TO EVALUATE THE QUALITY OF BLENDED FRUIT JUICE OF PASSION FRUIT AND OTHER FRUITS INCORPORATED WITH THE OIL AND DIETARY FIBRE EXTRACTS FROM THE SEEDS OF THE FRUIT, AND STUDY THE BIO-ACCESSIBILITY OF CAROTENOIDS.

CHAPTER 6 (SECTION A, B, C & D)

CHAPTER 6 SECTION A

EFFECT OF THERMOSONICATION TREATMENT ON PASSION FRUIT JUICE, ANN/GA OPTIMIZATION AND ITS PREDICTIVE MODELLING FOR SHELF LIFE AND QUALITY CHANGES DURING STORAGE

6A.1. Introduction

Pasion fruit juice (PFJ) is a very good source of polyphenols, carotenoids, and dietary fibre, and has well balanced minerals and low-fat content [17]. Passion fruit pulp exhibits antioxidant, antimicrobial, anti-diabetic properties, prevents chronic diseases, preserves visual activity and provides neuroprotective and immune protective effects [8,33].

Consumers prefer high quality fruit juice that is nutritious, contains minimal chemical preservatives, and maintains the natural characteristics while conforming to legislative requirements. PFJ may be extracted directly from the fruit, by squeezing from crushed material, which is enriched with pulp and other nutrients, but it is very perishable in nature and spoils very fast [17], so preservative techniques are used for the enhancement of the shelf-life. Generally conventional thermal treatments are used but the delicate flavour of PFJ is extremely sensitive to thermal treatment [10]. Additionally, higher thermal treatment of PFJ produces four toxic compounds including 5-hydroxymethyl-2-furfural [13]. Use of chemical preservative is nowadays less accepted by customers, and clarification techniques are associated with reduction of nutritional value as most of the nutrients remain in the colloidal suspension [38].

Ultrasonication (US) is an innovative preservation technique, which has been identified as a potential technology to meet the U.S. Food and Drug Administration requirement for juices [40]. Ultrasound-assisted processing enhances the content of phytochemicals and is a reliable, environment friendly technique that can be easily scaled up [6]. US has been used for bayberry [5], fruit and vegetables juices [19], and strawberry juice [6]. The combination of US and controlled temperature, termed as thermosonication (TS), has shown synergetic effect on food safety and quality attributes of different juices and has gained significant attention in enhancing the retention of bioactive phytochemicals and improving the shelf life of liquid food such as almond milk [24], hazelnut milk [3], spinach juice [25] etc.

Artificial neural network (ANN) is a computational and mathematical modelling technique that has been widely used for modelling of biochemical processes due to its flexibility and accuracy in prediction [22]. Optimization of TS process parameters plays a vital role in the efficient selection of variables. Response Surface Methodology has been extensively tested as an optimization tool, but it was suggested that ANN tool could be used for better prediction with higher accuracy than RSM, and ANN with genetic algorithm (GA) has also been used for identifying better optimization [7].

Shelf-life estimation of juices using traditional microbiological process is time consuming and expensive in nature [15]. Therefore, the interest for the development of mathematical model to predict the growth and deactivation of food spoilage microorganisms under diverse environmental conditions has been increasing. Predictive microbiology is a mathematical approach, through which the growth of microorganisms can be predicted in a quick, efficient and cost-effective manner as compared to traditional methods [20].

Although predictive microbiology is considered effective in preventing spoilage, but so far, studies on the predictive growth using total plate count (TPC) for various environmental factors of fresh and TS treated juice specially PFJ are limited. In predictive microbiology, mathematical models are mainly categorised as primary, secondary and tertiary. Primary growth models, namely Baranyi, Gompertz, and logistic models are used and the Gompertz model and logistics model are most commonly used in the development of the commercialized Pathogen Modeling Program [20]. But these models were not originally developed for bacterial growth modelling and therefore it remains a field of research and has not yet become a practical tool in food industries [15]. For these reasons Baranyi, a mechanistic model that could deal with varying environmental conditions, is the most extensively used primary model for describing growth of microorganism [20]. The maximum specific growth rate of microbial growth can be modelled by using the secondary models with extensive factors as functions. Among the secondary models, the Ratkowsky and Arrhenius models are most frequently used to determine temperature dependence of microbial growth and the combination of primary and secondary models forms the tertiary model [15]. The combination of Baranyi and Ratkowsky square-root model growth has been successfully demonstrated for prediction of microbial growth of *Escherichia coli* [37], *Saccharomyces cerevisiae* and *Byssochlamys fulva* in pomegranate juice [15], and spoilage microorganism in milk and orange juice [23]. Juneja et al. [18]

found Baranyi model as the best model among all the different primary prediction models. Hashemi and Roohi [15] successfully used the Baranyi model with root square technique for the efficient prediction of shelf life of TS pomegranate juice.

The aim of this Chapter was to evaluate the effect of TS on PFJ quality and optimize the process. Further, a predictive model was developed for quick and efficient prediction of the shelf life of the optimized TS treated sugar-added passion fruit juice (OPFJUS) under various temperature conditions.

6A.2. Material and Methods

The passion fruits were obtained from Bishnupur district, Manipur, India. Passion fruit juice was obtained from the same fruits used in **Chapter 3.** All other chemicals were of analytical grade.

6A.2.1. Juice preparation

After juice extraction (obtained from **Chapter 3**), 10 g of sucrose, which was already optimized as the ideal sucrose concentration for PFJ [32] was added to 100 mL PFJ and mixed properly and adjusted TSS of the juice to 13 ºBrix by adding drinking water and the sensory optimized untreated passion fruit juice (SPFJ) was thus obtained and was kept in amber glass bottle at -18ºC for further analysis.

6A.2.2. Thermosonication (TS) of SPFJ

TS treatment to SPFJ juice was applied in a sonication bath (Riviera Glass Pvt. Ltd., India, Power 230volt, 50 Hz). SFPJ sample (200 mL each) was carefully placed in a sonication bath. The TS treatment was carried out at different temperatures (30, 45, and 60 ℃) for different times (10, 20, and 30 min). To avoid any possible light and outside microbial interference, all sonication treatments were performed in sealed amber glass bottles. After treatment, all samples were immediately cooled in an ice water bath and analysed. Optimized TS treated SPFJ termed as OPFJUS.

6A.2.3. Physicochemical analysis of juices

6A.2.3.1. Yield determination

Yield was determined by the amount of juice/pulp per passion fruit.

6A.2.3.2. Proximate analysis

Proximate analysis of juice was investigated according to standard methods described in **Chapter 5B.**

6A.2.3.3. Total soluble solid (TSS) content

TSS content was determined by portable hand refractometer (Erma, Tokyo, Japan) by placing a drop of sample on its prism. The percentage of TSS was obtained from direct reading of refractometer (0-32 ^{o}Brix). Temperature correction method as described by Ranganna [30] was followed.

6A.2.3.4. pH value

This was determined directly by means of digital pH meter (PB 11, Sartorius).

6A.2.3.5. Total titratable acidity (TTA)

Percentage of titratable acidity was determined by titrating 5 mL of sample with 0.1 N NaOH solution, using 1% phenolphthalein indicator [30]. It is calculated by the following formulae (**Eq. 6A.1**):

$$\text{TTA (\%)} = \frac{(T \times N \times E \times 100)}{V \times 1000} \quad (6A.1)$$

Where, T= Titre value (mL), N= Normality of NaOH , E= Equivalent weight of acid (g), V= Volume of sample taken for estimation (mL).

6A.2.3.6. Fat content (Soluble)

It was determined by the method described by Chutia and Mahanta [7] with slight modification. Fat content was estimated by the batch method of two-phase liquid extraction technique. A definite volume of sample was repeatedly and vigorously shaken in separating funnel with a non-polar solvent (n-hexane, boiling point 60 ℃). The solvent layer from the separating funnel was collected in a pre-weighed flat-shaped dish and solvent was completely evaporated at 50 ℃ under vacuum. The quantum of fat was obtained in weights. Result was expressed in g/100 mL.

6A.2.3.7. Ascorbic acid (AA) content

AA content was estimated by 2,6-Dichlorophenol-indephenol dye method, described by Thimmaiah, [39], with slight modification. Dye solution used in this test was prepared by the following method: 21 mg sodium bicarbonate was dissolved in a small volume of distilled water and 26 mg 2,6- Dichlorophenol-indophenol was added in it and made up to 100 ml with distilled water. Stock standard solution was 100 mg L-ascorbic acid dissolved in 100 mL of 4 % oxalic acid solution (1 mg/mL) and 100 µg/mL that was prepared from the stock solution was used as working standard solution. **Procedure:** Briefly, 5 mL working standard and 10 mL of 4 % Oxalic acid solution was mixed and titrated against

the dye solution (V_1, mL). End point is appearance of a pink color which persists for a few minutes. Similarly, 5 mL sample and 10 mL of 4 % Oxalic acid solution was mixed and titrated against the dye solution (V_2, mL). The acid content was determined **Eq. 6A.2.**

$$\text{Ascorbic acid content} = \frac{0.5\ \text{mg} \times V_2 \times 100 \times 100}{V_1 \times 5 \times \text{Volume of sample}} \quad (6A.2)$$

6A.2.3.8. Total phenolic content

Total phenolic content in the juice samples was assessed following the Folin–Ciocalteau assay reported by Saikia et al. [34] with slight modification. The juice sample was extracted with 1:10 ratio of acetone and centrifuged at 15 °C at 5000×g for 20 min. For the analysis, 40 µL each of sample extract, gallic acid standard or blank as distilled water were taken in separate test tubes and to each 3.16 mL of distilled water and 200 µL of Folin–Ciocalteau reagent were added, shaken well and after 8 min, 600 µL of sodium carbonate (20 %) was added. The samples were vortexed immediately and incubated in the dark for 30 min at 40 ℃. Then the absorbance was measured with the help of UV-vis spectrophotometer (Agilent, Cary 60 UV-Vis) at 765 nm and total phenolic content was expressed in mg GAE/100 g.

6A.2.3.9. Total sugars content

Total soluble sugar estimation was carried out with modified Anthrone reagent method described by Chutia and Mahanta [7].

Reagents were prepared using the following procedures:

Anthrone reagents: Dissolved 200 mg anthrone in 100 mL of ice cold 95 % H_2SO_4.

Standard glucose solution: Dissolved 100 mg dextrose in 100 mL distilled water.

Working standard: 10 mL of stock was diluted to 100 ml with distilled water.

For analysis, first prepared the standards by taking 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1 mL of the working standard (‘0’ served as the blank) and made up the volume to 1 mL in all the test tube by adding distilled water. After that 4 mL anthrone reagent was added in all test tubes, then heated in boiling water bath for 8 min then cooled rapidly and read the green dark color at 630 nm. A standard graph was drawn by plotting concentration of standards on the X-axis versus absorbance on the Y-axis, and from the graph calculated the amount of total soluble sugar present in the sample tube. **Eq. 6A.3.** was used for calculation.

$$\text{Total soluble sugar (\%)} = \frac{\text{Sugar value from the graph (µg)} \times \text{Dilutionfactor}}{\text{Aliquot sample used (0.1 or 0.2)} \times 1000} \quad (6A.3)$$

6A.2.3.10. DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

DPPH free radical scavenging activity content was measured according to Chutia and Mahanta et al. [7] and details are already reported in **Chapter 3.**

6A.2.3.11. Total carotenoids content (TCC) and β-carotene content

TCC and β-carotene content was measured according to the methods reported in **Chapter 4.**

6A.2.3.12. Microbial analysis/ Total plate count (TPC)

Microbial load was measured using the method of Manzoor, et al. [24] . In brief, TPC (37 ℃ for 48 h) was assessed using plate count agar by the spread plate method. The obtained results are expressed as log CFU/mL. For easy calculation, serial dilution was performed.

6A.2.4. Storage study of juices

6A.2.4.1. Shelf-life estimation using predictive modelling

For the storage study, the parafilm sealed amber glass bottles of OPFJUS and SPFJ samples were stored at refrigerated (8±2 ℃) and room temperature (25±3 ℃) for 30 days. The microbial load test of SPFJ was carried out at an interval of one day up to seven days and after 7^{th} day, observation was made on day 14, 21 and 30. For the OPFJUS sample, microbial load (TPC) enumeration was carried out at every 3 days interval.

6A.2.4.2. Quality parameters during storage

The SPFJ and OPFJUS samples were kept at refrigerated temperature and at every 3 days interval, its physicochemical properties, phenolic content, β-carotene content, and sensory properties were studied until spoilage occurred.

6A.2.5. Artificial neural network (ANN) of TS of SPFJ

In this study, the prediction of input and output parameters of TS treatment was made with the help of ANN model and its schematic diagram is shown in **Fig. 6A.1**. The ANN modelling was performed in MATLAB software (MATLAB 2018). The number of data used for training was 13x3 or 39 individual values; the validation was done with 3 unused average data. With the help of the experimental TS data as input parameters, the suitable structure and the computed output values of output and hidden layer neurons were obtained by using the same methodology/procedure as described by Chutia and Mahanta [7].

Finally, the ANN model adequacies were validated by estimation of the mean relative deviation percentage (R_{dev}) as **Eq. 6A.4**.

$$R_{dev} = \frac{\sum |\frac{y_{Exp,i} - y_{Pred,i}}{y_{Exp,i}}|}{N} \times 100 \quad (6A.4)$$

Z_{Exp} and Z_{Pred} are the experimental and predicted values, respectively. N is the number of the studies.

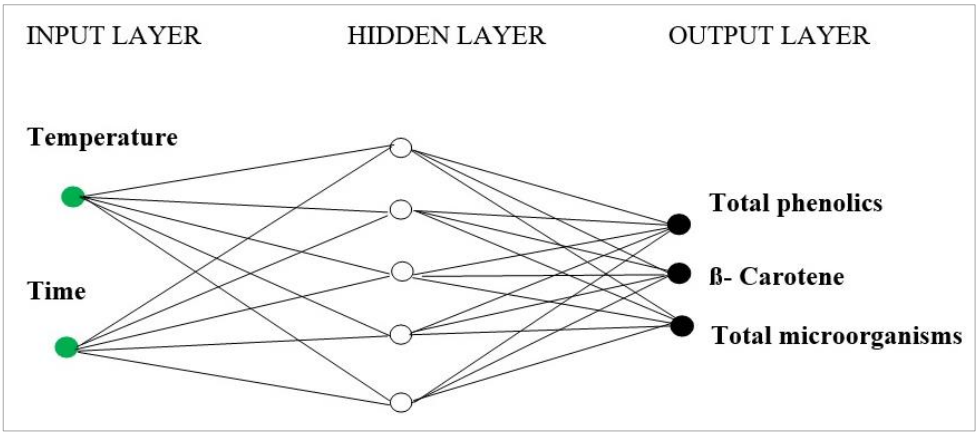

Fig. 6A.1. ANN structure of the TS treated SPFJ

6A.2.6. ANN/Genetic algorithm (GA) optimization of TS on SPFJ

Once an ANN-based model with high accuracy prediction was found and fitted, GA was used for the optimization. Briefly, the ANN structure was first developed as mentioned earlier. The independent variables were represented by an array of binary digits containing the numbers 0 and 1. Fitness values were calculated by using **Eq. 6A.5**, and the data were subjected to reproduction, crossover, and mutation (0 for 1 and vice versa) for final optimization by GA.

$$\text{Fitness function} = Y_{\text{phenolic content}} + Y_{\beta-\text{Carotene}} + \frac{1}{1+Y_{TPC}} \quad (6A.5)$$

6A.2.7. Predictive modelling / mathematical modelling for shelf-life prediction

The microbial growth was formulated using the Baranyi equation [15,18]

$$z(t) = z_0 + \mu_{max} f(t) - \ln\left(1 + \frac{e^{(\mu_{max} \times f(t))} - 1}{e^{(z_{max} - z_0)}}\right) \quad (6A.6)$$

$$\text{where, } f(t) = t + \frac{1}{\mu_{max}} \left(\ln\left(e^{-\mu_{max} \times t} - e^{-h_0} - e^{(-\mu_{max} \times t - h_0)}\right)\right) \quad (6A.7)$$

In (**Eq. 6A.6-7**), z(t) and z_0 and z_{max} is the microorganism population (log CFU/mL) at 't' time '0' and maximum microorganism population respectively. μ_{max} is represented the maximum specific growth rate (log CFU/mL) in one day and h_0 is directly related to the phase lag duration (λ) as $\mu_{max} \times \lambda$.

Moreover, to determine the temperature dependence of the obtained maximum growth rate (μ_{max}), the square root type model was applied (**Eq. 6A.8**).

$$\mu_{max} = b(T - T_{min}) \quad (6A.8)$$

where, b is a constant, T and T_{min} are the storage temperature and the minimum theoretical temperature at which $\mu_{max} \sim 0$, respectively.

To obtain the shelf-life values of the SPFJ and OPJFUS, the quantitative model reported by Hashemi and Roohi [15] was employed.

$$S_{life} = t_{lag} + (\ln 10)\left(\frac{\ln (N_S) - \ln (N_0)}{\mu_{max}}\right) \quad (6A.9)$$

In **Eq. 6A.9**, S_{life} and t_{lag} is the predicted shelf life and lag time in days respectively, N_S and N_0 is the minimum spoilage limit and initial microbial population, respectively.

Modified Arrhenius model equation related to specific shelf life was used to estimate the activation energy, **(Eq. 6A.10**)

$$\ln(S_{life}) = \ln\left(S_{life,ref}\right) - \frac{E_a}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T}\right) \quad (6A.10)$$

Where, $S_{life,ref}$ denotes the shelf life at absolute reference temperature ($T_{ref} = 273K$), E_a, T, and R is the activation energy (KJ/mol), temperature (K) and universal gas constant (8.314 J/mol K), respectively. The theoretical shelf-life values were compared to sensory analysis results to validate the modelling procedure.

Q_{10} is the temperature-sensitive parameter, and indicates the increase in the reaction rate due to increase in temperature by 10 ℃, which can be calculated by using **Eq. 6A.11** [26]

$$Q_{10} = \left(\frac{k_T}{k_r}\right)^{\frac{10}{T - T_r}} \quad (6A.11)$$

Where T and T_r is the storage and references temperature and k_r and k_T is the rate at the reference and storage temperature (T), respectively.

6A.2.8. Sensory evaluation

Sensory analysis was carried out by 10 semi trained consumers from the Department of Food Engineering and Technology, Tezpur University, Assam. The SPFJ and OPFJUS (20 mL) stored at 12±3 ℃ were offered to the consumers in paper cups. The samples were evaluated based on the 9-point hedonic scale method.

6A.2.9. Statistical analysis

All data were statistically analysed by ANOVA test in SPSS 24.0 (SPPS Inc., Chicago, IL, USA) and all the model parameters were estimated using non-linear least

squares regression using Microsoft Excel Solver (Microsoft office, USA) and confirmed by MATLAB (Release 2018). Various statistical parameters, including coefficient of determination (R^2) and root mean squared error (RMSE) was estimated.

6A.3. Results and Discussion

6A.3.1. Physicochemical parameters of SPFJ

The physicochemical composition of PFJ depends on seasonality of fruit production, variety, area, climate conditions, maturation stage, postharvest processing, and storage conditions [28]. The physicochemical properties of the SPFJ **(Table 6A.1)** are in reasonable agreement with the published data [4,8,31,35].

Table 6A.1. Physicochemical properties of SPFJ.

Parameters	Amount
Moisture (g/100 mL)	84.75±5.8
Carbohydrates (g/100 mL)	12.90 ± 2.29
Protein (g/100 mL)	0.63 ± 0.07
Fat (g/100 mL)	0.14 ± 0.06
Total dietary fibre (g/100 mL)	0.19 ± 0.01
Ash (g/100 mL)	0.5 ± 0.02
Ascorbic acid (mg/100 mL)	19.54 ± 1.26
Total carotenoids (µg/100 mL)	806.8 ± 54.34
ß-carotene (µg/100 mL)	589.5 ± 63.52
Total titratable acidity (g citric acid/100 mL)	3.98 ± 0.69
pH (at 25 ℃)	3.02 ± 0.3
TSS (at 25 ℃)	12.8 ± 0.87
Total phenolic content (mg GAE/100 mL)	33.5 ± 4.7
L*	41.05 ± 0.78
a*	8.29 ± 0.07
b*	22.82 ± 0.24
DPPH scavenging activity (%)	61.2 ± 5

The content of moisture, carbohydrates, protein, fat, total dietary fibre and ash was 84.75, 12.90, 0.63, 0.14, 0.19 and 0.5 g/100 mL, respectively for the SPFJ, which is in agreement with Biswas et al. [4], Corrêa et al. [8], and Ramaiya et al. [29]. The pH and TTA of SPFJ were 3.02 and 3.98 g citric acid/100 mL, respectively, which are within the acceptable limits for commercial PFJ (pH: 2.72-3.17 and TTA: 2.96-4.02 %) [10]. The AA

content was 19.24 mg/100 mL, in line with Ramaiya et al. [29]. The DPPH scavenging activity of SPFJ was 61.2 %, which may be due to the presence of AA, and other phenolic and carotenoids groups.

TCC content of SPFJ was 806.8 ± 54.34 µg/100 mL. The amount was slightly higher than the amount reported by Pertuzatti et al. [28] (207.51- 443.89 µg RAE/100 g) but lower than dos Reis et al. [31] (1785 µg/100 g d.w. pulp). The β-carotene content in the SPFJ was 589.5 ± 63.52 µg/100 mL juice, which contributes to 73 % of total carotenoids. Corrêa et al. [8] reported about 525 µg/100 g of β-carotene in yellow PFJ, whereas 1362 µg/100 g of β-carotene in passion fruit pulp was reported by da Silva et al. [35]. Biswas et al. [4] found β-carotene as the major carotenoid (743 µg/100 g) followed by β-cryptoxanthin (41 µg/100 g); β-carotene made up about 75 % of total carotenoids in yellow passion fruit pulp. In SPFJ, 33.5 ± 4.7 mg GAE/100 mL of total phenolic content was found, which is supported by Falguera et al. [9] and Ramaiya et al. [29].

6A.3.2. Effects of TS on total microbial/ TPC

As shown in **Fig. 6A.2a** and **2b**, the TPC content decreased with increasing treatment time up to approximately 20 min, after that became less effective, while time was kept constant. Similarly, with increase in treatment temperature, the TPC value decreased. The effect of time was more severe as compared to temperature. The combination of time and temperature effect on the TPC was found to be synergetic in nature **(Fig. 6A.2b)**. The inactivation may be due to generation of high temperature and pressure, disruption of cellular structure due to cavitation [27], generation of free radicals that injure the microbial cell walls and affect mitochondria activity [6]. Apart from the above, during TS treatment, acidity develops osmotic pressure causing release of nuclear compounds from microbial cells leading to cell degradation [24]. Overall, it was observed that TS synergistically increased the microbial inactivation. Similar results were reported in treated hazelnut milk [3], and spinach juice [25].

6A.3.3. Effect of TS on total phenolic content

As the temperature of TS increased from 30 to 50-55 ℃ **(Fig. 6A.2c** and **2d)**, a remarkable increase in phenolic content was observed **(Fig. 6A.2d)**, but with further increase a notable negative change in phenolic content was seen, when other parameters were kept unchanged. Increase in total phenolic content up to a certain temperature was also reported by Atalar et al. [3]. In TS treatment, increase in temperature enhanced the

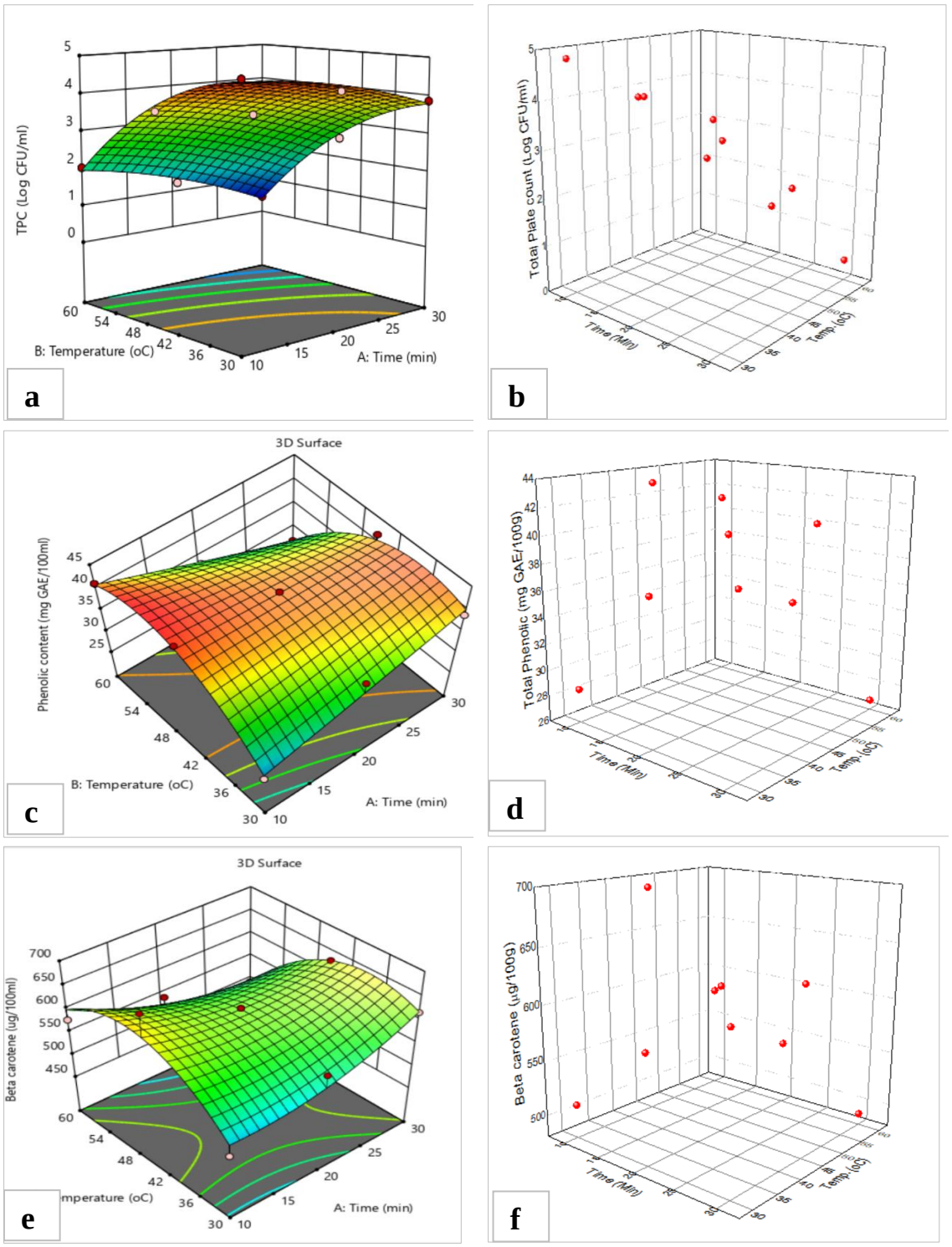

Fig. 6A.2. Effect of temperature and time on: (a) Total plate count (3D), (b) Total plate count (3D scatter), (c) Phenolic content, (3D), (d) Phenolic content (3D scatter), (e) β-carotene (3D), and (f) β-carotene (3D scatter).

fluidity, that would have facilitated the effective diffusivity in addition to the disruption of the cell wall material due to ultrasonication and as result better release of bioactive components occurred [27]. But, after 55 ℃, a further increase in temperature hampers the strength of cavitation process and also higher temperature degrades the bioactive compounds, and as a result total phenolic content decrease (**Chapter 3**). Further, total phenolic content increased as compared to SPFJ up to 10 min of ultrasonication and after that a decreasing trend was observed, which may be due to degradation of the polyphenols on longer exposure to ultrasonic waves [27].

