CHAPTER 3

OBJECTIVE 1

CHAPTER 3

CHARACTERIZATION OF TAMARILLO VARIETIES AND STUDY OF THE EFFECT OF ULTRASOUND AND HIGH PRESSURE HOMOGENIZATION ON BIOACTIVE COMPOUNDS PRESENT

3.1. Introduction

Tamarillo fruit variety is totally dependent upon the colour acquired by the fruit peel and pulp [21]. In early stages, the fruit is green in colour but as the maturity level of the fruit is reached green colour changes into red, yellow, purple colour depending upon the variety of fruit [3]. Different varieties of tamarillo possess different concentration of nutrients and antioxidant properties and vary according to geographical and climatic conditions. Vasco et al. [28], who studied the physical and chemical characteristics of two varieties from Ecuador and Spain, reported that Ecuador fruits were larger even though chemical composition was quite similar. Tamarillo is loaded with polyphenols and researchers have reported that these polyphenols possess countless health benefits like protection of low-density lipoprotein from oxidation [11]. Anthocyanins and carotenoids are two major colouring compounds present in the tamarillos and these compounds possess high antioxidant activity and decrease the risk of cardiovascular diseases, cancer, etc. [2].

Tamarillo can be consumed in fresh raw form or processed into juices, purees, ketchups, desserts etc., depending upon the variety [3]. Crushing is an important step in food processing; it helps in attaining uniform consistency while processing tamarillo into puree and juice. Therefore, researchers are studying the non-thermal processing techniques like ultrasonication (US) and high pressure homogenization (HPH) as an alternate to thermal processing, which is well known to cause drastic reduction in raw-like quality. Ultrasound assisted extraction (UAE) has gained in importance because it is known for effective extraction of bound phyto-compounds from the fruit sample matrix [19]. UAE technique involves the sound waves of frequency above 20 KHz, and the cavitation caused by ultrasound waves breaks the cell walls of the sample matrix and helps in releasing the bioactive compounds into the solvent extract [7]. HPH is a non-thermal technology known for producing a combination of cavitation and turbulence with hydraulic shear at 0-200 MPa pressure, which when applied to food sample cause changes in particle size and improves the emulsifying properties, and is used in the

processing of many food products [30]. The HPH and UAE techniques are cost efficient, energy saving, consumer friendly, and highly efficient techniques.

Tamarillo is gaining popularity in the global market because of its health beneficial properties and its unique sensorial characteristics but reported studies suggest wide variation in the composition of tamarillo from region to region. In India, all three varieties are available however; studies on their nutritional and phytochemical properties using non-thermal techniques are scanty. In order to better understand the three diverse tamarillo types from North-East India, we determined their proximate and biochemical characteristics as well as their antioxidant activities. We also looked at how the HPH and UAE affected their phenolic and antioxidant profiles.

3.2. Materials and methods

3.2.1. Raw sample collection

Fresh purple tamarillos were procured from the local market of Gangtok, Sikkim. Yellow and red tamarillos were procured from Dimapur, Nagaland, India. All the varieties were brought at Tezpur University, Assam. All the varieties of tamarillo were washed and kept at -20 °C in low-density polyethene zip pouch until further analysis.

3.2.2. Sample preparation for proximate & biochemical analysis

Moisture, crude protein, crude fat, crude fibre, total ash, and carbohydrate were calculated according to AOAC methods [8]. Pulp of the fruits was used to determine the biochemical activities of the tamarillos. The pH and TSS of tamarillo samples were analysed by pH meter (Eutech) and a hand refractometer (0-32 °Brix Erma, Japan), respectively [14]. Total titratable acidity (TTA) was analysed with respect to citric acid [14]. Vitamin C was determined by 2,6-dichloro-indophenol titration method [15].

3.2.3. Mineral content

The mineral content present in three varieties of tamarillo was calculated according to the method of Marboh and Mahanta [14] with some modification. 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 of individual minerals are expressed in mg/100 g.

3.2.4. 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 L* (dark to lightness), a* (green to red) and b*(blue to yellow).

3.2.5. Sample treatment

3.2.5.1. Conventional method

For conventional method, the sample was treated according to Marboh & Mahanta [14]. Each tamarillo sample was diluted ten times in distilled water and kept on magnetic stirrer (Remi, MS-500) for 30 min at 600 rpm. After treatment, the treated samples were placed in amber colour bottle and stored at -20°C until further analysis. Samples treated by conventional technique were coded as Control.

3.2.5.2. High pressure homogenization

Tamarillo samples were diluted 10 times with distilled water and subjected to high pressure homogenization (HPH) (GEA, Lab homogenizer Panda Plus 2000, Italy) at three different pressures (500, 750, and 1000 bar). After treatment the samples were placed in amber coloured bottles and stored at -20 °C until further analysis. Samples treated by HPH at 500, 750, and 1000 bar were coded as HPH-500, HPH-700, and HPH-1000, respectively.

3.2.5.3. Ultrasound-assisted treatment

Tamarillo samples were diluted 10 times with distilled water and subjected to ultrasonication in a ultrasound water bath (Bandelin Sonorex, Germany). The frequency of ultrasound was 30 kHz, amplitude: 100%, 100 W power and cut-off temperature of ultrasound was fixed at 40°C. The treatment was done for three different times (5, 10, and 15 min) in amber coloured glass bottle. Ultrasonicated samples in amber coloured bottles were stored at -20°C until further analysis. Samples treated by US for 5, 10, and 15 min were coded as US-5, US-10, and US-15, respectively.

3.2.6. Total monomeric anthocyanin content

The total monomeric anthocyanin content of tamarillo samples was calculated according to Ferreira et al. [5] using pH differential method. Two pH reagents were prepared (pH 1.0 using potassium chloride and pH 4.5 using sodium acetate buffer). The sample extract in triplicate was mixed with the above-mentioned reagents separately and absorbance was noted at wavelengths 520 and 700 nm (Thermo-Fischer Evolution A600). The anthocyanins of tamarillo juice were calculated using Eq. 1 and 2 below and reported as mg cyanidin-3-glucoside (C3G)/g of sample.

$$Anet = Abs510 - Abs700 \quad (1)$$

$$Anthocyanin\ \left(\frac{mg}{g}\right) = \frac{Anet}{26900}.MW.DF.\frac{V}{Wt} \quad (2)$$

here, *Anet* is net absorbance, *MW* (449.2) is molecular weight, *DF* stands for dilution factor, *V* stands for volume of sample, W_t stands for weight of initial sample taken for extraction, and 26900 is molar absorptivity value of cyanidin-3-glucoside.

3.2.7. Total carotenoids content

The total carotenoids content in the tamarillo sample was calculated according to the method adopted by Orqueda et al. [21]. Briefly, 1g of juice sample was mixed into 10 mL of hexane:acetone:ethanol (2:1:1 v/v/v) and centrifuged at 7000 x g for 10 min. The upper layer of mixture was separated carefully and adjusted to 10 mL using hexane. The absorbance of sample extracted in hexane was read at 450 nm (Thermo-Fischer Evolution A600) and values were reported as mg of β-carotene equivalents (mg β-CE)/ g of sample.

3.2.8. Sample extraction for phenolics and antioxidant activity

For phytochemical extraction and antioxidant activities, the tamarillo juice samples were mixed with extraction solvent (80:20 acetone: distilled 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 25 ±5 ℃. The supernatant was filtered through Whatman No. 4 (Whatman, India) and then stored at -20°C until further analysis [24]. All the extracts were prepared in duplicate and analysed in triplicate.

3.2.8.1. Total phenolic content

The total phenolic content (TPC) in tamarillo was determined according to Sakia et al. [24]. For the analysis, an aliquot of 0.5 mL of sample extracts was taken in a 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. The absorbance of the sample was noted in a UV-Vis spectrophotometer (Thermo-Fischer Evolution A600) after incubation time against the reagent blank mixture. Gallic acid was used as standard, and results are reporetd in mg GAE/100g.

