CHAPTER 3

DEVELOPMENT AND EVALUATION OF DEFATTED COCONUT MILK AND PINEAPPLE JUICE-BASED BLENDED BEVERAGE

Development and evaluation of defatted coconut milk and pineapple juice-based blended beverage

3.1. Introduction

When two or more plant sources are combined, a new product can be created that has distinct sensory and flavour attributes, additional vitamins and minerals, and a different composition than the original ingredients (Carvalho et al., 2006). To make non-dairy drinks, plant components including grains, nuts, and soy are extracted in water (Singhal et al., 2017). Coconut milk is an oil-in-water emulsion that is extracted from the white meat (flesh) of coconut with or without the addition of water. According to estimates, coconut milk accounts for 25% of the world's coconut production (Egidio et al., 2009). Coconut milk, unlike cow's milk, does not have allergens. The liquid obtained from the grated flesh of brown coconuts is called coconut milk. It has 3% of protein, 17%–24% fat, and 2% carbohydrates. It is free of cholesterol and is packed with minerals, vitamins, and electrolytes including chloride, calcium, and potassium (Benaissa et al., 2019). Coconut milk is bland in taste and used mainly in several cuisines. Fruit juice has become very popular among consumers as a beverage and the global exports of fruit and vegetable juice reached $926.7 Million in 2023 (United States Department of Agriculture, 2023). The rising popularity may be due in part to the growing awareness of the consumers about health and nutritional benefits (Tamuno and Monday, 2019), but also, to the reality that a variety of blended juice options are now available in the constantly growing fruit juice industry, satisfying a wide range of preferences and requirements (Khalid et al., 2016). Among the fruit juices, pineapple juice enjoys wide popularity among consumers. Pineapple (*Ananas comosus* var smooth cayennes) is widely grown in tropical and subtropical regions of the world. It is primarily consumed raw or juiced, and it has a good number of organic acids, vitamins, carbohydrate and phenols (Bamidele et al., 2017). Its juice has been used individually or combined with other fruit juices (Oyeleke et al., 2013). Since pineapple juice helps in the digestion of cholesterol and fat, it is one of the main drinks advised by dieticians for those trying to lose weight and it is useful in quick absorption of iron into the body (Khalid et al., 2016). Food industries are developing new products with high nutritional content, good sensory acceptability, and functional activity. So far, a complete study on a mixed beverage of coconut milk and pineapple juice is lacking. It is hypothesized that a blended beverage of defatted coconut milk and pineapple juice will have acceptable sensory attributes, and be a source for desirable nutrients and bioactive compounds. A study was, therefore, conducted to blend defatted coconut milk (aqueous phase) with pineapple juice and the

developed beverage was analyzed for sensorial, biochemical, and nutritional properties. This Chapter presents the work done and discusses the results obtained.

3.2. Materials and methods

Mature and fresh coconuts of Kamrupa variety (10–12 months) and ripe pineapples of Kew variety were procured from the local market. Standards were purchased from Sigma-Aldrich (USA) and TCI (Japan), solvents utilised for separation and identification were of HPLC grade, and chemicals were procured from Merck (Mumbai, India), Sisco Research Laboratory (Mumbai, India), and HiMedia (Mumbai, India).

3.2.1. Development of blended beverage of defatted coconut milk and pineapple juice

3.2.1.1. Extraction of pineapple juice

Pineapple was peeled, cut longitudinally into four halves and the inner core was removed. The fruit was cut into small pieces and juice was extracted using a mixer grinder. The pulp was pressed through a muslin cloth for further extraction of juice (Zheng et al., 2011). The pineapple juice was coded as P100.

3.2.1.2. Extraction of coconut milk

The outer fibrous cover of coconut was removed, and the nut was cut into two parts and the inside water was discarded. Each coconut half was scraped using 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. The Rose-Gottlieb method was used to determine the fat content of coconut milk (AOAC, 2000). The coconut milk (CM) was defatted by centrifugation (Eppendorf, model 5430 R, Germany) at 19630 × g for 15 min. Defatted coconut milk coded as C100.

3.2.1.3. Fat estimation in coconut milk

Fat content in CM and C100 was determined using Roese-Gottlieb (AOAC, 2000) method. For this, 10 g of the sample was transferred to a Mojonnier flask. In the same sample flask, 1.25 ml of ammonia and 10 ml of ethyl alcohol were added, and the mixture was thoroughly mixed. After mixing for 1 min, 25 ml of diethyl ether was added, and the mixture was mixed again for 1 min. Then, 25 ml petroleum ether (boiling point 40-60 °C) was added, and the mixture was mixed again for 1 min. The mixture was allowed to rest until the top ethereal layer was completely separated. A small amount of ethyl alcohol was added to aid in the separation of the layers if there was a propensity for an emulsion to develop. The separated clear ethereal

layer was then poured into the glass beaker. Using 15 ml of the solvent, the liquid remaining in the Mojonnier flask was extracted. The ethereal extract was transferred into the same glass beaker and allowed to fully evaporate at 102 ℃ for 2 h, cooled in a desiccator, and weighed. For 30 min, the flask was reheated in the oven at 102 ℃, weighed after cooling in a desiccator. Continued heating, cooling, and weighing was done until the difference between subsequent weights was less than 1 mg. Petroleum ether was used to gently remove the fat from the flask while ensuring that no insoluble residue remained. After heating the flask, it was dried and weighed again. The weight of the fat was noted. Eq. 3.1 was followed to determine the fat content.

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Weight of coconut milk}} \times 100 \qquad \text{(Eq. 3.1)}$$

3.2.1.4 Blending of defatted coconut milk and pineapple juice

C100 was blended with pineapple juice (P100) in different ratios: C20:P80, C30:P70, C40:P60, C50:P50, C60:P40, C30:P70, and C80:P20. All blended beverages were brought to 3.5 pH with citric acid and the TSS was increased to13 ^{o}Brix. The beverages were kept under refrigeration (4±1 ^{o}C) for further analysis.

3.2.2. Biochemical analysis

3.2.2.1. Determination of soluble solids

The blended beverage samples were thoroughly mixed and total soluble solids was determined using a refractometer. In the case of pure sucrose solutions, Brix is a measure of total soluble solids (TSS) (AOAC, 2000). A few drops of the sample are placed between the hand refractometer's prisms, and the reading at the demarcation line was noted.

