CHAPTER 5

OBJECTIVE 3

CHAPTER 5 SECTION I

STUDY THE EFFECT OF ADDITION OF OLIVE OIL AND THERMAL TREATMENT ON TAMARILLO PUREE

5.1.1. Introduction

Tamarillo or tree tomato (*Solanum betaceum*) is an edible fruit available in subtropical countries, that is attracting great interest among consumers and researchers due to the wide range of bioactive and flavor compounds present [1]. Tamarillo fruit is sweet-acidic in taste and is available in red, orange and purple colors [2]. These varieties are remarkably different in nutritional and phytochemical composition. Tamarillo is usually consumed in raw form, in salads, desserts, and beverages [18]. Tamarillo is rich in bioactive compounds especially phenolic acids, flavonoids, carotenoids, vitamin C, vitamin A, anthocyanins, etc. which are known to inhibit or decrease the risk of cancer, cardiovascular diseases, obesity etc. [12]. Carotenoids are the fat soluble compounds present in tamarillo, which are known to give typical colour to the fruit and exhibit various health benefits [14]. The major carotenoids reported in Brazilian cultivar were β-carotene and β-cryptoxanthin [21], while all-trans-β-cryptoxanthin esters and all-trans-β-carotene were found in tamarillo from Ecuador [15]. Tamarillo is a climacteric and seasonal fruit having high moisture content that makes it highly prone to microbial spoilage and degradation of bioactive compounds.

Thermal treatments like sterilization, pasteurization, cooking, and dehydration are widely applied techniques for the processing and preservation of food materials. Various changes in the concentration of phytochemicals due to thermal treatment have been reported in literature [13]. Thermal processing helps to extend shelf life of foods, for which selection of the right combination of temperature and time is required to obtain shelf stable products with maximum activity of the bioactive compounds [3]. Mertz et al. [14] studied the effect of heating of tamarillo pulp and reported that heating led to *cis*-isomerization of carotenoids and change in pulp colour.

Colle et al. [6] reported that addition of oil to tomato puree had positive effects on bioactive compounds during thermal processing and enhanced the bio-accessibility of carotenoids. Although tamarillo is loaded with health promising bioactive compounds, its commercial processing is limited. There are limited studies on thermal processing of tamarillo and its effect on bioactive compounds. The aim of this study was to know the effect of thermal processing of puree on the bioactive compounds and assess the effect of olive oil addition in puree on the concentration and activity of the bioactive compounds, which will be helpful for canning of tamarillo puree.

5.1.2. Materials and methods

5.1.2.1. Materials

Red coloured tamarillo was purchased from a local market in Kohima, Nagaland. Fresh tamarillos of similar size and firmness were sorted out for the study. Virgin olive oil (Del Monte Foods) was purchased from a local market in Tezpur, Assam.

5.1.2.2. Sample preparation

Fresh tamarillo were washed and blanched in boiling water for 90 s (Icier et al., 2006). The blanched tamarillos were peeled and cut into halves and processed into puree using mixer grinder (Philips HL Model No. 1632, India). Tamarillo seeds were removed from the puree using a standard stainless steel sieve, and 5% (v/v) of olive oil was added and blended in mixer-grinder for 120 s. All the experiments were done in a dark area to avoid the degradation of bioactive compounds.

5.1.2.3. Thermal processing of puree

The thermal processing of the puree was done in an oil bath (Ganesh Scientific Industries, India). One hundred gram of prepared puree was taken in a 500 ml beaker and covered with aluminum foil and kept in the oil bath for thermal treatment. The temperature and time combination followed was according to the method of Kang et al. [10] with some modification. The puree was subjected to 100º, 121º, 150º, 175º and 200ºC for 2, 4 and 6 min. After processing, treated puree was immediately cooled in an ice water bath. Treated puree was kept in airtight plastic container and kept under -20º C, until further analysis.

5.1.2.4. Biochemical analysis

The pH and total soluble solids (TSS) of tamarillo samples were analysed using a pH meter (Eutech) and a hand refractometer (0-32 °Brix Erma, Japan), respectively (Ranganna, 1986).

5.1.2.5. Total phenolic content

Th**e** total phenolic content (TPC) in tamarillo was determined according to Saikia and Mahanta [22]. For the analysis, an aliquot of 0.5 mL of diluted sample extracts was taken in test tubes 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 were expressed in mg GAE/100g.

5.1.2.6. Total flavonoid content

The total flavonoid content (TFC) in tamarillo samples was determined according to Saikia and Mahanta [22]. 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 read at 415 nm in UV-Vis spectrophotometer (Thermo-Fischer Evolution A600) against blank. Quercetin was used as the standard, and results were expressed in mg QE/100g.

5.1.2.7. DPPH radical scavenging activity

DPPH radical scavenging activity of tamarillos was calculated according to Saikia and Mahanta [22] with some modification. 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).

$$DPPH\ activity\ (\%) = \frac{A_o - A_s}{A_o} \times 100 \quad (1)$$

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

5.1.2.8. Total carotenoids content

The total carotenoids content in the tamarillo sample was calculated according to the method adopted by Rodriguez-Amaya et al. [21], with some modifications. 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 hexane containing carotenoids was read at 450 nm (Thermo-Fischer Evolution A600) and results were reported as mg of β-carotene equivalents (mg β-CE)/ g of sample.

5.1.2.9. 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). The Hue angle (color perception) was calculated according to Eq. 2 and Chroma (intensity of colour) of the puree samples was determined as mentioned in Eq. 3 [16].

$$Hue\ angle = tan^{-1}\frac{b^*}{a^*} \quad (2)$$

$$Chroma = \sqrt{(a^*)^2 + (b^*)^2} \quad (3)$$

5.1.2.10. Statistical analysis

All the experiments were done in triplicate and data are presented as mean ± standard deviation. Data was analyzed statistically by applying ANOVA (analysis of variance) and

Duncan's multiple range test with statistical significance ($p < 0.05$) using software (IBM SPSS Statistics Version 24.0, Armonk, NY: IBM Corporation,). The significant difference between puree without oil and puree with oil was calculated using student t-test.

5.1.3. Results and discussion

5.1.3.1. Effect of thermal processing and oil on pH and TSS

Temperature and heating time had significant and positive effect on the TSS, which increased in treated puree sample in comparison to control samples (untreated puree samples prepare with and without oil) (Table 5.1.1). TSS depends upon the moisture of sample and samples sealed with aluminum foil during treatment showed loss of moisture and increase in TSS [17] . The other reason for increase in TSS was heating, which enables de-polymerization of pectin substances and breakdown of insoluble polysaccharides (dietary fiber), causing a change in TSS value [24]. Similar trend for pH was observed and puree without oil showed greater increase in pH (Table 5.1.1). The increase in pH with increase in heating temperature and time may be associated with the evaporative effect of organic acids [4]. Therefore, addition of oil in the puree prevents the increase of pH during heating and helps in retaining the bioactive compounds. Vasco et al. [25] analysed same variety of tamarillo but cultivated in different countries and reported that pH ranged from 3.2- 3.5, vitamin C from 16-17 mg/100g, citric acid from 1.8-2.5 %, and mallic acid from 0.07-0.32 %. Mertz et al. [15] studied the thermal lability of vitamin C in yellow tamarillo fruit and found that increase in temperature from 80 to 95ºC caused loss in ascorbic and dehydroascorbic acid concentration. Addition of oil was therefore helpful in protecting the organic acids in tamarillo puree.

5.1.3.2. Effect of thermal processing and oil on total phenol content (TPC)

Puree without oil showed high TPC levels during thermal treatment at 150º and 175ºC, whereas in purees with oil, high TPC values were seen on treatment at 121º, 150º and 175ºC. TPC increased with time at lower temperature, but with further increase in temperature and time, a negative effect was observed (Table 5.1.2). The increase in phenolic content during heating was related to release of bound phenolic acids in , but after certain limit, excessive heating led to degradation of the phenolics. Addition of oil, therefore,

showed positive effect on TPC that are supported by findings on tomato puree [7]. Kim et al. [11] studied the effect of heating temperature (100 to 150ºC) on juices and reported 7 times more phenolic content in sample treated at 150ºC. Choi et al. [5] reported that increase in heating time and temperature from 100 to 121ºC and 10 to 30 min, respectively increased the free phenolic content by 1.9 times in mushroom. Rinaldi de Alvarenga et al. [20] reported that addition of olive oil helps in better extraction of phenolic compounds from sample matrix.

Table 5.1.1. Effect of thermal processing and addition of oil on TSS and pH of tamarillo puree

Temp (ºC)	Time (min)	TSS (º Brix)		pH	
		Without oil	With oil	Without oil	With oil
Control		$10.6 \pm 0.07^{m,A}$	$10.7 \pm 0.07^{f,A}$	$3.43 \pm 0.01^{k,A}$	$3.46 \pm 0.01^{k,A}$
100	**2**	$10.75 \pm 0.07^{l,A}$	$10.79 \pm 0.14^{f,A}$	$3.71 \pm 0.01^{j,A}$	$3.62 \pm 0.01^{i,B}$
	4	$11.05 \pm 0.14^{k,A}$	$11.05 \pm 0.07^{e,A}$	$3.76 \pm 0.02^{i,A}$	$3.64 \pm 0.02^{h,B}$
	6	$11.35 \pm 0.07^{j,A}$	$11.55 \pm 0.014^{de,A}$	$3.78 \pm 0.02^{h,A}$	$3.71 \pm 0.04^{g,B}$
121	**2**	$11.55 \pm 0.12^{i,A}$	$11.75 \pm 0.07^{d,A}$	$3.73 \pm 0.01^{i,A}$	$3.71 \pm 0.02^{g,A}$
	4	$12.15 \pm 0.14^{f,A}$	$12.25 \pm 0.07^{b,A}$	$3.79 \pm 0.02^{h,A}$	$3.76 \pm 0.01^{f,A}$
	6	$12.25 \pm 0.12^{e,A}$	$12.35 \pm 0.07^{b,A}$	$3.81 \pm 0.03^{g,A}$	$3.77 \pm 0.02^{e,B}$
150	**2**	$11.95 \pm 0.12^{h,A}$	$11.85 \pm 0.07^{e,A}$	$3.78 \pm 0.02^{h,A}$	$3.77 \pm 0.02^{e,A}$
	4	$12.45 \pm 0.07^{d,A}$	$12.55 \pm 0.14^{b,A}$	$3.82 \pm 0.01^{g,A}$	$3.78 \pm 0.02^{de,B}$
	6	$12.55 \pm 0.07^{c,B}$	$13.65 \pm 0.14^{a,A}$	$3.84 \pm 0.03^{f,A}$	$3.79 \pm 0.01^{d,B}$
175	**2**	$12.05 \pm 0.14^{g,A}$	$11.95 \pm 0.07^{c,A}$	$3.79 \pm 0.02^{h,A}$	$3.78 \pm 0.02^{de,A}$
	4	$12.65 \pm 0.14^{b,B}$	$13.55 \pm 0.14^{a,A}$	$3.88 \pm 0.04^{e,A}$	$3.79 \pm 0.01^{d,B}$
	6	$12.68 \pm 0.07^{b,B}$	$13.55 \pm 0.14^{a,A}$	$3.97 \pm 0.01^{c,A}$	$3.80 \pm 0.02^{c,B}$
200	**2**	$12.55 \pm 0.14^{c,A}$	11.95 ± 0.14^{c}	$3.93 \pm 0.02^{d,A}$	$3.78 \pm 0.01^{de,B}$
	4	$12.85 \pm 0.07^{a,B}$	$13.45 \pm 0.14^{a,A}$	$4.01 \pm 0.02^{b,A}$	$3.82 \pm 0.02^{b,B}$
	6	$12.85 \pm 0.14^{a,B}$	$13.45 \pm 0.07^{a,A}$	$4.11 \pm 0.03^{a,A}$	$3.85 \pm 0.04^{a,B}$

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 Duncan's multiple range test; capital letters (A & B) in superscript indicate significant difference ($P<0.05$) between samples without oil and with oil according to student t-test.

5.1.3.3. Effect of heating and addition of oil on total flavonoids content (TFC)

The effect of temperature and time on TFC was similar to TPC. Only difference was that TFC was retained at a higher level even at 121°C in purees without oil and with oil (Table 5.1.2). Dewanto et al. [7] reported an increase in TFC in tomato puree on heating. Higher temperature facilitates the release of bioactive material from cell tissue, and oil acts as a solvent for the flavonoids. Our results showing increase in the flavonoids content with time is supported by the study on mushroom, where increase in heating time from 15 to 30 min at 100ºC increased TFC from 0.8/100 g to 2.5 mg/100g [5].

5.1.3.4. Effect of heating and addition of oil on *in-vitro* antioxidant activity

The DPPH radical scavenging activity in the raw puree without and with oil was 24.23 and 24.96 %, respectively. Heating temperature and time had positive effect on DPPH activity, with maximum activity being exhibited at 175^{o}C in both type of purees (Table 5.1.2). The increase is related to the release of bound bioactive compounds from the sample cell matrix. Kang et al. [10] reported that thermal processing induces more antioxidant activity in ginseng as compared to raw. Ravichandran et al. [19] studied the impact of processing on red beetroot and reported that thermal processing (boiling, roasting and microwave treatments) helped in enhancing the antioxidant activity by 2 to 3 folds. Tamarillo puree with oil registered greater DPPH activity as oil acted as a better solvent for the phenolics and flavonoids. Our results are in agreement with Gärtner et al. [8], who observed 15% increases in antioxidant activity in tomato paste having olive oil with increase in cooking time.

5.1.3.5 Effect of addition of heating and addition of oil on total carotenoids content

The total carotenoids content in the raw sample puree prepared without and with oil was 0.63 and 0.65 mg β carotene/g, respectively. Carotenoids increased significantly with heating temperature and time and maximum levels were observed at 200^{o}C for heating time of 4 and 6 min in both forms of puree (Table 5.1.2). Addition of oil along with heat enhances the extraction of carotenoids because carotenoids naturally are oil soluble compounds and heating favors their solubility. Vallverdú-Queralt et al. [23] found that addition of 5% oil to tomato juice and increasing the cooking time had increased carotenoids

content. Gärtner et al. [8] also reported 2-3 folds increase in carotenoids concentration after cooking of tomato puree in oil. Olive oil improved carotenoids extractability from carrot [9].

5.1.3.6. Effect of heating time and addition of oil on colour values

Increase in heating temperature and heating time had significantly affected the colour values (Table 5.1.3). L* and a* values in puree had decreased on thermal treatment with time, and puree without oil exhibited greater changes than puree with oil. b* values (yellowness) increased with temperature and time of treatment, with puree with oil registering greater values than that without oil. The increase in yellowness of the puree is related to the carotenoids content (Table 5.1.2). The decrease in a* values may be related to degradation of bioactive compounds. Similar trend of decrease in L* and a* values after processing at 50-120ºC temperature for 0-60 min was reported for tomato puree [16]. The Hue angle refers to colour perception and was found to be amplified with processing parameters which may be attributed to the increase in carotenoids level. Chroma refers to the saturation of colour, and was found to be significantly higher in puree with oil. This finding suggests that addition of oil to tamarillo puree amplifies the saturation of colour and this is a desirable property in puree processing (Table 5.1.3).