3.2.8.2. Total flavonoid content

The total flavonoid content (TFC) in tamarillo samples was determined according to Saikia et al. [24]. For the analysis, an aliquot of 0.5 mL of sample was mixed, followed by addition of 1.5 mL of ethanol (95%), 0.1 mL of aluminium trichloride (10%), 0.1 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 noted at 415 nm using a UV-Vis spectrophotometer (Thermo-Fischer Evolution A600) against blank. Quercetin was used as standard, and results are reported in mg QE/100g.

2.8.3. DPPH radical scavenging activity

DPPH radical scavenging activity of tamarillos was calculated according to Saikia et al. [24] with some modification. In a test tube, 200µl of extract was taken in the test tube 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. 3).

$$DPPH\ activity\ (\%) = \frac{A_o - A_s}{A_o} \times 100 \quad (3)$$

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

3.2.8.4. ABTS radical scavenging activity

ABTS radical scavenging activity of tamarillo samples was calculated following the method of Saikia et al. [24]. 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 a diluent. In a test tube, 0.3 mL of sample extract was taken and 2.7 mL of ABTS solution was mixed. The radical scavenging activity was calculated using recorded absorbance of sample (Eq. 4).

$$ABTS\ activity\ (\%) = \left(\frac{A_o - A_s}{A_o}\right) \times 100 \qquad (4)$$

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

3.2.9. HPLC of phenolic acids

The phenolic acids of developed foam mat dried powder were identified and quantified using standards by UHPLC (Ultimate 3000, Thermo Scientific, USA). The identification of phenolic acids present in the tamarillo juice was done by RP-HPLC equipped with C18 column with a diode array detector. 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 solvent flow rate 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 [3].

3.2.10. HPLC of anthocyanins

The anthocyanins in tamarillo 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 extract was prepared according to the method adopted by Espin et al. [3] and sample was filtered through 0.45µm 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 solvent flow-rate 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.

3.2.11. HPLC of carotenoids

The carotenoids in the tamarillo 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 a calibration curve was developed. The sample extract was filtered using 0.45µm syringe filter prior to injecting. A gradient mode consisting of solvent A (water/acetonitrile/methanol 4:14:84, v/v/v) and solvent B (100 %, dichloromethane) at 25ºC was used. The flowrate 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 [19].

3.2.12. *In-vitro* digestibility study

The *in-vitro* digestion of phenolic content present in the tamarillo pulp was measured following the procedure of Kashyap et al. [10]. The oral mastication part was not done. The digestion of sample was done in three phases: oral, gastric, and intestinal phases. Concisely, 5 g of pulp was added with 5 mL of SSF (stimulated salivary fluid) (pH 7) and 7.5 mg of α-amylase and 25 µL of $CaCl_2$ (0.3 M), and pH was adjusted to 7.0 with continuous stirring. The solution was incubated at 37°C for 5 min. In the gastric phase, the sample was mixed with 5 mL of simulated gastric fluid (SGF) (20 mg of pepsin and 2.5 µl of $CaCl_2H_2O$) was added, and pH of the solution was adjusted to 3.0 using HCl. The sample mixture was incubated at 37 °C for 2 h with continuous stirring. Small aliquots were taken out from gastric solution and placed in an ice bath for 10 min followed by centrifugation at 7000g for 20 min. The supernatants were used for the analysis of TPC. In intestinal phase, 10 mL of (SIF) simulated intestinal fluid (37.5 mg of pancreatin and 40 mg of bile salts) was added into gastric phase mixture and pH of the

solution was adjusted to 7.0 using NaOH. The sample mixture was incubated at 37 °C for 2 h with continuous stirring. The crude extract of digesta was placed in an ice bath followed by centrifugation and filtration and stored at -20ºC until further analysis. The extract was analysed for TPC and DPPH scavenging activity.

3.2.13. Determination of bioaccessibility

The percentage bioaccessibility of the phenolic acids was calculated as follows (Eq. 5):

$$\textbf{IB\%} = (\text{ Bioaccessible content / Total initial content}) * 100 \quad (5)$$

Here, IB stands for *in-vitro* bioaccessibility for phenolic content

3.2.14. Statistical analysis

All the experiments were done in triplicate and experimental results are presented as mean ± S.D. (standard deviation). Experimental data were analysed using statistical tool SPSS version 24.0 (SPSS 24.0, IBM Corporation, USA) for ANOVA (analysis of variance) and the Duncan multiple range test with statistical significance ($p < 0.05$).

3.3. Results and discussion

3.3.1. Proximate and biochemical analysis

The proximate analysis of tamarillo varieties given in Table 3.1 show significant differences ($p < 0.05$) in the values for protein, fat, ash, and fibre. The moisture content varied from 89.36 ± 0.69 to 89.06 ± 0.74, which is within the range of 88.1-89.1% (w/w) reported in tamarillo harvested in New Zealand [21]. The crude protein was found to be highest in purple tamarillo and least in red tamarillo. Orqueda et al. [21] studied the chemical and functional characteristics of tamarillo juice and reported 8.84 g protein content /100g of dw in yellow-orange tamarillos from Argentina. The total ash was highest in yellow tamarillo and lowest in red tamarillo variety. High values of the ash content in the pulp indicate that it is a decent source of minerals. The fat and crude fiber content ranged from 0.42-0.25% and 14.30-15.28%. A higher amount of fibre content (19.04%) in tamarillo from Argentina was reported by Orqueda et al. [21]. The experimental results of the biochemical analyses of the samples are reported in Table 3.2. Tamarillo 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 [29]. The TSS of the tamarillo pulp varied from 9-9.8 °Brix. Tamarillo from the Ecuador region was reported to have 9-

11 °Brix TSS [28]. The titratable acidity of the tamarillos was found to range from 1.16-1.30% and agreed with the reported values of 1.03 -1.60 g/100g [29].

Table 3.1. Proximate composition of the pulp of three different tamarillo varieties.

Varieties	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 ± S.D. the values in the same row with different letters on are significantly different by ANOVA test ($p < 0.05$).

The concentration of vitamin C content was uppermost in the purple variety (17.11 mg/100g) trailed by yellow variety and least in the red variety. Our results compared well with the vitamin C content of 16-17 mg/100g reported in purple-red and yellow varieties from Ecuador [28]. However, tamarillos from New Zealand contained higher Vitamin C content in yellow and red varieties [29]. The mineral content determined in the three tamarillo varieties are presented in Table 3.2. Potassium was found in highest quantity in the three varieties and our results are found in the agreement with values reported by Vasco et al. [28]. Colour is one of the most important factors for fruits and vegetables that influences consumer mood towards consuming food. The highest L* (lightness) value was shown by yellow tamarillo (55.23), followed by red variety (48.96) and lowest lightness was depicted by purple tamarillo (Table 3.2). The high concentration of anthocyanins in the purple tamarillo, which imparts red-purple colour to the fruits, reduced the lightness of the sample. As anthocyanins were absent in yellow tamarillo, highest L* (55.23) value was shown by yellow tamarillo. The positive a* value (redness) of the sample indicates the redness of the sample and purple variety showed strong a* value (51.71), followed by red variety (12.44) and yellow tamarillo (8.43), in that order. The positive b* value indicates the yellowness of the sample and highest value was noticed for yellow variety (49.44). Mwithiga et al. [17] evalauted the

influence of ripeness in sensory properties of red tamarillo, found that increase in the ripeness were cause to change in L* (lightness) value which falls from 46 to 22, b* 28.3 to 4.9 and increase in a* value from -4.9 to 28.3 was reported.

Table 3.2. Biochemical analysis of tamarillo varieties

Parameters	Tamarillo varieties		
	Purple	Yellow	Red
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 with different letters are significantly different by ANOVA test ($p < 0.05$).