6A.3.4. Effect of TS on β- carotene content

Carotenoids are important natural colour pigments that act as provitamin A. They perform important functions in the human body as antioxidants, modulators of immune function, stimulators of cell-to-cell communication, etc. β-carotene is one type of biologically active carotenoid which comprises more than 70 % of total carotenoids in SPFJ. As the temperature of TS increased from 30 to 50 ℃ **(Fig. 6A.2e** and **2f),** increase in β-carotene content was observed**,** and further increase in temperature decreased β-carotene content, when other parameters were kept constant.

During the TS process, cell disruption takes place which may influence the change in microstructure, alter the exposition of hydrophilic structures, induce changes in the solvent accessibility to carotenoids located in the chromoplast [11], and as a result increase in carotenoids content occurs. Similar results were reported by Abid et al. [2] and Suo et al. [36].

6A.3.5. Optimization of TS on SPFJ

6A.3.5.1. ANN Prediction

By using the trial-and-error method, the ANN structure with lowest RMSE value was selected for the prediction TS effect on SPFJ. Using the experimental data, the RMSE value was lowest for three layered structures, with hidden layer containing 5 neurons. Therefore, 2-5-3 ANN structure (**Fig. 6A.3)** with learning rate of 0.5 and 30000 iterations for this data were selected for prediction. For the selected ANN structure, a good correlation during the training periods (R=0.99979), followed by validation of the structure (R=0.99989) and testing (R=0.99992) was observed between the target and output parameters **(Fig. 6A.3)**. The overall R=0.99981 implied a high correlation or good prediction by the selected structure.

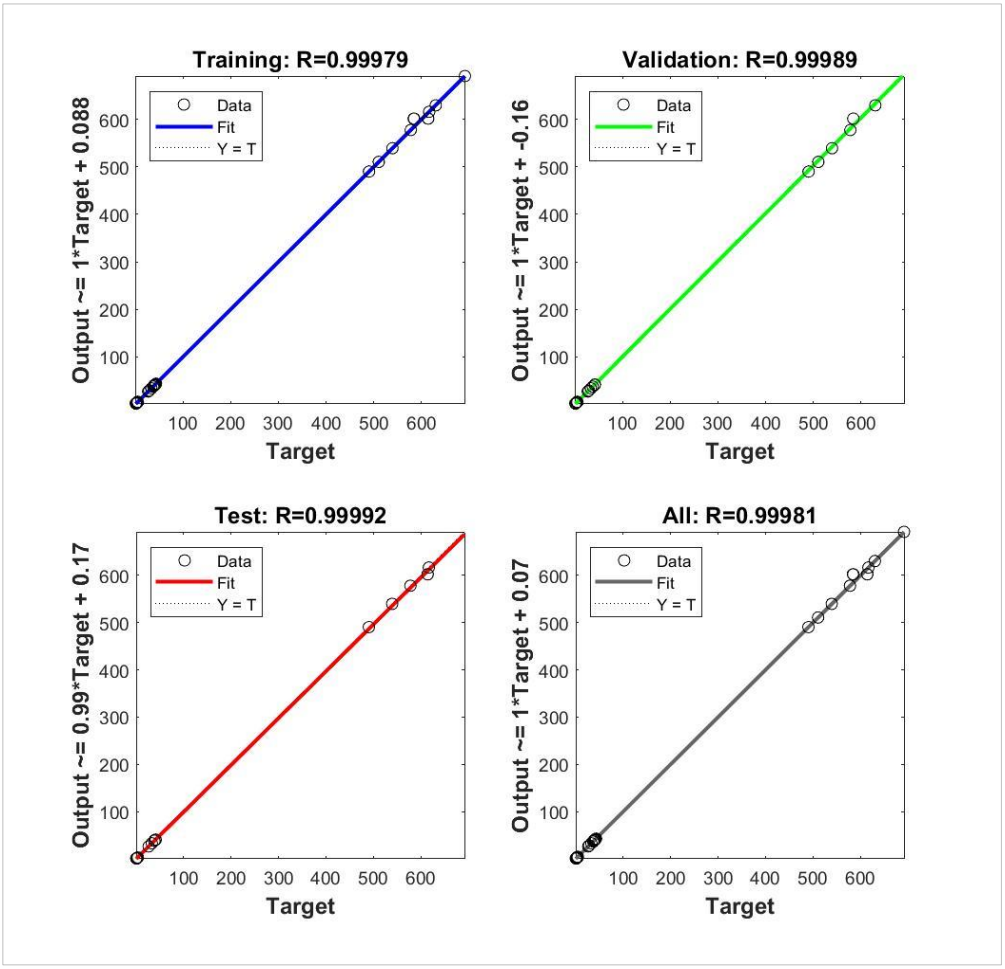

Fig. 6A.3. R value between Target and Output values of the selected ANN structure for Training set, Validation set, Testing set and Overall performance

The u, w, Y_h and Y_o values for the selected structure were estimated through random number generation by less relative deviation methods and final values of matrices are given below.

$$u = \begin{pmatrix} 2.8735 & 5.3642 & -1.8818 & 3.3146 & -0.8903 \\ -2.5449 & 1.0469 & -1.7472 & -2.7357 & 4.0213 \end{pmatrix}$$

$$w = \begin{pmatrix} 0.9649 & 0.6369 & 0.3484 \\ -1.6020 & -0.8389 & -0.5491 \\ -1.7781 & -1.0342 & 0.0345 \\ -1.0076 & -1.0597 & 0.1049 \\ -1.4591 & -1.4933 & -0.4460 \end{pmatrix}$$

$$T_h = \begin{pmatrix} -2.4942 \\ -0.4961 \\ -0.3577 \\ 2.5166 \\ -3.8608 \end{pmatrix} \qquad T_o = \begin{pmatrix} -0.1396 \\ -0.4491 \\ -0.0337 \end{pmatrix}$$

Table 6A.2. Relative influence of the independent variables on the responses.

Time (min)	Temp (℃)	Predicted			Experimental			R_{dev}			Over all R_d (%)
		Phenolic (mg GAE/100 mL)	β-carotene (µg/100 mL)	TPC (log CFU/mL)	Phenolic (mg GAE/100 mL)	β-carotene (µg/100 mL)	TPC (log CFU/mL)	Phenolic	β-carotene	TPC	
10	35	35.43	588.95	4.63	35.08	589.14	4.59				
25	45	40.83	614.53	3.15	40.53	644.25	2.97				
10	45	42.29	644.42	3.95	42.89	691.5	3.81	0.99	4.21	4.82	3.34
30	40	41.87	643.15	3.18	41.59	682.54	3.27				
20	60	33.62	518.76	1.37	33.24	539.375	1.24				

Table 6A.3. Response variables at optimized condition using ANN/GA model.

Time (min)	Temp (ºC)	Phenolic Content (mg GAE/100 mL)		β-Carotene (µg/100 mL)		Total plate count (Log CFU/mL)	
		Predicted Value	Experimental Value	Predicted Value	Experimental Value	Predicted Value	Experimental Value
10.15	56.60	41.76	42.35	621.54	623.40	2.42	2.50

The final values of u, w, T_h, and T_o were later converted to real values to obtain the corresponding predicted response.

6A.3.5.2. Validation of ANN

After finalizing the 2-5-3, structure, its validation was done by putting the new data and validated for the above specified optimized structure. **Table 6A.2** presents the prediction and validation of the model developed by ANN structure. ANN modelling for the three output parameters (phenolic content, β-carotene, TPC) can be predicted with overall 3.34% of R_{dev}.

6A.3.6. ANN/GA Optimization

The developed 2-5-3, ANN structure was subjected to optimization by GA, where phenolic content and β-carotene was maximized and TPC was minimized. In this process, the ANN output parameters u, w, T_h, and T_o were used as inputs for GA optimization process. Optimized condition of ANN/GA was selected on the basis of the highest fitness value **(Eq. 6A.5**). The optimal condition obtained from hybrid ANN/GA was time of 10.15 min and temperature 56.60 ℃ that gave phenolic content of 42.35 mg GAE/100 mL, β-carotene of 623.40 μg/100 mL and total microorganism load of 2.50 log CFU/mL. At optimized condition, the experiment was performed in triplicate and the average value is presented in **Table 6A.3.**

6A.3.7. Baranyi equation as predictive modelling

The parameters of the predictive model (Baranyi equation) are listed in **Table 6A.4.** For all storage conditions, $R^2 \geq 0.98226$, and RMSE ranging from 0.00122 to 0.17124 showed good accuracy of the theoretical model for prediction of microbial population during storage **(Fig. 6A.4).** The μ_{max} for SPFJ increased from 1.08983 to 2.44981 log CFU/mL/days when storage temperature increased from 8 ℃ to 25 ℃ (**Table 6A.4).** Similarly, for OPFJUS also, the μ_{max} value increased from 0.37687 to 0.73438. The specific growth rate increased and lag phase time decreased as the storage temperature increased (**Table 6A.4**). Similar trend was observed by Hashemi and Roohi [15] (for *Saccharomyces cerevisiae* and *Byssochlamys fulva* in pomegranate) and Kim et al. [20] for *A. hydrophila*. However, the μ_{max} increasing rate was different for juices stored at different temperatures as lower storage temperature slows down the growth of microorganisms [15]. The μ_{max} value decreased (65 % to 70 % reduction) and 'λ' value increased remarkably, after the optimized thermo-sonication treatment, which may be due

to the cellular injury of microorganisms and greater time required to recover from injury, respectively.

'λ' values in this study ranged between 1.44181and 11.53940 days, whereas Lee et al. [23] found 0.58 to 6.36 days as the phase duration of spoilage microorganisms in orange juice. The variation of 'λ' value from literature may be due to the pH as well as changes in composition, as Kim et al. [20] observed significant decrease of 'μ_{max}' value and significant increase of 'λ' value at low pH.

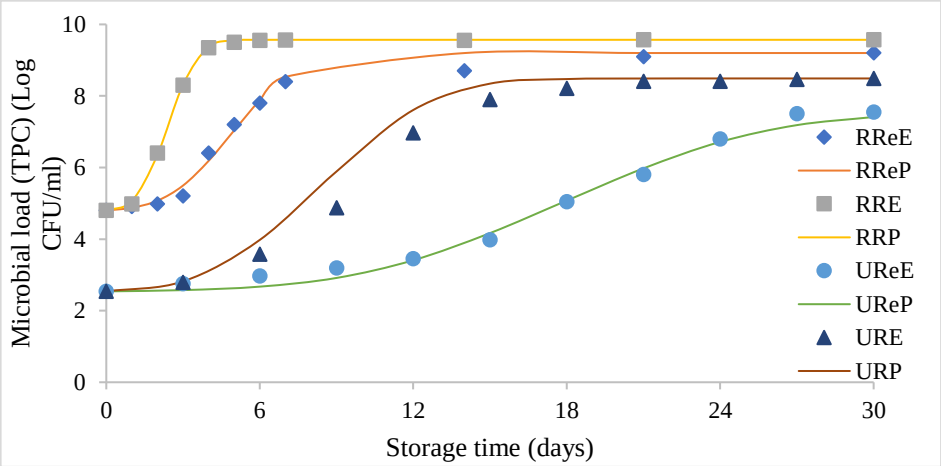

Fig. 6A.4. The variation of total microorganism population during storage as a function of storage temperature (RReE-SPFJ refrigerated experimental data; RReP- SPFJ refrigerated predicted data; RRE- SPFJ room temperature experimental data; RRP- SPFJ room temperature predicted data; UReE-OPFJ refrigerated experimental data; UReP- OPFJ refrigerated predicted data; URE- OPFJ room temperature experimental data; and URP- OPFJ room temperature predicted data).

The two main parameters of the Ratkowsky square type model, T_{min} and 'b' were calculated for both the juices and illustrated in **Table 6A.5**. The error estimation parameters (R^2 and RMSE) value for each juice was found in the acceptable range and this implied that square type model can predict thermal behaviour of μ_{max} with high accuracy.

Table 6A.4. The parameters of the Baranyi equation for total microorganisms (TPC)

Samples	Temp (°C)	μ_{max}/days	λ (days)	R^2	RMSE
SPFJ	8±2	1.08983	2.93511	0.98873	0.04537
	25±3	2.44981	1.44181	0.99971	0.00122
OPFJUS	8±2	0.37684	11.53940	0.99175	0.03536
	25±3	0.73438	4.38099	0.98226	0.17124

The T_{min} value of SPFJ and OPFJUS was predicted to be $-24.62346\ ^{o}C$ (with $R^2 = 0.99810$ and $RMSE = 0.10410$) and $-35.23059\ ^{o}C$ (with $R^2 = 0.97226$ and $RMSE = 0.14120$), respectively. Similarly, the 'b' value was found to be 0.03197 and 0.01420 for SPFJ and OPFJUS, respectively. The results agreed with the findings of Hashemi and Roohi [15] and Lee et al. [23]. Lee et al. [23] reported the 'b' value for milk and orange to range from 0.023 to 0.044 and 0.040 to 0.053, respectively; and T_{min} value to range between -31.50 and -85.25 for milk and -19.57 and -25.65 ^{o}C for orange juice.

Table 6A.5. Parameters and statistical analysis of the square root type model for the effect of temperature on the growth rate of TPC.

Samples	T_{min} (^{o}C)	b	R^2	RMSE
SPFJ	-24.62346	0.03197	0.99810	0.10410
OPFJUS	-35.23059	0.01420	0.97226	0.14120

Overall, the model could be used for efficient prediction and will additionally help to obtain data that can be used in developing critical limits for OPFJUS.

6A.3.8. Shelf-life estimation

The shelf life of SPFJ and OPFJUS, based on predictive modelling (theoretical) and sensory analysis at various storage temperatures are presented in **Table 6A.6**.

Table 6A.6. Shelf-life determination using mathematical equation and sensory analysis.

Samples	Temp. (^{o}C)	Calculated shelf life (days)	Experimental shelf life (days)
SPFJ	8±2	4.40	3.5
	25±3	1.90	1.5
OPFJUS	8±2	15.7	17
	25±3	6.50	8

The modelling can predict the sensorial observations with high accuracy ($R^2 = 0.962666$ and $RMSE = 2.2306$). The relative deviation from the sensory data lay between 7.8% and 27.7%. Increasing the temperature from 8 to 25 ℃, reduced the shelf life from 3.5 to 1.5 for SPFJ and 17 to 8 days for OPFJUS.

The E_a, $S_{life,ref}$ (at 273 k) and Q_{10} values for both SPFJ and OPFJUS are listed in **Table 6A.7**. The E_a value for SPFJ and OPFJUS was found to be 30.40 and 34.13 KJ/mol, respectively and '$S_{life,ref}$' was 6.39 and 24.06 days, respectively. Huang et al. [16] reported

about the inverse relation between rate of reaction and activation energy. Due to TS, injured microorganism may become resistant/lethal so higher energy was required [12]. Hashemi and Roohi [15] found the E_a and $S_{life,ref}$ to range from 28.90 to 30.16 KJ/mol and 14.78 to 38.93 days, respectively for *Saccharomyces cerevisiae* in pomegranate juice.

Table 6A.7. The activation energy (E_a) at reference temperature and Q_{10}.

Samples	Activation Energy (KJ/mol)	$S_{life,ref}$	Q_{10}
SPFJ	30.40	6.39	1.61040
OPFJUS	34.13	24.06	1.50950

Fitting the Q_{10} model to the microbial growth at optimized condition, value of SPFJ and OPFJUS was 1.509459 and 1.61040, respectively, which lay within the range (1.31 to 5.13) reported for various biological processes [26]. 'Q_{10}' was higher for the untreated juice as compared to treated one, which implied that greater increase in rate of reaction occurred with 10 ℃ increase in temperature for the untreated one. Hashemi and Roohi [15], reported the maximum Q_{10} value of 1.36 for *B. fulva* (fungi) and maximum 1.47 for *S. cerevisiae* (yeast) for the same treatment conditions. In our study, from the Q_{10} value, it can be deduced that the major portion of the tested microorganisms was yeast.

6A.3.9. Changes in quality parameters of SPFJ and OPFJUS during refrigerated storage

6A.3.9.1. Effect of TSS

As shown in **Fig. 6A.5a**, TSS value of OPFJUS was higher than SPFJ, indicating that TS treatment caused a slight increase in TSS value. PFJ contains high amount of pulp, and the sonication treatment which caused destruction of tissues and cell walls enabled more water to permeate into the cells and bring out more soluble solids [14]. A similar trend was observed by Tomadoni et al. [41]. For both SPFJ and OPFJUS, TSS increased up to a certain day (on day 12 for OPFJUS and day 3 for SPFJ) and after that decreased remarkably. The increase in TSS during the storage period may be due to the breakdown of complex molecules into simple form by microbial enzymes [19] and the later decrease was due to microbial utilization. The change in TSS of SPFJ was higher, which may be due to the higher initial microbial load.

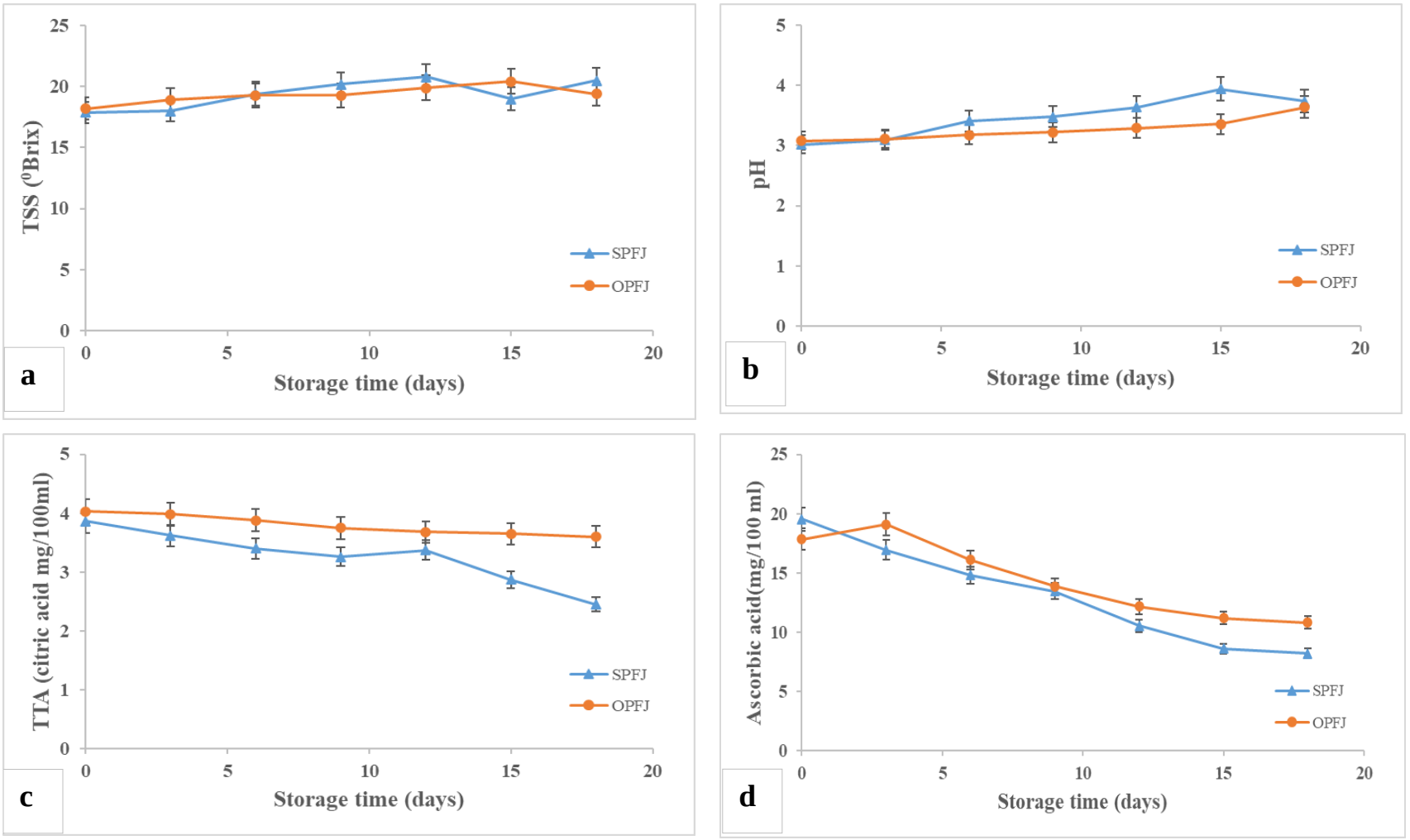

Fig. 6A.5. Changes in the quality parameters of SPFJ and OPFJUS during refrigerated storage: (a) TSS, (b) pH, (c) Total titratable acidity, (d) Ascorbic acid.

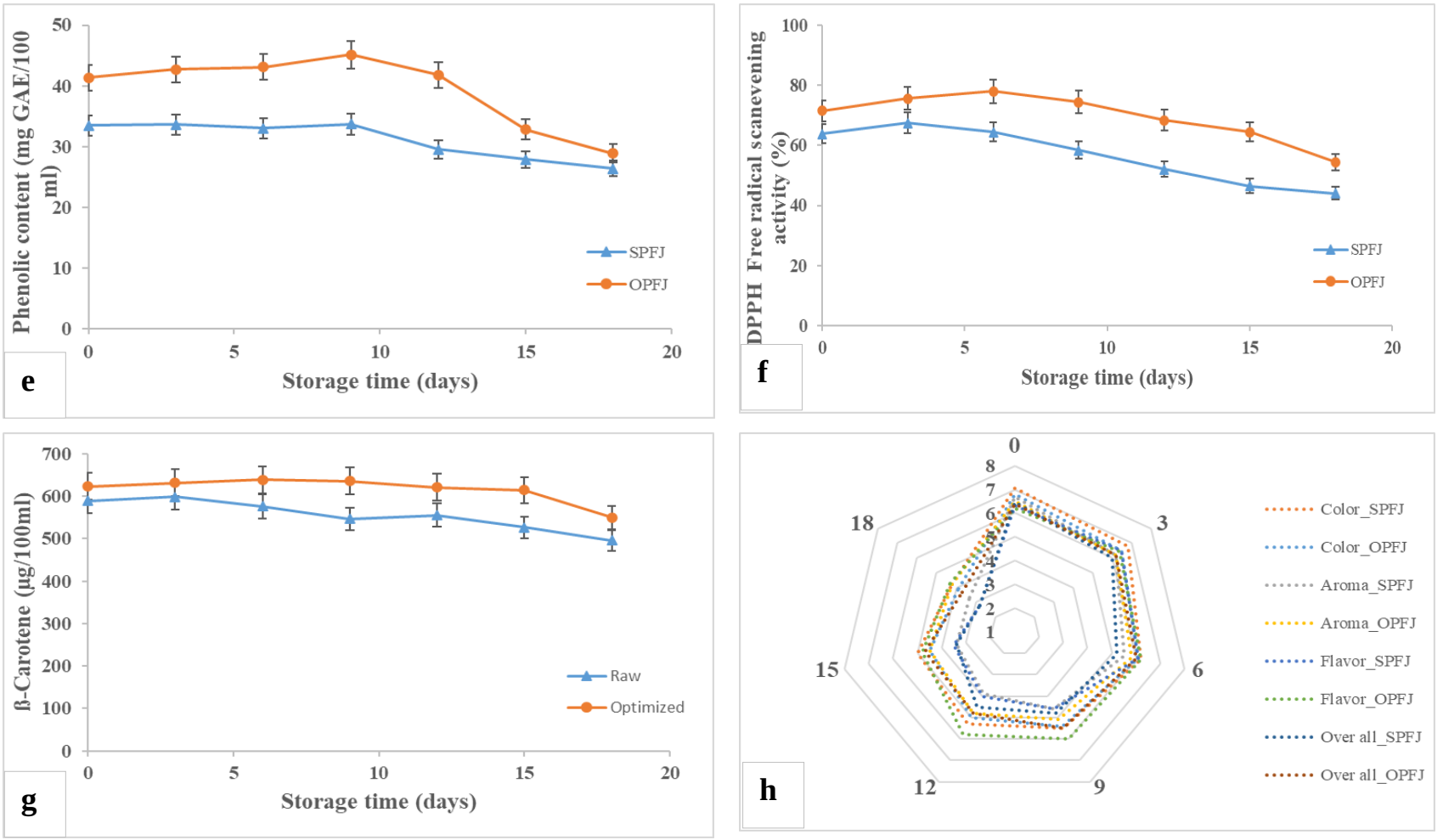

Fig. 6A.5. Changes in the quality parameters of SPFJ and OPFJUS during refrigerated storage: (e) Phenolic content, (f) DPPH free radical scavenging activity, (g) β-carotene and (h) Radar graph for sensory analysis.