Table 5.1.2. Effect of thermal processing and addition of oil on phenolic, flavonoids, in-vitro antioxidant and carotenoids content of tamarillo puree

Temp (^oC)	Time (min)	TPC (mg GAE/g)		TFC (mg QE/g)		DPPH activity (%)		Total carotenoids (mg β carotene/g)	
		Without oil	With oil	Without oil	With oil	Without oil	With oil	Without oil	With oil
Control		$5.15 \pm 0.05^{efg,A}$	$5.17 \pm 0.02^{d,A}$	$0.90 \pm 0.01^{h,A}$	$0.91 \pm 0.01^{g,A}$	$24.23 \pm 0.45^{de,A}$	$24.96 \pm 0.62^{e,A}$	$0.63 \pm 0.01^{i,A}$	$0.65 \pm 0.01^{i,A}$
100	**2**	$5.02 \pm 0.02^{g,B}$	$5.21 \pm 0.02^{d,A}$	$0.90 \pm 0.01^{h,A}$	$0.92 \pm 0.02^{g,A}$	$20.64 \pm 0.45^{g,B}$	$23.80 \pm 0.62^{f,A}$	$0.63 \pm 0.01^{i,B}$	$0.82 \pm 0.01^{h,A}$
100	**4**	$5.14 \pm 0.02^{efg,B}$	$5.23 \pm 0.03^{d,A}$	$0.91 \pm 0.02^{gh,A}$	$0.94 \pm 0.03^{fg,A}$	$21.82 \pm 0.71^{fg,B}$	$25.01 \pm 0.51^{e,A}$	$0.64 \pm 0.02^{i,B}$	$0.85 \pm 0.02^{gh,A}$
100	**6**	$5.15 \pm 0.02^{efg,B}$	$5.28 \pm 0.07^{d,A}$	$0.95 \pm 0.02^{def,A}$	$0.97 \pm 0.02^{ef,A}$	$24.17 \pm 1.14^{de,B}$	$25.95 \pm 0.42^{de,A}$	$0.67 \pm 0.01^{h,B}$	$0.87 \pm 0.01^{fg,A}$
121	**2**	$5.24 \pm 0.02^{def,B}$	$5.57 \pm 0.02^{bc,A}$	$0.93 \pm 0.02^{abc,A}$	$0.95 \pm 0.01^{fg,A}$	$23.55 \pm 0.60^{ef,B}$	$25.81 \pm 0.63^{de,A}$	$0.70 \pm 0.03^{g,B}$	$0.84 \pm 0.01^{h,A}$
121	**4**	$5.30 \pm 0.21^{bcd,B}$	$5.86 \pm 0.04^{a,A}$	$0.96 \pm 0.02^{bcd,A}$	$0.95 \pm 0.02^{fg,A}$	$26.52 \pm 0.28^{bc,A}$	$26.82 \pm 0.61^{bcde,A}$	$0.76 \pm 0.02^{f,B}$	$0.89 \pm 0.02^{f,A}$
121	**6**	$5.35 \pm 0.02^{abcd,B}$	$5.87 \pm 0.06^{a,A}$	$0.99 \pm 0.04^{ab,A}$	$0.98 \pm 0.01^{cdef,A}$	$28.32 \pm 0.94^{ab,A}$	$27.43 \pm 0.79^{abcd,B}$	$0.76 \pm 0.01^{f,B}$	$0.89 \pm 0.01^{f,A}$
150	**2**	$5.39 \pm 0.03^{abcd,B}$	$5.71 \pm 0.02^{abc,A}$	$0.98 \pm 0.02^{abc,A}$	$1.00 \pm 0.01^{bcde,A}$	$25.68 \pm 0.45^{cd,A}$	$26.31 \pm 1.14^{cde,A}$	$0.77 \pm 0.02^{e,B}$	$0.86 \pm 0.03^{g,A}$
150	**4**	$5.40 \pm 0.02^{abc,B}$	$5.90 \pm 0.11^{a,A}$	$0.98 \pm 0.02^{ab,A}$	$1.05 \pm 0.04^{a,B}$	$26.96 \pm 1.20^{abc,B}$	$29.02 \pm 0.42^{ab,A}$	$0.80 \pm 0.03^{d,B}$	$0.91 \pm 0.02^{de,A}$
150	**6**	$5.46 \pm 0.05^{ab,B}$	$5.87 \pm 0.08^{a,A}$	$0.92 \pm 0.06^{fgh,A}$	$1.01 \pm 0.01^{bcd,B}$	$27.30 \pm 1.33^{abc,B}$	$29.17 \pm 0.27^{ab,A}$	$0.81 \pm 0.01^{c,B}$	$0.93 \pm 0.01^{d,A}$
175	**2**	$5.42 \pm 1.04^{abc,B}$	$5.71 \pm 0.02^{abc,A}$	$0.97 \pm 0.07^{abc,A}$	$0.97 \pm 0.02^{def,A}$	$28.75 \pm 0.70^{a,B}$	$29.76 \pm 0.62^{a,A}$	$0.84 \pm 0.03^{b,B}$	$0.92 \pm 0.02^{de,A}$
175	**4**	$5.46 \pm 0.13^{a,B}$	$5.91 \pm 0.12^{a,A}$	$1.01 \pm 0.02^{a,B}$	$1.04 \pm 0.01^{a,A}$	$28.31 \pm 1.32^{ab,B}$	$29.17 \pm 1.14^{ab,A}$	$0.87 \pm 0.02^{a,B}$	$0.98 \pm 0.02^{c,A}$
175	**6**	$5.27 \pm 0.08^{cdef,B}$	$5.57 \pm 0.08^{bc,A}$	$0.96 \pm 0.02^{bcd,B}$	$1.02 \pm 0.02^{abc,A}$	$26.15 \pm 0.70^{c,B}$	$29.19 \pm 0.71^{ab,A}$	$0.87 \pm 0.02^{a,B}$	$1.07 \pm 0.01^{a,A}$
200	**2**	$5.32 \pm 0.08^{abcd,B}$	$5.78 \pm 0.15^{ab,A}$	$0.98 \pm 0.06^{abc,A}$	$1.01 \pm 0.03^{bcd,A}$	$23.78 \pm 0.65^{de,B}$	$28.42 \pm 0.76^{abc,A}$	$0.87 \pm 0.01^{a,B}$	$1.04 \pm 0.02^{b,A}$
200	**4**	$5.28 \pm 0.05^{cde,B}$	$5.58 \pm 0.01^{bc,A}$	$0.95 \pm .02^{cde,B}$	$1.01 \pm 0.01^{bcd,A}$	$23.58 \pm 0.21^{ef,B}$	$27.28 \pm 131^{bcde,A}$	$0.87 \pm 0.01^{a,B}$	$1.06 \pm 0.02^{ab,A}$
200	**6**	$5.12 \pm 0.02^{fg,B}$	$5.52 \pm 0.14^{c,A}$	$0.93 \pm 0.05^{fgh,B}$	$0.99 \pm 0.01^{bcde,A}$	$23.81 \pm 0.78^{de,B}$	$27.46 \pm 0.46^{abcd,A}$	$0.87 \pm 0.01^{a,B}$	$1.06 \pm 0.01^{ab,A}$

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 Duncan's multiple range test; capital letters (A & B) in superscript indicate significant difference ($P<0.05$) between samples without oil and with oil according to student t-test.

Table 5.1.3. Effect of thermal processing and addition of oil on colour parameters of tamarillo puree

Temp (°C)	Time (min)	L*		a*		b*		Hue (*H*°)		Chroma	
		Without oil	With oil	Without oil	With oil	Without oil	With oil	Without oil	With oil	Without oil	With oil
Control		54.66 ± 0.78^{a,A}	55.87 ± 0.33^{a,A}	10.44 ± 0.11^{a,B}	11.93 ± 0.06^{a,A}	27.78 ± 0.65^{f,A}	28.96 ± 0.89^{j,A}	69.40 ± 0.49^{h,A}	67.63 ± 0.72^{k,B}	29.68 ± 0.57^{f,B}	31.35 ± 0.81^{i,A}
100	**2**	50.76 ± 0.62^{b,B}	55.82 ± 0.17^{a,A}	10.27 ± 0.05^{a,B}	11.91 ± 0.02^{a,A}	28.46 ± 0.23^{f,B}	35.22 ± 0.14^{i,A}	70.17 ± 0.14^{g,B}	71.11 ± 0.11^{j,A}	30.27 ± 0.20^{e,B}	37.17 ± 0.12^{g,A}
100	**4**	50.73 ± 0.70^{b,B}	55.25 ± 0.14^{b,A}	10.14 ± 0.02^{a,B}	11.70 ± 0.14^{ab,A}	29.05 ± 0.09^{e,B}	36.27 ± 0.05^{h,A}	70.75 ± 0.01^{g,B}	71.12 ± 0.05^{j,A}	30.76 ± 0.11^{e,B}	38.12 ± 0.04^{fg,A}
100	**6**	50.23 ± 0.21^{bc,B}	55.21 ± 0.07^{b,A}	10.12 ± 0.02^{a,B}	11.68 ± 0.27^{ab,A}	29.30 ± 0.07^{de,B}	36.95 ± 0.21^{gh,A}	70.93 ± 0.01^{g,B}	72.29 ± 0.10^{i,A}	31.01 ± 0.09^{de,B}	38.41 ± 0.19^{fg,A}
121	**2**	50.82 ± 0.71^{b,B}	55.22 ± 0.44^{b,A}	10.19 ± 0.14^{a,B}	11.55 ± 0.22^{bc,A}	29.16 ± 0.08^{de,B}	36.88 ± 0.33^{gh,A}	70.75 ± 0.21^{g,B}	72.61 ± 0.12^{hi,A}	30.92 ± 0.12^{e,B}	38.51 ± 0.32^{fg,A}
121	**4**	49.94 ± 0.41^{c,B}	54.93 ± 0.24^{b,A}	9.66 ± 0.42^{b,B}	11.48 ± 0.32^{bc,A}	29.41 ± 0.07^{d,B}	37.16 ± 0.08^{g,A}	71.81 ± 0.78^{f,B}	72.83 ± 0.36^{gh,A}	30.95 ± 0.08^{de,B}	38.91 ± 0.06^{f,A}
121	**6**	49.84 ± 0.68^{c,B}	54.56 ± 0.09^{c,A}	9.38 ± 0.36^{b,B}	11.22 ± 0.02^{cd,A}	29.56 ± 0.08^{d,B}	37.92 ± 0.09^{g,A}	72.41 ± 0.69^{ef,B}	73.25 ± 0.10^{fg,A}	31.04 ± 0.03^{de,B}	38.94 ± 0.09^{f,A}
150	**2**	50.33 ± 0.96^{bc,B}	54.21 ± 0.19^{d,A}	8.99 ± 0.02^{c,B}	11.17 ± 0.26^{cd,A}	29.41 ± 0.07^{d,B}	38.18 ± 0.26^{f,A}	73.01 ± 0.08^{de,B}	73.69 ± 0.10^{f,A}	30.75 ± 0.06^{de,B}	39.78 ± 0.12^{f,A}
150	**4**	48.94 ± 1.02^{c,B}	54.23 ± 0.21^{d,A}	8.92 ± 0.04^{c,B}	11.13 ± 0.16^{cd,A}	29.81 ± 0.08^{c,B}	39.60 ± 0.34^{e,A}	73.31 ± 0.12^{cd,B}	74.29 ± 0.24^{e,A}	31.10 ± 0.06^{de,B}	41.14 ± 0.28^{e,A}
150	**6**	48.82 ± 0.65^{c,B}	53.95 ± 0.46^{e,A}	8.88 ± 0.01^{c,B}	11.06 ± 0.06^{de,A}	30.72 ± 0.01^{b,B}	40.22 ± 0.14^{e,A}	73.55 ± 0.04^{cd,B}	74.69 ± 0.13^{e,A}	31.35 ± 0.06^{cd,B}	41.69 ± 0.11^{e,A}

175	**2**	48.72 ± 0.56^{c,B}	54.17 ± 0.29^{d,A}	8.69 ± 0.08^{d,B}	10.67 ± 0.43^{ef,A}	30.82 ± 0.05^{b,B}	41.22 ± 0.08^{d,A}	73.88 ± 0.12^{c,B}	75.48 ± 0.26^{d,B}	31.31 ± 0.07^{cd,B}	42.57 ± 0.12^{d,A}
175	**4**	48.82 ± 0.77^{c,B}	53.35 ± 0.14^{e,A}	8.22 ± 0.14^{ef,B}	10.62 ± 0.04^{ef,A}	30.88 ± 0.33^{b,B}	44.28 ± 0.08^{c,A}	75.09 ± 0.39^{ab,B}	76.51 ± 0.05^{c,A}	31.96 ± 0.28^{ab,B}	45.53 ± 0.08^{c,A}
175	**6**	48.66 ± 0.93^{c,B}	53.37± 0.07^{e,A}	8.26 ± 0.20^{e,B}	10.55 ± 0.26^{f,A}	30.76 ± 0.55^{b,B}	45.96 ± 0.96^{b,A}	74.95 ± 0.09^{b,B}	77.09 ± 0.06^{bc,A}	31.81 ± 0.59^{ab,B}	47.18 ± 0.97^{b,A}
200	**2**	48.84 ± 0.73^{c,B}	53.28 ± 0.04^{e,A}	8.16 ± 0.07^{f,B}	10.67 ± 0.02^{de,A}	31.05 ± 0.09^{ab,B}	45.47 ± 0.21^{b,A}	75.27 ± 0.07^{ab,B}	76.79 ± 0.03^{b,A}	32.10 ± 0.11^{a,B}	46.70 ± 0.21^{b,A}
200	**4**	49.82 ± 0.71^{c,B}	52.65 ± 0.46^{f,A}	7.93 ± 0.06^{f,B}	10.31 ± 0.21^{ef,A}	31.25 ± 0.02^{a,B}	46.81 ± 0.62^{a,A}	75.75 ± 0.17^{a,B}	77.58 ± 0.42^{ab,A}	32.47 ± 0.13^{a,B}	47.93 ± 0.57^{a,A}
200	**6**	48.82 ± 0.71^{c,B}	51.75 ± 0.14^{g,A}	7.81 ±0.23^{f,B}	10.12 ± 0.16^{f,A}	30.72 ± 0.22^{b,B}	47.18 ± 0.10^{a,A}	75.72 ± 0.50^{a,B}	77.89 ± 0.21^{a,A}	31.89 ± 0.15^{ab,B}	48.26 ± 0.06^{a,A}

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 Duncan's multiple range test; capital letters (A & B) in superscript indicate significant difference ($P<0.05$) between samples without oil and with oil according to student t-test.

5.1.4. Conclusion

The phenolics and flavonoids content, and in vitro antioxidant activity were greatly influenced by processing parameters. Significant difference in the experimental results of phenolics, flavonoids, antioxidant activity, carotenoids, and colour values between samples prepared without oil and with oil at higher temperature and longer heat treatment time was observed. Addition of oil and application of thermal treatment increased the yield of carotenoids from the tamarillo puree. b* value, hue angle and chroma were enhanced with the addition of oil. Addition of oil, heating temperature and time of treatment enhanced the visual appearance of tamarillo puree. These results will be helpful in canning of oil-enriched tamarillo puree.

Bibliography

[1] Abad, J., Valencia-Chamorro, S., Castro, A., and Vasco, C., Studying the effect of combining two nonconventional treatments, gamma irradiation and the application of an edible coating, on the postharvest quality of tamarillo (*Solanum betaceum Cav.*) fruits. *Food Control,* 72:319-323, 2017.