3.3.2. Effect of HPH and US on the bioactive compounds in tamarillos

3.3.2.1. Total monomeric anthocyanin content (TMAC)

Anthocyanins were found in purple and red tamarillo varieties, and significant difference was found among control, HPH and US treated samples (Table 3.3). The anthocyanins content was high in control sample of purple tamarillo (0.25 C3G/g) in comparison to red variety (0.13 mg C3G/g). The yellow tamarillo did not have any anthocyanins. Vasco et al. [28] reported 0.38 mg (C3G)/g of anthocyanins content in Ecuadorean tamarillo. Increase in TMAC was found in US and HPS treated samples,

with higher concentration in US treated sample (Table 3.3). US treated samples did not show significant difference between 10 and 15 min of sonication time. Decrease in anthocyanins and colour stability were reported for blackcurrant fruit juice when the pressure of HPH was increased from 50 to 220 MPa [12]. Our results showed an initial increase in the anthocyanins content but when pressure was increased from 750 to 1000 bar, there was no significant impact on the anthocyanins release. Researchers have reported that attachment of a cooling system during HPH treatment will help to prevent the degradation of anthocyanins and phenolic compounds [9]. Our results suggested that US treatment for 10 min showed better stability of anthocyanins in purple and red tamarillos in comparison to HPH treated samples.

3.3.2.2. Total carotenoids content

The carotenoids content reported for purple, yellow and red tamarillos in Table 3.3 show significant differences among the control, HPH and US treated samples ($p > 0.05$). Among control samples, the carotenoids content in the yellow fruit pulp was highest followed by purple and least in red variety with a value of 0.63, 0.42 and 0.27 mg βCE/100g, respectively. Vasco et al. [28] reported that purple-red tamarillo has 5.2 mg/100g of β-carotene and yellow tamarillo has 3.2 mg/100g of β-carotene, but our results showed that yellow tamarillo had higher carotenoids content in comparison to purple tamarillo. The higher concentration of carotenoids gives the colour to yellow tamarillo, and the yellow colour in purple tamarillo was masked by the high concentration of anthocyanins in it. The carotenoids extracted from HPH and US were compared and investigated. The results infer that employing HPH up to 750 bar triggered a noteworthy increase in carotenoids content in contrast to the control sample but further increase in pressure up to 1000 bar had no significant effect ($p > 0.05$). US treatment showed an increase in carotenoids content in all varieties of tamarillo. The increase in carotenoids content after giving US and HPH treatments is related to rupture of the cell walls which enhances the release of the bioactive compounds. Similar increase in carotenoids content after giving US in comparison to control sample was reported for gooseberry juice [20].

Table 3.3. Effect of HPH and US treatment on total anthocyanins and total carotenoids present in tamarillo varieties.

Sample	Total monomeric anthocyanin content (mg C3G/g)			Total carotenoids content (mg βCE/100g)		
	Purple	Yellow	Red	Purple	Yellow	Red
Control	0.25 ± 0.01^{c}	N.D.	0. 13 ± 0.02^{c}	0.42 ± 0.04^{c}	0.63 ± 0.05^{d}	0.27 ± 0.02^{b}
HPH-500	0.28 ± 0.01^{c}	N.D.	0.18 ± 0.01^{b}	0.52 ± 0.01^{b}	0.72 ± 0.01^{c}	0.37 ± 0.02^{a}
HPH-750	0.29 ± 0.02^{c}	N.D.	0.21 ± 0.01^{a}	0.59 ± 0.02^{a}	0.78 ± 0.02^{a}	0.39 ± 0.04^{a}
HPH-1000	0.29 ± 0.03^{c}	N.D.	0.21 ± 0.01^{a}	0.57 ± 0.03^{a}	0.77 ± 0.01^{a}	0.40 ± 0.02^{a}
US-05	0.34 ± 0.02^{b}	N.D.	0.22 ± 0.02^{a}	0.51 ± 0.01^{b}	0.69 ± 0.01^{b}	0.35 ± 0.03^{a}
US-10	0.37 ± 0.01^{a}	N.D.	0.21 ± 0.02^{a}	0.53 ± 0.02^{b}	0.74 ± 0.03^{a}	0.36 ± 0.02^{a}
US-15	0.38 ± 0.01^{a}	N.D.	0.21 ± 0.02^{a}	0.52± 0.02^{b}	0.75 ± 0.04^{a}	0.36 ± 0.03^{a}

Values expressed as mean ± SD. Values in the same column with different letters are significantly different by ANOVA test ($p < 0.05$). N.D. means not detected.

3.3.2.3. Total phenolic content

The TPC of control, HPH and US treated samples was analysed, and significant difference was found ($p > 0.05$). The highest concentration of TPC was possessed by purple tamarillo trailed by yellow tamarillo and least by yellow variety (Fig. 1a). Vasco et al. [28] reported higher polyphenol content in purple red variety in comparison to the golden yellow variety of Ecuadorean tamarillo. The phenolic content in purple, yellow and red tamarillo was 6.13, 5.03 and 4.52 mg GAE/g of fresh fruits, and on HPH and US treatments, a rise in TPC was observed. In purple tamarillo, high phenolic content may be correlated with the presence of greater amount of anthocyanins (pelargonidin and delphinidin), which are absent in yellow tamarillo [15]. Increase in the HPH pressure from 750 to 1000 bar led to an increase in TPC, but no significant difference was found among the varieties of tamarillos. However, HPH-750 treatment of purple tamarillo resulted in 30% increase in the TPC and in comparison, 23% and 17% increase occurred

in yellow and red tamarillo, respectively. TPC improved with the increase in pressure from 500 to 750 bar (Fig. 3.1a), and a similar trend was reported in HPH pressure treated strawberry juice [30]. Increase in HPH pressure enhances the efficiency to disrupt the cell materials due to shear stress distribution, which in turn favours better release of the bioactive compounds, especially the bound phenolics and makes them to solubilize [30]. On the other hand, US treated samples showed an increase in TPC in comparison to control sample and extension of the US treatment time had positive impact on TPC. An increase in phenolic content with an increase in ultrasound time was found in yellow and red tamarillo to some extent but after giving further treatment, degradation in TPC was observed. Plazzotta and Manzocco [23] observed that better extraction of polyphenols from lettuce occurred using ultrasound in comparison to HPH but on the contrary, our results showed that application of HPH at 750 bar had the best positive effect on enhancing TPC release from purple, yellow, and red tamarillos.

3.3.2.4. Total flavonoids content

The TFC in different varieties of tamarillo was evaluated and significant difference was found in control, HPH and US treated samples (Fig. 3.1b). The TFC in control sample of purple, yellow and red varieties was 0.98, 0.89 and 0.88 mg QE/g of the fresh fruits sample, respectively. The reported value of quercetin was 4-6 mg/100g and myricetin was 1.2-1.4 mg/100g in purple red and golden-yellow tamarillos cultivated in Ecuador [28]. Application of HPH and US showed positive effect on the release of the flavonoids. When HPH was applied to purple tamarillo, the maximum level of flavonoids was released at 1000 bar, whereas 500 and 750 bar had no discernible impact. On the other hand, a small rise in TFC was seen in yellow and red tamarillo when pressure was increased from 750 to 1000 bar. Sentandreu et al. [26] observed that increase in pressure of HPH leads to a reduction in the particle size of the food sample which favours greater release of bioactive compounds. In US treated samples, treatment time had significant difference in purple and red tamarillo, except red tamarillo, even though an increase in TFC was noticed. An increase in ultrasound time favoured the extraction of bioactive compounds in red and yellow tamarillo fruit. In yellow tamarillo, significant increase in TFC occurred when US time was increased from 10 to 15 min. However, greater yield of TFC was attained by HPH because of the higher efficiency of extractability of bioactive compounds by disrupting plant tissues.