6A.3.9.2. Effect of pH

A drastic change in pH was observed in SPFJ after 3 days, whereas for OPFJUS, a gradual increase was observed during the entire shelf-life period **(Fig. 6A.5b)**. It may be attributed to the metabolites released by the microorganisms. After US treatment, gradual increase in pH for the citrus juice was reported by Khandpur and Gogate [19].

6A.3.9.3. Effect of total titratable acidity

The TTA of both juices decreased throughout the storage time **(Fig. 6A.5c),** which may be due to the increase in catabolism of citrate and malate during storage [19]. However, extent of change was more in SPFJ as compared to OPFJUS, which may be due to the higher initial microbial load.

6A.3.9.4. Effect of ascorbic acid (AA)

After the TS treatment, 8.64 % of AA loss was observed **(Fig. 6A.5d).** Loss of AA by ultrasound in strawberry was observed by Cao et al. [5]. But, despite the losses, the consumption of 200 mL of OPFJUS (1:8 juice to water dilution) would supply 9 % of recommended daily intake (45 mg) of vitamin C for adults.

During storage, AA content for OPFJUS increased up to 3 days, which could be directly due to the removal of entrapped oxygen by cavitation phenomenon and also the better release of components on account of cell disruption [13]. Decrease in AA may be due to microbial activity as well as the degradation effect due to factors like light, oxygen, temperature during the storage period. By the end of storage period, 58% and 39% of AA was lost in SPFJ and OPFJUS, respectively. Increase in AA in US treated passion fruit juice up to a certain storage time at refrigerated temperature followed by rapid degradation was observed by Gómez-López et al. [13].

6A.3.9.5. Effect of total phenolic content

After the TS treatment, the total phenolic amount had remarkably increased from 33.5 (SPFJ) to 41.32 (OPFJUS) mg of GAE/100 mL (**Fig. 6A.5e).** This increase might be attributed to the release of bound form of phenolic contents due to disruption of cell wall by TS treatment and also due to addition of hydroxyl groups produced by sonication to the aromatic ring of phenolic compounds [1].

Up to a certain period of storage (9 days for OPFJUS and 3 days for SPFJ), an increase in phenolic compounds was observed, which may be due to the decomposition of the cell structure and liberation of free phenolic acids [41], followed by degradation of

phenolics, which may be attributed to the rapid microbial growth. Degradation of SPFJ was higher than OPFJUS, due to higher initial microbial load and higher μ_{max} value.

6A.3.9.6. Effect of DPPH scavenging activity

Increased antioxidant activity (**Fig. 6A.5f**) can be associated with higher values of total phenolic content and TCC and also generation of hydroxyl radicals by hydroxylation of flavanols in TS treated juice [27]. DPPH scavenging activity decreased on storage, probably due to the reduction in the natural antioxidants and the growth of microorganism.

6A.3.9.7. Effect of β-carotene

A remarkable increase in β-carotene content (5.75%) was observed in OPFJUS as compared to SPFJ **(Fig. 6A.5g)** because of the mechanical rupture of the cell wall and organelles on TS that facilitated the release of compounds [36].

During storage for first 6 days for OPFJUS and 3 days for SPFJ, slight increase followed by a decrease in β-carotene was observed. TS treated juice showed higher carotenoids level than the untreated one. These results indicated that TS treatment helped to retain and stabilize bioactive compounds.

6A.3.9.8. Sensory analysis

Sensory characteristics of fruits juices are of utmost importance as it significantly contributes to their acceptance or rejection by consumers. Sensory evaluation of SPFJ and OPFJUS on storage was done and the scores of overall acceptability, colour, aroma, and flavour of SPFJ (6.45, 7.05, 6.7 and 6.3, respectively) and OPFJUS (6.38, 6.8, 6.5 and 6.2, respectively) are presented in **Fig. 6A.5h.** No remarkable change in sensory attributes was observed after TS [14]. During storage, a sharp increase in flavour intensity on 3rd day for both the juices followed by a gradual decrease was noticed (**Fig. 6A.5h)**, which may be due to the increase in the volatile compounds particularly esters in fruits [21], which with further storage were degraded due to microbial growth. Both colour and aroma decreased with storage time.

SPFJ can be stored only for 3 days at 8±2 ℃, whereas OPFJ maintained the optimum scores up to 15 days **(Fig. 6A.5h).** The results agree with the theoretical shelf life estimated by the predictive model.

6A.4. Conclusion

In this research, the passion fruit juice was treated with thermosonication to enhance the overall quality and predictive modelling was performed to determine shelf life.

Thermosonication process was optimized using ANN/GA optimization technique with β-carotene, phenolic content and total plate count as response parameters. Predictive modelling could predict the shelf life of both treated and untreated passion fruit juice with higher efficiency. Thermosonication increased the phenolic and β-carotene content, and maintained the TSS, pH, TTA, ascorbic acid content, and DPPH scavenging activity after treatment as well as during storage. Thermosonication of passion fruit juice can be a way forward to enhance the overall quality of the juice and leverage its economic worth over traditional processing techniques.

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CHAPTER 6 SECTION B

FUZZY LOGIC SENSORY OPTIMIZATION OF PASSION FRUIT-BASED BLENDED BEVERAGE

6B.1. Introduction

Although the pulp/juice of passion fruit is enriched with several health-benefiting compounds, its direct use is not popular among consumers because of its astringency and sour taste (high acidity) [1]. In order to balance the acidity and astringency, passion fruit juice is often mixed with juices of pineapple, mango, ginger, etc. [2]. The flavour of the final product can be enhanced taking into consideration the appropriate ratio of passion fruit juice to blended fruit juice , amount of added sucrose, and pH of the developed blended beverage [14]. Mango and guava juice can be blended with passion fruit juice to obtain better sensory quality beverage and can be optimized by Fuzzy logic. A passion fruit-based blended beverage having red colour and flavor (pleasant taste) was developed with optimized conditions of 1: 3 ratio of material to liquid, pH 4.0, and 8% sucrose by Zhu et al. [14]. The yellow coloured passion fruit (commercial) juice has a fairly acidic flavour, therefore, sweeteners need to be added to improve the flavour and Rocha and Bolini [9] found 9.4g/100 mL as equi-sweet concentration of sucrose in yellow coloured passion fruit (commercial) juice.

For the improvement of process control strategies of biological systems, intelligent soft computing helps to integrate study results with sensory evaluation for effective product development. Sensory evaluation is a scientific tool that measures, analyzes, and interprets sensory attributes perceived by human sensory organs. Without appropriate sensory evaluation, there is a great chance of market failure [13] because it is the primary criteria for acceptability of food [4,6]. Human perception is always fuzzy and assessors give their opinion in the linguistic form [5]. For analysis of human perception of sensory attributes, Fuzzy sets can be used, which give better result as compared to average scores of the attributes. Fuzzy logic is a computing-based approach which helps to interpret imprecise and objective interpretations, and conclusion are drawn on the acceptability or non-acceptability of the product. The developed product can also be ranked on the basis of strong and weak attributes [13]. Fuzzy logic has been successfully applied for the evaluation of different beverages such as coconut blended beverage [3] and mango drinks [7]. In the present chapter, Fuzzy logic tool was used to optimize blended beverage on the basis of sensory parameters and the beverage was evaluated.

6B.2. Materials and Methods

6B.2.1. Samples

The sensory optimized sugar added PFJ (SPFJ) was processed according to the method outlined in Chapter 6, section A. Ripe guavas and mangoes were procured from the local market.

6B.2.2. Juice extraction

Guava juice was extracted using the method described by Kadam et al. [8]. Briefly, A 2 % NaOH solution at 80 ℃ was used to lye-peel ripe, high-quality guava fruits for around 3 min. Guava fruits that were lye peeled were then neutralised with a 1 % solution of citric acid and washed in running water. Fruits were crushed using a crusher/slicer. Then, juice was extracted using a household juicer (Philips juicer) and passed through a stainless-steel screen (0.84 mm dia.) to separate the seeds, fibrous pieces, and pulp and pasteurised it immediately at 100 ℃ for 3 min after the centre point reached 100 ℃. The TSS of the juice was adjusted to 14 ºBrix [8]. Similar process was followed for processing mango juice.

6B.2.3. Sensory evaluation

The untreated SPFJ coded as SPFJU was blended with pasteurized guava juice and mango juice. Preliminary studies revealed that guava juice is more compatible than mango in terms of sensory. The five beverage samples were coded as S1 (100 % SPFJU juice), S2 (60 % SPFJU juice + 40 % guava juice), S3 (50 % SPFJU+ 50 % guava juice), S4 (1/3-part SPFJU juice, + 1/3-part guava juice+ 1/3-part mango juice) and S5 (70 % SPFJU+ 30 % guava juice).

Twenty-four semi-trained panel judges were instructed on the value of objective sensory evaluation, the description of quality characteristics to be evaluated, the content of the score sheet, and the scoring process for evaluating the sensory qualities of the blended beverages. Prior to tasting each item, the judges thoroughly washed their mouths with normal water. The judges were instructed to assess the sample's qualities and place a value on the fuzzy scale for each sensory feature.

The judges were told to categorize samples subjectively from ‘Not satisfactory’, ‘Fair’, ‘Medium’, ‘Good’ and ‘Excellent’ [3]. Fuzzy logic was used to analyse all the sets of data noted by the judges.

6B.2.4. Application of fuzzy logic on sensory data

To provide an output conclusion that helps establish whether the food's qualities are strong or weak and acceptable or not, Fuzzy logic employs imprecise and user-dependent data. Foods can be ranked based on the potency of their qualities. As followed by Jaya and Das [7], Sahu and Kadeppagari [11] and Chutia et al. [3], the developed blended beverage samples were ranked using triangular fuzzy membership distribution function. Fuzzy logic modelling follows the following steps for ranking of foods on the basis of sensory data [3,7]: (1) Formation of triplets from computed total sensory information of blended beverages; (2) Sensory evaluation of mixed beverages using a fuzzy membership function; (3) Fuzzy membership function is normalised; (4) On a conventional fuzzy scale, normalisation of fuzzy membership function matrix; (5) Matrix of the judgement membership function; (6) Judgment subset; (7) Rating of samples of blended beverages for quality; and (8) Ranking of the blended beverages. Ranking of generated beverage sample was done based on similarity values determined from triplets formed from the obtained sensory ratings. For calculating the fuzzy logic steps MATLAB (MATLAB R2012b, The MathWorks) program was developed and used. To obtain the triangle membership distribution pattern of the fuzzy set, a set of three numbers known as "triplets" was devised and 5-point sensory scale comprising of Not satisfactory/Not at all important (0,0,25), Fair/Somewhat important (25,25,25), Medium/Important (50,25,25), Good/Highly important (75,25,25) and Excellent/Extremely important (100,25,0) were taken [3] as per **Fig. 6B.1**. The value of the abscissa, if the value of the membership function was 1, is shown by the first number in the triplet. The second and third digits in the triplet show how far left and right of the first number, where the membership function is 0, respectively [3,4].

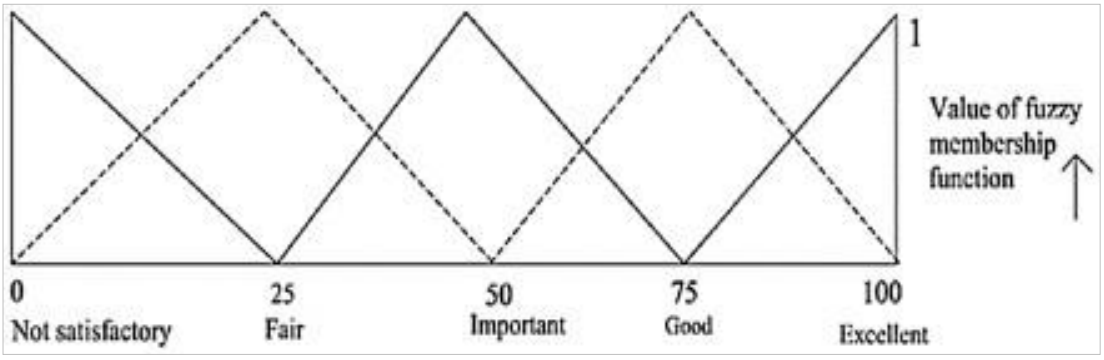

Fig. 6B.1. Representation of triangular membership function distribution pattern of the sensory scale [3].

The distribution pattern of the triangular membership function of the 6-point sensory scale, often known as the standard fuzzy scale, is shown in **Fig. 6B.2.,** where the sensory scales are represented as F1, F2, F3, F4, F5, and F6. The fuzzy scale for sensory analysis was linguistically rated on a scale of 1 to 5 as follows: Not satisfactory/Not at all necessary, Fair/Somewhat necessary, Satisfactory/Necessary, Good/Important, Very Good/Highly Important, and Excellent/ Extremely Important, according to Chutia et al. [3]. The triangle distribution pattern is followed by each membership function of the sensory fuzzy scale, with 1 being the maximum membership value.

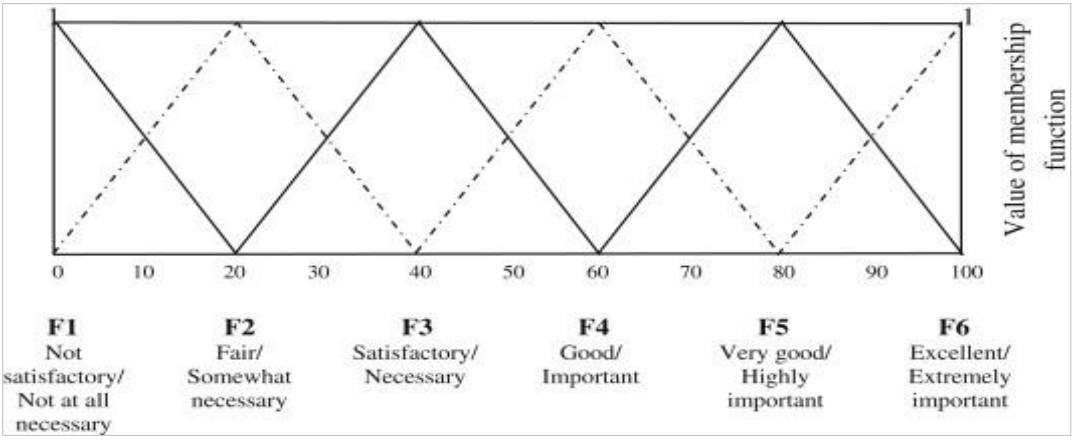

Fig. 6B.2. Standard Fuzzy Scale Used For Ranking Of The Blended Beverage Samples [3,7].

The overall membership function of the sensory score is shown graphically in **Fig. 6B.3** as a triplet (a,b,c). The graphic illustrates how the membership function has a value of 1 when the value of the abscissa is a, and a value of 0 when the value of the abscissa is greater than (a+c) or less than (a-b) [10].

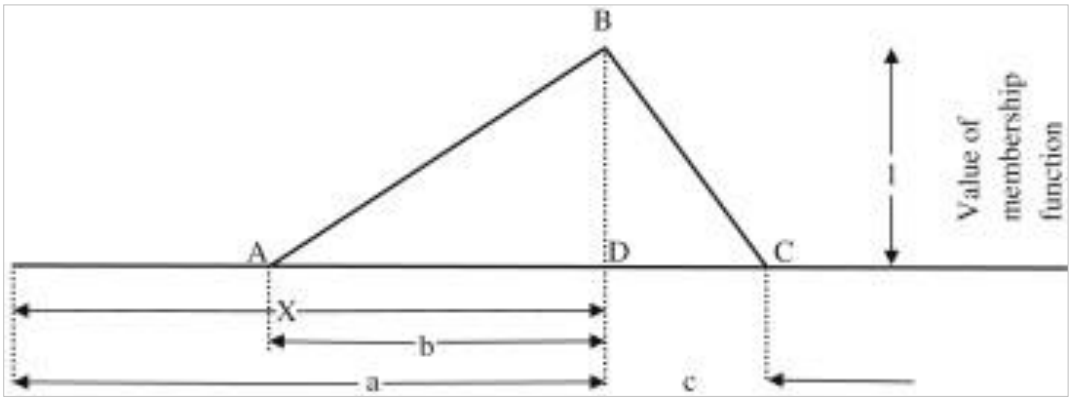

Fig. 6B.3. Graphical view of overall sensory score as triangle ABC and triplet ABC [7].

6B.3. Results and Discussion

6B.3.1. Sensory evaluation of blended juices

The direct use has fewer acceptances by customers because of its astringency and sour taste (high acidity) [1].

Table 6B.1. Sum of sensory scores for the quality attributes of blended beverage samples

Sensory Quality Parameters	Factors of Sensory Scale				
	Not Satisfactory	Fair	Medium	Good	Excellent
Colour					
S1	0	2	0	9	13
S2	0	0	1	8	15
S3	0	0	0	14	10
S4	0	0	2	9	14
S5	0	6	2	10	6
Flavour					
S1	0	2	0	16	6
S2	0	1	1	14	8
S3	0	1	3	12	8
S4	0	4	3	3	14
S5	2	4	2	9	7
Taste					
S1	1	1	0	16	6
S2	0	4	2	12	6
S3	2	0	1	15	6
S4	0	2	1	10	11
S5	4	2	2	14	2
Mouthfeel					
S1	0	2	2	16	4
S2	0	0	2	16	6
S3	0	2	4	10	8
S4	0	1	1	13	9
S5	5	1	2	14	2
Consistency					
S1	0	2	2	11	9
S2	0	0	2	12	10
S3	0	2	2	16	4
S4	0	1	4	10	9
S5	4	0	10	8	2
Overall acceptability					
S1	0	2	2	12	8
S2	0	1	3	14	6
S3	0	1	6	9	8
S4	0	1	2	10	11
S5	2	4	4	10	4

In order to balance the acidity and astringency, passion fruit juice is often mixed with juices of pineapple, mango, ginger, etc. [2]. The flavour of the final product is enhanced using appropriate ratio of material to liquid, amount of sucrose addition and pH as parameters of the developed blended beverages [14]. This fruit can, therefore, be used to develop blended beverages to enhance the taste. So, to overcome this problem, passion fruit juice was blended with mango and guava juice and fuzzy logic optimization was used for sensory optimization. **Table 6B.1.** presents the sensory scores given by the panel judges for blended beverage. The results of fuzzy logic analysis of sensory attributes of blended passion fruit beverage that was done according to Chutia et al. [3] are discussed in the coming sections.

6B.3.2. Triplets for sensory quality of blended beverage samples

According to Chutia et al. [3], a distribution of three numbers known as a triangular membership function was distributed from 0 to 100 on the sensory scale. Each sensory attribute evaluated by the sensory panellists received a triplet value to determine the numerical position of the quality attributes on the sensory scale

Triplets of the five samples' sensory qualities were assessed following assessment of (1) the judges' sensory evaluations that were added up. (**Table 6B.1**); (2) triplets associated with sensory scales; and (3) total number of panel of judges (i.e., 24). The blended beverage sample S1 triplet numbers for the sensory quality attribute of colour were computed, and the triplets value for colour (S1C) was established as follows (**Eq. 6B.2**).

$$S1C = \frac{0(0,0,25)+2(25,25,25)+0(50,25,25)+9(75,25,25)+13(100,25,0)}{0+2+0+9+13} \quad \text{6B.2)}$$

$$S1C = (84.3750 \quad 25.0000 \quad 11.4583)$$

where 0, 2, 0, 9, 13 represent the number of sensory panellists. The triple values in the matrix denote the distribution function of sensory panellist preference on the sensory scale [3]. Similarly, triplets value for S1 sample quality attributes for flavour (S1F), taste (S1T), mouthfeel (S1M), consistency (S1CO) and overall acceptability (S1OAA) were obtained as follows:

$$\begin{aligned} S1F &= (77.0833 \quad 25.0000 \quad 18.7500) \\ S1T &= (76.0417 \quad 23.9583 \quad 18.7500) \\ S1M &= (72.9167 \quad 25.0000 \quad 20.8333) \\ S1CO &= (78.1250 \quad 25.0000 \quad 15.6250) \\ S1OAA &= (77.0833 \quad 25.0000 \quad 16.6667) \end{aligned} \quad \text{(6B.3)}$$

Similarly, triplets for flavor, taste, mouthfeel, consistency and overall acceptability were estimated for S2, S3, S4 and S5, as given below:

S2C = (89.5833 25.0000 9.3750)
S2F = (80.2083 25.0000 16.6667)
S2T = (70.8333 25.0000 18.7500)
S2M = (79.1667 25.0000 18.7500)
S2CO = (83.3333 25.0000 14.5833)
S2OAA = (76.0417 25.0000 18.7500)
S3C = (85.4167 25.0000 14.5833)
S3F = (78.1250 25.0000 16.6667)
S3T = (73.9583 22.9167 18.7500)
S3M = (75.0000 25.0000 16.6667) (6B.4)
S3CO = (72.9167 25.0000 20.8333)
S3OAA = (75.0000 25.0000 16.6667)
S4C = (90.6250 26.0417 11.4583)
S4F = (78.1250 25.0000 10.4167)
S4T = (81.2500 25.0000 13.5417)
S4M = (81.2500 25.0000 15.6250)
S4CO = (78.1250 25.0000 15.6250)
S5OAA = (82.2917 25.0000 13.5417)
S5C = (66.6667 25.0000 18.7500)
S5F = (65.6250 22.9167 17.7083)
S5T = (58.3333 20.8333 22.9167)
S5M = (57.2917 19.7917 22.9167)
S5CO = (54.1667 20.8333 22.9167)
S5OAA = (60.4167 22.9167 20.8333)

6B.3.3. Determination of important quality attributes based on triplet values

Based on the sensory scores, the panellists' perception of the blended beverage samples' quality feature that was most crucial was selected. Colour, flavour, taste, mouthfeel, consistency and overall acceptability of the blended beverage samples were the quality attributes judged by the panellists **(Table 6B.2**). The weight the judges assigned to the qualities was based on (1) sensory scores scored by judges added up (**Table 6B.2);** (2) sensory scale triplets; and (3) total number of panellist/judges (i.e., 24), according to Chutia et al. [3].

