CHAPTER 4

DETERMINE THE EFFECT OF PASTEURIZATION AND HIGH-PRESSURE HOMOGENIZATION ON MICROBIAL STABILITY OF THE BLENDED BEVERAGE

Chapter 4

Determine the effect of pasteurization and high-pressure homogenization on microbial stability of the blended beverage

4.1. Introduction

Thermal pasteurization is an effective, economical, and environmentally friendly method, and it is frequently used in businesses to prevent the deterioration of juice. However, extensive heat treatment can result in unfavourable changes to phytonutrient, biochemical, and sensory profiles. Therefore, a suitable thermal inactivation procedure that optimizes the elimination of pathogenic and spoilage bacteria while minimizing concomitant damage to the product's flavour, colour, and nutritional attributes is essential (Deshaware et al., 2019). The characteristics of fruit juice and the composition of the growing medium may affect the heat tolerance of pathogens. Numerous investigations have shown that the decimal reduction time (D-value) of investigated pathogens is affected by acid adaptation, acid shock, storage conditions, and culture conditions (Topalcengiz, 2019). Adequate heat treatment is necessary to reduce pathogen counts and ensure fruit juice safety. It is recommended that fruit juices be pasteurized for 0.3–6 s at temperatures between 71.1 (160 °F) and 82.2 °C (180 °F) to achieve a 5-log decrease (5D) (FDA, 2004).

High-pressure homogenization (HPH) generates intense fluid-mechanical stresses on a liquid that is pressurized (up to 350 MPa) when it flows through a micrometric gap at high velocity; thus, HPH can be a financially advantageous method of reducing particle size. According to Maresca et al. (2011), juice homogenized at high pressure (100 MPa) had a longer shelf life and less microbial activity than juice homogenized at regular pressures. It was later demonstrated that HPH treatments were deactivating microbial flora that was either naturally occurring or intentionally introduced into fruit juices. The United States Food and Drug Administration (FDA) mandates juice processors to implement a mandatory juice Hazard Analysis and Critical Control Point (HACCP) program that assures the safety of fruit juice with a mandatory 5-log reduction of the most resistant microorganism of public health significance (pertinent microorganism). Fruit juices can be contaminated with foodborne pathogens, starting from fruit contamination in the field to the production facility (Topalcengiz, 2019). Many organisms use fruit as a substrate and induce spoilage, causing off flavors and odors as well as product discoloration. Acid-tolerant bacteria and fungi (yeasts and moulds) are excellent in this. If the infecting microbes are pathogens, they may be potentially harmful to humans (Velázquez et al., 2012).

Generally, pulp sedimentation is the cause of decreased cloudiness in juice. Fruit juice's inherent nature causes them to settle quickly. Juices exclude ingredients that slows down particle motion. Hydrocolloids are added to enhance the cloudy stability of the juice over extended periods (Silva et al., 2019). The food industry often uses sodium carboxymethylcellulose, xanthan, and guar gum as thickening agents (Liang et al., 2006). Guar gum, due to its low cost and high viscosity, is a naturally occurring non-ionic polysaccharide produced from guar beans and is used as a thickening ingredient in the food industry. Sedimenting particles in juice have no electrical charge; thus, it might be viewed as a technological challenge for the food sector (Liang et al., 2006).

The aqueous extract of coconut (*Cocos nucifera L.*) endosperm is known as coconut milk and is a common ingredient in many tropical countries. In essence, it is a coconut oil-in-water emulsion stabilized by phospholipids and coconut proteins. 11S globulin, also known as cocosin makes up the majority (65%) of the protein in coconut endosperm. It is believed that cocosin plays a more important role in governing the stability instead of albumin or the 7S globulin fraction (Tangsuphoom and Coupland, 2009). Pineapple juice is also a source of vitamins, phenols, organic acids, and carbohydrates. During storage, however, pineapple juice undergoes several deteriorative reactions (some nutrients degradation, microbial spoilage, development of off-flavour, changes in color and texture), resulting in quality degradation of the product (Zheng and Lu, 2011).

It is hypothesized that pasteurization and high-pressure homogenization will have a significant effect on the microbial stability of the blended beverage, and these treatments will reduce microbial load and extend the shelf life of the beverage without compromising the quality attributes. This chapter reports the findings of the study that was conducted to evaluate the effects of thermal and high-pressure homogenization on microbial growth, antioxidant activity, enzyme activity, and physicochemical parameters of the blended beverage of defatted coconut milk and pineapple juice (C50:P50). The effect of guar gum and storage time on the serum separation of the blended beverage was also evaluated.

4.2. Materials and methods

Coconuts and pineapples were collected as described in chapter 3. Chemicals were purchased from Sisco Research Laboratory (Mumbai, India), Merck (Mumbai, India), and HiMedia (Mumbai, India). Standards were purchased from Sigma-Aldrich (USA) and TCI (Japan).

4.2.1. Processing of blended beverage

The pineapple juice and defatted coconut milk was extracted and blended beverage (C50:P50) was prepared by the method described in section 3.2.1 of chapter 3.

4.2.2. Test bacteria standardization

The test bacteria included *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes*, and *Escherichia coli*. Each microorganism was separately inoculated for the respective determination of the D value rather than a mix (cocktail), ensuring that microbe to microbe susceptibility to treatment was not masked. Pure cultures of individual test bacteria were inoculated from nutrient agar slant stock culture into sterile 50 ml nutrient broth (Himedia, Mumbai, India) and incubated at 37 °C at 50 rpm for 18–20 h. After incubation, the bacteria were harvested by centrifugation of 1 ml of the nutrient broth suspension at 4000 rpm for 25 min at 25 °C. The cells were then washed twice with saline (0.9% w/v) and finally 1 ml of it was suspended in 10 ml of a clear, autoclaved blended beverage of coconut milk and pineapple juice (1:10 ratio was maintained) and kept for 30 min to acclimatize.

4.2.3. Thermal pasteurization of blended beverage

To conduct the thermal pasteurization of the blended beverage, the chosen temperatures were 60, 70, 80, and 90 °C while the time intervals chosen were 0, 5, 20, 40, 60, and 120 s. Inoculation of blended beverage with each bacterial culture (from section 4.22) was done individually and the effect of treatment was studied. The beverage obtained after inoculation had a bacterial concentration in the range of 10^7–10^8 CFU/ml. After incubation, grown colonies were counted and effect of heat on lethality of the microorganism under study in the blended beverage was studied. Sterile autoclaved beverage was inoculated with microbial culture and capped in glass test tubes. The inoculated tubes were completely submerged in the water bath and constantly agitated manually throughout the heating time to allow uniform heat contact. After completion of the respective experimental time and temperature combination, the tubes were removed and immediately immersed in an ice-cold water bath. All the heat treatments were performed in triplicates. After incubation, grown colonies were counted to determine the effect of thermal treatment on lethality of microorganism inoculated in the blended beverage (Topalcengiz, 2019).

4.2.4. Optimization of high-pressure homogenised blended beverage

Design-Expert Version 7.1.2 (Stat-Ease, Inc. MN), Response Surface Methodology (RSM) and face centred central composite design (FCCD) were used to optimize process conditions for the preparation of high-pressure homogenized blended beverage that was microbially safe and demonstrated peroxidase inactivation. Two independent parameters: the number of passes (2-6) and homogenization pressure (100-500 bar) were varied to see how they affected microbial growth and peroxidase inactivation in the blended beverage (Table 4.1). The *E. coli* bacterial culture was added to the blended beverage and acclimatised following the previously mentioned method in section 4.2.2 and subjected to high-pressure homogenization. ANOVA was performed to assess the importance of the model parameters under investigation. The significance level of $p \leq 0.05$ was used to determine its statistical significance.

Table 4.1: Independent factors and its range set in the experiment design

Experimental Variables	Codes	Coded levels		
		-1	0	+1
Homogenizing pressure (bar)	A	100	300	500
Number of passes	B	2	4	6

4.2.5. Stabilization and serum separation of the blended beverage

The blend (C50:P50) that was given the highest sensory score (from chapter 3, section 3.3.6.) was stabilized with guar gum at a concentration of 0.1%, 0.2% and 0.3% and coded as 1GGCP, 2GGCP, and 3GGCP. The C50:P50 without added guar gum was coded as Control. The guar gum concentration with lowest serum separation was added to the blended beverage and then subjected to high pressure homogenization and pasteurization separately. Blended beverage added with guar gum and subjected to optimized high-pressure homogenization was coded as 3GGHPH, blended beverage without guar gum and subjected to optimized high-pressure homogenization was coded as HPH, blended beverage added with guar gum and subjected to pasteurization was coded as 3GGPAS, and blended beverage without guar gum and subjected to pasteurization was coded as PAS. The blends were brought to with 3.5 pH and 13 ºBrix TSS following the method of (Teimouri et al., 2018) with some modifications. The height of the

upper layer of the samples after sedimentation was measured and divided by the total height of the sample and multiplied by 100 and expressed as the percent serum separation (SS%).

4.2.6. Determination of ascorbic acid content

Ascorbic acid content in treated blended beverages was determined by the method described in section 3.2.2.4. in chapter 3.

4.2.7. Phytochemical content and antioxidant properties of blended beverages

4.2.7.1. Determination of total phenolic content (TPC)

TPC of treated blended beverages was measured as per the method described in section 3.2.3.1. in chapter 3.

4.2.7.2. Determination of total flavonoid content (TFC)

The flavonoid content in treated blended beverage samples was determined following the method given in section 3.2.3.2. of chapter 3.

4.2.7.3. DPPH (2,2-diphenylpicrylhydrazyl) radical scavenging activity

Antioxidant activity of blended beverages was determined with stable radical DPPH using the method described in section 3.2.3.3. of chapter 3.

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

4.2.9. Peroxidase activity of 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. 4.1:

$$\text{Activity (U/ml)} = \frac{\text{Ab (sample)} - \text{Ab (blank)}}{0.001 \times t} \quad \text{(Eq. 4.1)}$$

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

4.2.10. Sensory analysis of freshly treated blended beverages

Sensory analysis of blended beverage treated with thermal pasteurization and high-pressure homogenization was determined using the nine-point hedonic scale following the method described in section 3.2.6. in chapter 3.

4.2.11. 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 ($p < 0.05$).

4.3. Results and discussion

4.3.1. Microbiological quality of thermally pasteurized blended beverage

A freshly prepared blended beverage of defatted coconut milk and pineapple juice (C50:P50) was loaded with four different microbes: *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes*, and *Escherichia coli*. Thermal treatment at 60, 70, 80, and 90 °C with time intervals of 0, 5, 20, 40, 60, and 120 s were used to analyse D_{10} value for different microbes. The D_{10} value calculated for *Staphylococcus aureus* at 60 ℃, 70 ℃, 80 ℃, and 90 ℃ was 71.0±1.30 s, 66.8±1.01 s, 49.9±0.28 s and 40.3±0.25 s, respectively. It is obvious that with increasing temperature, the D value decreased. Atalar et al. (2019) reported that the total aerobic mesophilic bacterial count in hazelnut beverage was significantly reduced by up to 2.41 log cycles after thermal treatment at 65 ℃ for 30 min. Increasing the thermal treatment temperature to 72 ℃ for 20 min caused complete microbial inactivation in hazelnut beverage. The D_{10} value for *Bacillus cereus* at 60 ℃, 70 ℃, 80 ℃, and 90 ℃ was 38.1±1.12 s, 33.9±0.72 s, 26.0±0.74 s, 22.7±0.88 s respectively; for *Listeria monocytogenes,* it was 41.3±0.18 s, 30.6±0.81 s, 21.6±1.97 s, 20.3±0.53 s; and for *E. coli,* it was 55.9±1.18 s, 41.2±1.27 s, 30.4±0.45 s, 21.1±0.19 s, respectively. To obtain the 5-log reduction, the D value was converted to 5D values (Fig. 4.1). The $5D_{10}$ value calculated for *Staphylococcus aureus* at 60 ℃, 70 ℃, 80 ℃, and 90 ℃ was 5.9±1.30 min, 5.5±1.01 min, 4.1±0.28 min and 3.3±0.25 min, respectively. 5D value for *Bacillus cereus* at 60 ℃, 70 ℃, 80 ℃ and 90 ℃ was 3.1±1.12 min,

2.8±0.72 min, 2.1±0.74 min, 1.8±0.88 min, respectively. Gabriel and Nakano (2011) reported that D value of *E. coli* of thermally treated apple juice was 25 s and 5.3 s at 55 ˚C. At temperature 60 ℃, 70 ℃, 80 ℃, and 90 ℃, 5D value for *Listeria monocytogenes* was 3.44±0.18 min, 2.5±0.81 min, 1.8±1.97 min, 1.6±0.53 min and for *E. coli* was 4.6±1.18 min, 3.4±1.27 min, 2.5±0.45 min, 1.7±0.19 min, respectively. Deshaware et al. (2019) reported that D value for native microflora was 19.7 s in bitter gourd juice. Therefore, a total time of 98.5 s was required to achieve a 5-log reduction in native microflora. Authors observed that *E. coli* was the most heat-sensitive pathogen and *Shigella boydii* was the most heat-resistant pathogen, with D_{10} value of 21.2 s and 42.8 s, respectively.

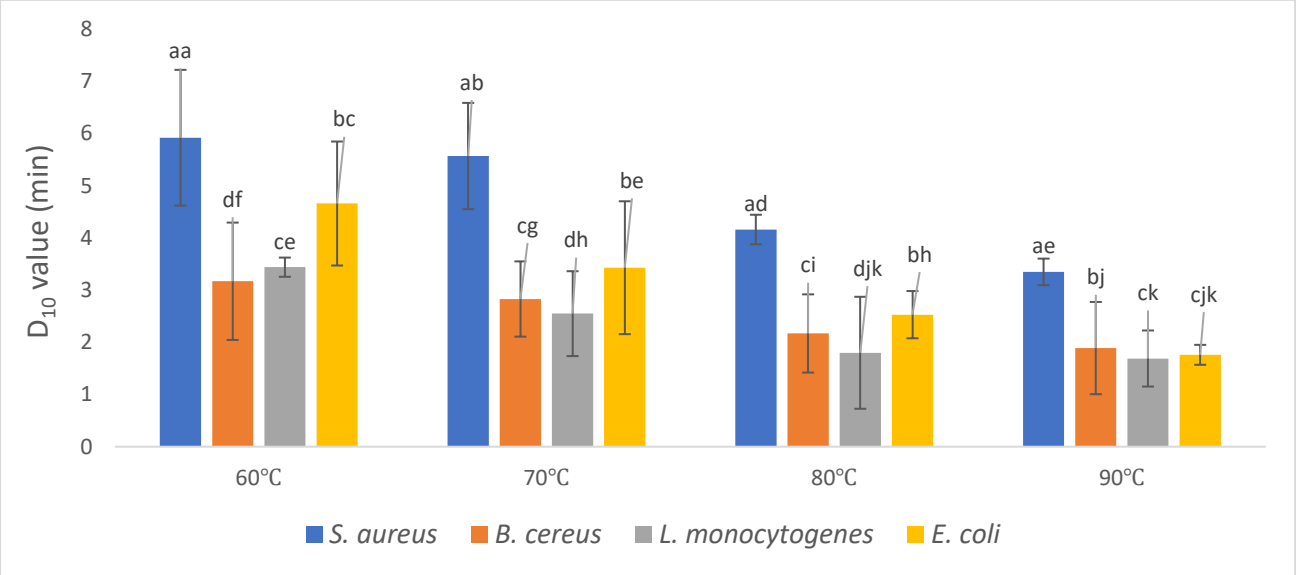


Fig 4.1: $5D_{10}$ (time) value for test organisms post thermal pasteurization at 60 ℃, 70 ℃, 80 ℃, and 90 ℃.

4.3.2. Effect of thermal treatment on enzyme activity of blended beverage

Thermal pasteurization resulted in significant ($p > 0.05$) changes in peroxidase activity. Fig. 4.2 reveals that POD was inactivated in samples thermally treated for different times and temperatures to fulfil 5-log reduction of the test microbes. Peroxidase, which is responsible for many oxidative changes and flavour alterations in fruits and vegetables, is generally considered the most thermostable enzyme in plants. Consequently, it has been widely used as an indicator of heat treatments in food processing (Hirsch et al., 2008). The POD activity decreased with the effect of temperature and time. At 60 ℃, and thermal treatment for 5.9 min, 3.1 min, 3.4 min, 4.6 min, the POD residual activity was 67.4±0.73%, 81.6±1.40%, 76.7±1.4% and 68.6±0.61%, respectively; at 70 ℃ time and treatment time of 5.5 min, 2.8 min, 2.5 min, 3.4 min, the residual activity was 53.4±1.26%, 62.8±1.06%, 76.9±0.25% and 64.2±0.18%,

respectively; at 80 ℃ with treatment time of 4.1 min, 2.1 min, 1.8 min, 2.5 min, the POD residual activity was 27.5±0.30%, 35.5±0.43%, 26.6±0.69% and 24.1±0.07%, respectively; and at 90 ℃ with treatment time of 3.3 min, 1.8 min, 1.6 min, 1.7 min, POD residual activity was 22.9±0.07%, 24.6±0.05%, 27.7±0.26 and 26.2±0.15%, respectively.. It was seen that with an increase in temperature and time, the residual activity of POD decreased. The blended beverage with pH of 3.5 and thermal treatment was effective in reducing the POD activity. Hirsch et al. (2008) reported residual POD activities of 49.2% and 25.8% after the thermal treatment of orange juice at 42°C and 52°C, respectively. Hun et al. (2009) reported that after thermal inactivation of pineapple homogenate for 15 min at 65 ℃ and 75 ℃, the residual POD activities were approximately 54% and 22%, respectively. When the temperature was reduced to 45 ℃ and 55 ℃, the residual activities of POD were 74% and 70%, respectively. Murtaza et al. (2020) reported that relative activity of POD in apple juice after thermal treatment at 45, 65, and 75 °C for 20 min was 115.3%, 94.6%, and 79.4%, respectively. Koo et al. (2023) reported that Bok choy juice with pH 4 and 6 had relative activities of 15.1 ± 0.9% and 87.5%. The low acid juice should be acidified to pH below 4.6 to prevent the growth of pathogenic *Clostridium botulinum* spores during storage (US FDA, 2007). The POD activity at 80 ℃ for 1.8 min and 4.1 min and at 90 ℃ for 1.6 min and 1.7 min showed insignificant difference and lower POD residual activity as compared to the activity levels at 60 ℃ and 70 ℃. The significantly lowest POD residual activity of 22.9±0.07% was recorded at 90 ℃ with treatment time of 3.3 min. With increase in time also, the POD residual activity had reduced.

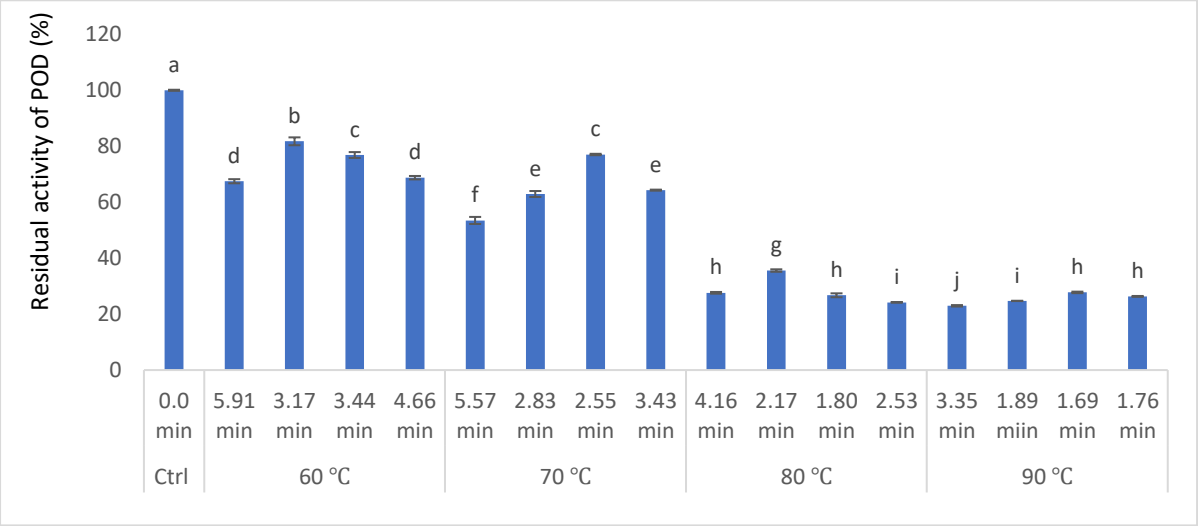


Fig 4.2: Effect of thermal pasteurization on peroxidase residual activity in blended beverage (C50:P50).