[2] Acosta-Quezada, P.G., Raigón, M.D., Riofrío-Cuenca, T., García-Martínez, M.D., Plazas, M., Burneo, J.I., Figueroa, J.G., Vilanova, S., and Prohens, J., Diversity for chemical composition in a collection of different varietal types of tree tomato (*Solanum betaceum Cav*.), an Andean exotic fruit. *Food Chemistry,* 169:327-335, 2015.

[3] Adefegha, S. and Oboh, G., Cooking enhances the antioxidant properties of some tropical green leafy vegetables. *African Journal of Biotechnology,* 10(4):632-639, 2011.

[4] Charles-Rodríguez, A., Nevárez-Moorillón, G., Zhang, Q., and Ortega-Rivas, E., Comparison of thermal processing and pulsed electric fields treatment in pasteurization of apple juice. *Food and Bioproducts Processing,* 85(2):93-97, 2007.

[5] Choi, Y., Lee, S., Chun, J., Lee, H., and Lee, J., Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. *Food Chemistry,* 99(2):381-387, 2006.

[6] Colle, I.J., Lemmens, L., Van Buggenhout, S., Met, K., Van Loey, A.M., and Hendrickx, M.E., Processing tomato pulp in the presence of lipids: The impact on lycopene bioaccessibility. *Food Research International,* 51(1):32-38, 2013.

[7] Dewanto, V., Wu, X., Adom, K.K., and Liu, R.H., Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry,* 50(10):3010-3014, 2002.

[8] Gärtner, C., Stahl, W., and Sies, H., Lycopene is more bioavailable from tomato paste than from fresh tomatoes. *The American Journal of Clinical Nutrition,* 66(1):116-122, 1997.

[9] Hornero-Méndez, D. and Mínguez-Mosquera, M.I., Bioaccessibility of carotenes from carrots: Effect of cooking and addition of oil. *Innovative Food Science & Emerging Technologies,* 8(3):407-412, 2007.

[10] Kang, K.S., Kim, H.Y., Pyo, J.S., and Yokozawa, T., Increase in the free radical scavenging activity of ginseng by heat-processing. *Biological and Pharmaceutical Bulletin,* 29(4):750-754, 2006.

[11] Kim, H.-Y., Woo, K.-S., Hwang, I.-G., Lee, Y.-R., and Jeong, H.-S., Effects of heat treatments on the antioxidant activities of fruits and vegetables. *Korean Journal of Food Science and Technology,* 40(2):166-170, 2008.

[12] Kou, M.-C., Yen, J.-H., Hong, J.-T., Wang, C.-L., Lin, C.-W., and Wu, M.-J., Cyphomandra betacea Sendt. phenolics protect LDL from oxidation and PC12 cells from oxidative stress. *LWT-Food Science and Technology,* 42(2):458-463, 2009.

[13] Lopez-Sanchez, P., De Vos, R., Jonker, H., Mumm, R., Hall, R., Bialek, L., Leenman, R., Strassburg, K., Vreeken, R., and Hankemeier, T., Comprehensive metabolomics to evaluate the impact of industrial processing on the phytochemical composition of vegetable purees. *Food Chemistry,* 168:348-355, 2015.

[14] Mertz, C., Brat, P., Caris-Veyrat, C., and Gunata, Z., Characterization and thermal lability of carotenoids and vitamin C of tamarillo fruit (*Solanum betaceum Cav.*). *Food Chemistry,* 119(2):653-659, 2010.

[15] Mertz, C., Gancel, A.-L., Gunata, Z., Alter, P., Dhuique-Mayer, C., Vaillant, F., Perez, A.M., Ruales, J., and Brat, P., Phenolic compounds, carotenoids and

antioxidant activity of three tropical fruits. *Journal of Food Composition and Analysis*, 22(5):381-387, 2009.

[16] Nisha, P., Singhal, R.S., and Pandit, A.B., Kinetic modelling of colour degradation in tomato puree (*Lycopersicon esculentum L.*). *Food and Bioprocess Technology*, 4(5):781-787, 2011.

[17] Pareek, S., Paliwal, R., and Mukherjee, S., Effect of juice extraction methods and processing temperature-time on juice quality of Nagpur mandarin (*Citrus reticulata Blanco*) during storage. *Journal of Food Science and Technology*, 48(2):197-203, 2011.

[18] Prohens, J. and Nuez, F., The tamarillo (*Cyphomandra betacea*) a review of a promising small fruit crop. *Small Fruits Review*, 1(2):43-68, 2001.

[19] Ravichandran, K., Saw, N.M.M.T., Mohdaly, A.A., Gabr, A.M., Kastell, A., Riedel, H., Cai, Z., Knorr, D., and Smetanska, I., Impact of processing of red beet on betalain content and antioxidant activity. *Food Research International*, 50(2):670-675, 2013.

[20] Rinaldi de Alvarenga, J.F., Quifer-Rada, P., Francetto Juliano, F., Hurtado-Barroso, S., Illan, M., Torrado-Prat, X., and Lamuela-Raventós, R.M., Using extra virgin olive oil to cook vegetables enhances polyphenol and carotenoid extractability: a study applying the sofrito technique. *Molecules*, 24(8):1555, 2019.

[21] Rodriguez-Amaya, D., Bobbio, P., and Bobbio, F., Carotenoid composition and vitamin A value of the brasilian fruit *Cyphomandra betacea*. *Food Chemistry*, 12(1):61-65, 1983.

[22] Saikia, S., Mahnot, N.K., and Mahanta, C.L., Phytochemical content and antioxidant activities of thirteen fruits of Assam, India. *Food Bioscience*, 13:15-20, 2016.

[23] Vallverdú-Queralt, A., Regueiro, J., Rinaldi de Alvarenga, J.F., Torrado, X., and Lamuela-Raventos, R.M., Carotenoid profile of tomato sauces: Effect of cooking time and content of extra virgin olive oil. *International Journal of Molecular Sciences*, 16(5):9588-9599, 2015.

[24] Van Buggenhout, S., Sila, D., Duvetter, T., Van Loey, A., and Hendrickx, M., Pectins in processed fruits and vegetables: Part III—Texture engineering. *Comprehensive Reviews in Food Science and Food Safety*, 8(2):105-117, 2009.

[25] Vasco, C., Avila, J., Ruales, J., Svanberg, U., and Kamal-Eldin, A., Physical and chemical characteristics of golden-yellow and purple-red varieties of tamarillo fruit (*Solanum betaceum Cav*.). *International Journal of Food Sciences and Nutrition,* 60(sup7):278-288, 2009.

STUDY THE EFFECT OF HIGH PRESSURE HOMOGENIZATION AND THERMAL PROCESSING OF TAMARILLO PUREE

5.2.1. Introduction

Tamarillo is native to the Andes region, and currently this fruit is cultivated worldwide for commercial production of variety-specific new food products [41]. Tamarillo is loaded with healthy nutrients and bioactive compounds, which are known for possessing various health benefits. However, with all of that, the fruit is neglected and not utilized as per its potential [27]. Functional foods with enhanced nutritional value and similarity to raw food in terms of freshness, quality, and safety have seen an increase in consumer demand in recent years [19]. It is well known that thermal treatment alters the natural flavour and texture of foods. Researchers and food industries have shown interest in using non-thermal techniques to process foods.

High pressure homogenization (HPH) is an innovative, environment friendly, and sustainable non-thermal technology, which is widely utilized by the industries for the processing of food samples [25]. In HPH, the food sample is passed through a small orifice between a small homogenizer valve and seat valve and the pressure attained can be in the range of 10-70 MPa [39]. Therefore, passing the liquid sample through a small gap enables the reduction of particle size of the macromolecules and cells. Fruits and vegetables processing by HPH is being done, because it enhances the adsorption properties of the product [13]. Hua et al. [12] studied the effect of HPH on tomato fibers and found that insoluble fibers present in tomato pulp were modified to soluble forms. Santiago et al. [33] studied the effect of HPH on tomato puree consistency and reported that HPH restored the consistency of the puree that was affected by pectinase. Food products especially purees, sauces and ketchup are subjected to homogenization because it helps to reduce the particle size of the food sample and enhance the food stability with better mixing of ingredients [39]. It has been reported that addition of oil helps in enhancing the bioaccessibility of carotenoids [21]. Many intrinsic and extrinsic factors such as phytochemicals of sample, bioavailability of compounds, human metabolism, and processing methods and their extent play important roles in determining the bioaccessibility [37]. However, the processing methods and degree of processing are very important factors that play important role in determining the bioaccessibility of phytochemicals [16]. As tamarillo is a seasonal fruit in

nature, we aimed to process tamarillo puree for extended shelf life with good retention of bioactive compounds using HPH and thermal treatment. This chapter focuses on the determination of the effect of HPH and thermal treatment on the physicochemical, morphological, *in-vitro* bioaccessibility, and storage studies of bioactive compounds present in tamarillo puree.

5.2.2. Materials and methods

Fresh yellow tamarillo were procured from a local market in Kohima, Nagaland and directly brought to Tezpur University, Assam without causing any physical damage. Tamarillos were washed with clean water, packed in polyethylene zip lock LDPE pouch and kept at -20ºC until further analysis.

5.2.2.1. Preprocessing

Tamarillos of similar maturity were selected for the processing of the puree. Briefly, the tamarillos were washed with clean water and kept for thawing at room temperature. Thawed tamarillos were blanched (90 s) using boiling water (95-100 ºC), and directly put in ice water bath. The blanched tamarillos were peeled and further processed into puree form using mixer grinder (Philips HL Model No. 1632). Then, extra virgin olive oil (5 % w/w) was added to 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 4-8 ºC until further analysis

5.2.2.2. High pressure homogenization

A high pressure homogenizer (GEA, Lab homogenizer Panda Plus 2000, Italy) was used for the treatment of tamarillo 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. The samples were coded as 500 bar for 1 pass (500-1P), 700 bar for 1 pass (700-1P) and 1000 bar for 1 pass (1000-1P).

5.2.2.3. Light microscopy

The microstructure of the tamarillo puree was observed according to the method of Tan and Kerr [39] using a light microscope. The HPH treated samples were diluted using distilled water (1:10) and then stained with methylene blue. The samples were placed on a glass slide and images were observed at magnification of 10x. A digital camera was used for capturing the image of the HPH treated samples.

5.2.2.4. Colour measurement

The colour values of the HPH treated puree were obtained 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 was noted. The total colour difference of the sample was calculated according to López-Gámez et al. [23].

5.2.2.5. Particle size analysis

The particle size analysis of the puree was determined according to the method adopted by Tan and Kerr [39] by laser diffraction method using a Malvern Mastersizer (Model MSS, Malvern Instruments Ltd.). The tamarillo puree samples were dispersed in MilliQ water for measurement.

5.2.2.6. Determination of bioactive compounds

For TPC and *in-vitro* antioxidant activities, 1 g of tamarillo 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 extract was stored at 4ºC until further analysis.

5.2.2.6.1 Total phenolic content

The total phenolic content of the HPH treated puree was calculated according to the method adopted by Saikia and Mahanta [32] with some modification. Briefly 0.5 ml of sample extract was taken and mixed with 2.5 mL of Folin-Ciocalteau reagent (1:10 in water). After 5 min, 2 ml of 7.5 % sodium carbonate was added to 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.

5.2.2.6.2. DPPH radical scavenging activity

The DPPH radical scavenging activity of the HPH treated sample was calculated according to the method adopted by Saikia and Mahanta [32] with some modification. Briefly, 0.2 ml of sample extract was mixed in 2.8 ml of DPPH reagent (10^{-4}). 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

5.2.2.6.3. Determination of total carotenoids concentration (TCC)

The total carotenoids of tamarillo puree was calculated according to the method adopted by Orqueda et al. [26] with slight modification. Briefly, 1 g of tamarillo puree was taken, followed by the addition 10 mL of extraction solvent (50% hexane, 25% acetone, 25% ethanol, containing 0.1% BHT). The solution was centrifuged at 6700 g at 10 min at 4 ºC, and the top layer of the solution was collected very carefully and made up to 10 mL with hexane. The results of TCC determined in tamarillo were expressed as mg β-CE (carotene equivalent)/100 g of puree.

5.2.2.7. HPLC of phenolic acids

The phenolic acids present in HPH treated puree were 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 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 [6].

5.2.2.8. HPLC of carotenoids

The carotenoids in tamarillo puree were identified and quantified using reverse phase in C30 column and diode array detector in a UHPLC (Ultimate 3000, Thermo Scientific, USA) with the help of internal standards and calibration curve. 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 [11].

5.2.2.9. Thermal inactivation studies

The test microorganisms included *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. 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, bacterial cells were harvested by centrifugation of 1 mL of nutrient broth suspension at 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 all the microbial cultures was adjusted to 0.400 Au at wavelength of 600 nm. The treated samples inoculated with microorganisms were plated in the luria bertani agar and incubated at 37°C for 24-48 h. All the experiments were conducted in triplicate and the mean values of log CFU/g samples were reported.

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. The determination of *D*-value (time required at a specific temperature to kill 90% of microorganism) of individual inoculated microorganism was done according to the method adopted by Fasogbon et al. [7] and 5D process was followed for the bottling of puree.

$$D\ value = \frac{H}{\log a - \log b}$$

Here, H stands for heating time, *a* denotes the initial microbial load and *b* denotes the microorganisms that survived the heating time (min).

5.2.2.10. *In-vitro* digestion of puree

The *in-vitro* digestion of phenolic content present in the tamarillo pulp was measured following the method of Kashyap et al. [15] with some modification. Briefly, 5 g of tamarillo 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). 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 samples were again kept for incubation for 30 min at 37ºC in dark. This step initiated 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. The TPC and TCC after digestion were measured as mentioned in Sec. 5.2.2.6.1 and 5.2.2.6.2, respectively, followed by HPLC.

5.2.2.11. Bioaccessibility of bioactive compounds

Bioaccessibility were calculated according to Corona-Leo et al. [4] with slight modification.

$$Bioaccesibility = \frac{X_1}{X_2} \times 100$$

Where, X_1 stands for bioactive compounds released intestinal digestion, X_2 stands for bioactive compounds present in food before digestion.

5.2.2.12. Storage study of puree

For storage study, the bottled puree was stored at 25 °C in an incubator and the changes in the pH, TSS, Vitamin C were calculated according to methods adopted by Orqueda et al. [26]. The TCC, TPC and *in-vitro* antioxidant property of the bottled tamarillo puree were calculated according to the method given in Sec. 5.2.2.6. For microbial analysis, the total microbial count (TMC) of the bottled puree was analyzed according to Marszałek et al. [24]. The results of microbial test were expressed as log CFU/g of tamarillo puree.

5.2.2.13. Statistical analysis

All experiments were performed in triplicate and experimental results are presented as mean ± 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 Duncan multiple range test with statistical significance ($p < 0.05$).

5.2.3. Results and discussion

5.2.3.1. Particle size

The particle size of the puree was analyzed and significant difference in the untreated and HPH treated puree was observed (Table 5.2.1). Particle size of control sample was 2048 nm, however after applying HPH, the particle size was found to be less than 1000 nm at all pressure levels and passes. Increase in the number of passes and pressure caused destruction of the cells resulting in reduced particle size of the puree. Similar results were reported for tomato puree [39, 44].