3.2.2.2. Determination of pH

Using a digital pH meter (pH-700, EUTECH), the juice's pH was measured using AOAC (2000) technique. The standard buffer solution with pH values of 4.0 and 7.0 were used to calibrate the pH metre. A beaker was filled with 50 ml of the blended beverage, and the pH was measured. Before taking readings, enough time was given for equilibration.

3.2.2.3. Determination of titratable acidity

As described by AOAC (2000), the blended beverage of 10 ml of was pipetted into a conical flask, along with distilled water (25 ml) was added. Then, 200 ml of 0.1M sodium hydroxide was transferred into a burette and titrated against the sample in the flask until a pink coloration

was noticed. Phenolphthalein (three drops) were used as an indicator. The burette reading was used to calculate titratable acidity using the following equation (Eq. 3.2):

$$\text{Titratable acidity (\%)} = \frac{\text{Titre} \times \text{blank} \times \text{normality of base} \times 0.06404}{\text{Weight of sample (ml)}} \quad \text{(Eq. 3.2)}$$

3.2.2.4. Determination of ascorbic acid content in blended beverage

Ascorbic acid content in treated blended beverage was determined by the method of Khaksar et al. (2019). Firstly, 50 µl of 3% metaphosphoric acid was combined with a 100 µl aliquot of each juice supernatant, and the mixture was incubated at RT for 10 min. After adding the mixture to 150 µl of 0.8 mM 2,6-dichloroindophenol (DCP), the absorbance was measured at 515 nm in 30 s using a blank solution comprising of 50 µl of 3% metaphosphoric acid and 150 µl of DCP mixture. Ascorbic acid content was determined using a standard curve generated from genuine L-ascorbic acid (1–50 mg/100 ml). The findings were given as mg of ascorbic acid per 100 ml of blended beverage.

3.2.3. Determination of phytochemical content and antioxidant properties

3.2.3.1. Total phenolic content (TPC) of blended beverages

TPC of all the blended beverages was measured as per the method of Saikia et al. (2016). For analysis 20 µl of the blended beverage sample, the blank was placed in separate test tubes. Next, 100 µl of the Folin-Ciocalteau reagent and 1.58 ml of distilled water were added to each test tube, thoroughly mixed, and allowed to sit for 8 minutes before 300 µl of sodium carbonate was added. Sample containing test tubes were vortexed and then placed in the dark at 40°C for 30 minutes. Using an Agilent Cary 60 UV-vis spectrophotometer, the absorbance at 765 nm was measured. The TPC of the blended beverage were calculated using a standard curve derived from gallic acid (0.2–4 mg GAE/100 ml) and were represented as mg gallic acid equivalent (GAE) per 100 ml fresh blended beverage

3.2.3.2. Total flavonoid content (TFC) of blended beverages

Using the aluminium trichoride method described by Saikia et al. (2016), the flavonoid content of the entire blended beverage sample was ascertained. To do this, 0.5 ml of the blended beverage sample was combined with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium trichloride, 0.1 ml of 1M potassium acetate, and 2.8 ml of deionized water. This mixture was then placed in a UV-vis spectrophotometer (Cary 60 UV-Vis, Agilent), the absorbance of the reaction mixture was measured at 415 nm against deionized water used as a blank after 40 min

of incubation at RT. The TFC of the blended beverage was calculated using a standard curve derived from quercetin (0.2–4 mg QE/100 ml).

3.2.3.3. DPPH (2,2-diphenylpicrylhydrazyl) radical scavenging activity of blended beverages

Antioxidant activity of blended beverage was analyzed with stable radical DPPH using the method of Saikia et al. (2016). Blended beverage of 100 µl was added to 1.4 ml of DPPH methanolic solution (10^{-4}M). The absorbance of DPPH and sample mixture was measured at 517 nm using UV-VIS spectrophotometer (Cary 60 UV-vis, Agilent).

Results were reported in terms of the DPPH radical scavenged by the sample.

$$\text{DPPH radical scavenging activity (\%)} = \frac{A1-A2}{A1} \times 100 \quad \text{(Eq. 3.3)}$$

Where, A2 is absorbance of sample and A1 is absorbance of control blank.

3.2.3.4. Ferric reducing antioxidant power assay (FRAP) of blended beverages

The Saikia et al. (2016) method was utilized to quantify the FRAP activity of all samples. To make the FRAP solution, 25 ml of 0.3 M acetate buffer (pH 3.6), 2.5 ml of 20 mM ferric chloride, and 2.5 ml of 10 mM 2,4,6-TPTZ [2,4,6-tri (2-pyridyl)-1,3,5 triazine] were combined with 40 mM hydrochloric acid. A UV-vis spectrophotometer (Cary 60 UV-Vis, Agilent) was used to measure the absorbance of the reaction solution at 593 nm after it had been incubated for 4 min at 37 °C. The FRAP solution of 3 ml was combined with a 40 µl sample of blended beverage that had been appropriately diluted. A calibration curve was prepared using ferrous sulfate (0.2–5 mg/100 ml). Equations for linear regression were then used to quantify the FRAP in the samples.

3.2.3.5. Metal chelation capacity (MCC) of blended beverages

For metal chelation estimation, 1.0 ml of 0.3125 mM ferrozine and 1.0 ml of 0.125 mM ferrous sulfate was added with 0.2 ml of blended beverage sample and mixture was equilibrated for 10 min at room temperature. The absorbance of mixture was measured at 562 nm using UV-VIS spectrophotometry (Cary 60 UV-Vis, Agilent). The control contained all reagents except sample and decreased absorbance of the reaction indicated increased activity.

$$\text{Metal chelation activity (\%)} = \frac{A0-Ac}{A0} \times 100 \quad \text{(Eq. 3.4)}$$

A0 indicated absorbance of control and Ac indicated absorbance of sample.

3.2.4. Color analysis of blended beverages

The color of the juice blends was measured using Hunter Lab Colorimeter (Ultrascan, VIS-Hunter Associates Lab.) that was calibrated with a white tile. The L*, a*, and b* values of blended beverage were obtained. Lightness L* has a value between 0 and 100, where 0 represents darkness and 100 represents whiteness. The red-green axis is represented by the a* scale, where positive values denote red and negative values denote green. The yellow-blue axis is represented by the b* scale, where positive values denote yellow and negative values denote blue.