Table 6B.2. Preferences to the importance of quality attributes of blended beverage

Sensory quality parameters	Factors of Sensory Scale				
	Not at all important	**Somewhat Important**	**Important**	**Highly Important**	**Extremely Important**
Colour	0	3	1	11	9
Flavour	0	4	2	8	10
Taste	0	0	4	9	11
Mouthfeel	1	3	4	10	6
Consistency	2	2	2	11	7
Overall acceptability	0	1	2	13	7

As explained below, the significance of the quality characteristic of colour (QC) was established (**Eq. 6B.5**)

$$QC = \frac{0(0,0,25)+3(25,25,25)+1(50,25,25)+11(75,25,25)+9(100,25,0)}{0+3+1+11+9} \quad (6B.5)$$

Where 0, 3, 1, 11, 9 at both numerator and denominator indicate the number of sensory panellists/judges. Similarly, triplets for sensory panellist's preference for the importance of flavour (QF), taste (QT), mouthfeel (QM), consistency (QC) and overall acceptability (QOOA) were obtained as below (**Eq. 6B.6**).

$$\begin{aligned} QF &= (77.0833 \quad 25.0000 \quad 15.6250) \\ QF &= (75.0000 \quad 25.0000 \quad 14.5833) \\ QT &= (82.2917 \quad 25.0000 \quad 13.5417) \\ QM &= (67.7083 \quad 23.9583 \quad 18.7500) \\ QCO &= (69.7917 \quad 22.9167 \quad 17.7083) \\ QOAA &= (75.0000 \quad 23.9583 \quad 16.6667) \end{aligned} \quad (6B.6)$$

6B.3.4. Triplets for overall sensory scores of the blended beverage samples

The triplets for the sensory quality values were multiplied by the triplets for significant quality characteristics as decided by the sensory panellists for each sample to arrive at the total sensory scores for the blended beverage samples. The multiplication of triplets (a, b, c) and (w, y, z) was done by the following **Eq. (6B.7).**

$$(a, b, c) \times (w, y, z) = (a \times wb \times y + w \times ba \times z + w \times c) \quad (6B.7)$$

In the above equation, if 'a' and 'w' value are between 0 and 100, multiplication product value 'a' and w (a × w) will be between 0 and 1,000. The overall sensory score's first digit will range from 0 to 40,000. Therefore, it is crucial to range the overall sensory score's initial digit from 0 to 100. In order to obtain this, as per Chutia et al. [3],

Eq. 6B.6 values were multiplied by the factor $1/Q_{sum}$, where Q_{sum} is the triplets' initial digit values added together. 'Relative weightage of the quality attribute' was expressed for colour as: $QC_{rel} = QC/Q_{sum}$; flavour: $QF_{rel} = QF/Q_{sum}$; taste: $QT_{rel} = QT/Q_{sum}$; mouthfeel: $QM_{rel} = QM/Q_{sum}$; consistency: $QOC_{rel} = QOC/Q_{sum}$ and for overall acceptability: $QOAA_{rel} = QOAA/Q_{sum}$. From **the Eq. (6B.8)**

$$Q_{Sum} = (77.0833 + 75.0000 + 82.2917 + 67.7083 + 69.7917 + 75.0000) \quad (6B.8)$$
$$= 446.8750$$

The next phase consisted of developing triplets to represent the relative weighting value for each quality attribute. The formula used to get the triplets' relative weightage value for colour is provided below. For colour relative weightage of triplet (QC_{rel}) was

$$QC_{rel} = \frac{QC}{Q_{Sum}} = (77.0833/446.8750 \quad 25.0000/446.8750 \quad 15.6250/446.8750) \quad (6B.9)$$

Similarly, for the other quality attributes, relative weightage of triplets were determined to be:

$$\begin{aligned} QC_{rel} &= (0.1725 \quad 0.0559 \quad 0.0350) \\ QF_{rel} &= (0.1678 \quad 0.0559 \quad 0.0326) \\ QT_{rel} &= (0.1841 \quad 0.0559 \quad 0.0303) \\ QM_{rel} &= (0.1515 \quad 0.0536 \quad 0.0419) \\ QCO_{rel} &= (0.1562 \quad 0.0512 \quad 0.0396) \\ QOAA_{rel} &= (0.1678 \quad 0.0536 \quad 0.0373) \end{aligned} \quad (6B.10)$$

The triplet multiplication approach (**Eq. 6B.11**) was used to calculate the overall sensory score (SO1) for S1, as shown below.

$$SO1 = S1C \times QC_{rel} + S1F \times QF_{rel} + S1T \times QT_{rel} + S1M \times QM_{rel} + S1CO \times QOC_{rel} + S1OAA \times QOAA_{rel}$$

$$SO1 = (77.6806 \quad 24.8082 \quad 16.94028) \quad (6B.11)$$

Similarly for others, the overall sensory scores were calculated

$$\begin{aligned} SO2 &= (79.7300 \quad 25.0000 \quad 16.1325) \\ SO3 &= (76.8041 \quad 24.6164 \quad 17.3417) \\ SO2 &= (82.0294 \quad 25.1797 \quad 13.2988) \\ SO2 &= (60.5354 \quad 22.0935 \quad 20.9742) \end{aligned} \quad (6B.12)$$

6B.3.5. Ranking of blended beverage on sensory fuzzy scale

The description of the standard sensory fuzzy scale is given by Chutia et al. [3] and Sakre et al. [12]. A six-point scale was used to classify the conventional sensory fuzzy

scale: Not satisfactory/Not at all necessary, Fair/Somewhat necessary, Satisfactory/ Necessary, Good/Important, Very Good/Highly Important, and Excellent/Extremely Important (Fig. 6B.2), according to Chutia et al. [3]. The triangle distribution pattern was followed by each membership function of the sensory fuzzy scale, with 1 being the maximum membership value [3]. In this phase, the typical fuzzy scale was used to compare the blended beverage sample samples' overall quality score. For that, the overall quality of the samples was determined based on the sensory scores of the blended beverage samples. The triplet (a, b, c) for overall quality of the blended beverage samples, was expressed by a triangle ABC, as per **Fig. (6B.3)**. Ranking of blended beverage samples was done by measuring where the centroid of the triangle ABC, represented by the triplet (a, b, c), was located [3]. Due to the fact that ABD and BDC are right-angled triangles, the relative areas of the triangles ABC, ABD, and BDC are 0.5(b + c), 0.5b, and 0.5c respectively. Thus, if X is the distance of the centroid of triangle ABC, its value is calculated as (**Eq. 6B.13**)

$$X = \frac{a-(b-c)}{3} \tag{6B.13}$$

$$X_{s1} = 23.2809$$

$$X_{s2} = 23.6208$$

$$X_{s3} = 23.1765 \tag{6B.14}$$

$$X_{s4} = 23.3829$$

$$X_{s5} = 19.8054$$

The developed blended beverage's overall quality was ranked from the X value mentioned above (**Eq. 6B.14**) in the following order:

S2>S4>S1>S3>S5

6B.3.6. Ranking of blended beverage on the basis of quality attributes

The developed blended beverage samples were graded in this phase based on their quality characteristics. **Eq. (6B.13)** was then filled in with the triplet values for importance of quality as determined by the sensory panellists' preferences. Accordingly, the relative importance of colour, flavour, taste, mouthfeel, consistency and overall acceptability, X_{QC}, X_{QF}, X_{QT}, X_{QM}, X_{QCO} and X_{QOAA}, of the blended beverage were (**Eq. 6B.15**)

$$X_{QC} = 22.5694$$

$$X_{QF} = 21.5278$$

$$X_{QT} = 23.6111$$

$$X_{QM} = 20.8333 \quad \text{(6B.15)}$$

$$X_{QCO} = 21.5278$$

$$X_{QOAA} = 22.5694$$

From the above values blended beverage were graded on the basis of quality attributes in this order: Taste > Overall acceptability = Color > Consistency = Flavour > Mouthfeel.

6B.3.7. Similarity analysis for blended beverage samples on standard fuzzy scale:

In order to rank and categorise sample attributes according to sensory scores in linguistic form, similarity analysis was used to depict the distribution pattern of the overall sensory score of samples using six sensory scales of the standard fuzzy scale. Membership function F1–F6 value of sensory scale expressed by set of 10 numbers [3,4] was between 0 and 10; between 10 and 20; between 20 and 30; between 30 and 40; between 40 and 50; between 50 and 60; between 60 and 70; between 70 and 80; between 80 and 90; and between 90 and 100.

Following (**Fig. 6B.3)**, the membership functions values F1, F2, F3, F4, F5 and F6 can be expressed as [3]:

Not satisfactory: F1 = [1, 0.5, 0, 0, 0, 0, 0, 0, 0, 0] 6B.16)

Fair: F2 = [0.5, 1, 1, 0.5, 0, 0, 0, 0, 0, 0]

Satisfactory: F3 = [0, 0, 0.5, 1, 1, 0.5, 0, 0, 0, 0]

Good: F4 = [0, 0, 0, 0, 0.5, 1, 1, 0.5, 0, 0]

Very good: F5 = [0, 0, 0, 0, 0, 0, 0.5, 1, 1, 0.5]

Excellent: F6 = [0, 0, 0, 0, 0, 0, 0, 0, 0.5, 1]

6B.3.8. Membership function of overall sensory scores on standard fuzzy scale

In this stage, the overall scores for the blended beverage sample sets were calculated using the standard fuzzy scales (values of F1-F6) membership function. In **Eq. 6B.13,** the sensory scores were shown as triplets. From **Fig. 6B.3**, for a given abscissa value "x", membership function value B_X can be calculated as

$$B_X = \frac{X-(a-b)}{b} \quad \text{for } (a-b) < X < a$$

$$= \frac{(a+c)-X}{c} \quad \text{for } a<X< (a+c) \quad \text{(6B,17)}$$

$$= 0 \text{ for all other values of X.}$$

In order to determine the membership function values of B_X for blended beverage samples with X equal to 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100, following **Eq.**

6B.17, was used according to Chutia et al. [3]. A set of 10 integers were used to identify the membership function value of the overall sensory scores of each created blended sample on the standard fuzzy scale, where the maximum values of B_X in the 10 intervals from 0 to 100 are in the specified range of X, outputs are presented below (**Eq. 6B.18**):

B1 = (0, 0, 0, 0, 0, 0.2873, 0.6903, 0.8633, 0.2740, 0)
B2 = (0, 0, 0, 0, 0, 0.2108, 0.6108, 0.9833, 0.3634, 0)
B3 = (0, 0, 0, 0, 0, 0.3174, 0.7236, 0.8157, 0.2391, 0) (6B.18)
B4 = (0, 0, 0, 0, 0, 0.1251, 0.5223, 0.9194, 0.4006, 0)
B5 = (0, 0, 0, 0.0705, 0.5231, 0.9758, 0, 0.5488, 0.0720, 0, 0)

where B1, B2, B3, B4 and B5 represents the overall sensory scores distribution pattern of S1, S2, S3, S4 and S5 on the standard fuzzy scale.

6B.3.9. Similarity values for blended beverage samples

The values of the membership function (B1, B2, B3, B4, B5) for each of the produced samples S1, S2, S3, S4 and S5 were expressed in **Eq. 6B.18,** and these values were compared with the equivalent membership function value of the conventional fuzzy scale (F1-F6) from **Eq. 6B.16**. Similarity value (S_m) for the samples were calculated as

$$S_m = \frac{F \times B^T}{\text{maximum of } (F1 \times F1 \text{ and } B1 \times B1^T)} \quad (6B.19)$$

The calculated similarity values for blended beverages samples are shown in **Table 6.3**. The samples were judged by the maximum similarity value.

Table 6B.3. Similarity values for blended beverage samples

Scale factor	S1	S2	S3	S4	S5
Not satisfactory	0.0000	0.0000	0.0000	0.0000	0.0000
Fair	0.0000	0.0000	0.0000	0.0000	0.0256
Satisfactory	0.1041	0.0764	0.1150	0.0453	0.7839
Good	1.0215	0.9519	1.0501	0.8024	1.3207
Very Good	1.0746	1.1975	1.0268	1.1461	0.2511
Excellent	0.0993	0.1317	0.0866	0.1452	0.0000

As seen from **Table (6B.3),** the similarity value and their categories for S1, S2, S3, S4 and S5 was 1.0746 (Very good), 1.1975 (Very good), 1.0501 (Good), 1.1461 (Very good), and 1.3207 (Good), respectively. Similarity values were used to rank the blended beverages as: S2 (Very good) > S4 (Very good) > S1 (Very good) >S5 (Good) > S3 (Good). According to fuzzy analysis, S2 showed the highest acceptability among these

five samples due to its highest similarity value. So, S2, which composed of 60 % SPFJU and 40 % guava juice was selected as optimized composition and used for further processing.

6B.4. Conclusion

The sensorial drawbacks associated with raw passion fruit juice, namely, astringency flavour, and sour taste can be overcome by blending with other fruit juice. The results implied that passion fruit juice can be blended with guava juice to obtain blended juice with better sensory attributes, which can be optimized by Fuzzy logic. Fuzzy logic sensory optimization technique was able to grade the importance of the quality attributes of passion fruit and guava juice based blended beverage as: Taste > Overall acceptability = Color > Consistency = Flavour > Mouthfeel. The blended beverage combination of sugar-added passion fruit juice and guava in the ration of 60 :40 showed the best sensory outcome.

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CHAPTER 6 SECTION C

DEVELOPMENT OF PICKERING NANOEMULSION FROM PASSION FRUIT SEED FIBRE AND SEED OIL AND PASSION FRUIT PEEL DERIVED CAROTENOIDS-ENRICHED OIL AND ITS OPTIMIZATION

6C.1. Introduction

Emulsion is a promising strategy to incorporate lipophilic bioactive compounds in a carrier system for retaining their bioactivity and enhancing their bioavailability. Apart from solubility, an emulsion is also being used to control the delivery of bioactive compounds and enhance the quality and shelf life of food products [2,16]. Passion fruit seed oil (extraction process given in **Chapter 5**) and carotenoids enriched oil (process given in **Chapter 3**) can be incorporated in a liquid medium through emulsification. Many researchers reported about the emulsification properties of fibres, and used different fibres such as orange pulp fibre [23], orange pulp and peel fibre [9], citrus fibres [20], bamboo shoot dietary fibres [7] in the emulsion development process. Additionally, studies have shown that dietary fibre has a variety of functional qualities, including the capacity to bind water, oil, minerals, and organic molecules, as well as the ability to form gels and be soluble in liquids. These qualities can help to improve the properties of foods, such as enhancing beverage emulsification and extending product shelf life [18]. There is a growing demand for natural and clean-label fibre products and HPH technology can be potentially applied in emulsified food systems to improve the functional properties of fibres [9], and meet the market demand.

The direct incorporation of fibre and oil in liquid sample is a challenging task, so production of an emulsion using HPH technique can be a way to introduce the fibre and oil in the liquid system. Again, the development of a stable emulsion depends on various manufacturing parameters such as the amount of oil and stabilizer/ surfactant used, and homogenization pressure [14]. Optimization of the process conditions is crucial in the creation of stable emulsions in order to preserve quality and minimise financial loss.

In the present chapter, effects of ultrasound extracted passion fruit seed based dietary fibre, oil phase (seed oil and passion fruit peel derived carotenoids enriched oil), and high pressure on size of emulsion and its optimized conditions for the development of Pickering emulsions were evaluated.

6C.2. Materials and methods

6C.2.1. Samples

Ultrasound extracted dietary fibre from passion fruit seeds was obtained according to the method given in Chapter 5. The oil phase, passion fruit seed oil, and passion fruit peel derived carotenoids enriched oil were obtained following processes given in **Chapter 5** and **Chapter 3,** respectively.

6C.2.2. Characterization of nanoemulsion

The average particle size of the prepared emulsions was measured through dynamic light scattering (DLS) using a Zeta potential and Nano particle size analyser (Nanoplus-3). Mean values of five readings are reported.

6C.2.3. Preparation of carotenoids enriched fibre-based Pickering nanoemulsion

Oil phase was developed by mixing equal amounts (50:50 ratio) of the optimized carotenoids enriched olive oil and optimized extracted passion fruit seed oil. Coarse emulsion was prepared by mixing the oil phase (5-25 %) with fibre using a T25 Ultra-Turrax (IKA Works, Inc., NC, USA) high-speed mixer at 12500 rpm for 5 min. The fibre range taken for nanoemuslion formation was 1-4 %. Other independent parameters selected in this study were concentration of oil (5-25 %) and homogenizing pressure (500-2000 bar). The experimental design is given in **Table 6C.1.** Initially, the number of passes used were 1, 3, 5, 7, and 10 and eventually 5 numbers of passes was selected based on particle size analysis, temperature of the emulsion, and separation of the emulsion.

Table 6C.1. Real and coded values of the independent parameters for the development of fibre-based Pickering emulsion.

Experimental Variables	Code	Coded levels		
		-1	0	+1
High pressure (bar)	A	500	1250	2000
Oil content (g/100 mL)	B	5	15	25
Fibre content (g/100 mL)	C	1	2.5	4

The well mixed coarse emulsion was thereafter homogenized in a two-stage high-pressure homogenizer (GEA, Lab homogenizer Panda Plus 2000, Italy) as per the experimental design **(Table 6C.1)**. Five hundred millilitres of coarse emulsion were processed for each experiment set, and pressure of the second stage was adjusted to

about 1/10 of that of the first stage [24], which is an industrial practice to achieve better homogenization.

6C.2.4. Experimental design for optimization

The designing of the experiments and optimization was performed using Response Surface Methodology (RSM). For modelling the experimental process, Box–Behnken design (BBD) was used with homogenized pressure, oil content, and fibre content as the three independent variables to predict the effect and optimize particle size of the Pickering nanoemulsion (dependent variable). A total of 17 experimental runs were carried out. The predicted model was fitted to a second-order quadratic polynomial equation. Design-Expert Version 7.1.2 (Stat-Ease, Inc. MN) was used for the experiment design.

The experiment variables, given in **Table 6C.1,** were coded according to **Eq. 6C.1** [22]

$$y_j = \frac{Y_j - Y_{j0}}{\Delta Y_j} \quad (6C.1)$$

where 'Y_j' and 'y_j' indicates the actual and coded values of the 'j' experimental variable, Y_{j0} is actual value of the 'j' experimental variable at the central point, and ΔY_j is the step change of the dimensionless value.

6C.2.5. Optimization

The variables were optimized on the basis of higher desirability value, details of which are already mentioned in **Chapter 4**.

6C.3. Results and discussion

6C.3.1. Effects of pressure on practice size

Previous studies done by various researchers have shown that the homogenization pressure and number of passes affect the size of the oil droplets greatly [23]. According to Fernández-Ávila et al. [4], solid polymers treated with HPH at 100 MPa and 200 MPa resulted in emulsions that were both more stable and had lower particle sizes [4]. The optimized condition of this chapter would be adopted in **Chapter 6D** to developed fibre-based Pickering emulsion based blended beverage, because of which high pressure homogenization pressure range varying from 500-2000 bar [12] was used for the development of emulsion, which aimed not only to enhance the emulsion stability, but also maintain the quality as well as control microbial growth (i.e. prolong the shelf life) [24].

The effect of pressure on particle size of emulsion was found to be non-significant (p=0.0528 > 0.05) **(Table 6C.2).** The particle size initially decreased as high pressure was increased to approximately 1300 bar **(Fig. 6C.1a** and **1b)** and then increased in size with further increase in pressure, while other parameters were kept constant. In HPH, initial size decrease happened due to breakdown of solid particles and droplets caused by combination of shearing, grinding, and cavitation [3], but too high pressure may have caused partial denaturation of protein present in the dietary fibre (aprriximaletly 16 %) and consequent agglomeration due to the high presssure and temperature generation [6]; also higher pressure caused significant structural damage to the fibres [9] due to which higher swelling and ultimately larger particle size may occur. According to Huang et al. [9], HPH treatment reduced the size of the fibre particles and increased the surface area of orange fibres, which may have served as a holding area for oil droplets to be placed on the fibres' surfaces, thereby reducing coalescence and improving emulsion stability.

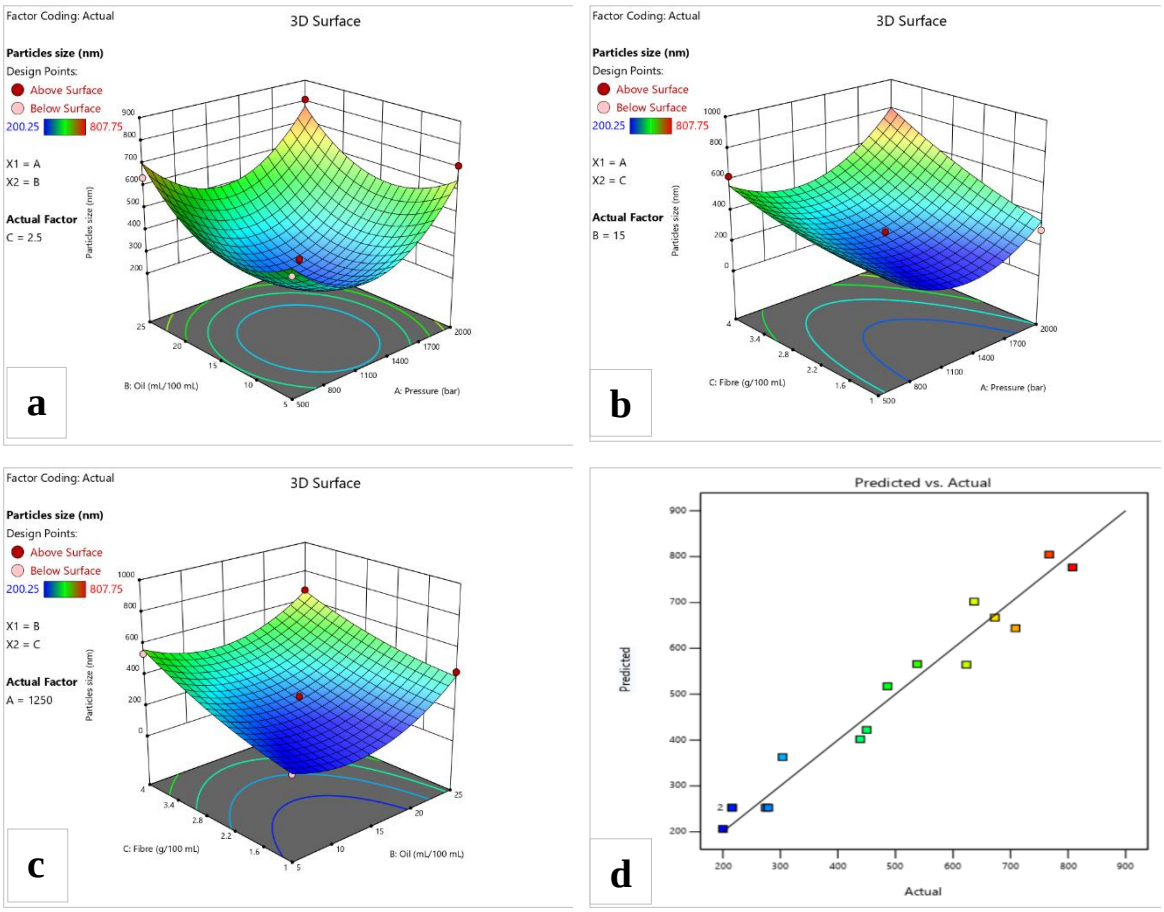

Fig. 6C.1. Effect of (a) Oil and pressure, (b) Fibre and pressure, (c) Fibre and oil, on particles size and (d) Actual vs predicted.

Similarly Zhang et al. [27] carried out tests with homogenising pressure up to 140 MPa and noticed that particle size decreased as the homogenizing pressure increased, but the lowest particle size was 4.79 nm at 100 MPa. Wallecan et al. [23] also developed Pickering emulsion using orange pulp fibre by applying HPH and they observed that HPH at 700 bar prior to drying helped in the development of significantly finer emulsion that showed no coalescence for 2 weeks [23].

6C.3.2. Effects of fibre content on particle size of the nanoemulsion

Fibre based Pickering nanoemulsion was developed with 1-4 g/100 mL of optimized extracted fibre. Slight increase in emulsion size initially followed by continuous rapid increment of emulsion size with an increase in fibre content was observed (**Fig. 6C.4a, 4c),** so optimized value of fibre content that was obtained was near the lowest fibre content. The effects of oil on particle size of emulsion were highly significant ($p=0.0002 < 0.05$) (**Table 6C.2)**. The combination effects of fibre and oil, and also oil and pressure were both found to be non-significant.

Table 6C.2. ANOVA table of fibre-based Pickering nanoemulsion optimization.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6.728E+05	9	74758.54	19.99	0.0003	Significant
A-Pressure	20275.95	1	20275.95	5.42	0.0528	
B-Oil	50323.78	1	50323.78	13.45	0.0080	
C-Fibre	1.825E+05	1	1.825E+05	48.79	0.0002	
AB	669.52	1	669.52	0.1790	0.6849	
AC	19460.25	1	19460.25	5.20	0.0566	
BC	3291.89	1	3291.89	0.8801	0.3794	
A^2	2.379E+05	1	2.379E+05	63.61	< 0.0001	
B^2	1.212E+05	1	1.212E+05	32.40	0.0007	
C^2	7816.98	1	7816.98	2.09	0.1915	
Residual	26183.50	7	3740.50			
Lack of Fit	21744.70	3	7248.23	6.53	0.0507	Not significant
Pure Error	4438.80	4	1109.70			
Cor Total	6.990E+05	16				

The extracted passion fruit seed dietary fibre contained significant amount of protein and pectin (approximately 25 % total) and the emulsification properties of pectin

[5] and protein [27] are already well established. The use of various fibres such as orange pulp fibres [23], citrus fibre [20], orange fibers [9], bamboo shoot fibres [7], citrus fiber [18] etc. as emulsifiers/stabilizers in emulsion systems has been studied by various researchers and it has been suggested that fibres can be used as novel green emulsifiers [20]. Qi et al.[18] had reported that 2% fibre with 25% oil gave stable fibre-based emulsion, and had observed that protein, pectin and cellulose are major components of orange fibre, and the authors found that protein-polysaccharide conjugates improved the emulsifying ability of the fibre.

They reported that proteins in orange fibres were bound to the cellulose particles, which may act as a physical barrier to protect oil droplets from flocculation and ensure the adequate stability over time. Similar to that report, in this study, defatted passion fruit seed portion contained significant amount of cellulose (approximately 41.43% cellulose) and protein (approximately 16.3% in defatted seed) and pectin (12.5% to 18.34%) [8, 26]. These components may help to give higher emulsification properties at low concentration.

It was reported that when fibre concentration was low (like 0.25%), the emulsion displayed no network formation in the continuous phase [18], whereas a slight network-like structure was formed when fibre concentration increased [13], and 2% concentration produced stable emulsion from citrus fibre [18]. Additionally, higher fibre concentration results in improved oil droplet surface covering, which shields them from re-coalescence [23]. 2% (W/V) citrus fibre-stabilized Pickering emulsion droplets were stable for 60 days of storage, since there was no evidence of creaming [25]. He et al. [7] observed 100 % emulsification index when the fibre content was increased to 0.3 % and showed that the amount of bamboo shoot dietary fibres in suspensions had a great influence on the formation of a stable emulsion.

The stability mechanism of Pickering emulsion using fibre and HPH could be divided into several steps [11]. High-pressure/shearing breaks down the large oil droplets into smaller ones, and fibres adsorbing at the oil-water contact stabilise the interface and create an emulsion. But when there isn't enough fibre to stabilise the growing oil-water interface, the oil droplets clump together to produce larger oil droplets, which reduce the surface area of the contact. Because of this, the emulsion's droplet size increases. This observation was also supported by the emulsion's projected surface coverage. With the improvement of the oil fraction phase, the theoretical surface coverage of the emulsion was reduced.

