ANNEXURE-C

FINAL PROJECT COMPLETION REPORT

1. Title of the project: **Evaluation of tree tomatoes (tamarillo) of Nagaland utilizing the pulp, peel and seeds for its commercialization**
2. Principal Investigator(s) and Co-Investigator(s):

PI (Name & Address):
Prof. Charu Lata Mahanta
Department of Food Engineering and Technology,
Tezpur University, Napaam, Assam 784028

Co-PI (Name & Address):
Dr. Dipankar Kalita
Department of Food Engineering and Technology,
Tezpur University, Napaam, Assam 784028

3. Implementing Institution(s) and other collaborating Institution(s):

Tezpur University, Napaam, Assam 784028

4. Date of commencement: **21/12/2018**

5. Planned date of completion: **20/12/2020**

6. Actual date of completion: **20/03/2022**

7. Objectives as stated in the project proposal:

i. To determine the nutritional, biochemical, phenolic, flavonoid, anthocyanins and carotenoid composition of the tree tomatoes grown in Nagaland.
ii. To develop carotenoids-enriched tree tomato canned puree and determine it's physicochemical, functional, and storage properties.
iii. To obtain antioxidant rich extract from the unutilized peel of tree tomato and determine its stability.
iv. To investigate the physical and chemical properties of astaxanthin based emulsion using tree tomato seed oil.

8. Deviation made from original objectives if any, while implementing the project and reasons thereof:

None

9. Experimental work giving full details of experimental set up, methods adopted, data collected supported by necessary table, charts, diagrams & photographs:

Objective wise experimental work

9.1.0 Objective 1:

To determine the nutritional, biochemical, phenolic, flavonoid, anthocyanins and carotenoid composition of the tree tomatoes grown in Nagaland

9.1.1 Introduction

Tree tomato also known as tamarillo, is an acidic fruit, cultivated in the sub-tropical countries and three prominent varieties (purple, yellow and red) are available. Fruit variety is totally dependent upon the colour acquired by the fruit peel and pulp (Fig. 1.1). Different varieties of tree tomato possess different concentration of nutrients and antioxidant properties and vary according to geographical and climatic conditions. Therefore, the aim of this objective was to determine the phytochemicals present in the different varieties of tree tomato.

Purple tree tomato Yellow tree tomato Red tree tomato

Fig. 1.1: Different varieties of tree tomato

9.1.2 Methods

9.1.2.1 Proximate analysis

Moisture, crude fat, crude protein, total ash and crude fibre were determined. Carbohydrate content was calculated by difference. For biochemical estimation, pulp of the fruit was used.

9.1.2.2 Biochemical analysis

The pH and TSS of tree tomato samples were analysed using a pH meter (Eutech) and a hand refractometer (0-32 °Brix Erma, Japan), respectively. Total titratable acidity (TTA) was analysed with respect to citric acid. Vitamin C was analysed using 2,6-dichloro-indophenol titration method.

9.1.2.3 Mineral content

The mineral content present in the three varieties of tree tomato was analyzed. Potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), phosphorus (P), iron (Fe), and zinc (Zn) were determined by atomic absorption spectrometry (Thermoscientific Model No. ICE3500, USA) and the results are expressed in mg/100 g.

9.1.2.4 Colour

Colour of the fruit pulp was analysed using Hunter colour spectrophotometer (Hunter Colour Lab Ultrascan Vis, USA). The instrument was standardized using the standards before measurement of the samples. Scale parameters for colour analysis were L* (0 for darkness and 100 for lightness), a* (negative for green to positive for red) and b* (blue for negative to yellow for positive).

9.1.2.5 Sample extraction for phenolics and antioxidant activity

For phytochemical extraction and antioxidant activities, the tree tomato juice samples were mixed with extraction solvent (80:20 acetone:distill water) in the ratio of (1:10) in a shaking incubator (Labtech) at 200 rpm and then centrifuged at 3000 x g (Eppendorf 5430 R) for 10 min at room temperature. The supernatant was filtered through Whatman No. 4 (Whatman, India) and then stored at -20 °C until further analysis. All the extracts were prepared in duplicates and analysed in triplicates.

9.1.2.6 Total phenolic content

Th**e** total phenolic content (TPC) in tree tomato was determined. For the analysis, an aliquot of 0.5 mL of diluted sample extracts was taken in test tube and mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10). For blank, sample extract was replaced with distilled water. After 5 min of incubation, 2 mL of sodium carbonate (7.5%) was added into each test tube, vortexed and kept for 2 h in a dark place at room temperature. Absorbance was read by UV-Vis spectrophotometer (Thermo-Fischer Evolution A600) after incubation time against the reagent blank mixture. Gallic acid was used as the standard, and results are expressed in mg GAE/100g.

9.1.2.7 Total flavonoid content

For the analysis, an aliquot of 0.5 mL of sample was mixed followed by the addition of 1.5 mL of ethanol (95%), 0.1 mL of aluminium-trichloride (10%), 0.1 mL of potassium acetate (1M) and 2.8 mL of deionized water. The test tube was vortexed and kept for 2 h in a dark

place at room temperature for 40 min. The absorbance of the sample was read at 415 nm in UV-Vis spectrophotometer (Thermo-Fischer Evolution A600) against blank. Quercetin was used as standard, and results are expressed in mg QE/100g.

9.1.2.8 DPPH radical scavenging activity

In a test tube, 200 µL of sample extract was taken followed by the addition of 2.8 mL of DPPH radical prepared in methanol, vortexed and kept for 30 min in a dark place for incubation. The absorbance of sample was read at 517 nm using UV-Vis spectrophotometer (Thermo-Fischer Evolution A600) against blank (Eq. 1.1).

$$DPPH\ activity\ (\%) = \frac{A_o - A_s}{A_o} \times 100 \quad (1.1)$$

Here, A_o is absorbance of control blank, and A_s is sample absorbance

9.1.2.9 ABTS radical scavenging activity

For preparation of fresh solution of ABTS solution, 2.45 mM of potassium acetate and 7 mM ABTS reagent were mixed in ethanol separately. The radical solution was prepared by mixing potassium acetate and ABTS solution in (1:1 v/v) and kept for incubation for 16 h at room temperature in dark. After incubation, the radical solution was read for absorbance of 734 nm using UV-Vis spectrophotometer (Thermo-Fischer Evolution A600) against blank. The absorbance value of solution was adjusted to 0.70 ± 0.05 using ethanol as diluent. In a test tube, 0.3 mL of tree tomato extract was taken and 2.7 mL of ABTS solution was mixed. The radical scavenging activity was calculated using recorded absorbance of sample (Eq. 1.2).

$$ABTS\ activity\ (\%) = \left(\frac{A_o - A_s}{A_o}\right) \times 100 \quad (1.2)$$

here, A_o and A_s stands for absorbance for control and sample values

9.1.2.10 HPLC of phenolic acids

The identification of phenolic acids present in the tree tomato juice was done by reverse phase HPLC in a C18 column with diode array detector (Ultimate 3000, Thermo Scientific, USA).. The sample extract was filtered through 0.45 µm syringe filter prior to injection. The gradient mode consisted of two solvents A (0.1% formic acid) and B (100% acetonitrile) at 35 °C. The flow rate of the solvent was kept at 0.5 mL/min at 330 nm wavelength and the gradient flow pattern was 15% B for 5 min, 20–35% B for 10 min, 35–50% B for 10 min, 50–60% B for 5 min, and 60% B for 5 min.

9.1.2.11 HPLC of anthocyanins

The anthocyanins in tree tomato juice were identified and quantified using UHPLC (Ultimate 3000, Thermo Scientific, USA) with the help of anthocyanin standards in reverse phase in C18 column using diode array detector and a calibration curve was developed. The sample was filtered through 0.45 µm syringe filter prior to injecting. A gradient mode consisting of two solvents, solvent A (0.1% triflouroacetic acid) and B (100% acetonitrile) at 35 ºC was used. The flow rate of the solvent was kept at 0.5 mL/min at a wavelength of 520 nm using gradient flow with 10% B for 3 min, 10–15% B over 12 min, 15% B for 5 min, 15–18% B over 5 min, 18–30% B over 20 min, 30–35% B over 5 min, and re-equilibration to initial solvent.

9.1.2.12 HPLC of carotenoids

The carotenoids in the tree tomato juice were identified and quantified using UHPLC (Ultimate 3000, Thermo Scientific, USA) with the help of internal standards in reverse phase in C30 column using diode array detector and calibration curve was developed. The sample extract was filtered using 0.45µm syringe filter prior to injecting. A gradient mode consisting of two solvent A (methanol/acetonitrile/water 84:14:4, v/v/v) and solvent B (dichloromethane) at 25ºC was used. The flow rate of the solvent was kept at 1 mL/min at a wavelength of 450 nm using gradient flow rate with 100% A and 0% B initially, raised to 10% B at 4 min, 18% B at 12 min, 21% B at 17 min, 30% B at 20 min and maintained until 25 min, increased further to 39% B at 28 min, 60% B at 40 min and returned re-equilibration to initial solvent parameters.

9.1.3 Results and discussion

9.1.3.1 Proximate analysis

The proximate compositions given in Table 1.1 show significant differences ($p < 0.05$) in the values for protein, fat, ash and fibre of tree tomato varieties. The moisture content varied from 89.36 ± 0.69 to 89.06 ± 0.74 that is within the range of 88.1-89.1% (w/w) reported tree tomato harvested in New Zealand.

The crude protein was found to be very high in purple tree tomato followed by yellow and least in red tree tomato. The total ash was highest in yellow tree tomato and lowest in red tree tomato variety. High values of the ash content in pulp indicate that the fruit pulp is a good source of minerals. The fat and crude fiber content ranged from 0.42-0.25% and 14.30-

15.28%. However, no significant difference was observed in the carbohydrate content (Table 1.1).

Table 1.1 Proximate composition of three different varieties of the tree tomato pulp

Variety	Crude protein (% db)	Total ash (% db)	Crude fat (% db)	Crude fibre (% db)	Carbohydrate by difference (% db)
Purple	9.39 ± 0.35^{a}	6.55 ± 0.14^{b}	0.42 ± 0.03^{a}	14.44 ± 0.32^{b}	68.16 ± 0.81^{a}
Yellow	8.21 ± 0.27^{b}	7.78 ± 0.11^{a}	0.25 ± 0.03^{b}	14.30 ± 0.13^{b}	69.43 ± 0.73^{a}
Red	6.61 ± 0.10^{c}	5.85 ± 0.16^{c}	0.25 ± 0.03^{b}	15.28 ± 0.32^{a}	67.96 ± 0.65^{a}

Values expressed as mean ± SD. Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$).

9.1.3.2 Biochemical analysis

The results of the biochemical analyses of the samples are reported in Table 1.2. Tree tomato fruits were found to be highly acidic in nature with pH ranging from 3.54-3.94, which is supported by literature values of 3.2-3.8. The TSS of the tree tomato pulp varied from 9-9.8 °Brix. Tree tomato from the Ecuador region was reported to have 9-11 °Brix TSS. The titratable acidity of the tree tomatoes was found to range from 1.16-1.30% and was in agreement with reported values of 1.03 -1.60 g/100g fw. The highest acidity was shown by the purple variety and least acidity was recorded by the yellow variety. However, no statistically significant difference was found among the tree tomato varieties for acidity and Vitamin C. The concentration of vitamin C content was highest in the purple variety (17.11 mg/100g) followed by yellow variety and least was possessed by the red variety. The mineral content determined in the three tree tomato varieties is presented in Table 1.2. Potassium was found in highest quantity in the three varieties among the minerals.

9.1.3.3 Colour analysis

Colour is one of the most important factors for fruits and vegetables since it is an important visual criterion which influences consumer mood towards consuming food and also reveals the fresh quality parameters of fruit. The highest L* (lightness) value was shown by yellow tree tomato (55.23), followed by red variety (48.96) and lowest lightness was depicted by purple tree tomato (Table 1.2). The high concentration of anthocyanins in the purple tree tomato, which imparts red-purple colour to the fruits, reduces the lightness of the sample. The concentration of anthocyanins was low in red tree tomato and absent in yellow tree tomato

and therefore, highest L* (55.23) value was shown by yellow tree tomato. The positive a* value indicates the redness of the sample and purple variety showed strong a* value (51.71), followed by red variety (12.44), and yellow tree tomato (8.43). The positive b* value indicates the yellowness of the sample and highest value was noticed for yellow variety (49.44). The colour of tree tomato helps to distinguish the varieties, which is influenced by the type and concentration of the pigments present.

Table 1.2. Biochemical analysis of tree tomato

Parameters	Purple variety	Yellow variety	Red variety
pH	03.94 ± 0.11^{a}	03.70 ± 0.14^{b}	03.74 ± 0.18^{b}
Total Soluble Solids (°Brix)	10.10 ± 0.25^{a}	09.80 ± 0.15^{a}	09.60 ± 0.25^{a}
Titratable Acidity (%)	01.16 ± 0.14^{a}	01.13 ± 0.14^{a}	01.12 ± 0.10^{a}
Vitamin C (mg ascorbic acid/100g)	17.11 ± 0.28^{a}	16.81 ± 0.42^{a}	16.78 ± 0.38^{a}
Potassium (mg/100g)	406.12 ± 0.45^{b}	432.12 ± 0.83^{a}	401.12 ± 0.36^{c}
Magnesium (mg/100g)	17.65 ± 0.31^{b}	19.36 ± 0.23^{a}	16.56 ± 0.19^{c}
Calcium (mg/100g)	24.23 ± 0.15^{b}	26.32 ± 0.36^{a}	21.23 ± 0.15^{c}
Sodium (mg/100g)	3.45 ± 0.21^{b}	3.65 ± 0.44^{a}	3.36 ± 0.25^{c}
Phosphorus (mg/100g)	0.13 ± 0.02^{b}	0.15 ± 0.02^{a}	0.15 ± 0.01^{a}
Iron (mg/100g)	0.17 ± 0.02^{a}	0.17 ± 0.03^{a}	0.16 ± 0.03^{a}
Zinc (mg/100g)	0.18 ± 0.03^{a}	0.19 ± 0.04^{a}	0.18 ± 0.03^{a}
L	12.71 ± 1.21^{c}	55.23 ± 1.91^{a}	48.96 ± 1.01^{b}
a*	51.71 ± 0.61^{a}	08.43 ± 1.69^{c}	12.44 ± 1.42^{b}
b*	16.71 ± 0.86^{c}	49.44 ± 2.24^{a}	27.31 ± 1.35^{b}

Values expressed as mean ± SD. Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$).

9.1.3.4 Total phenolic content (TPC)

Significant difference was found in phenolic content among the tree tomato varieties ($p > 0.05$). The highest concentration of phenolic content was possessed by purple tree tomato (6.13 mg GAE/g fw) followed by yellow tree tomato (5.03 mg GAE/g fw) and least by red variety (4.52 mg GAE/g fw) (Table 1.3).

9.1.3.5 Total flavonoids content

The total flavonoids content of different varieties of tree tomato was evaluated and significant difference was observed ($p > 0.05$). The TFC in the purple, yellow and red variety was 0.98,

0.89 and 0.88 mg QE/g of the fresh fruits sample, respectively (Table 1.3). The reported value of quercetin was 4-6 mg/100g and myricetin was 1.2-1.4 mg/100g in golden-yellow and purple red tree tomato.

9.1.3.6 DPPH radical scavenging activity

Significant differences were found in the DPPH radical scavenging activity among the tree tomato varieties. The highest activity of 89.14% was shown by purple followed by yellow 65.05% and least activity of 60.23% was possessed by red tree tomato (Table 1.3). High value of phenolic and flavonoid content were correlated with enhanced *in-vitro* antioxidant activity. The highest phenolic and flavonoid content found in purple followed by yellow and least in red tree tomato was reflected in DPPH scavenging activity.

9.1.3.7 ABTS radical scavenging activity

ABTS scavenging activity varied significantly among the tree tomato varieties. The highest activity was shown by the purple variety (92.32%), followed by yellow variety (70.20%), and least activity was shown by red tree tomato (64.11%) (Table 1.3). Due to the presence of high amount of phenolics, flavonoids and anthocyanin compounds in the purple tree tomato in comparison to other varieties, purple variety depicted highest ABTS radical scavenging activity. Literature reports reported ABTS scavenging activity of purple and yellow tree tomato to range from 70-89 and 22-45 Trolox equivalents/g, respectively.

Table 1.3. Polyphenols and in-vitro antioxidant activity

Variety	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH radical scavenging activity (%)	ABTS radical scavenging activity (%)
Purple	6.13 ± 0.12^{a}	0.98 ± 0.02^{a}	89.14 ± 1.23^{a}	92.32 ± 0.56^{a}
Yellow	5.03 ± 0.08^{b}	0.89 ± 0.03^{b}	65.04 ± 0.89^{b}	70.20 ± 0.89^{b}
Red	4.52 ± 0.09^{c}	0.88 ± 0.01^{b}	60.23 ± 1.01^{c}	64.11 ± 0.98^{c}

Values expressed as mean ± SD. Values in the same row followed by different letters are significantly different by ANOVA test ($p < 0.05$).

9.1.3.8 HPLC analysis of the phenolic acids

HPLC analysis of phenolic acids present in different tree tomato varieties was done. Six phenolic acids present in tree tomato juice were identified, namely, gallic acid, chlorogenic

acid, caffeic acid, p-coumaric acid, ferulic acid and rosmarinic acid (Fig. 1.2). The highest concentration of phenolic acids was found in purple variety, followed by the yellow and least concentration was shown in red tree tomato (Table 1.4).

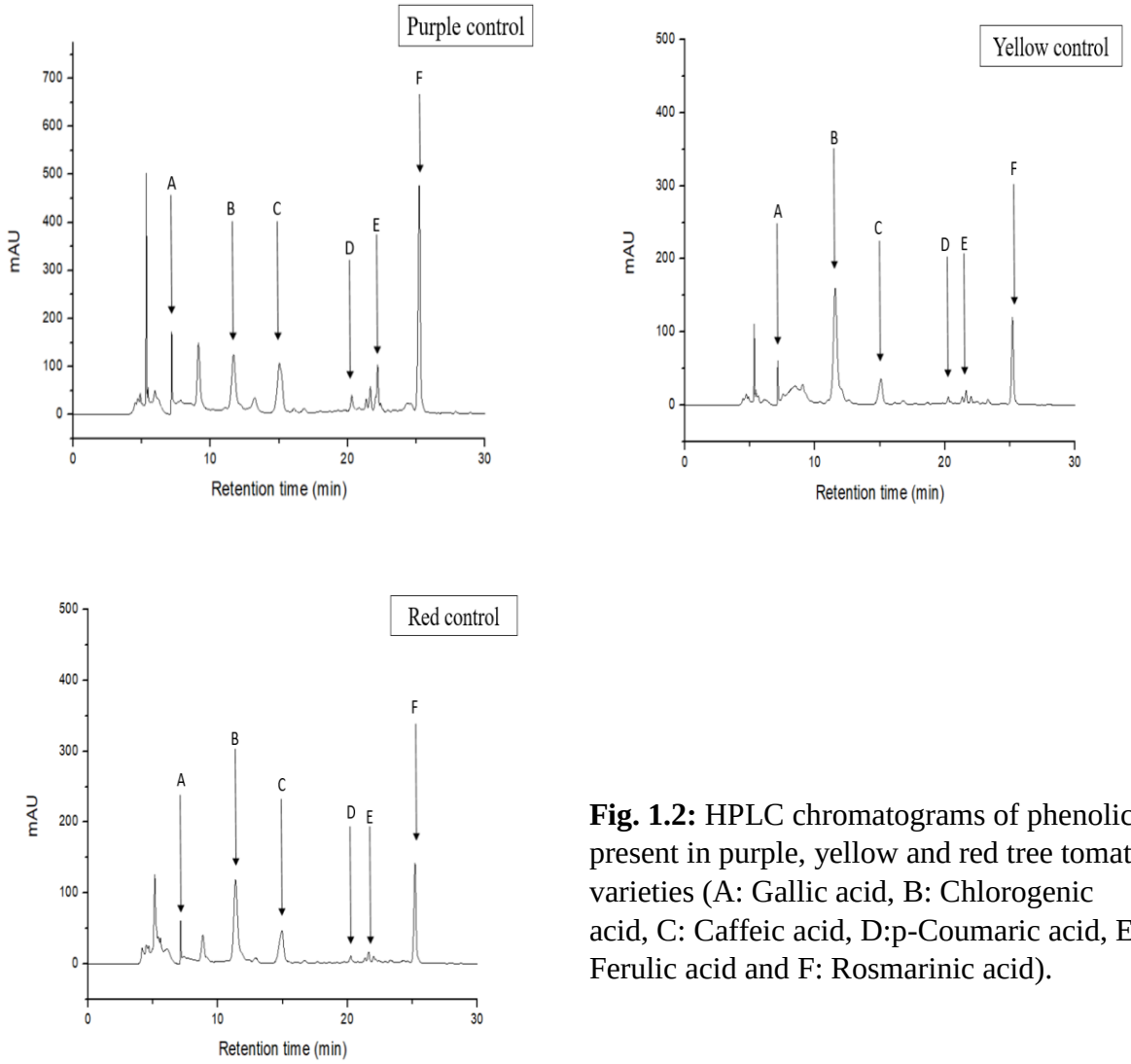


Fig. 1.2: HPLC chromatograms of phenolics present in purple, yellow and red tree tomato varieties (A: Gallic acid, B: Chlorogenic acid, C: Caffeic acid, D:p-Coumaric acid, E: Ferulic acid and F: Rosmarinic acid).

