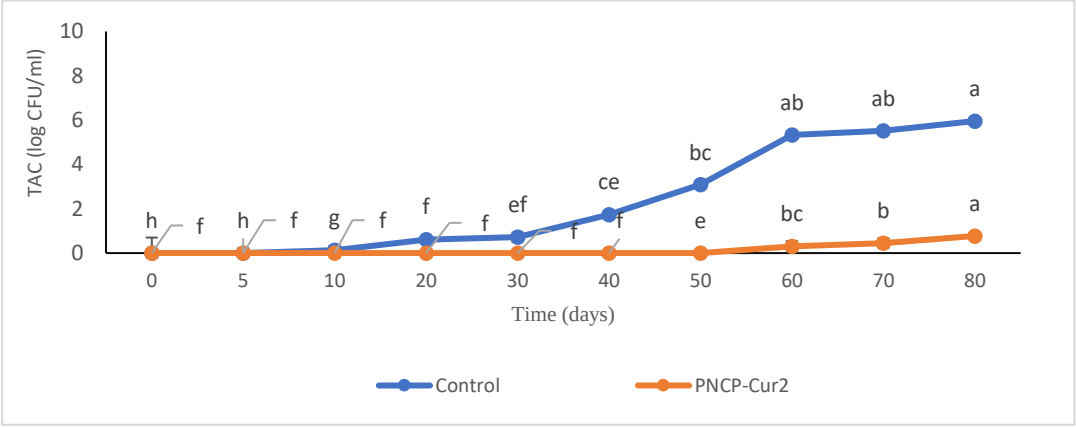
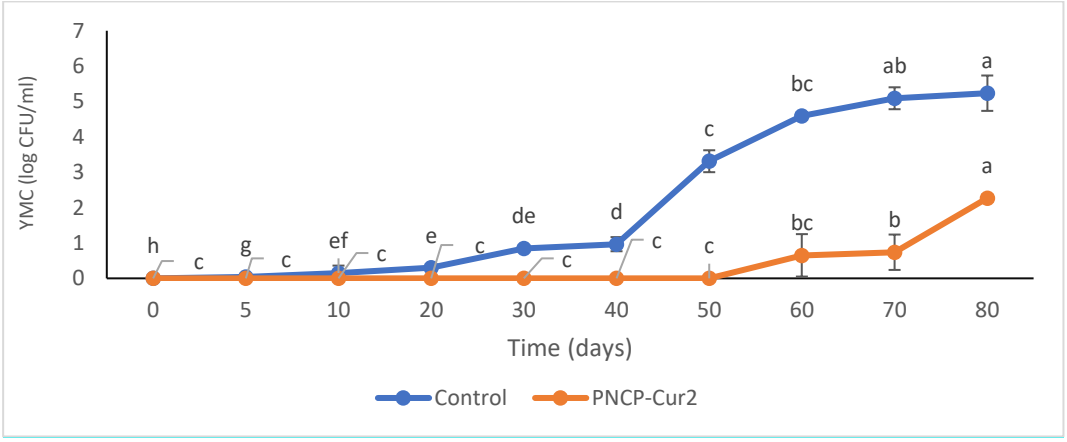


(b)

Fig 6B.5: Effect of curcumin on TAC of Control (pasteurized blended beverage C50:P50 without curcumin) and PNCP-Cur2 (pasteurized curcumin-enriched Pickering nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature

It can be seen from Fig. 6B.5 and Fig. 6B.6 that as the storage period at 25±2 ℃ increased, a gradual increasing trend was observed in the number of total aerobic counts, which increased in both control and PNCP-Cur2, but the sample without curcumin showed more microbial growth than the sample with curcumin. The TAC of blended beverage with curcumin in it did not exceed the maximum limit set by FSSAI. No evidence of yeast or mould growth was observed in PNCP-CUR2 up to day 50 of storage at 25±2 ℃ temperature. However, some yeast and mould growth were observed at 2.267 log CFU/ml on day 80, which was within the maximum limit recommended by FSSAI. As a natural food preservative with effective broad-spectrum antibacterial and antioxidant capabilities, curcumin is playing a crucial role in

preservation of food (Lan et al., 2023). Mane et al. (2019) reported that during the entire storage period at 25±2 ℃ and 4±2 ℃, there was no any evidence of yeast and mould, or coliform in their prepared beverage (fresh turmeric rhizome-based orange RTS), and hence, it could be stored without any detrimental changes for up to 90 days. However, there was a gradual increase in the total plate count, but below the limit specified by the FSSAI. Chia et al. (2012) reported that heat pasteurization reduced overall plate count and the counts of yeast and mould, resulting in a longer shelf life. Mwangi et al. (2019) also reported the unpasteurized juice increased in bacteria counts by 3 logs after being kept at 20°C for a day. The bacteria count in carrot juice surged tenfold even when refrigerated. At 20°C, the addition of curcumin (0.4 mM) in the form of curcumin-methyl-β-cyclodextrin inclusion complex stopped the growth of microorganisms in the juice. Due to encapsulation of curcumin in blended beverage (PNCP-Cur2), the antimicrobial property of beverage might have increased (Mwangi, et al., 2019).



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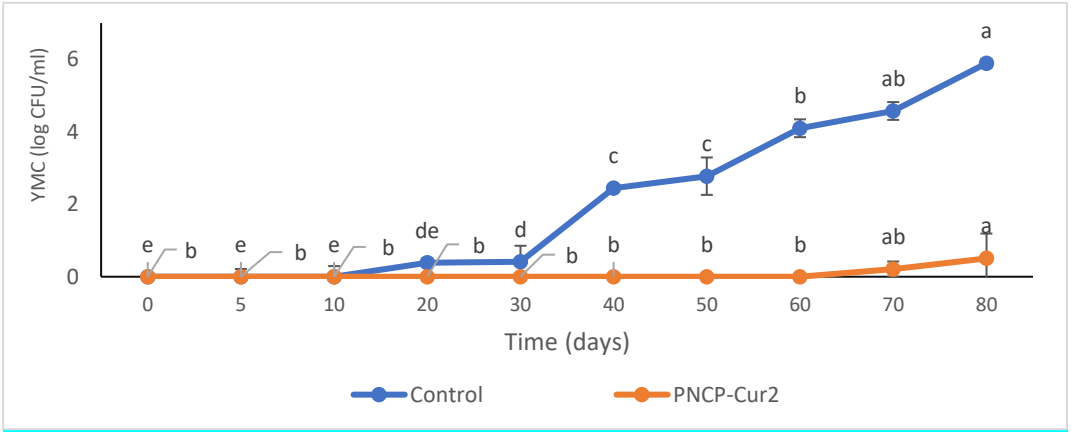