4.3.3. Effect of thermal treatment on antioxidant property of blended beverage

Initial DPPH radical scavenging activity of blended beverage was 86.4±0.15%. Thermal pasteurization resulted in a significant ($p > 0.05$) decrease in DPPH activity, as shown in Fig. 4.3. The DPPH activity decreased when subjected to different temperatures and times. DPPH activity after thermal treatment at 60℃ for 5.9 min, 3.1 min, 3.4 min, 4.6 min, was 63.4±0.65%, 76.1±0.14%, 69.1±0.27% and 68.3±0.51%, respectively. DPPH activity was 59.0±0.06%, 63.4±0.67%, 65.1±0.14% and 70.2±0.31%, respectively at 70 ℃ when thermally treated for 5.5 min, 2.8 min, 2.5 min, 3.4 min. The DPPH activity in the beverage when thermally treated at 80℃ for 4.1 min, 2.1 min, 1.8 min, 2.5 min was 36.2±0.31%, 65.4±0.67%, 69.4±0.56% and 58.0±0.05%, respectively, whereas at 90 ℃, DPPH activity was 31.2±0.32%, 44.2±0.40%, 55.3±0.45% and 52.3±0.50% respectively on thermal treatment for 3.3 min, 1.8 min, 1.6 min, 1.7 min With increase in temperature and time of pasteurisation, residual activity of POD also decreased. Bavisetty and Venkatachalam (2021) reported that the DPPH activity of waxed apple juice decreased upon thermal pasteurization. Similar results of decreased DPPH activity with increase in temperature and time was observed in the blended beverage. Thermal treatment for 1.8 min at 80 ℃ showed significantly higher DPPH activity as compared to other treatment times at 80 ℃ and 90 ℃. The DPPH activity at 70 ℃ and 80 ℃ with treatment time of 3.4 min and 1.8 min was 70.2±0.31%, and 69.4±0.56%, respectively, and the difference was insignificant (Fig 4.3). Benattouche et al. (2021) reported that DPPH activity of untreated orange juice was 61.5% and after thermal treatment at 90 ℃ for 1 min was reduced to 58.9%. Due to use of heat during thermal pasteurization the antioxidant quality of pasteurized blended beverage may be adversely affected. This decline is attributed to the degradation of heat-sensitive bioactive compounds, which are key contributors to antioxidant activity (Bavisetty and Venkatachalam, 2021). The extent of antioxidant degradation during pasteurization is influenced by the temperature and duration of the heat treatment. Higher temperatures and longer exposure times are associated with greater losses in antioxidant compounds (Pérez et al., 2021).

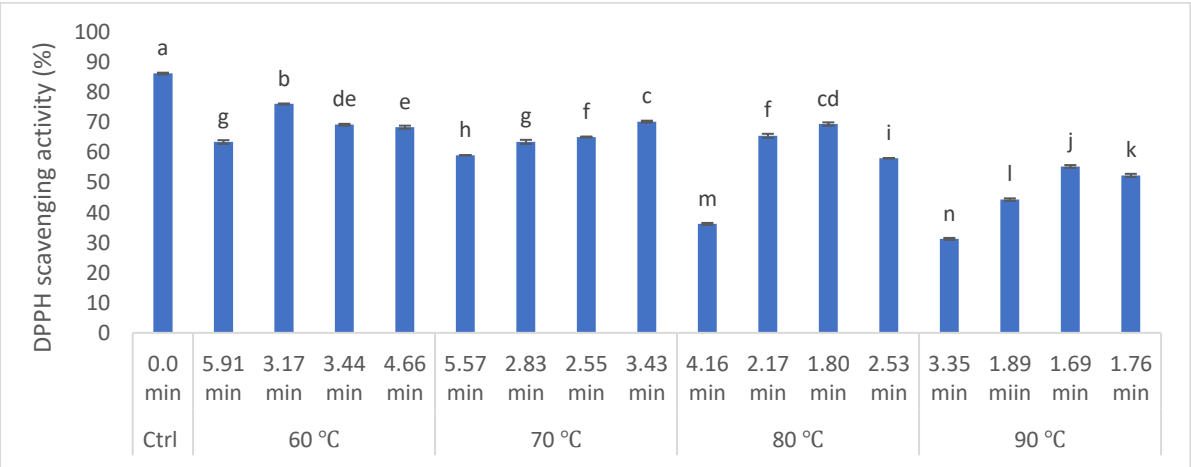


Fig 4.3: Effect of thermal pasteurization treatment on DPPH scavenging activity of blended beverage (C50:P50).

4.3.4. Optimization of high-pressure homogenization of blended beverage

The effect of high-pressure homogenization (HPH) of blended beverages containing defatted coconut milk and pineapple juice are shown in Fig. 4.5. The impact of HPH pressure and number of passes on microbial counts and POD activity were analysed. The pressure and number of passes were thus changed using an FCCD design model to mix distinct ratios of the pressure and passes. The *E. coli* strains were inoculated into the blended beverage, and the microbial count of the beverage was 5.47 log CFU/ml. The microbial count decreased with increasing homogenization pressure and passes. Microbial count was reduced to 1.10 log CFU/ml when the beverage was homogenized at maximum pressure of 500 bar for 6 passes. From the ANOVA results, the overall effect of homogenization pressure and the number of homogenizing passes was found to be significant for reduction of microbial load ($p< 0.0001$) and POD residual activity ($p< 0.0001$) (Tables 4.3 and 4.4). The R^2 value for POD residual activity and microbial load was 0.9617 and 0.9721, respectively. This validated the models, meaning that they could effectively explain the experimental data. Pressure was significantly more effective than the number of passes (Table 4.3, 4.4 and Fig. 4.5) in the reduction of microbial count and residual activity of POD in the blended beverage. Fig. 4.5 (a) shows the effect of increasing the homogenizing pressure on reducing the lethality of *E. coli* in the blended beverage. However, the number of passes did not considerably reduce the lethality of *E. coli* (Fig. 4.5 (b) and Table 4.4). Food items that are safe for consumption often have naturally occurring spoilage and pathogenic microbe counts of less than 10^6 CFU/ml (Chen et al., 2014). The maximum lethality of *E. coli* (1.1 log CFU/ml) was observed at a pressure of 490 bar and 6 number of passes. Welti et al. (2009) reported both the number of passes (0-5) and pressure (50-250 MPa) affected the microbial load in homogenized orange juice. The microbial load decreased as the number of passes increased. The microbial count in orange juice homogenized for 5 cycles was 8.7×10^3 CFU/ml for mesophile and 1.85×10^3 CFU/ml for yeasts and moulds. Tables 4.3 and 4.4 shows that the lack of fit of the model was not significant relative to the pure error. The F-values (0.57 and 0.19) for the regression and lack of fit are also provided in Tables 4.3 and 4.4. Thus, the model was found to be adequate for prediction over the range of tested variables.

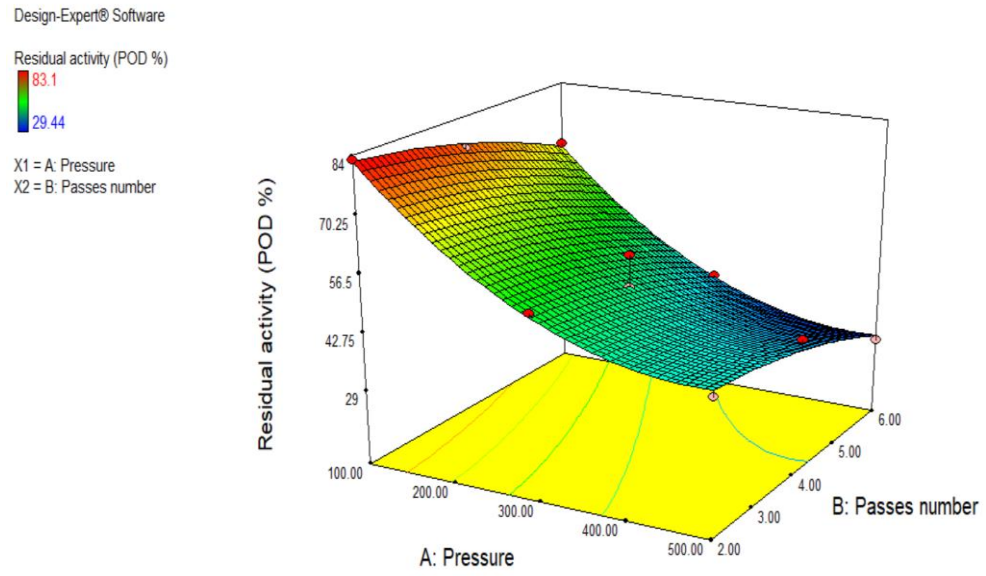
Maresca et al. (2011) reported the potential of HPH treatment at pressures between 200 and 300 MPa for inactivating pathogenic and spoilage microflora as an alternative to thermal treatments for prolonging the juice shelf life. For instance, the application of HPH treatment to

orange juice (300 MPa in a two stage-homogenization system) significantly decreased *E. coli.* Finally, the optimum parameters selected were: homogenizing pressure of 490 bar and 6 number of passes. As shown in Fig. 4.5 (a), with an increase in homogenizing pressure and the number of passes, the POD activity decreased. Fig. 4.6 (a and b) shows the validation of the adequacy of the model, in which all data points were found to be quite close to the predicted line, indicating excellent fitness of the model. The correlation coefficients (R^2) between the predicted and observed values were 0.9617 and 0.9721 ($P< 0.0001$). Thus, the accuracy and reliability of the proposed model were validated for reducing *E. coli* survival and POD residual activity in blended beverage of defatted coconut milk and pineapple juice after high-pressure homogenization. Analysing the initial microbial counts and predominant species present in C50:P50 allowed us to develop a method to inactivate bacteria using RSM to optimize sterilization conditions. Based on maximum desirability level of 0.99, optimized conditions that were obtained were: 490 bar pressure and 6 number of passes (Table 4.5). Under these conditions, the predicted lethality of *E. coli* and residual POD activity was 1.1 log CFU/ml and 30.5%, respectively. Szczepanska et al. (2022) reported that the effects of multi-pass HPH may also be attributed to the ability of HPH to break down molecular aggregates in the homogenizing valve. Codina et al. (2017) reported a 20% decrease in POD activity under high-pressure homogenization treatment of tiger nut milk at an inlet temperature of 40 ◦C.

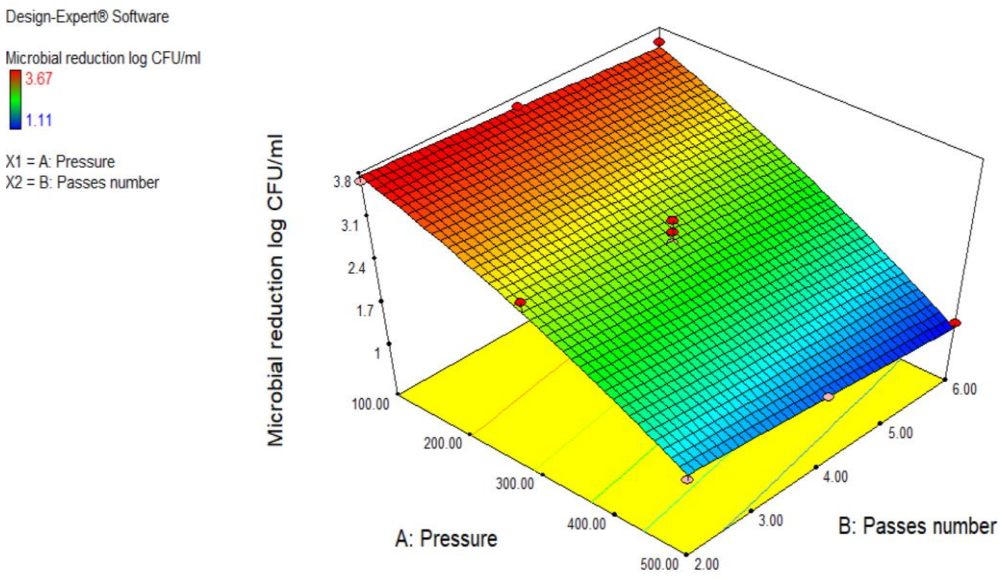
Table 4.2: Experimental design matrix for the effect of high-pressure homogenization pressure and number of passes on residual activity of POD and lethality of *E. coli*

Experimental run	Independent variables		Responses	
	Factor 1 Pressure (bar)	**Factor 2 Passes number**	**Response 1 Residual activity POD (%)**	**Response 2 Lethality of *E. coli* (log CFU/ml)**
1	100	2	83.1	3.6
2	100	4	76.3	3.6
3	100	6	68.2	3.5
4	300	2	54.7	3.0
5	300	4	47.2	2.7
6	300	6	39.3	2.3
7	300	4	56.2	2.9
8	300	4	48.3	2.6
9	300	4	47.4	2.4

10	300	4	42.8	2.4
11	500	6	29.4	1.1
12	500	4	42.7	1.3
13	500	2	43.6	1.4

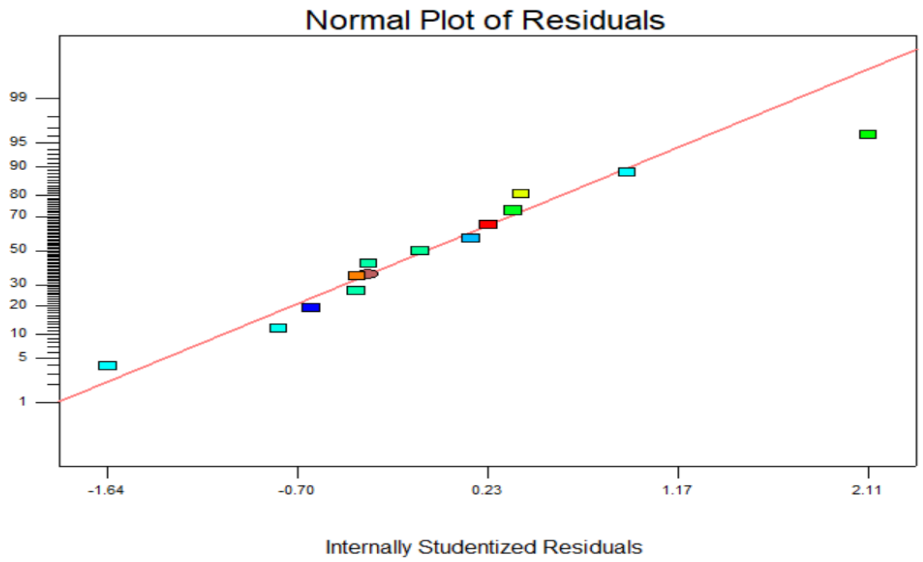


(a)

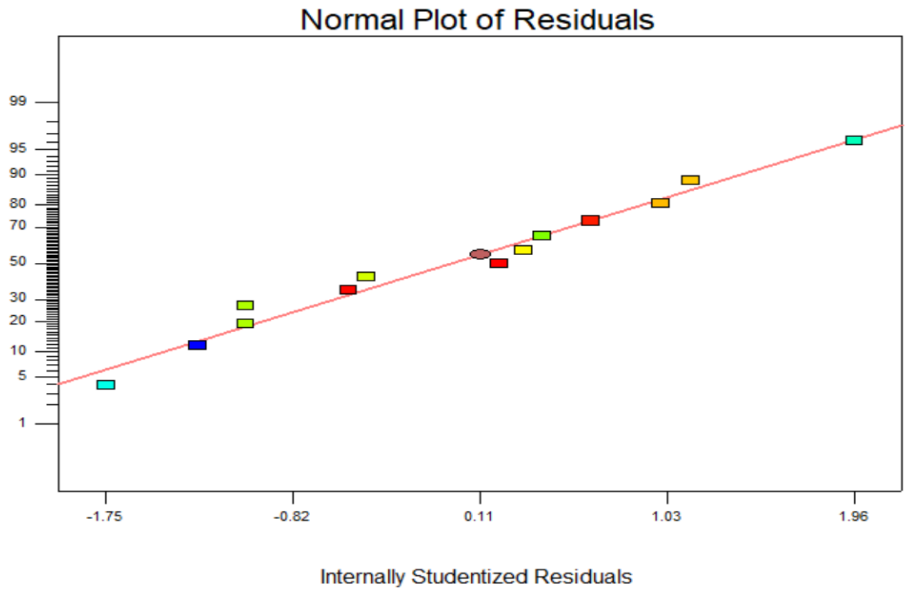


(b)

Fig 4.4: Response surface plots: (a) POD residual activity (homogenization pressure and number of passes) and (b) lethality of *E. coli* (homogenization pressure and number of passes)



(a)



(b)

Fig 4.6: Scatter plot: (a) POD residual activity (homogenization pressure and number of passes) and (b) lethality of *E. coli* (homogenization pressure and number of passes).

4.3.4.1. Modelling and validation

Design-Expert (Design-Expert Version 7.1.2 software, Stat-Ease) was used to analyse statistically all the experimental data, that were determined in accordance with FCCD design. The quadratic polynomial equations (Eq. 4.1 and 4.2) fitted with all the experimental data explained the effect of homogenizing pressure, number of passes on microbial reduction, and residual POD activity.

Microbial reduction = + 2.65 – 1.17*A – 0.19*B – 0.065*A*B – 0.19*A^2 – 0.011*B^2 Eq. 4.1

Residual POD activity = + 48.71 – 18.62*A – 7.43*B + 0.017*A*B + 10.13*A^2 – 2.38*B^2 Eq. 4.2

R^2 values obtained were 0.97 and 0.96 for lethality of *E. coli* and residual POD activity of homogenized blended beverage, respectively (Table 4.3 and 4.4). The quadratic model having higher R^2 indicates a good correlation between responses and input parameters. The CV% score of less than 10% indicated well fit of model (Table 4.3 and 4.4). The models were validated (Table 4.6) by non-significant lack of fit for responses. The model's high level of significance with $p<0.0001$ for lethality of *E. coli* and $p<0.0001$ for residual POD activity, explained the high efficiency of experimental data.