9.1.3.9 HPLC analysis of the anthocyanins

HPLC analysis of anthocyanins present in purple and red varieties of tree tomato was done. The anthocyanins present in control sample of purple tree tomato were delphinidin 3-O-rutinoside, cyanidin-3-O-rutinoside and pelargonidin-3-O-rutinoside. No anthocyanin was detected in yellow tree tomato samples (Fig. 1.3). The concentration of anthocyanins was substantially higher in purple variety in comparison to red variety (Table 1.4).

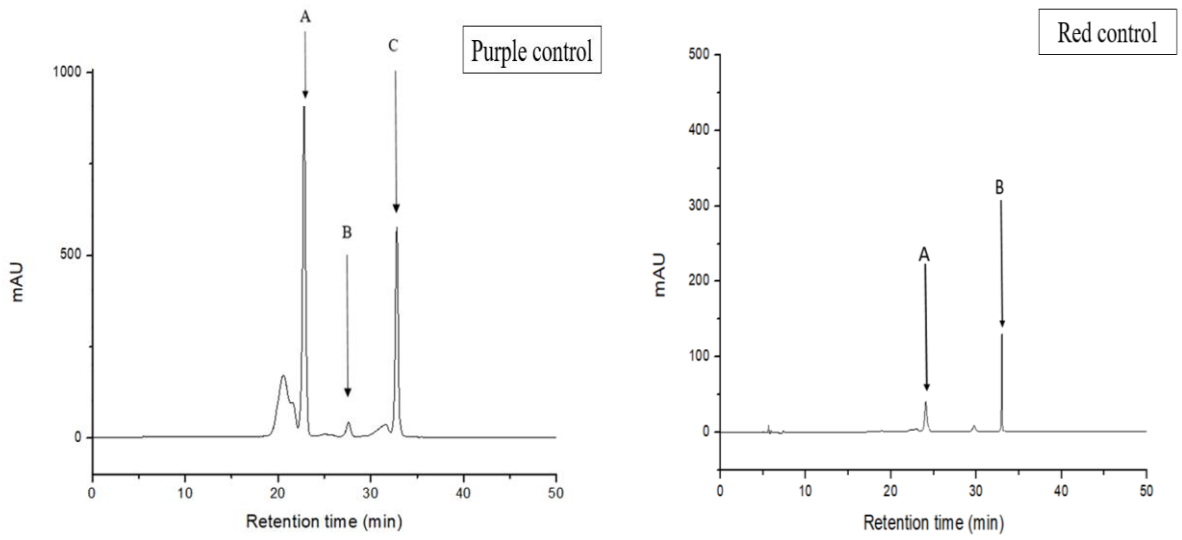
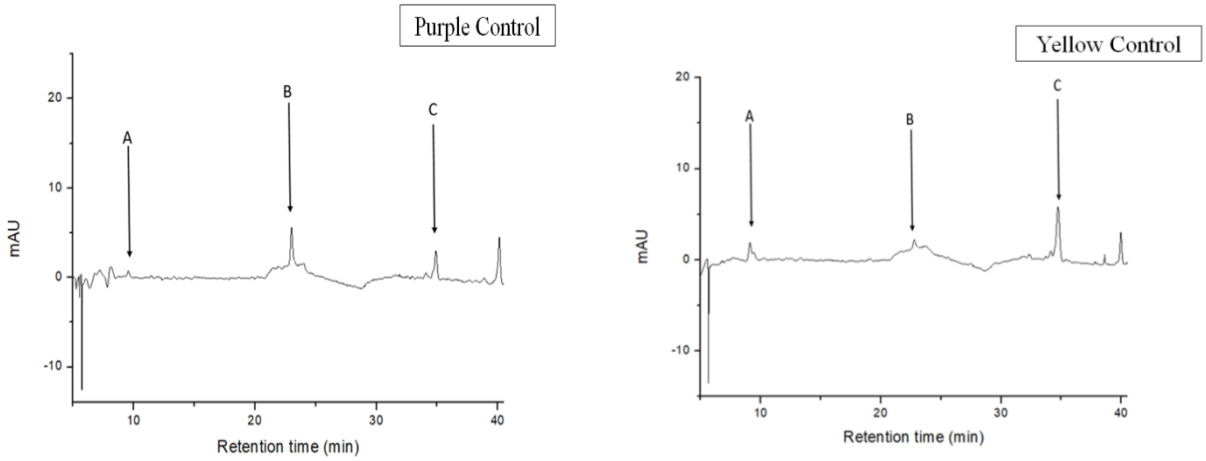


Fig. 1.3: HPLC chromatograms of anthocyanins present in purple and red tree tomato (A: Delphinidin 3-rutinoside, B: cyanidin-3-O-rutinoside and C: pelargonidin-3-O-rutinoside)

9.1.3.10 HPLC analysis of the carotenoids

Results of the HPLC analysis of carotenoids present in yellow and red varieties of tree tomato are shown in chromatographs given in Fig. 1.4. In the yellow and purple tree tomato varieties, three carotenoid compounds viz. zeaxanthin, β-crptoxanthin and β-carotene were identified, while only β-crptoxanthin and β-carotene were identified in the red variety. The concentration of β-carotene in purple, yellow and red tree tomato control samples was found



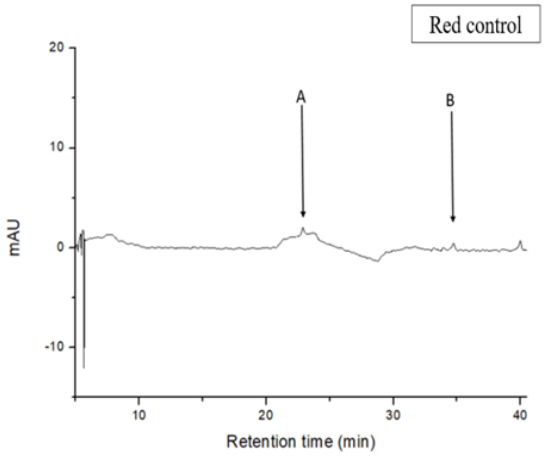


Fig. 1.4: HPLC chromatogram of carotenoids found in purple, yellow and red tree tomato (A: zeaxanthin, B: β-crptoxanthin and C: β-carotene).

to be dominant with the value of 199.71, 253.29 and 169.32 µg/100g, respectively (Table 1.4). Six carotenoids were reported to be present in Brazilian tree tomato, with β-cryptoxanthin and β-carotene being the major carotenoids.

Table 1.4: Concentration of bioactive compounds determined in tree tomato using HPLC

Identified bioactive compounds		**Purple tree tomato**	**Yellow tree tomato**	**Red tree tomato**
Phenolic acids (µg/g)	Gallic acid	238.18	153.9	67.95
	Chlorogenic acid	124.88	92.17	79.24
	Caffeic acid	41.13	22.8	23.84
	p-coumaric acid	4.57	3.73	3.24
	Ferulic acid	98.08	68.08	60
	Rosmarinic acid	89.15	53.7	47.68
Anthocyanin (µg/g)	Delphinidin 3-O-rutinoside	21.03	ND	4.05
	Cyandin-3-O-rutinoside	7.08	ND	ND
	Pelargonidin 3-O-rutinoside	23.32	ND	8.05
Carotenoids (µg/100g)	Zeaxanthin	42.26	63.65	ND
	β-cryptoxanthin	85.14	72.61	53.32
	β-carotene	199.71	253.29	169.32

ND means not identified

9.1.4 Conclusion

Differences in proximate composition, and biochemical and antioxidant properties were observed in purple, yellow and red tree tomato varieties. Varietal differences were also seen in the composition and concentration of phenolic acids, anthocyanins and carotenoids. Purple

tree tomato was rich in anthocyanins and yellow variety was rich in carotenoids. Further, both anthocyanins and carotenoids were present in purple and red varieties.

9.2.0 Objective 2:

To develop carotenoids-enriched tree tomato bottled puree and determine its physicochemical, functional, and storage properties.

9.2.1 Introduction

Yellow tree tomato is rich in bioactive compounds especially phenolic acids, flavonoids, carotenoids, vitamin C, vitamin A, anthocyanins etc. which are known to inhibit or decreases the risk of cancer, cardiovascular diseases, obesity etc. Tree tomato is usually consumed in raw form, in salads, desserts and beverages. Therefore, the main aim of this objective is to develop the carotenoids-enriched tree tomato bottled puree. Schematic of the tree tomato processing is given below (Fig. 2.1).

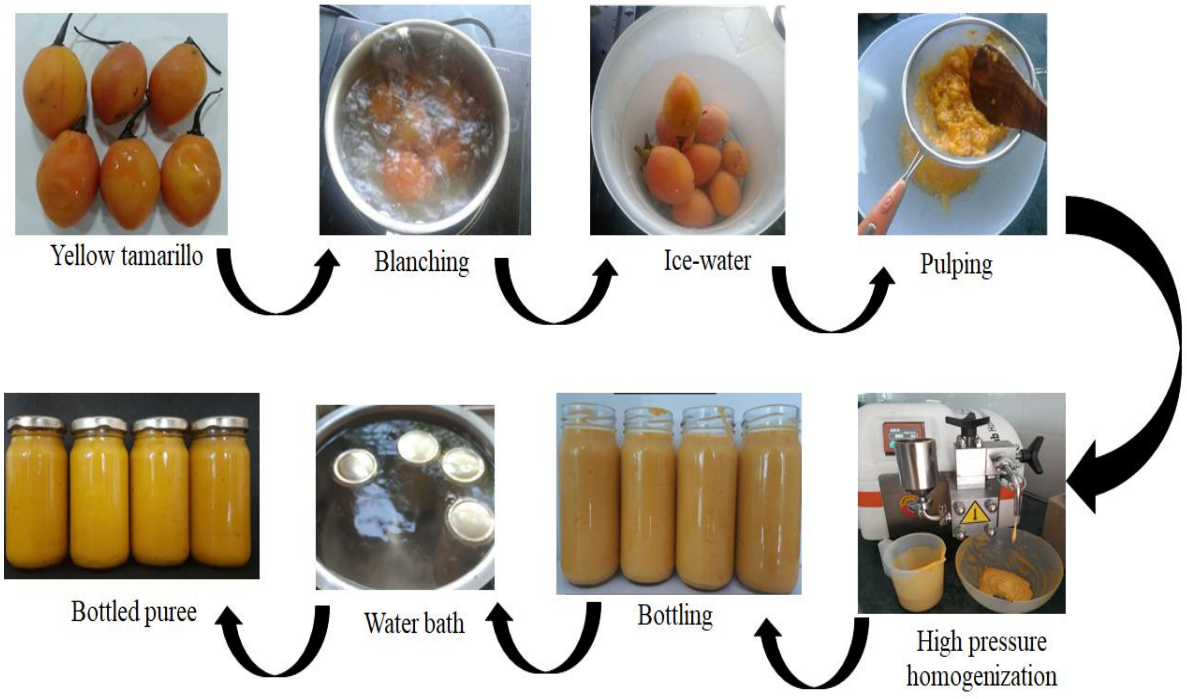


Fig. 2.1: Schematic diagram of process flow chart of bottled tree tomato puree

9.2.2 Methods

9.2.2.1 Preprocessing

Tree tomatoes of similar maturity were selected for the processing of the puree. Briefly, the tree tomatoes were washed with clean water and kept for thawing at room temperature.

Thawed tree tomatoes were blanched (90 s) using boiling water (95-100 ºC), and directly put in ice water bath. The blanched tree tomatoes were peeled and further processed into puree form using mixer grinder (Philips HL Model No. 1632). Then, the extra virgin olive oil (5 % w/w) was added into the puree and the mixture was blended properly into puree in a mixer grinder. The prepared puree was immediately collected in glass bottles and kept at 4-8ºC until further analysis.

9.2.2.2 High pressure homogenization (HPH)

A high pressure homogenizer (Panda 2K, Gea Niro Soavi, Mechelen, Belgium) was used for the treatment of tree tomato puree. The homogenizer was equipped with two valves; by adjusting these two valves the pressure of the homogenizer was set. Puree was subjected to three passes in the homogenizer at three different pressures (500,700, and 1000 bar). The HPH treated puree was collected in clean bottles for further analysis. Fresh puree sample was prepared prior to each experiment.

9.2.2.3 Light microscopy

The microstructure of the tree tomato puree was observed using a light microscope. The HPH treated samples was diluted using distilled water (1:10) and staining of the cells was done using methylene blue. The samples were placed on a glass slide and observed at magnification of 10x. A digital camera was used for the capturing the image of the HPH treated samples.

9.2.2.4 Colour measurement

The colour of the HPH treated puree was noted using Hunter colorimeter (Ultrascan, VIS-Hunter Associates Lab, USA). The L* (lightness), a* (red-green) and b* (yellow-blue) values of non-treated and HPH treated samples were recorded.

9.2.2.5 Particle size analysis

The particle size analysis of the puree was determined using laser diffraction method using a Malvern Mastersizer (Model MSS, Malvern Instruments Ltd.). The tree tomato puree samples were dispersed in distilled water and measurement was recorded.

9.2.2.6 Sample preparation for antioxidant properties

For antioxidant activities, 1 g of tree tomato puree was mixed into 80% of acetone and stirred for 30 min. After 30 min, the sample extract was filtered using Whatman filter paper no 4 and the extract was stored at 4ºC until further analysis.

9.2.2.7 Total phenolic content

Briefly, 0.5 mL of sample extract was taken and mixed with 2.5 mL of Folin-Ciocalteau (1:10 in water). After 5 min, 2 mL of 7.5 % sodium carbonate was added in the test tube and kept for incubation for 2 h at room temperature in dark. The absorbance of the sample was read at 725 nm using spectrophotometer. Gallic acid was used as the standard and results were expressed in mg GAE (gallic acid equivalent)/100g of the sample.

9.2.2.8 DPPH radical scavenging activity

The DPPH radical scavenging activity of the HPH treated sample was determined. Briefly, 0.2 mL of sample extract was mixed with 2.8 mL of 10^{-4} M DPPH reagent. The sample was kept for incubation for 30 min at room temperature and absorbance of the sample was read at 517 nm.

$$\boldsymbol{DPPH\ radical\ scavenging\ activity} = \frac{A_c - A_s}{A_c} \times 100$$

here, A_c stands for absorbance of control and A_s stands for absorbance of sample

9.2.2.9 Determination of total carotenoids concentration

Briefly, 1 g of tree tomato puree was taken, followed by the addition 10 mL of extraction solvent (50% hexane, 25% acetone, 25% ethanol, containing 0.1% BHT) were added. The solution was centrifuged at 6700 g at 10 min at 4ºC, the top layer of the solution was collected very carefully and adjusted to 10 mL with hexane. The results of total carotenoids content in the tree tomato was expressed as mg β-carotene (mg β-CE)/100 g of fruit sample.

9.2.2.10 HPLC of phenolic acids

The phenolic acids present in HPH treated puree was identified and quantified using UHPLC (Ultimate 3000, Thermo Scientific, USA), C18 column, and diode array detector at multiple wavelengths. The sample extract was filtered through 0.45 µm syringe filter prior to the injection. The gradient mode consisting of two solvents: A (0.1 % formic acid) and B (100 % acetonitrile) at 35 °C was used for identification. The flow rate of the solvent was kept at 0.5 mL/min at 330 nm wavelength and the gradient flow pattern was 15% B for 5 min, 20–35% B for 10 min, 35–50% B for 10 min, 50–60% B for 5 min, and 60% B for 5 min and re-equilibration to initial solvent parameter.

9.2.2.11 HPLC of carotenoids

The carotenoids in the tree tomato puree were identified and quantified using UHPLC (Ultimate 3000, Thermo Scientific, USA) with the help of internal standards in reverse phase in C30 column and diode array detector and calibration curve was developed. The sample extract was filtered through 0.45 µm syringe filter prior to injecting. A gradient mode consisting of two solvents, A (methanol/acetonitrile/water 84:14:4, v/v/v) and solvent B (dichloromethane) at 25ºC was used. The flow rate of the solvent was kept at 1 mL/min at a wavelength of 450 nm using gradient flow rate with 100% A and 0% B initially, raised to 10% B at 4 min, 18% B at 12 min, 21% B at 17 min, 30% B at 20 min and maintained until 25 min, increased further to 39% B at 28 min, 60% B at 40 min and returned to initial solvent parameters.

9.2.2.12 Thermal pasteurization of puree

The prepared culture was mixed with sterile autoclaved puree (1:10) and kept for 30 min. For thermal pasteurization studies, the temperatures chosen were 65, 75, 85 and 95 °C while the time intervals chosen were 0, 5, 10, 15 and 20 min. Digital thermometer and water bath were used for determination of the D value.

Test microorganisms for the tree tomato puree

The test microrganisms included *E. coli, L. monocytogenes, S. aureus and B. cereus*. Each microorganism was separately inoculated for respective determination of D value. Pure culture of individual test microorganism was inoculated from stock culture into sterile 50 mL nutrient broth with the help of a sterile loop and incubated at 37 °C at 50 rpm for 18–20 h. After incubation, the bacteria were harvested by centrifugation of 1 mL of nutrient broth suspension at 4000 rpm (1700 x g) for 25 min at 25 °C. The cells were then washed twice with saline water (0.9% w/v) and absorbance of the all the microbial cultures was adjusted to 0.400 Au at wavelength of 600 nm.

9.2.2.13 In-vitro digestion of puree

Briefly, 5 g of tree tomato puree was taken in 50 mL falcon tube, followed by the addition of 5 mL NaCl and ascorbic acid solution (0.9% NaCl and 1% ascorbic acid in water) and 5 mL of stomach electrolyte (0.3% NaCl, 0.15% $CaCl_2.2\ H_2O$, 0.11% KCl, 0.05% KH_2PO_4, 0.07% $MgCl_2.6\ H_2O$ in water) was added. After this, 5 mL of pepsin solution (0.52% pepsin from porcine gastric mucosa in electrolyte) was added. The pH of the solution was adjusted to 4.0 ± 0.05 and headspace was flushed with nitrogen and sample was incubated at 37 ºC for 30

min in dark and sample was stirred continuously. Change in pH was adjusted to 2 ± 0.05 and sample was again kept for incubation for 30 min at 37ºC in dark. This step initiates the second part of the digestion in the small intestine, here the pH of the solution was adjusted to 7.00 by the addition of 3 mL of pancreatin/bile solution (0.4% porcine pancreas pancreatin, 0.2% porcine pancreas lipase, 2.5% porcine bile extract, 0.5% pyrogallol,1% α-tocopherol in water) and samples were kept for incubation for 2 h at 37 °C.

9.2.2.14 Recovery index and bioaccessibility

The recovery index of phenolics and bioaccessibility was calculated. The recovery index and bioaccessibility was calculated, respectively.

$$Recovery = \frac{A}{C} \times 100$$

$$Bioaccessibility = \frac{B}{C} \times 100$$

Here, *A* stands for bioactive compounds released after gastric digestion,
B stands for bioactive compounds released after intestinal digestion, and
C stands for bioactive compounds present in the food before digestion.