3.3.2.5. DPPH radical scavenging activity

DPPH radical scavenging activity varied significantly among the tamarillo varieties. The maximum activity was shown by purple followed by yellow and least activity was possessed by red tamarillo (Fig.3.1c). It was reported that purple tamarillo possesses higher phenolic content in comparison to golden-yellow tamarillo and shows high antioxidant activity [28]. High value of phenolics and flavonoids content were correlated with enhanced *in-vitro* antioxidant activity [24]. The HPH treated samples showed high DPPH scavenging activity which directly correlated with our results of high extraction of phenolics and flavonoids from the tamarillo samples. Samples treated with HPH at 750 bar showed 10-18% increase in radical scavenging activity, while the US treated sample for 10 min showed an increase of 6-11% activity. The radical scavenging activity of US treated samples being lower in comparison to HPH treated samples can be attributed to factors like ultrasound power, amplitude, and frequency [18]. Our findings are in agreement with Zhang et al. [31], who reported lower scavenging activity in US treated tomato juice than HPH treated juice.

3.3.2.6. ABTS radical scavenging activity

ABTS scavenging activity varied significantly among the tamarillo 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 tamarillo (64.11%) (Fig.3.1d). Application of HPH and US to tamarillo enhanced the antioxidant activity, however the increment was in the range from 4-10% in HPH and 2-9% in US treated samples. ABTS scavenging activity of purple and yellow tamarillo was reported to range from 70-89 and 22-45 Trolox equivalents/g, respectively [3], which was also supported by Wang et al. [29]. Increase in the pressure and time of HPH and US had positive impact on the ABTS activity. In purple and red varieties, increase in US treatment time beyond 10 min had no significant difference on ABTS scavenging activity, however a decreasing effect on yellow tamarillo was found. These results support the observation of Zhang et al. [31] that US treatment of tomato juice caused reduction in carotenoids content. Among all the tamarillo samples, HPH at 750 bar attained maximum antioxidant activity.

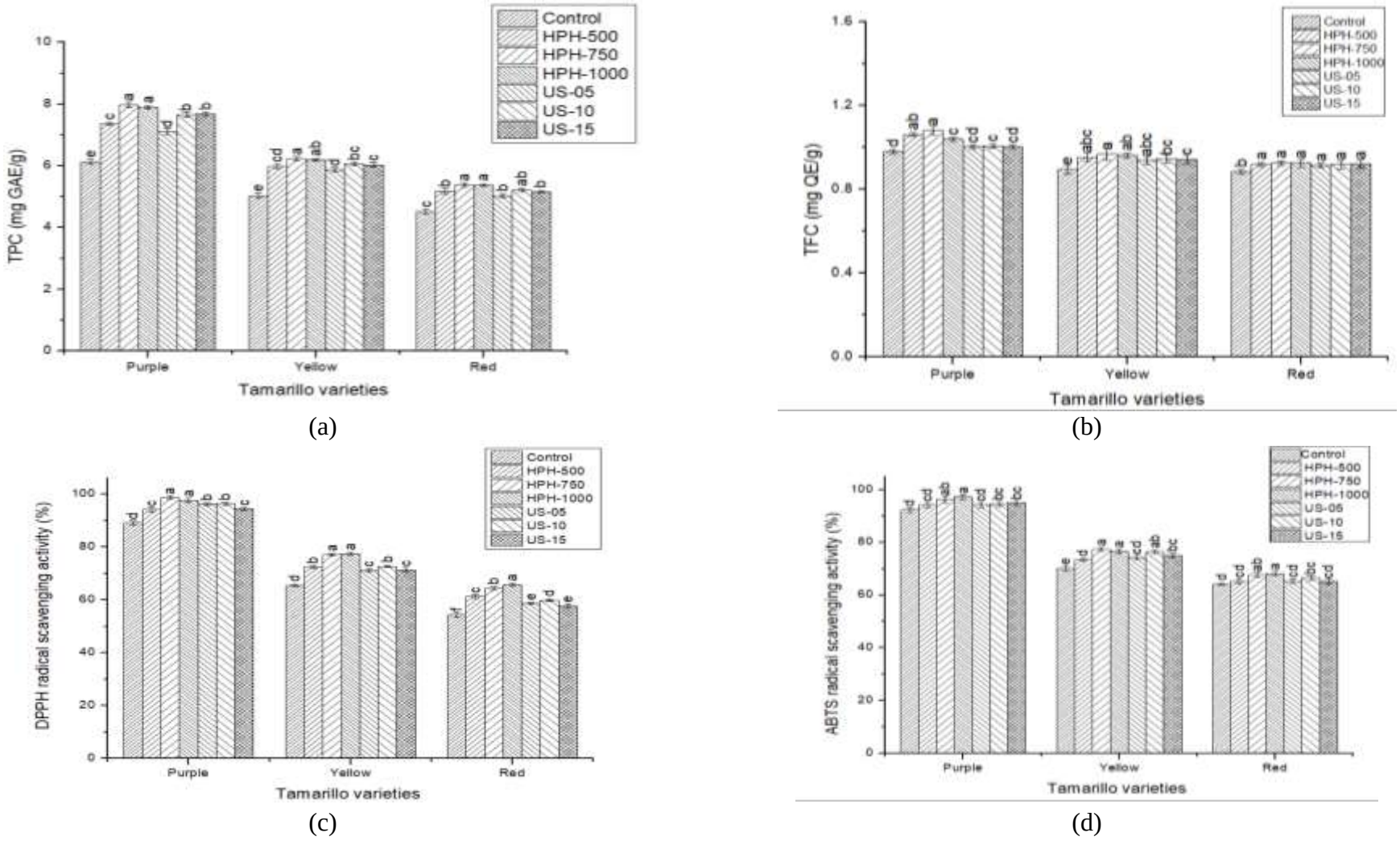


Fig. 3.1. Effect of HPH and US treatment on (a) TPC, (b) TFC, (c) DPPH radical scavenging activity, and (d) ABTS radical activity in tamarillo varieties. Values are expressed in mean ± standard deviation, small alphabets in superscript indicate significant differences ($P<0.05$) between means among columns across samples according to DMRT.

3.3.3. HPLC analysis of the phenolic acids in control, HPH, and US-treated tamarillo samples

The HPLC analysis of phenolic acids present in HPH-750 and US-10 treated tamarillo samples was done, as these samples exhibited maximum phenolics content. Raw sample was taken as the control. Six phenolic acids present in tamarillo juice were identified, namely, gallic acid, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, and rosmarinic acid (Fig. 3.2). The highest concentration of phenolic acids was found in purple variety, followed by the yellow and least concentration was shown in red tamarillo. Espin et al. [3] did not find gallic acid in Ecuadorean tamarillo, while Diep et al. [2] detected gallic acid in tamarillo cultivated in New Zealand.

However, of the six phenolic acids present in untreated sample, only four phenolics, namely gallic acid, caffeic acid, chlorogenic acid, and p-coumaric acid were present in HPH and US treated samples. Ferulic and rosmarinic acid were absent in the HPH and US treated samples. Researchers have reported that rosmarinic acid is an ester of caffeic acid [25] and application of HPH and US in tamarillo may have caused hydrolysis of the ester linkages. Like rosmarinic acid, ferulic acid also gets converted to caffeic acid [25]. Choulitoudi et al. [1] studied the effect of rosmarinic acid in an emulsion and reported that rosmarinic acid degraded faster in comparison to total phenolic acids. The concentration of the all the phenolic acids present in the control, HPH-750 and US-10 treated samples of the three tamarillo varieties are reported in Table 3.4. Treated samples showed an increase in phenolic concentration in comparison to the control, which can be attributed to the better extraction by the HPH and US treatments. The absence of ferulic and rosmarinic acid may be due to several factors like temperature, oxygen, mechanical stress, extraction process, change in pH, etc. The increase in the concentration of the p-coumaric acid in HPH and US treated samples correlated with the increase in temperature during processing; similar increase in p-coumaric acid has been reported by Lu et al. [13].