5.2.3.2. Microstructure

Fig. 5.2.1 clearly illustrates the difference in the microstructure of control and HPH treated samples. Increase in homogenising pressure helps to disintegrate the cell structure which is directly related to decrease in particle size that facilitates better release of the bioactive compounds from the cells. HPH treatment cuts the large elongated cells into smaller pieces, and repetition of the process at the same pressure further helps in cellular disintegration [44]. However, subsequent passes at constant pressure showed reduction/disruption in the particle size by disintegrating the cells of tamarillo puree (Fig. 5.2.1). Continuous passes were reported to decreases the particle size and yield better consistency of the final product [31]. While full intact cells were observed in the control sample, complete disintegration of all the cells were found in HPH treated samples. Tan and Kerr [39] reported that increase in HPH pressure was found to disintegrate the tomato juice cells. Increase in pressure from 500 to 700 bar showed more disruption, however their subsequent passes also disrupted the particle in a more uniform way. The micrographs of the puree treated at 2^{nd} and 3^{rd} pass at 1000 bar were quite similar in observation of reducing the particle size. Tan et al. [39] reported that through HPH treatment, a uniform microstructure was obtained with gradual increase in pressure. Therefore, pressure and passes play important roles in reducing the particle size and disintegrating the microstructure, which enhances the bioaccessibility of bioactive compounds present in the puree. The microstructures of the homogenized puree samples as

assessed by light microscopy were found to be in accordance with the results of decrease in particle size with increasing pressure and passes.

5.2.3.3. Colour analysis

The colour analysis of HPH treated puree is given in Table 5.2.1, and data showed significant differences ($p < 0.05$) among the samples. Increase in the pressure and pass was seen to increase the lightness of the puree. The L*value of control sample was 55.32, whereas it ranged from 76.44-79.39 in HPH-treated puree samples. The increase in lightness was therefore severe after treatment, but at 1000 bar pressure after 2^{nd} and 3^{rd} passes, the change in lightness of the puree was non-significant ($p < 0.05$). The results of lightness were found to agree with results reported for processing of pumpkin puree using high pressure [9]. The change in the colour values is related to the pressure and passes because increases in pressure helps to reduce the particle size and increase the viscosity of the product due to uniform distribution of oil droplets in the particles. Similar results of change in lightness were reported for sesame vegetable cream [2]. The decrease in the a* value is attributed to heating factor, because temperature increased as the pressure of the puree was increased, which caused fractional loss of the major pigment. Decrease in the redness values was supported by the results reported for pumpkin puree [9] and blackcurrant juice [18] . The yellowness present in tamarillo puree was due to the presence of the carotenoids in tamarillo puree [41], and the effect of HPH treatment on b* was similar to that of L*. The increase in b* value may be due to the release of the carotenoids from the cells and their uptake by the olive oil that was added to the puree prior to high pressure homogenization. These results of cell wall disruption and decrease in the size of the cell was confirmed by the results of particle size analysis and light microscopy, respectively. The total colour difference (ΔE) was calculated for the HPH treated tamarillo puree samples and it was observed that HPH led to an increase in the ΔE values. ΔE increased with homogenization pressure, but subsequent passes did not have pronounced effect. At 1000 bar, highest ΔE was observed but statistically no significant difference was observed at 1000-2P and 1000-3P. Change in the colour parameters of the HPH treated puree is related to the higher compressibility, which cause in the reduction of particle size and favors the uniform emulsification of oil in the puree [35].

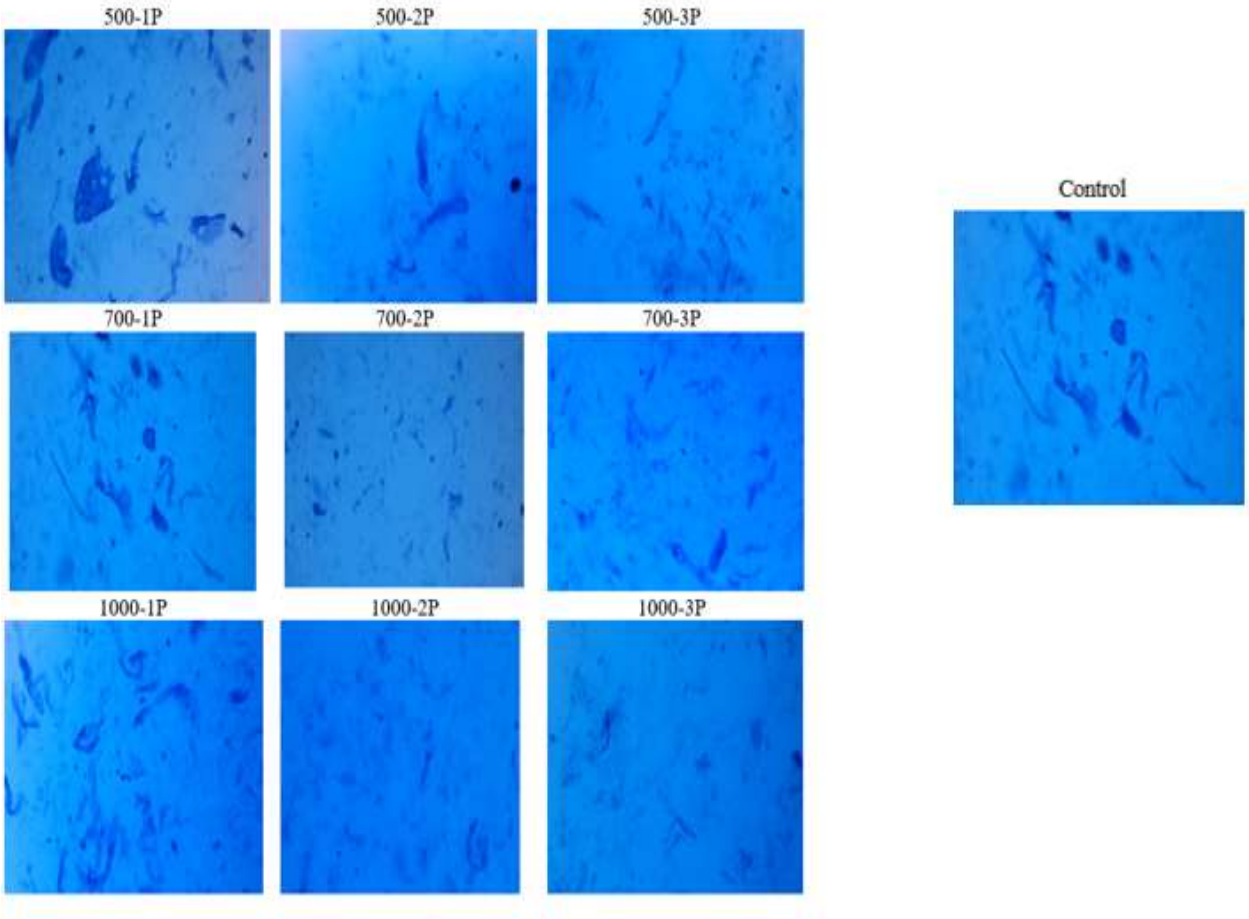


Fig 5.2.1. Light microscopy images of tamarillo puree given HPH treatment at different pressures and different passes.

5.2.3.4. Total phenolic content

The total phenolic content of the puree was analyzed and significant difference in TPC was observed (Table 5.2.1). The phenolic content in the control puree was 5.40 mg GAE/g, which increased to 5.88 mg GAE/g in HPH treated puree at 500 bar pressure. Total phenolic content of puree increased further at higher pressures. However, repeat passes appeared to have no effect on TPC. As the pressure was increased to 1000 bar the increase in phenolic content was found but no statistical difference in the phenolic content was observed after 2nd and 3rd passes. Similar results of increasing phenolic content from 582 to 662 mg GAE/100g in two passes was reported for strawberry juice as the homogenizing pressure was increased from 60 to 100 MPa [14]. The distortion and cell rupture during HPH helped to increase the release of bioactive compounds in the extraction solvents [29]. The enhanced release of phenolic content observed in the samples is desirable for better release of phytochemicals during digestion.

5.2.3.5. DPPH radical scavenging activity

Significant difference in the DPPH radical scavenging activity was noticed in the puree samples (Table 5.2.1). The radical activity in the control puree was 23.62 %, which increased gradually with increase in homogenizing pressure. Highest radical scavenging activity was found in the sample treated at 1000 bar for 3 passes. This increase in antioxidant activity was in agreement with the study done on strawberry juice [14]. The pressure caused disruption of the cellular matrix and released the bioactive compounds in both free and bound forms, therefore the DPPH activity was found to increase gradually. Further increase in pressure from 750 to 1000 bar led to an increase in the bioactive compounds initially, but researchers have claimed that increase in pressure more than required cause degradation of bioactive compounds [22]. Samples with high TPC showed high scavenging activity.

5.2.3.6. Total carotenoids content (TCC)

The total carotenoids content of the control and HPH treated sample was analyzed, and significant difference between the samples was observed ($p < 0.05$) (Table 5.2.1). Increase in pressure and passes indicated to have positive impact on enhancing the TCC value. In control puree, the TCC was 0.65 mg βCE/100g whereas, at 1000 bar, the TCC value was 0.79 mg βCE/100g. An increase of 21% in TCC was observed after applying HPH, but after three passes at 1000 bar (1000-3P), the TCC value decreased to 0.77 mgβ CE/100g. Increase in the number of passes at 500 and 700 bar was found to be in agreement with the results reported on rosehip nectar by Saricaoglu et al. [34]. Zhang et al. [45] reported that optimal pressure for lycopene extraction using HPH was 200 bar, after that reduction was observed. Similarly, in our case, the increase in pressure from 500 to 1000 bar led to an increase in TCC value by disruption of cells, but after further passing the puree in HPH at 1000 bar, no significant difference was observed. In HPH, pressure with passes helps in release of the

5.2.3.7. HPLC of phenolic acids

The HPLC profile of phenolic acids in the HPH treated samples is presented in Fig. 5.2.2. The main phenolic acids found in control and HPH treated samples were gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid. Similar phenolic acids were found in tamarillo from New Zealand, as reported by Diep et al. [5]. It was observed that increase in pressure from 500 to 1000 bar showed an increase in the phenolic acid concentration.

Increase in pressure results in the reduction of the particle size, ultimately enhancing the solubility of phenolic acids in the extraction solvent [44]. The highest concentration of chlorogenic acid, caffeic acid and p-coumaric acid was found at 1000-1P, with values of 254, 62 and 103 µg/g, respectively. After increase in number of passes at 1000 bar, there was a decrease in concentration and the phenolic acids concentration agreed with the experimental results of TPC. Another main reason for increase in phenolic content was related to the release of bound phenolic compounds due to HPH treatment. It was reported that, in native form some phenolic compounds are bound to the cell wall of the sample, therefore less phenolic components were released from the sample [29]. Therefore, use of 1000 bar pressure was recommended for processing of tamarillo puree for enhancing the concentration of extractable phenolic acids in the puree.

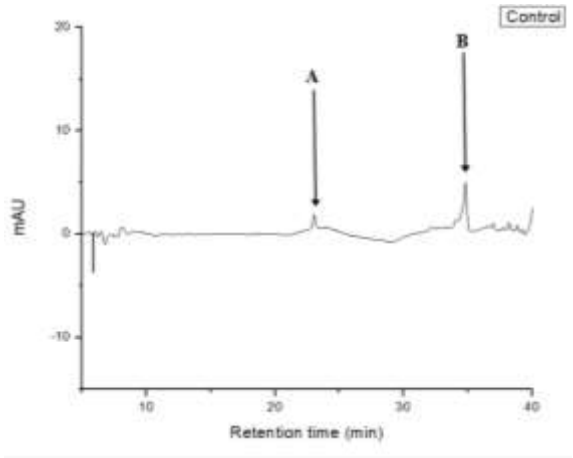
5.2.3.8. HPLC of carotenoids

The HPLC profile of carotenoids in the HPH treated samples was shown in Fig. 5.2.3. The identified carotenoids were β-cryptoxanthin and β-carotene in the tamarillo puree. Similar results of carotenoids were reported for β-cryptoxanthin and β-carotene being the major carotenoids in tamarillo puree [10]. Increase in pressure with passes showed positive impact on the carotenoids, however pressure had more positive impact on enhancing the TCC yield, as was also reported bt researchers [34]. At 700-3P, the concentration of β-cryptoxanthin and β-carotene was 150 and 287, however at 1000-1P, it was 144 and 299, respectively (Table 5.2.2). Increase in homogenization pressure and repeated passes facilitated the release of more bioactive compounds by destroying the cell structure of the sample matrix [39]. Therefore, 1000 bar can be recommended for processing of tamarillo puree to get enhanced level of TCC.

Table 5.2.1. Effect on particle size, colour, TPC, *in vitro* antioxidant activity, and TCC of HPH treated tamarillo puree

Sample code	Particle size (nm)	L*	a*	b*	ΔE	TPC (mg GAE/g)	DPPH radical scavenging activity (%)	TCC (mgβ CE/100g)
Control	2804	55.32 ± 0.16^{c}	10.25 ± 0.11^{a}	52.31 ± 0.15^{a}	0	5.40 ± 0.41^{b}	23.62 ± 0.25^{d}	0.65 ± 0.02^{f}
500 -1P	790	76.44 ± 0.16^{f}	9.49 ± 0.14^{b}	53.31 ± 0.14^{b}	21.33 ± 0.15^{e}	5.88 ± 0.45^{ab}	28.55 ± 0.28^{c}	0.69 ± 0.02^{e}
500-2P	635	77.04 ± 0.12^{e}	9.77 ± 0.18^{c}	53.32 ± 0.15^{b}	21.92 ± 0.11^{d}	5.89 ± 0.54^{ab}	29.42 ± 0.32^{b}	0.71 ± 0.02^{de}
500-3P	587	77.61 ± 0.32^{d}	9.64 ± 0.16^{c}	54.45 ± 0.21^{c}	22.13 ± 0.29^{d}	5.95 ± 0.48^{ab}	29.99 ± 0.12^{bc}	0.73 ± 0.01^{bcd}
700-1P	609	78.26 ± 0.25^{c}	9.53 ± 0.11^{c}	54.94 ± 0.08^{d}	23.28 ± 0.23^{c}	6.01 ± 0.41^{ab}	30.38 ± 0.23^{ab}	0.76 ± 0.02^{abc}
700-2P	541	78.36 ± 0.14^{bc}	8.71 ± 0.12^{d}	55.61 ± 0.14^{e}	25.31 ± 0.11^{c}	5.94 ± 0.41^{ab}	30.48 ± 0.23^{ab}	0.76 ± 0.01^{abc}
700-3P	519	78.63 ± 0.36^{b}	8.49 ± 0.09^{de}	57.64 ± 0.26^{f}	24.18 ± 0.28^{b}	5.98 ± 0.32^{ab}	30.64 ± 0.21^{ab}	0.73 ± 0.02^{bcd}
1000-1P	523	78.62 ± 0.26^{b}	8.29 ± 0.11^{de}	57.93 ± 0.33^{f}	24.25 ± 0.16^{b}	6.19 ± 0.15^{a}	30.77 ± 0.54^{a}	0.79 ± 0.02^{a}
1000-2P	500	79.33 ± 0.26^{a}	8.09 ± 0.18^{de}	59.66 ± 0.15^{g}	25.41 ± 0.19^{a}	6.17 ± 0.24^{a}	30.88 ± 0.71^{a}	0.79 ± 0.02^{a}
1000-3P	499	79.39 ± 0.25^{a}	7.94 ± 0.11^{e}	59.87 ± 0.33^{g}	25.54 ± 0.13^{a}	6.21 ± 0.30^{a}	31.04 ± 0.46^{a}	0.77 ± 0.03^{ab}