9.2.2.15 Statistical analysis

All the experiments were done in triplicate and results are expressed in mean ± standard deviation. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were conducted using SPSS 8.0 (SPSS, INC., USA). Results were considered to be significantly different at $p < 0.05$.

9.2.3 Results and discussion

9.2.3.1 Particle size

The particle size of the puree was analyzed and it was found that increase in pressure had significant impact on the particle size (Table 2.1). It was found that the particle size of the puree taken as control sample was 2048 nm, which decreased to 790 nm after HPH treatment at 500 bar. It was clearly observed that increase in pressure and consecutive passes of the puree through homogenizer showed decrease in particle size.

This clearly indicates that application of HPH treatment helped in breaking the cells and causing decrease in size. Further, increase in pressure of homogenization and consecutive

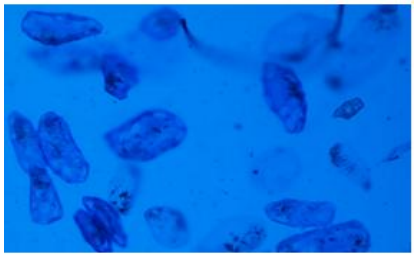
passes at constant pressure showed positive effect on particle size reduction of the puree. Increase in the number of passes and pressure cause a decrease in the particle size of the tree tomato puree which is desirable and required for better bioaccessibility of phytochemicals.

Table 2.1: Mean particle diameter of HPH treated tree tomato puree

Sample code	Particle size (nm)
Control	2804
500 -1P	790
500-2P	635
500-3P	587
700-1P	609
700-2P	541
700-3P	519
1000-1P	523
1000-2P	500
1000-3P	499

9.2.3.2 Microstructure

In Fig. 2.2, the microstructure of control (untreated) tree tomato puree and samples treated with HPH. A clear difference in the sample can be observed between the samples treated with HPH and control samples. Increase in pressure helps to degrade the cell structure, which is directly related to decrease in particle size, and helps in enhanced release of bioactive compounds from the cells. Similar results of the decrease in intact cells with increase in the pressure were reported for tomato puree. Pressure of 1000 bar and 3 passes helped in attaining the desired particle size which is required to enhance the bioaccessibility of bioactive compounds of puree.



Control

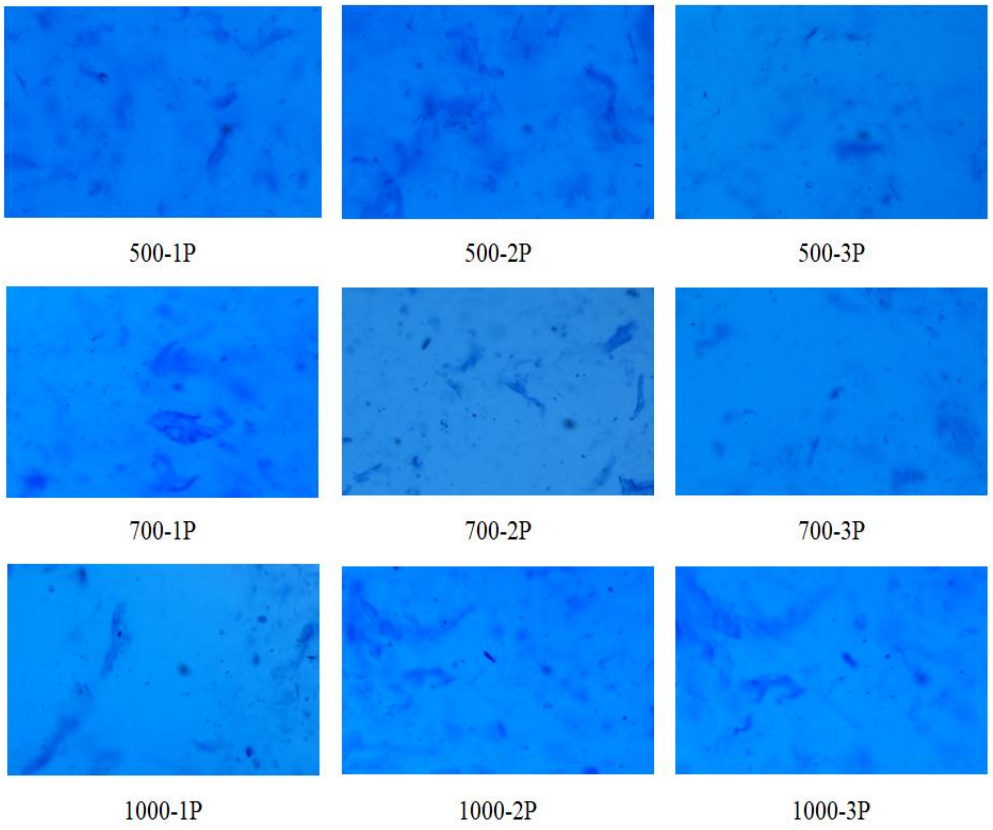


Fig. 2.2: Light microscopy of HPH treated tree tomato puree

9.2.3.3 Colour analysis

The colour analysis of HPH treated puree is reported in Table 2.2. Significant difference ($p < 0.05$) was observed among the samples. Increase in the pressure caused changes in the lightness of the puree, as in control sample the lightness was 55.32 and puree subjected to HPH at 500 bar changes it to 76.44. The increase in the lightness is clearly subjected to however, in the case of 1000 bar for 2nd & 3rd pass, the lightness of the puree was found to non-significant ($p < 0.05$). The results of lightness were found with the correlation of study reported for processing of pumpkin puree using high pressure. The redness of the puree was found to be decreased with the HPH treatment parameters values were increased. The decrease in the redness may be due to heating factor, because as the pressure of the puree was increased, increase in the temperature was there inside the instruments which leads to decrease in the redness of the puree. Our results of decrease in the redness values was found to be correlated with the results reported for pumpkin puree, in which highest redness value was shown by control sample and puree treated at a combination of low pressure and low temperature. The yellowness of the puree was due to the presence of the carotenoids in the

tree tomato of the puree, and by increase the processing parameters (pressure and passes) positive results in the yellowness was found. The increase in b* value may be to the addition of oil which helps in emulsification and cause to amplify the carotenoids concentration by breakdown of the cell tissue and solubilizing the carotenoids in whole puree from the inside of cell. Our results of disruption of cell wall and decrease in the diameter of cell were confirmed by the results of particle size and light microscopy respectively.

Table 2.2. Colour values of HPH treated puree

Sample code	L*	a*	b*	ΔE
Control	55.32 ± 0.16^{c}	10.25 ± 0.11^{a}	52.31 ± 0.15^{a}	0
500 -1P	76.44 ± 0.16^{f}	9.49 ± 0.14^{b}	53.31 ± 0.14^{b}	21.33 ± 0.15^{e}
500-2P	77.04 ± 0.12^{e}	9.77 ± 0.18^{c}	53.32 ± 0.15^{b}	21.92 ± 0.11^{d}
500-3P	77.61 ± 0.32^{d}	9.64 ± 0.16^{c}	54.45 ± 0.21^{c}	22.13 ± 0.29^{d}
700-1P	78.26 ± 0.25^{c}	9.53 ± 0.11^{c}	54.94 ± 0.08^{d}	23.28 ± 0.23^{c}
700-2P	78.36 ± 0.14^{bc}	8.71 ± 0.12^{d}	55.61 ± 0.14^{e}	25.31 ± 0.11^{c}
700-3P	78.63 ± 0.36^{b}	8.49 ± 0.09^{de}	57.64 ± 0.26^{f}	24.18 ± 0.28^{b}
1000-1P	78.62 ± 0.26^{b}	8.29 ± 0.11^{de}	57.93 ± 0.33^{f}	24.25 ± 0.16^{b}
1000-2P	79.33 ± 0.26^{a}	8.09 ± 0.18^{de}	59.66 ± 0.15^{g}	25.41 ± 0.19^{a}
1000-3P	79.39 ± 0.25^{a}	7.94 ± 0.11^{e}	59.87 ± 0.33^{g}	25.54 ± 0.13^{a}

9.2.3.4 Total phenolic content

Significant differences in TPC were observed (Table 2.3). The phenolic content in the control puree was 5.40 mg GAE/g whereas, HPH treated puree at 500 bar recorded 5.88 mg GAE/g. It was clear that application of HPH in the puree helped to increase the phenolic content of the puree. However, no difference ($p < 0.05$) was observed after treatment at 700 bar pressure up to 3 passes. But, as the pressure was increased to 1000 bar, there was an increase in the phenolic content; however, increase in number of passes at 1000 bar pressure did not show any statistically different phenolic content. Similar results of increasing phenolic content from 582 to 662 in two passes was reported for strawberry juice as the pressure of was increased from 60 to 100 MPa. The increase in the phenolic content with increase in pressure and passes is related to the distortion and cell rupture, which helps in increasing the release of bioactive compounds in the extraction solvent.

9.2.3.5 DPPH radical scavenging activity

The DPPH radical scavenging activity of the HPH treated puree was analyzed, and significant difference in the radical scavenging activity was noticed (Table 2.3). The radical activity in the control puree was found to be 23.62 % and after applying HPH, the radical activity of the puree had increased gradually with increase in pressure and number of passes. Highest radical scavenging activity was found in the sample treated at 1000 bar with 3 passes. Our results of increase in antioxidant activity were found to correlate with the study done on strawberry juice, lettuce waste and wheat bran.

9.2.3.6 Total carotenoids content

Significant difference in TCC was observed (Table 2.3) in the total carotenoids content of the HPH treated puree. Increase in pressure and number of passes showed positive impact on the carotenoids content. Pressure helped in the breakdown of tissues into smaller particles that favored the release of bioactive compounds in extraction solvents. Puree subjected to 1000 bar pressure showed maximum extraction of total carotenoids content.

Table 2.3. Phenolic, in vitro-antioxidant capacity and total carotenoids content of HPH treated tree tomato puree

Sample code	TPC (mg GAE/g)	DPPH radical scavenging activity (%)	Total carotenoids content (mgβCE/100g)
Control	5.40 ± 0.41^{b}	23.62 ± 0.25^{d}	0.65 ± 0.02^{f}
500 -1P	5.88 ± 0.45^{ab}	28.55 ± 0.28^{c}	0.69 ± 0.02^{e}
500-2P	5.89 ± 0.54^{ab}	29.42 ± 0.32^{b}	0.71 ± 0.02^{de}
500-3P	5.95 ± 0.48^{ab}	29.99 ± 0.12^{bc}	0.73 ± 0.01^{bcd}
700-1P	6.01 ± 0.41^{ab}	30.38 ± 0.23^{ab}	0.76 ± 0.02^{abc}
700-2P	5.94 ± 0.41^{ab}	30.48 ± 0.23^{ab}	0.76 ± 0.01^{abc}
700-3P	5.98 ± 0.32^{ab}	30.64 ± 0.21^{ab}	0.73 ± 0.02^{bcd}
1000-1P	6.19 ± 0.15^{a}	30.77 ± 0.54^{a}	0.79 ± 0.02^{a}
1000-2P	6.17 ± 0.24^{a}	30.88 ± 0.71^{a}	0.79 ± 0.02^{a}
1000-3P	6.21 ± 0.30^{a}	31.04 ± 0.46^{a}	0.77 ± 0.03^{ab}

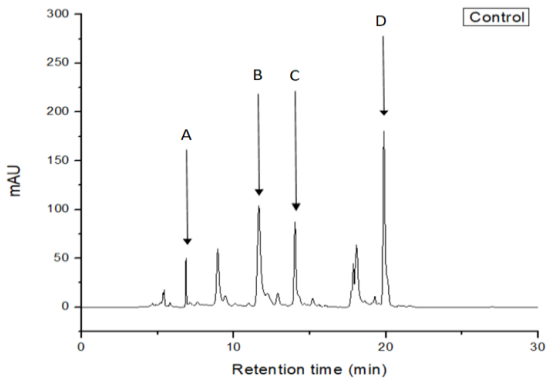
9.2.3.7 HPLC analysis of the phenolic acids

The concentration of the phenolic acids identified in the puree is given in Table 2.4. The identified phenolic acids in the puree were gallic acid, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, and rosmarinic acid. It was found that increase in pressure led to

an increase in the phenolic acid concentration, which can be attributed to the reduction of particle size and thereby easy extractability of the bioactive compounds (Fig. 2.3). Another reason for the increase in phenolic content may be due to the release of bound phenolic compounds after HPH treatment.

Table 2.4. Phenolic acid concentration in the HPH treated tree tomato puree

Sample code	Phenolic acids (µg/g)			
	Gallic acid	Chlorogenic acid	Caffeic acid	p-coumaric acid
Control	85.77	172.07	47.42	50.67
500-1P	128.68	230.41	56.68	81.70
500-2P	126.10	223.09	56.42	80.80
500-3P	54.74	221.06	57.60	87.51
700-1P	117.82	246.47	58.96	92.91
700-2P	58.36	243.82	61.70	100.67
700.-3P	110.07	252.65	60.04	95.41
1000-1P	112.14	254.26	62.54	103.30
1000-2P	112.14	233.43	60.04	100.67
1000-3P	117.82	249.01	56.45	90.15



HPLC analysis of the phenolic acids present in untreated tree tomato puree

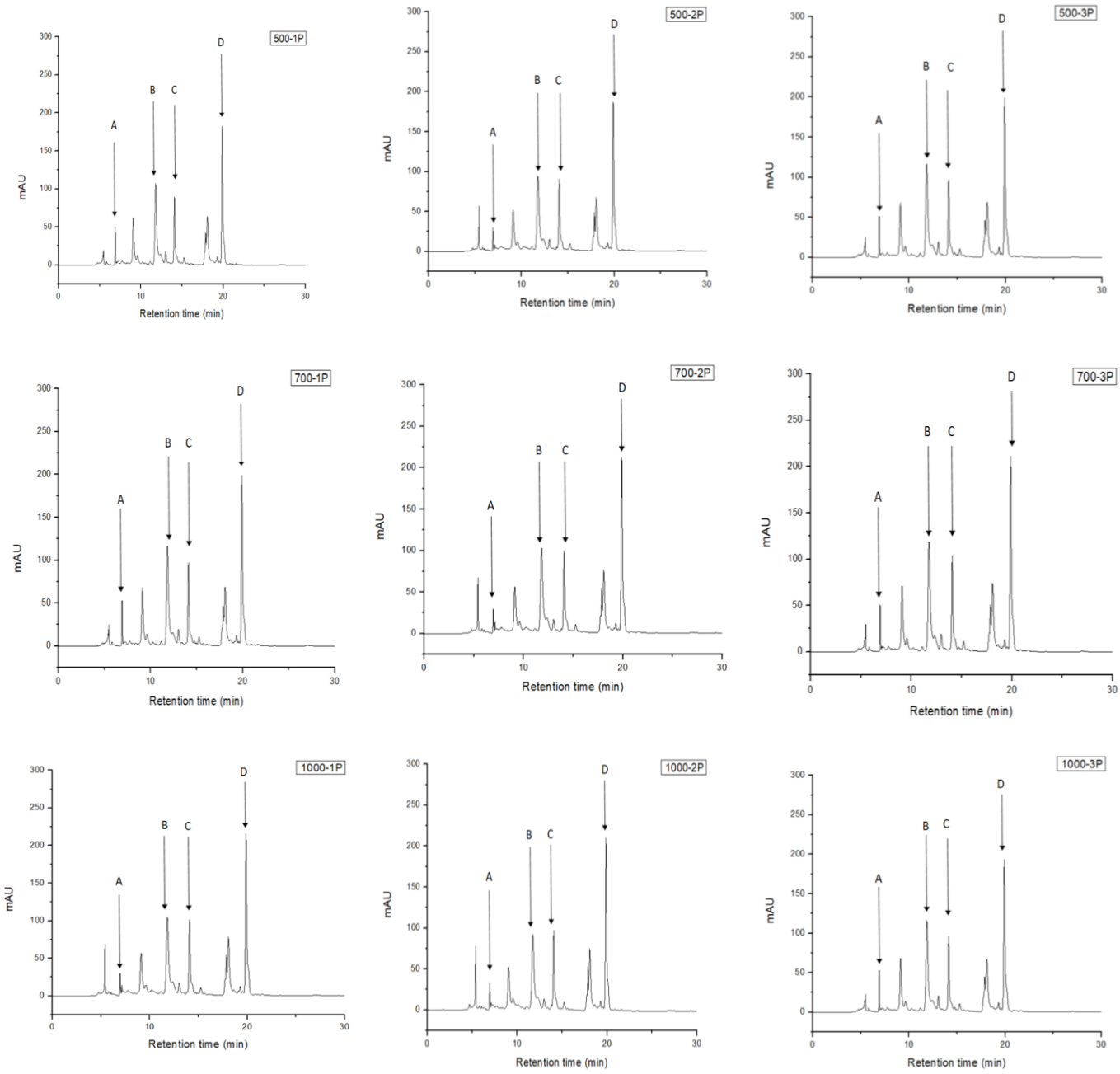


Fig. 2.3: HPLC analysis of the phenolic acids present in HPH treated tree tomato puree

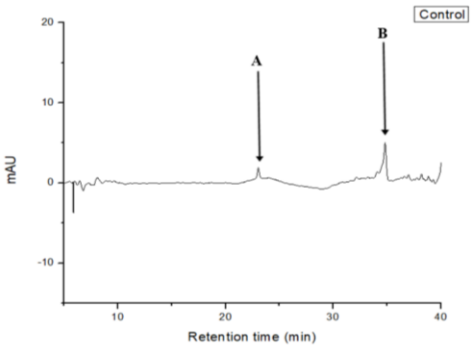
9.2.3.8 HPLC analysis of carotenoids

It was observed that HPH favored the release of carotenoids from the sample matrix. The identified carotenoids found in the tree tomato puree were β-cryptoxanthin and β-carotene (Fig. 2.4). It was observed that with an increase in pressure and number of passes, carotenoids content increased in comparison to the control sample. The highest concentration of β-cryptoxanthin was 150 µg/100g in 700-3P, and β-carotene was 299 µg/100g in 1000-1P

(Table 2.5). But the highest release of phenolic acids and β-carotene (major carotenoid) was in 1000-1P. Thus, pressure of 1000 bar was selected for processing of tree tomato puree.