3.3.4. HPLC analysis of the anthocyanins in control, HPH, and US-treated tamarillo samples

HPLC analysis of anthocyanins present in purple, red, and yellow varieties of tamarillo was done. The anthocyanins present in control sample of purple tamarillo were cyanidin-3-O-rutinoside, delphinidin-3-O-rutinoside, and pelargonidi-3-O-rutinoside. No anthocyanin was detected in yellow tamarillo samples. Vasco et al. [28] also observed

that anthocyanins were present in purple tamarillo but absent in yellow tamarillo. The concentration of anthocyanins was substantially higher in purple variety in comparison to red variety (Table 3.4). Wang and Zhu [29] reported cyanidin-3-glucoside, pelargonidin-3-rutinoside, cyanidin-3-rutinoside, delphinidin-3- glucoside delphinidin-3-rutinoside, and pelargonidin-3-glucoside as the anthocyanins present in tamarillo fruit. In our study, control and US-10 treated purple tamarillo had three anthocyanin compounds, while HPH-750 sample showed only two anthocyanin compounds (Fig. 3.3). Frank et al. [6] studied the stability of anthocyanins in high pressure homogenization and reported that up to 1500 bar there was no degradation of anthocyanins but further increase in temperature had negative impact on the anthocyanins. However, the increase in temperature may be the cause of degradation of the anthocyanins in HPH-750 sample, as our equipment was not equipped with a temperature controller. In US-10, increase in the anthocyanins was found; this increase was related to the improved solubilisation of the anthocyanins in contrast to the control. The variation in the composition of anthocyanins depends upon many factors like cultivar, location, variety, method of extraction and identification, etc. [29]. It is reported that degradation of anthocyanins as correlated with an increase in ultrasound temperature [32]. Our results of TMAC were found to correlate with the HPLC analysis of the anthocyanins.

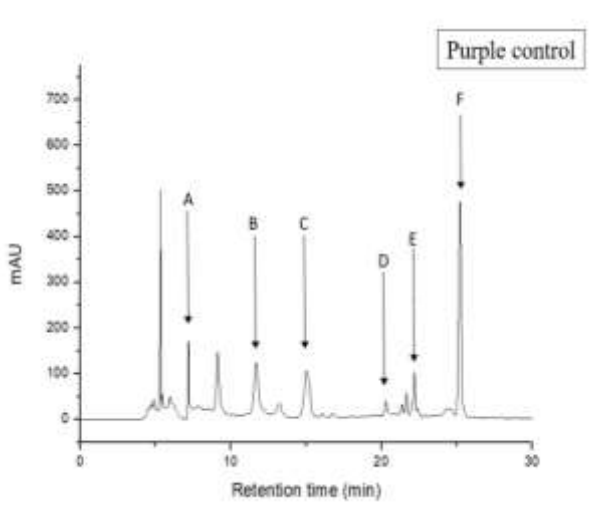
3.3.5. HPLC analysis of the carotenoids in control, HPH, and US-treated tamarillo samples

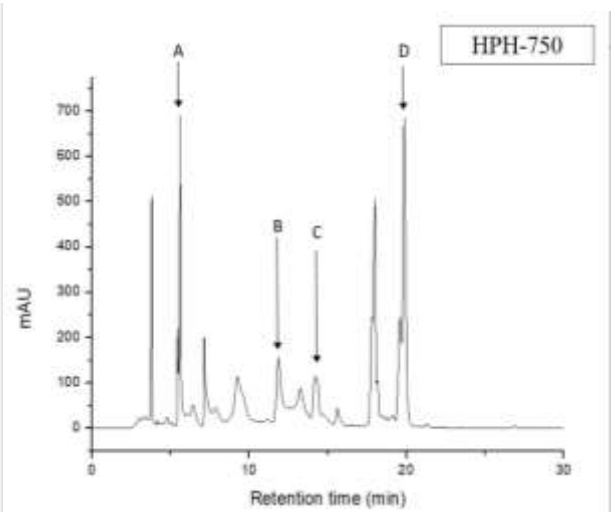
HPLC analysis of carotenoids present in yellow and red varieties of tamarillo was performed (Fig.3.4). In the purple and yellow tamarillo varieties, three carotenoid compounds viz. zeaxanthin, β-crptoxanthin and β-carotene were identified, while zeaxanthin was absent in the red variety. The concentration of β-carotene in the control samples of purple, yellow and red tamarillos was found to be dominant with a value of 199.71, 253.29 and 169.32 µg/100g, respectively (Table 3.4). Mertz et al. [15] studied the stability of carotenoids in tamarillo from the Ecuador region and reported that β-carotene and β-cryptoxanthin are the major carotenoids present. Application of HPH and US caused an increase in the carotenoids content of the samples. However, higher concentration of carotenoids was found in HPH-750 in comparison to US-10 sample. Zhang et al. [31] studied the effect of HPH and ultrasound on tomato juice and reported that maximum extraction of lycopene in HPH was achieved at 200 bar, and further increase in pressure led to carotenoids loss. However, our results indicated that

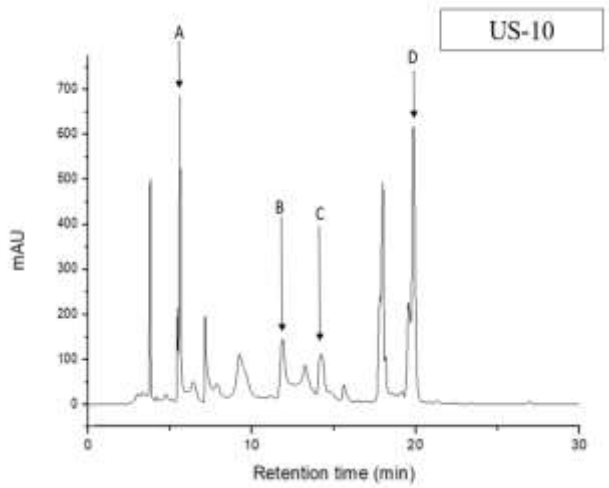
application of up to 750 bar pressure during HPH gave better results for β-cryptoxanthin and β-carotene compounds in all the tamarillo varieties. Mutsokoti et al. [16] studied the effect of HPH with pressure ranging from 10-100 MPa on tomato and carrot-based products and observed that carotenoids were stable and an increase in bioaccessibility was discovered. Ultrasonication was the next finest technique for the extraction of carotenoids. Extraction time, temperature, amplitude imparts major role in extracting the bioactive compounds from the plant matrix [20]. Our results of total carotenoids were found to correlate with the HPLC analysis of the carotenoids.

3.3.6 *In-vitro* digestion of control, HPH, and US treated tamarillo samples

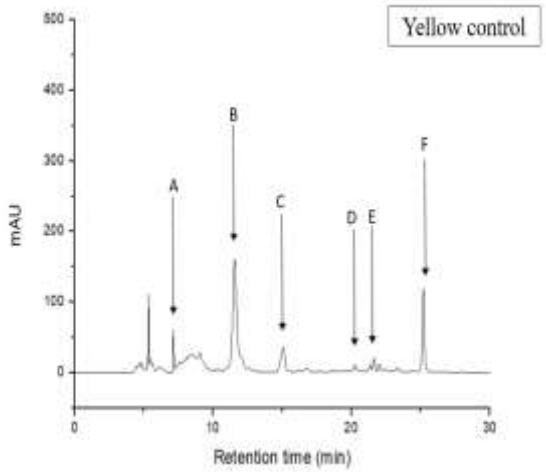
In Fig. 3.5, the TPC and DPPH radical scavenging activity of different varieties of tamarillos in undigested and digested material in the gastric and intestinal phases during *in-vitro* digestion are reported. The bioaccessible fraction in the control, HPH-750, and US-10 treated samples was determined for the three tamarillo varieties. It was clear that application of HPH and US increased the phenolic content at initial level in comparison to the conventional (control) samples. It was noticed that TPC and DPPH radical scavenging activity in the filtrate was found to decrease from gastric to intestinal phase. Kashyap et al. [10] studied the *in-vitro* digestion of phenolic content of Meghalayan cherry and reported that the reduction in phenolic concentration was dependent upon many factors like change in pH and photolytic enzyme action on the sample. Therefore, enzymatic action and change in pH from oral to gastric phase leads to loss of the phenolic compounds [22].