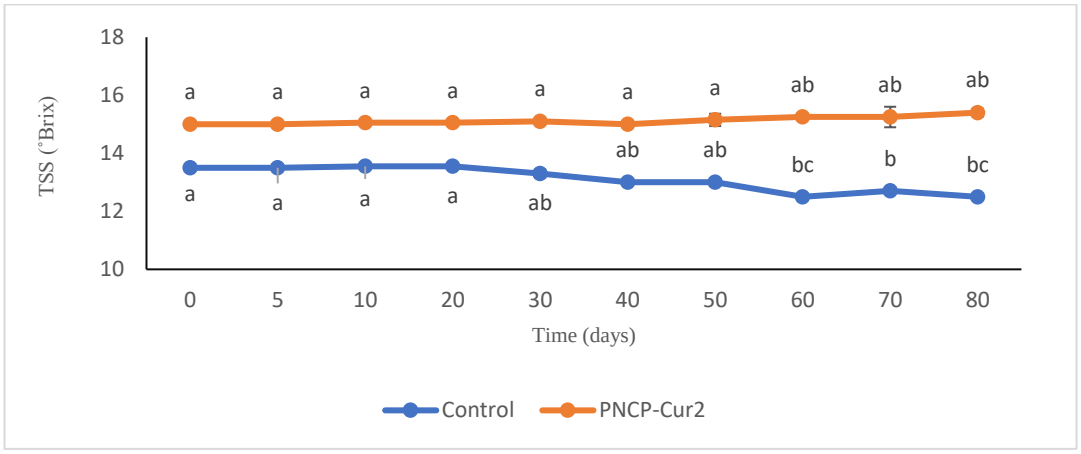
(b)

Fig 6B.6: Effect of curcumin on YMC of Control (pasteurized blended beverage C50:P50 without curcumin) and PNCP-Cur2 (pasteurized curcumin-enriched Pickering nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

6B.3.8. Effect of processing treatments on physicochemical properties of curcumin-enriched Pickering nanoemulsified blended beverage during storage

Fig. 6B.7 shows the effect of storage time on the TSS and pH values of curcumin-enriched Pickering nanoemulsified blended beverages. The initial TSS value of Control and PNCP-Cur2 was 13±0.07 ºBrix and 15±0.14 ºBrix, and on 80 days of storage at 25±2 ℃ the TSS value was 12.5±0.35 ºBrix and 15.4±0.07 ºBrix, respectively. No significant changes in TSS were observed in pasteurized PNCP-Cur2 sample during storage period of 80 days at 25±2 ℃. Chia et al. (2012) reported that the thermally pasteurised pineapple juices did not show significant changes in TSS during storage of 13 weeks. However, in the Control sample, TSS significantly decreased with storage time. According to Rivas et al. (2006), the bacteria that cause the fruit juice to deteriorate as a result of sugar fermentation is responsible for the change in total soluble solids. The method by which bacteria cause fermentation is the breakdown of glucose through biochemical pathway. On the other hand, on storage at 4±2 ℃, the TSS value decreased in Control (12.3±0.07 ºBrix) and no significant change was observed for PNCP-Cur2 (15.2±0.12 ºBrix), as shown in Fig. 6B.7.b on day 80 of storage.

The initial pH values of the Control and PNCP-Cur2 samples was 3.5±0.21 and on 80 day of storage was 2.0±0.07 and 3.5±0.13, respectively. The PNCP-Cur2 sample showed no significant difference in pH at 25±2 ℃ storage temperature. The pH of the Control and PNCP-Cur2 samples at 4±2 ℃ before storage was 3.5±0.01, whereas on 80 day of storage 4.5±0.01 and 3.6±0.21, respectively. The pH increase in Control can be related to TSS decrease, illustrated in Fig. 6B.7 and Fig. 6B.8. The TSS decrease may be due to the microorganisms that cause juice spoilage (Chia et al., 2012). Shukla et al. (2017) also reported that the pH of pasteurized mango-based dairy beverage decreased from 6.57 to 5.99 within 10 days of storage at 5 ℃. The sugar present in beverage, may be a source of microbial spores. During storage, these spores and the bacteria converted the reducing sugars into acid. Igual et al. (2010) had reported that in pasteurized grape juice, the °Brix and pH remained stable during storage for 60 days at 4 ℃, indicating that the juice was not affected by storage time at low temperature.



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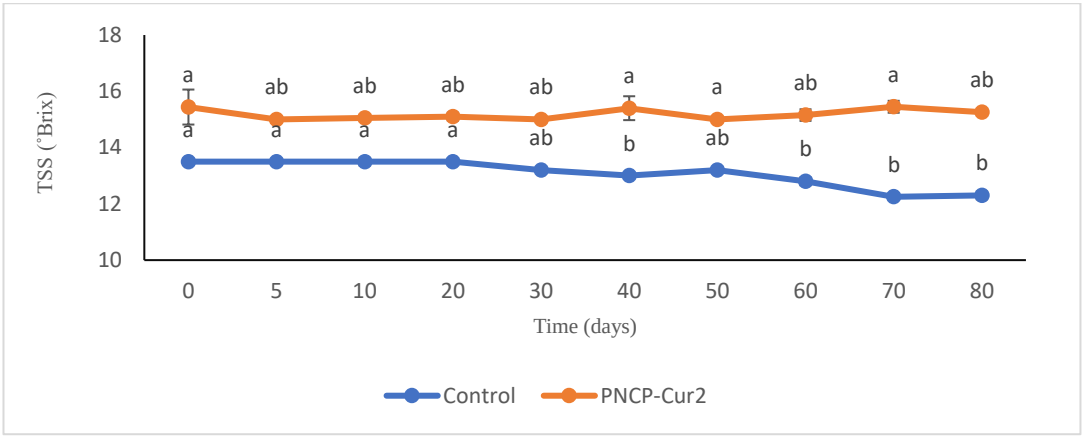
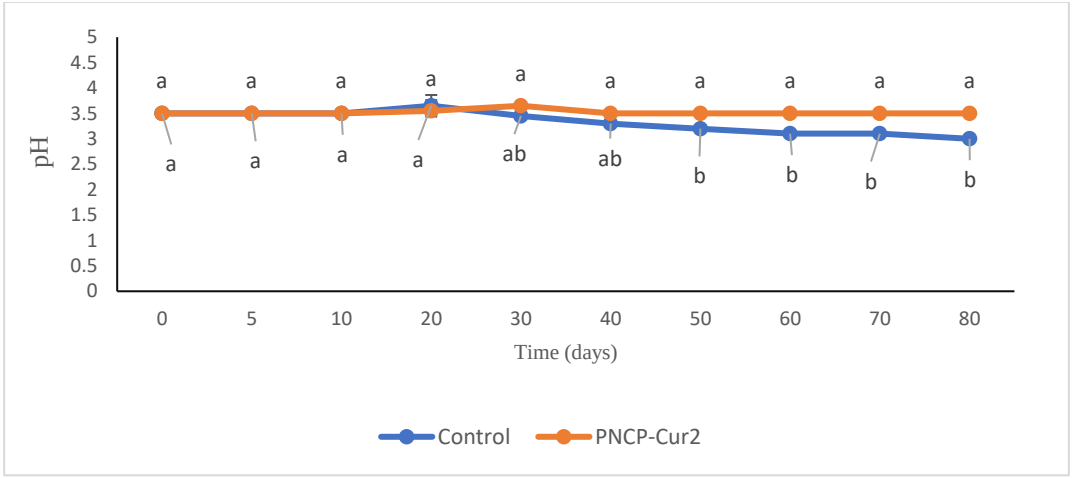


(b)

Fig 6B.7: Effect of curcumin on TSS of Control (pasteurized blended beverage C50:P50 without curcumin) and PNCP-Cur2 (pasteurized curcumin-enriched Pickering nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.