Table 2.5: Carotenoids concentration in the HPH treated tree tomato puree

Sample code	Carotenoids (µg/100g)	
	β-cryptoxanthin	β-carotene
Control	74.27	209.01
500-1P	78.42	222.57
500-2P	80.50	287.57
500-3P	76.35	306.86
700-1P	140.66	266.14
700-2P	134.44	280.43
700.-3P	150.41	287.76
1000-1P	144.18	299.83
1000-2P	134.03	284.47
1000-3P	142.27	256.69



HPLC analysis of carotenoids present in untreated tree tomato puree

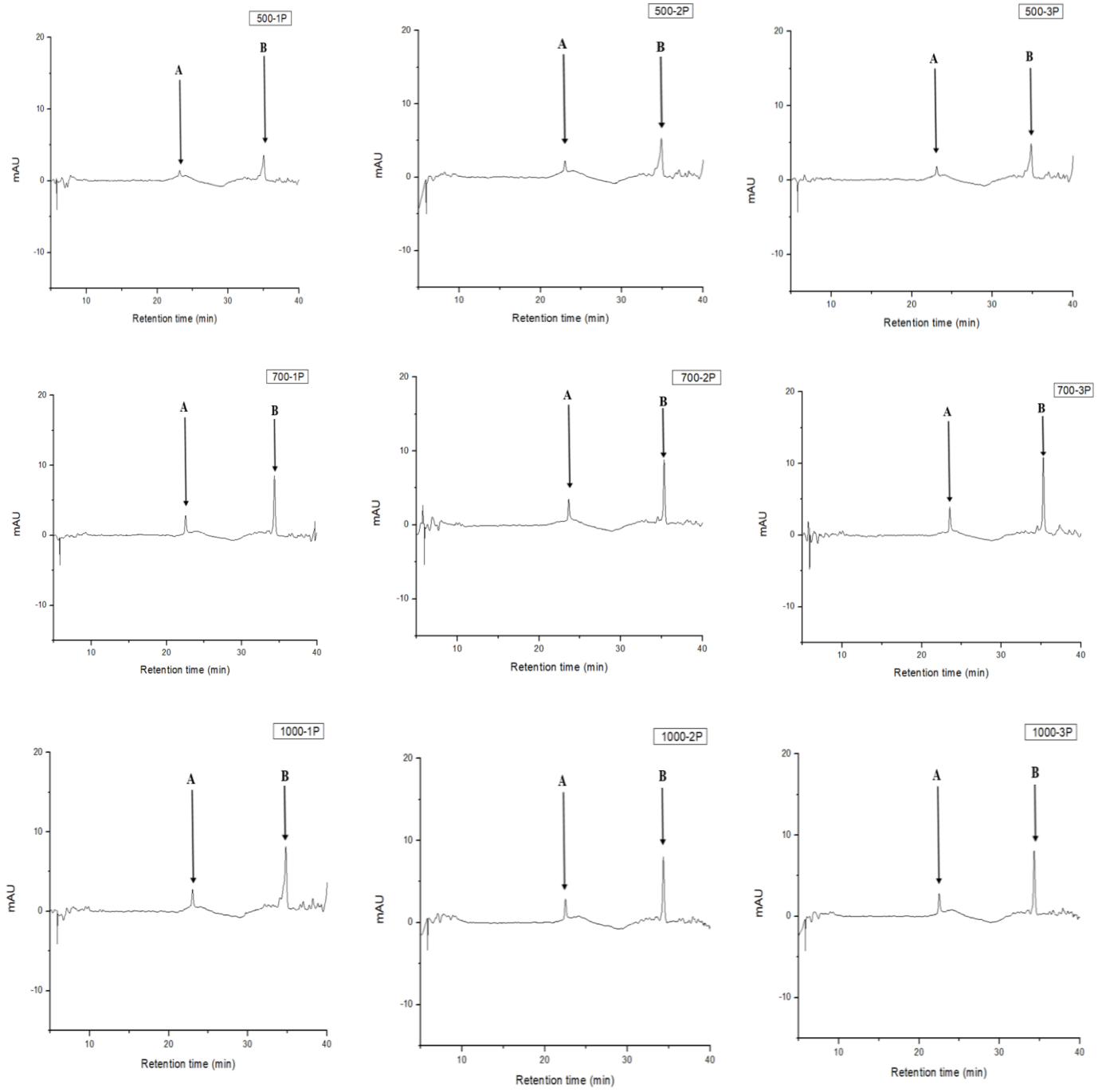


Fig. 2.4: HPLC analysis of carotenoids present in HPH treated puree

9.2.3.9 Thermal death time (D-value)

In Table 2.6, the thermal reduction time (D-value) of the different microorganisms is presented. Three different microorganisms were cultured and inoculated in the puree for determination of the D value. Each microorganism has different thermal death time; therefore, it is important to calculate the D value. It was found that an increase in the temperature of the thermal treatment led to a decrease in the D value of the product, which may be due to the combined effect of acids present in the puree and treatment temperature.

L.monocytogenes showed highest D value followed by *B.cereus*, *S.aureus*, and *E. coli*, in that order. Higher temperature causes minimum damage to the product because of their less holding time. Therefore, based on D values, 95 °C temperature was selected for the thermal treatment of the tree tomato puree.

Table 2.6: Determination of D value of the tree tomato puree

	D value (min)			
Microorganism	**65 °C**	**75 °C**	**85 °C**	**95 °C**
Escherichia coli	7.17	5.02	3.91	2.64
Staphylococcus aureus	7.20	6.25	3.44	2.70
Listeria monocytogenes	10.92	7.69	3.88	3.22
Bacillus cereus	5.61	5.18	3.58	3.12

9.*2.3.10 In-vitro* digestion of tree tomato puree

The *in-vitro* digestion of the HPH treated tree tomato puree skipping the mastication part was done using INFOGEST method. It was observed that concentration of phenolic acids decreased from gastric to intestinal phase, while the carotenoids were found to increase gradually from gastric to intestinal phase. Researchers explained that factors such as pH, temperature, enzymes and experimental surrounding conditions play important role in affecting the release of the bioactive compounds. Initially, action of enzymes giver higher yields of phenolic acids in gastric phase whereas, carotenoids are pH sensitive therefore low release of the carotenoids content in gastric phase was observed. But, in intestinal phase (pH 7.0) reduces the release of phenolic acids but, carotenoids being stable at neutral pH are released in greater amounts. Further, the bioaccessibility of both the phenolic acids as well as the carotenoids was observed to be lower in the treated samples.

9.2.3.11 Storage study of the puree

The storage study of 1000-1P thermally treated at 95 °C was stored at 25 °C. Initially, up to 60 days no growth was observed in the processed tree tomato puree. Researchers claimed that the acceptable CFU recommended for the tomato products is 10^3 CFU/mL, however our puree shows and found to be acceptable. As seen from Table 2.7, the content of phenolic

compounds, flavonoids and carotenoids, and antioxidant activity content were gradually reduced with increase in the storage period at 25 °C.

Table 2.7. Effect of storage on phytochemicals content and microbial load in the tree tomato puree.

Storage time (days)	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	Total carotenoids content (mg βCE/100g)	DPPH radical scavenging activity (%)	Total plate count (Log CFU/mL)
0	5.85 ± 0.09^{a}	1.01 ± 0.01^{a}	1.02 ± 0.02^{a}	29.23 ± 0.21^{a}	1.30
30	4.89 ± 0.07^{b}	0.81 ± 0.03^{b}	0.89 ± 0.03^{b}	23.16 ± 0.22^{b}	1.30
60	4.03 ± 0.09^{c}	0.68 ± 0.02^{c}	0.84 ± 0.03^{c}	21.62 ± 0.16^{c}	1.78
90	3.54 ± 0.12^{d}	0.61 ± 0.02^{c}	0.81 ± 0.02^{c}	18.56 ± 0.27^{c}	2.60
120	3.17 ± 0.07^{e}	0.54 ± 0.03^{e}	0.68 ± 0.03^{d}	15.15 ± 0.16^{e}	2.90

9.2.4 Conclusion

Our result suggested that HPH at 1000 bar with single pass followed by thermal treatment of 95 °C will be most suitable for processing of tree tomato puree. Increase in HPH pressure had positive impact on phytochemicals and addition of oil to the puree enhanced the retention of carotenoids content. The processed tree tomato puree was found to be microbiological acceptable up to 4 months and had better *in- vitro* bioaccessibility of carotenoids.

9.3.0 Objective 3

To obtain antioxidant rich extract from the unutilized peel of tree tomato and determine its stability

9.3.1 Introduction

Antioxidant rich extract was extracted from tree tomato peel using two techniques, namely supercritical fluid extraction, and ultrasound assisted extraction. The extraction parameters for both the parameters were optimized. The extracts were analyzed for total phenolic content and total anthocyanins content and antioxidant activity.

9.3.2 Methods

9.3.2.1 Sample preparation

Tree tomato was procured from the local market of Gangtok, Sikkim, India and transported to Tezpur University. Fruits were sealed in low-density polyethene zip pouch and stored in a plastic container in a deep freezer (-20 ºC). Freeze-dried powder was prepared just before extraction. For freeze-dried powder preparation, frozen fruits were allowed to thaw at RT (room temperature) and rinsed with clean water. The pulp of tree tomato was scraped off from the peel with the help of a spoon. In the process, some pulp remains adhered to the peel. Peel with the attached pulp of tree tomato was freeze-dried (Lyo Lab, Lyophilisation Systems, Inc.) for 12 h. The freeze-dried peel was powdered using household mixer grinder (Crompton Greeves), passed through a standard sieve mesh (425 μm) and stored in an air-tight container at -20 °C until further analysis.

9.3.2.2 Supercritical fluid extraction (SCFE)

SCFE was carried out in Waters (SCF 100) system. The modifier (acidified co-solvent; mixture of ethanol and distilled water) that was brought to pH 2.0 with citric acid was used in the ratio of 1:1. Sample weight taken was 2g. The flow rate of modifier was 1 mL/min and flow rate of CO_2 was 5mL/min, and was kept constant throughout the study.

In SCFE, major parameters that play important role in extracting the extractable compounds from the sample matrix are time, temperature, pressure, and the flow rate of supercritical CO_2 and modifier. For determining the time for SCFE, 2 g sample was taken and temperature and pressure were kept at 50 °C and 165 bar, respectively. For determination of temperature, the time and pressure were kept at 45 min and 165 bar, respectively. For pressure, the time and temperature were kept at 45 min and 50 °C, respectively. The limits of all the parameters were selected on the basis of maximum total phenolic content obtained in the extract.

9.3.2.2 Ultrasound-assisted extraction (UAE)

UAE was carried out in a probe type ultrasonicator (TAKASHI Model No U-500, JAPAN) with power of 500 W, and amplitude range from 0-100 %, at constant frequency of 20.5 KHz. For extraction, 2 g of freeze dried powder sample was added to 50 mL of acidified co-solvent mixture (pH 2.0).

Time, temperature, and amplitude were the independent variables selected for optimization. For determining the time, 1g sample was mixed in 25 mL of extraction solvent and the mixture was treated for 5-40 min and temperature and amplitude were kept at 50 °C and 50%, respectively. For determining the temperature, sample was mixed in extraction

solvent (1:25), the temperature was varied from 35-65 °C, and time and amplitude was 15 and 50%, respectively. For amplitude, extraction was done for 15 min at 50 °C and the amplitude was varied from 10-80%. The limits of all the three independent variables were decided on the basis of total phenolic content.

9.3.2.3 Extraction of bioactive compounds

A three level Box-Behnken design with three independent variables was used for the optimization of parameters for extraction of anthocyanins and phenolic compounds by SCFE and UAE methods. Total phenolic content and total monomeric anthocyanins content were taken as the responses for optimization of extraction of bioactive compounds using SCFE and UAE.

9.3.2.4 Total phenolic content (TPC)

An aliquot of 0.5 mL of sample extract was taken in a test tube followed by addition of 2.5 mL of Folin Ciocalteu reagent (1:10 diluted with distilled water) and allowed to stand for 5 min, then 2 mL of sodium carbonate anhydrous (7.5%) was added to each of the test tubes and then vortexed. The absorbance was noted down after 2 h of incubation in the dark (test tubes were covered by aluminum foil) to avoid contact with air using UV-Visible spectrophotometer (CECIL Aquaris 7400) against a reagent blank at 725 nm. Gallic acid was used as the standard and amount of total phenolic content was expressed as mg GAE (Gallic acid equivalents) /g of tree tomato fruit.

9.3.2.5 Total monomeric anthocyanin content (TMAC)

Total monomeric anthocyanin content was measured by the pH differential method. One reagent of pH 1.0 using potassium chloride (1.86 g/L di-ionized water) and another reagent of sodium acetate buffer of pH 4.5 (32.8 g/L of di-ionized water) were prepared. The sample extract was added to the above-prepared reagents; dilution factor was noted while adding the extract. The absorbance of each sample mixture was taken at wavelengths 520 and 700 nm in triplicate. Anthocyanins content was calculated as mg cyanidin-3-glucoside (C3G)/g of dry material using Eq. 3.1 and 3.2.

$$Anet = Abs510 - Abs700 \tag{3.1}$$

$$Anthocyanin\ \left(\frac{mg}{g}\right) = \frac{Anet}{26900}.MW.DF.\frac{V}{Wt} \tag{3.2}$$

Where, *Anet* is net absorbance, *MW* (449.2) is molecular weight, *DF* is dilution factor, *V* is volume of sample, Wt is weight of sample used for extraction, and 26900 is molar absorptivity of cyanidin-3-glucoside (C3G).

9.3.2.6 HPLC analysis of phenolic acids

The phenolic acids in the sample extract were identified and quantified using reverse phase UHPLC (Utimate 3000, Thermo Scientific, USA) with the help of standards in C18 (4.6 × 250 mm) column and diode array detector. The sample extract was filtered through 0.45µm syringe filter prior to injecting. A gradient mode consisting of two solvents, A (0.1% formic acid) and B (acetonitrile) at 35 ºC was used. The flow rate of the solvent was kept at 0.5 mL/min and wavelength was kept at 330 nm and the gradient flow pattern was 15% B for 5 min, 20–35% B for 10 min, 35–50% B for 10 min, 50–60% B for 5 min, and 60% B for 5 min.

9.3.2.7 HPLC analysis of anthocyanins

The anthocyanins in the sample extract were identified and quantified using UHPLC (Utimate 3000, Thermo Scientific, USA) with the help of anthocyanin standards in reverse phase in C18 column using diode array detector and a calibration curve was developed. The sample extract was filtered through 0.45 µm syringe filter prior to injecting. A gradient mode consisting of two solvents, solvent A (0.1% triflouroacetic acid) and B (acetonitrile) at 35ºC was used. The flowrate of the solvent was kept at 0.5 mL/min at a wavelength of 520nm using gradient flow with 10% B for 3 min, 10–15% B over 12 min, 15% B for 5 min, 15–18% B over 5 min, 18–30% B over 20 min, 30–35% B over 5 min, and re-equilibration to initial solvent parameters.

9.3.2.8 Stability of the anthocyanin

The thermal stability of the optimized extracts using UAE and SCFE were tested. The optimized extract was kept in a falcon tube (50 mL) and heated in a water bath at 50, 70 and 90 °C, and sample was collected at 0.5,1, 2, 3, 4, 5 and 6 h intervals and placed in ice cooled bath to stop further degradation. The analysis was done in triplicate and the amount of anthocyanins present was determined.

9.3.2.9 Scanning electron microscopy

The residue remaining after SCFE and UAE extraction were collected and dried at 40ºC in a hot air oven. The sample was placed on the SEM stubs using double-sided tape and coated

with a thin layer of gold. The SEM images were captured using JSM -6390LV scanning electron microscope (174 JEOL, Japan) at 15kV at 500 and 5500 magnifications.

9.3.3 Results and discussion

9.3.3.1 Determination of the range for independent variables of SCFE

The range of the independent variables was selected based on the effect of experiments trials on TPC (Fig. 3.1). Highest extraction of phenolics that was significantly different was found at 50 min of extraction followed by 55 and 60 min (Fig. 3.1a). Decrease in the TPC occurred at 65 min, probably due to the degradation of phenolic compounds. Highest phenolic content was reported at 55 °C and lowest at 70 °C (Fig. 3.1b). There was significant difference in the phenolic content as the temperature increased from 35 °C to 55°C, which may be due to release and solubility of phenolic compounds in the extracting solvent. Phenolic content increased as pressure was raised to 165 bar and decreased beyond it (Fig. 3.1c). Increase in pressure during extraction causes disintegration of the sample matrix that helps to release the bioactive compounds; however, excessive pressure causes degradation. Therefore, for optimization of bioactive compounds using SCFE, the selected range for time was 30-60 min, temperature was 40-60 °C, and pressure was 150-180 bar.

9.3.3.2 Effect of independent variables on responses using SCFE

The Box-Behnken design was used to optimize TPC and TMAC using supercritical extraction technique. Seventeen experimental conditions and their responses are given in Table 3.1. The phenolic content ranged from 11.65 -16.05 mg GAE/g of dry powder sample. The interaction between the independent variables during TPC extraction is presented in Figure 2 (X1, X2 and X3). It was observed that increase in time from 30 to 45 min showed a positive impact on TPC as also increase in pressure up to a certain extent had a positive impact. TPC increased as the temperature increased from 40 °C to 50 °C and thereafter decreased.

In Eq. 3.3 the interaction of time, temperature, and pressure on TPC are given. In SCFE, modifier plays an important role in extracting the extractable compounds from the sample because it increases the solvating power which ultimately increases the extraction efficiency. Linear effect of all independent variables had a positive impact on extraction of phenolic compounds. The interaction term of time and pressure only had positive effect, whereas

interaction term of time with temperature and temperature with pressure showed negative effects on TPC.

$$TPC_{SCFE} = +15.73 + 0.44 \times A + 0.42 \times B + 0.32 \times C - 0.12 \times A \times B + 0.46 \times A \times C - 0.72 \times B \times C - 0.95 \times A^2 - 1.87 \times B^2 - 0.093 \times 6C^2 \quad (3.3)$$

$$TMAC_{SCFE} = +.0.62 + 0.018 \times A + 02.500\text{E} - 003 \times B + 0.030 \times C + 5.000\text{E} - 003 \times A \times B - 5.000\text{E} - 003 \times A \times C - 0.015 \times \text{B} \times \text{C} - 0.012 \times A^2 - 0.052B^2 + 0.016 \times C^2 \quad (3.4)$$

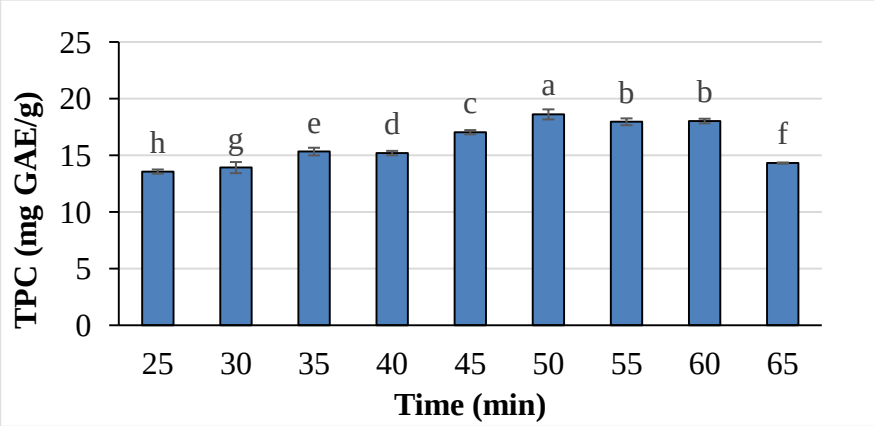
TMAC varied from 0.50-0.63 mg C3G/g dry sample. As seen in Eq. 3.4, during SCFE, pressure and time had positive effects on anthocyanins extraction and temperature showed negative impact. In Fig. 3.2 (Y1, Y2 and Y3), illustrates the effect of the independent variables on the response. However, in Fig. 3.2 Y2 and Y3, increase in pressure with time and temperature increased anthocyanins content. SCFE at higher pressure and temperature not only increases concentration but also stability of anthocyanins. Anthocyanins extraction decreased at 60 °C. High pressure increases the solvating power due to increase in density which leads to an increase extracted anthocyanins.

9.3.3.3 Verification of model for SCFE

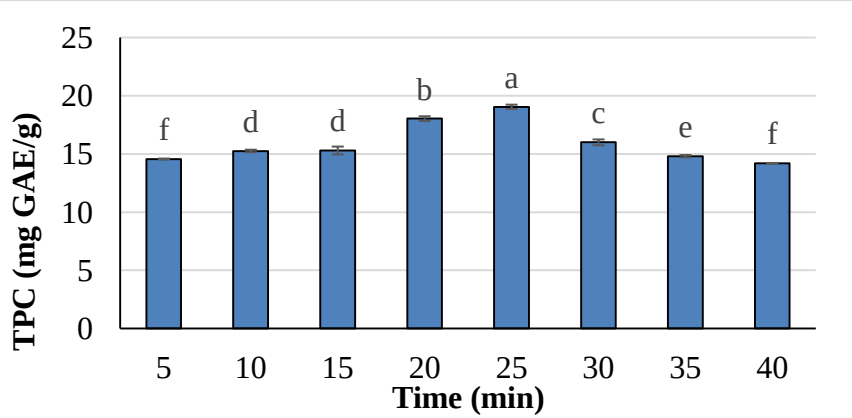
Four different models (Linear, 2 Fi, Quadratic and Cubic) were applied for the optimization of the TPC and TMAC extraction from purple tree tomato using SCFE. Table S2 presents coefficients of regression R^2, Adequate R^2, Predicted R^2 and *p* value of all the models. The adequacy of the individual model was tested using Design Expert 7.0 software. Among the models, Quadratic model was found best for the optimization of extraction of phenolic compounds and anthocyanins content with R^2 value of 0.97 and 0.98, respectively and *p* values being less than 0.0001. A high R^2 value with low *p* value is required for fitting of the model. Even though cubic model showed highest R^2 value, its F value was high and, therefore, quadratic model was selected for optimization. The interactions and effect of independent variables on the response with quadratic model are given in Table S3. The coefficient of variance (CV) values of the model for TPC and TMAC was 2.29 and 1.27 %, respectively in SCFE; CV values being less than 10% indicates that the model satisfies the fitting paramters.