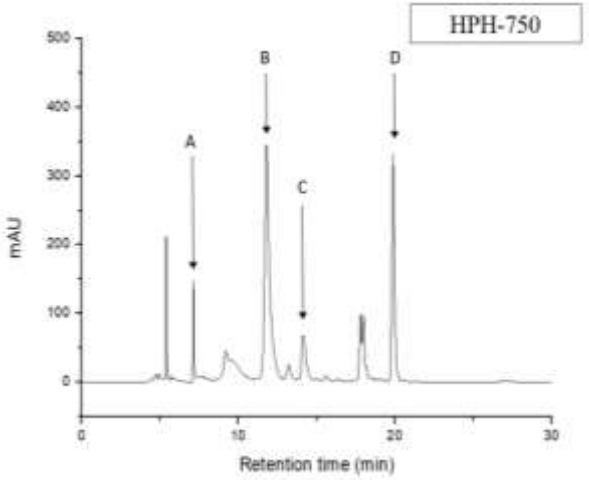


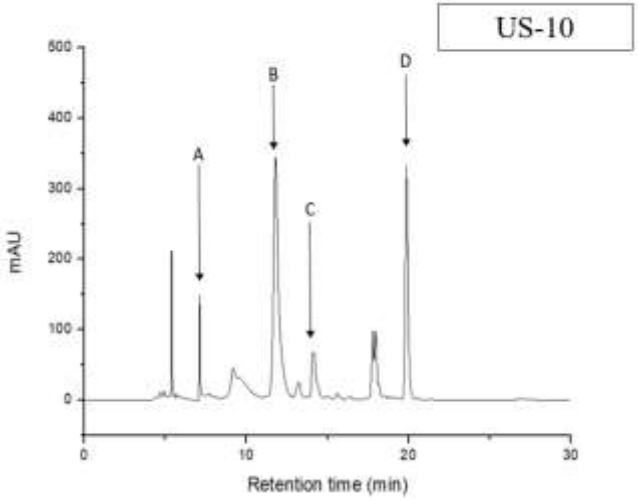




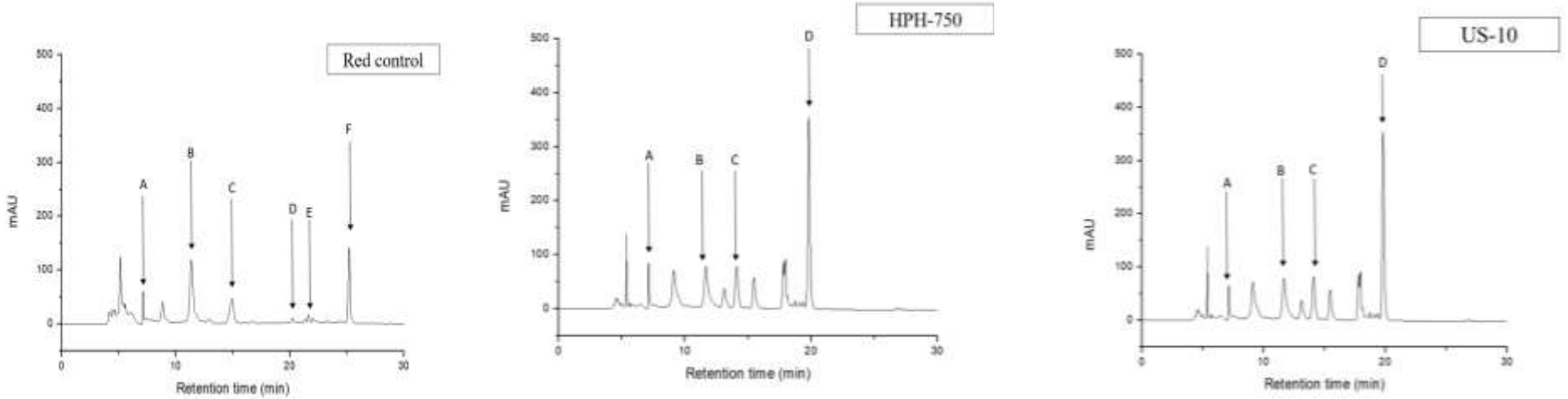
Purple tamarillo







Yellow tamarillo



Red tamarillo

Fig. 3.2. HPLC chromatograms of phenolic acids present in purple, yellow and red tamarillo.