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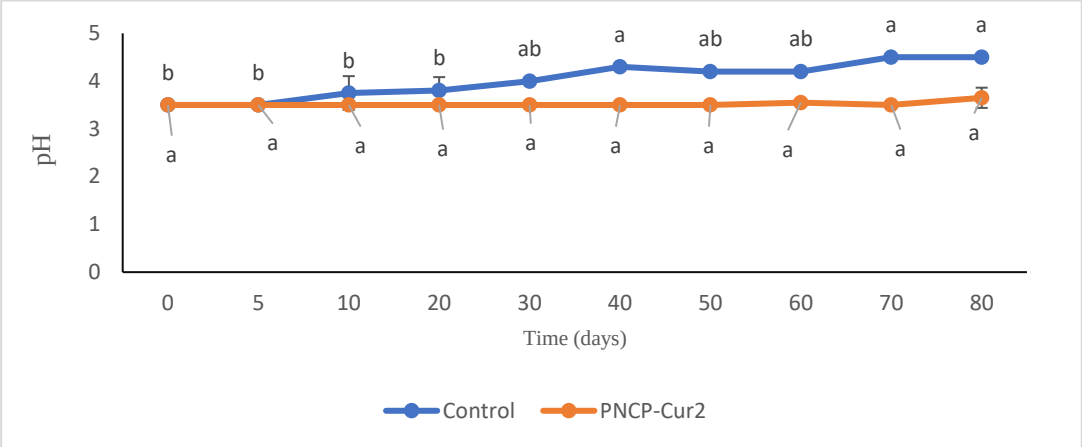


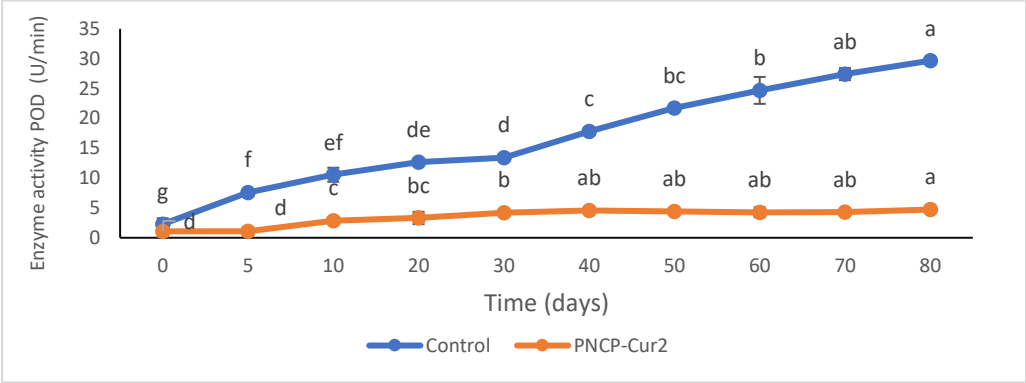
(b)

Fig 6B.8: Effect of curcumin on pH of Control (pasteurized blended beverage C50:P50 without curcumin) and PNCP-Cur2 (pasteurized curcumin-enriched Pickering nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

6B.3.9. Effect of processing treatments on POD activity curcumin-enriched Pickering nanoemulsified blended beverage during storage

The initial POD at 25±2 ℃ of Control and PNCP-Cur2 after pasteurization was 2.28±0.98 U/min and 1.07±0.96 U/min, respectively, and on 80 days of storage significantly increased to 29.66±0.14 U/min and 4.72±0.22 U/min, respectively. The initial POD activity at 4±2 ℃ of Control and PNCP-Cur2 was 2.45±0.32 U/min and 1.68±0.20 U/min, respectively, and on day 80 of storage was 28.45± 0.13 U/min and 3.34± 0.28 U/min, respectively (Fig 6B.9. a-b). The POD activity significantly increased in Control and PNCP-Cur2, but Control has higher POD activity as compared to PNCP-Cur2. POD gets regenerated with time and causes browning and oxidation of natural compounds present in juice (Shaik and Chakraborty, 2023). POD is a significant food quality-related enzyme that has been linked to alterations in the nutritional value, color, and flavour of food (Tao et al., 2019). The POD activity of PNCP-Cur2 was lower as compared to the Control. At 25±2 ℃, Control and PNCP-Cur2 samples showed 12 and 3.4-fold increase in POD activity, respectively on day 80 of storage. The POD activity increases for Control and PNCP-Cur2 samples at 4±2 ℃ was 10.6 and 0.98-fold on day 80 of storage. The study results revealed that the POD of PNCP-Cur2 at 25±2 ℃ was higher as compared to 4±2 ℃ during the entire period of storage. Addition of curcumin and increasing its concentration in PNCP-Cur2 (12.6 mg/100ml) as compared to CP-Cur (0.95 mg/100 ml from Chapter 6A) along with thermal pasteurization had positive effects on reducing the POD activity of blended beverages PNCP-Cur2. The peroxidase activity increase in CP-Cur was 5.5 and 4.3-fold on day 80 of storage at 25±2 ℃ and at 4±2 ℃, respectively (section 6A, subsection 6A.3.4). Curcumin as a

natural food preservative with effective broad-spectrum antibacterial qualities is crucial to the preservation of food (Lan et al., 2023). Deng et al. (2023) reported that curcumin is a promising therapeutic agent due to its natural antioxidant properties and strong anti-inflammatory and antimicrobial functions. The authors opined that a possible explanation for curcumin ability to suppress enzymatic browning is because it increases antioxidant capacity.



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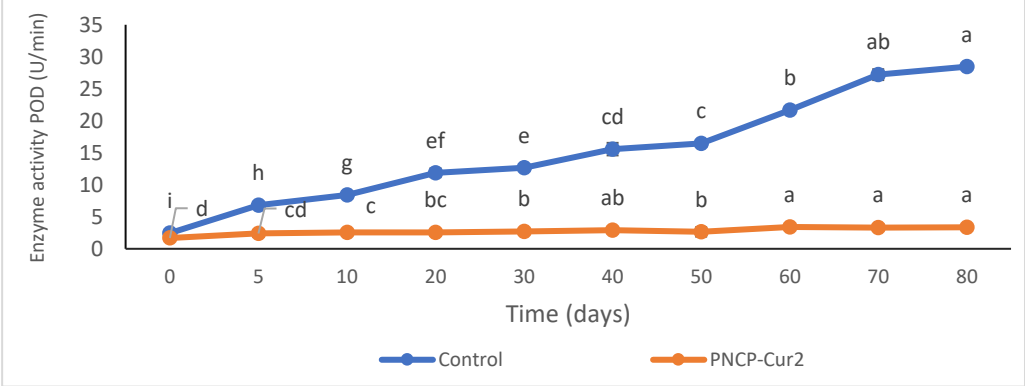


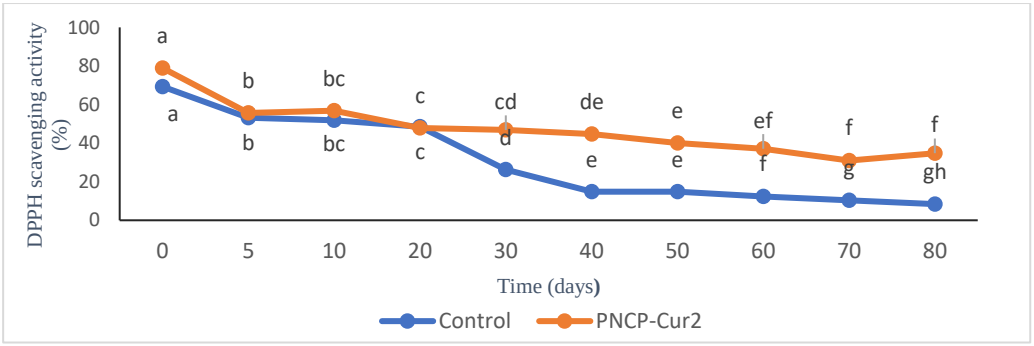
(b)