Table 3.1: Experimental data of the responses for SCFE and UAE in BOX-Behnken design.

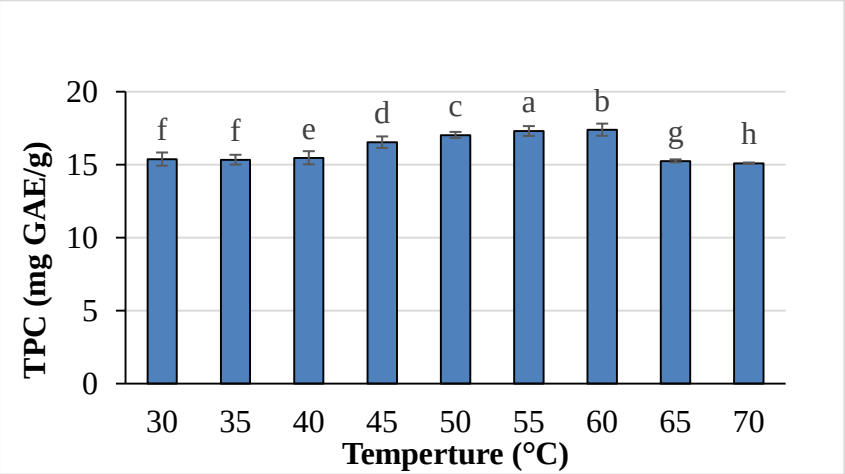
	Supercritical fluid extraction					Ultrasound assisted extraction				
Run	**A Time (min)**	**B Temperature (°C)**	**C Pressure (bar)**	**TPC (mg GAE/g)**	**TMAC (mg C3G/g)**	**A Time (min)**	**B Temperature (°C)**	**C Amplitude (%)**	**TPC (mg GAE/g)**	**TMAC (mg C3G/g)**
1	30 (-1)	40 (-1)	165 (0)	11.65	0.54	10 (-1)	40 (-1)	40 (0)	16.81	0.54
2	60 (+1)	40 (-1)	165 (0)	13.05	0.57	30 (+1)	40 (-1)	40 (0)	16.32	0.59
3	30 (-1)	60 (+1)	165 (0)	13.02	0.53	10 (-1)	60 (+1)	40 (0)	18.45	0.70
4	60 (+1)	60 (+1)	165 (0)	13.95	0.58	30 (+1)	60 (+1)	40 (0)	19.25	0.59
5	30 (-1)	50 (0)	150 (-1)	14.65	0.54	10 (-1)	50 (0)	20 (-1)	17.22	0.69
6	60 (+1)	50 (0)	150 (-1)	14.32	0.58	30 (+1)	50 (0)	20 (-1)	18.96	0.73
7	30 (-1)	50 (0)	180 (+1)	14.15	0.61	10 (-1)	50 (0)	60 (+1)	20.32	0.71
8	60 (+1)	50 (0)	180 (+1)	15.65	0.63	30 (+1)	50 (0)	60 (+1)	18.49	0.69
9	45 (0)	40 (-1)	150 (-1)	12.36	0.50	20 (0)	40 (-1)	20 (-1)	16.38	0.67
10	45 (0)	60 (+1)	150 (-1)	14.32	0.54	20 (0)	60(+1)	20 (-1)	20.73	0.72
11	45 (0)	40 (-1)	180 (+1)	14.65	0.59	20 (0)	40 (-1)	60 (+1)	18.96	0.63
12	45 (0)	60 (+1)	180 (+1)	13.75	0.57	20 (0)	60 (+1)	60 (+1)	20.38	0.74
13	45 (0)	50 (0)	165 (0)	15.65	0.62	20 (0)	50 (0)	40 (0)	20.87	0.69
14	45 (0)	50 (0)	165 (0)	15.36	0.61	20 (0)	50 (0)	40 (0)	20.34	0.68
15	45 (0)	50 (0)	165 (0)	16.05	0.63	20 (0)	50 (0)	40 (0)	20.77	0.68
16	45 (0)	50 (0)	165 (0)	15.96	0.61	20 (0)	50 (0)	40 (0)	21.04	0.69
17	45 (0)	50 (0)	165 (0)	15.64	0.62	20 (0)	50 (0)	40 (0)	19.84	0.71


TPC (mg GAE/g)
25
20
15
10
5
0
h g e d c a b b f
25 30 35 40 45 50 55 60 65
Time (min)

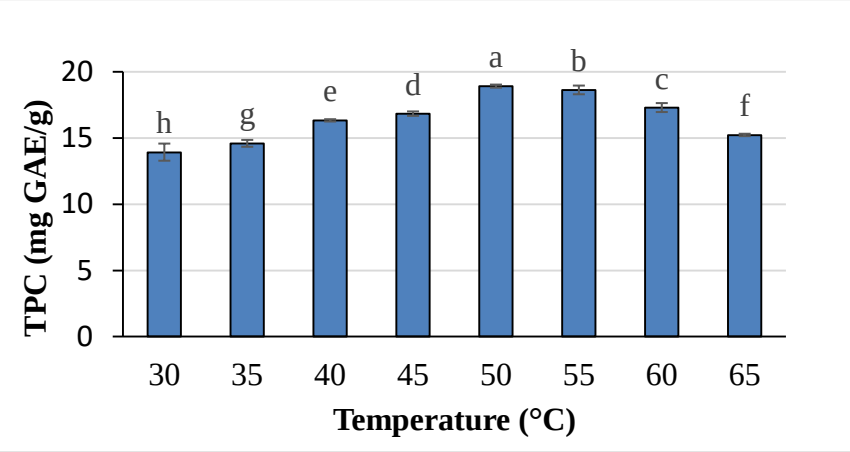
(a)


TPC (mg GAE/g)
25
20
15
10
5
0
f d d b a c e f
5 10 15 20 25 30 35 40
Time (min)

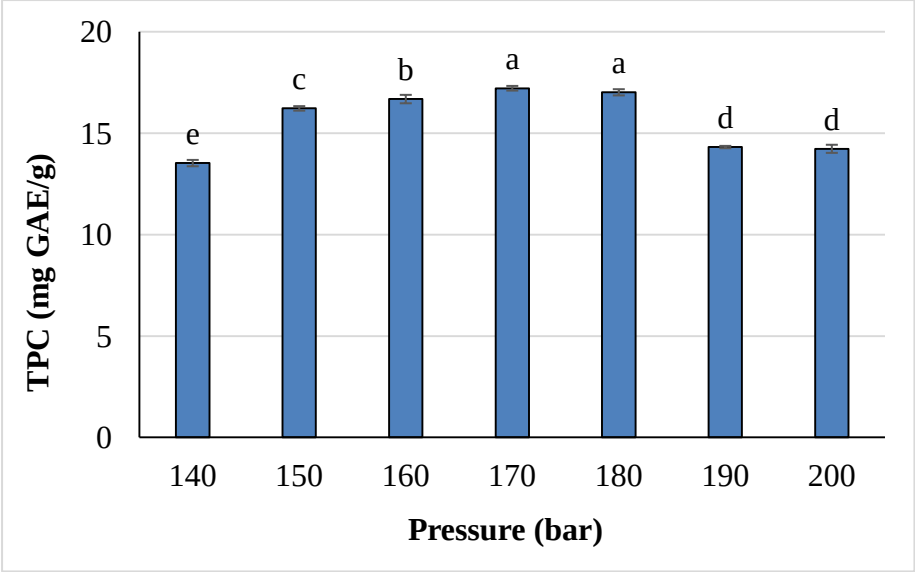
(x)


TPC (mg GAE/g)
20
15
10
5
0
f f e d c a b g h
30 35 40 45 50 55 60 65 70
Temperture (°C)

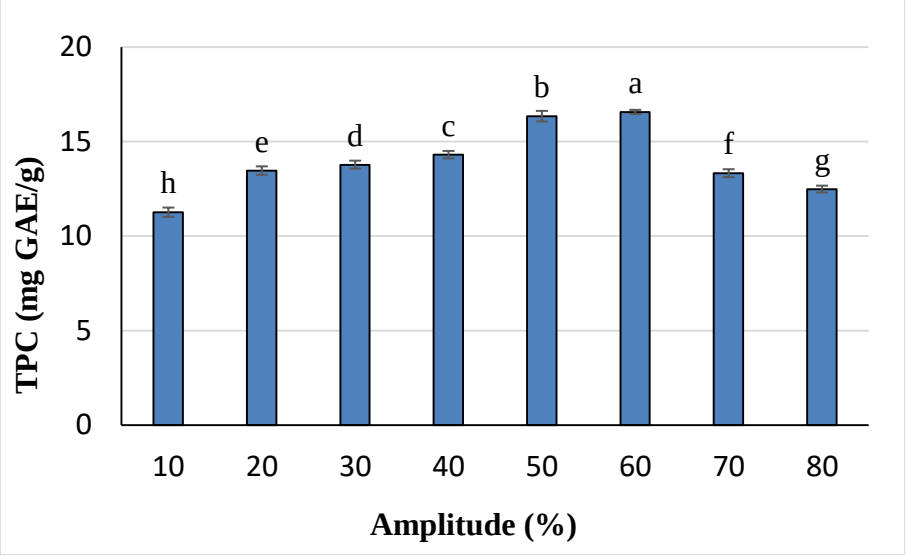
(b)


TPC (mg GAE/g)
20
15
10
5
0
h g e d a b c f
30 35 40 45 50 55 60 65
Temperature (°C)

(y)



(c)



(z)

Fig. 3.1: Selection of experimental range for independent variables for the extraction of bioactive compounds

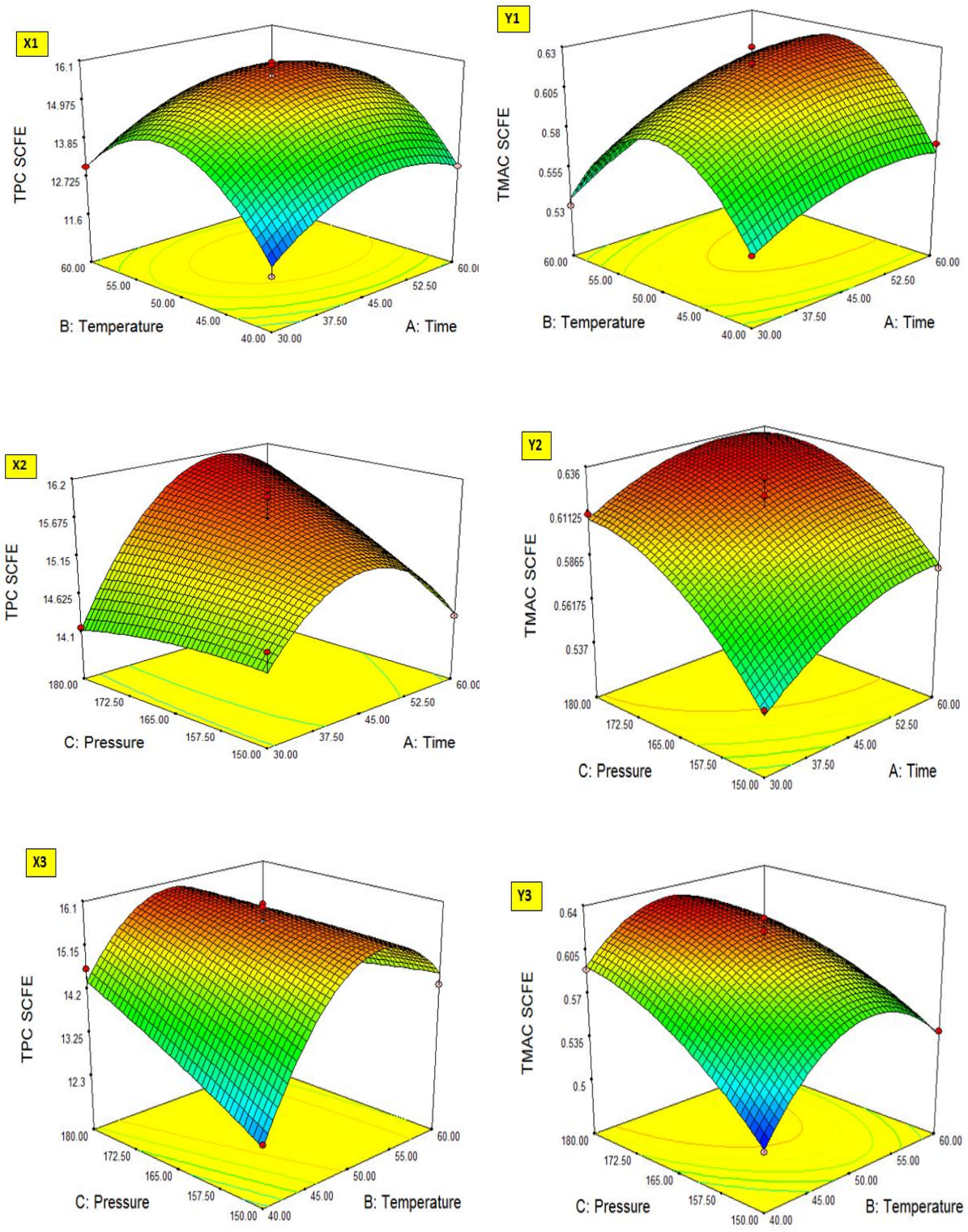


Fig. 3.2: Response surface graphs for optimization using SCFE

9.3.3.4 Determination of the limits of independent variables of UAE

For optimization using UAE, three independent variables were decided (time, temperature, and amplitude) and based on TPC limits the range of independent variables were decided

(Fig. 3.1 X-Z). For the determination of time range, time varied from 5-40 min while temperature and amplitude were kept constant at 50 °C and 50%, respectively. Significant difference ($p < 0.05$) was found in TPC and maximum extraction occurred at 25 min. The decrease in TPC was due to degradation of bioactive compounds on prolonged treatment (Rohilla and Mahanta, 2021). In determining the limits of temperature, it was noticed that as the temperature was increased from 40 to 50 °C, increase in the extraction of phenolics occurred that however decreased at higher temperature due to degradation of phenolic compounds. For amplitude, the highest phenolic content was observed at 60% followed by 50% due to the degradation of phenolic compounds.

9.3.3.5 Effect of independent variables on response using UAE technique

In Table 1, all the experimental trials and their specific extraction parameters with their respective response are given. The phenolic content from the sample extract ranged from 16.32-21.04 mg GAE/g of sample. The best response was recorded with processing parameters of 20 min time, 50 °C temperature and 50% amplitude. In Fig. 3.3 (A1-A3), the 3D response surface graphs are given with interactions of the independent variables in terms of response. The responses for the experimental conditions were fitted in the second-order polynomial equations to understand the interactions between independent variables and response. In Eq. 3.5, individually time, temperature and amplitude showed positive effect on the phenolic content, however only interaction terms of time and temperature showed positive effect. The TPC was highly affected by ultrasound temperature and amplitude. The quadratic terms A^2, B^2 and C^2 were highly significant ($p < 0.01$). The interaction for AB, CA was non-significant at $p < 0.05$. Increase in time from 10 to 20 min and temperature from 40 to 50 °C led to an increase in TPC thereafter there was gradual decrease, which may be due to excessive heat generation that will lead to degrading of the bioactive compounds. Higher extraction temperature and time helps to extract the extractable compounds from the sample matrix and increase the solubility with enhanced mass transfer, but beyond limits concentration decreases due to degradation. Amplitude shows a positive effect on the extraction of the phenolic compounds because the increase in the amplitude is directly related to enhanced diffusion and increased mass transfer of bioactive compounds into the extracting solvent.

$$TPC_{UAE} = +20.57 + 0.028 \times A + 1.10 \times B + 0.66 \times C + 0.32 \times A \times B - 0.89 \times A \times C - 0.13 \times B \times C - 1.17 \times A^2 - 1.70 \times B^2 - 0.66 \times C^2 \quad (3.5)$$

$$TMAC_{UAE} = +0.71 + 1.250\text{E}-003 \times A + 7.500\text{E}-003 \times B + 1.250\text{E}-003 \times C - 0.013 \times A \times B - 0.015 \times A \times C - 2.500\text{E}-003 \times B \times C - 0.073 \times A^2 - 0.055 \times B^2 - 2.500\text{E}-003 \times C^2 \quad (3.6)$$

TMAC ranged between 0.54 and 0.74 C3G/g of dry weight of the sample. The second-order polynomial equations for response TMAC using UAE is mentioned in Eq. 3.6 that helps to determine the interactions between independent variables on response. In Fig. 3.3 (B1-B3), the 3D response surface graphs are given with interactions of the independent variables in terms of response. In Fig. 3.3 (B1), an increase in the temperature and time caused an increase in the TMAC and after reaching the maximum, it decreased gradually. However, an increase in the amplitude showed an increase in the anthocyanin content in the sample extract. Similar results were obtained in the case of extraction of anthocyanin using ultrasound from jabuticaba epicarp. Acidified solvent enhances anthocyanin extraction and improves stability of anthocyanin at acidic pH (2.0-2.3).

9.3.3.6 Verification of model for UAE

Four different models (Linear, 2 Fi, Quadratic and Cubic) were applied for the optimization of UAE for extraction of TPC and TMAC from purple tree tomato fruit. Quadratic model was selected because of its high R^2, Adequate R^2 and less p-value, which are desirable conditions for model fitting. The R^2, Adequate R^2 and p-values for the quadratic model applied in TPC were 0.97, 0.94 and < 0.0001 and for TMAC 0.97, 0.94 and < 0.0001, respectively. The model was found to be significant at $p<0.05$ as lack of fitness was non-significant and residual values were low. The CV for TPC_{UAE} and $TMAC_{UAE}$ was 1.96 and 1.86, respectively.

9.3.3.7 Optimization and comparison in techniques

In Table 3.2, the optimized conditions for supercritical and ultrasounds are mentioned. In both the techniques, all the independent variables were kept in range and responses was maximized. The desirability for SCFE and UAE was 1.00 and 0.95, respectively, and experimental results were found to be near to the predicted model. In SCFE, the experimental values for TPC and TMAC were found to be 16.12 ± 0.05 mg GAE/g and 0.62 ± 0.02 mg C3G/g, respectively. In UAE, the experimental values for TPC and TMAC were found to be 21.07 ± 0.14 mg GAE/g and 0.71 ± 0.03 mg C3G/g, respectively.