Table 4.3: Regression coefficients and ANOVA estimated for POD residual activity of the high pressure homogenized blended beverage

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remarks
Model	2702.3	5	540.46	35.19	< 0.0001	Significant
A-Pressure	2080.97	1	2080.97	135.5	< 0.0001	
B-Passes number	330.78	1	330.78	21.54	0.0024	
AB	0.11	1	0.11	7.09E-03	0.9352	
A^2	283.34	1	283.34	18.45	0.0036	
B^2	15.6	1	15.6	1.02	0.3471	
Residual	107.5	7	15.36			
Lack of Fit	13.5	3	4.5	0.19	0.8971	Not significant
Pure Error	94	4	23.5			
Cor Total	2809.81	12				

R^2	0.9617
CV%	7.50

Table 4.4: Regression coefficients and ANOVA determined for lethality of *E. coli* of the high pressure homogenized blended beverage

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remarks
Model	8.55	5	1.71	48.71	< 0.0001	Significant
A-Pressure	8.19	1	8.19	233.36	< 0.0001	
B-Passes number	0.22	1	0.22	6.39	0.0179	
AB	0.017	1	0.017	0.48	0.0864	
A^2	0.095	1	0.095	2.71	< 0.0001	
B^2	3.16E-04	1	3.16E-04	8.99E-03	0.9271	
Residual	0.4	7	0.035			
Lack of Fit	0.23	3	0.025	0.57	0.6615	
Pure Error	0.17	4	0.043			Not significant
Cor Total	14.62	12				
R^2			0.9721			
CV%			7.31			

Table 4.5: Desirability values at optimized parameters

Homogenizing pressure (bar)	Number of passes	Lethality of E. coli (log CFU/ml)	POD activity of (%RA)	Desirability
490.16	6	1.1	30.51	0.99
492.22	6	1.1	30.52	0.99
495.07	6	1.1	30.53	0.99

Table 4.6: Validation of optimization conditions of HPH

		Predicted		Experimental		R_{dev}(%)		
Pressure (bar)	Number of Passes	log CFU/ml	POD activity of (%RA)	log CFU/ml	POD activity of (%RA)	log CFU/ml	POD activity of (%RA)	Overall R_{dev}(%)
490	6	1.10	30.51	1.07	29.34	2.80	3.98	3.39

4.3.5. Serum separation (SS) of blended beverage

Guar gum is a naturally occurring non-ionic polysaccharide produced from guar beans and is utilised as a stabilizer in the food industry to improve the stability of juice. The influence of guar gum at different concentrations (0.1%, 0.2% and 0.3%) was ascertained individually and in combination with high pressure homogenization and pasteurization on the stabilization of C50:P50. The results are presented in Fig 4.4. Ruihuan et al. (2017) reported that orange juice with 0.5% guar gum affected the taste and made it thick. In line with their observations, guar gum concentrations below 0.5% were selected for addition in the blended beverage to reduce serum separation. Significant differences between the samples and the Control were identified using analysis of variance (ANOVA) $P<0.01$. The Control with no stabilizer showed serum separation after 1 h of blended beverage preparation, i.e. 23.63±1.4% and no further change in SS% occurred on day 15 of storage. With increasing guar gum concentration, the SS% decreased ($P<0.05$). The SS% values of samples 1GGCP, 2GGCP, and 3GGCP showed no separation after 2 h. However, after the first day of storage, the blended beverage containing 0.1% guar gum had an SS% of 3.6±0.05%, whereas the blended beverages containing 0.2% and 0.3% guar gum remained stable. On day 5 of storage, 2GGCP exhibited a separation of 2.30±0.02%, whereas no separation was observed in 3GGCP. The beverage added with 0.3% guar gum (3GGCP) registered serum separation after day 5, but recorded SS% of 12.6±0.05% on day 15. A previous study of a milk and sour cherry juice mixture reported by Teimouri et al. (2018) found that by increasing the concentration of inulin, the SS% significantly decreased. The authors reported that a considerably greater amount of inulin (10% w/v) was required to restrict serum separation to 12% in the juice mixture. The SS% values of 3GGPAS and PAS

after 1 h of storage was 11.0±0.14% and 15.0±1.5%, respectively. The HPH blended beverage after 1 h of storage showed SS% of 20±1.4%. Maximum SS% of 60.0±0.05% was observed in 3GGHPH (blended beverage with 0.3% guar gum and subjected to high-pressure homogenization) and HPH (high-pressure homogenization blended beverage without guar gum) on day 15 of storage, whereas the SS% of Control sample was 23.63±1.4%. In 3GGPAS and PAS samples, the sediment content increased over time till 15 days attaining an SS% of 42.5±0.35% and 55.0±1.5%, respectively on day 15. Thus, increasing the guar gum concentration had a positive impact on SS%, whereas a reverse trend was observed for high-pressure homogenization. Serum separation that occurred due to the precipitation under the influence of gravity of some insoluble materials present in blended beverage (Ruihuan et al., 2017). The steric repulsion decreased during homogenization, resulting in the destabilization of the dispersed material (Teimouri et al., 2018).

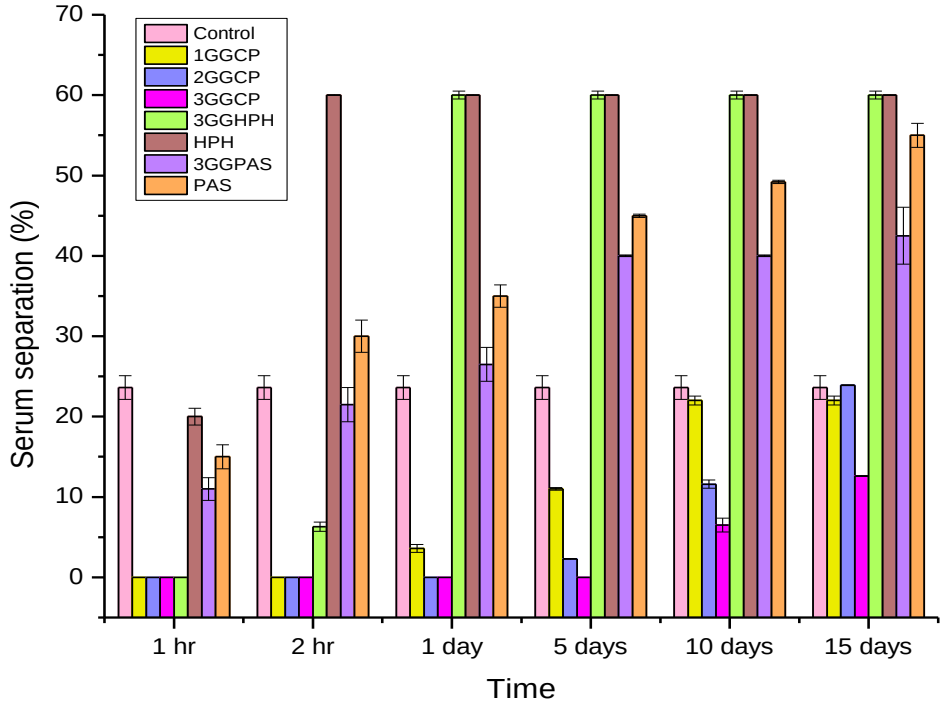


Fig 4.4: Serum separation of (a) Control without gum; (b) 1GGCP: blended beverage (C50:P50) treated with 0.1% guar gum; (c) 2GGCP: blended beverage (C50:P50) treated with 0.2% guar gum; (d) 3GGCP: blended beverage (C50:P50) treated with 0.3% guar gum; (e) 3GGHPH: combination of 0.3% guar gum and high-pressure homogenization; (f) HPH: high-pressure homogenisation of blended beverage (C50:P50); (g) 3GGPAS: combination of 0.3% guar gum and pasteurization; and (h) PAS: pasteurized blended beverage.

Atalar et al. (2019) reported that the colloidal stability of hazelnut beverage samples was reduced by thermal treatment. In comparison to the Control and long temperature long time treated hazelnut beverage, particle sedimentation of high temperature short time beverage

occurred quickly. This may be connected to protein denaturation during heat treatment, which causes phase separation during storage. Following heat treatment, particle sedimentation increases due to the increased particle size of beverages (Atalar et al., 2019). pH is an important factor in serum separation. At the isoelectric point, aggregation and precipitation occur because of the reduction in the repulsive electrostatic force and the predominance of attraction forces. At isoelectric points, the positive and negative charges are balanced, and most proteins have isoelectric points in the pH range of 4 to 7 (Novák and Havlíček, 2016). Tangsuphoom and Coupland (2008) reported that the isoelectric point of coconut milk protein is pH 3.5 to 4. According to our study results, addition of 0.3% guar gum to blended beverage of defatted coconut milk and pineapple juice

4.3.6. Phytochemical and antioxidant properties of treated blended beverage

The blended beverage that was pasteurized at 80 ℃ for 1.80 min was used for further studies due to its lower POD residual activity and maximum DPPH scavenging activity. Along with it, optimized high-pressure homogenised blended beverage was used for antioxidant and phytochemical studies. The total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity in pasteurized and high-pressure homogenized blended beverages are given in Table 4.6. The TPC, TFC, and DPPH activity of untreated blended beverage (Control) was 30.6±0.53 mg/100 ml, 18.7±0.12 mg/100 ml and 86.0±0.52%, respectively. TPC content in 3GGPAS and 3GGHPH were significantly lower i.e. 21.4±0.09 mg/100 ml and 19.3±0.31 mg/100 ml than in Control ($p<0.05$), and the retention of TPC was higher in 3GGPAS than in 3GGHPH. As shown in Table 4.6, the TFC and DPPH radical scavenging activity of 3GGPAS was 12.2±0.32 mg/100 ml and 69.4±0.4%, respectively, which was significantly higher than the values for 3GGHPH i.e. 9.8±0.26 mg/100 ml and 52.5±0.7%, respectively. He et al. (2016) reported that thermal treatment disrupts cell walls and promotes the release of bound phenolic compounds from fruit cells, which increases their concentration in the extracts. High pressure homogenization ruptures the fruit cellular structure and may cause the release of cytoplasmic polyphenol oxidase, resulting in the oxidative degradation of the phenolic compounds. The enhancement of TPC, TFC, and DPPH radical scavenging properties of 3GGPAS was greater than that of 3GGHPH. Similar results were obtained by He et al. (2016), who reported that thermally treated grape juice has a higher total phenolic content than high-pressure homogenized grape juice. Kruszewski et al. (2021) reported that both the inlet temperature and homogenization pressure were crucial for the maintenance of juice antioxidant activity. The heat breakdown of compounds after one minute of pasteurization resulted in a 26.6% reduction in the total phenolic concentration of fresh blackcurrant juice.

High-pressure homogenization with 20 ℃ inlet temperature exhibited 86% reduction in total phenolic content in black currant juice (Kruszewski et al., 2021). During the HPH treatment, the degradation of antioxidant property may be due to oxidation reaction with the introduced oxygen brought to the juice by the turbulent process of homogenization (Kruszewski et al., 2021). From our study we can say that the impact of mechanical stress along with heat generated during high pressure homogenization affect the antioxidant property of blended beverage more as compared to thermally treated blended beverage.

4.3.7. Ascorbic acid content of treated blended beverages

The ascorbic acid concentration (Table 4.6) in the Control (untreated blended beverage) was 35.6±0.09 mg/100 ml. The ascorbic acid content in 3GGHPH (high-pressure homogenized and gum treated blended beverage) was significantly lower than that of the Control sample ($p<0.05$) Even the retention of ascorbic acid was higher ml in 3GGPAS (27.7±0.38 mg/100 ml) than 3GGHPH (22.8±0.21 mg/100 ml). Ascorbic acid is known to be sensitive and unstable to temperature (Kruszewski et al. 2021); even if only a few seconds is spent in a high-pressure homogenization valve, its content may deteriorate. Chaikham and Apichartsrangkoon (2012) reported two main causes for ascorbic acid reduction: oxidative reactions by enzymes such as cytochrome oxidase and ascorbic acid oxidase. High pressure affects the secondary, tertiary, and quaternary structures of proteins; such conformational changes can enhance enzyme activity by revealing active sites, thereby facilitating catalytic conversion. However, it is difficult to prevent ascorbic acid loss during processing, since ascorbic acid stability largely depends on the oxygen concentration.

4.3.8. Peroxidase activity (POD) of treated blended beverages

The data on the POD residual activity are presented in Table 4.6. There was a statistically significant decrease ($p<0.05$) in the residual activity of peroxidase in 3GGPAS (26.8±0.54%) and 3GGHPH (30.2±0.04%) and in comparison, to the untreated Control sample. (99.0±0.89%). Szczepańska et al. (2021) reported that high-pressure homogenization decreased the POD activity of apple juice by 16.4% compared to that of untreated fresh juice. This phenomenon may be due to mechanical damage to the fruit juice cells caused by high-pressure homogenization. The damage may then enable the enzymes contained in plant vacuoles to release, rendering the enzymes susceptible to inactivation (Szczepańska et al., 2021).

Table 4.6: Antioxidant and phytochemical properties of treated blended beverage

Samples	Total phenolic content (mg GAE/100 ml)	Total flavonoid content (mg QE/100 ml)	DPPH activity (%)	Residual activity of POD (%)	Ascorbic acid (mg/100 ml)
Control	30.6±0.53[a]	18.7±0.12[a]	86.0±0.52[a]	99.0±0.89[a]	35.6±0.09[a]
3GGHPH	19.3±0.31[c]	9.8±0.26[c]	52.5±0.7[c]	30.2±0.04[b]	22.8±0.21[c]
3GGPAS	21.4±0.09[b]	12.2±0.32[b]	69.4±0.4[b]	26.8±0.54[c]	27.7±0.38[b]

[a-c] Values with different superscripts vary significantly within a column at $p<0.05$

4.3.9. Sensory analysis of treated blended beverages

In the present study, the panellists evaluated blended beverage of defatted coconut milk and pineapple juice treated with thermal and high-pressure homogenization in terms of color, aroma, taste, consistency and overall acceptability. According to Fig. 4.7 and Table 4.7, the average overall acceptability was higher in Control and 3GGPAS as compared to 3GGHPH. Differences in relation to the Control and treated blended beverages were observed in terms of aroma, colour, consistency, with the blended beverage 3GGPAS showing higher score than 3GGHPH and Control, as shown in Fig 4.7. The Control and 3GGPAS scored higher on taste then 3GGHPH. Geraldi et al. (2021) found that thermally processed jabuticaba juice scored higher on overall liking, appearance, aroma, flavour and mouth feel as compared to Control sample. Velázquez et al. (2019) reported that during sensory analysis in terms of sweetness, flavour, acidity, and overall acceptability, no significance difference was observed between pasteurised and high-pressure homogenised orange juice, but colour of high-pressure homogenized sample scored worst as compared to pasteurized and Control orange juice. Among the three samples that were evaluated, thermally treated 3GGPAS got maximum scores for all sensory attributes.

Table 4.7: Sensory evaluation of treated blended beverages using 9-point hedonic scale

Samples	Average score				
	Aroma	Taste	Colour	Consistency	Overall acceptability
Control	7.4±0.15[b]	8.0±0.11[a]	7.3±0.07[c]	7.3±0.12[bc]	8.0±0.12[a]
3GGHPH	7.3±0.08[b]	7.5±0.07[b]	7.8±0.02[ab]	7.5±0.01[ab]	7.6±0.21[ab]
3GGPAS	7.7±0.20[a]	8.0±0.12[a]	8.0±0.07[a]	7.8±0.20[a]	8.0±0.14[a]

[a-c] Values with different superscripts vary significantly within a column at $p<0.05$

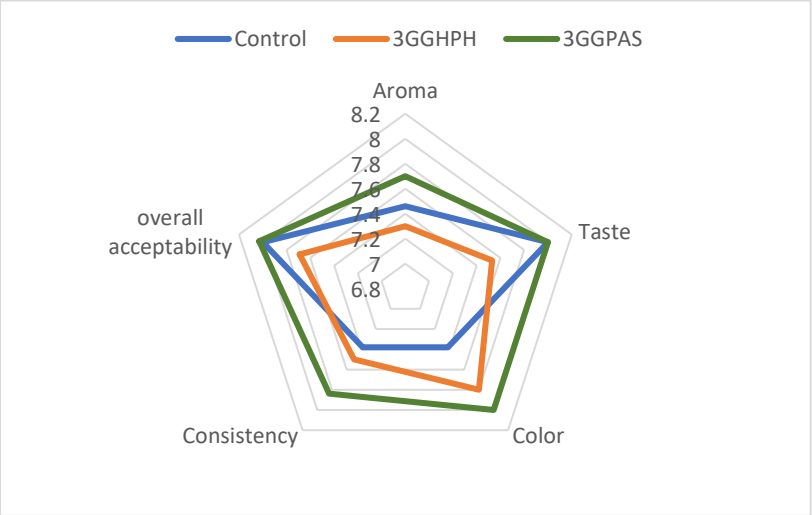
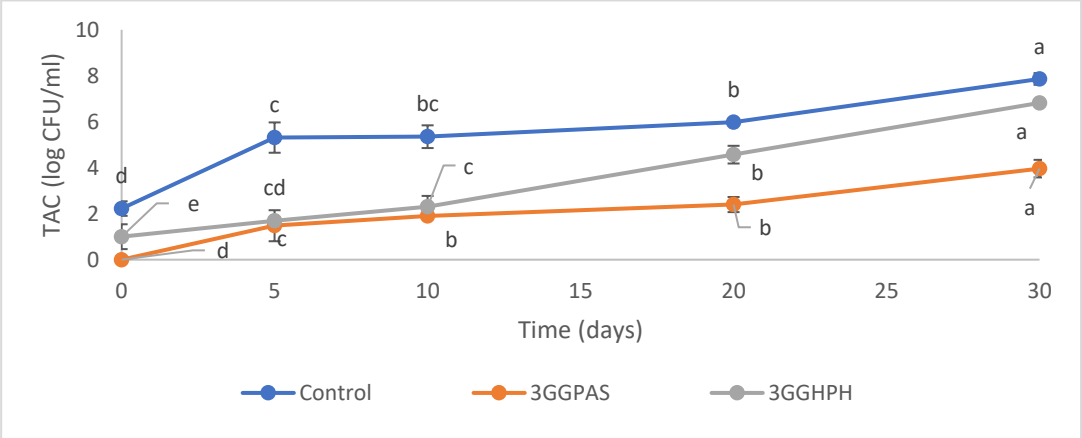


Fig 4.7: Sensory analysis of treated blended beverages.

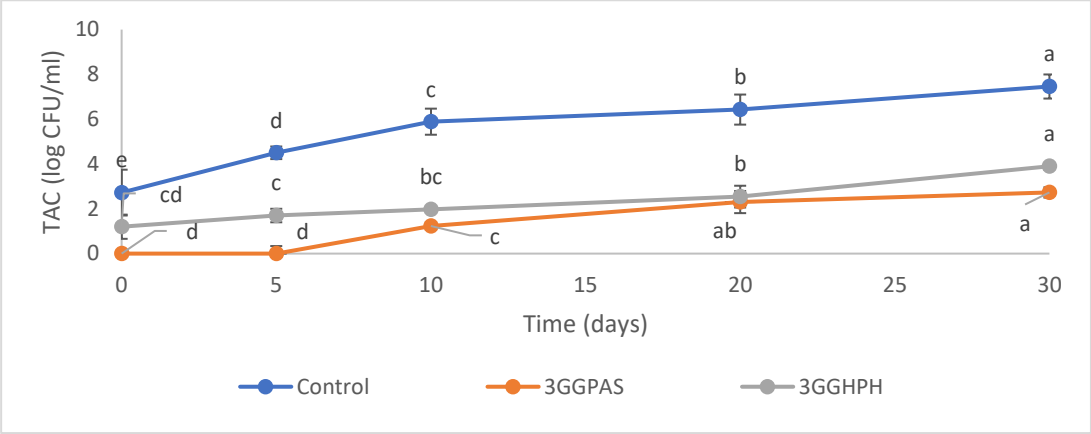
Control - Blended beverage (C50:P50); 3GGHPH- blended beverage with 0.3% guar gum and high-pressure homogenization; 3GGPAS- blended beverage with 0.3% guar gum and pasteurization.

4.3.10. Microbial safety of treated blended beverages during storage

The initial total aerobic count in Control was 2.7±0.31 log CFU/ml (Fig 4.8). No growth was observed for total plate count in 3GGPAS directly after pasteurization. During the entire storage period of 30 days at 25±2 ℃ and 4±2 ℃, Control beverage showed maximum microbial count followed by 3GGHPH and 3GGPAS, in that order. Hirsch et al. (2008) reported no growth of microorganism in thermally treated orange juice. However, 3GGHPH showed 1.2 log CFU/ml of growth after processing. Pasteurized blended beverage 3GGPAS registered colony growth at 25±2 ℃ and 4±2 ℃ storage temperature on day 30 of storage; its total plate count increased up to 3.9±0.38 log CFU/ml and 2.7±0.22 log CFU/ml as shown in Fig 4.8 (a and b). According to Hirsch et al. (2008), the initial total viable counts increased during storage up to a maximum of approximately 10^4 CFU/ml in thermally treated orange juice on day 30. At 25±2 ℃ and 4±2 ℃ storage temperature, total plate count in 3GGHPH sample showed microbial population of 6.8±0.08 log CFU/ml and 3.8±0.14 log CFU/ml on day 30 of storage. Cheng et al. (2020) reported that untreated mandarin juice sample had total aerobic bacterial count of 3.7 log CFU/ml, while thermally pasteurized and high-pressure processed juice contained lower bacterial count of 1.0 log CFU/ml. One possible explanation for higher TAC in 3GGHPH is that the juice becomes aerated during homogenization, and homogenizer valve design, and seals and unsealed valve in a high-pressure homogenized device have impact on juice quality (Kruszewki et al 2021).