3.2.5. Minerals content in blended beverages

A solution comprising of 5 ml of sample, 2 ml of 30% hydrogen peroxide, and 1 ml of 65% nitric acid was heated to a temperature of 75 °C in a thermodigester block until the discoloration of sample. The liquid was then made up to 20 ml with ultrapure water and filtered through qualitative Whatman No. 44 filter paper, and further diluted for the determination of sodium (Na), potassium (K), iron (Fe), zinc (Zn), calcium (Ca), manganese (Mn), and magnesium (Mg) following the method of Dutra et al. (2018) using a flame atomic absorption spectrophotometer (ThermoFisher, ICE 3500).

3.2.6. Sensory analysis of blended beverages

A panel of 10 semi-trained panellists (25 participants, 25-35 years old) were given the coded samples of pineapple juice, coconut milk, and blended beverages to assess the sensory characteristics using the nine-point hedonic scale. The sensory attributes of colour, taste, aroma (in terms of fruity smell), and overall acceptability were assessed by the panel, with 1 representing the lowest value and 9 the highest. All samples were served at room temperature. The beverage samples were labelled with random codes and offered in clear glasses in a random order and were protected with glass covers.

3.2.7. RP-HPLC of phenolic compounds

The standards used for identification were gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid and catechin. An HPLC unit (ThermoFisher Ultimate 3000, United States of America), C18 column (5µm, 120A, 4.6X 250 mm) and UV-vis detector were used for the separation of compounds. For HPLC identification and quantification of individual phenolic compounds in the extract of blended beverages, the gradient elution method of Espin et al. (2016) was followed. The mobile phase comprised of A- 0.1% formic acid and B- acetonitrile. The solvent elution was 15% B for 5 min, 15–20% B for 5 min, 20–35% B for 10 min, 35–

50% B for 10 min, 50–60% B for 5 min, and 60% B for 5 min. For separation, 20 µl of extracted sample volume was injected and UV-vis spectra were recorded at 254 nm. By comparing the retention time of blended beverage sample peaks, absorption spectra, and mass spectra with reference standards, the gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid and catechin concentrations was determined. Acetonitrile was used to generate six distinct concentrations of gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid and catechin standards, which were 0.1, 0.5, 1, 5, 10, and 15 µg/ ml. Equations for linear regression were then used to quantify the phenolic compounds in the samples.

3.2.8. Fourier transform-infrared spectroscopic (FTIR) analysis

The infrared absorbance spectra of the blended beverages were recorded on an FTIR spectrophotometer (Perkin Elmer, spectrum 100, United Kingdom). The spectra of the powdered samples of blended beverages were obtained in the wave number range from 4000–400 cm^{-1} with a resolution of 2 cm^{-1}.

3.2.9. Statistical analysis

All analyses are expressed as the mean±standard. Data was analysed by One-way analysis of variance (ANOVA) using IBM SPSS 20.0. The data were statistically analysed by Duncan's multiple range tests at $p \leq 0.05$ significance level.

3.3. Results and discussion

3.3.1. Fat content in coconut milk

Coconut milk in this study had a fat content of 21.2±0.03% (Table 3.1). Coconut oil tends to solidify at 15℃ (Abeysundara et al., 2001; Marina et al., 2009) due to the nature of the fatty acids present. Lauric acid is the predominant fatty acid in coconut fat (Jadhav and Annapure, 2023). Presence of fat in the coconut milk-based beverage will cause either separation (at higher temperature) or solidification (at lower temperature). Therefore, the milk was defatted by centrifugation. The defatted coconut milk had a fat content of 0.3±0.02% (Table 3.1). Furthermore, fat removal makes the beverage a heathy drink since fat intake is associated with health problems like coronary heart disease and obesity (Khuenpet et al., 2016). The cream that was removed by centrifugation from the coconut milk prior to incorporation in blends is a by-product, which can be used to extract virgin coconut oil.

Table 3.1. Fat content in coconut milk

Sample	Fat (%)
Coconut milk	21.2±0.03[a]
Defatted coconut milk	0.3±0.02[b]

3.3.2. Biochemical analysis of the blended beverages

The biochemical properties of blended beverage before pH and TSS adjustment with acid and sugar (as mentioned in section 3.2.1), respectively are presented in Table 3.2. The pH of the blended beverage differed considerably ($p < 0.05$) and ranged from 3.75 in P100 to 6.05 in C100. Blending in different ratios gave in-between pH values. Tangsuphoom and Coupland (2005) also reported that the pH of coconut milk extract ranges from 6.1 to 6.3. The pH value is very important for the preservation of juices and beverages, as bacterial growth is restricted in an acidic pH environment. It plays a crucial role in the prevention of bacterial spoilage (Rincon et al., 2020). The blends, therefore, needs acidification to improve their shelf stability. The TSS of blended beverage was between 11.6 ^{o}Brix for C100 and 10.8 ^{o}Brix for P100. Beegum et al. (2022) reported 12.1^{o}Brix for coconut milk. This may be due to the removal of cream phase from coconut milk, which probably also removed some milk proteins. The TSS of all the blended beverages increased with increasing concentration of defatted coconut milk. The titratable acidity (TA) of all the samples ranged from 0.4 to 0.8%, with P100 having the highest (0.8%) and C100 having the least (0.4%) value (Table 3.2). Pineapple juice addition raised the TA level in the blended beverages. Bamidele and Fasogbon (2017) reported 0.73% titratable acidity in pineapple juice. The TA of C70:P30 and C60:P40 were not significantly affected with the increase in pineapple juice concentration. The ascorbic acid content ranged between 2.4-45.0 mg/100 ml for all the samples and P100 had the highest content (Table 1). As reported by Achinewhu and Hart (1994), ascorbic acid content in pineapple juice was 47.8 mg/100 g. In comparison, ascorbic acid content of C100 was 2.41 mg/100 ml. Omotosho and Odeyemi (2012) reported 0.46 mg/1000 ml of vitamin C in coconut milk.