Fig 6B.9: Effect of curcumin on POD of Control (pasteurized blended beverage C50:P50 without curcumin) and PNCP-Cur2 (pasteurized curcumin-enriched Pickering nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

6B.3.10. Effect of processing treatments on antioxidant properties curcumin-enriched Pickering nanoemulsified blended beverage during storage

The total antioxidant activity that assessed through DPPH radical scavenging activity assay of defatted coconut milk and pineapple juice blends at different treatment conditions like without curcumin (Control) and with curcumin (PNCP-Cur) after pasteurization. The initial DPPH scavenging activity was 69.2± 0.75% and 78.9± 0.11%, respectively and after 80 days of storage at 25±2 ℃ was 8.4±0.49% and 34.7±1.88%, respectively, registering a drop of 87.8% and 56%. The antioxidant activity decreased significantly during the storage period. Correspondingly, initial DPPH scavenging activity of Control and PNCP-Cur was 69.1±0.79%

and 78.7±0.12% at 4±2 ℃. The DPPH activity of Control and PNCP-Cur was 10.0±0.38% and 50.4±0.20% on 80 days of storage at 4±2 ℃, suggesting a drop by 85.5% and 35.9% (Fig. 6B.10. a-b). Amanina et al. (2019) reported that the DPPH activity of pineapple-mango juice blends after thermal treatment was considerably reduced after 9 weeks of storage. No significant difference in DPPH activity was observed during the refrigeration storage (4±2 ℃) from day 10 to day 70 of storage of PNCP-Cur2. The DPPH of PNCP-Cur2 dropped from initial value of 78.9% to 55.7% on day 5 of storage at 25±2 °C. During storage at 25±2 °C, change in DPPH activity in PNCP-Cur2 was insignificant from day 5 to day 10. Khaksar et al. (2019) reported that on day 6 of storage, the DPPH activity values of pineapple, guava, carrot, and white dragon juices drastically decreased at RT (~28 ℃). whereas no significant difference in DPPH activity was observed during the refrigeration storage (4±2 ℃) on day 6 of storage by the authors. DPPH activity of PNCP-Cur2 significantly decreased with storage time at 25±2 °C and on day 80 the activity was 34.7±1.88%. During the entire storage period at both temperatures, DPPH activity of PNCP-Cur2 remained higher than the Control sample, which indicated that the antioxidant property of curcumin was retained to a great extent.



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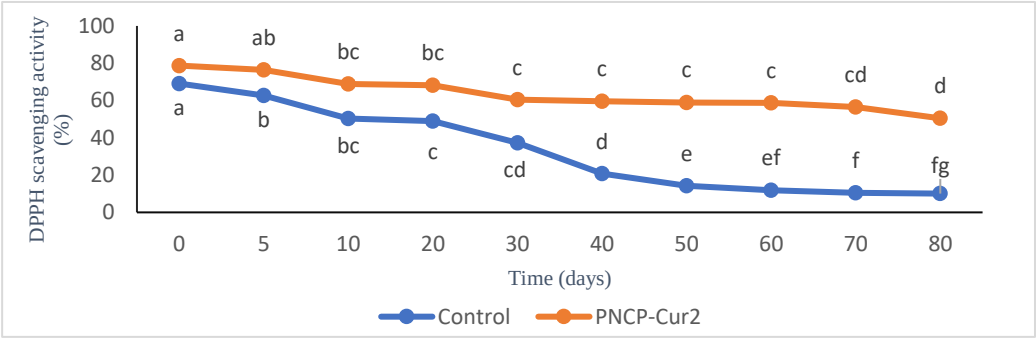


(b)

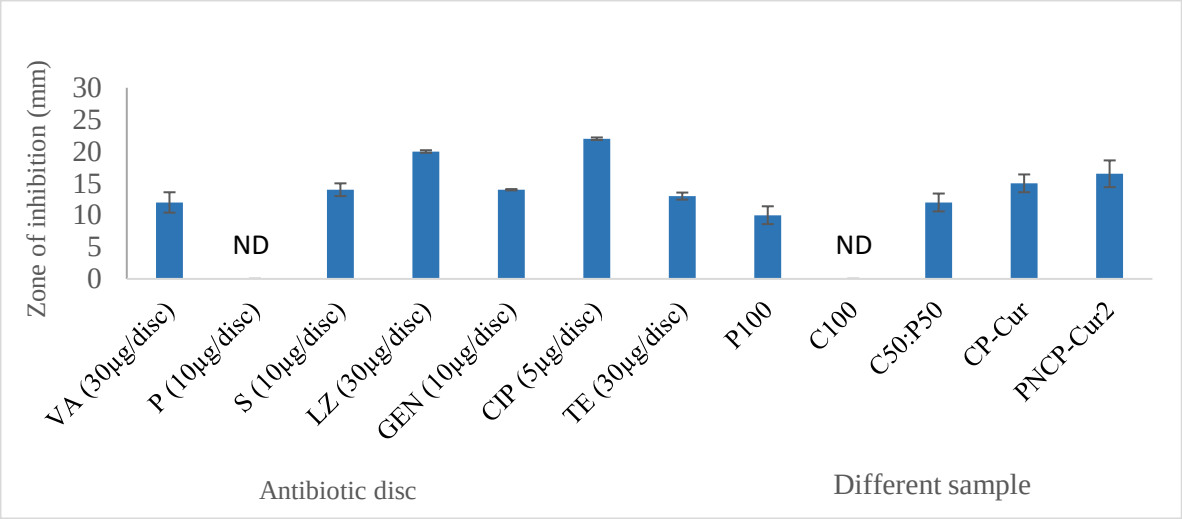
Fig 6B.10: Effect of curcumin on DPPH free radical scavenging activity of Control (pasteurized blended beverage C50:P50 without curcumin) and PNCP-Cur2 (pasteurized curcumin-enriched Pickering nanoemulsified blended beverage) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

6B.3.11. Antimicrobial activity blended beverages

Impact of antibiotics and curcumin-enriched blended beverage on the growth of *E. coli* and *S. aureus*