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


Control
A
B
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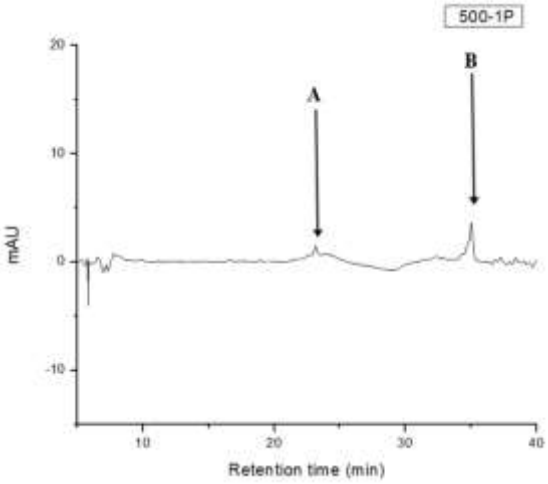
Retention time (min)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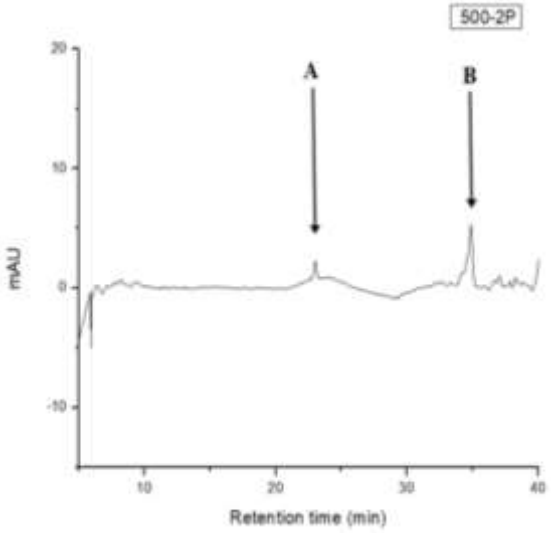
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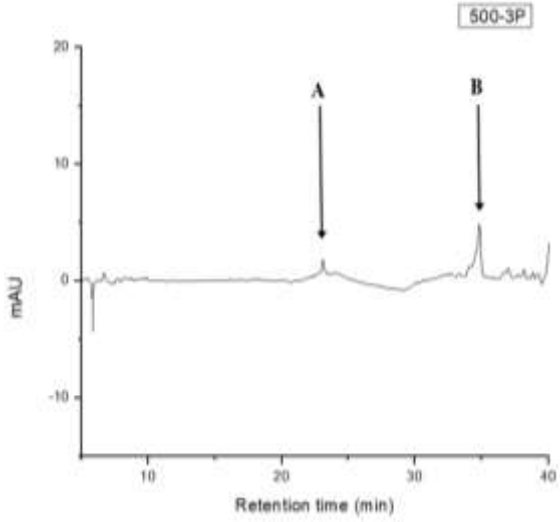
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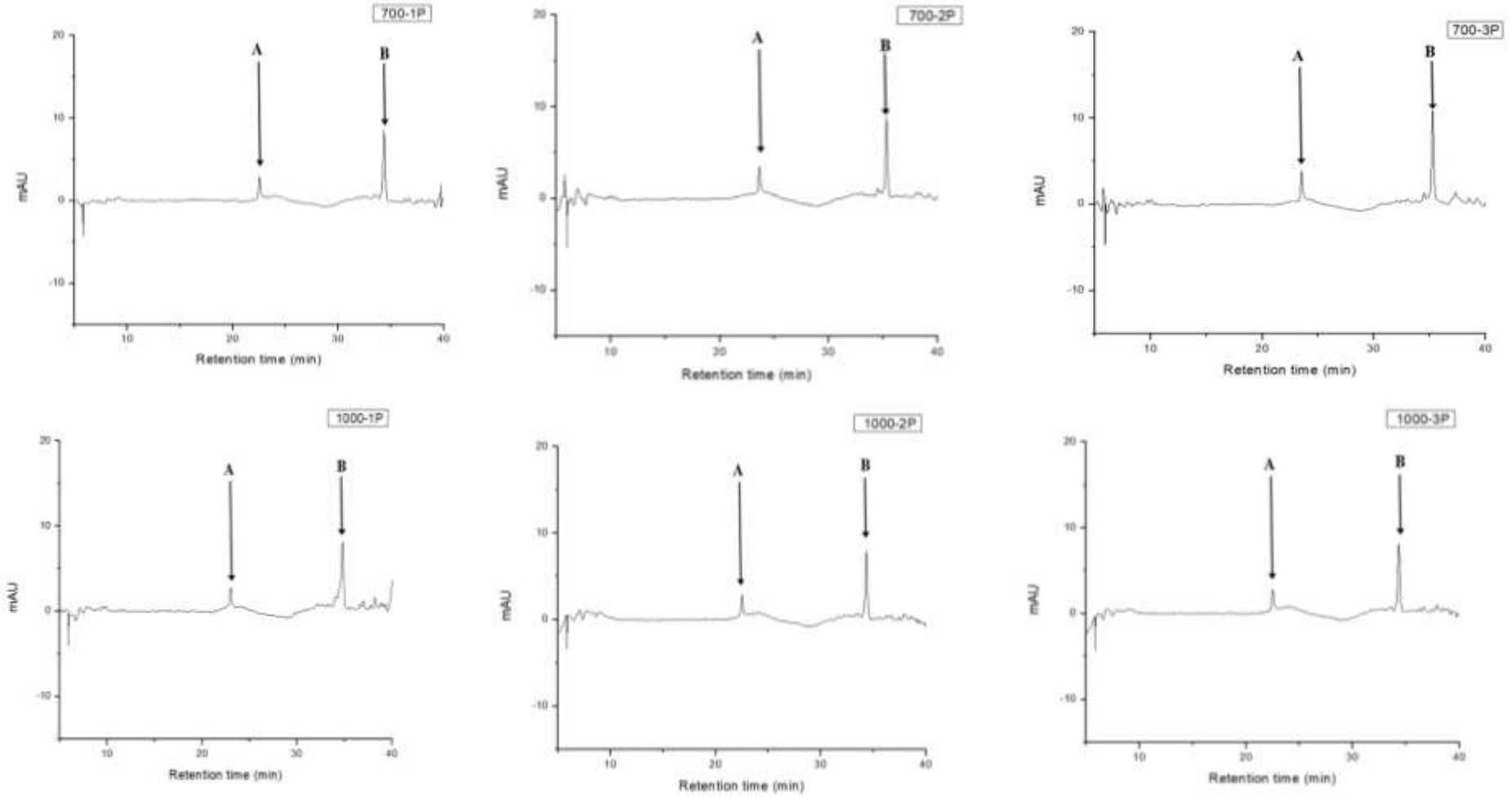
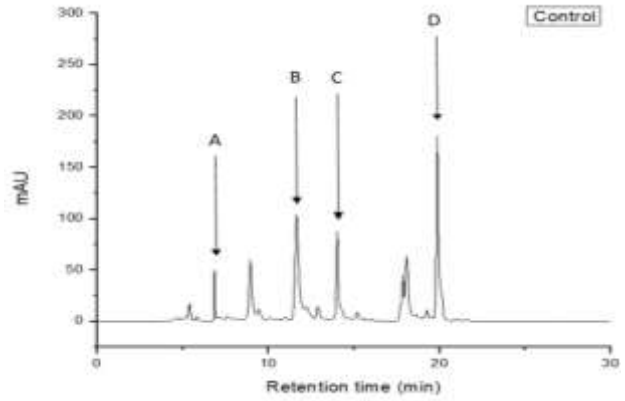


Fig. 5.2.2. HPLC analysis of carotenoids present in HPH treated puree


Control
A
B
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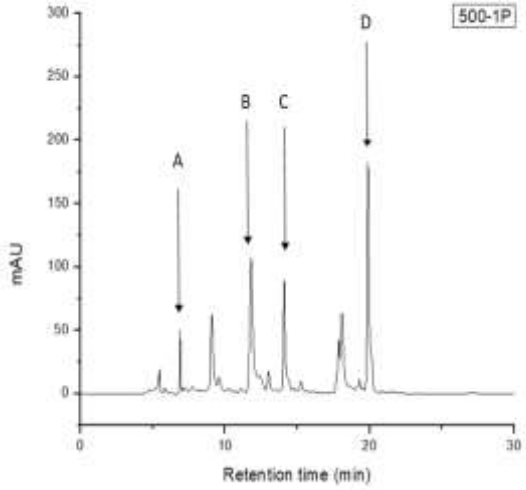
Retention time (min)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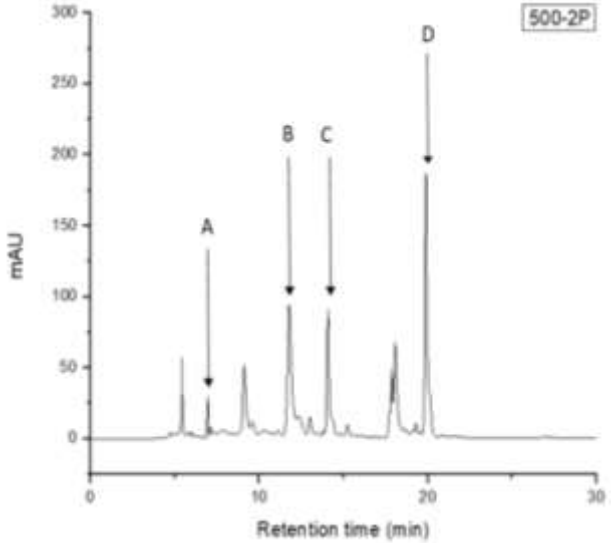
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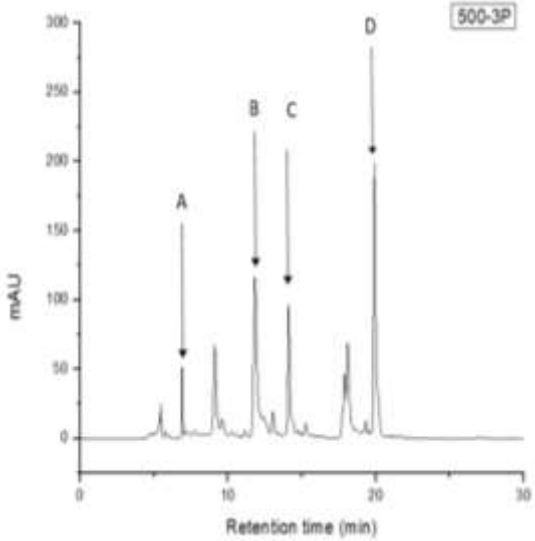
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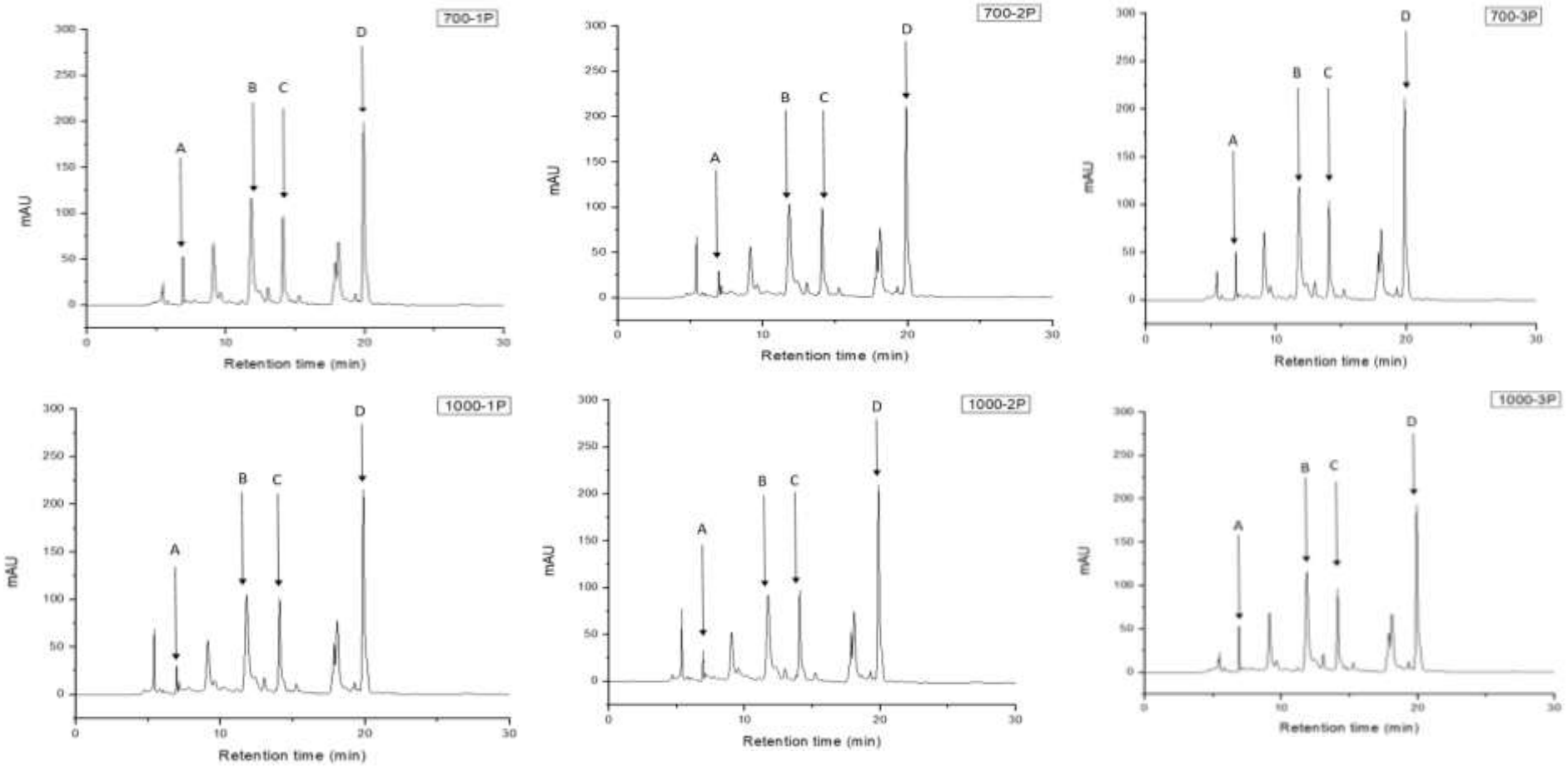


Fig. 5.2.3. HPLC analysis of the phenolic acids present in HPH treated tamarillo puree

Table 5.2.2. Phenolic acid and carotenoids concentration in the HPH treated tamarillo puree and HPH processed thermally treated bottled tamarillo puree