3.3.3. Phytochemical content and antioxidant properties of the blended beverages

3.3.3.1 Total phenolic content (TPC)

The TPC of blended beverages brought to 3.5 pH and 13˚Brix TSS are given in Table 3.3. Among all the samples, P100 contained higher amount of TPC (38.4 mg GAE/100 ml) than C100 (32.4 mg GAE/100 ml). TPC of all the blended samples varied from 28.4 - 36.4 mg

GAE/100 ml and showed significant differences. Nadeeshani et al. (2015) reported that TPC of thick and thin coconut milk ranged from 816 to 2040 µg/ 100 ml.

3.3.3.2 Total flavonoids content (TFC)

The flavonoids are a group of polyphenols consisting of anthocyanins, isoflavones, flavones, flavanones, flavanols and flavanols that are mostly found in plants. As seen from Table 3.3, the amount of TFC was higher in C100 (23.9 mg QE/100 ml) than P100 (20.5 mg QE/100 ml). The blended samples showed lower values than C100 and P100, suggesting pH induced degradation of flavonoids. Arivalagan et al. (2018) reported that TPC and TFC in different solvent extracts of defatted coconut testa ranged from 4.9-167 mg GAE/g and 8.84-115 mg QE/g, respectively. Marina et al. (2009) observed that TPC was more in the oil fraction than the aqueous fraction of coconut milk, and, therefore, TPC in the coconut milk reduces after virgin oil is extracted.

3.3.3.3 Antioxidant properties

The antioxidant properties of the samples were measured by determining the DPPH radical scavenging activity, MCC, and FRAP activity. As presented in Table 3.3, all the samples possessed antioxidant activities. Fruits and vegetables' ability to scavenge free radicals and maintain their antioxidant capacity is mostly dependent on phenolic components. These compounds possess the capacity to scavenge harmful free radicals and reduce the possibility of certain illnesses caused by oxidative stress (Alam et al., 2023). Antioxidant activity in all the activity tests were highest in P100 and lowest in C100. The blended samples of C20:P80, C30:P70, C40:P60, C50:P50, C60:P40, C70:P30, C80:P20 showed considerably good antioxidant properties. DPPH radical scavenging activity of blended beverage ranged between 66.8±0.01% and 88.6±0.03%; with increase in the concentration of pineapple juice the DPPH radical scavenging activity also increased. The metal chelation activity and FRAP also increased with increase in the concentration of pineapple juice, ranging from 22.9±0.01% to 52.1±0.01% for MCC and 14.9±0.01 mg/100 ml to 16.4±0.01 mg/100 ml for FRAP. Owolade et al. (2016) reported 81.1% DPPH radical scavenging activity of 100% pineapple juice and for 20.6% for 100% carrot juice and the blend with equal concentration of carrot and pineapple juice showed more DPPH as compared to blend having more concentration of carrot juice. Alyaqoubi et al. (2015) reported that FRAP and DPPH scavenging activity of coconut milk is 610.19±2.54 mg/100 g and 68.39±1.30%, respectively. When comparing the results of defatted coconut milk to the stated values of whole coconut milk, the DPPH scavenging activity and FRAP are somewhat lower. The variation in results could be due to genetic, cultivar, agronomic

and environmental factors, and also as a result of the cream removal procedure used during defatting (Moussa et al., 2014).

Table 3.2. Physicochemical analysis of blended beverages.

Sample	pH	ºBrix	Titratable acidity (%)	Ascorbic acid (mg/100 ml)
C100	6.05±0.02^{a}	11.6±0.00^{c}	0.40±0.35^{g}	2.41±0.01^{i}
C80:P20	5.49±0.00^{ab}	10.9±0.00^{f}	0.41±0.00^{g}	10.35±0.07^{h}
C70:P30	5.33±0.00^{c}	11.2±0.07^{d}	0.42±0.07^{f}	19.95±0.07^{g}
C60:P40	5.26±0.21^{d}	11.3±0.21^{d}	0.43±0.00^{f}	33.10±0.14^{f}
C50:P50	4.85±0.07^{e}	12.5±0.35^{a}	0.49±0.00^{e}	35.65±0.21^{e}
C40:P60	4.61±0.14^{f}	11.0±0.07^{e}	0.51±0.01^{d}	37.55±0.70^{d}
C30:P70	4.58±0.01^{f}	11.9±0.00^{b}	0.54±0.00^{c}	38.95±0.07^{c}
C20:P80	4.45±0.00^{g}	10.8±0.07^{f}	0.57±0.00^{b}	40.00±0.63^{b}
P100	3.75±0.07^{h}	10.8±0.00^{f}	0.80±0.00^{a}	45.00±1.41^{a}

$^{a-i}$ Values with different superscripts vary significantly within a column at p <0.05.

Table 3.3. Phytochemical content and antioxidant activity of blended beverages

Coconut milk: pineapple juice)	Total phenolic content (mg GAE/100 ml)	Total flavonoid content (mg QE/100 ml)	DPPH radical scavenging activity (%)	MCC (%)	FRAP (mg /100 ml)
C100	32.4±0.05^{d}	23.9±0.04^{a}	66.8±0.01^{i}	22.9±0.01^{i}	14.9±0.01^{d}
C80:P20	28.4±0.07^{i}	21.4±0.17^{b}	69.5±0.30^{h}	23.2±0.18^{h}	11.3±0.39^{g}
C70:P30	29.3±0.13^{h}	20.2±0.11^{d}	76.6±0.02^{g}	30.4±0.26^{g}	12.3±0.08^{f}
C60:P40	29.9±0.04^{g}	19.5±0.05^{e}	85.3±0.11^{f}	36.5±0.10^{f}	13.2±0.00^{e}
C50:P50	30.6±0.05^{f}	18.7±0.05^{f}	86.0±0.02^{e}	44.9±0.01^{e}	14.7±0.03^{d}
C40:P60	31.8±0.07^{e}	18.9±0.04^{f}	86.5±0.01^{d}	47.7±0.10^{d}	14.1±0.03^{d}
C30:P70	33.2±0.40^{c}	18.3±0.08^{g}	87.0±0.06^{c}	49.7±0.20^{c}	15.6±0.35^{c}
C20:P80	36.4±0.06^{b}	20.0±0.06^{d}	87.7±0.42^{b}	51.1±0.11^{b}	16.1±0.06^{b}
P100	38.4±0.06^{a}	20.5±0.02^{c}	88.6±0.03^{a}	52.1±0.01^{a}	16.4±0.01^{a}