(a)



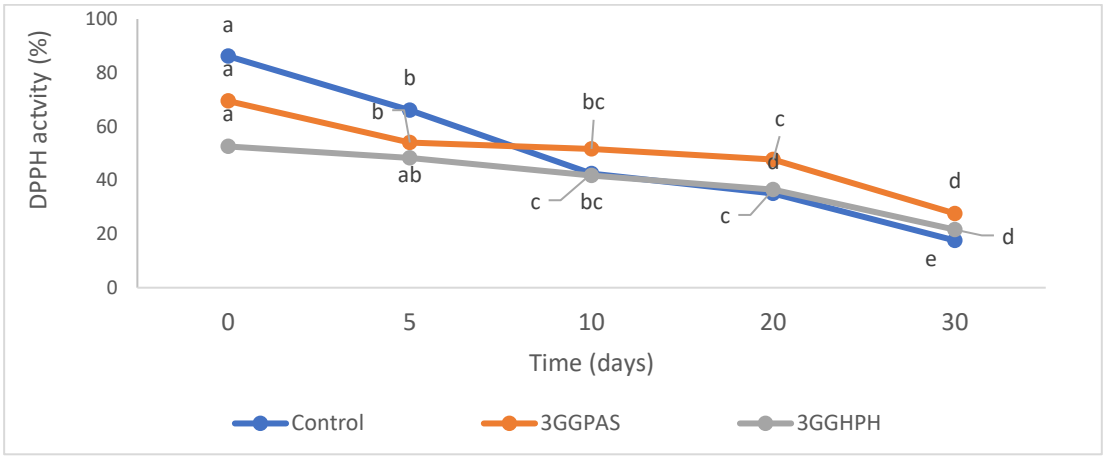
(b)

Fig. 4.8: Effect of thermal treatment and high-pressure homogenization on total aerobic count of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with 0.3% guar gum and pasteurization) and 3GGHPH (blended beverage with 0.3% guar gum and high-pressure homogenization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

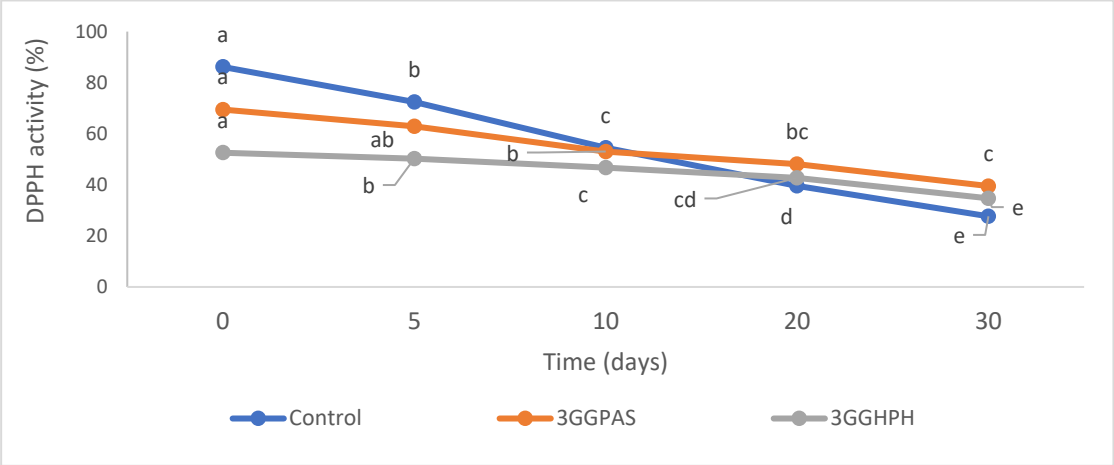
4.3.11. Antioxidant property of treated blended beverages during storage

The initial antioxidant capacity (DPPH scavenging activity) in Control (untreated blended beverage) was 86.1±0.09%, and a significantly ($p<0.05$) decreased activity of69.4±0.48% and 52.5±0.78% determined in 3GGPAS and 3GGHPH, respectively immediately after processing. It should be noted that the DPPH activity of 3GGPAS processed samples remained higher than 3GGHPH during the entire 30 days of storage at 25±2 ℃ and 4±2 ℃. DPPH scavenging activity of 3GGPAS was 27.6±0.22% and 3GGHPH was 21.6±0.24% at 25±2 ℃ on day 30. DPPH activity of 3GGPAS and 3GGHPG at 4±2 ℃ on day 30 of storage was 39.5±0.36% and 34.7±0.22%. According to Shaik and Chakraborty (2023), antioxidant breakdown is accelerated by storage temperature.

Increased humidity is detrimental to the quality of antioxidants because it accelerates the rate at which organic acids dissolves and makes antioxidants more prone to deterioration. The DPPH activity decreased on day 30 of storage was 17.5±0.33% as compared to treated blended beverages in Control at 25±2 ℃, as shown in Fig. 4.9 (a). According to He et al. (2016), thermal treatment breaks down the cell walls of fruit cells, promoting the release of bound phenolic compounds and increasing their concentration in extracts. Although high-pressure homogenization obviously breaks down the fruit's cellular structure, 3GGHPH showed a declining trend in DPPH activity. These results are similar to those reported by Suárez et al. (2011). In every antioxidant test conducted, the antioxidant capacity of pasteurized apple juice at 90 ℃ for 4 min was significantly (5–19%) greater than that of high-pressure homogenized apple juice treated at 100, 200, and 300 MPa. Up to 10 days of storage, DPPH activity in Control was higher than the pasteurized and homogenized beverages and thereafter the pasteurized beverage exhibited greater DPPH activity. The DPPH activity in untreated blended beverage (Control) stored at 25±2 ℃ was higher than the treated blended beverages, namely, 3GGHPH and 3GGPAS till 5 days of storage and thereafter a gradual fall in the activity occurred and was almost similar to 3GGHPH over the remaining period till day 30. One possible explanation is that the juice becomes aerated during homogenization, and mechanical forces and the turbulent process generate heat and a higher temperature at the valve, which encourages oxidation (Kruszewski et al., 2021). This significantly affects DPPH activity in 3GGHPH. Shaik and Chakraborty (2023) reported that thermally treated lime juice has a lower antioxidant activity than untreated lime juice. During storage for 46 days at 4 ℃, the antioxidant property of the samples was reduced from 16.9 ± 0.7 g GAEAC/L to 11.5 ± 0.8 g GAEAC/L.



(a)

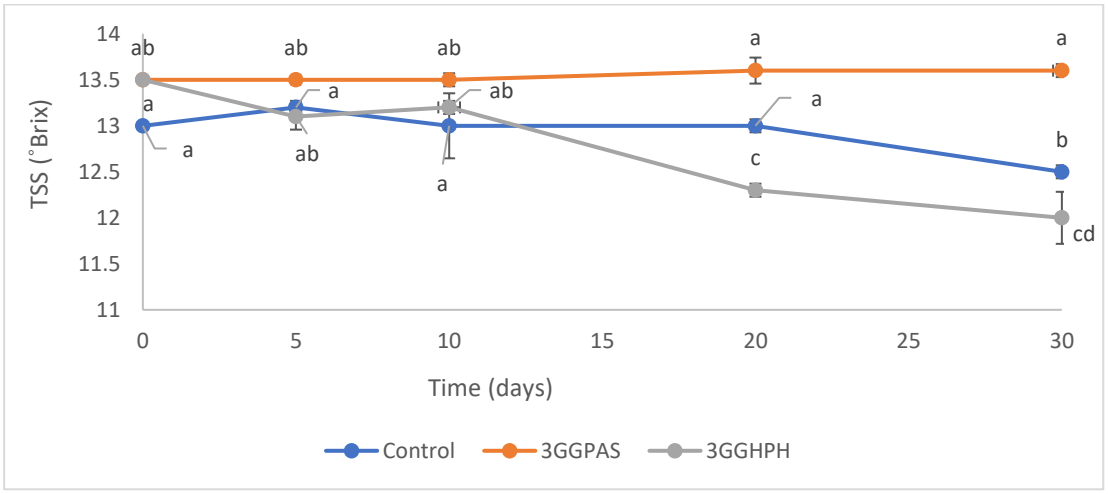


(b)

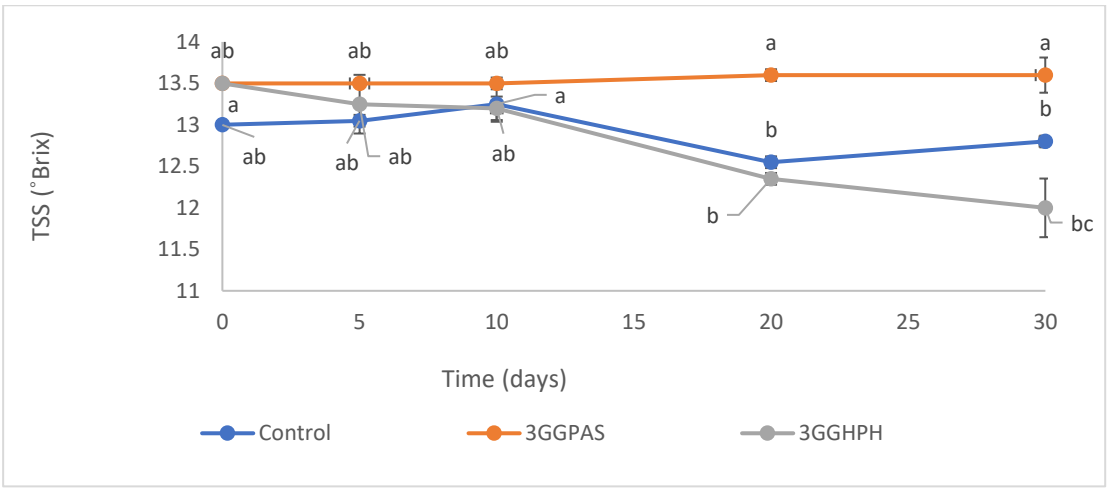
Fig. 4.9: Effect of thermal treatment and high-pressure homogenization on DPPH free radical scavenging activity of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with 0.3% guar gum and pasteurization) and 3GGHPH (blended beverage with 0.3% guar gum and high-pressure homogenization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

4.3.12. Physicochemical properties of treated blended beverages during storage

To evaluate the impact of pasteurization and high-pressure homogenization on blended beverages (3GGPAS and 3GGHPH), total soluble solids (TSS) and pH were measured at 25±2 °C and 4±2 °C The initial TSS value of Control was 13 ºBrix, whereas TSS of 3GGPAS and 3GGHPH was 13.5. The TSS of the Control sample stored at 4±2 °C and 25±2 °C decreased to 12.8±0.07 ºBrix and 12.5±0.28 ºBrix on day 30. A significant difference was not observed in the TSS of 3GGPAS ranges from initial value 13.5±0.01 ºBrix to 13.6±0.02 ºBrix on day 30 of storage at both of the storage temperatures (4±2 °C or 25±2 °C), as shown in Fig 4.10 (a and b). The TSS decline shown in may be connected to the increase in pH. The microorganism that causes juice to deteriorate were the source of the decrease in TSS (Chia et al., 2012). The 3GGHPH blended beverage TSS decreased at both the temperature 4±2 °C and 25±2 °C on day 30 of storage was 12±0.35 ºBrix and 12±0.28 ºBrix, respectively as shown in Fig 4.10. Kruszewski et al. (2021) reported that high- pressure homogenization results in a decrease in TSS due to the precipitation and aggregation of some soluble substances caused by harsh processing conditions, such as increased shear stress, cavitation, and valve temperature.

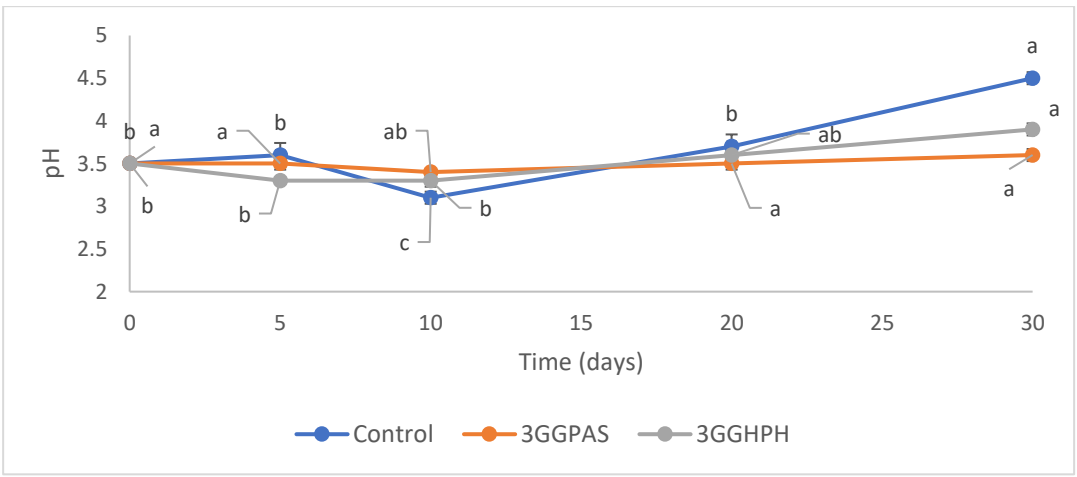


(a)

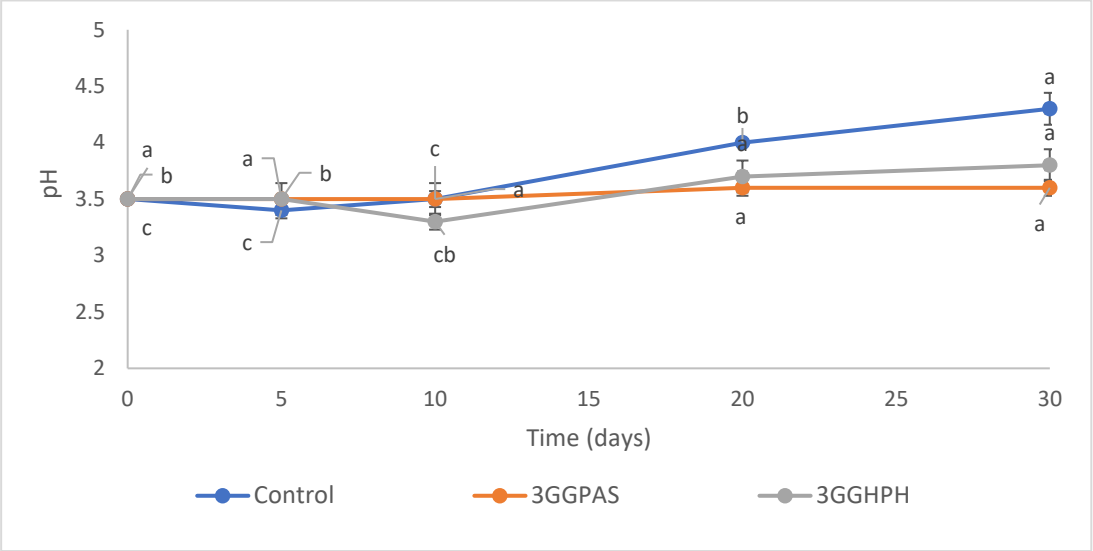


(b)

Fig. 4.10: Effect of thermal treatment and high-pressure homogenization on TSS of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with guar gum 0.3% and pasteurization) and 3GGHPH (blended beverage with guar gum 0.3% and high-pressure homogenization) on storage at (a) 25±2 °C and (b) 4±2 °C temperature.



(a)



(b)

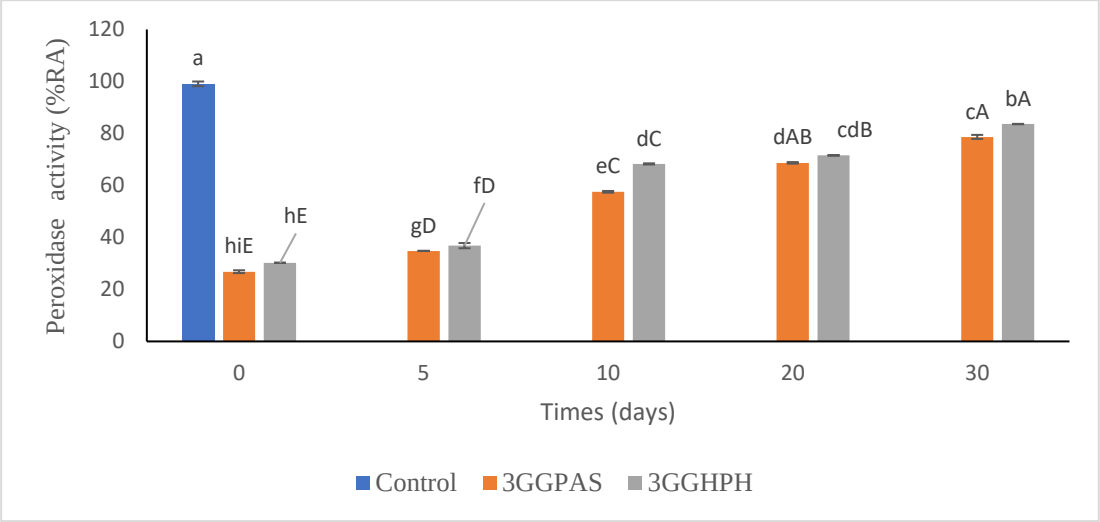
Fig. 4.11: Effect of thermal treatment and high-pressure homogenization on pH of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with guar gum 0.3% and pasteurization) and 3GGHPH (blended beverage with guar gum 0.3% and high-pressure homogenization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

A comparison of the results obtained by 3GGPAS and 3GGHPH showed that there were no statistically significant differences in the pH values immediately after processing compared with the Control sample i.e. 3.5 as shown in Fig. 4.11. Kruszewski et al. (2021) reported that the pH of blackcurrant juice was unaffected by high-pressure homogenization processing, as well as thermal pasteurization, and that no significant difference was observed compared with the Control sample. During the subsequent storage of the 3GGPAS samples at 25±2℃ and 4±2 ℃, there was no significant difference in the pH of the 3GGPAS samples on day 30 of storage at either temperature (Fig. 4.11 (a and b). Chia et al. (2012) observed that the pH of thermally pasteurized pineapple juice was not significantly altered by storage for 13 weeks at 4±2 ℃. The pH of the Control samples varied; it drops to 3.1±0.01 after day 10 of storage and rises to 4.5±0.07 at 4±2 ℃ on day 30. On day 30 of storage, the Control sample pH increases to 4.3±0.14 at 25±2℃. Chia et al. (2012) reported similar results; untreated pineapple juice showed a statistically significant pH increase during 13 weeks of storage. According to Rivas et al. (2006), there were no pH changes in thermally treated blended juices of orange and carrot stored at 2 ℃ and 12 ℃. On day 30 of storage, the 3GGHPH pH increased in both the 4±2 ℃ and 25±2 ℃ storage temperature ranges (3.9±0.07 and 3.8±0.14) respectively. Similar results were reported by Cortes et al. (2008), who found that after seven weeks of storage at 2 ℃ and 10 ℃, the pH increased considerably in fresh, high-pressure homogenized, and pasteurized

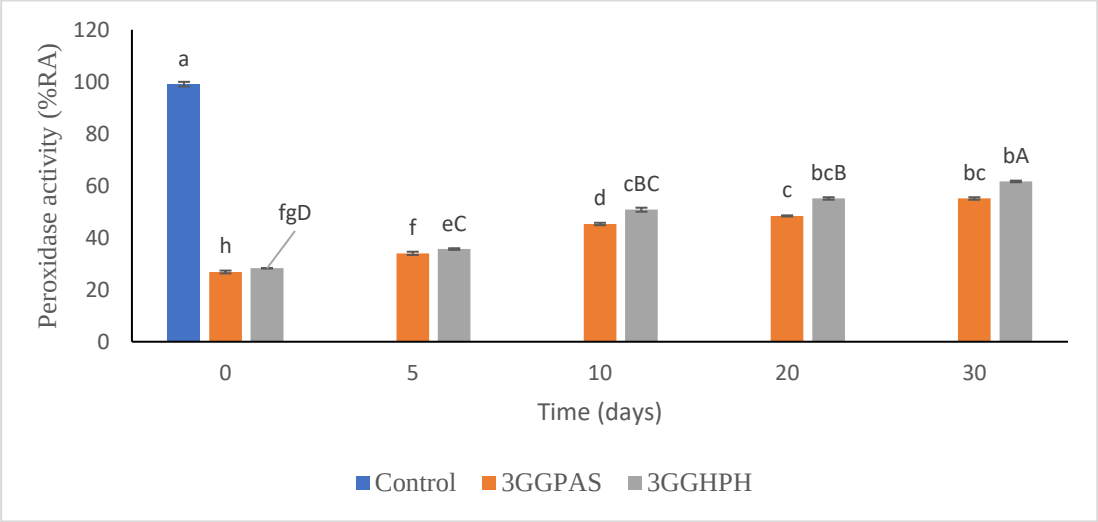
orange juice. The microorganisms responsible for the juice spoiling were responsible for the higher pH levels in these juices (Cortes et al., 2008).