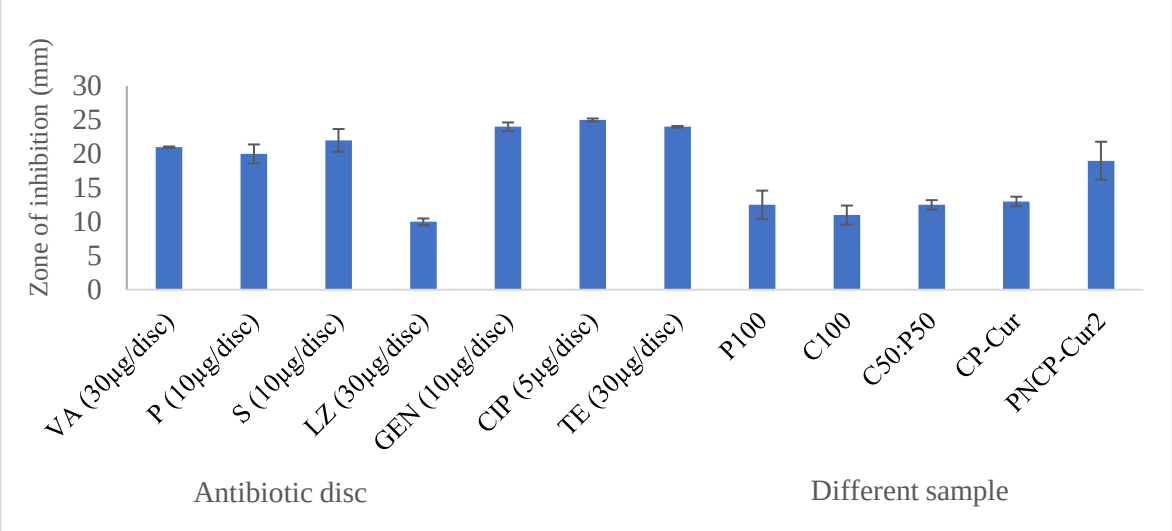
Fig 6B.11 presents the antimicrobial susceptibility of blended beverage and curcumin-enriched blended beverages samples for *E. coli.* From the antibiotic susceptibility patterns, *E. coli* was found to be susceptible to Vancomycin (VA), Streptomycin (S), Linezolid (LZ), Gentamycin (GEN), Ciprofloxacin (CIP), and tetracycline (TE), and the zones of inhibition was 12±1.6 mm, 14±1.0 mm, 20±0.2 mm, 14±0.1 mm, 22±0.2 mm, 13±0.5 mm, respectively as shown in Fig.6B.11. It was also observed that P100, C50:P50, CP-Cur, and PNCP-Cur2 were susceptible to *E. coli,* and the zones of inhibition were 10.0±1.4 mm, 12.0±1.4 mm, 15.0±1.4 mm, and 16.5±2.1mm; however, C100 was resistant as it did not show any zone of inhibition against *E. coli*. The maximum inhibition zone was observed for PNCP-Cur2>CP-Cur>C50:P50>P100, indicating that the presence of curcumin provided inhibitory property against *E. coli*. Hussain et al. (2022) reported that curcumin has antimicrobial properties.

Staphylococcus aureus was found to be susceptible to the following antibiotics: vancomycin (VA), Penicillin (P), Streptomycin (S), Linezolid (LZ), Gentamycin (GEN), Ciprofloxacin (CIP), and tetracycline (TE), and the zones of inhibition were 21±0.1 mm, 20±1.4 mm, 22±1.6 mm, 10±0.5 mm, 24±0.6 mm, 25±0.2 mm, and 24±0.1 mm, respectively (Fig.6B.12). For *S. aureus* sample, the zones of inhibition of P100, C100, C50:P50, CP-Cur, and PNCP-Cur2 were 12.5±2.1 mm, 11.0±1.4 mm, 12.5±0.7 mm 13.0±0.7 mm, and 19.0±2.8 mm, respectively. Singh et al. (2016) observed the antimicrobial activities of whey-based fermented soy beverages supplemented with curcumin, and found maximum inhibitory zone against *E. coli*, followed by *S. aureus*. When the curcumin concentration was increased, the inhibition zone spanned from15–24 mm. Curcumin, a naturally occurring phenylpropanoid derivative that is edible and GRAS (generally recognised as safe), has drawn interest as a possible "green" antibacterial agent (Mwangi, et al., 2019). The present study reiterates the antimicrobial efficiency of curcumin contained in blended beverages CP-Cur and PNCP-Cur2 in nanoemulsified form. The curcumin-enriched beverages could inhibit the growth of *E. coli* and *S. aureus*.



VA- Vancomycin, P- Penicillin, S- Streptomycin, LZ- Linezolid, GEN-Gentamycin, CIP-Ciprofloxacin, TE- Tetracycline, ND- Not detected.

Fig 6B.11: Zone of inhibition for *E. coli* growth by antibiotics and curcumin-enriched blended beverage.



VA- Vancomycin, P- Penicillin, S- Streptomycin, LZ- Linezolid, GEN-Gentamycin, CIP-Ciprofloxacin, TE- Tetracycline.

Fig 6B.12: Zone of inhibition for *Staphylococcus aureus* growth by antibiotics and curcumin-enriched blended beverage.

6B.4. Conclusion

This study showed that physically stable curcumin-enriched Pickering nanoemulsified blended beverage can be prepared using 0.1% nanocellulose and 0.1% Tween 80. The curcumin-enriched blended beverages stabilized with nanocellulose and Tween 80 provided good encapsulation property, had small droplet size, showed long-term stability, and demonstrated good *in vitro* bioaccessibility. The microbial stability of PNCP-Cur2 (curcumin-enriched) was

better than that of blended beverages without curcumin. The results showed that PNCP-Cur2 was able to restrict microbial growth because of which total aerobic count (TAC) and yeast and mould count (YMC) during storage at 25±2℃ and 4±2℃ remained below FASSI specifications. Using a blended beverage as the aqueous phase increased the concentration of curcumin that could be incorporated (~12.63 mg/100ml). In section 6A, only 10% of the curcumin-enriched Pickering nanoemulsion was added to a blended beverage and the nanoemulsified beverage (CP-Cur) contained ~0.95 mg/100 ml of curcumin. Due to the increase in the concentration of curcumin in PNCP-Cur2 the storage properties of PNCP-Cur2 also improved. PNCP-Cur2 registered lower microbial load, higher antioxidant property, reduced peroxidase activity and better physicochemical properties as compared to CP-Cur. The *in vitro* bioaccessibility of PNCP-Cur2 was superior to CP-Cur, with greater amount of curcumin being released in the intestinal phase of digestion. These results have important implications for the design of functional blended beverage of defatted coconut milk and pineapple juice to encapsulate and release curcumin in intestinal phase and increase the storage stability of blended beverages due to the antimicrobial and antioxidant property of curcumin in it.

Bibliography

Aditya, N. P., Aditya, S., Yang, H. J., Kim, H. W., Park, S. O., Lee, J., and Ko, S. (2015). Curcumin and catechin co-loaded water-in-oil-in-water emulsion and its beverage application. *Journal of Functional Foods*, *15*:35–43.

Adsare, S. R., and Annapure, U. S. (2021). Microencapsulation of curcumin using coconut milk whey and Gum Arabic. *Journal of Food Engineering*, *298*:110502.

Ahmed, K., Li, Y., McClements, D. J., and Xiao, H. (2012). Nanoemulsion-and emulsion-based delivery systems for curcumin: Encapsulation and release properties. *Food Chemistry*, *132*(2):799–807.

Alzate-Arbeláez, A. F., Dorta, E., López-Alarcón, C., Cortés, F. B., and Rojano, B. A. (2019). Immobilization of Andean berry (Vaccinium meridionale) polyphenols on nanocellulose isolated from banana residues: A natural food additive with antioxidant properties. *Food Chemistry*, *294*:503–517.

Amanina, A. K. Z., Rosnah, S., Noranizan, M. A., and Alifdalino, S. (2019). Comparison of UV-C and thermal pasteurisation for the quality preservation of pineapple-mango juice blend. *Food Research*, *3*:362–372.

Aw, Y. Z., Lim, H. P., Low, L. E., Singh, C. K. S., Chan, E. S., and Tey, B. T. (2022). Cellulose nanocrystal (CNC)-stabilized Pickering emulsion for improved curcumin storage stability. *LWT-Food Science and Technology*, *159*:113249.

Baek, E. J., Garcia, C. V., Shin, G. H., and Kim, J. T. (2020). Improvement of thermal and UV-light stability of β-carotene-loaded nanoemulsions by water-soluble chitosan coating. *International Journal of Biological Macromolecules*, *165*:11561–163.