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

3.3.4. Colour analysis of blended beverages

Table 3.4 gives the L*, a*, b* values of the samples. The lightness values increased significantly with the increase in the concentration of defatted coconut milk. C100 registered the maximum L* value of 75.1±0.04, as was also observed by Rincona et al. (2020). The ratio of lightness and yellowness are the major color parameters for the determination of quality of pineapple juice (Assawarachan and Noomhorm, 2010). Results showed that when pineapple juice concentration increased, there was a substantial ($p<0.05$) increase in a* negative value and b* positive value (Table 3.4) but L* value decreased. The L* value of P100 was 29.9±0.92. The a* negative ranged from -3.9±0.25 to -1.2±0.00; negative a* value indicates greenness and with increase in the concentration of pineapple juice negative value also increased. The b* positive value was maximum in P100 with a value of 6.75±0.04 and it significantly decreased in the order C20:P80 > C30:P70 > C40:P60 > C50:P50 > C60:P40 > C70:P30 > C80:P20. The higher a* negative and b* positive values in P100 was due to the yellowish pigments present (Joomwong and Sornsrivichai, 2006). The a* values significantly decreased and the b* values significantly increased as the percentage of pineapple juice increased in the blended beverages.

Table 3.4. Colour values of the blended beverages

Samples	L*	a*	b*
C100	75.1±0.04^{a}	-1.2±0.00^{g}	2.2±0.02^{g}
C80:P20	48.3±0.17^{f}	-1.4±0.36^{f}	4.2±0.07^{f}
C70:P30	49.3±0.18^{e}	-1.9±0.04^{d}	4.6±0.02^{e}
C60:P40	55.7±0.01^{b}	-2.7±0.00^{c}	6.0±0.02^{d}
C50:P50	55.7±0.02^{b}	-3.2±0.01^{b}	6.2±0.02^{c}
C40:P60	53.8±0.00^{c}	-3.6±0.00^{a}	6.2±0.01^{c}
C30:P70	51.3±0.29^{d}	-3.8±0.02^{a}	6.4±0.06^{b}
C20:P80	44.4±0.30^{g}	-3.8±0.07^{a}	6.6±0.01^{a}
P100	29.9±0.92^{h}	-3.9±0.25^{e}	6.7±0.04^{a}

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

3.3.5. Mineral content in blended beverage

The mineral composition of C100, C60:P40, C50:P50, C40:P60, and P100 are presented in Table 3.5. Ca, Fe, Zn, Mn, and Mg were higher in P100 than C100. Na and K were highest in C100. Their blends, therefore, had intermediate values depending on the level of substitution. Pineapple juice contains calcium (15.8 mg/100 ml), iron (0.2 to 0.7 mg/100 ml), magnesium

(12.0 mg/100 ml), sodium (0.8 mg/100 ml), potassium (128.0 mg/100 ml), and zinc (0.5 mg/100 ml) (Sairi et al., 2004; Afolabi et al., 2015). Our results on minerals content are in agreement with the reported results (Sairi et al., 2004; Afolabi et al., 2015; Appaiah et al., 2015). Tulashie et al. (2022) reported that coconut milk contains calcium (92.5 mg/l), potassium (805 mg/l), magnesium (279.45 mg/l), iron (149 mg/l), manganese (2330 mg/l), copper (2.5 mg/l) and zinc (71 mg/l). Appaiah et al. (2015) reported that the mature coconut kernel contains calcium, sodium, potassium, iron, and zinc ranging from 4.9-18.1, 21.6 -31.9, 122.1-154.6, 4.0-7.9, and 0.2-2.2mg/l, respectively. Defatted coconut milk had lower minerals and trace elements as compared to whole coconut milk, which might be due to the removal of the cream layer during defatting process. The blends contained good amount of K, Mg, and Zn. The potassium content in C100 was higher (132.6±0.01 mg/100 ml) than P100 (123.2±0.02 mg/100 ml). Blended beverages are good sources of K and moderately good sources of Mg and Zn. Mineral content of fruits largely depends on many factors, for example, soil type, phase of development, assortment of cultivars, geology, and other topographical elements. Magnesium has a wide variety of roles in biochemical and physiological activities. Potassium is known for its role in nerve impulse transmission and fluid homeostasis (Ani and Abel, 2018). It has been reported that zinc is a component of over 70 different enzymes that play a variety of roles in cell metabolism, such as the metabolism of proteins, lipids, and carbohydrates. It is also reported that Zn can interact with necessary elements like Cu and Fe to reduce their levels in tissues and slow down oxidative processes (Dani et al., 2012).

Table 3.5. Mineral content in the blended beverages (mg/100 ml)

Samples	Na	Ca	K	Mg	Zn	Fe	Mn
C100	7.8± 0.02^{a}	2.9± 0.05^{e}	132.6± 0.01^{a}	12.5± 0.50^{e}	1.0± 0.68^{d}	0.1± 0.01^{d}	0.2± 0.04^{e}
C60:P40	5.8± 0.01^{b}	7.8± 0.01^{d}	116.6± 0.30^{c}	13.5± 0.00^{d}	1.1± 0.01^{c}	0.2± 0.01^{c}	0.5± 0.01^{d}
C50:P50	5.5± 0.05^{c}	10.4± 0.03^{c}	109.5± 0.70^{d}	14.6± 0.09^{c}	1.2± 0.06^{c}	0.3± 0.01^{b}	0.7± 0.46^{c}
C40:P60	4.7± 0.01^{d}	12.1± 0.23^{b}	96.4± 0.56^{e}	17.5± 0.01^{b}	1.8± 0.01^{b}	0.3± 0.01^{b}	0.9± 0.00^{b}
P100	3.4± 0.01^{e}	17.2± 0.28^{a}	123.2± 0.02^{b}	22.8± 0.01^{a}	1.9± 0.01^{a}	0.4± 0.01^{a}	1.1± 0.07^{a}