4.3.13. Peroxidase (POD) activity of treated blended beverages during storage

Both high-pressure homogenization and pasteurization affected the enzyme activity of the blended beverage (Fig. 4.12). The initial residual activity (POD) was 99.0±0.89% in Control sample, but pasteurization and high-pressure homogenization of the blended beverage (3GGPAS and 3GGHPH, respectively) caused a significant decrease in the residual activity of POD with value of 26.8±0.54% and 28.2±0.04%, respectively. The POD residual activity of 3GGPAS significantly increased to 78.6±0.78% and 55.1±0.48% at 25±2 °C and 4±2 °C, respectively on day 30. The POD activity of 3GGPAS increased up to 1.9 and 1.0-fold at 25±2 °C and 4±2 °C, respectively on day 30. According to Marszałek et al. (2017), in comparison with PPO, POD proved to be more resistant to heat and pressure. The impact of the residual activity (POD) of 3GGHPH on the day 30 of storage is shown in Fig., where it significantly increased by 83.6±0.07% and 61.6±.31% at 25±2 °C and 4±2 °C, respectively. The POD activity of 3GGHPH increased by 1.9 and 1.1-fold at 25±2 °C and 4±2 °C, respectively on day 30 of storage. However, the 3GGPAS sample showed lower residual activity than 3GGHPH during the entire storage period of 30 days. Beegum et al. (2018) reported that the residual activity of thermally treated watermelon juice increased by up to 50.9% on day 90 of storage at 4 °C storage. The maximum efficiency in inactivating POD was observed in thermally pasteurized juice. Yi et al. (2017) observed that a rise in residual activity may be due to an increase in extractability, and alterations to the secondary and tertiary structures of these enzymes via HPH. These conformational changes can either decrease or increase enzyme activity by altering substrate specificity and functional modification.



(a)



(b)

Fig. 4.12: Effect of thermal treatment and high-pressure homogenization on POD activity of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with 0.3% guar gum and pasteurization) and 3GGHPH (blended beverage with 0.3% guar gum and high-pressure homogenization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

4.4. Conclusion

Standardization of process conditions for thermal pasteurization and high-pressure homogenization of defatted coconut milk and pineapple juice was studied. Both pasteurized and high-pressure homogenized blended beverages were able to achieve considerable peroxidase inactivation and microbiological safety. Pasteurization conditions of 80 ℃ temperature and 1.8 min time for blended beverage of defatted coconut milk and pineapple juice were able to lower POD residual activity and retain DPPH activity to a large extent. The inclusion of guar gum to blended beverages decreased serum separation, according to our study. While guar gum and high-pressure homogenization had a detrimental impact on serum separation, guar gum and thermal pasteurization restricted serum separation. When compared to homogenized 3GGHPH, the pasteurized 3GGPAS demonstrated reduced serum separation properties. During storage, it was observed that 3GGPAS had better properties than 3GGHPH in terms of sensory properties, physicochemical properties, microbial growth, peroxidase activity and antioxidant activity at 25±2 ℃ and 4±2 ℃ across the storage period of 30 days.

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4.2.6. Determination of ascorbic acid content

Ascorbic acid content in treated blended beverages was determined by the method described in section 3.2.2.4. in chapter 3.

4.2.7. Phytochemical content and antioxidant properties of blended beverages

4.2.7.1. Determination of total phenolic content (TPC)

TPC of treated blended beverages was measured as per the method described in section 3.2.3.1. in chapter 3.

4.2.7.2. Determination of total flavonoid content (TFC)

The flavonoid content in treated blended beverage samples was determined following the method given in section 3.2.3.2. of chapter 3.

4.2.7.3. DPPH (2,2-diphenylpicrylhydrazyl) radical scavenging activity

Antioxidant activity of blended beverages was determined with stable radical DPPH using the method described in section 3.2.3.3. of chapter 3.

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

4.2.9. Peroxidase activity of 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. 4.1:

$$\text{Activity (U/ml)} = \frac{\text{Ab (sample)} - \text{Ab (blank)}}{0.001 \times t} \qquad \text{(Eq. 4.1)}$$

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

4.2.10. Sensory analysis of freshly treated blended beverages

Sensory analysis of blended beverage treated with thermal pasteurization and high-pressure homogenization was determined using the nine-point hedonic scale following the method described in section 3.2.6. in chapter 3.

4.2.11. 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 ($p < 0.05$).

4.3. Results and discussion

4.3.1. Microbiological quality of thermally pasteurized blended beverage

A freshly prepared blended beverage of defatted coconut milk and pineapple juice (C50:P50) was loaded with four different microbes: *Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes*, and *Escherichia coli*. Thermal treatment at 60, 70, 80, and 90 °C with time intervals of 0, 5, 20, 40, 60, and 120 s were used to analyse D_{10} value for different microbes. The D_{10} value calculated for *Staphylococcus aureus* at 60 ℃, 70 ℃, 80 ℃, and 90 ℃ was 71.0±1.30 s, 66.8±1.01 s, 49.9±0.28 s and 40.3±0.25 s, respectively. It is obvious that with increasing temperature, the D value decreased. Atalar et al. (2019) reported that the total aerobic mesophilic bacterial count in hazelnut beverage was significantly reduced by up to 2.41 log cycles after thermal treatment at 65 ℃ for 30 min. Increasing the thermal treatment temperature to 72 ℃ for 20 min caused complete microbial inactivation in hazelnut beverage. The D_{10} value for *Bacillus cereus* at 60 ℃, 70 ℃, 80 ℃, and 90 ℃ was 38.1±1.12 s, 33.9±0.72 s, 26.0±0.74 s, 22.7±0.88 s respectively; for *Listeria monocytogenes,* it was 41.3±0.18 s, 30.6±0.81 s, 21.6±1.97 s, 20.3±0.53 s; and for *E. coli,* it was 55.9±1.18 s, 41.2±1.27 s, 30.4±0.45 s, 21.1±0.19 s, respectively. To obtain the 5-log reduction, the D value was converted to 5D values (Fig. 4.1). The $5D_{10}$ value calculated for *Staphylococcus aureus* at 60 ℃, 70 ℃, 80 ℃, and 90 ℃ was 5.9±1.30 min, 5.5±1.01 min, 4.1±0.28 min and 3.3±0.25 min, respectively. 5D value for *Bacillus cereus* at 60 ℃, 70 ℃, 80 ℃ and 90 ℃ was 3.1±1.12 min, 2.8±0.72 min, 2.1±0.74 min, 1.8±0.88 min, respectively. Gabriel and Nakano (2011) reported that D value of *E. coli* of thermally treated apple juice was 25 s and 5.3 s at 55 ˚C. At

temperature 60 °C, 70 °C, 80 °C, and 90 °C, 5D value for *Listeria monocytogenes* was 3.44±0.18 min, 2.5±0.81 min, 1.8±1.97 min, 1.6±0.53 min and for *E. coli* was 4.6±1.18 min, 3.4±1.27 min, 2.5±0.45 min, 1.7±0.19 min, respectively. Deshaware et al. (2019) reported that D value for native microflora was 19.7 s in bitter gourd juice. Therefore, a total time of 98.5 s was required to achieve a 5-log reduction in native microflora. Authors observed that *E. coli* was the most heat-sensitive pathogen and *Shigella boydii* was the most heat-resistant pathogen, with D_{10} value of 21.2 s and 42.8 s, respectively.

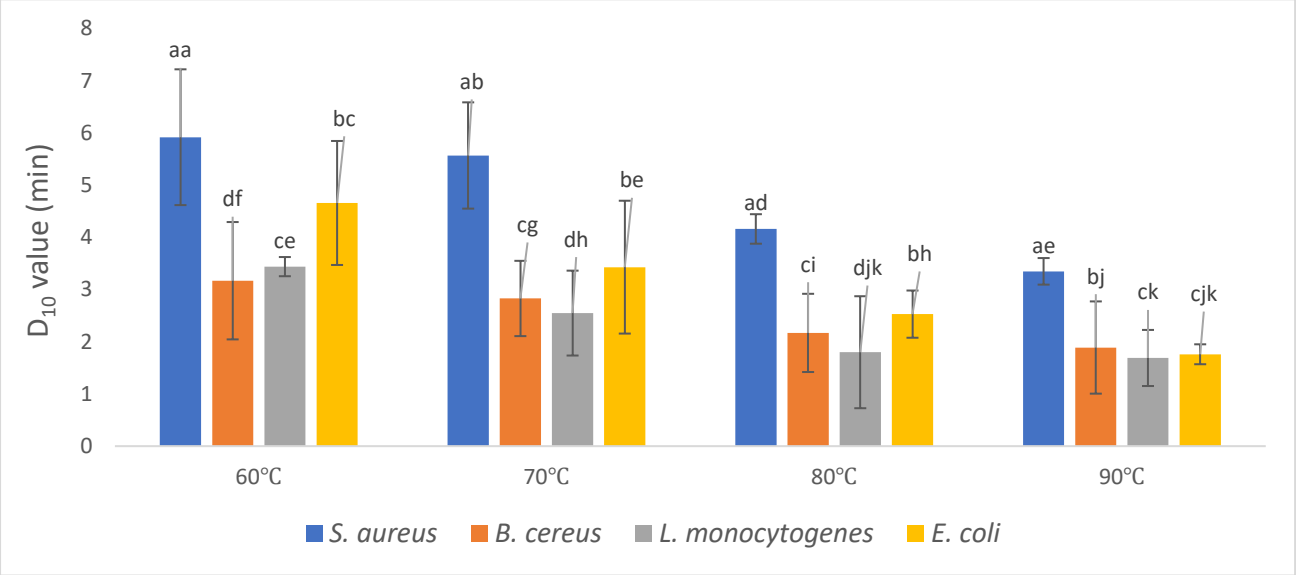


Fig 4.1: $5D_{10}$ (time) value for test organisms post thermal pasteurization at 60 °C, 70 °C, 80 °C, and 90 °C.

4.3.2. Effect of thermal treatment on enzyme activity of blended beverage

Thermal pasteurization resulted in significant ($p > 0.05$) changes in peroxidase activity. Fig. 4.2 reveals that POD was inactivated in samples thermally treated for different times and temperatures to fulfil 5-log reduction of the test microbes. Peroxidase, which is responsible for many oxidative changes and flavour alterations in fruits and vegetables, is generally considered the most thermostable enzyme in plants. Consequently, it has been widely used as an indicator of heat treatments in food processing (Hirsch et al., 2008). The POD activity decreased with the effect of temperature and time. At 60 °C, and thermal treatment for 5.9 min, 3.1 min, 3.4 min, 4.6 min, the POD residual activity was 67.4±0.73%, 81.6±1.40%, 76.7±1.4% and 68.6±0.61%, respectively; at 70 °C time and treatment time of 5.5 min, 2.8 min, 2.5 min, 3.4 min, the residual activity was 53.4±1.26%, 62.8±1.06%, 76.9±0.25% and 64.2±0.18%, respectively; at 80 °C with treatment time of 4.1 min, 2.1 min, 1.8 min, 2.5 min, the POD residual activity was 27.5±0.30%, 35.5±0.43%, 26.6±0.69% and 24.1±0.07%, respectively;

and at 90 ℃ with treatment time of 3.3 min, 1.8 min, 1.6 min, 1.7 min, POD residual activity was 22.9±0.07%, 24.6±0.05%, 27.7±0.26 and 26.2±0.15%, respectively.. It was seen that with an increase in temperature and time, the residual activity of POD decreased. The blended beverage with pH of 3.5 and thermal treatment was effective in reducing the POD activity. Hirsch et al. (2008) reported residual POD activities of 49.2% and 25.8% after the thermal treatment of orange juice at 42°C and 52°C, respectively. Hun et al. (2009) reported that after thermal inactivation of pineapple homogenate for 15 min at 65 ℃ and 75 ℃, the residual POD activities were approximately 54% and 22%, respectively. When the temperature was reduced to 45 ℃ and 55 ℃, the residual activities of POD were 74% and 70%, respectively. Murtaza et al. (2020) reported that relative activity of POD in apple juice after thermal treatment at 45, 65, and 75 °C for 20 min was 115.3%, 94.6%, and 79.4%, respectively. Koo et al. (2023) reported that Bok choy juice with pH 4 and 6 had relative activities of 15.1 ± 0.9% and 87.5%. The low acid juice should be acidified to pH below 4.6 to prevent the growth of pathogenic *Clostridium botulinum* spores during storage (US FDA, 2007). The POD activity at 80 ℃ for 1.8 min and 4.1 min and at 90 ℃ for 1.6 min and 1.7 min showed insignificant difference and lower POD residual activity as compared to the activity levels at 60 ℃ and 70 ℃. The significantly lowest POD residual activity of 22.9±0.07% was recorded at 90 ℃ with treatment time of 3.3 min. With increase in time also, the POD residual activity had reduced.

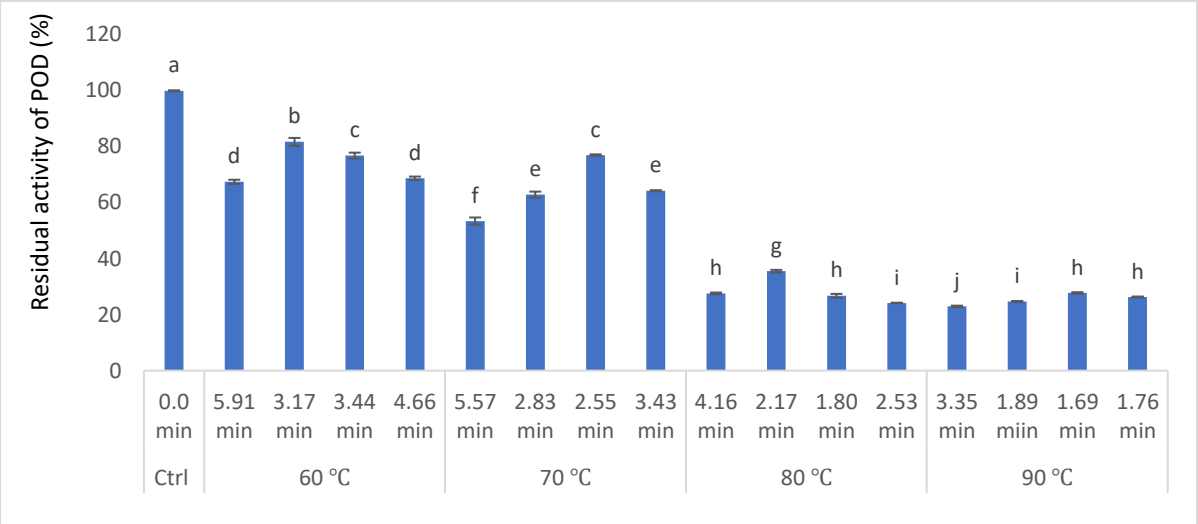


Fig 4.2: Effect of thermal pasteurization on peroxidase residual activity in blended beverage (C50:P50).

4.3.3. Effect of thermal treatment on antioxidant property of blended beverage

Initial DPPH radical scavenging activity of blended beverage was 86.4±0.15%. Thermal pasteurization resulted in a significant ($p > 0.05$) decrease in DPPH activity, as shown in Fig. 4.3. The DPPH activity decreased when subjected to different temperatures and times. DPPH

activity after thermal treatment at 60℃ for 5.9 min, 3.1 min, 3.4 min, 4.6 min, was 63.4±0.65%, 76.1±0.14%, 69.1±0.27% and 68.3±0.51%, respectively. DPPH activity was 59.0±0.06%, 63.4±0.67%, 65.1±0.14% and 70.2±0.31%, respectively at 70 ℃ when thermally treated for 5.5 min, 2.8 min, 2.5 min, 3.4 min. The DPPH activity in the beverage when thermally treated at 80℃ for 4.1 min, 2.1 min, 1.8 min, 2.5 min was 36.2±0.31%, 65.4±0.67%, 69.4±0.56% and 58.0±0.05%, respectively, whereas at 90 ℃, DPPH activity was 31.2±0.32%, 44.2±0.40%, 55.3±0.45% and 52.3±0.50% respectively on thermal treatment for 3.3 min, 1.8 min, 1.6 min, 1.7 min With increase in temperature and time of pasteurisation, residual activity of POD also decreased. Bavisetty and Venkatachalam (2021) reported that the DPPH activity of waxed apple juice decreased upon thermal pasteurization. Similar results of decreased DPPH activity with increase in temperature and time was observed in the blended beverage. Thermal treatment for 1.8 min at 80 ℃ showed significantly higher DPPH activity as compared to other treatment times at 80 ℃ and 90 ℃. The DPPH activity at 70 ℃ and 80 ℃ with treatment time of 3.4 min and 1.8 min was 70.2±0.31%, and 69.4±0.56%, respectively, and the difference was insignificant (Fig 4.3). Benattouche et al. (2021) reported that DPPH activity of untreated orange juice was 61.5% and after thermal treatment at 90 ℃ for 1 min was reduced to 58.9%. Due to use of heat during thermal pasteurization the antioxidant quality of pasteurized blended beverage may be adversely affected. This decline is attributed to the degradation of heat-sensitive bioactive compounds, which are key contributors to antioxidant activity (Bavisetty and Venkatachalam, 2021). The extent of antioxidant degradation during pasteurization is influenced by the temperature and duration of the heat treatment. Higher temperatures and longer exposure times are associated with greater losses in antioxidant compounds (Pérez et al., 2021).

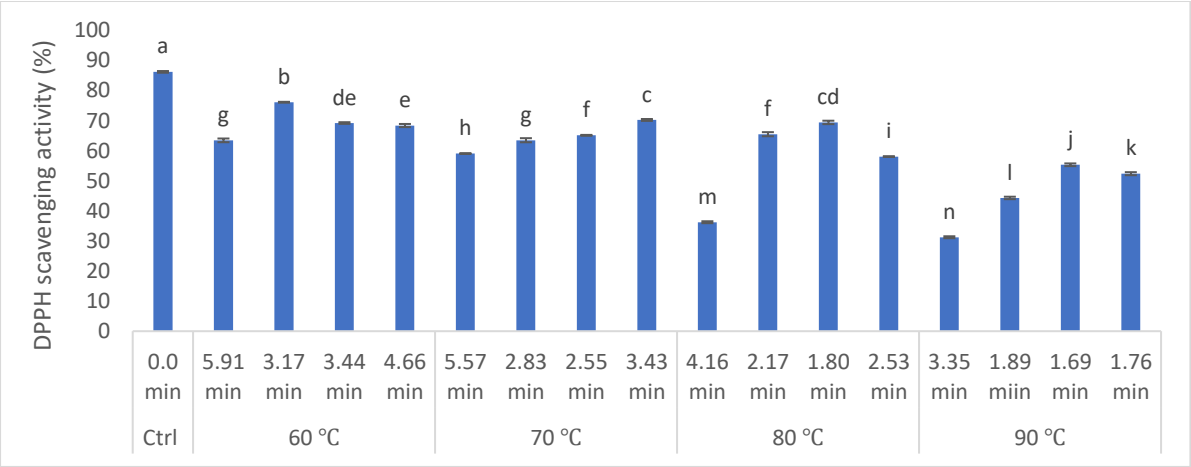


Fig 4.3: Effect of thermal pasteurization treatment on DPPH scavenging activity of blended beverage (C50:P50).