Baljeet, S. Y., Ritika, B. Y., and Sarita, R. (2013). Studies on development and storage of whey-based pineapple (Ananas comosus) and bottle gourd (Lagenaria siceraria) mixed herbal beverage. *International Food Research Journal*, *20*(2).

Bavisetty, S. C. B., and Venkatachalam, K. (2021). Physicochemical qualities and antioxidant properties of juice extracted from ripe and overripe wax apple as affected by pasteurization and sonication. *Journal of Food Processing and Preservation*, *45*(6):e15524.

Benaissa, Y., Addou, S., Dib, W., Benhatchi, S., Mehidi, A., Kheroua, O., & Saidi, D. (2019). Effect of *in vitro* hydrolysis on coconut milk digestibility. *Revue Française d'Allergologie*, *59*(5):380–384.

Biswas, S., Masih, D., Singh, M., and Sonkar, C. (2016). Development and quality evaluation of Aloe vera and pineapple juice blended beverage. *International Research Journal Engineering Technology*, *3*(10):214–220.

Busquets, S., Marmonti, E., Oliva, F., Simoes, E., Luna, D., Mathisen, J. S., and Argilés, J. M. (2018). Omega-3 and omega-3/curcumin-enriched fruit juices decrease tumour growth and reduce muscle wasting in tumour-bearing mice. *JCSM Rapid Communications*, *1*(1):1–10.

Cahyana, Y., Putri, Y. S. E., Solihah, D. S., Lutfi, F. S., Alqurashi, R. M., and Marta, H. (2022). Pickering emulsions as vehicles for bioactive compounds from essential oils. *Molecules*, *27*(22):7872.

Chaikham, P., Apichartsrangkoon, A., and Seesuriyachan, P. (2014). Physical and biochemical qualities of pressurized and pasteurized longanjuices upon storage. *Emirates Journal of Food and Agriculture*, 218–228.

Chia, S. L., Rosnah, S., Noranizan, M. A., and Ramli, W. W. (2012). The effect of storage on the quality attributes of ultraviolet-irradiated and thermally pasteurised pineapple juices. *International Food Research Journal*, *19*(3):1001.

Csáki, K. F. (2011). "Synthetic Surfactant Food Additives Can Cause Intestinal Barrier Dysfunction." *Medical Hypotheses* 76(5):676–81.

Dehghan, B., Kenari, R. E., and Amiri, Z. R. (2022). Stabilization of whey-based pina colada beverage by mixed Iranian native gums: a mixture design approach. *Journal of Food Measurement and Characterization*, 1–9.

Deng, B., Zhao, J., He, M., and Tian, S. (2023). Curcumin treatment enhances bioactive metabolite accumulation and reduces enzymatic browning in soybean sprouts during storage. *Food Chemistry,* 17:100607.

Deshaware, S., Gupta, S., Singhal, R., and Variyar, P. S. (2019). Influence of different pasteurization techniques on antidiabetic, antioxidant and sensory quality of debittered bitter gourd juice during storage. *Food Chemistry*, 285:156–162.

Fitri, I. A., Mitbumrung, W., Akanitkul, P., Rungraung, N., Kemsawasd, V., Jain, S., and Winuprasith, T. (2022). Encapsulation of β-carotene in oil-in-water emulsions containing nanocellulose: Impact on emulsion properties, in vitro digestion, and bioaccessibility. *Polymers*, *14*(7):1414.

Food and Drug Administration (June 2023). Food additive permitted for direct addition to food for human consumption. Retrieved on 20 Nov. 2023 from https://www.accessdata.fda.gov.

Food Safety and Standards Authority of India (2006). APPENDIX B: Microbiological Requirements. Retrieved on 18 Dec. 2024 from https://www.fssai.gov.in.

Goel, A., Kunnumakkara, A. B., and Aggarwal, B. B. (2008). Curcumin as "Curcumin": from kitchen to clinic. *Biochemical Pharmacology*, *75*(4):787–809.

Golding, Matt, and Tim J. Wooster. 2010. "The Influence of Emulsion Structure and Stability on Lipid Digestion." *Current Opinion in Colloid & Interface Science* 15(1–2):90–101.

Gonçalves, R. F., Vicente, A. A., and Pinheiro, A. C. (2023). Incorporation of curcumin-loaded lipid-based nano delivery systems into food: Release behaviour in food simulants and a case study of application in a beverage. *Food Chemistry*, *405*:134740.

Hirsch, A. R., Förch, K., Neidhart, S., Wolf, G., and Carle, R. (2008). Effects of thermal treatments and storage on pectin methylesterase and peroxidase activity in freshly squeezed orange juice. *Journal of Agricultural and Food Chemistry*, *56*(14):5691–5699.

Hussain, Y., Alam, W., Ullah, H., Dacrema, M., Daglia, M., Khan, H., and Arciola, C. R. (2022). Antimicrobial potential of curcumin: therapeutic potential and challenges to clinical applications. *Antibiotics*, *11*(3):322.

Inal, A., Yenipazar, H., and Şahin-Yeşilçubuk, N. (2022). Preparation and characterization of nanoemulsions of curcumin and echium oil. *Heliyon*, 8:(2).

Igual, M. G. M. E., García-Martínez, E., Camacho, M. M., and Martínez-Navarrete, N. (2010). Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. *Food Chemistry*, *118*(2):291–299.

Iqbal, R., Mehmood, Z., Baig, A., and Khalid, N. (2020). Formulation and characterization of food grade O/W nanoemulsions encapsulating quercetin and curcumin: Insights on enhancing solubility characteristics. *Food and Bioproducts Processing*, *123*:304–311.

Islam, M. Z., Tabassum, S., Harun-ur-Rashid, M., Vegarud, G. E., Alam, M. S., and Islam, M. A. (2021). Development of probiotic beverage using whey and pineapple (Ananas comosus) juice: Sensory and physico-chemical properties and probiotic survivability during in-vitro gastrointestinal digestion. *Journal of Agriculture and Food Research*, *4*: 100144.

Jaguezeski, A. M., Perin, G., Bottari, N. B., Wagner, R., Fagundes, M. B., Schetinger, M. R. C., and Da Silva, A. S. (2018). Addition of curcumin to the diet of dairy sheep improves health, performance and milk quality. *Animal Feed Science and Technology*, *246*:144–157.

Jiménez Saelices, C., and Capron, I. (2018). Design of Pickering micro-and nanoemulsions based on the structural characteristics of nanocelluloses. *Biomacromolecules*, *19*(2):46–469.

Jirapeangtong, K., Siriwatanayothin, S., and Chiewchan, N. (2008). Effects of coconut sugar and stabilizing agents on stability and apparent viscosity of high-fat coconut milk. *Journal of Food Engineering*, *87*(3):422–427

Joung, H. J., Choi, M. J., Kim, J. T., Park, S. H., Park, H. J., and Shin, G. H. (2016). Development of food-grade curcumin nanoemulsion and its potential application to food

beverage system: antioxidant property and in vitro digestion. *Journal of Food Science*, *81*(3):N745–N753.

Jusril, N. A., Bakar, S. I. A., Khalil, K. A., Saad, W. M. M., Wen, N. K., and Adenan, M. I. (2022). Development and optimization of nanoemulsion from ethanolic extract of Centella asiatica (NanoSECA) using D-optimal mixture design to improve blood-brain barrier permeability. *Evidence-based Complementary and Alternative Medicine: eCAM*, 483511:18.