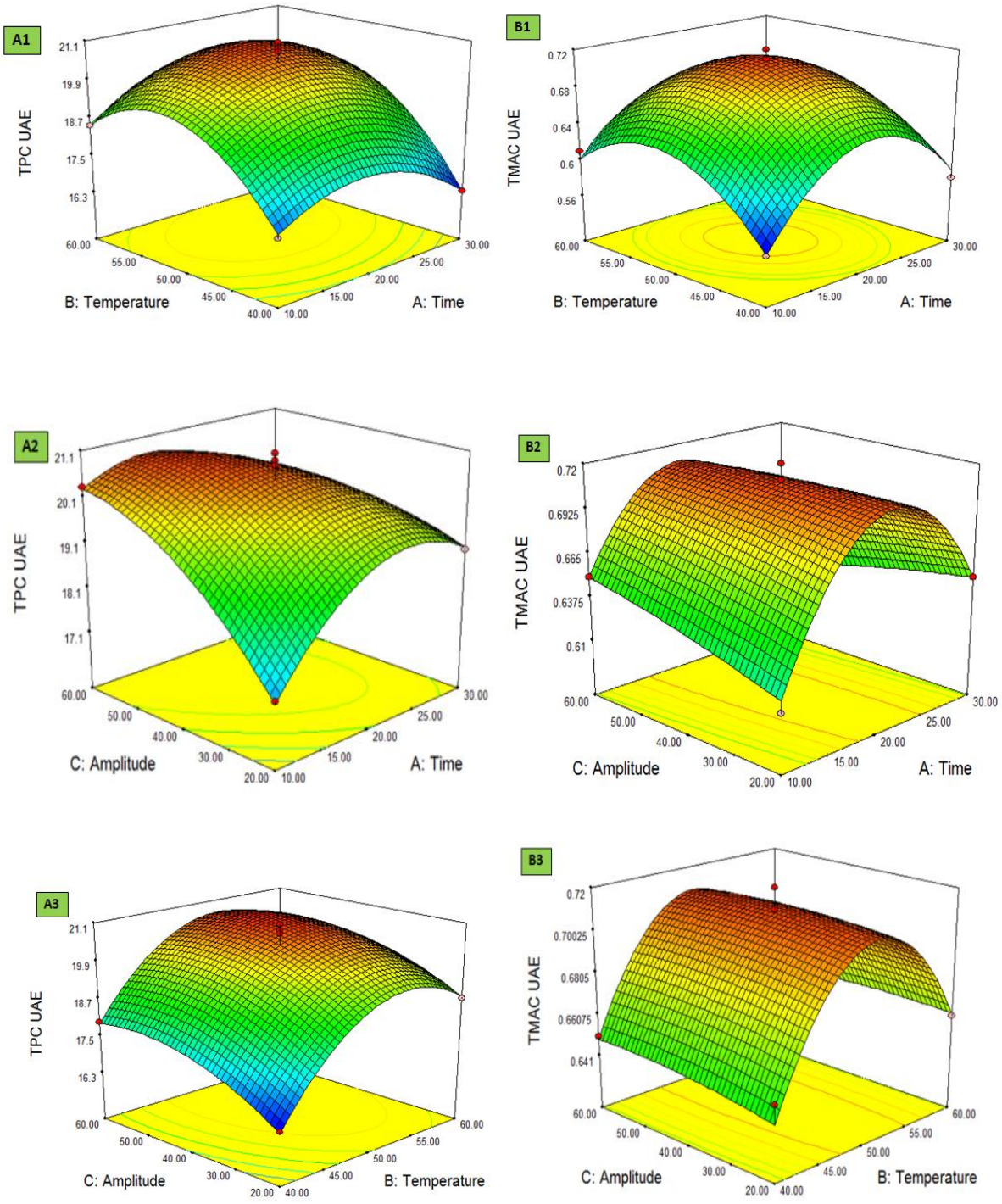


Fig. 3.3: Response surface graphs for optimization by UAE

Table 3.2. Optimized conditions for SCFE and UAE with predicted and experimental values.

Supercritical fluid extraction						
			Predicted value		Experimental value	
Time (min)	Temperature (°C)	Pressure (bar)	TPC **(mg GAE/g)**	TMAC **(mg C3G/g)**	TPC **(mg GAE/g)**	TMAC **(mg C3G/g)**
49.42	49.28	176.63	16.08	0.63	16.12 ± 0.05	0.62 ± 0.02
Ultrasound assisted extraction						
			Predicted value		Experimental value	
Time (min)	Temperature (°C)	Amplitude (%)	TPC **(mg GAE/g)**	TMAC **(mg C3G/g)**	TPC **(mg GAE/g)**	TMAC **(mg C3G/g)**
19.14	51.53	50.53	20.87	0.70	21.06 ± 0.14	0.71 ± 0.03

The validation of the experimental and predicted values was done using relative deviation. In SCFE, the relative deviation for TPC and TMAC was found to be 0.25 and 1.63%, respectively. In UAE, the relative deviation for TPC and TMAC was 0.90 and 1.63%, respectively. Therefore, the model was found to be satisfactory for optimization of extraction conditions.

The TPC and TMAC values of the extract obtained from SCFE and UAE techniques using optimized conditions are shown in Table 3.2. UAE showed higher yield of TPC and TMAC than SCFE. In the UAE, ultrasonic waves are powerful for generating microjets from bubbles that disintegrate sample matrix. This disruption enhances the extraction process and increases the concentration of bioactive compounds in the solvent. In SCFE, lower extraction yield may depend on factors like the solubility of polyphenols, temperature, extraction time, pressure, modifier concentration, flow rate, etc.

9.3.3.8 HPLC profile of phenolic acids

The HPLC profile of the phenolic acids of the extracts from SCFE and UAE techniques extracted under optimized conditions are presented in Fig. 3.4. The main phenolic acids identified in the extract of purple tree tomato extract were gallic acid, chlorogenic acid, caffeic acid and p-coumaric acid. The concentration of phenolic acids was higher in UAE extract than SCFE (Table 3.3). The highest concentration was seen for gallic acid with

2100.84 and 1572.63 µg/g in UAE and SCFE extracts, respectively, followed by chlorogenic acid, caffeic acid and p-coumaric acid.

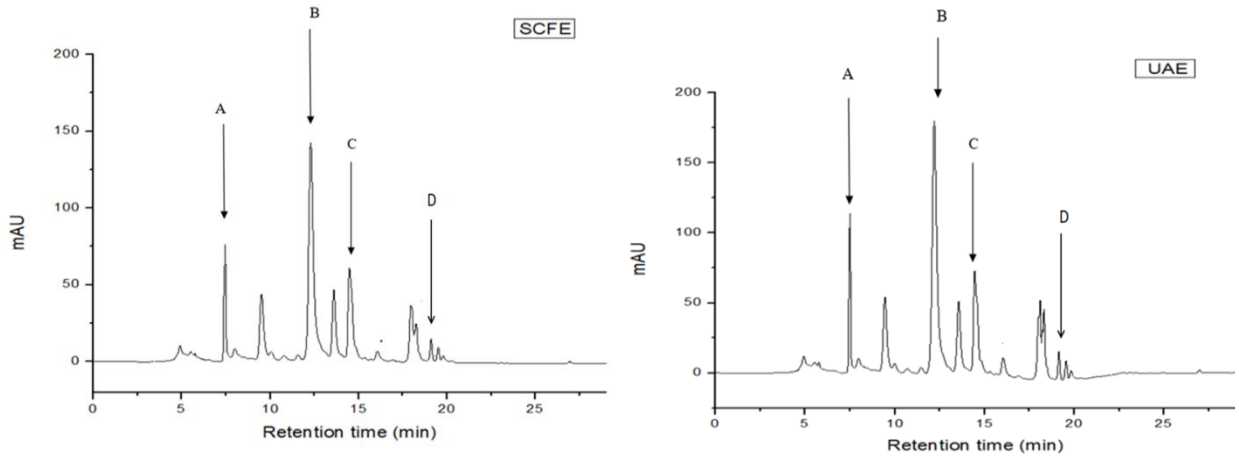


Fig. 3.4: HPLC of phenolic acids present in purple Tree tomato extracted using SCFE and UAE

9.3.3.9 HPLC profile of anthocyanins

Delphinidin 3-O-rutinoside, cyanidin-3-O-rutinoside and pelargonidin-3-O-rutinoside were the three anthocyanins identified in the extract of SCFE (Fig. 3.5) and UAE . Pelargonidin-3-O-rutinoside content was 308.44 µg/g and 213.78 µg/g in UAE and SCFE extracts, respectively (Table 3.3).

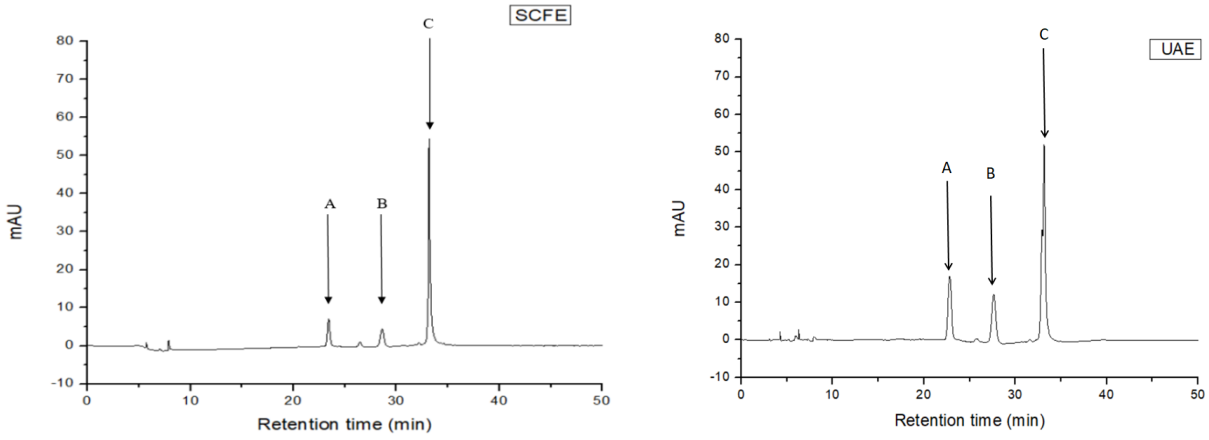


Fig. 3.5: HPLC analysis of the anthocyanin present in purple tree tomato extracted using SCFE and UAE

Table 3.3. Phenolic acids and anthocyanins content present in optimized extract of SCFE and UAE extracts of purple tree tomato.

Peak order	Compounds	SCFE (µg/g)	UAE (µg/g)
	Phenolic acids		
1	Gallic acid	1572.63	2100.84
2	Chlorogenic acid	1277.53	1481.43
3	Caffeic acid	136.92	140.35
4	p-Coumaric acid	57.07	106.01
	Anthocyanins		
1	Delphinidin 3-O-rutinoside	41.26	90.67
2	Cyanidin-3-O-rutinoside	38.27	58.87
3	Pelargonidin-3-O-rutinoside	213.78	308.44

9.3.3.10 Anthocyanin stability

The thermal degradation of anthocyanin was studied at three different temperatures for different intervals of time. In Fig. 3.6, the degradation of anthocyanins with respect to temperature and exposure time is indicated. It was found that highest degradation of anthocyanin was found at 90 °C followed by 70 °C and very less degradation of anthocyanin was found at 50 °C. No difference in the degradation of anthocyanins in UAE and SCFE extracts was observed.

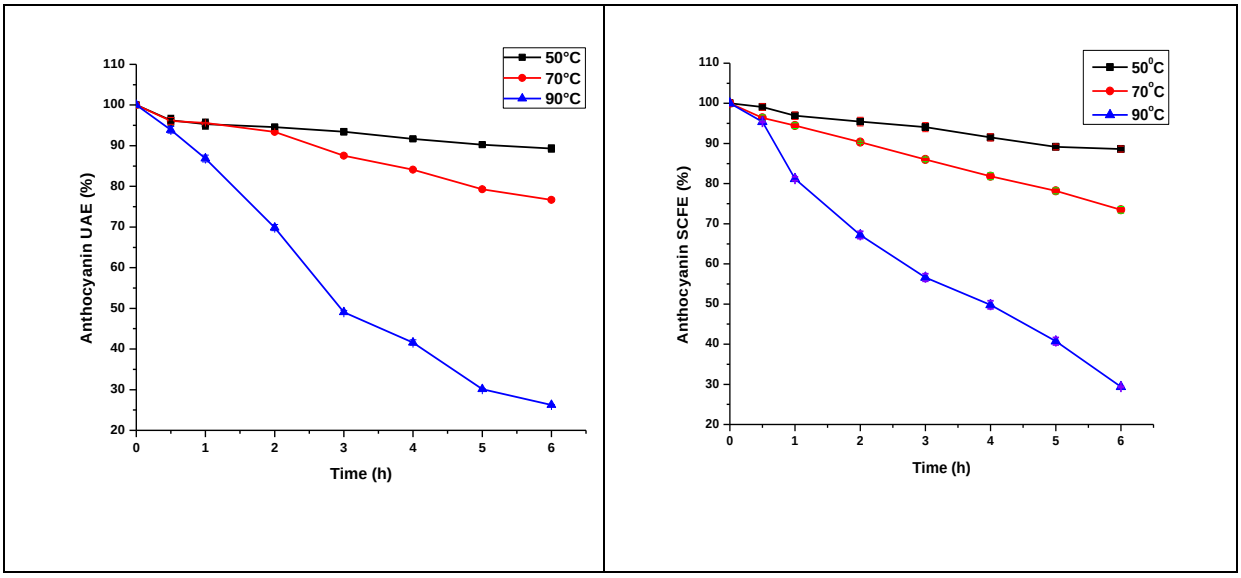


Fig. 3.6: Stability of anthocyanin extracted against different temperature w.r.t time

9.3.3.11 Scanning electron microscopy

The tree tomato residue left after SCFE and UAE were observed for morphological differences at ×500 and ×5500 magnifications (Fig. 3.7). Visible difference was found in the control and samples subjected to SCFE and UAE. UAE caused greater structural damage and crack formation than SCFE, which supports the higher release of TPC and TMAC from the sample matrix.

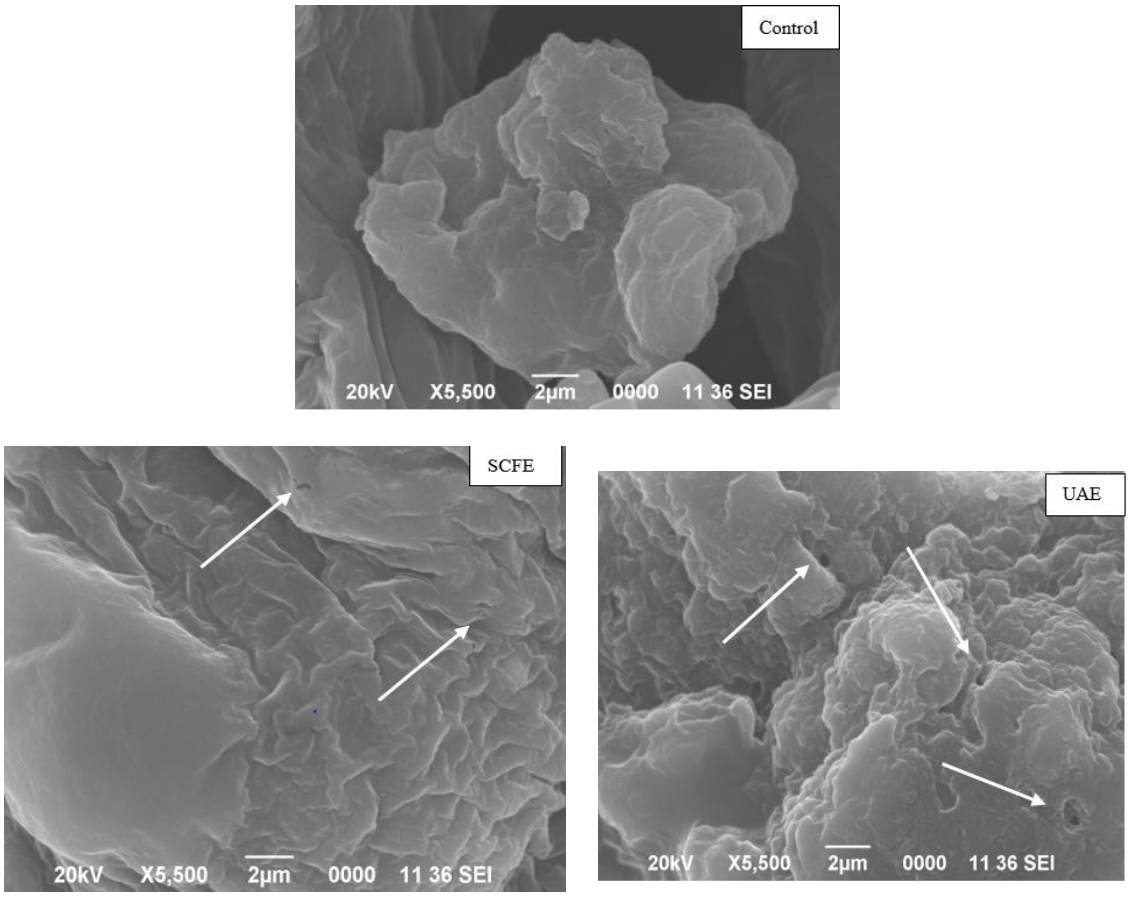


Fig. 3.7: SEM images of control, UAE and SCFE treated samples

9.3.4 Conclusion

Optimization of the extraction process for purple freeze-dried tree tomato was successfully done using response surface methodology for the extraction of total phenolics and anthocyanins content. Among the two techniques studied, UAE extracted higher amount of phenolics and anthocyanins than SCFE. Gallic acid was the major phenolic acid and pelargonidin-3-O-rutinoside was the major anthocyanin in purple tree tomato, as identified by HPLC. To the best of our knowledge, this is the first report on the comparison of UAE and SCFE for the extraction of phenolic compounds from the purple Tree tomato and the results indicated that UAE technique allows for greater extraction of phenolic acids.

9.4.0 Objective 4:

To investigate the physical and chemical properties of astaxanthin based emulsion using tree tomato seed oil

9.4.1 Introduction

Tree tomato seed oil was extracted and an emulsion incorporating astaxanthin was prepared using ultrasonication method. Astaxanthin (**Fig. 4.1**) is a carotenoid with antioxidant, anti-inflammatory and anti-apoptic properties. Tree tomato seed oil was extracted by soxhlet extraction method. Developed emulsions were characterized based on their particle size, zeta potential, optical density, centrifugal stability, effect of gastro-intestinal treatment on particle size, rheological properties (frequency sweep and viscosity), and cell line toxicity and permeability.

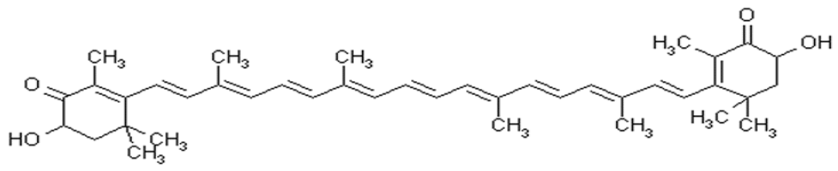


Fig. 4.1: Structure of the astaxanthin.

9.4.2 Methods

9.4.2.1 Development of emulsion

Oil in water based emulsion was prepared with oil extracted from tree tomato seeds using Tween 80 as surfactant (1%). Astaxanthin at concentrations of 1 mg, 5 mg and 10 mg was incorporated into 1 mL of oil each and sonicated for 3 min. Astaxanthin incorporated oil was dropwise introduced to 25 mL of surfactant and stirred for 4 h. Mixed solution was than sonicated using probe sonicator (300 W for 5 min). Developed nanoemulsions were characterized.

9.4.3 Results and discussion

9.4.3.1 Particle size and zeta-potential

Hydrodynamic particle size and zeta potential were estimated for all the prepared samples using Dynamic Light Scattering. Particle size (**Fig. 4.2**) of nanoemulsions were in the range

of 200-300 nm. Zeta potential (**Fig. 4.3**) value ranged from -25 to -35 mV, which indicated the high stability of the developed emulsions.

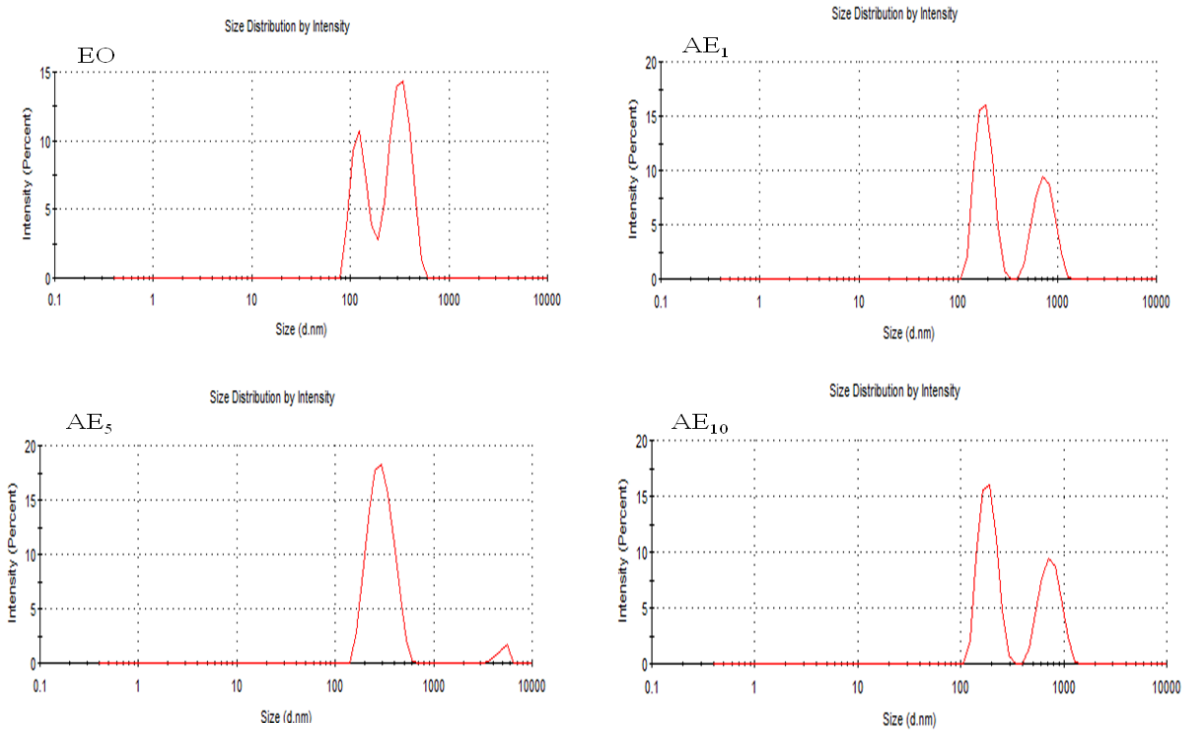


Fig. 4.2: Particle size of the nanoemulsions.