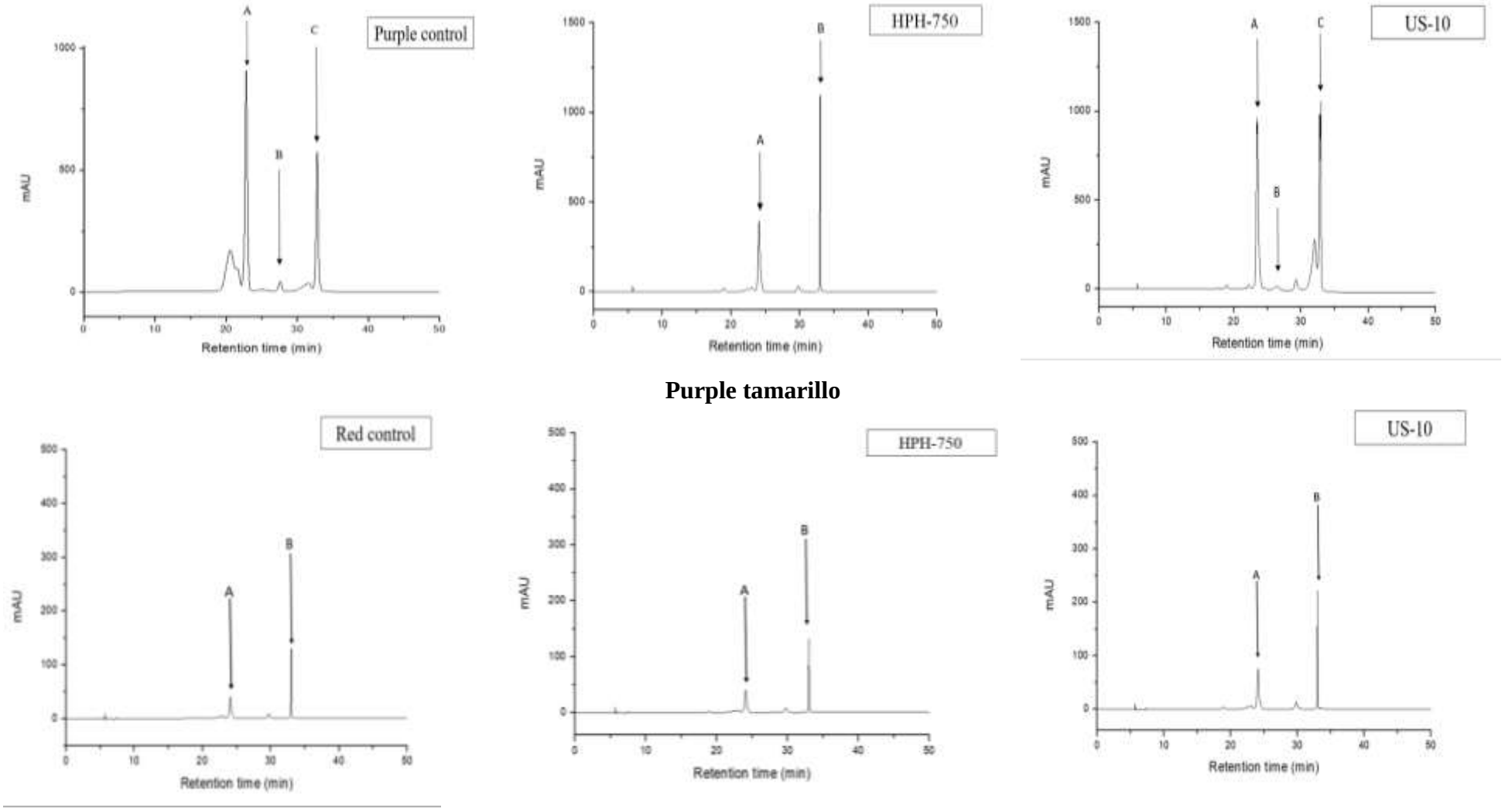
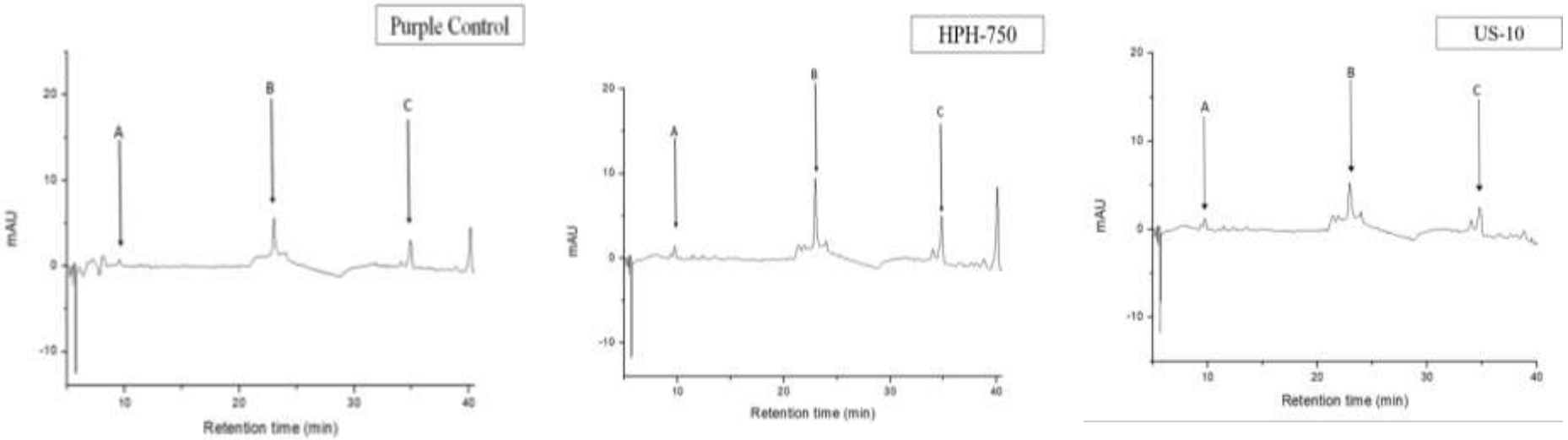
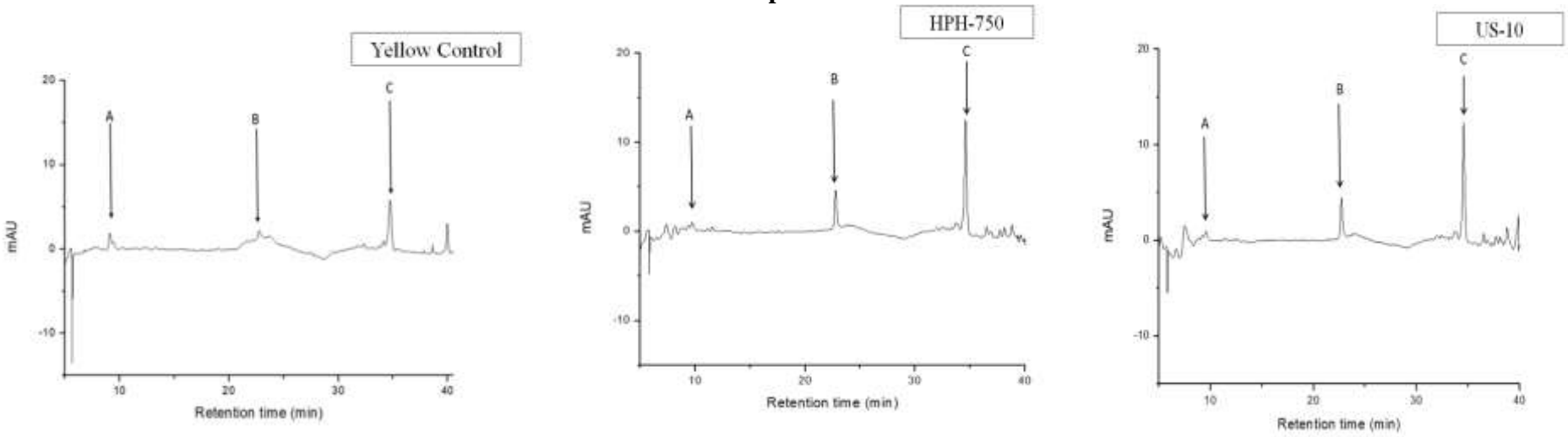


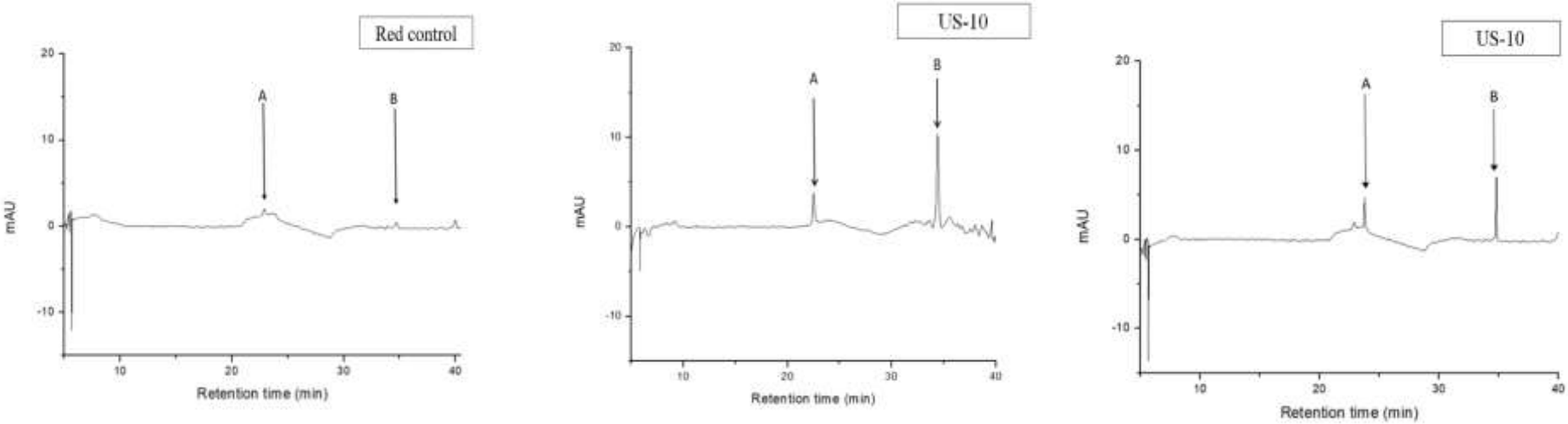
Fig. 3.3. HPLC chromatograms of anthocyanins present in purple and red tamarillo.



Purple tamarillo



Yellow tamarillo



Red tamarillo

Fig. 3.4. HPLC chromatograms of carotenoids found in purple, yellow, and red tamarillo.

Table 3.4. Phenolic acids, anthocyanins, and carotenoids in HPH and US treated tamarillo varieties quantified by HPLC.