	Phenolic acids (µg/g)				Carotenoids (µg/100g)	
Sample code	**Gallic acid**	**Chlorogenic acid**	**Caffeic acid**	**p-coumaric acid**	**β-cryptoxanthin**	**β-carotene**
Control	85.77	172.07	47.42	50.67	74.27	209.01
500-1P	128.68	230.41	56.68	81.70	78.42	222.57
500-2P	126.10	223.09	56.42	80.80	80.50	287.57
500-3P	54.74	221.06	57.60	87.51	76.35	306.86
700-1P	117.82	246.47	58.96	92.91	140.66	266.14
700-2P	58.36	243.82	61.70	100.67	134.44	280.43
700.-3P	110.07	252.65	60.04	95.41	150.41	287.76
1000-1P	112.14	254.26	62.54	103.30	144.18	299.83
1000-2P	112.14	233.43	60.04	100.67	134.03	284.47
1000-3P	117.82	249.01	56.45	90.15	142.27	256.69

Table 5.2.3. Phenolic acid and carotenoids concentration present in i*n-vitro* digestion of HPH processed thermally treated bottled tamarillo puree

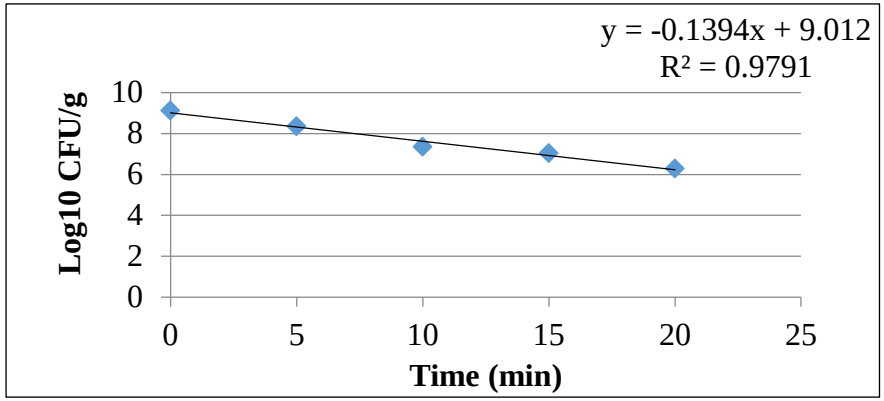
	Phenolic acids (µg/g)				Carotenoids (µg/100g)	
Sample code	**Gallic acid**	**Chlorogenic acid**	**Caffeic acid**	**p-coumaric acid**	**β-cryptoxanthin**	**β-carotene**
Initial	143.71	151.86	34.02	82.48	131.54	217.36
Gastric	26.6	116.17	22.75	38.97	77.59	95.21
Intestinal	48.31	113.83	27.46	38.74	87.97	150.57

5.2.3.9. D value of puree

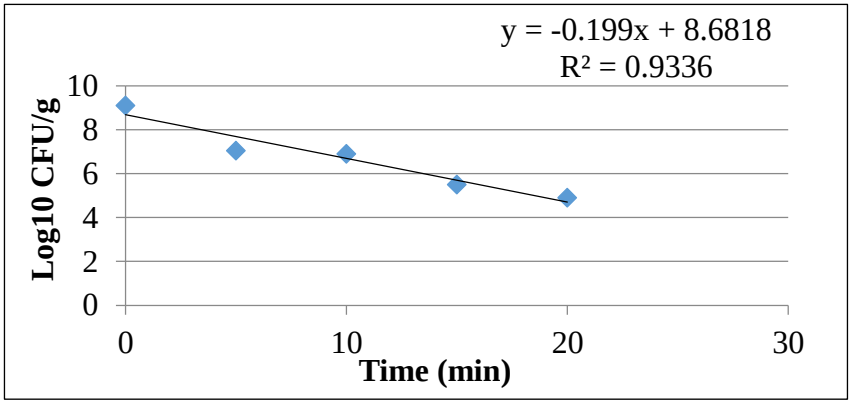
The thermal resistance study is very important in determining the shelf life of the hermetically sealed food products. The thermal treatment of tamarillo puree at 4 different temperatures (65, 75, 85, and 95 °C) and time (5, 10, 15, and 20 min) was studied and viability of microbes are mentioned in Fig. 5.2.4-Fig. 5.2.7. The initial no of microorganisms inoculated in the sample was log 10^7 CFU. It was found that the log cycle counts of *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, and Bacillus cereus* were reduced gradually with the increase in heating time. In Table 5.2.4, the D-value of all the microbes at respective time and temperature are mentioned. The D-value indicates the one-log reduction of microorganism population at a certain temperature and is an important parameter while determining the total process time [28]. The D-value of *Escherichia coli, Staphylococcus aureus, Listeria monocytogenes, and Bacillus cereus* at 95°C was 2.64, 2.70, 3.22 and 3.12 min, respectively. Fasogbon et al. [7] reported that 5D process is required in foods, therefore, 5D of tamarillo puree was attained after 16 min of thermal treatment at 95°C.

Table 5.2.4. Calculation of D values of different microbes evaluated in tamarillo 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



(a)



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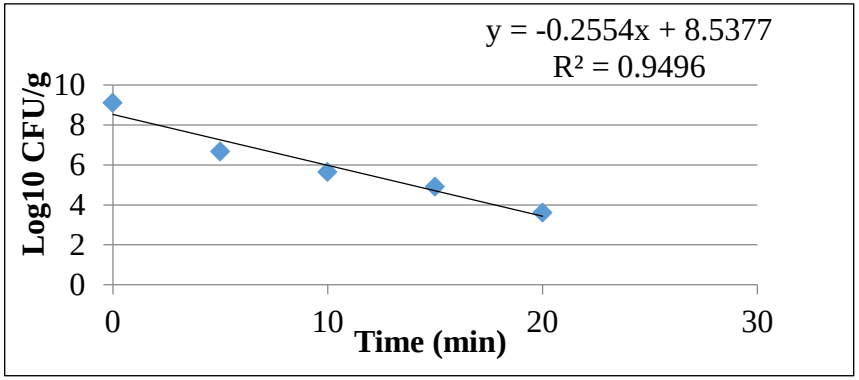

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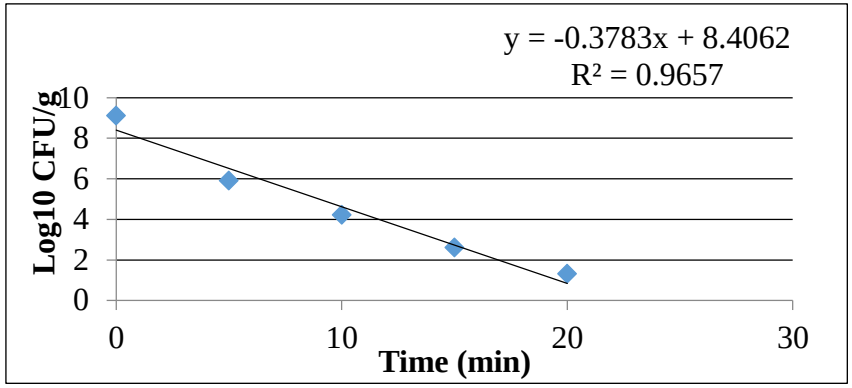
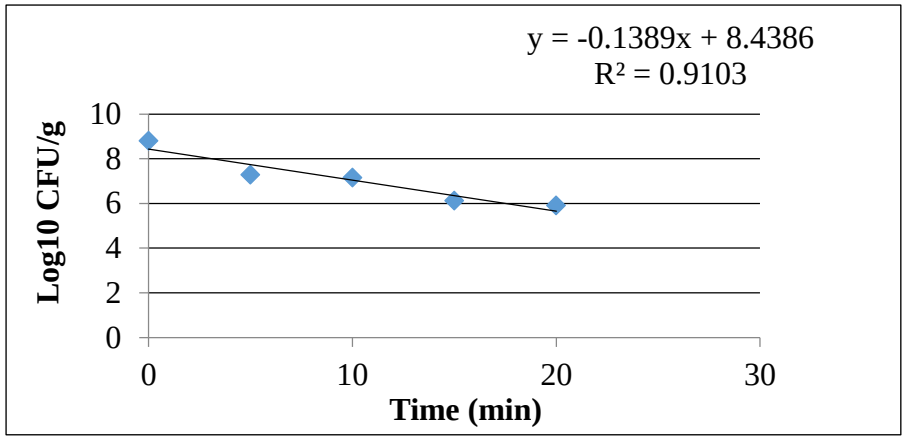
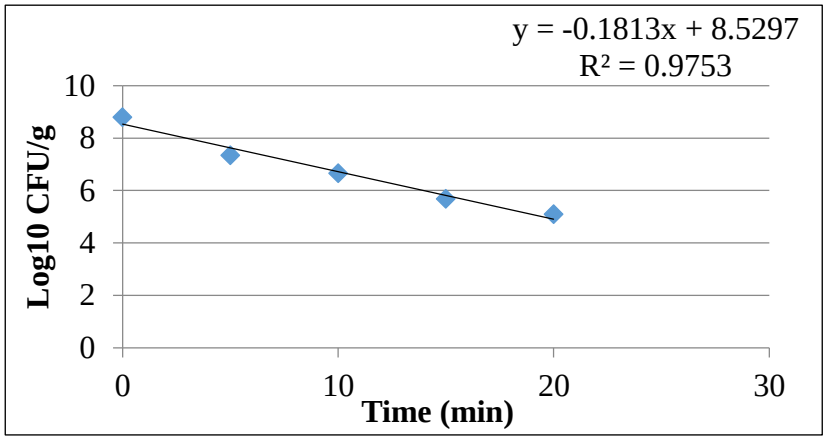


(d)

Fig. 5.2.4. *Escherichia coli* survival curve after heating in tamarillo puree at different temperature, a (65°C), b (75°C), c (85°C), and d (95°C)



(a)



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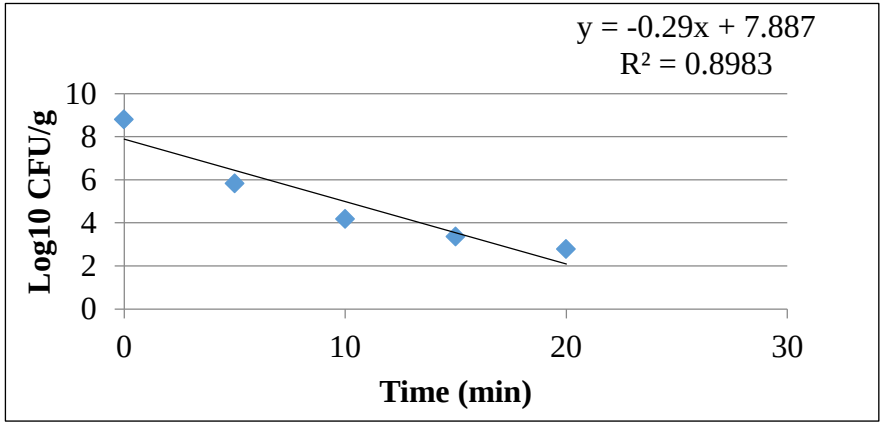

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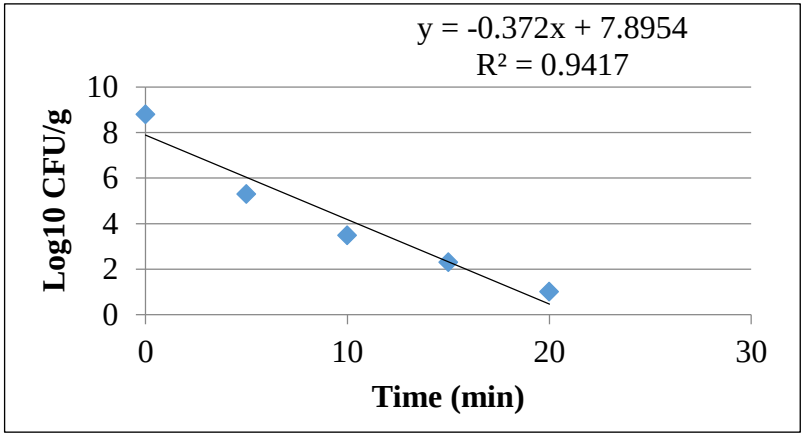


(d)

Fig. 5.2.5. *Staphylococcus aureus* survival curve after heating in tamarillo puree at different temperature a (65°C), b (75°C), c (85°C), and d (95°C)

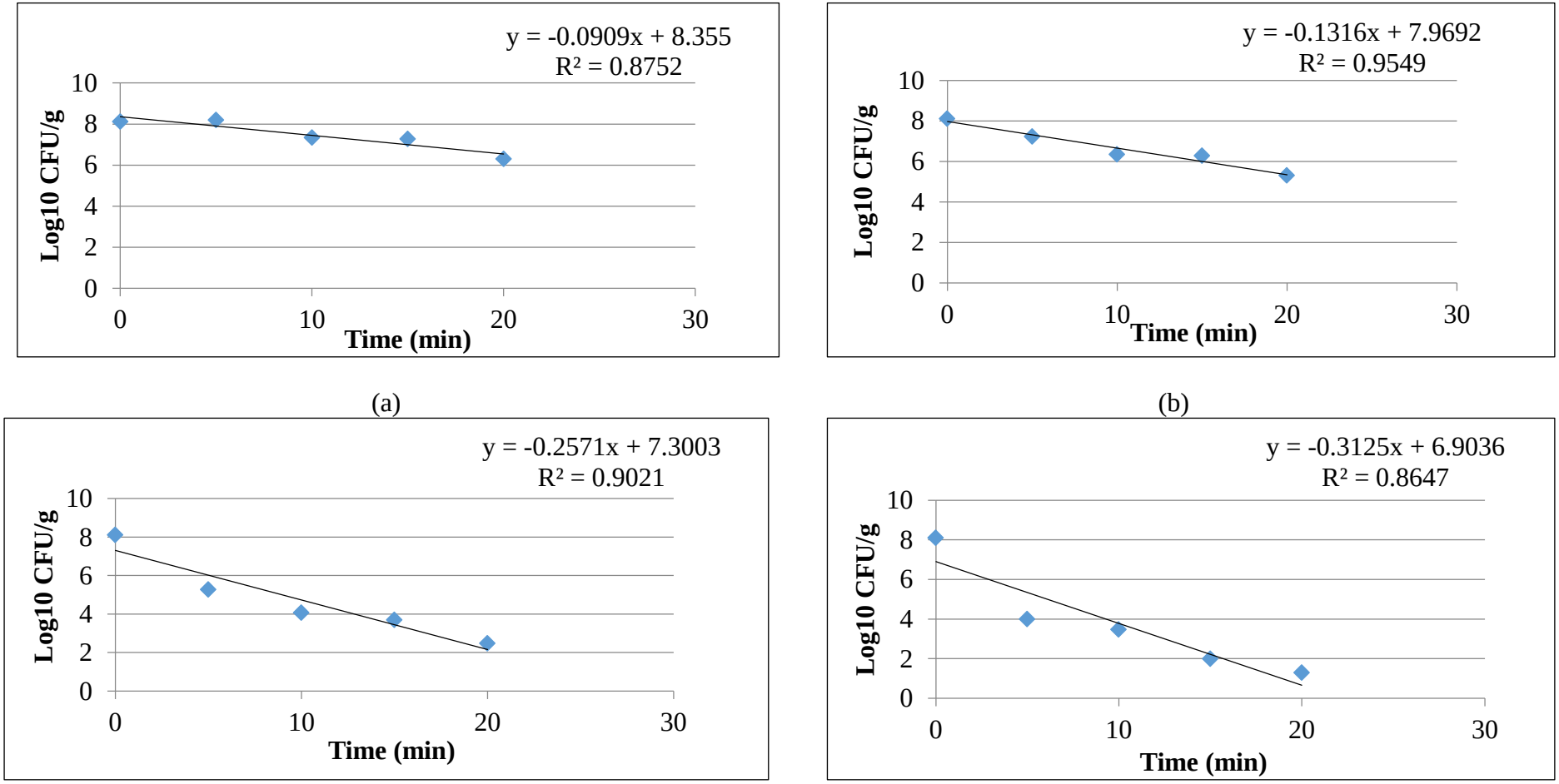
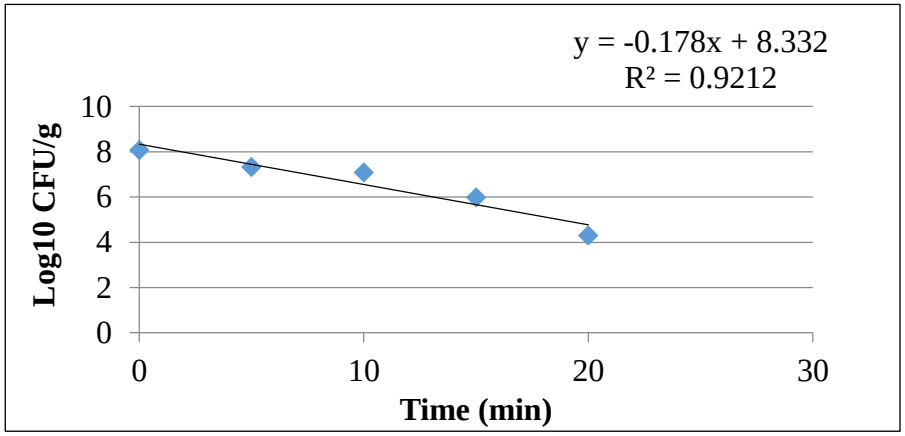


Fig. 5.2.6. *Listeria monocytogenes* survival curve after heating in tamarillo puree at different temperature, a (65°C), b (75°C), c (85°C), and d (95°C).