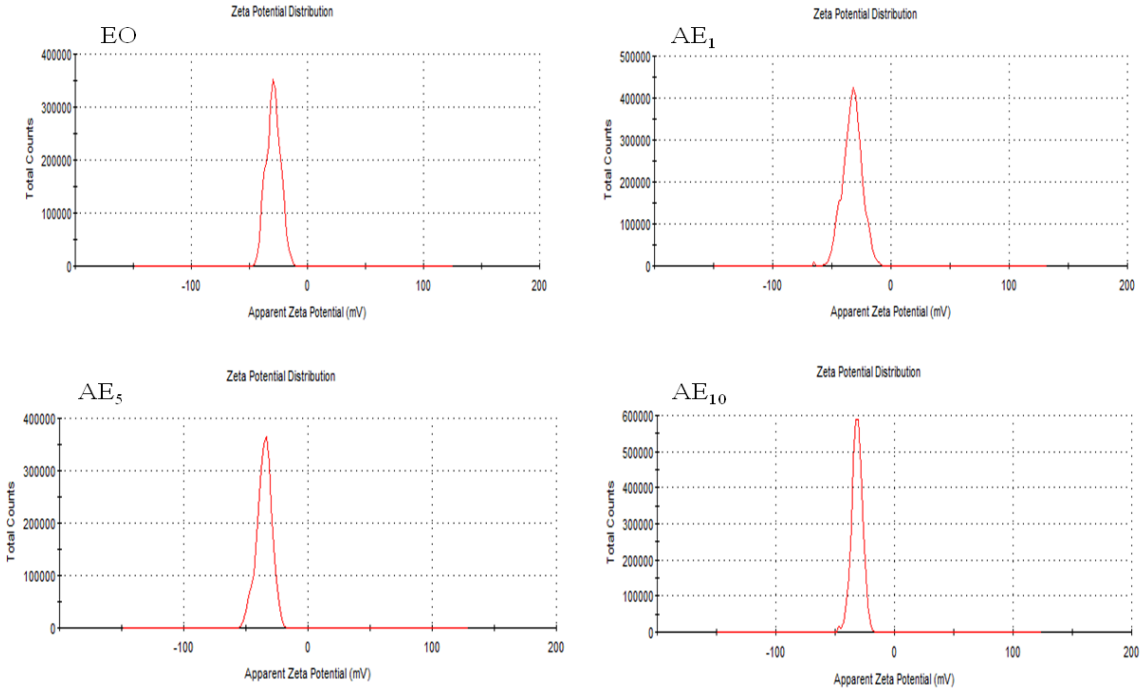


Fig. 4.3: Zeta potential of the nanoemulsions.

9.4.3.2 Light microscopy

Emulsions were further observed with an optical microscope (BX50, Olympus, Japan). The samples were examined under 40X magnification. One drop of sample was diluted with five drops of water on a glass slide. Microscopic observation revealed globular structure of the emulsions. The light microscope images (**Fig. 4.4**) revealed that flocculation occurred in the emulsions when astaxanthin was incorporated. Further, the microstructure indicated that droplet flocculation increased with increasing Astaxanthin concentration and due to smaller microglobule structure.

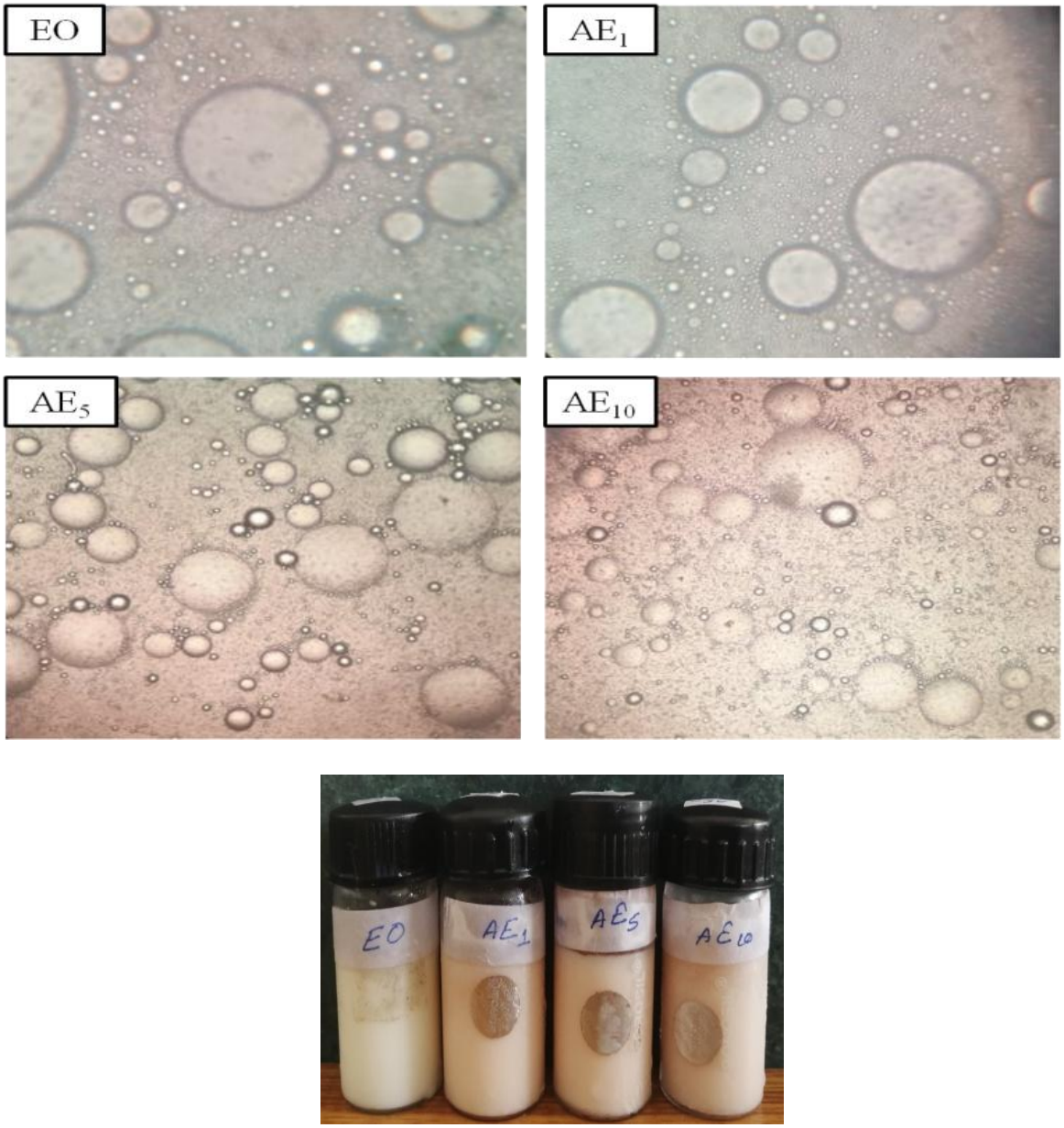


Fig. 4.4: Optical light microscopic images (40x) and developed nanoemulsions.

9.4.3.3 Emulsification index and centrifugal stability

The emulsification index (EI) is the ratio of the volume of the emulsion layer and the volume of the whole sample. In the present study, the centrifugal stability for 5, 10, 15, 20, 25 and 30 min was studied. There is a very less change in the emulsion after different time intervals of centrifugation, which indicated the stability of the emulsions (**Fig. 4.5**).

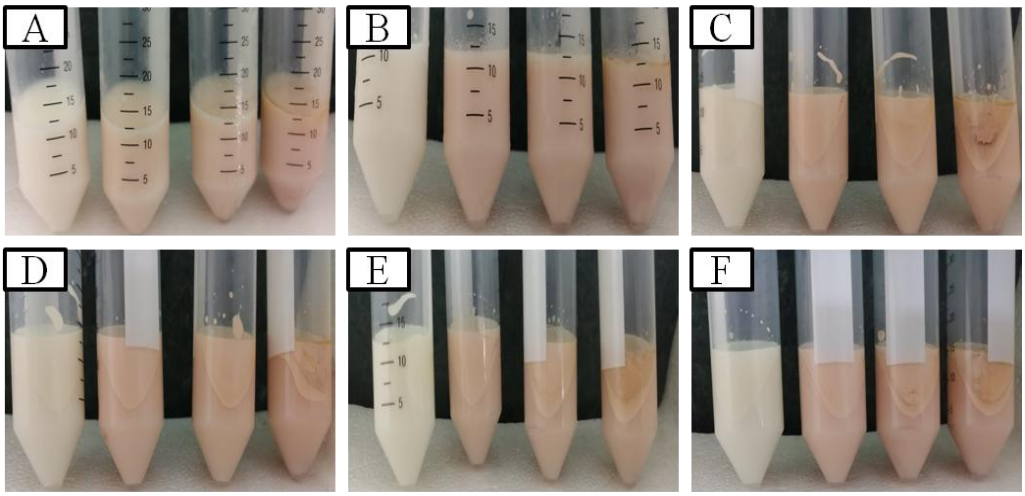


Fig. 4.5: Centrifugal stability for different time intervals.

9.4.3.4 Rheological behavior of nanoemulsion

The viscosity curves (**Fig. 4.6**) revealed that all developed emulsions were non-Newtonian and thixotropic. The emulsions were classified as non-Newtonian fluids because their viscosity varied with shear rate.

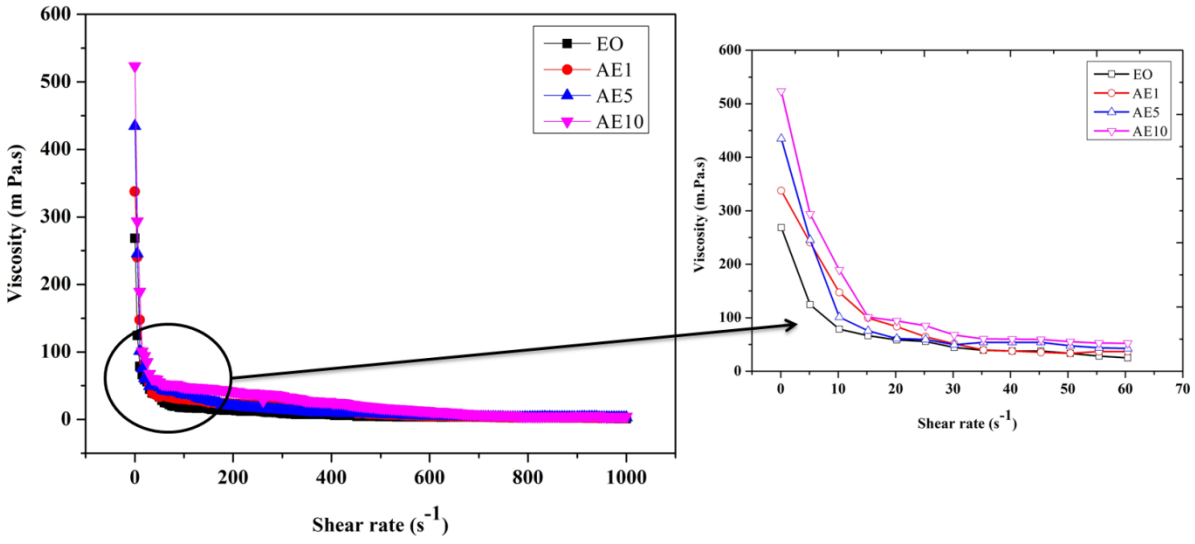


Fig. 4.6: The rheological flow curves for the nanoemulsions.

Frequency sweep curves can provide rheological information about how a product will behave during storage and application. **Fig. 4.7** depicts the storage modulus (G') and loss modulus (G") values of the nanoemulsion. All emulsions had a value of G' that was an order of magnitude greater than G" in the frequency range of 10-30 Hz, indicating the presence of a gel network. The samples exhibited yield stress, which is a characteristic of colloidal particle dispersion, and as a result, they required an initial force to initiate flow. The formulations showed a significant hysteresis point 0-10 rad/s, with the exception of formulation EO, which showed a decrease in thixotropy in comparison to AE1, AE5 and AE10 nanomeulsions. A larger hysteresis point indicated an increase in the microstructure of the liquid crystalline net, which improves dispersion and acts as a stabilizer against coalescence processes.

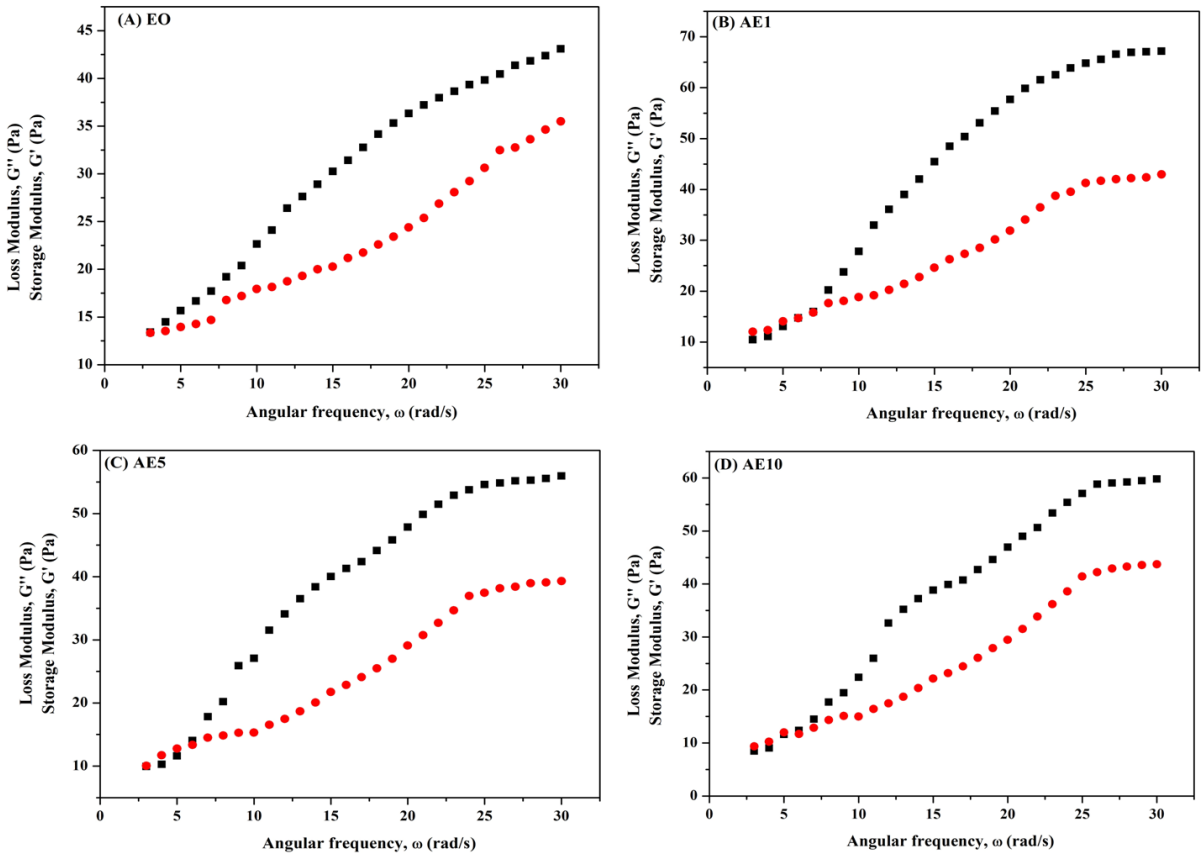


Fig. 4.7: The storage modulus (G') and loss modulus (G") values of the nanoemulsions

9.4.3.5 Gastro-intestinal release model

The astaxanthin-loaded emulsions were subjected to an in vitro digestion model that included simulated mouth, stomach, and small intestinal phases, as described in INFOGEST protocol, with minor modifications. After passing through the simulated oral and gastric conditions, the

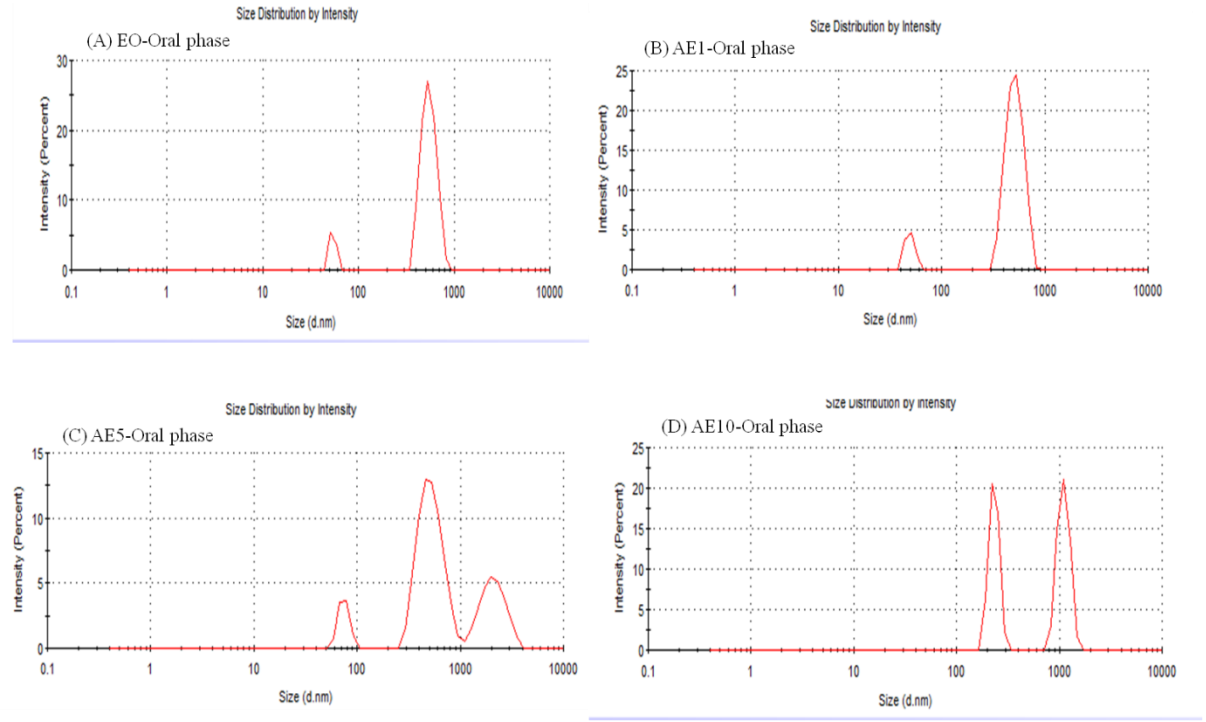


Fig. 4.8A: Particle size analysis for nanoemulsions treated with simulated oral solution.