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

3.3.6. Sensory analysis of the blended beverages

Sensory evaluation results of the freshly prepared blended beverages of defatted coconut milk and pineapple juice are given in Fig 3.1 and Table 3.6. The blended beverages in different ratios scored higher than C100 and P100 for colour. C100, C60:P40, C50:P50, C40:P60 C30:P70, and P100 scored almost similar scores for aroma. C100, P100 and C60:P40 were scored above 8.0 for the attribute of aroma; there was insignificant difference among them ($p<0.05$) for aroma. C80:P20 with higher concentration of defatted coconut milk was given the lowest score and C20:P80 beverage having higher concentration of pineapple juice scored higher for aroma than C80:P20, which may be due to the sweet and strong aroma of pineapple juice. Porto et al. (2023) reported that pineapple juice contains higher concentration of methyl hexanoate and thioesters which bring sweet and strong aroma to it. Maximum score for taste and overall acceptability of blended beverage was observed for sample C50:P50, having equal concentration of defatted coconut milk and pineapple juice. In comparison, the blended beverage sample C70:P30 scored minimum for overall acceptability and taste. All the beverage samples scored well for the attribute of colour, except for C100 and P100. C50:P50, C60:P40, and C40:P60 scored highly on the hedonic scale for aroma, taste, colour, and overall acceptability and were in the order C50:P50 > C60:P40 > C40:P60. Sensory results indicated that there is ground for developing blended beverage of coconut milk and pineapple juice.

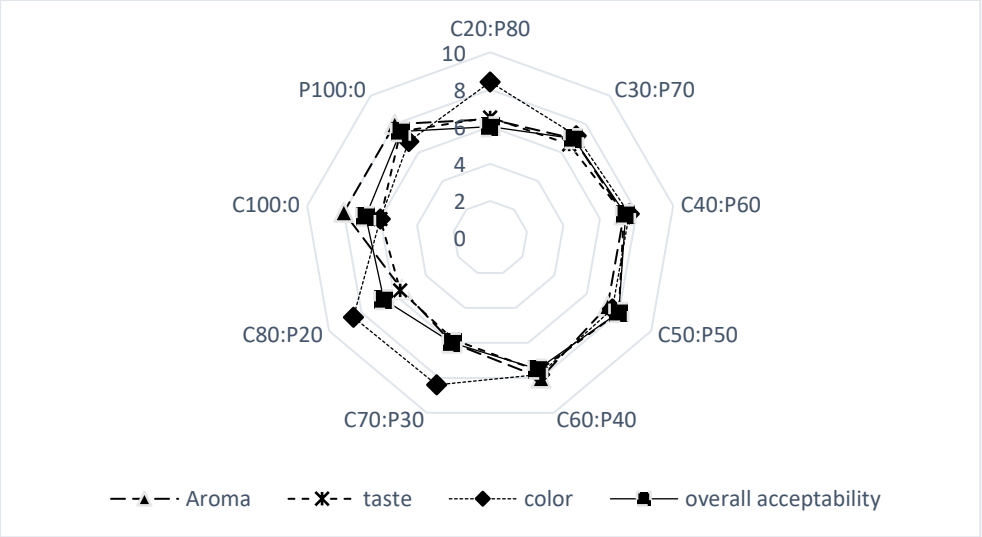


Fig 3.1: Radar graph showing the sensory scores of the blended beverages.

Table 3.6. Sensory scores obtained on a 9-point hedonic scale

Samples	Aroma	Taste	Color	Overall acceptability
C100	8.0±0.07^{a}	6.2±0.28^{c}	6.0±0.07^{f}	6.7±0.07^{de}
C80:P20	5.6±0.14^{f}	5.7±0.14^{d}	8.4±0.09^{a}	6.5±0.14^{ef}
C70:P30	6.0±0.07^{e}	5.4±0.07^{d}	8.3±0.07^{a}	6.1±0.14^{g}
C60:P40	8.1±0.14^{a}	7.7±0.14^{b}	7.8±0.07^{bc}	7.6±0.12^{b}
C50:P50	7.5±0.28^{b}	8.2±0.28^{a}	7.9±0.08^{b}	8.0±0.07^{a}
C40:P60	7.4±0.01^{bc}	7.5±0.28^{b}	7.6±0.07^{c}	7.5±0.16^{b}
C30:P70	7.1±0.14^{c}	6.6±0.07^{c}	7.1±0.07^{d}	7.0±0.07^{cd}
C20:P80	6.5±0.21^{d}	6.6±0.21^{c}	8.3±0.14^{a}	6.1±0.21^{fg}
P100	8.1±0.14^{a}	7.3±0.21^{b}	6.9±0.14^{e}	7.3±0.28^{bc}

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

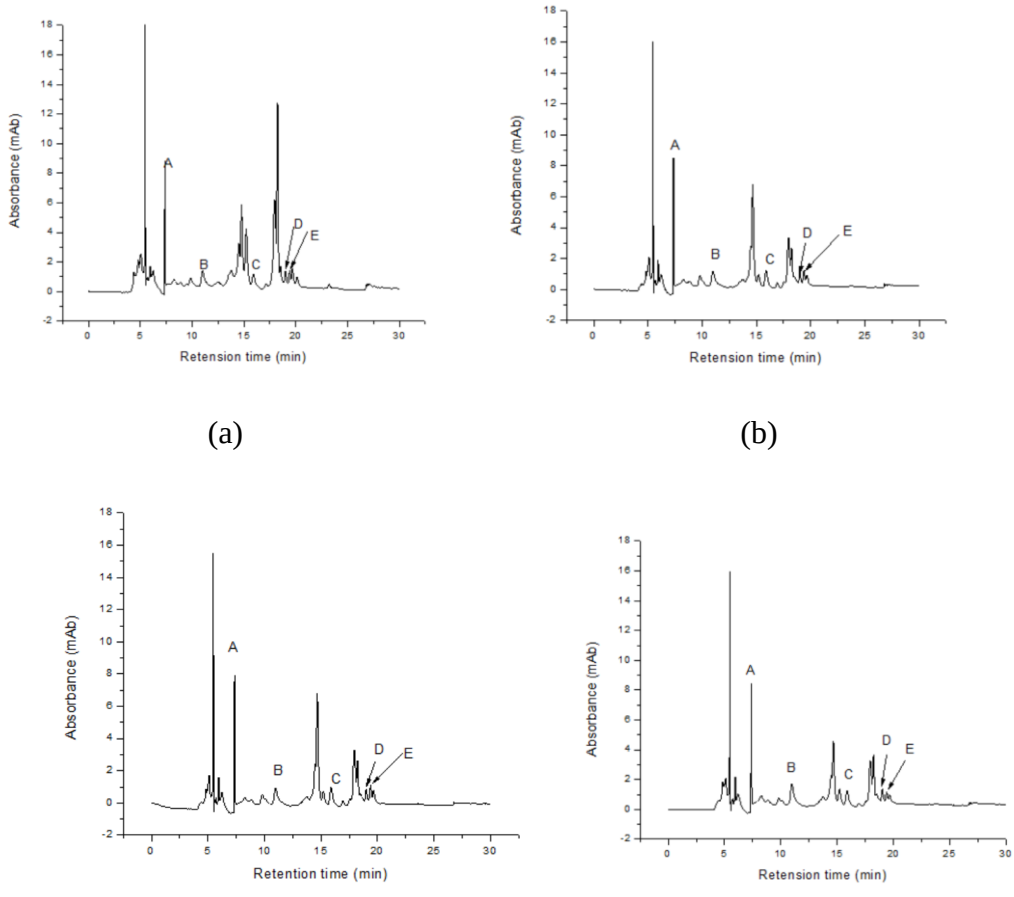
3.3.7. RP-HPLC analysis of phenolic compounds in blended beverages