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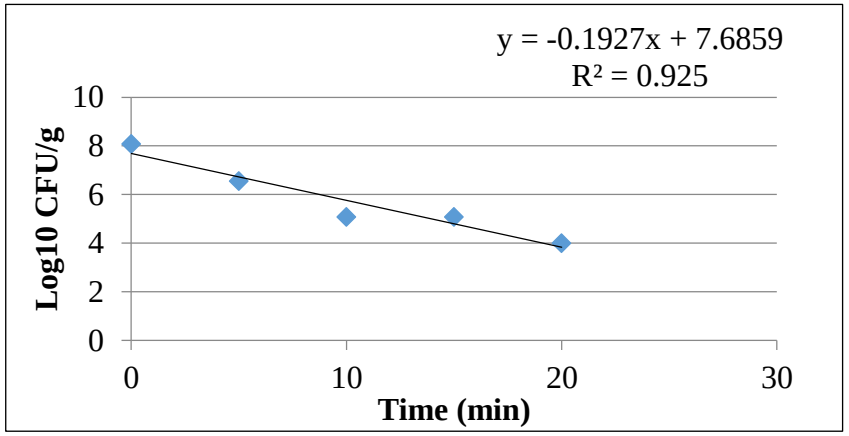

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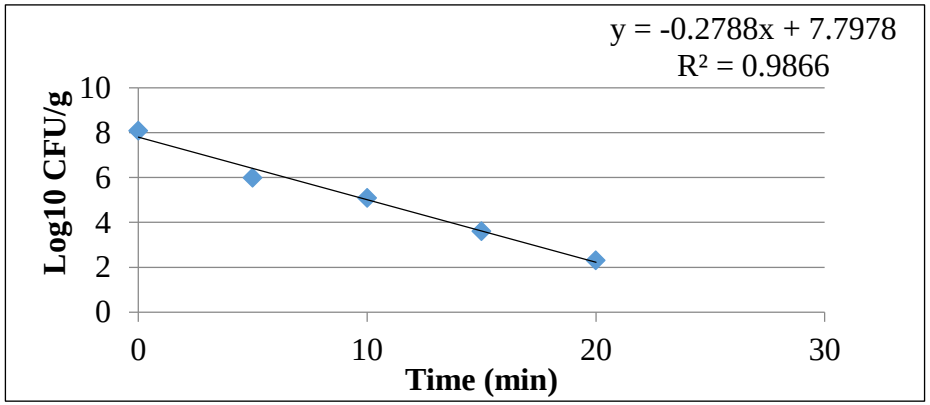

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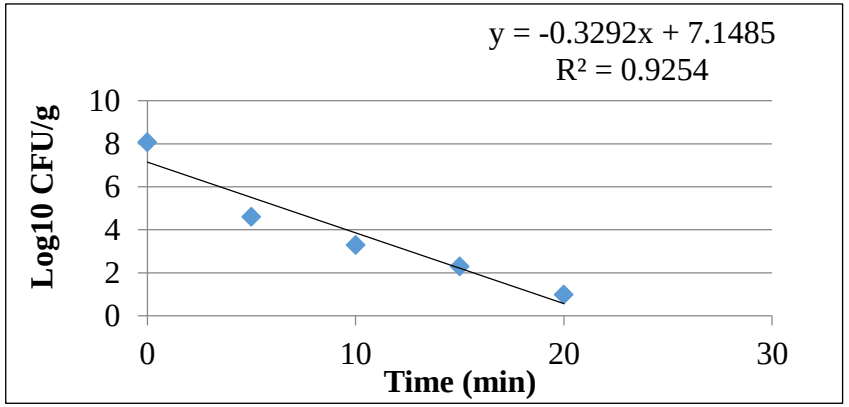

(d)

Fig. 5.2.7. *Bacillus cereus* survival curve after heating tamarillo puree at different temperatures, a (65°C), b (75°C), c (85°C), and d (95°C).

5.2.3.10. Bioaccessibility of TPC and phenolic acids of tamarillo puree

The *in-vitro* digestion of TPC of tamarillo puree was studied at different stages of the digestion, and results are presented in Fig. 5.2.5 (X1). It was observed that, TPC in undigested form was 5.11 mg GAE/g, however, in gastric phase, the TPC was 3.01 mg GAE/g which clearly showed that the bioaccessibility of TPC was 58% in the gastric phase. Tenore et al. [40] studied the *in-vitro* digestion of tea polyphenol and found that reduction in TPC was observed in gastric phase due to action of the enzymes and acidic pH. The bioaccessibility of TPC in intestinal phase was 72%, as seen in Fig. 5.2.8 X1. In intestinal phase, the increase in TPC was related to the change in pH and effect of processing , and similar results of high release of TPC in intestinal was reported for barley [43].

The HPLC profile of *in-vitro* digestion of the phenolic acids is given in Fig. 5.2.5 for initial, gastric and intestinal phases (X2, X3 and X4). The concentration of phenolic acids was found to be in the agreement with the results of TPC. All the phenolic acids were found to be increased in intestinal phase except chlorogenic acid (Table 5.2.3). Our results of decrease of phenolic acids in gastric and increase in intestinal phase are in concurrence with the results for Meghalayan cherry [15].

5.2.3.11 Bioaccessibility of TCC and carotenoids present in tamarillo pure

In Fig. 5.2.5 (Y1), the *in-vitro* digestion of TCC of tamarillo puree was studied at different stages of digestion. In the undigested sample, the TCC of the tamarillo puree was found to be 1.02 mg βCE/100 g, however at gastric level the TCC was found to be decreased, thus the bioaccessibility of the puree was 50%. Researchers cited that in gastric phase, action of digestive enzymes and the acidic environment cause a decrease in TCC [38]. At intestinal level, the carotenoids concentration was found to be increased to 0.72 mg βCE/100 g and the bioaccessibility was 74%. Our results of more bioaccessibility at intestinal phase was found by Kirkhus et al. [16]. Researchers reported that increase of TCC in the intestinal phase was related to the lipid digestion and also to greater release of carotenoids at neutral pH (6-7) [16].

The HPLC profile of *in-vitro* digestion of the carotenoids is shown in Fig. 5.2.5 for initial, gastric and intestinal level (Y2, Y3 and Y4). The concentration of carotenoids was found to be in the agreement with the results for TCC. Researchers reported that higher concentration of carotenoids release was found to be in intestinal phase than gastric phase [8].

The concentration of β-cryptoxanthin and β-carotene in gastric phase was 88 and 150 µg/100 g, respectively and intestinal phase was 77 and 95 µg/100 g, respectively (Table 5.2.3).

5.2.3.12 Effect on pH, TSS, vitamin C, TPC, DPPH scavenging activity, TCC, and total microbial load during storage of HPH processed heat treated bottled tamarillo puree

From Table 5.2.5, the effect of storage on the bioactive compounds present in tamarillo puree stored at 25 °C can be observed. It was noticed that the pH and TSS of freshly bottled HPH and heat processed puree was 3.78 and 12.20, respectively. It was found that pH and TSS was found to increase with storage time. Wani et al. [42] studied the shelf stability of peach puree and reported that increase in pH and TSS was related to breakdown of complex sugar compounds into simpler sugars.

The initial content of vitamin C was 12.32 mg/100g, TPC was 5.85 mg GAE/g, DPPH scavenging activity was 29 %, and TCC was 1.02 mg βCE/100 g of HPH and thermally processed bottled tamarillo puree (Table 5.2.5). A significant difference in the degradation of vitamin C, TPC, DPPH scavenging activity and TCC was observed with an increase in the storage time. As all the bottled were sealed, anaerobic degradation of the product may have occurred. The degradation of vitamin C in the food products is related to oxidative deterioration and tends to decrease gradually with storage time [17]. Researchers claimed that many factors such as light, non-enzymatic anaerobic reactions, storage temperature and time play important roles in degradation [3]. After 4 months of storage, the decrease was: 11% of vitamin C, 46% of TPC, 56% of in-vitro antioxidant activity and 33% decrease of TCC. Similar decrease in the TPC and TCC was reported for apricot puree [20], and degradation was attributed to oxidation reactions during storage. DPPH activity is mainly due to the TPC, and our results showed decrease in TPC and DPPH scavenging activity.

The TMC of the bottled puree was studied, and the initial TMC was 1.30 Log CFU/g, which increased to 1.78 Log CFU/g after 60 days of storage and further increased to 2.90 Log CFU/g after 120 days of storage. The factors like increase in pH due to non-enzymatic reactions and degradation of bioactive compounds were responsible for the growth of TMC [30]. However, tamarillo puree was found to be acceptable microbiologically level up to 120 days of storage, because researchers have claimed that microbial load of more than 4 Log CFU/g should not be considered as acceptable in the pasteurized fruits and vegetables products [36].

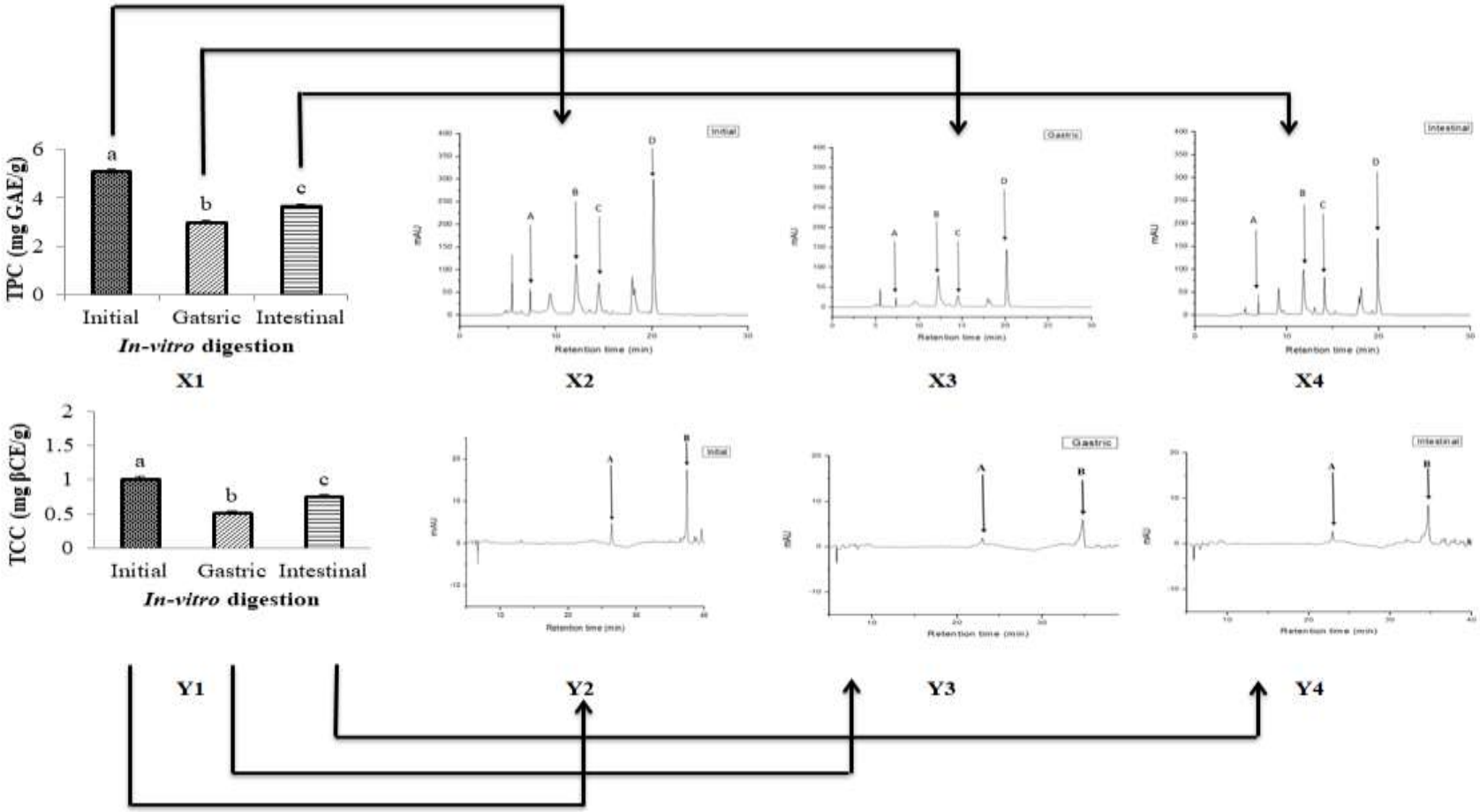

Fig 5.2.8. *In-vitro* digestion of the (X1) TPC and (Y1) TCC; X2, X3 and X4 represent the HPLC of phenolic acids in initial, gastric and intesntinal phases, respectively (A: gallic acid, B: chlorogenic acid, C: caffeic acid and D: p-coumaric acid) and Y2, Y3 and Y4 represents the concentration of individiual carotenoids using HPLC in initial, gastric, and intestinal phases (A: β-cryptoxanthin and B: β-carotene), respectively.