6C.3.3. Effects of oil amount on emulsion size

It is already well established that size of emulsion depends on polarity of the used oil and smaller droplets are produced when the polarity of the used oil phase is high, by reducing the interfacial tension at the oil/water interface [21], so to maintain the polarity of the dispersed phase, mixture of oils (equal amount) was used. Also, the extracted passion fruit seed oil **(**described in **Chapter 5**) is enriched with unsaturated fatty acids specially linoleic acid, which are prone to oxidative deterioration [17,19], and phenolic acids, but negligible amount of carotenoids are there, so to overcome this problem equal proportion of carotenoids enriched olive oil (obtained from **Chapter 3**) was added, and the mixture of oil was used as dispersed phase in Pickering emulsions. Emulsion formation of the enriched oil is desirable for wider food applications and so optimization of oil content in the emulsion is very important.

In this study, the oil content was varied between 5 to 25 g/100 mL and fibre content varied between 1-4 %. There may be an excess of non-adsorbed surfactant molecules in the bulk aqueous phase when the surfactant concentration is higher than the oil concentration, which may in turn stimulate the production of surfactant micelles. Due to depletion flocculation, which eventually results in droplet coalescence and a subsequent increase in droplet size following emulsification, these non-adsorbed surfactant micelles may produce an increase in the attraction forces between the droplets [15]. Again Kirkhus et al. [10] reported that higher amount of oil was needed because as the homogenization pressure increased from 100 to 1500 bar, more total carotenoids were released from the plant matrix into the oil droplets. Again, higher amount of oil implied higher carotenoids as well lipophilic bioactive compounds in the micelles, which probably make them more bio-accessible [10].

As shown in **Table. 6C.2.,** the oil content shows significant effect on emulsion size ($p=0.0080<0.050$). The size of the emulsions was found to decrease with an increase in oil content up to approximately 12.5 ml/100 ml, thereafter size increased with further increase in oil content (**Fig. 6C.4a & 4c.)**. Reduced size may be due to the disintegration of fibre as well as oil droplets. Another possibility for explaining the reduced droplet size observed is the existence of additional surface-active molecular species, such as phenolic and carotenoid compounds, which may adsorb to the oil-water interface and contribute to the total droplet size decrease during emulsification [15]. Generally, during HPH, fibres may absorb water and swell as the temperature rises, but in this study the swelling process was hindered may be due to coating with oil [24]. Therefore, increase in oil

content up to a certain level decreases the overall size of emulsion droplets but beyond the critical limit, no further disintegration occurs and an increase in size takes place probably because the excessive oil deposits as a thick layer on the surface [3]. Further, the total interfacial area of emulsion droplets increases as oil fraction is elevated, because fibre particles are not sufficient enough to fully stabilize the extra oil droplets, which results in loss of original equilibrium [25]. Wei et al. [25] observed increased volume based diameter [d(4,3)] from 17.44 ± 0.34 μm to 32.86 ± 1.77 μm, when oil fraction increased from 5 % to 35 % (V/V) and they observed slight coalescence in the emulsions when oil fraction increased to 25 % (V/V).

6C.3.4. Modeling and validation

All the experimental data obtained from BBD design were statistically analyzed (Design-Expert Version 7.1.2, software, Stat-Ease, Inc) and fitted to the quadratic model equations (**Eq. 6C.2)** and to explain the effect of input parameters**.**

$$\text{Particle size} = 252.4 \times 5 + 50.34 \times \text{A} + 79.31 \times \text{B} + 151.03 \times \text{C} - 12.94 \times \text{AB} + 69.75 \times \text{AC} - 28.69 \times \text{BC} + 237.71 \times \text{A}^2 + 169.65 \times \text{B}^2 + 43.09 \times \text{C}^2 \quad \textbf{(6C.2)}$$

As shown in **Fig. 6C.4d,** there was a good correlation between the predicted values calculated by using the model equation and experimental values, which indicates the good fitting of the model. R^2 value was 0.9625. Lack of fit was found to be non-significant (p=0.0507>0.05) and the model was highly significant (P=0.0003<0.05), implying the validation of model.

6B.3.5. Optimization of responses

As shown in ANOVA table **(Table 6C.3),** input parameters of oil content and fibre content with a probability value of 0.0080 and 0.0002, respectively were found to be the significant factors.

Table 6C.3. Optimization table of fibre-based Pickering nanoemulsion

Sl. No.	Pressure (Bar)	Oil (mL/100 mL)	Fibre (g/100 mL)	Particles size(nm)	Desirability	
1	1435.305	12.313	1.115	145.100	1.000	Selected
2	1201.926	12.140	1.816	179.296	1.000	
4	1154.630	7.877	1.128	165.314	1.000	

Although input parameter of pressure was non-significant (p=0.0528>0.05), second order effect of pressure (A^2) and oil content (B^2) were found to be highly significant with p value <0.0001 and 0.0007, respectively, whereas fibre content (C^2) value was found to be non-significant (p=0.1915>0.05). Combined effects of all input parameters were found be non-significant (for all p value > 0.05). The optimum conditions for size of fibre-based Pickering emulsion were predicted on the basis of maximum desirability [1]. Optimized conditions consisting of 1435.305 bar pressure, 12.313 mL/100 mL oil and 1.115 g/100 mL of fibre gave desirability level of 1.0 **(Table 6C.3).** Predicted value of particle size at optimized condition was 145.100 nm whereas experimental value was 151.20 nm (experimental conditions were performed at 1440 bar pressure, 12.5 % oil, and 1.2 % fibre content).

6C.4. Conclusion

Optimized development conditions yield a nanoemulsion with good emulsification properties. Passion fruit seed extracted dietary fibre can be suggested for use as a novel green emulsifier.

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CHAPTER 6 SECTION D

EFFECT OF THERMAL PASTEURIZATION AND HIGH-PRESSURE HOMOGENIZATION ON QUALITY OF BEVERAGES AND DEVELOPMENT AND EVALUATION OF AN OPTIMIZED PASSION FRUIT BASED-BEVERAGE ENRICHED WITH PICKERING NANOEMULSION OF FIBRE AND OIL EXTRACTED FROM ITS BYPRODUCTS

6D.1. Introduction

Due to lack of awareness about the health benefits of passion fruit, people have not been able to extract the full benefits of the fruit. In recent years, the fruit is being widely studied for its desirable chemical composition [60,63]. This fruit, especially the pulp appears to be an excellent source ofvitamins and minerals that are essential for life [42]. Consumers prefer high quality fruit juice that is nutritious, contains minimal chemical preservatives, and maintains natural characteristics while conforming to legislative requirements. Passion fruit juice (PFJ) may be extracted directly from the fruit, by squeezing from crushed material, which is enriched with pulp and other nutrients, but it is very perishable in nature and spoils very fast [2]. The high acidic juice spoils easily due to the growth of yeast and bacteria. Normally, at room temperature, raw passion fruit juice undergoes alcoholic fermentation by yeast followed by the oxidation of alcohol by bacteria [2]. PFJ having below 4% sugar preservative has been reported to spoil after three days [2]. Therefore, preservation techniques are used for the enhancement of the shelf-life. Generally conventional thermal treatments are used but the delicate flavour of PFJ is extremely sensitive to thermal treatment. Additionally, higher thermal treatment of PFJ produces four toxic compounds including 5-hydroxymethyl-2-furfural [19]. Use of chemical preservative is nowadays less accepted by customers, and clarification techniques are associated with reduction of nutritional value as most of the nutrients remain in the colloidal suspension.

High pressure homogenization (HPH) is a novel technology that has recently been researched for applications in the food industry to change the viscosity of fluids, primarily focussing on the fluid's physical changes, which also has been shown to be an effective method for microbial inactivation [13,53], enzyme inactivation, and improvement of techno-functional properties of food components and enhancement of overall functional properties [39,97].

Emulsions play an important role in food processing industries as emulsions act as excellent delivery systems for bioactive chemicals by increasing the permeability of substances in the stomach and through epidermal barriers that facilitates their bioavailability [22,71,86], and also act as effective carriers of lipophilic compounds. Drinks frequently employ emulsions to give the finished goods the proper flavour and look. One of the major difficulties is to prevent ring formation in emulsion-containing beverages during thermal processing and storage [8]. So, novel nonthermal treatment such as HPH [13,44,75], ultrasonication [1,43], and emulsification [11,41,65] can be used to enhance the quality and shelf life of food products. But as presented in **Chapter 6A**, ultrasonication was able to increase the shelf-life of PFJ only for some days i.e., ultrasonication is not sufficient to prolong the shelf life of PFJ [19], so combination of different techniques such as HPH and emulsification can be used.

Food industries have focussed on adding natural ingredients, particularly lipophilic compounds extracted from fruit and vegetables to produce healthier products that have desirable qualities for consumers [47]. Direct addition of lipophilic compounds to liquid foods cannot be done due to their insolubility. Use of emulsion technique can address this problem. In **Chapter 6B**, blended beverage of untreated sugar added sensory optimized PFJ (SPFJU) and guava juice was developed and characterized. No study reported about the shelf-life enhancement of PFJ and the sensory optimized beverage added with fibre extracted from seed and peel and carotenoids extracted from seed oil and developed into an emulsion using HPH technique. So, the main aim of this study was to valorize passion fruit and develop an optimized beverage enriched with bioactive compounds from its peel and seed using a process that has scope for industrial application.

6D.2. Materials and Methods

The passion fruit sample was collected from a market in Bishnupur, Manipur. Ripe guava was collected from the local market near Tezpur University. All the HPLC grade chemicals were purchased from Sigma-Merck and all others chemical were of analytical grade. All the analytical experiments were performed in triplicate.

6D.2.1. Sample preparation

Untreated sugar added sensory optimized PFJ (SPFJU) was processed according to the procedure outlined in **Chapter 6A**. Similarly, untreated optimized blended beverage (BSPFJU) was obtained following the procedure mentioned in **Chapter 6B**.

6D.2.2. HPH treatment of samples

The well mixed optimized blended beverages and SPFJU were homogenised at the optimized pressure (1440 bar) in a two-stage high-pressure homogenizer (GEA, Lab homogenizer Panda Plus 2000, Italy). Briefly, 500 mL of sample was processed and pressure of the second stage was adjusted to about 1/10 of that of the first stage [87], which is an industrial practice to achieve better homogenization. The HPH treated blended beverage is termed as BSPFJT, the HPH treated SPFJU is termed as SPFJT, and the untreated optimized blended beverage is termed as BSPFJU.

6D.2.3. Heat treatment (HT) of blended juice

For this, 200 mL of BSPFJU sample taken in a glass bottle was heated in a temperature-controlled water bath (LabTech) until the centre temperature reached 90 ℃, then the sample was kept at 90 ℃ for 5 min, following the method of Guan et al. [20]. The time needed for the samples to reach the process temperature was around 7 min. After treatment, the samples were immediately kept for cooling in an ice water bath for 10 min. The whole process of HT was performed in a sterile environment. The thermal pasteurized BSPFJU is termed as BSPFJTT,

6D.2.4. Blended beverage added with carotenoids enriched fibre-based Pickering nanoemulsion

The optimized conditions of carotenoids enriched fibre-based Pickering nanoemulsion preparation process that are stated in **Chapter 6C** were used for incorporation in the blended beverage. The method and preparation process was same only water phase was replaced by the blended beverage, i.e. 12.5 mL of oil and 1.2 % fibre were added to 100 mL optimized blended beverage and mixed properly using T25 Ultra-Turrax (IKA Works, Inc., NC, USA) high-speed mixer at 12500 rpm for 5 min and thereafter homogenized in a two-stage high-pressure homogenizer (GEA, Lab homogenizer Panda Plus 2000, Italy) at 1440 bar pressure for 5 passes. The developed blended beverage is termed as BNESPFJT.

6D.2.5. Physicochemical properties of treated and untreated beverages

The physicochemical properties of SPFJU, SPFJT, BSPFJU, BSPFJT, BSPFJTT and BNESPFJT such as total soluble solid (TSS), pH, total titratable acidity (TTA), ascorbic acid (AA), total phenolic content, total sugar, DPPH free radical activity, total

carotenoids content (TCC), microbial load (Total plate count, TPC) were determined according to the respective method mentioned in **Chapter 6A.**

6D.2.5.1. ABTS [2,2'-Azinobis (3-Ethylbenzothiazoline-6-Sulphonic Acid)] free radical scavenging activity assay of samples

ABTS scavenging activity was carried out using the modified method of Shah and Modi [70]. Equal parts of the ABTS aqueous solution (7 mM) and the potassium persulfate aqueous solution (2.45 mM) were mixed to develop the ABTS stock solution, which was then left to stand at room temperature for 12-16 h in the dark. The stock solution of ABTS was diluted in methanol to produce the working solution, which had an absorbance of 0.70±0.02 at 734 nm. After that, 1 mL of the aqueous extracts (0.5–5.0 mg/mL concentration) was combined with 2.0 mL of the ABTS solution. The mixture was then incubated for precisely 10 min in the dark at room temperature. To make the control, 2.0 mL of ABTS solution and 1 mL of double-distilled water were mixed. Using a spectrophotometer (Cary 60 UV-Vis, Agilent) the absorbance was measured at 734 nm in comparison to a blank. BHT was used as the standard. The percentage of ABTS scavenging activity of each sample was calculated as inhibition percentage (**Eq. 6D.1).**

$$\text{Antioxidant activity (ABTS \%)} = \left(1 - \frac{A_{\text{Sample}}}{A_{\text{Control}}}\right) \times 100 \quad \text{(6D.1)}$$

A_{Sample} , A_{Control} is absorbance of the sample and control respectively.

6D.2.6. Suspension stability / Cloudiness

Suspension stability was measured using the methods reported by Velázquez-Estrada et al. [85] and Liu et al [39] with slight modification. Briefly, 30 mL sample was centrifuged at 1520×g for 10 min at 6 ℃ and the absorbance was measured before ($A_{0,660}$) and after ($A_{t,660}$) centrifugation at wave length of 660 nm using a spectrophotometer (Cary 60 UV-Vis, Agilent) and expressed by **Eq. 6D.2**.

$$\text{Suspension stability/ Cloudiness} = \frac{A_{t,660}}{A_{0,660}} \quad \text{(6D.2)}$$

High cloudiness is correlated with high absorption measurements.

6D.2.7. Particle size

The area-based mean particle diameter (D[3,2]) and volume-based mean diameter (D[4,3]) of the samples was measured through dynamic light scattering (DLS) using a Zeta potential and Nano particle size analyser (Nanoplus-3). All the experiments were performed for five times.

6D.2.8. HPLC analysis of carotenoids of samples

Five millilitres of sample were added to 10 mL HPLC grade hexane and mixed vigorously, then subjected to ultrasonication in a sonication bath (Riviera Glass Pvt. Ltd., India, Power 230volt, 50 Hz) for 5 min followed by centrifugation (15 min at 3000 rpm and 4 ℃) and supernatant was then collected. For a thorough extraction of carotenoids, 5 mL of hexane was added to the residue, and same process was repeated. The extract was collected in an amber flask for HPLC analysis after undergoing syringe filtration.

Carotenoids content were analysed according to the method described by Hsu et al. [25] with slight modifications. In brief, a binary solvent system of (A) methanol/acetonitrile/water (84:14:4, v/v/v) and (B) dichloromethane with different gradient conditions, and C30 column of HPLC system (UltiMate 3000, Thermo Scientific) with DAD detector were used for detection at 450 nm and 25 ℃. Column temperature increase at the rate of 1 mL/min flow rate. Gradient condition was made as follows: initially 100 % A and 0 % B was raised to 10 % B at 4 min, 18 % B at 12 min, 21 % B at 17 min, 30 % B at 20 min and kept constant till 25 min, increased further to 39 % B at 28 min, 60 % B at 40 min and returned to initial condition at 41 min. The peaks were identified by comparing with the standards of β-carotene, β-cryptoxanthin, lutein, zeaxanthin, lycopene, ζ-carotene, α-carotene, and quantified by comparing area of specified absorption spectra with those of reference standards (**Eq. 6D.3**)

$$\text{Carotenoids (µg/100 mL)} = \frac{A_x \times C_s \times 10^4}{A_s \times V} \times 100 \qquad (6D.3)$$

A_x, A_s and C_s are peak area, area from standard curve, and concentration from standard curve, respectively of the specified carotenoids.

6D.2.9. HPLC analysis of phenolic acid of samples

Extraction procedure as given in 6D.2.8 was followed; only in place of hexane ethanol was used as the solvent. The phenolic acids were determined according to the method described by Espin et al. [15] using the HPLC instrument (Ultimate 3000, Themo Fisher Scientific) with C18 column at column temperature of 35 ℃. Solvents used were 0.1 % formic acid (A) and acetonitrile (B). The elution gradient established was isocratic 15 % B for 5 min, 15–20 % B over 5 min, 20–35 % B over 10 min, 35–50 % B over 10 min, 50–60 % B over 5 min, isocratic 60 % B for 5 min. Overall, the flow rate was 0.5 mL/min and detection was performed at 280, 330 and 370 nm as the preferred wavelengths. Phenolic acids were identified against standards and quantified by using same formula as mentioned above (used for HPLC of carotenoids).

6D.2.10. Storage study of samples

The BSPFJT and BNESPFJT were directly packed in small amber glass bottles and sealed with parafilm. Two batches of BSPFJT, BSPFJTT and BNESPFJT samples were separately stored in the dark at 5±2 ℃ and 25±2 ℃ (room temperature). Samples used for microbial load, TSS, pH, TTA, total sugar, and Colour were analyzed immediately after storage at 0, 7, 14, 21, 28, 35, 42, 49, and 56 days. Samples used for measurements of AA. TCC, total phenolic content and antioxidant capacity were stored at −40 ℃.

6D.2.11. Dynamic *In-vitro* digestion model of samples

The dynamic *In-vitro* digestion model described by Qian [59] and Zhu et al. [98] was used with slight modification. Each sample was passed through a three-step simulated GI model that consisted of a mouth phase, a gastric phase, and a small intestinal phase.

6D.2.11.1. Mouth phase

Simulated saliva fluid (SSF), containing mucin and various salts, was prepared and the composition used is mentioned in **Table 6D.1** [59.68**]**.

Table 6D.1. Chemical composition of artificial saliva.

Chemical name	Chemical formula	Concentration(g/L)
Sodium chloride	NaCl	1.594
Ammonium nitrate	NH_4NO_2	0.328
Potassium phosphate	KH_3PO_4	0.636
Potassium chloride	KCl	0.202
Potassium Citrate	$K_3C_6H_5O_7.\ H_2O$	0.308
Urea	H_2NCONH_2	0.198
Lactic acid sodium salt	$C_3H_5O_3Na$	0.146
Porcine gastric amylase		0-30

For this, 10 mL of samples was mixed with 10 mL of simulated artificial saliva in a beaker, the pH of the combination was adjusted to 6.8, and the mixture was shaken continuously at 100 rpm for 5 min on a magnetic hot plate (IKA C-MAG HS-7) at a controlled temperature of 37 ℃.

6D.2.11.2. Simulated gastric fluid phase:

Prepared simulated gastric fluid (SGF) that contained 2 g of NaCl and 7 mL of HCl (1.0 M HCl), 3.2 g of pepsin and diluted to 1 L, pH-adjusted to 1.2 using 1.0 M HCl

[69]. The sample from the mouth phase was then mixed with SGF at a 50:50 mass ratio. The mixture was then brought to pH 2.5 by adding 1 M NaOH and shaken vigorously on a magnetic hot plate for 2 h at 37 ℃.

6D.2.11.3. Simulated Intestinal phase:

Briefly, 30 mL of digesta sample from the SGF model was taken and put in a clean beaker. The beaker was then incubated in a water bath (37 ℃) for 10 min while being pH-adjusted with NaOH solution to 7. The 30 ml digesta was then added while stirring with 4.0 mL of a bile extract solution containing 187.5 mg of bile extract (pH 7.0, PBS) and resultant mixture was then adjusted to pH 6.8. A pH of 7.0 was then achieved by adding 1.0 mL of $CaCl_2$ solution, which contained 110 mg of $CaCl_2$ (pH 7.0, PBS). The mixture was then given a final addition of 2.5 mL of newly prepared pancreatin solution containing 60 mg of lipase (pH 7.0, PBS). The concentration of the bile solution and lipase solution was 5.0 and 1.6 mg/mL, respectively. Then, the final pH of the solution was adjusted to pH 7. Digestion experiments were performed for 2 h using agitation (200 rpm) with a magnetic stirrer at controlled temperature [59].

6D.2.11.4. Bio-accessibility determination

For this, 10 mL of the sample was taken after the *In-vitro* digestion, and centrifuged for 40 min at 25 ℃ and 4000 rpm. Three phases of centrifuged samples separated out with the transparent micelle phase in the centre, an oily or creamed phase at the top, and an opaque sediment phase at the bottom. The bioactive component was thought to be solubilized in mixed micelles in the intermediate phase. Aliquots (5 ml) were collected directly from the middle phase in the centrifuge tube.

Carotenoids content was measured according to the method described in **Chapter 6A,**

The bio-accessibility was calculated using the following equation (**Eq. 6D.4**)

$$\text{Bio-accessibility (\%)} = \frac{C_{Digested}}{C_{Sample}} \times 100 \qquad (6D.4)$$

Where, $C_{Digested}$ and C_{Sample} is the concentration of carotenoids in the digested material and the sample, respectively.

6D.2.12. Statistical analysis

All measurements reported were the arithmetic mean and standard deviation of triplicate experiments unless stated. An analysis of variance (ANOVA) of the data was performed, and a least significant difference (LSD) with a confidence interval of 95 %

was used to compare the means. All statistical analysis was performed employing SPSS (version 21.0, Chicago, the United States) and Excel (version 2019, Microsoft Corporation, Washington DC, USA).

6D.3. Results and Discussion

6D.3.1. Changes in quality parameters after treatments

Changes in quality parameters of SPFJ and BSPFJ after the HPH and HT and also after addition of fibre and oil treatment were evaluated. The quality parameters of SPFJU, SPFJT, BSPFJT, BSPFJT, BSPFJTT and BNESPFJT were analysed and results are presented in **Table 6D.2** and **6D.3.**

6D.3.1.1. pH value

As shown in **Table 6D.2.,** pH of the BSPFJU (3.51) increased significantly as compared to SPFJU (3.02), indicating the effect of blending. This may be due to the higher pH value of guava juice (4.12) than the passion fruit juice (3.02). Oranusi et al. [51] reported the nutritional composition of commercial guava juice as pH-3.98, TTA - 0.48 %, soluble solid - 8.60 (%), sugar (% sucrose) - 7.60, total solid (mg/kg) - 42.35, ascorbic acid (mg/100 mL) - 35.22 ,and benzoic acid - 122.04 ug/L.

After HPH treatment, slight decrease in pH was observed but changes were non-significant. Homogenization may breakdown the cells/cell walls, which enhanced the extraction of organic materials dissolved in organic acids and other substances and reduced the pH value [39,88]. Similar results have been reported by various researchers for pear juice [39], kiwifruit juice [53], and orange juice [85]. However, some researchers reported about slight increase in pH for mango juice [97], mixed juice [88] etc. Therefore, effects of HPH on pH of juice could be an increase or decrease, which depends on food matrices and treatments [39]. After the HT treatment, (BSPFJTT) slightly increased in pH, which may be due to organic acid degradation by thermal treatment [85]. Velázquez-Estrada et al. [85] observed an increase in the pH of orange juice from 3.188 to 3.249 after thermal pasteurization, whereas reduction in the pH value from 3.188 to 3.170 occurred when treated with HPH at 200 MPa and 20 ℃. After the HPH treatment, the changes in pH were higher for BSPFJT as compared to BNESPFJT, which may be due to the effect of oil and fibre. Also, developed emulsion protected the degradation rate of organic acids.

Table 6D.2. Changes in quality parameters after the treatments.