Phenolic compounds are among the most significant naturally occurring plant-based antioxidants in food. The results of RP-HPLC identification and quantification of individual phenolic compounds in blended beverages are given in Fig. 3.2 and Table 3.7. The phenolic acids that could be identified were gallic acid, chlorogenic acid, caffeic acid, coumaric acid, and catechin in C100, P100, and their blends. The phenolic acids in blended beverage were identified based on their retention times. Previous studies (Nadeeshani et al., 2015; Karunasiri et al., 2020) reported the presence of phenolic acids like gallic acid, chlorogenic acid, caffeic acid, catechin, and p-coumaric acid in coconut milk. Gallic acid was the major phenolic acid in C100 (154.5 µg/ml**)** and chlorogenic acid was the major one in P100 (147.5 µg/ml). Therefore, while gallic acid content reduced with the increase in pineapple juice in the blends, chlorogenic acid content increased. The content of caffeic acid, catechin and coumaric acid did not differ substantially in P100, C100, and their blends. Caffeic acid, p-coumaroylquinic acid, gallic acid, catechin acid, trans-cinnamic acid, chlorogenic acid, p-coumaroyl glucose, p-coumaroylquinic acid, di-p-coumaroylquinic acid, caffeoyl glucose, feruloylglucose, sinapoyl glucose, salicylic acid, p-hydroxybenzoic aldehyde, tannic acid, tyramine and myricetin were detected in pineapple fruits and pineapple juice (Arampath and Dekker, 2020; Wen and Wrolstad, 2002).

Table 3.7. RP-HPLC analysis of phenolic acids in blended beverages

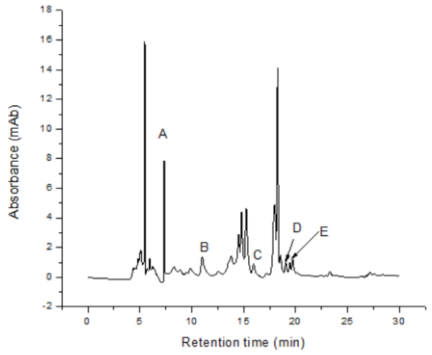
Phenolic acids	Retention time (min)	Concentration (µg/ml)				
		C100	C60:P40	C50:P50	C40:P60	P100
Gallic acid	07.00	154.56±0.0[a]	116.45±0.07[b]	114.24±0.01[c]	105.89±0.01[d]	105.06±0.08[e]
Chlorogenic acid	11.00	137.98±0.02[d]	141.12±0.01[c]	141.155±0.10[c]	142.27±0.01[b]	147.55±0.07[a]
Caffeic acid	16.83	62.38±0.01[c]	62.95±0.04[b]	63.16±0.01[a]	62.96±0.04[b]	63.15±0.07[a]
Coumaric acid	19.00	8.200±0.50[b]	7.96±0.02[e]	8.07±0.01[d]	8.09±0.01[c]	8.30±0.01[a]
Catechin	19.17	5.89±0.01[c]	5.94±0.05[bc]	5.80±0.13[c]	5.58±0.02[d]	6.11±0.01[a]

[a-e] Values with different superscripts vary significantly within a row at p <0.05.



(a) (b)

(c) (d)



(e)

Fig 3.2: HPLC chromatograms showing phenolic compounds present in blended beverages (a) C100, (b) C60:P40, (c) C50:P50, (d) C40:P60, (e) P100.
The identified phenolic compounds are: A= Gallic acid, B= Chlorogenic acid, C=Caffeic acid, D= Coumaric acid, E= Catechin

3.3.8. Fourier Transform Infrared spectroscopic (FTIR) analysis of blended beverages

FTIR peaks of CM, C100, P100 and C50:P50 (blend with highest overall acceptability) are shown in Fig 3.3. There are mainly eight peaks in sample CM (A, B, C, D, E, F, G and H), six peaks in C100 (A, D, E, F, G, H), and eight peaks in P100 (A, C, D, E, F, G, H and I) and C50:P50 (A, C, D, E, F, G, H and I). CM is the whole milk with fat. The band at 2920, 2920, 2929 and 2910 cm^{-1} (A) observed for CM, C100, P100 and C50:P50, respectively are assigned to the methylene and symmetric and asymmetric stretching vibration. The band peaks between the wavenumbers of 1800-750 cm^{-1} show the biochemical composition, mainly the carbohydrates, proteins, lipids, and polyphenols in plant (Leopold et al., 2011). The peaks at wavenumber 2859 cm^{-1} is assigned to minor CH symmetric stretch in CM, which is not observed in C100, and may be due to the removal of fat from coconut milk. The FTIR of vegetable oils show major peaks at 2937, 2856, 1749, 1454, 1166 and 709 cm^{-1}, which represent the triglyceride functional group (Yang et al., 2005). The minor peak obtained at 1736 and 1705 cm^{-1} (C) in samples (CM, P100, and C50:P50) can be ascribed to aldehyde (C = O) variable angle vibration. Most bands for phenolic compounds are seen between 3000-2960 cm^{-1}, 2280-1717 cm^{-1}, and 1543-966 cm^{-1} that are signs of antioxidant compounds. In C100 and coconut blends, the amide I band (within the range of wavenumber 1600-1700 cm^{-1}), the amide II band (wavenumber 1530-1550 cm^{-1}) and amide III band (within a wavenumber of 1260-1300 cm^{-1}) exist, which denote the presence of proteins (Sun et al., 2022). Sitorus et al. (2021) reported that the spectral data of unprocessed coconut milk from the traditional market had three absorbance peaks, specifically in the wavelength ranges of 2985-3000 cm^{-1}, 3418-3420 cm^{-1}, and 3449-3504 cm^{-1}. The wavenumber area somewhere in the range from