Kanchanathawornviboon, X., Monton, C., and Urairong, H. (2021). Microwave-assisted extraction of curcuminoids from organic Curcuma longa L. in different oil types for cosmetic purpose: An optimization approach. *Journal of Current Science and Technology*, *11*(1):71–89.

Khaksar, G., Assatarakul, K., and Sirikantaramas, S. (2019). Effect of cold-pressed and normal centrifugal juicing on quality attributes of fresh juices: do cold-pressed juices harbor a superior nutritional quality and antioxidant capacity. *Heliyon*, *5*(6).

Kharat, M., Du, Z., Zhang, G., and McClements, D. J. (2017). Physical and chemical stability of curcumin in aqueous solutions and emulsions: impact of pH, temperature, and molecular environment. *Journal of Agricultural and Food Chemistry*, *65*(8):1525–1532.

Kumar, D. D., Mann, B., Pothuraju, R., Sharma, R., and Bajaj, R. (2016). Formulation and characterization of nanoencapsulated curcumin using sodium caseinate and its incorporation in ice cream. *Food & Function*, *7*(1):417–424.

Kumara, A., Mahagamage, M. G. Y. L., and Arampath, P. C. (2019). Development of ready-to-serve pineapple juice with coconut milk. *The North African Journal of Food and Nutrition Research*, *3*(6):214–218.

Kunitake, M. T., Ditchfield, C., Silva, C. O., and Petrus, R. R. (2014). Effect of pasteurization temperature on sensory stability of an acidified sugarcane juice beverage. *Food Processing and Technology*, 5:12.

Lan, X., Liu, Y., Wang, L., Wang, H., Hu, Z., Dong, H., and Yuan, Y. (2023). A review of curcumin in food preservation: Delivery system and photosensitization. *Food Chemistry*, *424*:136464.

Lee, T. H., Chua, L. S., Tan, E. T. T., Yeong, C., Lim, C. C., Ooi, S. Y., and Sarmidi, M. R. B. (2009). Kinetics of thermal inactivation of peroxidases and polyphenol oxidase in pineapple (Ananas comosus). *Food Science and Biotechnology*, *18*(3):661–666.

Lei, Y., Zhang, X., Li, J., Chen, Y., Liang, H., Li, Y., and Liu, S. (2022). Nanocellulose from bamboo shoots as perfect Pickering stabilizer: Effect of the emulsification process on the interfacial and emulsifying properties. *Food Bioscience*, *46*:101596.

Li, J., Hwang, I. C., Chen, X., and Park, H. J. (2016). Effects of chitosan coating on curcumin loaded nano-emulsion: Study on stability and in vitro digestibility. *Food Hydrocolloids*, *60*:138–147.

Liu, J., Pedersen, H. L., Knarreborg, L., Ipsen, R., and Bredie, W. L. (2020). Stabilization of directly acidified protein drinks by single and mixed hydrocolloids—combining particle size, rheology, tribology, and sensory data. *Food Science & Nutrition*, *8*(12):6433–6444.

Liu, J., Pedersen, H. L., Knarreborg, L., Ipsen, R., and Bredie, W. L. (2020). Stabilization of directly acidified protein drinks by single and mixed hydrocolloids—combining particle size, rheology, tribology, and sensory data. *Food Science & Nutrition*, *8*(12):6433–644.

Liu, L., Kerr, W. L., and Kong, F. (2019). Characterization of lipid emulsions during in vitro digestion in the presence of three types of nanocellulose. *Journal of Colloid and Interface Science*, *545*, 317-329.

Lu, P. S., Inbaraj, B. S., and Chen, B. H. (2018). Determination of oral bioavailability of curcuminoid dispersions and nanoemulsions prepared from Curcuma longa Linnaeus. *Journal of the Science of Food and Agriculture*, *98*(1):51–63.

Mandha, J., Shumoy, H., Matemu, A. O., and Raes, K. (2023). Characterization of fruit juices and effect of pasteurization and storage conditions on their microbial, physicochemical, and nutritional quality. *Food Bioscience*, *51*:102335.

Mane, R. P., Pawar, V. S., Kshirsagar, R. B., and Nisar, M. (2019). Studies on development of process technology for formulation, sensory evaluation and storage studies of fresh turmeric rhizome juice-based orange RTS beverage. *International Journal of Chemical Studies*, *7*(1):1704–1709.

Marina, A. M., Che Man, Y. B., Nazimah, S. A. H., and Amin, I. (2009). Antioxidant capacity and phenolic acids of virgin coconut oil. *International Journal of Food Sciences and Nutrition*, *60*(sup2):114–123.

Moigradean, D., Poiana, M. A., Alda, L. M., and Gogoasa, I. (2013). Quantitative identification of fatty acids from walnut and coconut oils using GC-MS method. *Journal of Agroalimentary Processes and Technologies*, *19*(4):459–463.

Mwangi, E., Shlar, I., Horev, B., Vinokur, Y., and Rodov, V. (2019). Antimicrobial effect of methyl-β-cyclodextrin-curcumin complex as a potential food preservative. In *IV International Conference on Fresh-Cut Produce: Maintaining Quality and Safety 1319*:81–88).

Ngwabebhoh, F. A., Erdem, A., and Yildiz, U. (2018). A design optimization study on synthesized nanocrystalline cellulose, evaluation and surface modification as a potential biomaterial for prospective biomedical applications. *International Journal of Biological Macromolecules*, *114:*536–546.

Niu, F., Li, M., Huang, Q., Zhang, X., Pan, W., Yang, J., and Li, J. (2017). The characteristic and dispersion stability of nanocellulose produced by mixed acid hydrolysis and ultrasonic assistance. *Carbohydrate Polymers*, *165*:197–204.

Nokthai, P., Lee, V. S., and Shank, L. (2010). Molecular modeling of peroxidase and polyphenol oxidase: substrate specificity and active site comparison. *International Journal of Molecular Sciences*, *11*(9):3266–3276.

Ntazinda, A., Cheserek, M. J., Sheng, L. X., Meng, J., and Lu, R. R. (2014). Combination effect of sodium carboxymethyl cellulose and soybean soluble polysaccharides on stability of acidified skimmed milk drinks. *Dairy Science & Technology*, *94*:283–295.

Pala, Ç. U., and Toklucu, A. K. (2013). Microbial, physicochemical and sensory properties of UV-C processed orange juice and its microbial stability during refrigerated storage. *LWT-Food Science and Technology*, *50*(2):426–431.

Pengon, S., Chinatangkul, N., Limmatvapirat, C., and Limmatvapirat, S. (2018). The effect of surfactant on the physical properties of coconut oil nanoemulsions. *Asian Journal of Pharmaceutical Sciences*, *13*(5):409–414.

Peng, J., Tang, J., Barrett, D. M., Sablani, S. S., Anderson, N., and Powers, J. R. (2017). Thermal pasteurization of ready-to-eat foods and vegetables: Critical factors for process design and effects on quality. *Critical reviews in food science and nutrition*, *57*(14):2970–2995.

Petruzzi, L., Campaniello, D., Speranza, B., Corbo, M. R., Sinigaglia, M., and Bevilacqua, A. (2017). Thermal treatments for fruit and vegetable juices and beverages: A literature overview. *Comprehensive Reviews in Food Science and Food Safety*, *16*(4):668–691.

Raghavendra, S. N., and Raghavarao, K. S. M. S. (2011). Aqueous extraction and enzymatic destabilization of coconut milk emulsions. *Journal of the American Oil Chemists' Society*, *88*:481–487.