4.3.4. Optimization of high-pressure homogenization of blended beverage

The effect of high-pressure homogenization (HPH) of blended beverages containing defatted coconut milk and pineapple juice are shown in Fig. 4.5. The impact of HPH pressure and number of passes on microbial counts and POD activity were analysed. The pressure and number of passes were thus changed using an FCCD design model to mix distinct ratios of the pressure and passes. The *E. coli* strains were inoculated into the blended beverage, and the microbial count of the beverage was 5.47 log CFU/ml. The microbial count decreased with increasing homogenization pressure and passes. Microbial count was reduced to 1.10 log CFU/ml when the beverage was homogenized at maximum pressure of 500 bar for 6 passes. From the ANOVA results, the overall effect of homogenization pressure and the number of homogenizing passes was found to be significant for reduction of microbial load ($p< 0.0001$) and POD residual activity ($p< 0.0001$) (Tables 4.3 and 4.4). The R^2 value for POD residual activity and microbial load was 0.9617 and 0.9721, respectively. This validated the models, meaning that they could effectively explain the experimental data. Pressure was significantly more effective than the number of passes (Table 4.3, 4.4 and Fig. 4.5) in the reduction of microbial count and residual activity of POD in the blended beverage. Fig. 4.5 (a) shows the effect of increasing the homogenizing pressure on reducing the lethality of *E. coli* in the blended beverage. However, the number of passes did not considerably reduce the lethality of *E. coli* (Fig. 4.5 (b) and Table 4.4). Food items that are safe for consumption often have naturally occurring spoilage and pathogenic microbe counts of less than 10^6 CFU/ml (Chen et al., 2014). The maximum lethality of *E. coli* (1.1 log CFU/ml) was observed at a pressure of 490 bar and 6 number of passes. Welti et al. (2009) reported both the number of passes (0-5) and pressure (50-250 MPa) affected the microbial load in homogenized orange juice. The microbial load decreased as the number of passes increased. The microbial count in orange juice homogenized for 5 cycles was 8.7×10^3 CFU/ml for mesophile and 1.85×10^3 CFU/ml for yeasts and moulds. Tables 4.3 and 4.4 shows that the lack of fit of the model was not significant relative to the pure error. The F-values (0.57 and 0.19) for the regression and lack of fit are also provided in Tables 4.3 and 4.4. Thus, the model was found to be adequate for prediction over the range of tested variables.

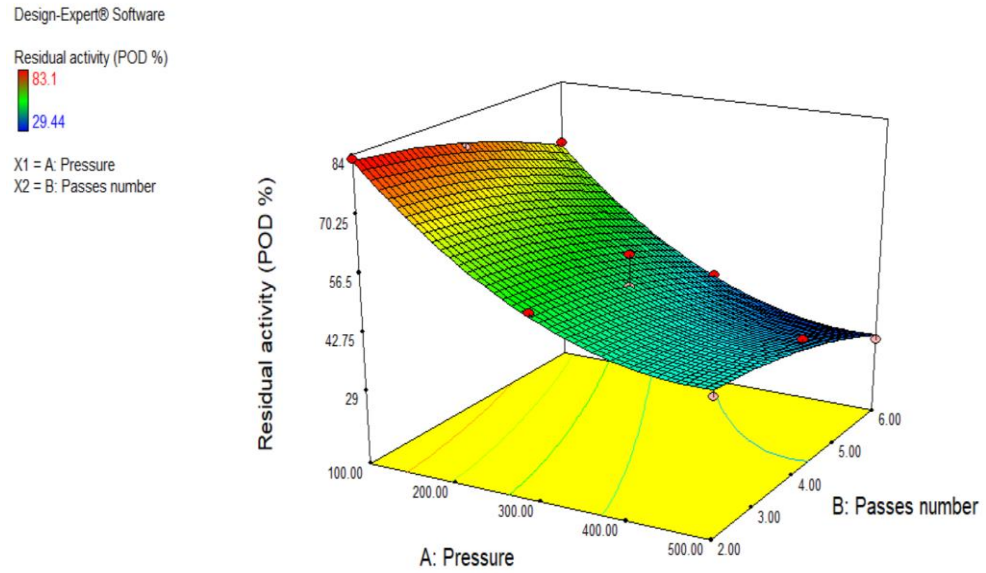
Maresca et al. (2011) reported the potential of HPH treatment at pressures between 200 and 300 MPa for inactivating pathogenic and spoilage microflora as an alternative to thermal treatments for prolonging the juice shelf life. For instance, the application of HPH treatment to orange juice (300 MPa in a two stage-homogenization system) significantly decreased *E. coli.* Finally, the optimum parameters selected were: homogenizing pressure of 490 bar and 6 number of passes. As shown in Fig. 4.5 (a), with an increase in homogenizing pressure and the

number of passes, the POD activity decreased. Fig. 4.6 (a and b) shows the validation of the adequacy of the model, in which all data points were found to be quite close to the predicted line, indicating excellent fitness of the model. The correlation coefficients (R^2) between the predicted and observed values were 0.9617 and 0.9721 ($P< 0.0001$). Thus, the accuracy and reliability of the proposed model were validated for reducing *E. coli* survival and POD residual activity in blended beverage of defatted coconut milk and pineapple juice after high-pressure homogenization. Analysing the initial microbial counts and predominant species present in C50:P50 allowed us to develop a method to inactivate bacteria using RSM to optimize sterilization conditions. Based on maximum desirability level of 0.99, optimized conditions that were obtained were: 490 bar pressure and 6 number of passes (Table 4.5). Under these conditions, the predicted lethality of *E. coli* and residual POD activity was 1.1 log CFU/ml and 30.5%, respectively. Szczepanska et al. (2022) reported that the effects of multi-pass HPH may also be attributed to the ability of HPH to break down molecular aggregates in the homogenizing valve. Codina et al. (2017) reported a 20% decrease in POD activity under high-pressure homogenization treatment of tiger nut milk at an inlet temperature of 40 ◦C.

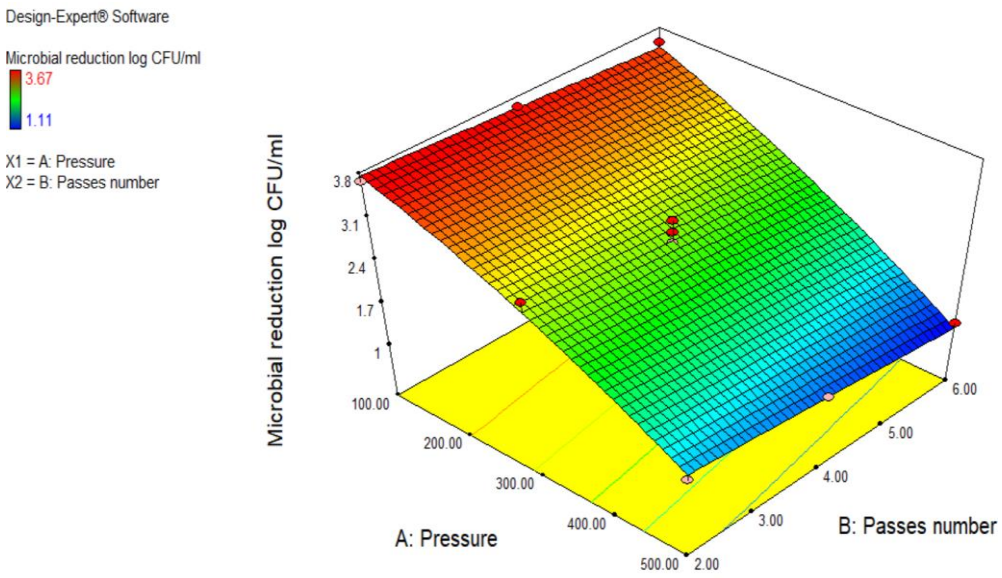
Table 4.2: Experimental design matrix for the effect of high-pressure homogenization pressure and number of passes on residual activity of POD and lethality of *E. coli*

Experimental run	Independent variables		Responses	
	Factor 1 Pressure (bar)	**Factor 2 Passes number**	**Response 1 Residual activity POD (%)**	**Response 2 Lethality of *E. coli* (log CFU/ml)**
1	100	2	83.1	3.6
2	100	4	76.3	3.6
3	100	6	68.2	3.5
4	300	2	54.7	3.0
5	300	4	47.2	2.7
6	300	6	39.3	2.3
7	300	4	56.2	2.9
8	300	4	48.3	2.6
9	300	4	47.4	2.4
10	300	4	42.8	2.4
11	500	6	29.4	1.1

12	500	4	42.7	1.3
13	500	2	43.6	1.4

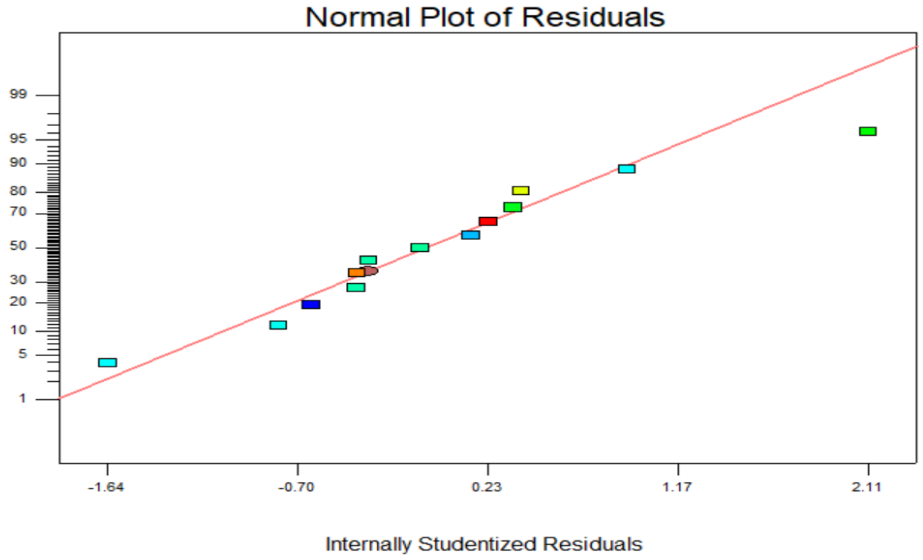


(a)

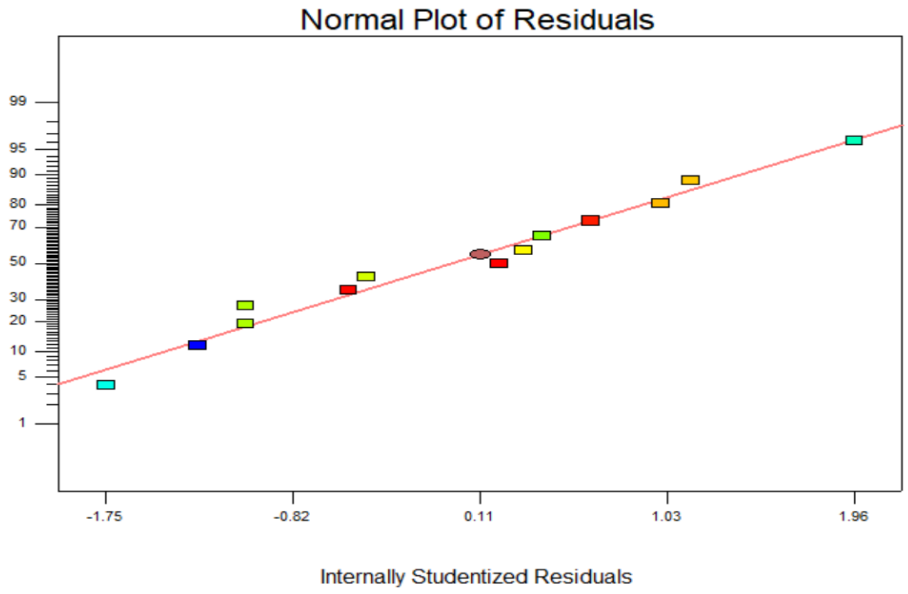


(b)

Fig 4.4: Response surface plots: (a) POD residual activity (homogenization pressure and number of passes) and (b) lethality of *E. coli* (homogenization pressure and number of passes)



(a)



(b)

Fig 4.6: Scatter plot: (a) POD residual activity (homogenization pressure and number of passes) and (b) lethality of *E. coli* (homogenization pressure and number of passes).

4.3.4.1. Modelling and validation

Design-Expert (Design-Expert Version 7.1.2 software, Stat-Ease) was used to analyse statistically all the experimental data, that were determined in accordance with FCCD design. The quadratic polynomial equations (Eq. 4.1 and 4.2) fitted with all the experimental data

explained the effect of homogenizing pressure, number of passes on microbial reduction, and residual POD activity.

Microbial reduction = + 2.65 – 1.17*A – 0.19*B – 0.065*A*B – 0.19*A^2 – 0.011*B^2 Eq. 4.1

Residual POD activity = + 48.71 – 18.62*A – 7.43*B + 0.017*A*B + 10.13*A^2 – 2.38*B^2 Eq. 4.2

R^2 values obtained were 0.97 and 0.96 for lethality of *E. coli* and residual POD activity of homogenized blended beverage, respectively (Table 4.3 and 4.4). The quadratic model having higher R^2 indicates a good correlation between responses and input parameters. The CV% score of less than 10% indicated well fit of model (Table 4.3 and 4.4). The models were validated (Table 4.6) by non-significant lack of fit for responses. The model's high level of significance with $p<0.0001$ for lethality of *E. coli* and $p<0.0001$ for residual POD activity, explained the high efficiency of experimental data.

Table 4.3: Regression coefficients and ANOVA estimated for POD residual activity of the high pressure homogenized blended beverage

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remarks
Model	2702.3	5	540.46	35.19	< 0.0001	Significant
A-Pressure	2080.97	1	2080.97	135.5	< 0.0001	
B-Passes number	330.78	1	330.78	21.54	0.0024	
AB	0.11	1	0.11	7.09E-03	0.9352	
A^2	283.34	1	283.34	18.45	0.0036	
B^2	15.6	1	15.6	1.02	0.3471	
Residual	107.5	7	15.36			
Lack of Fit	13.5	3	4.5	0.19	0.8971	Not significant
Pure Error	94	4	23.5			
Cor Total	2809.81	12				
R^2				0.9617		
CV%				7.50		

Table 4.4: Regression coefficients and ANOVA determined for lethality of *E. coli* of the high pressure homogenized blended beverage

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	Remarks
Model	8.55	5	1.71	48.71	< 0.0001	Significant
A-Pressure	8.19	1	8.19	233.36	< 0.0001	
B-Passes number	0.22	1	0.22	6.39	0.0179	
AB	0.017	1	0.017	0.48	0.0864	
A^2	0.095	1	0.095	2.71	< 0.0001	
B^2	3.16E-04	1	3.16E-04	8.99E-03	0.9271	
Residual	0.4	7	0.035			
Lack of Fit	0.23	3	0.025	0.57	0.6615	
Pure Error	0.17	4	0.043			Not significant
Cor Total	14.62	12				
R^2			0.9721			
CV%			7.31			

Table 4.5: Desirability values at optimized parameters

Homogenizing pressure (bar)	Number of passes	Lethality of E. coli (log CFU/ml)	POD activity of (%RA)	Desirability
490.16	6	1.1	30.51	0.99
492.22	6	1.1	30.52	0.99
495.07	6	1.1	30.53	0.99

Table 4.6: Validation of optimization conditions of HPH

Pressure (bar)	Number of Passes	Predicted		Experimental		R_{dev}(%)		Overall R_{dev}(%)
		log CFU/ml	POD activity of (%RA)	log CFU/ml	POD activity of (%RA)	log CFU/ml	POD activity of (%RA)	
490	6	1.10	30.51	1.07	29.34	2.80	3.98	3.39

4.3.5. Serum separation (SS) of blended beverage

Guar gum is a naturally occurring non-ionic polysaccharide produced from guar beans and is utilised as a stabilizer in the food industry to improve the stability of juice. The influence of guar gum at different concentrations (0.1%, 0.2% and 0.3%) was ascertained individually and in combination with high pressure homogenization and pasteurization on the stabilization of C50:P50. The results are presented in Fig 4.4. Ruihuan et al. (2017) reported that orange juice with 0.5% guar gum affected the taste and made it thick. In line with their observations, guar gum concentrations below 0.5% were selected for addition in the blended beverage to reduce serum separation. Significant differences between the samples and the Control were identified using analysis of variance (ANOVA) $P<0.01$. The Control with no stabilizer showed serum separation after 1 h of blended beverage preparation, i.e. 23.63±1.4% and no further change in SS% occurred on day 15 of storage. With increasing guar gum concentration, the SS% decreased ($P<0.05$). The SS% values of samples 1GGCP, 2GGCP, and 3GGCP showed no separation after 2 h. However, after the first day of storage, the blended beverage containing 0.1% guar gum had an SS% of 3.6±0.05%, whereas the blended beverages containing 0.2% and 0.3% guar gum remained stable. On day 5 of storage, 2GGCP exhibited a separation of 2.30±0.02%, whereas no separation was observed in 3GGCP. The beverage added with 0.3% guar gum (3GGCP) registered serum separation after day 5, but recorded SS% of 12.6±0.05% on day 15. A previous study of a milk and sour cherry juice mixture reported by Teimouri et al. (2018) found that by increasing the concentration of inulin, the SS% significantly decreased. The authors reported that a considerably greater amount of inulin (10% w/v) was required to restrict serum separation to 12% in the juice mixture. The SS% values of 3GGPAS and PAS after 1 h of storage was 11.0±0.14% and 15.0±1.5%, respectively. The HPH blended beverage after 1 h of storage showed SS% of 20±1.4%. Maximum SS% of 60.0±0.05% was observed in 3GGHPH (blended beverage with 0.3% guar gum and subjected to high-pressure

homogenization) and HPH (high-pressure homogenization blended beverage without guar gum) on day 15 of storage, whereas the SS% of Control sample was 23.63±1.4%. In 3GGPAS and PAS samples, the sediment content increased over time till 15 days attaining an SS% of 42.5±0.35% and 55.0±1.5%, respectively on day 15. Thus, increasing the guar gum concentration had a positive impact on SS%, whereas a reverse trend was observed for high-pressure homogenization. Serum separation that occurred due to the precipitation under the influence of gravity of some insoluble materials present in blended beverage (Ruihuan et al., 2017). The steric repulsion decreased during homogenization, resulting in the destabilization of the dispersed material (Teimouri et al., 2018).