Table 5.2.5. Effect on biochemical, polyphenols, carotenoids content and *in-vitro* antioxidant properties of tamarillo puree during storage (25°C)

Storage time (days)	pH	TSS (° Brix)	Vitamin C (mg/100g)	Total phenolic content (mg GAE/g)	DPPH radical scavenging activity (%)	Total carotenoids content (mg βCE/100 g)	Total microbial count (Log CFU/g)
0	3.78 ± 0.07^{c} (00.00 %)	12.20 ± 0.20^{b} (00.00 %)	12.32 ± 0.20^{a} (00.00 %)	5.85 ± 0.09^{a} (0.00 %)	29.23 ± 0.21^{a} (00.0 %)	1.02 ± 0.02^{a} (00.0 %)	1.30
30	3.80 ± 0.07^{c} (00.52 %)	12.40 ± 0.40^{b} (01.61 %)	11.88 ± 0.21^{b} (03.57 %)	4.89 ± 0.07^{b} (16.41 %)	23.16 ± 0.22^{b} (20.92 %)	0.89 ± 0.03^{b} (12.74 %)	1.30
60	4.20 ± 0.09^{b} (11.11 %)	12.60 ± 0.40^{ab} (03.27 %)	11.22 ± 0.19^{c} (08.92 %)	4.03 ± 0.09^{c} (31.11 %)	21.62 ± 0.16^{c} (26.92 %)	0.84 ± 0.03^{c} (17.64 %)	1.78
90	4.40 ± 0.20^{ab} (16.40 %)	12.80 ± 0.40^{ab} (04.91 %)	11.01 ± 0.21^{c} (10.63 %)	3.54 ± 0.12^{d} (39.48 %)	18.56 ± 0.27^{c} (36.50 %)	0.81 ± 0.02^{c} (20.58 %)	2.60
120	4.70 ± 0.30^{a} (24.33 %)	13.20 ± 0.20^{a} (08.19 %)	10.87 ± 0.25^{c} (11.76 %)	3.17 ± 0.07^{e} (45.81 %)	15.15 ± 0.16^{e} (55.91 %)	0.68 ± 0.03^{d} (33.32 %)	2.90

Values in superscript indicates significant difference ($p<0.05$) using Duncan's multiple range test, Value in parenthesis indicates % change as compared to 0 day value.

5.2.4. Conclusion

HPH helps in decreasing the particle size, enhancing the colour values, total polyphenols by 14%, total carotenoids by 21% and in-vitro antioxidant activity by 30%. A thermal treatment of 16 min was calculated and given to achieve the 5D log process for processing of tamarillo puree. HPLC results four major phenolic acids and two carotenoids were present in the tamarillo puree. Decrease in the concentration of bioactive compounds was found in tamarillo puree, however, bottled puree was microbiological acceptable for consumption for 4 months at 25 °C.

Bibliography

[1] Acosta-Quezada, P.G., Raigón, M.D., Riofrío-Cuenca, T., García-Martínez, M.D., Plazas, M., Burneo, J.I., Figueroa, J.G., Vilanova, S., and Prohens, J., Diversity for chemical composition in a collection of different varietal types of tree tomato (*Solanum betaceum* Cav.), an Andean exotic fruit. *Food Chemistry*, 169:327-335, 2015.

[2] Amiri Samani, S. and Naji, M.H., Effect of homogenizer pressure and temperature on physicochemical, oxidative stability, viscosity, droplet size, and sensory properties of Sesame vegetable cream. *Food Science & Nutrition*, 7(3):899-906, 2019.

[3] Andrés, V., Villanueva, M.J., and Tenorio, M.D., The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry,* 192:328-335, 2016.

[4] Corona-Leo, L.S., Meza-Márquez, O.G., and Hernández-Martínez, D.M., Effect of in vitro digestion on phenolic compounds and antioxidant capacity of different apple (*Malus domestica*) varieties harvested in Mexico. *Food Bioscience*, 43:101311, 2021.

[5] Diep, T., Pook, C., and Yoo, M., Phenolic and anthocyanin compounds and antioxidant activity of tamarillo (*Solanum betaceum Cav*.). *Antioxidants*, 9(2):169, 2020.

[6] Espín, S., Gonzalez-Manzano, S., Taco, V., Poveda, C., Ayuda-Durán, B., Gonzalez-Paramas, A.M., and Santos-Buelga, C., Phenolic composition and antioxidant capacity of yellow and purple-red Ecuadorian cultivars of tree tomato *(Solanum betaceum Cav*.). *Food Chemistry,* 194:1073-1080, 2016.

[7] Fasogbon, B.M., Adebo, O.A., Adeniran, H.A., and Taiwo, K.A., Thermal processing of canned dika kernel (ogbono) soup and the neural prediction of its canning parameters. *Journal of Food Process Engineering,* 45(9):e14122, 2022.

[8] Fernandes, A.S., Nascimento, T.C., Pinheiro, P.N., Vendruscolo, R.G., Wagner, R., de Rosso, V.V., Jacob-Lopes, E., and Zepka, L.Q., Bioaccessibility of microalgae-based carotenoids and their association with the lipid matrix. *Food Research International,* 148:110596, 2021.

[9] García-Parra, J., González-Cebrino, F., Delgado, J., Cava, R., and Ramírez, R., High pressure assisted thermal processing of pumpkin purée: Effect on microbial counts, color, bioactive compounds and polyphenoloxidase enzyme. *Food and Bioproducts Processing,* 98:124-132, 2016.

[10] García, J.M., Giuffrida, D., Dugo, P., Mondello, L., and Osorio, C., Development and characterisation of carotenoid-rich microencapsulates from tropical fruit by-products and yellow tamarillo (*Solanum betaceum Cav*.). *Powder Technology,* 339:702-709, 2018.

[11] Hsu, B., Pu, Y., Inbaraj, B.S., and Chen, B., An improved high performance liquid chromatography–diode array detection–mass spectrometry method for determination of carotenoids and their precursors phytoene and phytofluene in human serum. *Journal of Chromatography B,* 899:36-45, 2012.

[12] Hua, X., Xu, S., Wang, M., Chen, Y., Yang, H., and Yang, R., Effects of high-speed homogenization and high-pressure homogenization on structure of tomato residue fibers. *Food Chemistry,* 232:443-449, 2017.

[13] Huang, X., Yang, Y., Liu, Q., and He, W.-Q., Effect of high pressure homogenization on sugar beet pulp: Physicochemical, thermal and structural properties. *LWT,* 134:110177, 2020.

[14] Karacam, C.H., Sahin, S., and Oztop, M.H., Effect of high pressure homogenization (microfluidization) on the quality of Ottoman Strawberry *(F. Ananassa*) juice. *LWT-Food Science and Technology,* 64(2):932-937, 2015.

[15] Kashyap, P., Riar, C.S., and Jindal, N., Effect of extraction methods and simulated in vitro gastrointestinal digestion on phenolic compound profile, bio-accessibility, and antioxidant activity of Meghalayan cherry (*Prunus nepalensis*) pomace extracts. *LWT,* 153:112570, 2022.

[16] Kirkhus, B., Afseth, N.K., Borge, G.I.A., Grimsby, S., Steppeler, C., Krona, A., and Langton, M., Increased release of carotenoids and delayed in vitro lipid

digestion of high pressure homogenized tomato and pepper emulsions. *Food Chemistry,* 285:282-289, 2019.

[17] Koh, E., Charoenprasert, S., and Mitchell, A.E., Effects of industrial tomato paste processing on ascorbic acid, flavonoids and carotenoids and their stability over one-year storage. *Journal of the Science of Food and Agriculture,* 92(1):23-28, 2012.

[18] Kruszewski, B., Zawada, K., and Karpiński, P., Impact of high-pressure homogenization parameters on physicochemical characteristics, bioactive compounds content, and antioxidant capacity of blackcurrant juice. *Molecules,* 26(6):1802, 2021.

[19] Kubo, M.T.K., Atribst, A., and Augusto, P.E.D., High pressure homogenization in fruit and vegetable juice and puree processing: effects on quality, stability and phytochemical Profile. *Innovative Food Processing Technologies: A Comprehensive Review,* 3, 2021.

[20] Le Bourvellec, C., Gouble, B., Bureau, S., Reling, P., Bott, R., Ribas-Agusti, A., Audergon, J.-M., and Renard, C.M., Impact of canning and storage on apricot carotenoids and polyphenols. *Food Chemistry,* 240:615-625, 2018.

[21] Liu, J., Bi, J., Liu, X., Zhang, B., Wu, X., Wellala, C.K.D., and Zhang, B., Effects of high pressure homogenization and addition of oil on the carotenoid bioaccessibility of carrot juice. *Food & Function,* 10(1):458-468, 2019.

[22] López-Gámez, G., Elez-Martínez, P., Martín-Belloso, O., and Soliva-Fortuny, R., Pulsed electric field treatment strategies to increase bioaccessibility of phenolic and carotenoid compounds in oil-added carrot purees. *Food Chemistry,* 364:130377, 2021.

[23] Lopez-Sanchez, P., De Vos, R., Jonker, H., Mumm, R., Hall, R., Bialek, L., Leenman, R., Strassburg, K., Vreeken, R., and Hankemeier, T., Comprehensive metabolomics to evaluate the impact of industrial processing on the phytochemical composition of vegetable purees. *Food Chemistry,* 168:348-355, 2015.

[24] Marszałek, K., Mitek, M., and Skąpska, S., The effect of thermal pasteurization and high pressure processing at cold and mild temperatures on the chemical composition, microbial and enzyme activity in strawberry purée. *Innovative Food Science & Emerging Technologies,* 27:48-56, 2015.

[25] Martínez-Monteagudo, S.I., Yan, B., and Balasubramaniam, V., Engineering process characterization of high-pressure homogenization—from laboratory to industrial scale. *Food Engineering Reviews,* 9(3):143-169, 2017.

[26] Orqueda, M.E., Rivas, M., Zampini, I.C., Alberto, M.R., Torres, S., Cuello, S., Sayago, J., Thomas-Valdes, S., Jiménez-Aspee, F., and Schmeda-Hirschmann, G., Chemical and functional characterization of seed, pulp and skin powder from chilto (*Solanum betaceum*), an Argentine native fruit. Phenolic fractions affect key enzymes involved in metabolic syndrome and oxidative stress. *Food Chemistry,* 216:70-79, 2017.

[27] Orqueda, M.E., Zampini, I.C., Torres, S., Alberto, M.R., Ramos, L.L.P., Schmeda-Hirschmann, G., and Isla, M.I., Chemical and functional characterization of skin, pulp and seed powder from the Argentine native fruit mistol (*Ziziphus mistol*). Effects of phenolic fractions on key enzymes involved in metabolic syndrome and oxidative stress. *Journal of Functional Foods,* 37:531-540, 2017.

[28] Peng, J., Tang, J., Barrett, D.M., Sablani, S.S., Anderson, N., and Powers, J.R., Thermal pasteurization of ready-to-eat foods and vegetables: Critical factors for process design and effects on quality. *Critical Reviews in Food Science and Nutrition,* 57(14):2970-2995, 2017.

[29] Plazzotta, S. and Manzocco, L., Effect of ultrasounds and high pressure homogenization on the extraction of antioxidant polyphenols from lettuce waste. *Innovative Food Science & Emerging Technologies,* 50:11-19, 2018.

[30] Pokhrel, P.R., Boulet, C., Yildiz, S., Sablani, S., Tang, J., and Barbosa-Cánovas, G.V., Effect of high hydrostatic pressure on microbial inactivation and quality changes in carrot-orange juice blends at varying pH. *LWT,* 159:113219, 2022.

[31] Qian, C. and McClements, D.J., Formation of nanoemulsions stabilized by model food-grade emulsifiers using high-pressure homogenization: Factors affecting particle size. *Food Hydrocolloids,* 25(5):1000-1008, 2011.

[32] Saikia, S., Mahnot, N.K., and Mahanta, C.L., Phytochemical content and antioxidant activities of thirteen fruits of Assam, India. *Food Bioscience,* 13:15-20, 2016.

[33] Santiago, J.S.J., Kermani, Z.J., Xu, F., Van Loey, A.M., and Hendrickx, M.E., The effect of high pressure homogenization and endogenous pectin-related

enzymes on tomato purée consistency and serum pectin structure. *Innovative Food Science & Emerging Technologies,* 43:35-44, 2017.

[34] Saricaoglu, F.T., Atalar, I., Yilmaz, V.A., Odabas, H.I., and Gul, O., Application of multi pass high pressure homogenization to improve stability, physical and bioactive properties of rosehip (*Rosa canina L.*) nectar. *Food Chemistry,* 282:67-75, 2019.

[35] Sentandreu, E., Stinco, C.M., Vicario, I.M., Mapelli-Brahm, P., Navarro, J.L., and Meléndez-Martínez, A.J., High-pressure homogenization as compared to pasteurization as a sustainable approach to obtain mandarin juices with improved bioaccessibility of carotenoids and flavonoids. *Journal of Cleaner Production,* 262:121325, 2020.

[36] Starek, A., Pawłat, J., Chudzik, B., Kwiatkowski, M., Terebun, P., Sagan, A., and Andrejko, D., Evaluation of selected microbial and physicochemical parameters of fresh tomato juice after cold atmospheric pressure plasma treatment during refrigerated storage. *Scientific Reports,* 9(1):1-11, 2019.

[37] Stinco, C.M., Sentandreu, E., Mapelli-Brahm, P., Navarro, J.L., Vicario, I.M., and Meléndez-Martínez, A.J., Influence of high pressure homogenization and pasteurization on the in vitro bioaccessibility of carotenoids and flavonoids in orange juice. *Food Chemistry,* 331:127259, 2020.

[38] Szabo, K., Teleky, B.E., Ranga, F., Simon, E., Pop, O.L., Babalau-Fuss, V., Kapsalis, N., and Vodnar, D.C., Bioaccessibility of microencapsulated carotenoids, recovered from tomato processing industrial by-products, using in vitro digestion model. *LWT,* 152:112285, 2021.

[39] Tan, J. and Kerr, W., Rheological properties and microstructure of tomato puree subject to continuous high pressure homogenization. *Journal of Food Engineering,* 166:45-54, 2015.

[40] Tenore, G.C., Campiglia, P., Giannetti, D., and Novellino, E., Simulated gastrointestinal digestion, intestinal permeation and plasma protein interaction of white, green, and black tea polyphenols. *Food Chemistry,* 169:320-326, 2015.

[41] Wang, S. and Zhu, F., Tamarillo (*Solanum betaceum*): Chemical composition, biological properties, and product innovation. *Trends in Food Science & Technology,* 95:45-58, 2020.

[42] Wani, S., Bakshi, R.A., Khan, Z.S., Fayaz, S., Muzaffar, K., and Dar, B., Physiochemical, sensorial and rheological characteristics of puree developed from

Kashmiri peaches: influence of sugar, KMS and storage conditions. *Heliyon*, 7(8):e07781, 2021.

[43] Xiang, Z., Deng, J., Yang, K., Zhu, Y., Xia, C., Chen, J., and Liu, T., Effect of processing on the release of phenolic compounds and antioxidant activity during in vitro digestion of hulless barley. *Arabian Journal of Chemistry*, 14(12):103447, 2021.

[44] Yi, J., Kebede, B., Kristiani, K., Grauwet, T., Van Loey, A., and Hendrickx, M., Minimizing quality changes of cloudy apple juice: The use of kiwifruit puree and high pressure homogenization. *Food Chemistry*, 249:202-212, 2018.

[45] Zhang, A., Wang, L., Song, T., Yu, H., Wang, X., and Zhao, X.-h., Effects of high pressure homogenization on the structural and emulsifying properties of a vegetable protein: *Cyperus esculentus L*. *LWT*, 153:112542, 2022.