Parameters	**SPFJU**	**SPFJT**	**BSPFJU**	**BSPFJT**	**BSPFJTT**	**BNESPFJT**
pH (25-27℃)	3.02 ± 0.04^{b}	2.96 ± 0.06^{b}	3.51 ± 0.09^{a}	3.46 ± 0.08^{a}	3.64 ± 0.08^{a}	3.48 ± 0.04^{a}
TTA (%)	3.98 ± 0.07^{a}	4.06 ± 0.06^{a}	2.78 ± 0.05^{b}	2.85 ± 0.04^{b}	2.51 ± 0.09^{c}	2.69 ± 0.07^{b}
TSS (^{o}Brix)	12.8 ± 0.16^{c}	13.21 ± 0.15^{abc}	13.12 ± 0.16^{abc}	13.46 ± 0.14^{a}	12.98 ± 0.17^{bc}	13.35 ± 0.15^{ab}
Total sugar (g/100 mL)	10.46 ± 0.19^{c}	10.18 ± 0.20^{c}	11.99 ± 0.21^{a}	11.78 ± 0.19^{a}	11.05 ± 0.18^{b}	11.86 ± 0.17^{a}
Ascorbic acid (mg/100 mL)	19.54 ± 0.82^{b}	13.98 ± 0.91^{cd}	24.74 ± 1.02^{a}	15.64 ± 0.92^{cd}	13.42 ± 0.90^{d}	16.5 ± 1.12^{c}
Total phenolic acids (mg GAE/100 mL)	33.50 ± 0.91^{c}	38.54 ± 1.10^{b}	29.92 ± 1.12^{d}	33.85 ± 1.05^{c}	27.54 ± 1.14^{d}	84.54 ± 1.34^{a}
Total carotenoids (µg/100 mL)	806.80 ± 12^{a}	792.8 ± 16^{a}	673.76 ± 19^{bc}	661.50 ± 15^{bc}	634.5 ± 14^{c}	698.50 ± 23^{b}
β- carotene (µg/100 mL)	589.50 ± 34^{a}	575.40 ± 14^{a}	364.25 ± 19^{b}	349.40 ± 18^{b}	331.45 ± 17^{b}	373.80 ± 18^{b}
DPPH scavenging activity (%)	61.2 ± 2.10^{bc}	65.04 ± 1.82^{ab}	59.85 ± 2.14^{bc}	63.50 ± 1.90^{bc}	57.87 ± 1.91^{c}	70.21 ± 2.41^{a}
ABTS activity (%)	72.4 ± 1.84^{cd}	78.54 ± 1.95^{ab}	67.82 ± 1.80^{de}	74.24 ± 1.82^{bc}	64.80 ± 1.24^{e}	81.85 ± 1.22^{a}
L*	65.85 ± 0.67^{b}	67.14 ± 0.48^{b}	62.15 ± 0.66^{d}	63.87 ± 0.37^{c}	62.97 ± 0.47^{cd}	71.47 ± 0.66^{a}
a*	2.24 ± 0.42^{a}	1.96 ± 0.62^{ab}	0.97 ± 0.22^{bc}	0.24 ± 0.11^{c}	0.12 ± 0.05^{c}	2.06 ± 0.78^{ab}
b*	19.82 ± 0.44^{a}	17.65 ± 0.42^{b}	12.27 ± 0.63^{c}	11.18 ± 0.64^{cd}	10.45 ± 0.59^{d}	21.36 ± 0.74^{a}
ΔE	-	2.55 ± 0.09^{e}	8.47 ± 0.13^{c}	9.04 ± 0.16^{b}	$9.99\pm0.0.9^{a}$	5.83 ± 0.09^{d}
Suspension stability	0.38 ± 0.07^{d}	1.76 ± 0.19^{b}	0.42 ± 0.08^{d}	1.82 ± 0.17^{ab}	1.21 ± 0.11^{c}	2.15 ± 0.17^{a}
Microbial load (TPC) (Log CFU/mL)	4.63 ± 0.07^{a}	1.76 ± 0.06^{b}	4.79 ± 0.06^{a}	1.87 ± 0.04^{b}	0.84 ± 0.02^{c}	0.89 ± 0.08^{c}

6D.3.1.2. Total titratable acidity (TTA)

After the blending, TTA of BSPFJU (2.78) decreased significantly than SPFJU (3.98) (**Table 6D.2**), which may be due to the lower TTA value of guava. As pH is inversely related to TTA, same trend was observed for pH. After HPH treatment, non-significant increase in TTA for all samples was observed, which may be due to the dissolved organic acids and other substances released on disruption of cell during HPH treatment [39].

6D.3.1.3. Total soluble solid (TSS)

The TSS content of BSPFJU was higher than SPFJU (**Table 6D.2),** which indicated that addition of guava juice enhanced the TSS content of mixed juices as TSS of guava juice (13.4 ^{o}Brix) was higher than the passion fruit juice (12.8 ^{o}Brix). Gill [18] reported that guava juice is associated with high amount of TSS including sugars and organic acids, and therefore, it could be used in taste adjustment of mixed/blended juices. After blending, although the TSS content had increased, the increment was non-significant.

After the HPH treatment, for all samples, non-significant increase in TSS content was noticed (**Table 6D.2).** The HPH treatment elevates temperature in the valve, and together with higher shear forces and cavitation may break down the particles that enhanced the TSS value [97]. On the other hand, after HT treatment, non-significant reduction of TSS was observed, which may be due to the breakdown of soluble components by high temperature [85]. After addition of oil and fibre, the beverage emulsion developed using HPH treatment (BNESPFJT) showed slightly increased TSS.

Changes in TSS after HPH treatment was also observed for mango juice [97], blackcurrant juice [34], mixed juices [88], and pear juice [39], which agreed with the observed results of this study. Significant increase of TSS value from 15.83 to 16.50 ^{o}Brix was observed by Kruszewski et al. [34] for blackcurrant juice after HPH treatment at 150 MPa for 3 passes. Similarly, TSS value increased from 11.27 to 11.70 ^{o}Brix, when pear juice was treated with HPH at 100 MPa at an inlet temperature of 4 ℃.

6D.3.1.4. Total sugar

The total sugar content of BSPFJU was significantly higher than SPFJU (**Table 6D.2)** which indicated that addition of guava juice enhanced the total sugar content of blended juice as sugar content in guava juice (14.4% sugar content) was higher than SPFJU (10.46%). After the blending, total sugar content increased significantly, which

enhanced the sensory quality of blended juice; this was confirmed by sensory analysis (Fuzzy logic optimization). After the HPH treatment, for all samples, nonsignificant decrease of sugar content was observed. The elevated temperature in the valve, high shear forces, and cavitation during HPH treatment may break down the sugar particles [85,92]. On the other hand, after the HT treatment, significant reduction of total sugars was observed, which may be due to the breakdown of soluble component by high temperature [85]. After the HPH treatment of oil and fibre added blended juice (BNESPFJT), the sugar content had also decreased from 11.99 g/100 mL to 11.86, but degradation was less as compared to BSPFJT (11.78 g/100 mL), which may be because fibre and developed emulsion may entrap some sugar molecules in their moieties.

Velázquez-Estrada et al. [85] reported about the reduction of the total sugar content in orange juice from 13.973 to 10.969 (g of glucose/100 mL) after pasteurization and to 13.393 (g of glucose/100 mL) after HPH treatment at 100 MPa and 20 ℃ inlet temperature. Similarly, after the HPH treatment reduction of total sugars content was also observed by Yi et al. [92] in a mixture of cloudy apple juice and kiwifruit puree.

6D.3.1.5. Ascorbic acid (AA)

The initial content of AA in SPFJU had increased from 19.54 mg/100 mL to 24.74 mg/100 mL (BSPFJU) after blending with guava juice (**Table 6D.2**). This may be due to the higher amount of AA in guava juice (35.1 mg/100 mL). Hassimotto et al. [23] reported about the higher ascorbic acid content in guava juice, which was four times more than that in orange, whereas almost five times more vitamin C in guava juice than orange juice was reported by Vasanthakaalam et al. [82]. Oranusi et al. [51] reported 35.22 mg/100 mL ascorbic acid in commercial guava juice [51].

After the treatments (HPH and HT), significant decrease in ascorbic was observed, but HPH treated juices retained significantly higher content of ascorbic acid than the thermally treated one. After the HPH treatment, as shown in **Table 6D.2,** 71.54% of AA in SPFJ and 63.26 % of AA in BSPFJ was retained, whereas only 54.24% was retained after the HT treatment. It implied that ascorbic acid degradation was more significant in HT treatment as compared to HPH. In the BNESPFJT juice, 66.7% ascorbic acid was retained. Although the mean maximum temperature of HPH was high, deterioration was still lower than it was for HT treatments, indicating that temperature may not have been the only factor affecting vitamin C in the case of the HPH treatments [84]. Again, at same HPH conditions, treated BSPFJT and BNESPFJT showed different retention

amount of ascorbic acid, which confirmed the significant effect of environmental conditions on ascorbic acid.

The loss of ascorbic acid during HPH processing showed significant difference in different juices and several authors have reported about it. Velázquez-Estrada et al. [84] reported that the HPH processing pressure level had a substantial impact on the ascorbic acid content in orange juice. They observed that samples treated at 100 and 200 MPa showed a decrease of about 2% and 5%, respectively, while samples treated at 300 MPa had a significantly higher decrease of about 11%, whereas after pasteurization (90 ℃, 1 min), the orange juice retained only 82.6% of its original L-ascorbic acid content [84]. Only 63.8% and 38.2% of L-dehydroascorbic acid were retained in apple juice treated with HPH at 100 MPa and 200 MPa, respectively [75]. Yu et al. [95] also noted a decrease of AA by 58.7% in juices treated at 200 MPa.

AA may have undergone oxidation and degradation as a result of certain processing conditions, such as presence of oxygen (deaerated juice before processing), high temperature, and shear in the homogenising valve [75]. Some authors also reported that the presence of traces of metal alloy (such as Be-Cu), which comes from the erosion of the seals of the HPH equipment, may contribute to the oxidation of vitamin C [80].

6D.3.1.6. Total phenolic content and polyphenol profile

Numerous studies have shown that cloudy juices have a higher polyphenol content than clear juices [45], and SPFJU was more cloudy than guava juice, and because of this a higher amount of phenolic content was observed for SPFJU as compared to BSPFJU after the HPH treatment **(Table 6D.2).** The total phenolic content in SPFJU was found to be 33.50 mg GAE/100 mL (SPFJU), which significantly decreased to 29.92 mg GAE/100 mL (BSPFJU) after blending with guava juice. The significant reduction of total phenolic content in BSPFJU may be due to the low phenolic content in guava juice (25.4 mg GAE/100 mL). Yousaf et al. [94] reported that in guava total phenolic content ranged from 94.06 to 190.64 mg GAE/100 g, which was diluted four to five times for commercial juice manufacturing.

In SPFJU, 33.5 mg GAE/100 of total phenolic content was found, which is supported by Falguera et al. [16] and Ramaiya et al. [61]. Gallic acid (peak-1, approximately 25 % of total) followed by p-coumaric acid (peak-5, 19%) and vanillic acid (peak-3, 13%) were identified in SPFJU as the major phenolic acids **(Fig. 6D.1a)** that is supported by Ramaiya et al. [61]. Chlorogenic acid (peak-2) and caffeic acid

(peak-4) were minor phenolics in SPFJU. On the other hand, Rotta et al. [64] found vanillic acid and quercetin as major phenolic compounds (41 % of total) in PFJ, whereas syringic acid, ferulic acid, p-coumaric acid were reported as majors phenolics by Talcott et al., [77] in ethyl acetate extracts. Apart from the sample variation, phenolics detection also depends on the solvent used, method of extraction, HPLC protocol, and wavelength used [15].

After the HPH treatments, total phenolic acid significantly increased by 15.04 % in SPFJT (**Fig. 6D.1a** and **1b)** and 13.13 % in BSPFJT (**Fig. 6D.1c** and **1d**). On the contrary, HT treatment caused significant loss of 8% phenolic content in BSPFJ (**Fig. 6D.1e, Table 6D.2).** The increased phenolic content for HPH treatment may be due to the decreased dominance of enzymatic reaction [39], and also might be due to release of more content of polyphenols from vacuoles during intensive cell disruption by HPH treatment [88]. Another reason given is the formation of derivatives of already present phenolic compounds and/or new ones through the hydrolysis and depolymerization of complexes that was induced directly by high mechanical forces during the HPH process [39]. Moreover, HPH could enhance the degree of methyl esterification of pectin which promotes hydrophobic interactions between pectin and polyphenols enhancing polyphenol stability, which also may enhance the phenolic content [80].

But for HT treatment, the significant reduction of phenolic content may be due to a heat-induced degradation of polyphenols and also oxidation due to presence of oxygen [90]. After HPH treatment, increase in phenolic content was also observed for mixed juice [88], apple juice [75], strawberry [29] etc. Almost 20 % increase of total phenolic content in apple juice after the HPH treatment at 150 MPa was observed by Szczepańska et al. [75], with particular increase recorded for quercetin (52 %) and phloridzin (47 %), respectively [75]. Similarly, an increase in the total phenolic content of 11.5% was also observed by Karacam et al. [29] in strawberry juice using HPH at 100 MPa. Whereas, Velázquez-Estrada et al. [84] reported that total phenolic content in orange juice, decreased maximally by 6.6 % using pressure of up to 300 MPa.

Pasteurization at 90 ℃ for 1 min reduced the content of total phenolics by 26.6 % due to the thermal degradation of compounds whereas HPH processing caused a slight change of about ± 5 % of the original value in the TPC of cloudy blackcurrant juice [34]. After the addition of fibre and oil, significant increase in phenolic content in BNESPFJT was observed that was confirmed by HPLC analysis (**Fig. 6D.1f**).

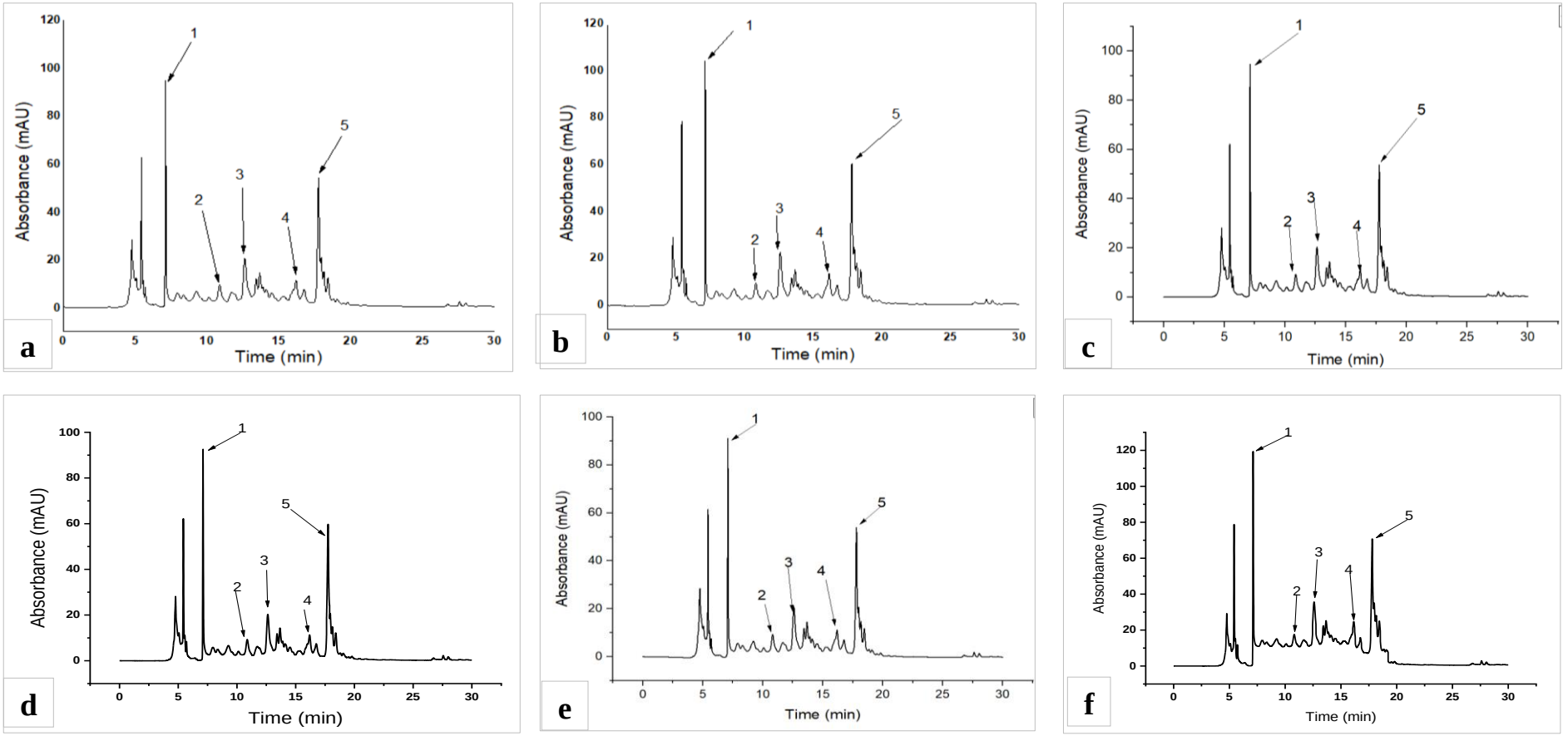


Fig. 6D.1. HPLC analysis of phenolic acids in (a) SPFJU, (b) SPFJT, (c) BSPFJU, (d) BSPFJT, (e) BSPFJTT and (f) BNESPFJT.

This increase was approximately 2.82 times of blended juice, which may be due to the higher phenolic content of passion fruit seed oil and fibres. In BNESPFJT, gallic acid and p-coumaric acid increased significantly and predominantly (**Fig, 6D.1f**). Total phenolic content in passion fruit seed oil was found to be 33.57 mg GAE/g of oil. Purohit et al. [57]. reported 36.02-39.11 mg GAE/g of total phenolic content in yellow passion fruit seed oil from North East region. The total phenolic content in passion fruit seed oil was 26.28 GAE/g [73]. Moreover, HPH could enhance the degree of methyl esterification of pectin, which promoted hydrophobic interactions between pectin and polyphenols enhancing polyphenol stability, and may explain for the enhanced phenolic content [6,88] as added fibre also enriched the pectin (approximately 12.5 %) and protein (16.3 %) content. Again, HPH increased the extractability of polyphenols from tissues or caused depolymerization of the complexes [75]. This explains why fibre and oil added nanoemulsion based blended juice showed the maximum phenolic content.

6D.3.1.7. Total carotenoids content (TCC) and β-carotene content and carotenoids profiles

TCC content of SPFJU was 806.8 ± 54.34 µg/100 mL. The amount was slightly higher than the amount reported by Pertuzatti et al. [54] (207.51- 443.89 µg RAE/100 g) but lower than dos Reis et al. [63] (1785 µg/100 d.w. pulp). HPLC analysis (**Fig. 6D.2a)** identified the presence of β-carotene (peak-5), β-cryptoxanthin (peak-1), lycopene (peak-2), ζ-carotene (peak-3), and α-carotene (peak-4) in SPFJU, which are concurrent with dos Reis et al. [63] and Pertuzatti et al. [54]. From the HPLC analysis (**Fig. 6D.2a)**, the β-carotene content in the SPFJU was 589.5 ± 63.52 µg/100 mL juice, which contributes to 73% of total carotenoids. Corrêa et al. [10] reported about 525 µg/100 g of β-carotene in yellow PFJ, whereas 1362 µg/100 g of β-carotene in passion fruit pulp was reported by da Silva et al.[72]. Biswas et al. [4] found β-carotene as the major carotenoid (743 µg/100 g) followed by β-cryptoxanthin (41 µg/100 g); β-carotene made up about 75 % of total carotenoids in yellow passion fruit pulp. BSFPJU, BSPFJT, BSPFJTT, and BNESPFJT containing guava juice showed a peak for lutein (**Fig. 6D.2c-f**). Chandrika et al. [7] identified lycopene (45.3±8.0 µg/g fresh weight (f.w)), lutein (2.1±0.6µg/g f.w.), β-carotene (2.0±0.2µg/g f.w.) and β-cryptoxanthin in guava juice.

TCC content of SPFJU (806.8 µg/100 mL) changed significantly after blending with guava (BSPFJU, 623.76 µg/100 mL), which may be due to the significantly low amount of TCC content in guava juice (440.5 µg/100 mL) (**Table 6D.2**).

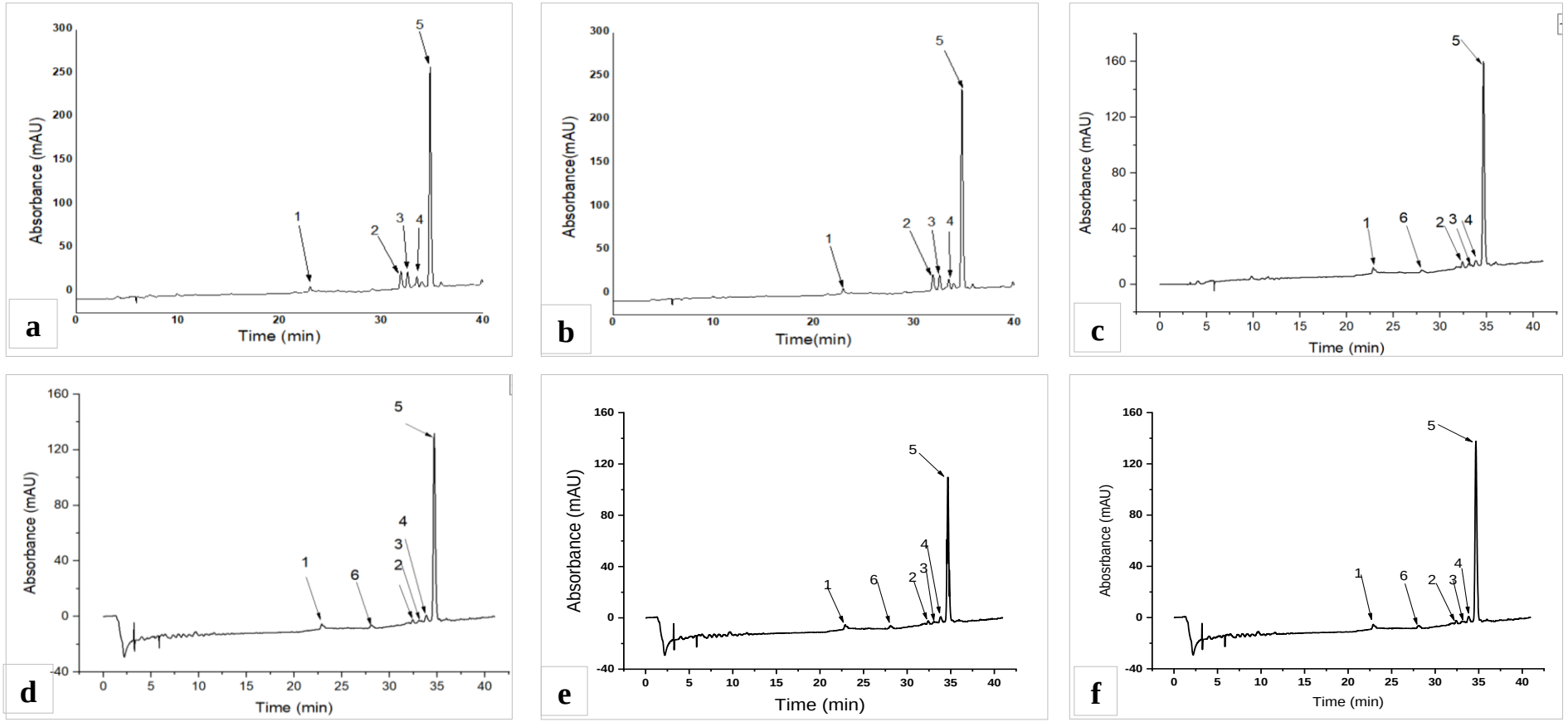


Fig. 6D.2. HPLC analysis of carotenoids: (a) SPFJU, (b) SPFJT, (c) BSPFJU, (d) BSPFJT, (e) BSPFJTT, and (f) BNESPFJT.

Similarly, after blending the β-carotene content significantly decreased from 73.07 % (SPFJU) to 54.06 % of total carotenoids in the blended beverage (BSPFJU). The decrease in β-carotene content may be due to the low amount of β-carotene content in guava juice, as was reported by Chandrika et al. [7].

After the HPH treatment, TCC content of SPFJT and BSPFJT decreased non-significantly from 806.8 to 792.80 µg/100 mL and 673.76 to 661.50 µg/100 mL respectively, but after the thermal treatment, the TCC value decreased significantly **(Table 6D.2)**. After HPH, the decreased TCC content may be due to degradation of carotenoids on exposure to oxygen [52,88], and also due to the high temperature generated in the two stage HPH system. Velázquez-Estrada et al. [85] observed that after the HPH treatment at 209.8 MPa, the inlet temperature of orange juice increased from 19.0 to 72.1 ℃ before the second homogenization valve. In thermal treatment (BSPFJT), the high temperature caused significant degradation of TCC [30]. Guan et al. [20] also observed a significant decrease in carotenoids, and phenolic and ascorbic acids after heat treatment of mango juices.

BNESPFJT sample that was a homogenised blended juice added with fibre and oi, showed significant increase in the TCC content. This may be due to the addition of carotenoids enriched oil. The emulsion produced by HPH (fibre and oil based) has the potential to protect the lipophilic compounds from the environment and also retain the bioactive compounds specially lipophilic compounds [66] such as carotenoids. Wellala et al. [88] observed significant decreased in TCC content in mixed juices after the HPH treatment. Velázquez-Estrada et al. [84] also observed significant decrease in TCC and β- carotenoids content of orange juice after the HPH treatment. Similar trend was observed for β- carotene also. But Guan et al. [20] observed increase in carotenoids content after HPH treatment, which may be due to the release of carotenoids at the temperature used.

6D.3.1.8. Antioxidant activity

Due to the complex nature of phytochemicals present in plant extracts, antioxidant activity analysis by two or more methods are recommended [88]. The antioxidant activity of all juices was evaluated using both DPPH and ABTS assays and results are presented in **Table 6D.2.** The antioxidant capacity analysis by the ABTS assay is higher for fruits, vegetables, and drinks compared to that by the DPPH assay due to highly pigmented and hydrophilic antioxidants present there [17] and also their better reflection

in ABTS assay than DPPH assay [17,63]. Reis et al. [63] reported significantly higher ABTS activity than DPPH activity for passion fruit pulp i.e. up to 4.1 times higher ABTS activity than DPPH activity.

It has been reported that the antioxidant capacity of fruit juice is closely related to the bioactive components such as total phenolics, ascorbic acid, carotenoids, etc.[21], However, in addition to the bioactive compounds, antioxidant rely on their structure, mode of interaction, and concentration [55]. As shown in the **Table 6D.2**, after blending with guava juice, the DPPH activity of SPFJU increased non-significantly for BSPFJU, whereas ABTS assay value increased significantly, which may be due to significant changes in hydrophilic antioxidants such as ascorbic acid, polyphenols etc, [17], and also colourful pigmented compounds such as carotenoids. After blending, the changes in antioxidant activity may be due to lower antioxidant activity of guava juice as compared to SPFJU. This is corroborated by Yousaf et al. [94] who reported about 27.70-78.15% of radical scavenging activity in guava.