1200 cm^{-1} and 950 cm^{-1} contains functional groups mainly ascribed to carbohydrates. Vardin et al. (2008) reported that the C=O stretching vibration, C–O acid stretching, and O–H deformation of acids stretch was due to the peaks at 1715, 1255, and 915 cm−1, respectively. C–O and –OH deformation peaks occur at 1150, 1100 and 1050 cm^{-1}. The peaks at 1634 cm^{-1} (D), 1461, 1410, 1410, 1420, 1410 cm^{-1} (E), 1052, 1043, 1062 cm^{-1} (G), 991, 930, 920, 940, 920 cm^{-1} (H), 828 and 869 cm^{-1} (I) bands of CM, C100, P100 and C50:P50, respectively, are mainly because of C=O, CH_2, C-O, OH, C=C stretching, respectively. Egidio et al. (2009) ascribed peaks observed for fresh cut pineapple juice to the presence of different compounds in the juice; peaks at 1639-1500 cm^{-1} were due to water present, peaks in the range of 1120-995 cm^{-1} (coupled C-C and C-O stretching vibration) indicated the presence of organic acids and sugars. The absorption peak at 1246 cm^{-1} (F) was only seen for samples P100 and C50:P50 and occurred due to C-O acid stretch. Bhushan et al. (2023) reported that absorbance at 1246 cm^{-1} was attributed to the characteristic flavonoid compound stretching of the pyran rings. CM and C100 showed peak at 1052 cm^{-1} (G) (Fig. 3.3), which indicated the presence of fat. Man (2013) reported that virgin coconut oil has one peak in the frequency region of 1120 - 1090 cm^{-1}. As the samples CM contained 21.2% fat and C100 contained 0.3% fat, a peak at 1052 cm^{-1} was recorded, which is in agreement with Man (2013).

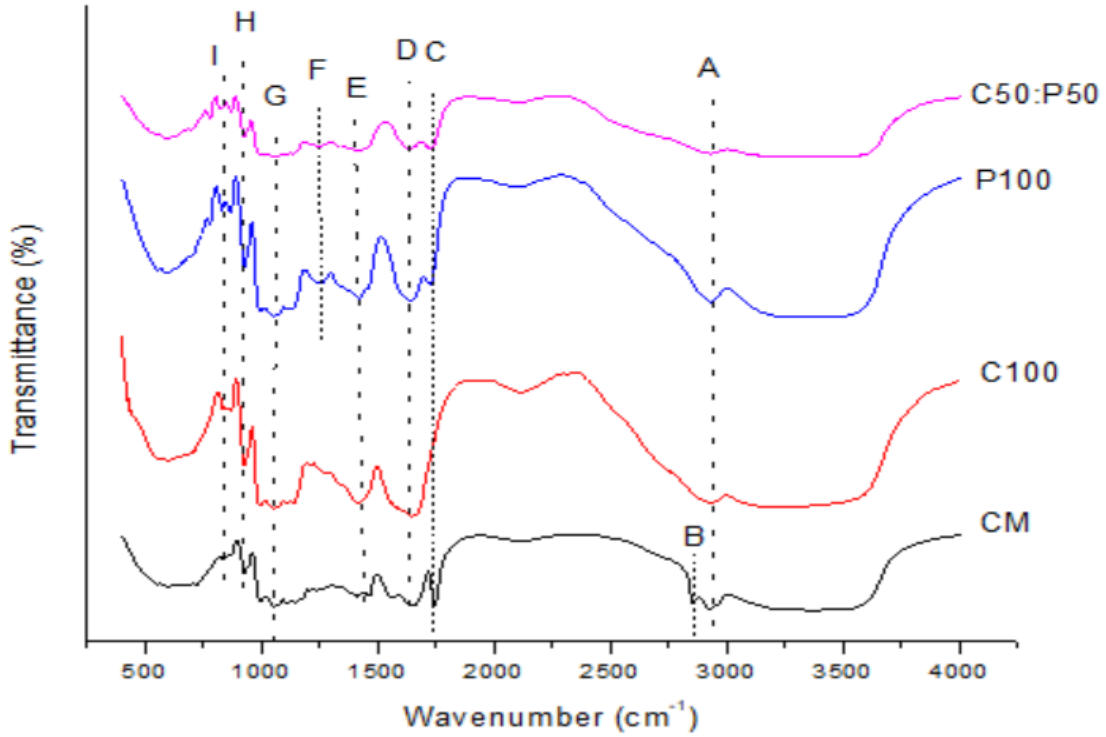


Fig 3.3. FTIR spectra of coconut milk (CM), defatted coconut milk (C100), pineapple juice (P100), and blended beverage (C50:P50).

3.4. Conclusion

The analysis of different parameters of blended beverages revealed good mineral and phytochemical consumption. The blends exhibited good levels of TFC and possessed strong antioxidant properties. The addition of defatted coconut milk and pineapple juice contributed to acceptable blended beverage in terms of aroma colour, and taste by the sensory panellists. The blended beverages (C60:P40, C50:P50, C40:P60) were liked for their sensorial properties. Based on sensory evaluations, it was found that while some of the blended beverages had an unfavourable taste, the defatted coconut milk and pineapple juice mix (C50:P50) was generally favoured and had the highest overall ranking. Findings of this study showed that blending of defatted coconut milk with pineapple juice offer interesting and novel means to develop a blended beverage of coconut milk with pineapple juice. Thus, there is scope of diversified use of coconut milk after virgin oil extraction for commercial purpose.

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