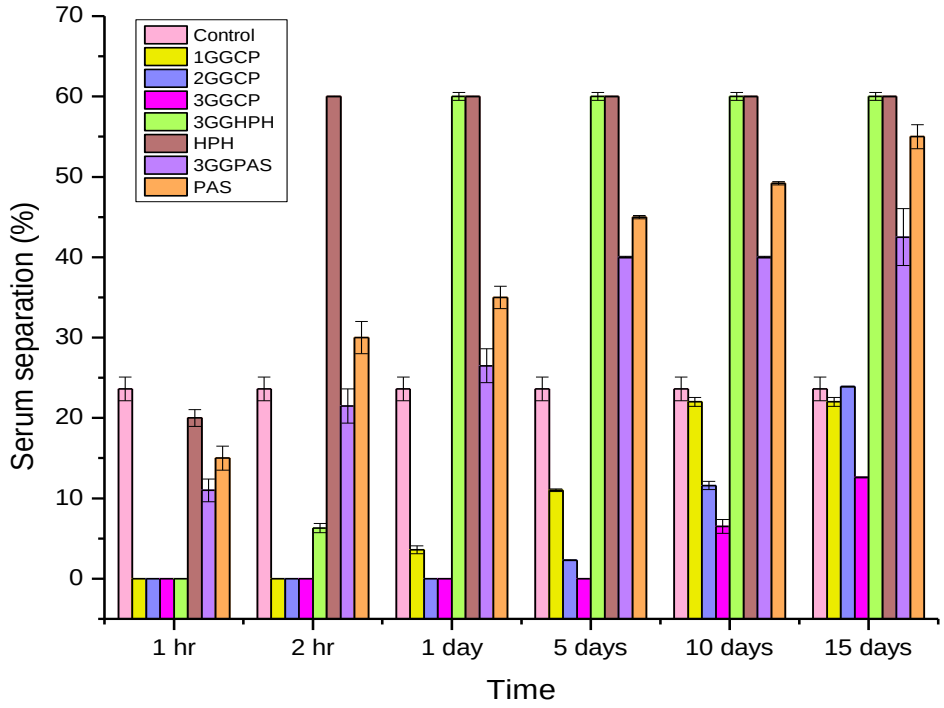


Fig 4.4: Serum separation of (a) Control without gum; (b) 1GGCP: blended beverage (C50:P50) treated with 0.1% guar gum; (c) 2GGCP: blended beverage (C50:P50) treated with 0.2% guar gum; (d) 3GGCP: blended beverage (C50:P50) treated with 0.3% guar gum; (e) 3GGHPH: combination of 0.3% guar gum and high-pressure homogenization; (f) HPH: high-pressure homogenisation of blended beverage (C50:P50); (g) 3GGPAS: combination of 0.3% guar gum and pasteurization; and (h) PAS: pasteurized blended beverage.

Atalar et al. (2019) reported that the colloidal stability of hazelnut beverage samples was reduced by thermal treatment. In comparison to the Control and long temperature long time treated hazelnut beverage, particle sedimentation of high temperature short time beverage occurred quickly. This may be connected to protein denaturation during heat treatment, which causes phase separation during storage. Following heat treatment, particle sedimentation increases due to the increased particle size of beverages (Atalar et al., 2019). pH is an important

factor in serum separation. At the isoelectric point, aggregation and precipitation occur because of the reduction in the repulsive electrostatic force and the predominance of attraction forces. At isoelectric points, the positive and negative charges are balanced, and most proteins have isoelectric points in the pH range of 4 to 7 (Novák and Havlíček, 2016). Tangsuphoom and Coupland (2008) reported that the isoelectric point of coconut milk protein is pH 3.5 to 4. According to our study results, addition of 0.3% guar gum to blended beverage of defatted coconut milk and pineapple juice

4.3.6. Phytochemical and antioxidant properties of treated blended beverage

The blended beverage that was pasteurized at 80 ℃ for 1.80 min was used for further studies due to its lower POD residual activity and maximum DPPH scavenging activity. Along with it, optimized high-pressure homogenised blended beverage was used for antioxidant and phytochemical studies. The total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical scavenging activity in pasteurized and high-pressure homogenized blended beverages are given in Table 4.6. The TPC, TFC, and DPPH activity of untreated blended beverage (Control) was 30.6±0.53 mg/100 ml, 18.7±0.12 mg/100 ml and 86.0±0.52%, respectively. TPC content in 3GGPAS and 3GGHPH were significantly lower i.e. 21.4±0.09 mg/100 ml and 19.3±0.31 mg/100 ml than in Control ($p<0.05$), and the retention of TPC was higher in 3GGPAS than in 3GGHPH. As shown in Table 4.6, the TFC and DPPH radical scavenging activity of 3GGPAS was 12.2±0.32 mg/100 ml and 69.4±0.4%, respectively, which was significantly higher than the values for 3GGHPH i.e. 9.8±0.26 mg/100 ml and 52.5±0.7%, respectively. He et al. (2016) reported that thermal treatment disrupts cell walls and promotes the release of bound phenolic compounds from fruit cells, which increases their concentration in the extracts. High pressure homogenization ruptures the fruit cellular structure and may cause the release of cytoplasmic polyphenol oxidase, resulting in the oxidative degradation of the phenolic compounds. The enhancement of TPC, TFC, and DPPH radical scavenging properties of 3GGPAS was greater than that of 3GGHPH. Similar results were obtained by He et al. (2016), who reported that thermally treated grape juice has a higher total phenolic content than high-pressure homogenized grape juice. Kruszewski et al. (2021) reported that both the inlet temperature and homogenization pressure were crucial for the maintenance of juice antioxidant activity. The heat breakdown of compounds after one minute of pasteurization resulted in a 26.6% reduction in the total phenolic concentration of fresh blackcurrant juice. High-pressure homogenization with 20 ℃ inlet temperature exhibited 86% reduction in total phenolic content in black currant juice (Kruszewski et al., 2021). During the HPH treatment, the degradation of antioxidant property may be due to oxidation reaction with the introduced

oxygen brought to the juice by the turbulent process of homogenization (Kruszewski et al., 2021). From our study we can say that the impact of mechanical stress along with heat generated during high pressure homogenization affect the antioxidant property of blended beverage more as compared to thermally treated blended beverage.

4.3.7. Ascorbic acid content of treated blended beverages

The ascorbic acid concentration (Table 4.6) in the Control (untreated blended beverage) was 35.6±0.09 mg/100 ml. The ascorbic acid content in 3GGHPH (high-pressure homogenized and gum treated blended beverage) was significantly lower than that of the Control sample ($p<0.05$) Even the retention of ascorbic acid was higher ml in 3GGPAS (27.7±0.38 mg/100 ml) than 3GGHPH (22.8±0.21 mg/100 ml). Ascorbic acid is known to be sensitive and unstable to temperature (Kruszewski et al. 2021); even if only a few seconds is spent in a high-pressure homogenization valve, its content may deteriorate. Chaikham and Apichartsrangkoon (2012) reported two main causes for ascorbic acid reduction: oxidative reactions by enzymes such as cytochrome oxidase and ascorbic acid oxidase. High pressure affects the secondary, tertiary, and quaternary structures of proteins; such conformational changes can enhance enzyme activity by revealing active sites, thereby facilitating catalytic conversion. However, it is difficult to prevent ascorbic acid loss during processing, since ascorbic acid stability largely depends on the oxygen concentration.

4.3.8. Peroxidase activity (POD) of treated blended beverages

The data on the POD residual activity are presented in Table 4.6. There was a statistically significant decrease ($p<0.05$) in the residual activity of peroxidase in 3GGPAS (26.8±0.54%) and 3GGHPH (30.2±0.04%) and in comparison, to the untreated Control sample. (99.0±0.89%). Szczepańska et al. (2021) reported that high-pressure homogenization decreased the POD activity of apple juice by 16.4% compared to that of untreated fresh juice. This phenomenon may be due to mechanical damage to the fruit juice cells caused by high-pressure homogenization. The damage may then enable the enzymes contained in plant vacuoles to release, rendering the enzymes susceptible to inactivation (Szczepańska et al., 2021).

Table 4.6: Antioxidant and phytochemical properties of treated blended beverage

Samples	Total phenolic content (mg GAE/100 ml)	Total flavonoid content (mg QE/100 ml)	DPPH activity (%)	Residual activity of POD (%)	Ascorbic acid (mg/100 ml)

Control	30.6±0.53[a]	18.7±0.12[a]	86.0±0.52[a]	99.0±0.89[a]	35.6±0.09[a]
3GGHPH	19.3±0.31[c]	9.8±0.26[c]	52.5±0.7[c]	30.2±0.04[b]	22.8±0.21[c]
3GGPAS	21.4±0.09[b]	12.2±0.32[b]	69.4±0.4[b]	26.8±0.54[c]	27.7±0.38[b]

[a-c] Values with different superscripts vary significantly within a column at $p<0.05$

4.3.9. Sensory analysis of treated blended beverages

In the present study, the panellists evaluated blended beverage of defatted coconut milk and pineapple juice treated with thermal and high-pressure homogenization in terms of color, aroma, taste, consistency and overall acceptability. According to Fig. 4.7 and Table 4.7, the average overall acceptability was higher in Control and 3GGPAS as compared to 3GGHPH. Differences in relation to the Control and treated blended beverages were observed in terms of aroma, colour, consistency, with the blended beverage 3GGPAS showing higher score than 3GGHPH and Control, as shown in Fig 4.7. The Control and 3GGPAS scored higher on taste then 3GGHPH. Geraldi et al. (2021) found that thermally processed jabuticaba juice scored higher on overall liking, appearance, aroma, flavour and mouth feel as compared to Control sample. Velázquez et al. (2019) reported that during sensory analysis in terms of sweetness, flavour, acidity, and overall acceptability, no significance difference was observed between pasteurised and high-pressure homogenised orange juice, but colour of high-pressure homogenized sample scored worst as compared to pasteurized and Control orange juice. Among the three samples that were evaluated, thermally treated 3GGPAS got maximum scores for all sensory attributes.

Table 4.7: Sensory evaluation of treated blended beverages using 9-point hedonic scale

Samples	**Average score**				
	Aroma	**Taste**	**Colour**	**Consistency**	**Overall acceptability**
Control	7.4±0.15[b]	8.0±0.11[a]	7.3±0.07[c]	7.3±0.12[bc]	8.0±0.12[a]
3GGHPH	7.3±0.08[b]	7.5±0.07[b]	7.8±0.02[ab]	7.5±0.01[ab]	7.6±0.21[ab]
3GGPAS	7.7±0.20[a]	8.0±0.12[a]	8.0±0.07[a]	7.8±0.20[a]	8.0±0.14[a]

[a-c] Values with different superscripts vary significantly within a column at $p<0.05$

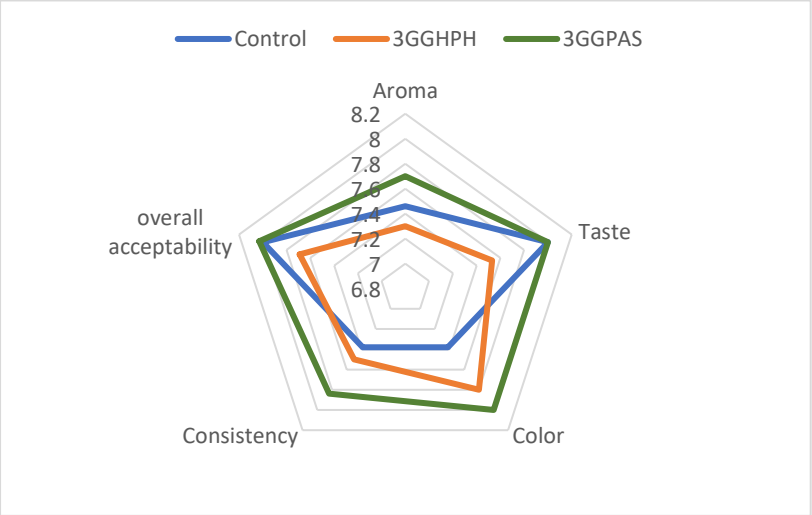
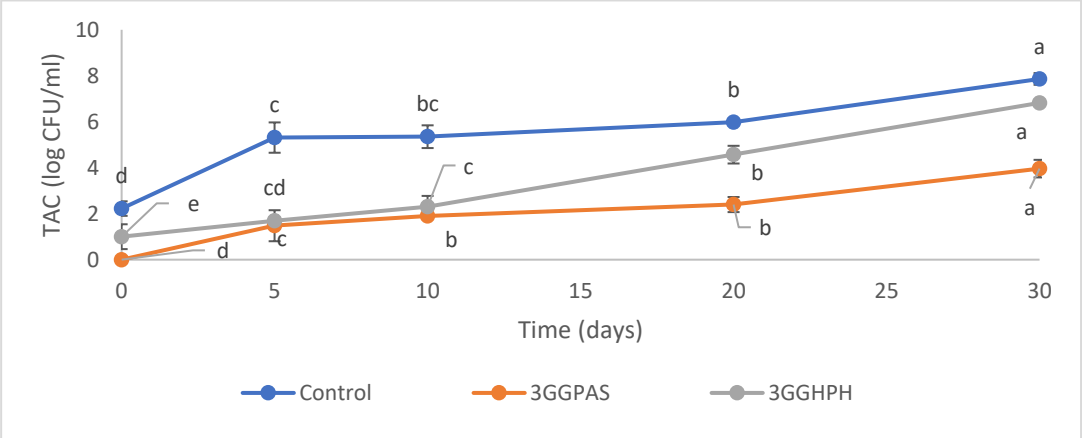


Fig 4.7: Sensory analysis of treated blended beverages.

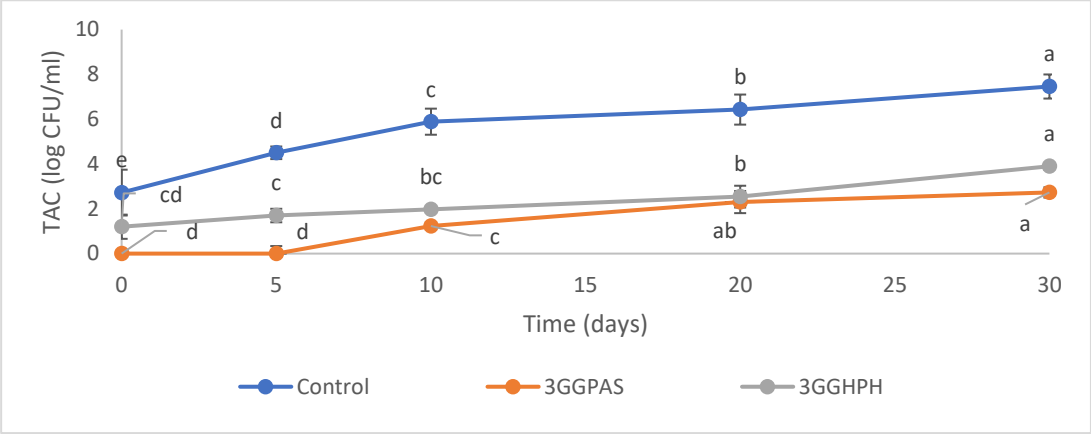
Control - Blended beverage (C50:P50); 3GGHPH- blended beverage with 0.3% guar gum and high-pressure homogenization; 3GGPAS- blended beverage with 0.3% guar gum and pasteurization.

4.3.10. Microbial safety of treated blended beverages during storage

The initial total aerobic count in Control was 2.7±0.31 log CFU/ml (Fig 4.8). No growth was observed for total plate count in 3GGPAS directly after pasteurization. During the entire storage period of 30 days at 25±2 ℃ and 4±2 ℃, Control beverage showed maximum microbial count followed by 3GGHPH and 3GGPAS, in that order. Hirsch et al. (2008) reported no growth of microorganism in thermally treated orange juice. However, 3GGHPH showed 1.2 log CFU/ml of growth after processing. Pasteurized blended beverage 3GGPAS registered colony growth at 25±2 ℃ and 4±2 ℃ storage temperature on day 30 of storage; its total plate count increased up to 3.9±0.38 log CFU/ml and 2.7±0.22 log CFU/ml as shown in Fig 4.8 (a and b). According to Hirsch et al. (2008), the initial total viable counts increased during storage up to a maximum of approximately 10^4 CFU/ml in thermally treated orange juice on day 30. At 25±2 ℃ and 4±2 ℃ storage temperature, total plate count in 3GGHPH sample showed microbial population of 6.8±0.08 log CFU/ml and 3.8±0.14 log CFU/ml on day 30 of storage. Cheng et al. (2020) reported that untreated mandarin juice sample had total aerobic bacterial count of 3.7 log CFU/ml, while thermally pasteurized and high-pressure processed juice contained lower bacterial count of 1.0 log CFU/ml. One possible explanation for higher TAC in 3GGHPH is that the juice becomes aerated during homogenization, and homogenizer valve design, and seals and unsealed valve in a high-pressure homogenized device have impact on juice quality (Kruszewki et al 2021).



(a)



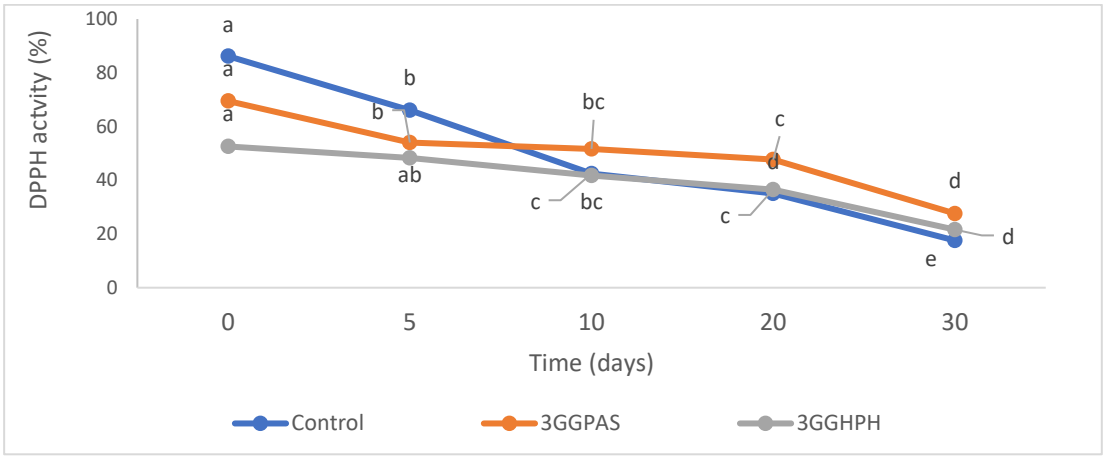
(b)

Fig. 4.8: Effect of thermal treatment and high-pressure homogenization on total aerobic count of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with 0.3% guar gum and pasteurization) and 3GGHPH (blended beverage with 0.3% guar gum and high-pressure homogenization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

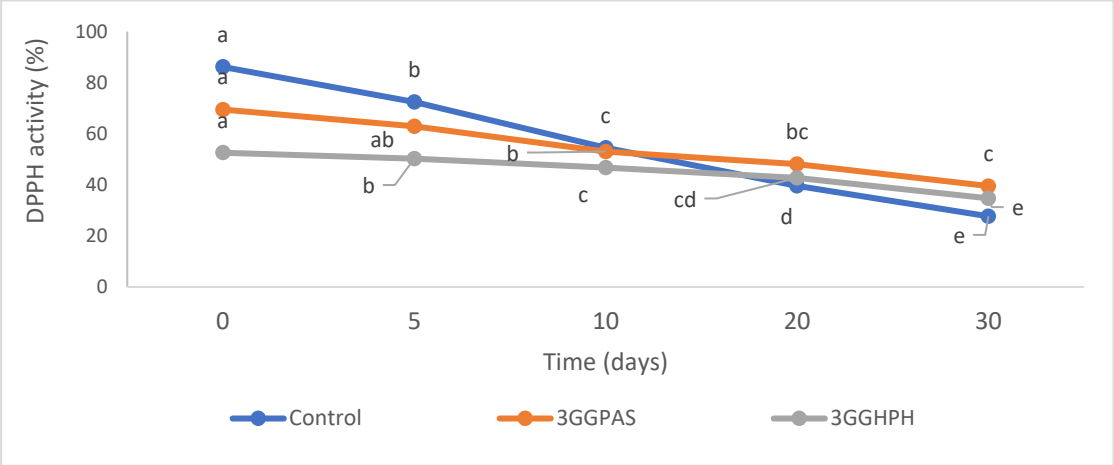
4.3.11. Antioxidant property of treated blended beverages during storage

The initial antioxidant capacity (DPPH scavenging activity) in Control (untreated blended beverage) was 86.1±0.09%, and a significantly ($p<0.05$) decreased activity of69.4±0.48% and 52.5±0.78% determined in 3GGPAS and 3GGHPH, respectively immediately after processing. It should be noted that the DPPH activity of 3GGPAS processed samples remained higher than 3GGHPH during the entire 30 days of storage at 25±2 ℃ and 4±2 ℃. DPPH scavenging activity of 3GGPAS was 27.6±0.22% and 3GGHPH was 21.6±0.24% at 25±2 ℃ on day 30. DPPH activity of 3GGPAS and 3GGHPG at 4±2 ℃ on day 30 of storage was 39.5±0.36% and 34.7±0.22%. According to Shaik and Chakraborty (2023), antioxidant breakdown is accelerated by storage temperature.