After the HPH treatment, significant increase in ABTS antioxidant activity but non-significant increment for DPPH antioxidant activity was observed (**Table 6D.2**). The enhancement of antioxidant activity might be due to the release of greater content of polyphenols from vacuoles during intensive cell disruption [88]. Again, HPH enhances the degree of methyl-esterification, which promotes hydrophobic interactions between pectin and polyphenols enhancing polyphenol stability [88]. But for HT treated juice (BSPFJT), decreased values of ABTS and DPPH activity was observed, which may be due to reduction of bioactive (total phenolic acids, ascorbic acid, and carotenoids) compounds after the HT treatment. Among the juices, highest antioxidant activity was observed for NEBSPFJT (**Table 6D.2**), which may be due to the significantly higher total phenolics and carotenoids present in oil and the phenolic compounds present in fibre. Further, the fibre contains pectin which promotes hydrophobic interactions between pectin and polyphenols that enhances polyphenol stability [88].

Several studies also have shown that phenolic compounds exhibit a strong antioxidant capacity [29], and release more amount of polyphenols during intensive cell disruption caused by homogenization, and this enhances the antioxidant capacity. Such observation was made for mulberry juice [95], mango juice [20], mixed juice of carrot, pear and apple juice [88], strawberry juice [29] etc. However, significantly decreased value was observed for blackcurrant juice [34] and apple juice [75] after the HPH treatment. After the HPH treatment (at 140 MPa and inlet temperature 25 ℃ for 1 pass),

the antioxidant activity (DPPH) was approximately 3 times for carrot juice and 2 times for mixed juice of carrot, apple and pear juice used in the ratio of 40:40:20, as observed by Wellala et al. [88]. Significant increase in DPPH and ABTS activities of mango juice after HPH treatment was observed by Guan et al. [20], whereas significant reduction of both the antioxidant activity assays was observed for HT treated juice.

6D.3.1.9. Colour value

The colour parameters of juices, L* (lightness), a* (redness: green to red), b* (yellowness: blue to yellow), and colour differences were characterized and results are shown in **Table 6D.2**. L* value of SPFJU (65.85) decreased to 62.15 after blending with guava (BSPFJU). Similarly, a* and b* value of BSPFJU also decreased significantly. The decreased value may be due to the lower L*, a* and b* values of guava [14] as compared to SPFJU.

HPH treatment increased L* values for juices causing brighter colour. Similar to HPH treatment, HT treated juice (BSPFJTT) registered non-significant increase in L* value, but BSPFJT showed significant increase. Juice lightness increases, most likely as a result fragmentation of the suspended particles, which is evident in the form of size reduction and shape change in the particles [34]. HPH reduced the particle size, and the small sized particles had higher abilities to scatter light resulting [39] in increased L* values. Velázquez-Estrada et al. [85] observed positive correlation between L* and cloudiness and a negative correlation with the particle size values. Wellala et al. [88] observed an increase in L* value of mixed juice of carrot, apple and pear juice after the HPH treatment. Similarly, Liu et al. [39] also reported increase in L* value of pear juice after HPH treatment.

HPH treatment decreased a* value of all samples (**Table 6D.2).** The decreased a* value may be due to the degradation of carotenoids as strong correlation between Colour value a* (colour direction in red or green) and total carotenoids was observed by Itle and Kabelka [27]. Velázquez-Estrada et al. [85] also reported decrease in a* value after HPH treatment with pressure of 100, 200 and 300 MPa. For all samples (SPFJT, BSPFJT, and BNESPFJT), HPH treatment significantly increased the b* value (**Table 6D.2**). The breakdown of the cells caused the dissolution of pigments, which could be responsible for the enhancement of b* values. Increases in b* value can be caused by an increase in the activity of browning-related enzymes including polyphenol oxidase and peroxidase

[39]. After HPH treatment, increased L* and b* values were observed by Wellala et al. [88] for pear juice.

For HT treatment, L* value was slightly increased and a* and b* decreased for BSPFJTT (**Table 6D.2)**. The decreased value may be due to degradation of bioactive compounds. Additionally, during high temperature treatment, a decrease in the activities of browning-related enzymes could lead to a decrease in b* values [39]. Similar results were observed by Velázquez-Estrada et al. [85] for orange juice. Increased L* and b* values but decreased a* value compared to untreated juice after HPH was observed for mixed juice of apple juice, kiwi fruit puree [92], and orange juice[85].

For BNESPFJT juice, all the Colour parameters (L*, a* and b*) increased significantly. During the processing, due to the production of emulsion (addition fibre and oil), there may be a protective effect on the bioactive compounds (specially carotenoids) [79] for which overall retention of colour parameters was noticed. Again, it is well known that oil droplet significantly influences the optical properties of emulsions [46]. After the HPH treatment, BNESPFJT developed kinetically stable emulsion with small size, and small particles scatter the light less intensely than the bigger one, which causes an increase in the lightness, opacity, and whiteness index of emulsions based juice [67].

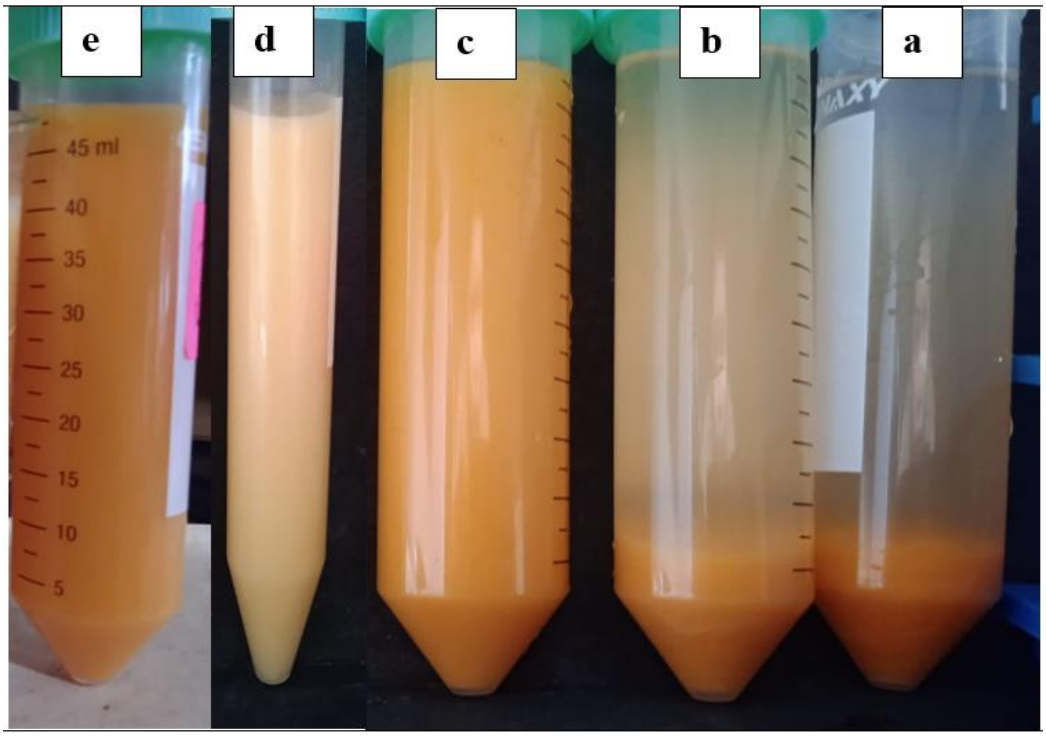


Fig. 6D.3. Images of juice after 1 h of storage at room temperature: (a) SPFJU, (b) BSPFJU, (c) BSPFJT, (d) BNESPFJT, and (e) BSPFJTT.

The ΔE, as an indicator of total Colour difference, reflects a noticeable visual difference when the value is not less than 2 [85]. In all samples, ΔE values increased and were higher than 2, which indicated that there were noticeable changes observed in the Colour of treated samples in comparison to the untreated ones (**Fig. 6D.3).** Highest ΔE was observed for BSPFJTT (**Table 6D.2),** which indicated that HPH treatment possessed strong ability to maintain the Colour of the juice. Velázquez-Estrada et al. [85] reported significant Colour difference in ΔE value between untreated and HPH treated orange samples and the values varied from 3.69 to 5.81 when treated in the pressure range of 100-300 MPa , whereas Karacam et al. [29] observed ΔE value of 14.61 for Ottoman Strawberry juice after HPH treatment.

6D.3.1.10. Suspension stability/ cloudiness

Cloudiness/ suspension stability of juice are typically indicators of the presence of insoluble pectin and other polysaccharide particles scattered in suspension, which have significant functional effects on the stability and rheology of juice [88] and also on the consumers acceptance of the juice. Cloud stability of cloudy mixed juices may be impacted by interactions with the serum phase and particle size [88]. Moreover, Yi et al. [92] stated that the homogenised cloudy mixed juice with particle sizes more than 100 μm were unstable and lost cloud stability. In the present study, all samples before being given any treatment had particles size larger than 200 μm, which indicated high susceptibility to sediment.

Cloud instability is caused by activity of some microorganism, by activity of pectin methyl-esterase, and due to particle size [85]. As shown in **Table 6D.2,** the suspension stability of SPFJU (0.38) increased non-significantly after blending with guava juice (BSFJU, 0.42). After HPH treatment, the value of SPFJT and BSPFJT, increased significantly to 1.76 and 1.82, which indicated the significant improvement in juice stability by HPH treatment. After HPH treatment, increased suspension stability was also observed for orange juice [85], pear juice [39], mixed juices of carrot, apple, and peach juice [88].

Gravity has a tendency to cause larger, coarser particles to settle to the bottom, however HPH treatments break these particles into smaller fragments [88], including tissue portions (such as cell walls and organelles) and insoluble polymer clusters, which are primarily responsible for cloud stability [39,85]. HPH also caused enzyme inactivation due to shearing and rise in temperature, which may lead to better stability.

Additionally, HPH may release serum components that bind to particles and to other molecules in the serum phase, making the dispersion more stable [88]. In other words, HPH increased the cloud stability significantly.

Cloud stability was also increased significantly after HT treatment, which may be due to the inactivation of enzymes such as pectin methyl-esterase [38]. However, juice stability does not depend entirely on enzymes inactivation but also depends on particle size and structural changes of pectin and other suspended materials, and therefore, stability value after HT treatment was less as compared to HPH.

The highest suspension stability was observed for BNESPFJT, which may be due to the developed emulsion [26] and fibre adsorbed at the oil–water interface of the emulsion droplets [58]. The juice behaved like a gel [58] and enhanced stability. Apart from it, due to fibre addition in BNESPFJT, the pectin content was increased, which may result in higher methyl-esterification that may have favored non-covalent hydrophobic interactions in the serum phase leading to higher stability [88].

6D.3.1.11. Microbiological analysis (Total plate count)

It has already been reported that inactivation effect of total plate count (TPC) by HPH treatment is less as compared to mould and yeast i.e. mould and yeast were more sensitive to HPH treatment [20]. As shown in **Table 6D.2**, the initial TPC of SPFJU and BSPFJU was found to be 4.63 and 4.79 log CFU/mL, respectively. In this study after addition of guava juice, the total plate count increased slightly, but after HPH treatment the microbial count decreased significantly.

After the treatments, the microbial load (TPC) (Log CFU/mL) of SPFJT, BSPFJT, BSPFJTT, and NEBSPFJT was found to be 1.76, 1.87, 0.84 and 0.89, respectively. HPH significantly reduced the TPC. It is believed that the mechanical loss of microbial cell integrity is brought on by many mechanisms, including shear stress, spatial pressure and velocity gradients, turbulence, and cavitation that occur in liquids during HPH treatment [20,39]. Also these physical phenomena of HPH could increase the permeability or rupture of the cell membrane causing cell death [39]. Again during the two stage HPH treatment, temperature of the samples was also increased as Velázquez-Estrada et al. [85] observed that the inlet temperature of orange juice increased from 19.0 ℃ to 72.1 ℃ before the second homogenisation valve during HPH treatment at 209.8 MPa. HPH has also been demonstrated to preserve the colour, flavour, and fragrances of original juice, to inactivate or modify the activity of enzymes that induce phase separation in fruit or

vegetable juices, and, finally, to maintain the nutritional and functional properties of the treated matrices [53]. After the HT treatment (BSPFJTT), lowest microbial load was observed **(Table 6D.2**). Despite the lowest microbial load for HT treatment, substantial heat degradation of bioactive substances was seen. Moreover, panellists gave HT juice the lowest sensory acceptance score.

Bioactive compounds such as phenolic compounds, carotenoids, ascorbic acid, etc. also show antimicrobial activity [21]. Among the HPH treated sample, lowest microbial load was observed for BNESPFT (**Table 6D.2),** which may be due to the higher amount of phenolic content (36.02-39.11 mg GAE/g of oil) in passion fruit seed oil [56] and carotenoids in olive oil [31] that was used in emulsion development process. Moreover, passion fruit seed fibre, which was used in the development of emulsion also showed significant antimicrobial activity.

Several studies have reported about the reduction of microorganisms by using the HPH treatments in liquids like apple juice [75], water melon juice [37], kiwifruit juice [53], mulberry Juice [99], mango nectar [80], orange juice [83], commercial fruit juices (orange, red orange, pineapple) [44] etc. and this demonstrates its potential to be used as a novel pasteurization/sterilization technique. Complete inactivation of moulds and yeasts and TPC count below 2.0 $\log_{10}$ CFU/mL (3.21 $\log_{10}$ CFU/mL reduction) was reported in mango juice by Guan et al. [20] with HPH at 190 MPa for 3 passes and 60 ℃ inlet temperature. A reduction of 3.67 and 3.19 log CFU per ml of *E.coli* present in milk and apple juice, respectively was seen after HPH treatment at 200 MPa with the inlet temperature at 40 ℃ by Dong et al. [13]. Tahiri et al. [76] also reported about the significantly higher reduction of *E. coli* O157:H7, i.e., around 6.0 $\log_{10}$ CFU/mL after 5 passes of HPH at 200 MPa and 25 ℃, while only 2.0 $\log_{10}$ CFU/mL reduction was noticed after 1 pass of HPH.

6D.3.1.12. Particle size

Derived measurements of area-based mean particle diameter (D[3,2]) and volume-based mean diameter (D[4,3]) were evaluated and data are presented in **Table 6D.3.** SPFJU showed a bimodal distribution. As expected, HPH significantly decreased the particle diameter of SFPJU, since the volume peak of PSD had moved from 210.52 to 7.25 µm and became monomodal (**Table 6D.3)** after treatment (SPFJT). In BSPFJU also bimodal distribution was observed which may be due to two different types of juices present (passion fruit and guava).

Table 6D.3. Changes in particle size and bio-accessibility after the treatments.

Samples	Bio-accessibility (%)	D[4, 3] (µm)	D[3, 2] (µm)	Ratio (D[4,3]/D[3,2])
SPFJU	7.29±0.54^{a}	210.52±10.20^{a}	35.78±2.42^{ab}	5.88
SPFJT	18.47±0.57^{b}	7.25±0.90^{c}	2.61±0.51^{c}	2.78
BSPFJU	8.58±0.45^{a}	218.42±11.01^{a}	41.64±3.25^{a}	6.21
BSPFJT	20.10±0.64^{b}	8.40±1.10^{c}	2.10±0.64^{c}	4.01
BSPFTT	16.48±014^{b}	185.40±10.01^{b}	32.81±4.21^{b}	5.65
BNESPFJT	30.29±0.84^{c}	9.51±0.80^{c}	3.52±0.51^{c}	2.70

The splitting of bigger particles into smaller ones by the turbulence and shear force created during HPH treatment resulted in a significant reduction in mean particle diameter after treatment [88]. Similar to HPH, HT treatment also significantly decreased the mean particle diameter but HPH was found to be much more effective than HT. This result is compatible with results reported for orange juice [85], mixed juice, cloudy apple juice and kiwifruit juice [92] and mango juice [97]. After 1 HPH pass at 20 ℃ from 40 to 190 MPa, mango juice particle size decreased significantly from 138 to 6 µm,as observed by Zhou et al. [97]. Whereas, Wellala et al. [88] observed significant decrease in particle size from 532.4 to 7.3 µm in pure pear juice.

It is already well established that surface weighted mean diameter (D[3,2]) is more influenced by the smaller particles, while the volume weighted mean diameter (D[4,3]) is more influenced by the larger ones [97]. Here, D[4,3] value was almost 5.88 and 6.21 times higher than the D[3,2] for SPFJU, and BSPFJU juice, respectively (**Table 6C.3**), but after the HPH treatment, its value decreased by 2.78 (SPFJT), 4.01 (BSPFJT), and 2.70 times (BNESPFJT), which confirmed that there were a higher amount of bigger particles than smaller ones in the untreated juice. Zhou et al. [97] reported that the D[4,3] value was almost eight times higher than the D[3,2] value for the untreated mango samples and approximately 3.5 times higher for HPH treated mango juice at 190 MPa/3. Velázquez-Estrada et al. [85] also observed approximately 7.6 times higher D[4,3] value than the D[3,2] value for the untreated orange juice.

6D.3.1.13. *In-vitro* bio-accessibility of carotenoids

Carotenoid *In-vitro* bio-accessibility depends on various product and process related factors such as the crystalline nature of the carotenoids and their entrapment within chromoplasts [66], food matrix structure that depend on tissue type [66], processing condition [66], oil content [62], other bioactive compounds [62], micelle

formation [49], and particle/droplets size. These factors on an average, do not allow for the absorption of more than 5–30 % of the ingested amount of carotenoids [48,89].

The bio-accessibility of carotenoids of all the developed juices are evaluated and presented in **Table 6D.3.** The carotenoids bio-accessibility of SPFJU was found to be 7.29 %, which increased non-significantly to 8.58 % after blending with guava. The high degree of carotenoids entrapment in the tissue cells is presumably the cause of the reduced bio-accessibility. Carotenoids must first cross the chromoplast (the innermost physical barrier), the cell wall, and the aqueous environment surrounding the cells in order to be isolated from the matrix. This additional physical barrier may prevent carotenoid incorporation into the oil phase [66], and hence lower bio-accessibility occurs.

After the HPH treatment, the bio-accessibility increased significantly to 18.47 % (SPFJT) and 20.10 % (BSPFJT). Mechanical homogenization may induce the breakdown of the plant tissue matrix, facilitating the diffusion and release of carotenoids from chromoplasts and make it more accessible for overall increased bio-accessibility [33,66]. Again, high mechanical shear stress and increased temperature may enhance the diffusivity as well as change the crystallinity, which may positively affect bio-accessibility. After the HT treatment, the bio-accessibility of BSPFJTT increased to 16.48 %, which was less than the HPH treatment; this may be due to the lesser structural disruption by HT treatment compared to HPH. Additionally, the use of heat treatment may weaken the cell wall structure, enabling the diffusion and release of carotenoids from chromoplasts, and this improved their bio-accessibility [66].

Again, during the blending of juice with oils and fibres using high pressure homogenization, emulsion was developed, and this increased the bio-accessibility further to 30.29%, as in BNESPFJT. Carotenoids being lipophilic in nature, their solubility and extractability from the cell chromoplast are enhanced in oil. Apart from the effects of oil droplets, mechanical homogenization processing also similarly induces diffusion and release of carotenoids from chromoplasts. Also there is in addition, the effect of reduced particle size that help to increase bio-accessibility [33,66]. Again the developed emulsion contains significant amount of fibre, which is rich in pectin and it has been reported that emulsion with pectin significantly enhances the overall bio-accessibility of carotenoids [79].

Salvia-Trujillo et al. [66] found increase in lycopene (carotenoids) bio-accessibility of raw tomato juice from 7.5 % to 12.5 % by adding small emulsion droplets whereas,

only 2.5 % increment in bio-accessibility was obtained by emulsions containing large droplets (10.0 %). Hedrén et al. [24] observed significantly inceased release of total β-carotene present in carrot from 3 % (raw carrots in pieces) to 21 % (pulped) after homogenization. In that study, cooking the pulp with and without oil increased the accessibility to 39 % and 27 %, respectively. Although HPH dramatically boosted the release and micellar integration of α and β-carotene in carrot emulsions by 1.5 to 1.6 folds, it had no discernible effect on the retention of carotenes or ascorbic acid [74]. Maximum bio-accessibility of ≈36 % of β-carotene in nanoemulsion with 2 % of pectin was observed by Teixé-Roig et al. [79]. They also reported that nanoemulsions presented greater stability and lower β-carotene degradation over time in comparison with coarse emulsion, which highlights the potential of adding pectin to β-carotene nanoemulsions to enhance their functionality by efficiently preventing the compound degradation and increasing the in vitro bio-accessibility [79].

6D.3.1.14. Sensory parameters

HPH is regarded as one of the most promising new liquid food processing technologies due to recent advancements made to high-pressure homogenizers and the growing acceptance of pressure-processed foods by customers [97]. The results of sensory evaluation are shown in **Fig. 6D.4.** Even though significant difference was not observed ($p>0.05$) for the external quality attributes of Colour, taste, smell, consistency among juice samples, lowest overall sensory value was observed for HT treated BSPFJ.

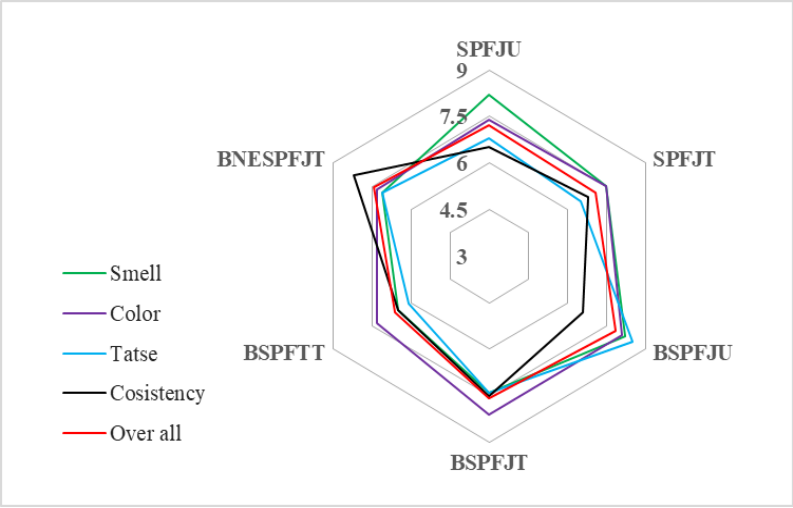


Fig. 6D.4. Sensory radar chart of all juices.

For all the treatments, the sensory value specially the colour, taste and over all acceptability decreased slightly, whereas consistency value increased which may be due

to the breakdown of the particles and emulsion by the combination of oil and fibre, as for BNESPFJT. After the blending of fruit juices, sugar to acid ratio increased from 2.628 (SPFJU) to 4.312 (BSPFJU), and BSPFJU scored the highest sensorial acceptability value. It is well reported that the sugar and acid balance affects taste [88], and higher the sugar and acid balance, better is the taste. After the HPH treatment, the sugar to TTA ratio had decreased and this may have decreased the sensory scores. Nonetheless, all the values were in the acceptable limit. Similar kind of sensory data was observed for mixed juice[88], grape must [40], orange juice[85] after HPH treatment.

For all treated juices, the consistency score was high. The consistency is a measure of cloudiness, and the level of cloudiness is judged from visual appearance of consistency in the sensory test (**Fig. 6D.4)**. The suspension stability/ cloudiness value of BNESPFJT was 8.2 followed by BSPFJT (7.5) and SPFJT (6.8), implying the presence of higher content of solubles in these samples. The addition of fibre makes the products thicker (**Fig. 6D.4)**, which may help to perceive sensorily that BNESPFJT has the thickest consistency. Blending guava juice with SPFJU made the colour more attractive for which highest score for Colour was attained by BSPFJU. For overall acceptability, the highest score was given to BSPFJU. After the HPH treatment, the overall acceptability of BNESPFJT was slightly higher than SPFJT.

6D.3.1.15. Cytotoxicity MTTS assay

Cytotoxicity studies are beneficial for identifying baseline cytotoxic processes shared by a variety of cells, but not for identifying toxins that are specific to an organ [91]. To evaluate the cytotoxicity of SPFJU, BSPFJU, BSPFJT, BSPFJTT, and BNESPFJT **(Fig. 6D.5)**, THP-1 macrophage was treated with increasing concentration of the samples in triplicates.

As shown in **Fig. 6D.5**, with an increase in concentration, there was a minor effect of the samples on cell viability. Maximum cell death was observed for SPFJU at 500 μg/mL concentration, while lowest cell death was observed for BNESPFJT. For all samples, within the studied concentration, the cell viability values were higher than 90%, which implies that all the samples are consumable and had negligible toxicity. Extracts that allow cell viability lower than 80% can be considered as toxic [36]. The acute toxicity study by Am et al. [3] of passion fruit seed extract revealed that the extract was safe up to 5000 mg/kg, and from the results of sub chronic toxicity study, the authors observed that 3000 mg/kg was safe for 28 days.