Raghavendra, S. N., and Raghavarao, K. S. M. S. (2010). Effect of different treatments for the destabilization of coconut milk emulsion. *Journal of Food Engineering*, *97*(3):341–347.

Ribeiro, A., Gonçalves, R. F., Pinheiro, A. C., Manrique, Y. A., Barreiro, M. F., Lopes, J. C. B., and Dias, M. M. (2022). In vitro digestion and bioaccessibility studies of vitamin E-loaded nanohydroxyapatite Pickering emulsions and derived fortified foods. *LWT-Food Science and Technology*, 154:112706.

Rincon, L., Botelho, R. B. A., and de Alencar, E. R. (2020). Development of novel plant-based milk based on chickpea and coconut. *LWT-Food Science and Technology, 128*:109479.

Rivas, A., Rodrigo, D., Martínez, A., Barbosa-Cánovas, G. V., and Rodrigo, M. (2006). Effect of PEF and heat pasteurization on the physical–chemical characteristics of blended orange and carrot juice. *LWT-Food Science and Technology*, *39*(10):1163–1170.

Saffarionpour, S. (2020). Nanocellulose for stabilization of Pickering emulsions and delivery of nutraceuticals and its interfacial adsorption mechanism. *Food and Bioprocess Technology*, *13*(8):1292–1328.

Saikia, S., Mahnot, N. K., and Mahanta, C. L. (2016). Phytochemical content and antioxidant activities of thirteen fruits of Assam, India. *Food Bioscience*, 13:15–20.

Salvia-Trujillo, L., Qian, C., Martín-Belloso, O., and McClements, D. J. (2013). Influence of particle size on lipid digestion and β-carotene bioaccessibility in emulsions and nanoemulsions. *Food Chemistry*, *141*(2):1472–1480.

Sari, T. P., Mann, B., Kumar, R., Singh, R. R. B., Sharma, R., Bhardwaj, M., and Athira, S. (2015). Preparation and characterization of nanoemulsion encapsulating curcumin. *Food Hydrocolloids*, *43*:540–546.

Shaik, L., and Chakraborty, S. (2023). Effect of different storage conditions on the quality attributes of sweet lime juice subjected to pulsed light and thermal pasteurization. *Sustainable Food Technology*, *1*(5):722–737.

Shukla, P., Bajwa, U., and Bhise, S. (2017). Effect of storage on quality characteristics of pasteurized mango-based milk beverage. *International Journal Current Microbiological Applied Science*, *6*(11):3446–3456.

Silva, L. F. R., Gomes, A. D. S., Castro, D. R. G., Souza, F. D. C. D. A., Mar, J. M., da Silva, L. S., and Campelo, P. H. (2019). Ultrasound-assisted homogenization and gum Arabic combined to physicochemical quality of cupuaçu juice. *Journal of Food Processing and Preservation*, *43*(9):e14072.

Singh, D., Vij, S., and Singh, B. P. (2016). Antioxidative and antimicrobial activity of whey based fermented soy beverage with curcumin supplementation. *Indian Journal Dairy Sci*ence, 69(2):171–177.

Souza, A. G., Ferreira, R. R., de Oliveira, E. R., Kato, M. M., Mitra, S. K., and Rosa, D. D. S. (2022). Chemical stabilization behind cardamom pickering emulsion using nanocellulose. *Polysaccharides*, *3*(1):200–216.

Sumesh, K. R., Kanthavel, K., and Kavimani, V. (2020). Peanut oil cake-derived cellulose fiber: Extraction, application of mechanical and thermal properties in pineapple/flax natural fiber composites. *International Journal of Biological Macromolecules*, *150*:775–785.

Tadros, T., Izquierdo, P., Esquena, J., and Solans, C. (2004). Formation and stability of nano-emulsions. *Advances in Colloid and Interface Science*, *108*:303–318.

Tang, Y., Yang, S., Zhang, N., and Zhang, J. (2014). Preparation and characterization of nanocrystalline cellulose via low-intensity ultrasonic-assisted sulfuric acid hydrolysis. *Cellulose*, *21*:335–346.

Tangsuphoom, N., and Coupland, J. N. (2005). Effect of heating and homogenization on the stability of coconut milk emulsions. *Journal of Food Science*, *70*(8):e466–e470.

Tao, R., Zhang, F., Tang, Q. J., Xu, C. S., Ni, Z. J., and Meng, X. H. (2019). Effects of curcumin-based photodynamic treatment on the storage quality of fresh-cut apples. *Food Chemistry*, *274*:415–421.

Tapal, A., and Tiku, P. K. (2012). Complexation of curcumin with soy protein isolate and its implications on solubility and stability of curcumin. *Food Chemistry*, *130*(4):960–965.

Tikekar, R. V., Pan, Y., and Nitin, N. (2013). Fate of curcumin encapsulated in silica nanoparticle stabilized Pickering emulsion during storage and simulated digestion. *Food Research International*, *51*(1):370–377.

Tsai, Y. M., Chien, C. F., Lin, L. C., and Tsai, T. H. (2011). Curcumin and its nano-formulation: the kinetics of tissue distribution and blood–brain barrier penetration. *International Journal of Pharmaceutics*, *416*(1):331–338.

Van Aken, G. A., Bomhof, E., Zoet, F. D., Verbeek, M., and Oosterveld, A. (2011). Differences in *in vitro* gastric behaviour between homogenized milk and emulsions stabilised by Tween 80, whey protein, or whey protein and caseinate. *Food Hydrocolloids*, *25*(4):781–788.

Wang, W., Chen, H., Ke, D., Chen, W., Zhong, Q., Chen, W., and Yun, Y. H. (2020). Effect of sterilization and storage on volatile compounds, sensory properties and physicochemical properties of coconut milk. *Microchemical Journal*, *153*:104532.

Wen, C., Yuan, Q., Liang, H., and Vriesekoop, F. (2014). Preparation and stabilization of d-limonene Pickering emulsions by cellulose nanocrystals. *Carbohydrate Polymers*, *112*:695–700.

Winuprasith, T., and Suphantharika, M. (2015). Properties and stability of oil-in-water emulsions stabilized by microfibrillated cellulose from mangosteen rind. *Food Hydrocolloids*, *43*:690–699.

World Health Organization. (1974). *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition, Meetings Report Series, No. 53A, 1974 WHO Food Additives Series, No. 5: 1974. WHO Geneva.

Yuan, Y., Liu, Q., Huang, Y., Qi, M., Yan, H., Li, W., and Zhuang, H. (2022). Antibacterial Efficacy and Mechanisms of Curcumin-Based Photodynamic Treatment against Staphylococcus aureus and Its Application in Juices. *Molecules*, *27*(20):7136.

Yuliarti, O., Mei, K. H., Ting, Z. K. X., and Yi, K. Y. (2019). Influence of combination carboxymethylcellulose and pectin on the stability of acidified milk drinks. *Food Hydrocolloids*, *89*:216–223.

Zheng, H., and Lu, H. (2011). Use of kinetic, Weibull and PLSR models to predict the retention of ascorbic acid, total phenols and antioxidant activity during storage of pasteurized pineapple juice. *LWT-Food Science and Technology*, *44*(5):1273–1281.

Zhou, H., Zheng, B., and McClements, D. J. (2021). Encapsulation of lipophilic polyphenols in plant-based nanoemulsions: Impact of carrier oil on lipid digestion and curcumin, resveratrol and quercetin bioaccessibility. *Food & Function*, *12*(8):3420–3432.