Increased humidity is detrimental to the quality of antioxidants because it accelerates the rate at which organic acids dissolves and makes antioxidants more prone to deterioration. The DPPH activity decreased on day 30 of storage was 17.5±0.33% as compared to treated blended beverages in Control at 25±2 ℃, as shown in Fig. 4.9 (a). According to He et al. (2016), thermal treatment breaks down the cell walls of fruit cells, promoting the release of bound phenolic compounds and increasing their concentration in extracts. Although high-pressure homogenization obviously breaks down the fruit's cellular structure, 3GGHPH showed a declining trend in DPPH activity. These results are similar to those reported by Suárez et al. (2011). In every antioxidant test conducted, the antioxidant capacity of pasteurized apple juice at 90 ℃ for 4 min was significantly (5–19%) greater than that of high-pressure homogenized apple juice treated at 100, 200, and 300 MPa. Up to 10 days of storage, DPPH activity in Control was higher than the pasteurized and homogenized beverages and thereafter the pasteurized beverage exhibited greater DPPH activity. The DPPH activity in untreated blended beverage (Control) stored at 25±2 ℃ was higher than the treated blended beverages, namely, 3GGHPH and 3GGPAS till 5 days of storage and thereafter a gradual fall in the activity occurred and was almost similar to 3GGHPH over the remaining period till day 30. One possible explanation is that the juice becomes aerated during homogenization, and mechanical forces and the turbulent process generate heat and a higher temperature at the valve, which encourages oxidation (Kruszewski et al., 2021). This significantly affects DPPH activity in 3GGHPH. Shaik and Chakraborty (2023) reported that thermally treated lime juice has a lower antioxidant activity than untreated lime juice. During storage for 46 days at 4 ℃, the antioxidant property of the samples was reduced from 16.9 ± 0.7 g GAEAC/L to 11.5 ± 0.8 g GAEAC/L.



(a)

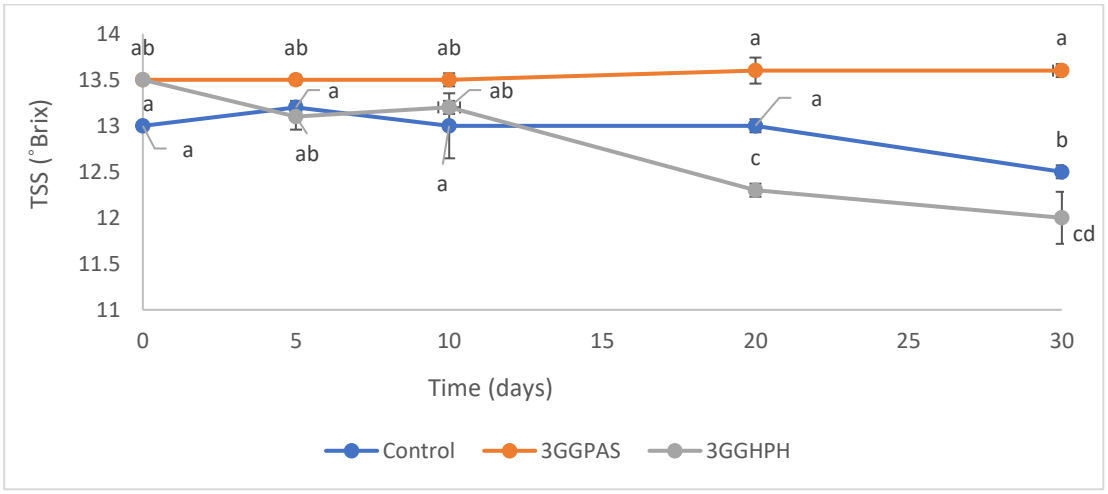


(b)

Fig. 4.9: Effect of thermal treatment and high-pressure homogenization on DPPH free radical scavenging activity of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with 0.3% guar gum and pasteurization) and 3GGHPH (blended beverage with 0.3% guar gum and high-pressure homogenization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

4.3.12. Physicochemical properties of treated blended beverages during storage

To evaluate the impact of pasteurization and high-pressure homogenization on blended beverages (3GGPAS and 3GGHPH), total soluble solids (TSS) and pH were measured at 25±2 °C and 4±2 °C The initial TSS value of Control was 13 ºBrix, whereas TSS of 3GGPAS and 3GGHPH was 13.5. The TSS of the Control sample stored at 4±2 °C and 25±2 °C decreased to 12.8±0.07 ºBrix and 12.5±0.28 ºBrix on day 30. A significant difference was not observed in the TSS of 3GGPAS ranges from initial value 13.5±0.01 ºBrix to 13.6±0.02 ºBrix on day 30 of storage at both of the storage temperatures (4±2 °C or 25±2 °C), as shown in Fig 4.10 (a and b). The TSS decline shown in may be connected to the increase in pH. The microorganism that causes juice to deteriorate were the source of the decrease in TSS (Chia et al., 2012). The 3GGHPH blended beverage TSS decreased at both the temperature 4±2 °C and 25±2 °C on day 30 of storage was 12±0.35 ºBrix and 12±0.28 ºBrix, respectively as shown in Fig 4.10. Kruszewski et al. (2021) reported that high- pressure homogenization results in a decrease in TSS due to the precipitation and aggregation of some soluble substances caused by harsh processing conditions, such as increased shear stress, cavitation, and valve temperature.



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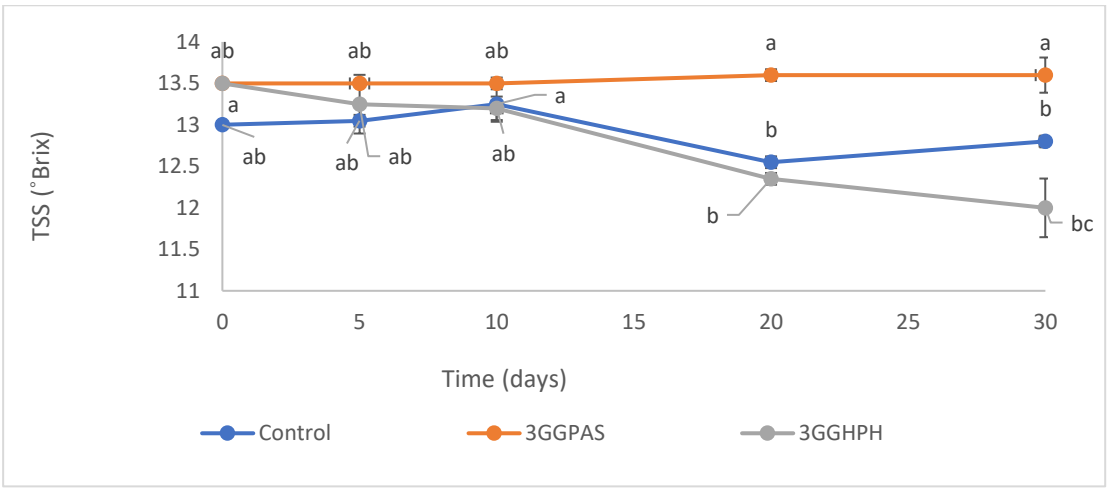
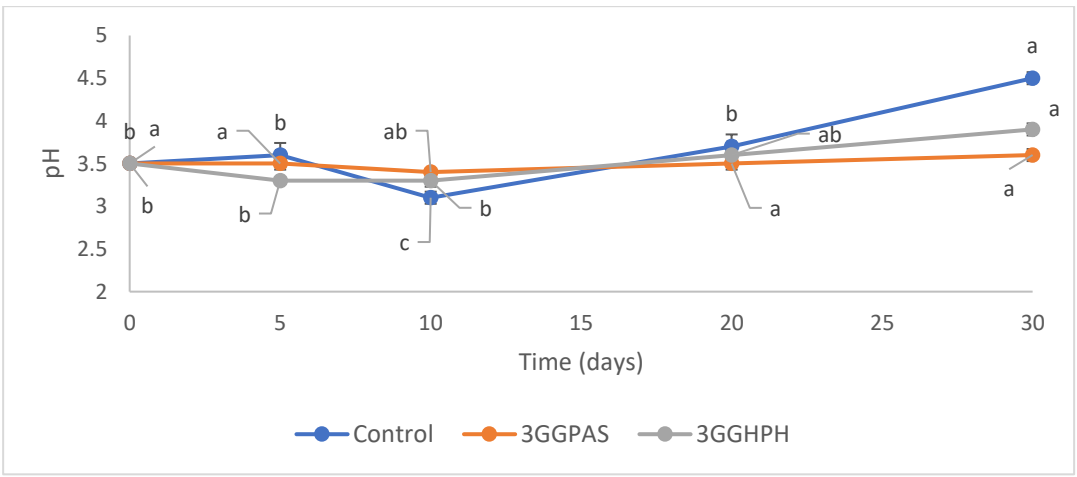
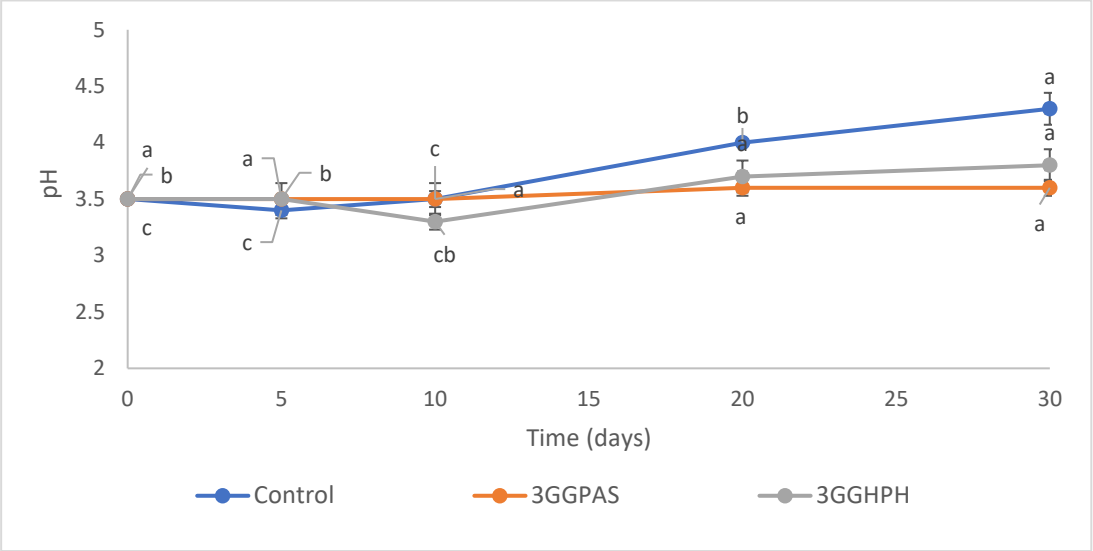


(b)

Fig. 4.10: Effect of thermal treatment and high-pressure homogenization on TSS of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with guar gum 0.3% and pasteurization) and 3GGHPH (blended beverage with guar gum 0.3% and high-pressure homogenization) on storage at (a) 25±2 °C and (b) 4±2 °C temperature.



(a)



(b)

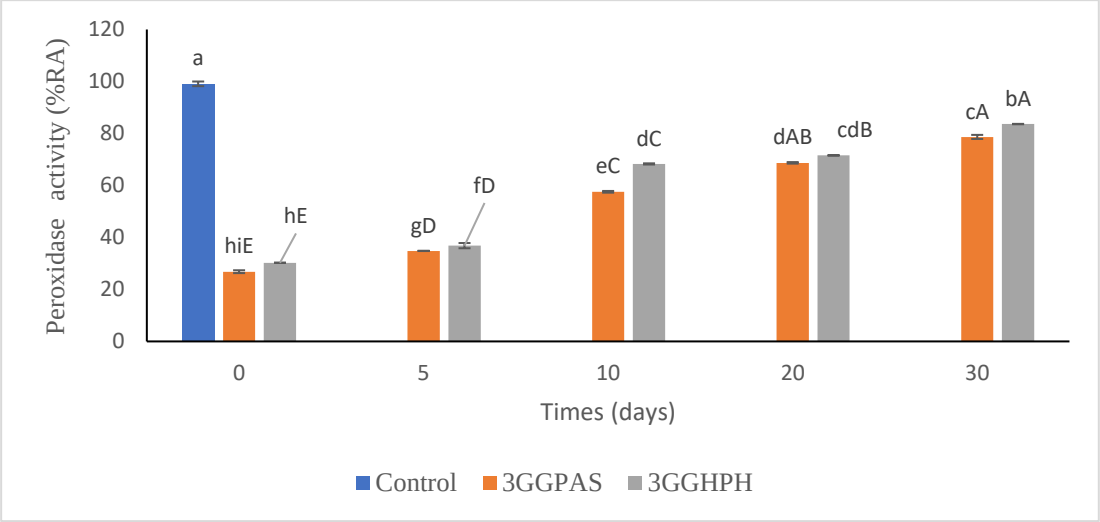
Fig. 4.11: Effect of thermal treatment and high-pressure homogenization on pH of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with guar gum 0.3% and pasteurization) and 3GGHPH (blended beverage with guar gum 0.3% and high-pressure homogenization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

A comparison of the results obtained by 3GGPAS and 3GGHPH showed that there were no statistically significant differences in the pH values immediately after processing compared with the Control sample i.e. 3.5 as shown in Fig. 4.11. Kruszewski et al. (2021) reported that the pH of blackcurrant juice was unaffected by high-pressure homogenization processing, as well as thermal pasteurization, and that no significant difference was observed compared with the Control sample. During the subsequent storage of the 3GGPAS samples at 25±2℃ and 4±2 ℃, there was no significant difference in the pH of the 3GGPAS samples on day 30 of storage at either temperature (Fig. 4.11 (a and b). Chia et al. (2012) observed that the pH of thermally pasteurized pineapple juice was not significantly altered by storage for 13 weeks at 4±2 ℃. The pH of the Control samples varied; it drops to 3.1±0.01 after day 10 of storage and rises to 4.5±0.07 at 4±2 ℃ on day 30. On day 30 of storage, the Control sample pH increases to 4.3±0.14 at 25±2℃. Chia et al. (2012) reported similar results; untreated pineapple juice showed a statistically significant pH increase during 13 weeks of storage. According to Rivas et al. (2006), there were no pH changes in thermally treated blended juices of orange and carrot stored at 2 ℃ and 12 ℃. On day 30 of storage, the 3GGHPH pH increased in both the 4±2 ℃ and 25±2 ℃ storage temperature ranges (3.9±0.07 and 3.8±0.14) respectively. Similar results were reported by Cortes et al. (2008), who found that after seven weeks of storage at 2 ℃ and 10 ℃, the pH increased considerably in fresh, high-pressure homogenized, and pasteurized

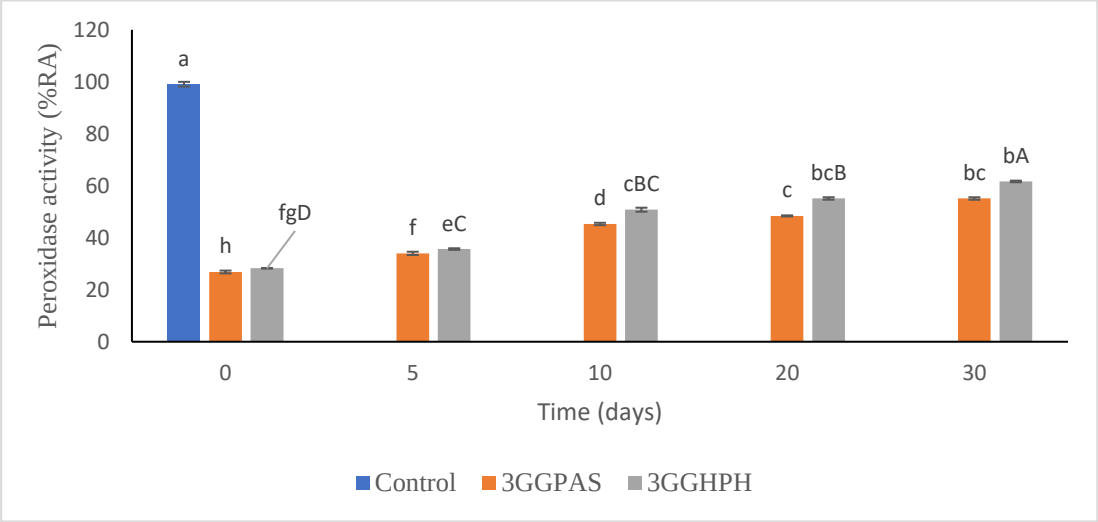
orange juice. The microorganisms responsible for the juice spoiling were responsible for the higher pH levels in these juices (Cortes et al., 2008).

4.3.13. Peroxidase (POD) activity of treated blended beverages during storage

Both high-pressure homogenization and pasteurization affected the enzyme activity of the blended beverage (Fig. 4.12). The initial residual activity (POD) was 99.0±0.89% in Control sample, but pasteurization and high-pressure homogenization of the blended beverage (3GGPAS and 3GGHPH, respectively) caused a significant decrease in the residual activity of POD with value of 26.8±0.54% and 28.2±0.04%, respectively. The POD residual activity of 3GGPAS significantly increased to 78.6±0.78% and 55.1±0.48% at 25±2 °C and 4±2 °C, respectively on day 30. The POD activity of 3GGPAS increased up to 1.9 and 1.0-fold at 25±2 °C and 4±2 °C, respectively on day 30. According to Marszałek et al. (2017), in comparison with PPO, POD proved to be more resistant to heat and pressure. The impact of the residual activity (POD) of 3GGHPH on the day 30 of storage is shown in Fig., where it significantly increased by 83.6±0.07% and 61.6±.31% at 25±2 °C and 4±2 °C, respectively. The POD activity of 3GGHPH increased by 1.9 and 1.1-fold at 25±2 °C and 4±2 °C, respectively on day 30 of storage. However, the 3GGPAS sample showed lower residual activity than 3GGHPH during the entire storage period of 30 days. Beegum et al. (2018) reported that the residual activity of thermally treated watermelon juice increased by up to 50.9% on day 90 of storage at 4 °C storage. The maximum efficiency in inactivating POD was observed in thermally pasteurized juice. Yi et al. (2017) observed that a rise in residual activity may be due to an increase in extractability, and alterations to the secondary and tertiary structures of these enzymes via HPH. These conformational changes can either decrease or increase enzyme activity by altering substrate specificity and functional modification.



(a)



(b)

Fig. 4.12: Effect of thermal treatment and high-pressure homogenization on POD activity of Control (blended beverage without pasteurization), 3GGPAS (blended beverage with 0.3% guar gum and pasteurization) and 3GGHPH (blended beverage with 0.3% guar gum and high-pressure homogenization) on storage at (a) 25±2 ℃ and (b) 4±2 ℃ temperature.

4.4. Conclusion

Standardization of process conditions for thermal pasteurization and high-pressure homogenization of defatted coconut milk and pineapple juice was studied. Both pasteurized and high-pressure homogenized blended beverages were able to achieve considerable peroxidase inactivation and microbiological safety. Pasteurization conditions of 80 ℃ temperature and 1.8 min time for blended beverage of defatted coconut milk and pineapple juice were able to lower POD residual activity and retain DPPH activity to a large extent. The inclusion of guar gum to blended beverages decreased serum separation, according to our study. While guar gum and high-pressure homogenization had a detrimental impact on serum separation, guar gum and thermal pasteurization restricted serum separation. When compared to homogenized 3GGHPH, the pasteurized 3GGPAS demonstrated reduced serum separation properties. During storage, it was observed that 3GGPAS had better properties than 3GGHPH in terms of sensory properties, physicochemical properties, microbial growth, peroxidase activity and antioxidant activity at 25±2 ℃ and 4±2 ℃ across the storage period of 30 days.

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