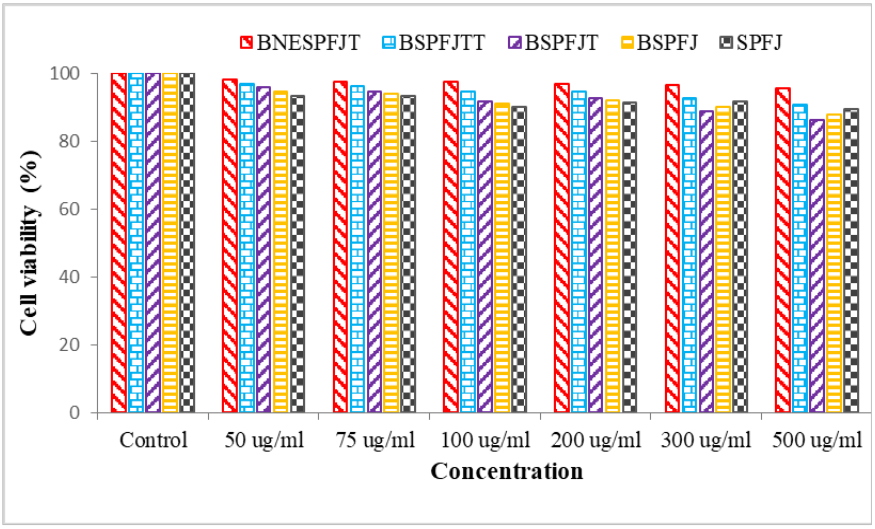


Fig. 6D.5. Cytotoxicity MTTS assay of SPFJU, BSPFJU, BSPFJT, BSPFJTT and BNESPFJT

6D.3.2. Storage study of BSPFJT, BSPFJTT, and BNESPFJT

The BSPFJT, BSPFJTT, and BNESPFJT samples were packed in 10 mL amber glass vials and stored at 5±2 ℃ and 25 ±2 ℃ and their physicochemical analyses were performed and the observed changes are reported in **Fig. 6D.6.**

6D.3.2.1. pH and total titratable acidity (TTA)

As shown in **Fig. 6D.6a**, during the initial storage period slight reduction in pH value was observed which may be due to the release of organic acid from the HPH disrupted cells [39], but in the later stage slight increase in pH value was observed, which may be attributed to the metabolites released by the microorganisms [83]. At low pH, when the juice is held at temperatures permitting their growth, microorganism such as acetic acid bacteria, yeasts and molds can grow [44] and degrade the organic acid during the storage period. A decreasing trend was therefore observed for BSPFJTT during the entire storage period **(Fig. 6D.6a**). The microbial growth rate was higher at room temperature compared to refrigeration temperature, and higher pH changes at higher storage temperatures were observed. However, lowest change was observed for BNESPFJT (**Fig. 6D.6a**), which may be due to the protective effect of developed emulsions as they prevent the growth of microorganisms.

During the initial period of storage, TTA in BSPFJT, BSPFJTT and BNESPFJT slightly increased, which may be due to the leaching out of organic acids and also by the organic acids developed by microbial growth, and this was followed by a decrease in TTA in the later stage (**Fig. 6D.6b**). The increase in TTA at 25 ℃ was greater than that

in 5℃, which may be brought on by the development and reproduction of microorganisms. Some bacteria were able to produce considerable amount of acetic acid and propionic acid, and this resulted in an increase in the acidity level and rancid smell [37]. The increase in TTA could probably be due to the accumulation of organic acids from sugars produced by metabolic activities of microorganisms [28]. TTA of all juices decreased throughout the storage time which may be due to the increase in catabolism of citrate and malate during storage [32]**.** Zhong et al. [96] observed considerable decrease in TTA of kiwifruit wine during storage, which was caused by fermentation by yeast and bacteria that increased the breakdown of organic acids (degraded citric acid) [81].

6D.3.2.2. Total sugar and TSS

Sample (BSPFJT, BSPFJTT and BNESPFJT) stored at 5 and 25 ℃ (**Fig. 6D.6c)** caused a decrease in total sugar level The decrease in total sugars could probably be due to the metabolic activities of microorganisms as they convert the sugars to organic acids. Microorganisms like yeasts not only ferment the sugars but also cause spoilage in juices [28].

The TSS values of BSPFJT, BSPFJTT and BNESPFJT juice after treatment showed a decreasing trend over the storage period (**Fig. 6D.6d**) probably due to the increasing microbial growth [44] and also possibly due to the effect of residual hydrolysis by pectinase [37]. In both temperatures, less reduction was observed for BNESPFJT, which may be due to less microbial growth. Similar results were reported by Guan et al. [20] for mango juice stored at room and refrigerated temperatures and Liu et al. [37] for watermelon juice.

6D.3.2.3. Ascorbic acid (AA)

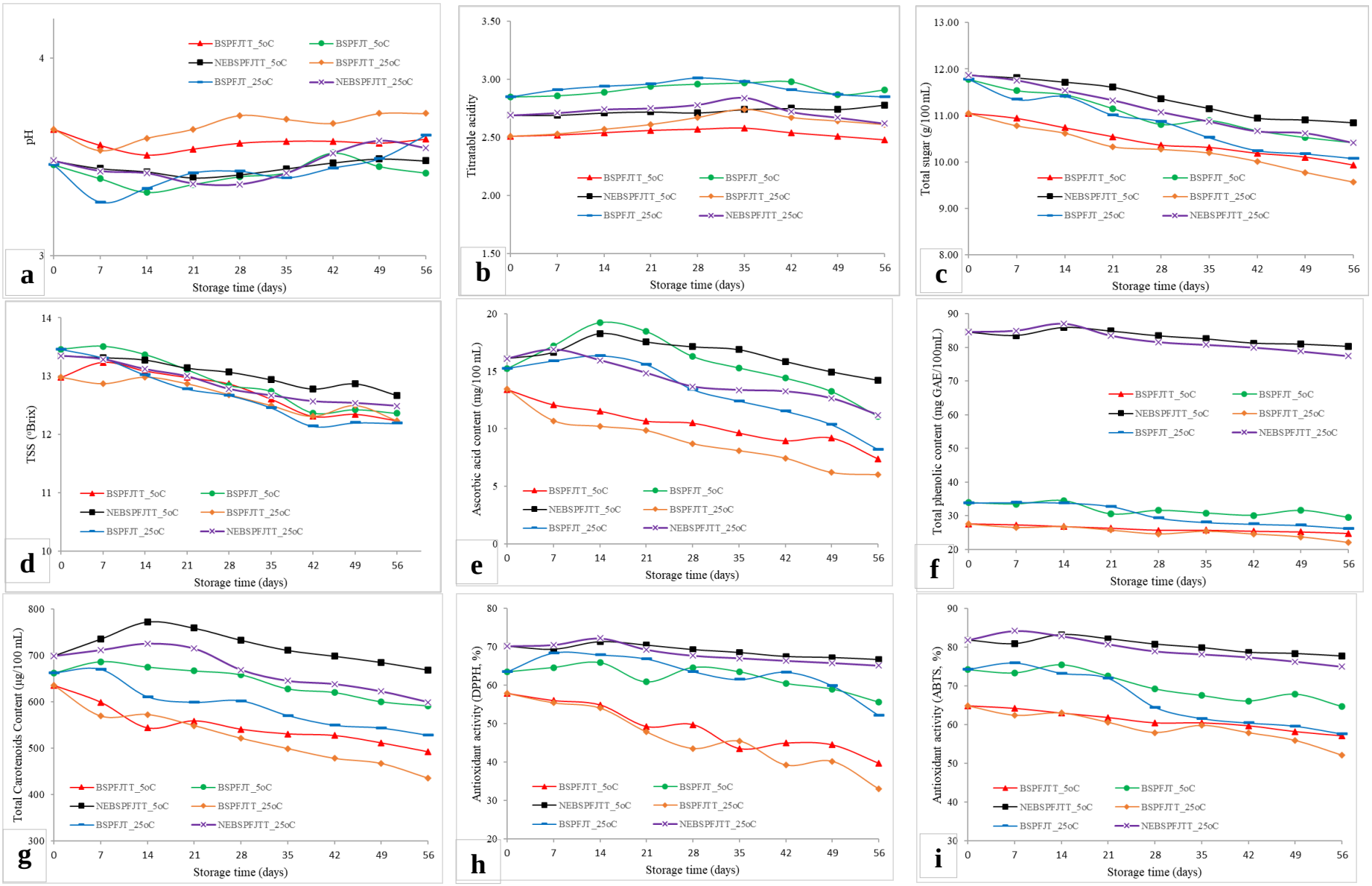
HPH treatment was found to better preserve AA in the blended juice samples than HT (**Fig. 6D.6e)**. The quantifiable amount of ascorbic acid in BSPFJT and BNESPFJT on HPH treatment showed an increasing trend up to first 14 days of storage at 5 ℃, whereas, BNESPFJT stored at 25 ℃ showed an increasing trend up to 7 days. However, a decreasing trend was observed thereafter. Continuous decrease of AA amount in HT treated beverage (BSPFJTT) was observed during the entire storage period (**Fig. 6D.6e**). Similar results were reported by Guan et al. [20] for mango juice, where mango juice treated with 3 HPH passes exhibited a detectable amount of AA that increased during the first 12 days of storage at 4 ℃ before showing a downward trend. Apple juice stored at 4 ℃ for 28 days showed that AA content increased during the first 7 days by 138.39%

before gradual decline with further storage [44]. It was concluded that the rise in the quantity of bioactive compounds at the beginning of storage was likely caused by the fact that the compounds were more easily removed and released from the cells damaged by HPH treatment [20]. Later decrease in ascorbic acid content may be due to the degradation of AA by oxygen, temperature, light, metals, microorganisms and enzymes [20]. After 8 weeks of storage, the retention of ascorbic acid stored at 5 ℃ was 55.14%, 72.92% and 88.25% for BSPFJT, BSPFJTT and BNESPFJT, respectively, whereas ascorbic acid of the respective juices stored at 25 ℃ was 44.82 %, 55.91 % and 69.56 %, respectively. The higher degradation of AA for BSPFJTT may be due to the high temperature as heat can speed up the oxidation process of the compound [20]. For both storage temperatures, nanoemuslion added beverage (BNESPFJT) showed better retention of AA, which may be due to the protective effect of bioactive compounds within the emulsion [33,50]**.** After 60 days of storage at 4 ℃ and room temperature, Guan et al. [20] reported a retention of 75.52% and 50.75%, respectively of AA.

6D.3.2.4. Total phenolic content

Fig. 6D.6f represents the changes in total phenolic content in BSPFJT, BSPFJTT and BNESPFJT beverages stored at 5 ℃ and 25 ℃. During the storage (at both temperatures) HPH treated BSPFJT and BNESPFJT juices showed a slight increase in phenolic content at initial stage followed by a decrease. The initial (up to 14 days) higher phenolic content may be due to the higher extractability effect of HPH. During HPH treatment intensive cell disruption occurred [88], which enhanced the leaching of polyphenols during the initial stage, and higher storage temperature may improve the effective diffusivity, which also causes release of more polyphenols from vacuoles. The decreasing trend in the later stage may be explained by the rapid activity of polyphenol oxidase and peroxidase enzymes that are known to cause degradation of phenolic compounds as partially inactivated enzymes recover their property activity [5,9].

As usual, compared to beverage stored at 5 ℃, both HPH treatment and HT stored at 25 ℃ showed lower quantifiable amount of bioactive compounds. For example, 89.83 % retention of total phenolic content was found in HT sample (BSPFJTT) stored for 56 days at refrigerated temperature (5 ℃), whereas only 80.31 % was retained when stored at room temperature (25 ℃) **(Fig. 6D.6f)**. After 56 days of storage, 87.06 % of total phenolic content in BSPFJT stored at 5 ℃ was retained while only 77.59 % was retained


a
pH
Storage time (days)
b
Titratable acidity
Storage time (days)
c
Total sugar (g/100 mL)
Storage time (days)
d
TSS (°Brix)
Storage time (days)
e
Ascorbic acid content (mg/100 mL)
Storage time (days)
f
Total phenolic content (mg GAE/100mL)
Storage time (days)
g
Total Carotenoids Content (µg/100 mL)
Storage time (days)
h
Antioxidant activity (DPPH, %)
Storage time (days)
i
Antioxidant activity (ABTS, %)
Storage time (days)
BSPFJTT_5oC
BSPFJT_5oC
NEBSPFJTT_5oC
BSPFJT_25oC
BSPFJT_25oC
NEBSPFJTT_25oC

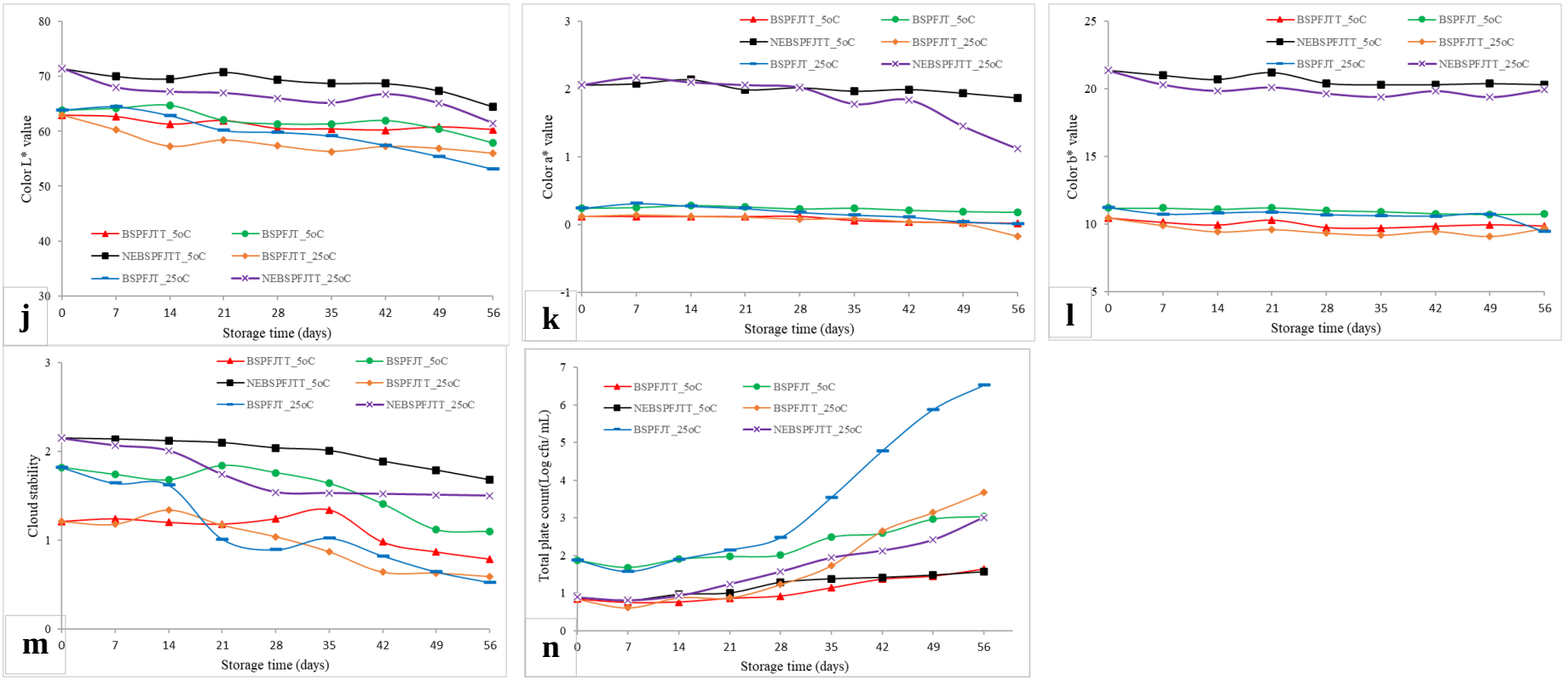


Fig. 6D.6. Storage study of BSPFJT, BSPFJTT and BNESPFJT stored at 5 and 25 ℃ of (a) pH, (b) TTA, (c) Total sugar, (d) TSS, (e) Ascorbic acid, (f) Total phenolic, (g) Total carotenoids, (h) Antioxidant activity (DPPH %), (i) Antioxidant activity (ABTS %), (j) Colour L*, (k) Colour a*, (l) Colour c*, (m) Cloud stability, (n) Microbial load (TPC).

when stored at 25 ℃. For BNESPFJT, 94.91 % and 91.57 % of total phenolic acid was retained when stored at 5 ℃ and 25 ℃, respectively. Although HT treatment recorded higher retention percentage of total phenolic content as compared to HPH treatment, it was observed after 56 days of storage that in terms of actual amount HPH was able to maintain the phenolic content in an efficient manner.

The breakdown of cell clusters into individual cells and/or cell fragments is facilitated by HPH. Pectin and proteins, which are components of cell walls, are released and solubilized, and help to improve particle interactions with polyphenols [53], and therefore, there is a higher phenolic content [6,88]. Similar storage study result was observed for kiwifruit juice [53] and mango juice [20]. Among all three types of blended juice, BNESPFJT showed higher retention of phenolic content, which may be due to the protective effect of bioactive compounds by the developed emulsion. Also, the added fibre enhances the formation of stable interaction.

Patrignani et al. [53] observed that kiwifruit juices treated with HPH showed a considerable increase in total phenol concentration, rising from 35 to 42 mg/100 mL. Additionally, they noted that phenolic content decreased slightly during storage at 5°C, primarily during the first 15 days, even though samples treated at 200 MPa for three cycles did not exhibit any discernible differences. Further, they noted that during storage at higher temperatures, TPC values decreased in all of the samples, even though the highest retention was found in HPH samples [53].

6D.3.2.5. Total carotenoids content

HPH treated blended juice (BSPFJT) retained bioactive compounds better than HT treated juice (BSPFJTT) not only immediately after treatment but also during their storage at 5 ℃ and 25 ℃ (room temperature) (**Fig. 6D.6g**). Similarly, Guan et al. [20] observed higher retention of carotenoids after the HPH treatment as compared to heat treatment of mango juice.

After 8 weeks of storage, 77.56 % and 68.64 % of the carotenoids content remained in BSPFJTT when stored at 5 ℃ and 25 ℃, respectively. After the HPH treatment (BSPFJT), 89.26 % (storage at 5 ℃) and 79.665 % (storage at 25 ℃) of total carotenoids remained after 8 weeks of storage period. In BNESPFJT beverage, 95.59 % and 85.71 % of total carotenoids remained after 8 weeks of storage at 5 and 25 ℃, respectively. The higher retention of carotenoids for BNESPFJT may be due to the protective effect of lipophilic bioactive compounds in the nanoemulsion [74,79] and also

because of the fibre present, which can entrap the carotenoids and prevent degradation by the environmental factors [79]**.**

As shown in the **Fig. 6C.6G** for BSPFJT (HPH treated blended beverage), the carotenoids content increased up to a few days followed by a gradual reduction. It was deduced that the increase of bioactive compounds in the beginning of storage was probably due to their easy extraction and release from cells after disintegration by HPH treatment [52]. The oxidation and/or destruction of bioactive substances by light, metals, enzymes and rapid growth of microorganisms caused the subsequent decline in carotenoids content [20].

6D.3.2.6. Storage of antioxidant

As shown in **Fig 6C.6h** and **6i**, during storage for 56 days at 5 ℃ and 25 ℃, the antioxidant capacity (loss of DPPH scavenging activity and ABTS activity) of BSPFJT, BSPFJTT, and BNESPFJT samples showed similar pattern as for bioactive compounds, which justify the strong corelation between antioxidant activities and bioactive compounds present, specially ascorbic acid, carotenoids, and phenolics [84,95]. However, the antioxidant capacity of juice could also be due to the synergistic action of complicated bioactive compounds, and it would be difficult to define the contribution of these bioactive compounds to antioxidant capacity.

The changes in pattern of ABTS antioxidant activity during storage period was almost same with polyphenols and ascorbic acid, which implies that hydrophilic antioxidant activity was better reflected by ABTS assay than DPPH scavenging assay [17], while some deviation was observed for DPPH antioxidant activity. During the storage period, the better retention of antioxidant activity of BNESPFJT may be due to the retention of bioactive compounds as HPH promoted hydrophobic interactions between pectin and polyphenols that provided polyphenol stability [88].

6D.3.2.7. Colour value

The L*****(**Fig. 6D.6j**), a* (**Fig. 6D.6k**), and b* (**Fig. 6D.6l**) values of BSPFJT, BSPFJTT and BNESPFJT beverages decreased over entire storage period at 5 ℃ and 25 ℃, meanwhile ΔE values significantly increased. The colour values of samples stored at 25 ℃, showed a greater decrease than the ones stored at 5 ℃, which may be induced by higher browning reactions at higher temperature. When compared to BSPFJT, BSPFJTT had greater alterations that could be attributed to the Maillard reaction and/or pigment degradation brought on by thermal treatment [20]. During storage, the decreased values

of L*, a* and b* may be due to interactions of environmental factors such as oxygen, temperature, etc. with the pigments and also due to the effect of enzymes and microorganisms present in the beverages [53]. These changes may have resulted from oxidation of the bioactive compounds (carotenoids) by the dissolved oxygen in the beverages, since this pigment is responsible for the red Colour. HPH increases the exposure of the carotenoids, and larger area makes it more susceptible to oxidation [35]. Guan et al. [20] reported decrease in L*, a*, and b* values of mango juice after HPH and HT treatments during storage at 4 ℃ and room temperature for 60 days. Similar changes in pattern were also observed by Kubo et al. [35] and Patrignan et al. [53] for tomato juice and kiwifruit, respectively during storage after HPH treatment.

6D.3.2.8. Storage stability and cloud stability

As shown in the **Fig.6D.6m,** during storage, the cloud stability decreased for all beverages, this may be caused by particle agglomeration (which results in particle sedimentation) as well as microbial and enzyme growth during storage, which over all resulted in juice instability [93]. Storage, at high temperature cause higher instability as compared to low storage temperature because of the higher growth rate of microorganisms [78]. Among the three beverages, highest stability was observed for BNESPFJT, which may be due to the protective effect of fibre and oil in the Pickering nanoemuslion developed with the help of HPH treatment. In addition, pectin combined with oil produces higher metyl-esterification that promotes stability of pectin and cloudy dispersion [38].

During storage, higher cloud stability value was observed for BNESPFJT, which may be attributed to the higher retention of antioxidant and phenolic compounds in the nanoemulsion that hindered the growth of microorganism. Yi, et al. [93] also observed cloud stability of mixed juice up to 42 days of storage at 4 ℃ . In light of this, it can be said that HPH is a physical pre-processing step that can be taken into account to reduce the cloud loss in hazy fruit juice [92].

6D.3.2.9. Microbial load and shelf life

It is already well reported that as inactivation effect of total plate count (TPC) by HPH treatment is higher than mould and yeast, mould and yeast are more sensitive to HPH treatment [20]. Therefore, in this study the shelf life was expressed in terms of TPC.

According to Codex Alimentarius Commission (CAC) of the Food and Agricultural Organization (FAO) (2003), the permitted level of TPC for juice samples is 10^5 CFU/mL [28]. Furthermore, the borderline of acceptable level of microorganisms for pasteurised foods including fruit juice was about the $10^4 - 10^7$ cfu/mL under ready-to-eat foods category [12].

In this study, as shown in **Fig. 6D.6n**., the microbial growth rate in beverages stored at 25 ℃ was higher as compared to refrigerated storage (5 ℃). As seen in **Fig. 6D.6n,** the lowest microbial growth rate was observed in BNESPFJT because of the protection provided by bioactive compounds in an emulsified system and also because passion fruit seed oil (enriched with phenolic compounds) and passion fruit seed fibre shows good antimicrobial activity [29, 38,44,60]. BNESPFJT could deliver the properties in a controlled manner. Therefore, oil and fibre added and HPH treated passion fruit juice (BNESPFJT) showed highest antimicrobial activity. During the storage of BSPFJTT, BSPFJT, and NEBSPFJT at 5 ℃, the TPC increased from 0.84 to 1.64, 1.87 to 3.04 and 0.89 to 1.59, respectively, i.e., microbial load increased by 1.95, 1.62 and 1.79 times of the initial load while on storage at 25 ℃ , the microbial load increased by 4.38, 3.49 and 3.38 times, respectively. The TPC of all samples showed no significant change up to 21 days. However, after 21 days significant microbial growth was observed for BSPFJT and BSPFJTT samples stored at 25 ℃, but TPC in BSPFJTT was lower than BSPFJT. Guan et al. [20] found that TPC growth in mango juice treated with HPH was greater than that treated with HT up to 36 days. Due to the intense heat treatment used, HT samples were able to attain a better level of microbiological stability but their sensory quality significantly decreased [20].

For all HPH-treated beverages stored at 5 ℃ for 8 weeks, the TPC counts were below the permitted level, indicating HPH treatment could provide microbial stability in blended beverages at refrigerated temperature. After 42 days, BSPFJU juice stored at 25 ℃ exceeded the permitted level, but BNESPFJT remained below the permitted level during the entire storage period for both temperatures. Similarly, Szczepańska et al. [75] observed that after 21 days of homogenization process at 150 MPa, the total number of microorganisms increased and exceeded the initial level determined in fresh apple juice stored at 4 ℃.

During the storage period, TTA also increased and the increase of titratable acidity at 25 °C was greater than 5 °C, which might be caused by the growth and reproduction of

microorganisms. Because some bacteria could produce a large amount of acetic acid and propionic acid, acidity level increased and rancid smell appeared [37]. No differences were found between orange juice treated with HPH at 200 MPa and samples that had been pasteurised (90 °C for 1 or 2 min) during storage at 4 ℃ for 50 days, as reported by Velázquez-Estrada et al. [83]. Neither the microbiological count nor the PME activity increased during that time [83]. As the sample storage temperature increased, a decrease in shelf-life of the juice was observed [53].

6D.4. Conclusion

Raw passion fruit juice is less accepted by customers due to its astringency and sour taste. This problem can be addressed by blending the juice with other beverages. Although sensorial problem can be resolved by blending, but shelf life of blended juice is still an issue. Thermal and chemical treatments have their own drawbacks. So, HPH could be used to modify several physical characteristics of the fluid, like reducing particle size, reducing phase separation, and improving texture uniformity. Emulsion based blended beverages showed better quality retention as well as longer shelf life as compared to only thermal and high-pressure treatment. This study revealed that Pickering emulsion incorporated with materials having natural antimicrobial properties and subjected to high pressure homogenization can be used as novel preservation technique.

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