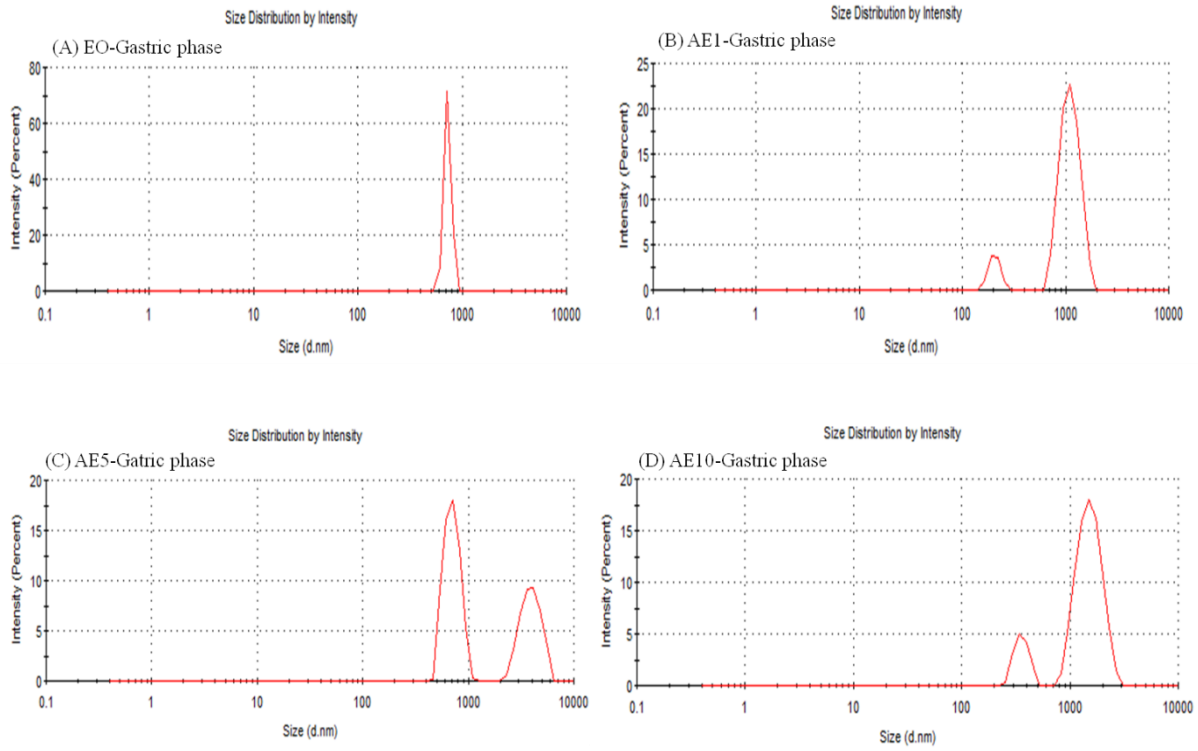


Fig. 4.8B: Particle analysis for nanoemulsion treated with simulated gastric solution.

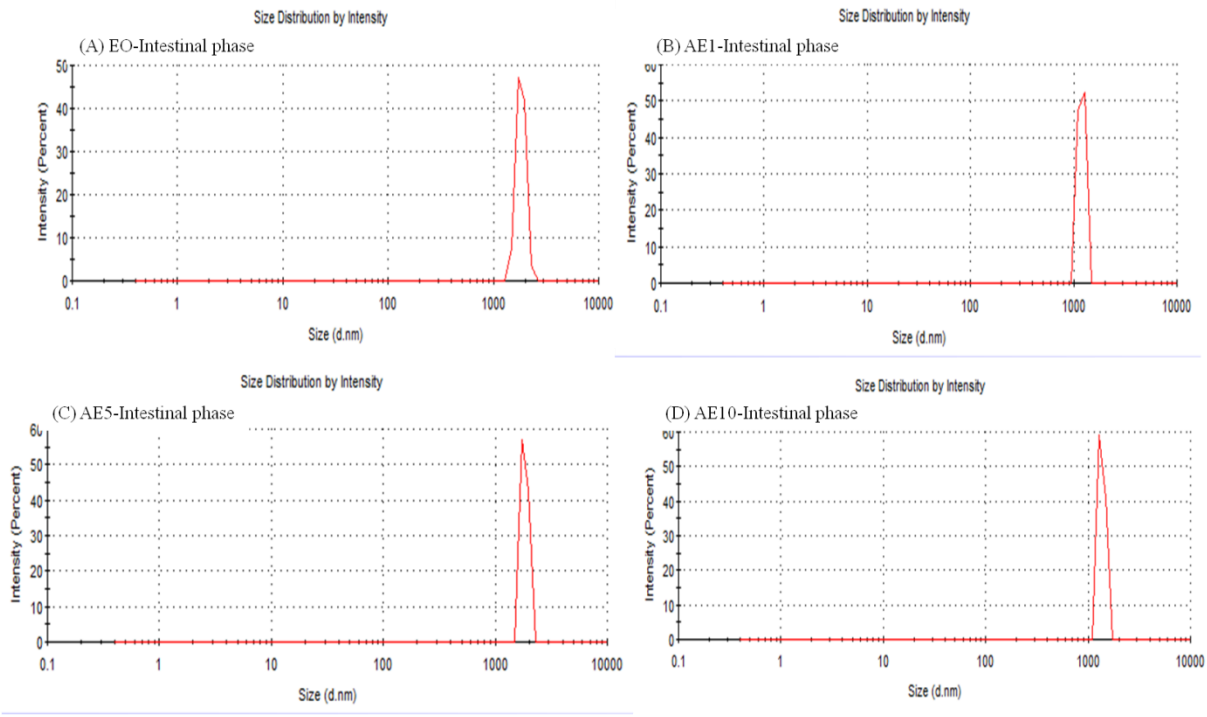


Fig. 4.8C: Particle analysis for nanoemulsion treated with simulated intestinal solution.

particle size of the emulsion systems increased significantly (Fig. 4.8). The increase in mean droplet diameter could be due to a variety of physicochemical mechanisms, including the highly ionic strength of gastric fluid decreasing electrostatic repulsion between droplets surfaced, (ii) protein hydrolysis by pepsin decreasing droplet stability from aggregation, and (iii) some protein-coated droplets being replaced by other surface molecules present in the system.

9.4.3.6 Cell line toxicity and permeation

HepG2 cell line cytotoxicity and permeability tests were done for nanoemulsions. Cytotocxicity test revealed an increase in toxicity with increase in concentration of asthaxanthin (**Fig. 4.9A).** However, cell death percentage was less than 50. Additionally, when FITC tagged nanoemulsions were incubated with HepG2 cell line for 24 h for permeability test (**Fig. 4.9B**), it was found that with increase in concentration of astaxanthin, permeation level was decreased which might be due to the higher toxicity level.

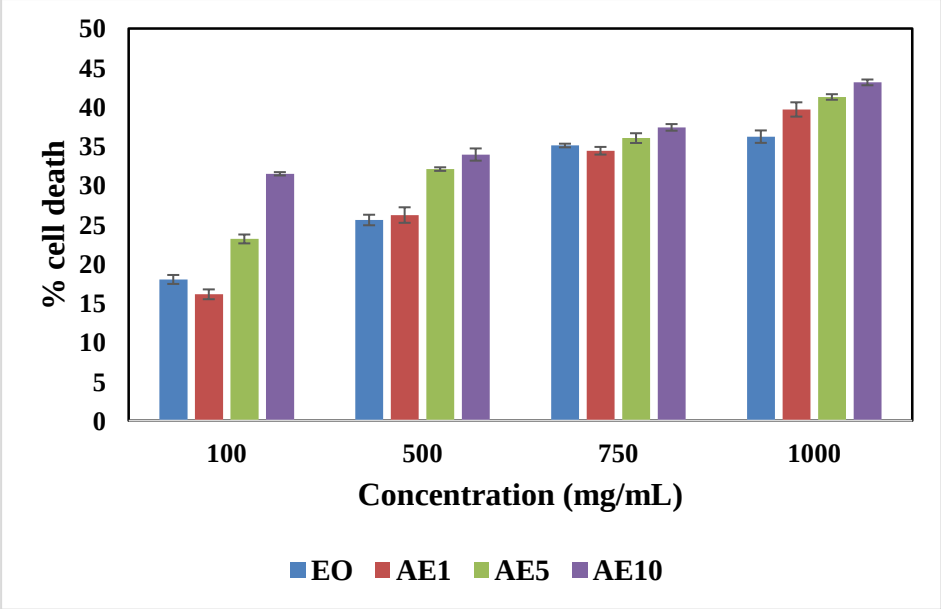


Fig. 4.9A: HepG2 cell line toxicity test

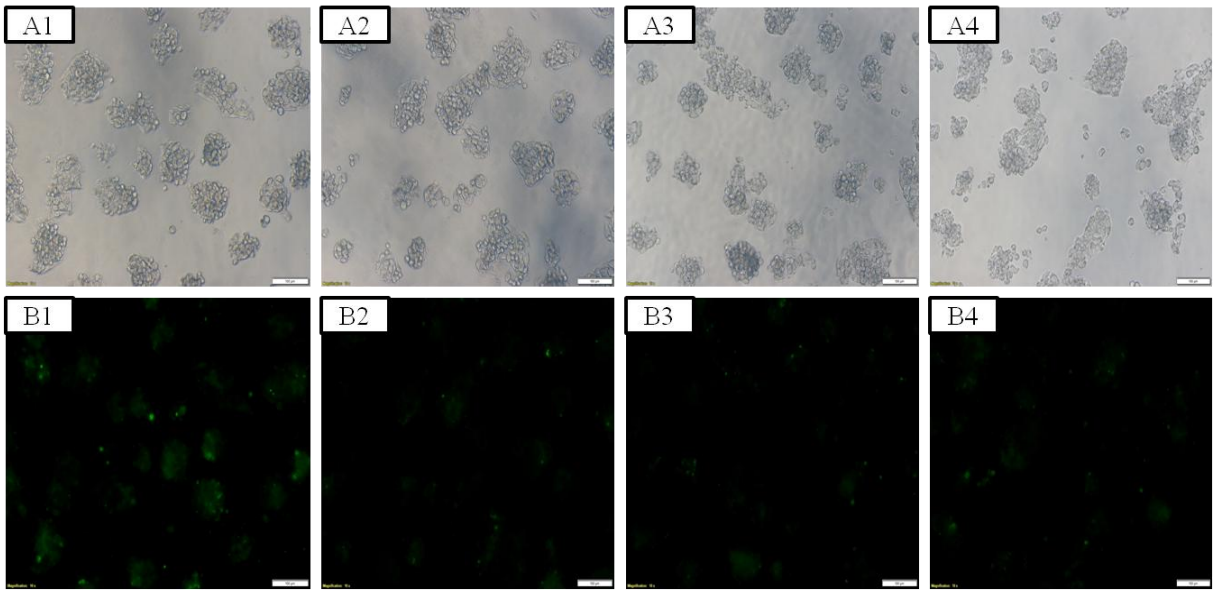


Fig. 4.9B: Cell permeability test under Fluorescence microscope

9.4.4 Conclusion

Astaxanthin loaded nanoemulsion using tree tomato seed oil was done using ultrasound as the fabrication technique. Nanoemulsions incorporated with astaxanthin were created in three different concentrations: 1, 5, and 10 mg. The incorporation of astaxanthin improved gastro-intestinal stability, but there was an increase in viscosity with increasing concentration, which also resulted in flocculation.

10.Detailed analysis of results indicating contributions made towards increasing the state of knowledge in the subject:

Detailed results are given for each objective in the previous section. The results have been discussed for each experiment and in the discussion itself, the increase in the state of knowledge can be seen. Results indicated that the three varieties of tree tomato are rich in bioactive compounds. The pulp, peel, and the seed oil of the tree tomato can be used for formulation of novel food products.

11.Conclusions summarising the achievements and indication of scope for future work:

i. Varietal difference exists in proximate composition, and biochemical and antioxidant properties, composition and concentration of phenolic acids, anthocyanins and carotenoids in purple, yellow and red tree tomato varieties. Purple tree tomato was rich in anthocyanins and yellow variety was rich in carotenoids. Both anthocyanins and carotenoids were present in purple and red varieties.

ii. Addition of olive oil to tree tomato puree and high pressure homogenisation at 1000 bar with single pass followed by thermal treatment of 95 °C yielded good quality tree tomato puree that was microbiologically acceptable up to 4 months and had improved *in- vitro* bioaccessibility of carotenoids.

iii. Ultrasound assisted extraction technique extracted higher amount of phenolics and anthocyanins than supercritical fluid extraction technique. HPLC study could identify gallic acid as the major phenolic acid and pelargonidin-3-O-rutinoside as the major anthocyanin in purple tree tomato. This is the first report on the comparison of UAE and SCFE for the extraction of phenolic compounds from the purple tree tomato.

iv. Astaxanthin can be loaded in nanemuslion of trre tomato oil which showed gastro-intestinal stability. However, further work on its storage stability is required, which is beyond the scope of the objective.

12. S&T benefits accrued:

i. List of Research publications

S No	Authors	Title of paper	Name of the Journal	Volume	Pages	Year
1.	Rohilla, S. & Mahanta, C.L.	Optimization of extraction conditions for ultrasound-assisted extraction of phenolic compounds from fruit	Journal of Food Measurement and Characterization	15	1763–1773	2021

		(*Solanum betaceum*) using response surface methodology.				
2.	Rohilla, S., Bora, J., and Mahanta, C. L.	Effect of thermal treatment and addition of olive oil on the antioxidant properties of puree.	Journal of Food Science and Technology. (Ms. No. JFST-D-21-01031, Accepted May 2022).			
3	Rohilla, S., Chutia, H., Marboh, V., and Mahanta, C. L.	Ultrasound and supercritical fluid extraction of phytochemicals from purple: Optimization, comparison, kinetics and thermodynamics studies.	Applied Food Research. (MS. No. AFRES-D-22-00222, Accepted August 2022).			

ii. Manpower trained on the project

a) Research Scientists or Research Associates : **01 JRF**
b) No. of Ph.D. produced: **01**
c) Other Technical Personnel trained: **01**
iii. Patents taken, if any :

Two technologies were transferred to M/S Tsuipu Food Product, MSME, Dimapur. The technologies were:

i. **Instant tree tomato soup powder**
ii. **Bottled tree tomato puree**

13.Financial Position:

No	Financial Position/ Budget Head	Funds Sanctioned (1^{st} +2^{nd} installments)	Expenditure	% of Total cost
I	Salaries/ Manpower costs	739200	674102	91.19
II	Equipment	1414289	1360477	96.19
III	Consumables	775000	754427	97.35
IV	Contingencies	-	-	-
V	Travel	75000	67758	90.34
VI	Institutional Charges	300000	301500	100.5
	Total	3303489	3158264	95.6%

14 Procurement/ Usage of Equipment

a)

S No	Name of Equipment	Make/ Model	Cost (FE/ Rs)	Date of Installation	Utilisation Rate (%)	Remarks regarding maintenance/ breakdown
1.	High pressure homogeniser	GEA, Italy PandaPlus2000	1341000.00	08/08/2019	100	Working satisfactorily
2	Pressure vessel with thermocouple		19477.00	22/08/2019	100	Working satisfactorily

b) Plans for utilising the equipment facilities in future:

The equipments are being used by the Research Scholars of the Department for their PhD research.

Name and Signature with Date

Charu Lata Mahanta 21/09/2022
Charu Lata Mahanta
(Principal Investigator)

Dipankar Kalita 21.09.2022
Dipankar Kalita
(Co-Investigator)

21/9/22
Head of Institute/Organization

Registrar
Tezpur University
Napaam, Tezpur

Date:

14.Procurement/ Usage of Equipment

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The equipments are being used by the Research Scholars of the Department for their PhD research.

Name and Signature with Date

Charu Lata Mahanta
(Principal Investigator)

Dipankar Kalita
(Co-Investigator)

Date: Head of Institute/Organization

Statement of Expenditure (01-04-2021 to 20-03-2022)

S. No.	Particulars	Grant Sanctioned (Rs.)	Amount Sanctioned (Rs.) 1st installment	Amount Sanctioned (Rs.) 2nd installment	Expenditure (Rs.)				Total Expenditure (Rs.)	Balance amount to be received as 3rd installment (Rs.)
					2018-2019	2019-2020	2020 - 2021	01-04-2021 to 20-03-2022		
1	Equipments	14,14,289/-	14,14,289 /-	NIL	-	13,60,477	-	-	13,60,477	NIL
2	Salaries	7,39,200/-	3,69,600 /-	263131	34,821	1,02,097	2,00,133	3,37,051	6,74,102	22,857
3	Consumables	7,75,000/-	3,87,500 /-	238061	3,60,523	-	8,850	3,85,054	7,54,427	77,500
4	TA/DA	75,000/-	37,500 /-	26379	-	19,163	14,716	33,879	67,758	18,864
5	Institutional charges	3,00,000/-	1,50,000/-	120000	93,750	-	57,750	1,50,000	3,01,500	32,583
	Grand Total	**33,03,489/-**	**23,58,889/-**	**6,47,571/-**	**4,89,094**	**14,81,737**	**2,81,449**	**9,05,984**	**31,58,264**	**1,51,804**

Total interest earned: Rs. 29640.00
Interest refunded: Rs. 29248.00+ Rs. 392.00 = Rs. 29640.00

Charu Lata Mohanta

Name and Signature of Principal Investigator

Date: 10/02/2023

14.2.23

Signature of Competent financial/ audit authority with seal

Finance Officer
Tezpur University

Date:

For SURAJIT CHAKRABORTY & CO.
CHARTERED ACCOUNTANTS

17.02.2023.

CA. SURAJIT CHAKRABORTY
(Proprietor)
Membership No.- 305054

UTILISATION CERTIFICATE
(for the FY 01-04-2021 to 20-3-2022)

(Rs. in lakhs)

1.	Title of the project/scheme :	Evaluation of tree tomatoes (tamarillo) of Nagaland utilising the pulp, peel and seeds for its commercialisation
2.	Name of the Organization	Tezpur University , Assam
3.	Principal Investigator :	Prof. Charu Lata Mahanta
4.	Sanction order No. & date of sanctioning the project	F. No. **Q-11/25/2018-R&D, dtd.21-08-2018**
5.	Amount brought forward from the previous financial year quoting letter No. & date in which the authority to carry forward the said amount was given :	**Rs. 1,35,857/-**
6.	Amount received from MoFPI during the financial year (Please give No. and dates of sanction orders showing the amounts paid) :	**Rs.6,47,571/-** **(F. No. Q-11/25/2018-R&D, dtd.14-09-2021)**
7.	Other receipts/interest earned, if any, on the grants :	**Rs. 392/-**
8.	Total amount that was available for expenditure during the financial year (Sl. nos. 5, 6 and 7) :	**Rs 7,83,820/-**
9.	Actual expenditure (excluding commitments) incurred during the financial year (statement of expenditure is enclosed) :	**Rs. 9,05,984/-**
10.	Unspent balance refunded, if any (Please give details of cheque No. etc.):	**Interest refunded=Rs.29,248/-** (in favour of PAO, Ministry of Food Processing Industries, vide Cheque No. 535266, dtd. 12/08/2021)
11.	Balance amount available at the end of the financial year :	**Rs 392/- (interest amount to be refunded)**
12.	Amount allowed to be carried forward to the next financial year vide letter No. & date	**NIL**

1. Certified that out of **Rs. 6,47,571/-** of Grants- in-aid sanctioned during the years 2021-2022 in favour of Registrar, Tezpur University, Napaam-784028, Assam under this Ministry letter No. **F. No. Q-11/25/2018-R&D, dtd.14-09-2021**, and **Rs 29,248/-** refunded as interest, a sum of **Rs. 9,05,984/-** has been utilized for the purpose it was sanctioned and that the balance of **Rs 392/-** being the interest amount will be refunded.

2. Certified that I have satisfied myself that the conditions on which the grants- in-aid was sanctioned have been duly fulfilled/are being fulfilled and that I have exercised that following check s to see that the money was actually utilized for the purpose for which it was sanctioned.

Kinds of checks exercised:

1. Accounts audited by qualified Chartered Accountant appointed by this University as Internal Auditor.
2. The AG (Audit), Guwahati has already audited the account.
3. All the instrument(s), chemicals, consumables, etc purchased from the grant are entered in the log book.

Charu Lata Mahanta
Signature of the Principal Investigator

14/2/23
Signature of Finance Officer with seal

Finance Officer
Tezpur University

For SURAJIT CHAKRABORTY & CO.
CHARTERED ACCOUNTANTS

17.02.2023
CA. SURAJIT CHAKRABORTY
(Proprietor)
Membership No.- 305054

Signature of the Head of the Institute with seal

Registrar
Tezpur University