Variety		Phenolic acids (µg/g)						Anthocyanins (µg/g)			Carotenoids (µg/100g)		
		Gallic acid	Chlorogenic acid	Caffeic acid	p-coumaric acid	Ferulic acid	Rosmarinic acid	Delphinidin 3-rutinoside	Cyandin-3-rutinoside	Pelargonidin 3-O-rutinoside	Zeaxanthin	β-cryptoxanthin	β-carotene
Purple	**Control**	238.18	124.88	41.13	4.57	98.08	89.15	21.03	7.08	23.32	42.26	85.14	199.71
	HPH-750	264.59	169.43	47.17	11.92	N.D.	N.D.	24.25	N.D.	27.11	78.12	97.34	231.44
	US-10	261.7	163.86	46.77	11.62	N.D	N.D.	45.34	6.65	86.22	72.31	87.08	215.11
Yellow	**Control**	153.9	92.17	22.8	3.73	68.08	53.7	N.D.	N.D.	N.D.	63.65	72.61	253.29
	HPH-750	205.28	104.43	22.19	10.99	N.D.	N.D	N.D.	N.D.	N.D.	81.12	90.05	295.52
	US-10	202.88	100.98	22.95	10.35	N.D.	N.D.	N.D.	N.D.	N.D.	79.24	81.02	273.36
Red	**Control**	67.95	79.24	23.84	3.24	60	47.68	4.05	N.D.	8.05	N.D.	53.32	169.32
	HPH-750	88.84	82.15	17.95	9.83	N.D.	N.D.	4.93	N.D.	8.79	N.D.	89.61	174.65
	US-10	88.84	84.64	16.42	9.28	N.D.	N.D.	5.83	N.D.	9.88	N.D.	81.20	166.16

N.D. stands for not detected.

In HPH-750 treated samples, the IB% of gastric phase of purple, yellow, and red tamarillo was found to be 86, 79, and 88%, respectively. However, the IB% of gastric phase in US treated sample of purple, yellow, and red tamarillo was found to be 88, 73, and 89%, respectively (Table 3.5). The higher IB% in US treated sample in comparison to HPH treated sample might be due to anthocyanin stability in gastric phases and US favours anthocyanin stability more than HPH [4]. The initial activity in gastric phase was related to the release of the bioactive compounds, specifically the anthocyanins from the sample matrix into the extraction solvents. Sollano-Mendieta et al. [27] studied the *in vitro* digestion of plum and reported that after digestion, anthocyanins were degraded in the intestinal phase, and carotenoids were not released during gastric digestion but released in the intestinal fluid. Therefore, release of anthocyanins and carotenoids was found in the HPH and US treated samples and our results suggested that processing aids in enhancing the bioaccessibility of bioactive compounds in tamarillo fruit.

Table 3.5. *In-vitro* bioaccessibility of control, HPH, and US treated tamarillo varieties

Samples	Purple		Yellow		Red	
	Gastric (IB %)	**Intestinal (IB %)**	**Gastric (IB %)**	**Intestinal (IB %)**	**Gastric (IB %)**	**Intestinal (IB %)**
Control	83.52	69.66	74.35	72.56	86.06	76.33
HPH-750	86.09	75.31	79.94	74.64	88.85	84.39
US-10	88.63	76.86	73.43	68.48	89.44	83.30

IB % stands for *in-vitro* bioaccessibility

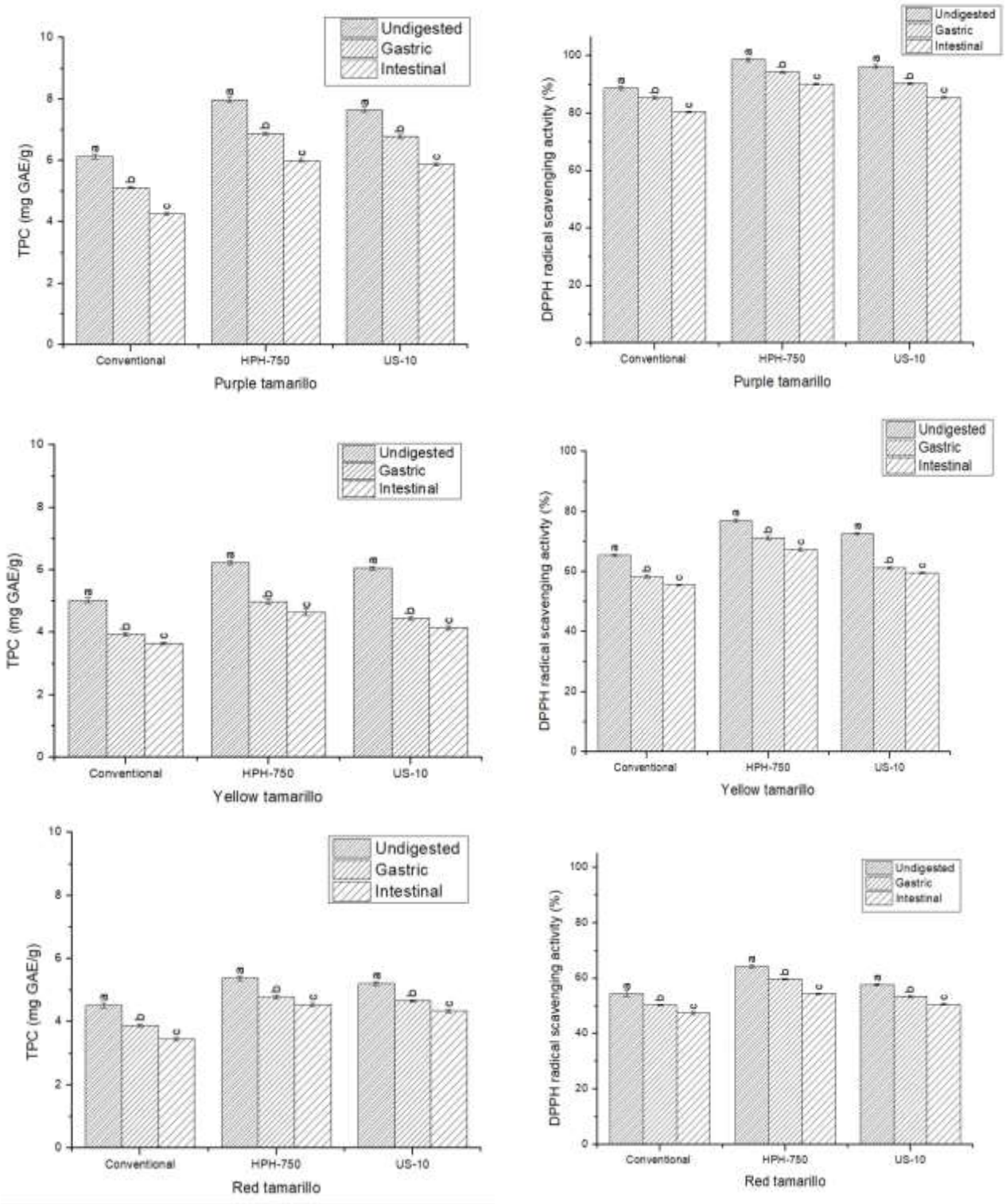


Fig. 3.5. Impact of *in-vitro* digestion on TPC and DPPH radical scavenging activity of Control, HPH-750, and US-10 treated tamarillo samples.

3.3 Conclusion

A difference in the concentration of proximate, biochemical, and antioxidant properties and phenolic profile was found among purple, yellow, and red tamarillos. Purple tamarillo was rich in anthocyanins and yellow variety was rich in carotenoids, however both anthocyanins and carotenoids were present in purple and red varieties. Our results suggested that application of HPH and US enhanced the extraction of bioactive compounds from the tamarillos and made it more bioaccessible than untreated sample. For processing, HPH at a pressure of 750 bar is recommended for carotenoids-rich tamarillo and US for 10 min is suggested for anthocyanins rich tamarillo.

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