CHAPTER 8

CONLUSION AND FUTURE SCOPE OF WORK

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Summary, conclusions and future scope

8.1 Salient findings

The research work presented the phytochemical profiling of purple, red, and yellow varieties of tamarillo cultivated in North-East India. From purple tamarillo, the anthocyanins rich polyphenols extract was obtained using ultrasound as an extraction technique. In yellow tamarillo, high pressure homogenization and thermal processing was applied for the development and processing of carotenoids rich tamarillo puree. In red tamarillo, foam mat dried tamarillo powder was developed using different foaming agents. From the waste, extraction of carotenoids was done using high shear disperser and olive oil was used as the extraction medium and fabrication of carotenoids loaded nanoemulsion was done using ultrasound. The effect of various techniques on puree, powder and emulsion quality was assessed.

8.1.1. Analysis of the biochemical properties, nutrient composition, and antioxidant activities of the red, yellow, and purple varieties of tamarillos

- The pulp showed good amount of protein, fat, and crude fibre. The pH and TSS of the tamarillos were found to range from 3.70-3.94 and 9.6-10.2°Brix, respectively. Vitamin C was highest in purple tamarillo. Potassium was the major mineral in all the varieties. Anthocyanins were majorly found in purple tamarillo (0.35 mg C3G/g), while carotenoids were maximum in yellow tamarillo (0.63 mg βCE/100g). However, both anthocyanins and carotenoids pigments were found in purple and red tamarillo varieties. Through HPLC analyses, six phenolic acids were identified and among them, gallic, chlorogenic, ferulic and rosmarinic acids were the major ones. Pelargonidin-3-O-rutinoside was the major anthocyanin in purple (86.22 µg/g) and red (9.88 µg/g) tamarillo. Three different carotenoids, viz. zeaxanthin, β-cryptoxanthin, and β-carotene were present in all the varieties of tamarillo. HPH and UAE treatments had significant impact on the phytochemical composition in the treated sample. HPH at 750 bar and ultrasound for 10 min showed higher phenolics, anthocyanins and carotenoids in the

tamarillo varieties and these techniques are recommended for processing of tamarillos.

8.1.2. Optimization of the conditions of ultrasonication and supercritical fluid extraction of polyphenols from tamarillo pulp and identification of polyphenols in the optimized extracts

- The ultrasound-assisted extraction of phenolic compounds was optimized using response surface methodology as the optimization tool. Initially, four solvents, namely, distilled water, ethanol, acetone, and methanol at 100% concentration level were tested for extraction of total phenolics, and acetone was found to be the best solvent. For optimization of red and yellow tamarillo using UAE, Box-Behnken design with three independent variables of extraction time (5-15 min), ultrasound amplitude (20-60%), and solvent concentration (50-80%) were taken to maximize total phenolic content, total flavonoids content and DPPH radical scavenging activity, that were taken as dependent variables. Extraction with acetone at 73 and 78 % concentration and ultrasound amplitude of 43 and 46 % were optimum for red and yellow tamarillo, respectively. Optimized extraction time was found to be 12 min that provided maximum phenolic content, flavonoids content, and DPPH radical scavenging activity with a desirability of 0.94 and 0.97 for yellow and red tamarillo, respectively.

- The parameters for extraction of phenolic compounds and anthocyanins in purple tamarillo using supercritical fluid (SCFE) and ultrasound-assisted extraction (UAE) techniques were optimized. Optimization of extraction techniques was done using Box-Behnken design. The experimental range for independent variables in SCFE was 30-60 min time, 40-60 °C temperature and 150-180 bar pressure, which were determined based on total phenolic content (TPC). Similarly, for UAE, the selected independent variables were 10-30 min time, 40-60 °C temperature, and 20-60% amplitude. The optimized conditions for SCFE were determined to be 49.42 min, 49.28 °C and 176.63 bar pressure, and at the optimized parameters, the TPC was 16.12 mg GAE/g and anthocyanins was 0.62 mg C3G/g in the extract. The optimized parameters for UAE were 19.14 min, 51.53 °C and 50.53 % amplitude, which gave 21.06 mg GAE/g TPC and 0.71 mg C3G/g anthocyanins. Four phenolic acids (gallic acid, chlorogenic acid, caffeic

and p-coumaric acid) and three anthocyanins (delphinidin-3-O-rutinoside, cyanidin-3-O-rutinoside and pelargonidin-3-O-rutinoside) were identified by HPLC in both the extracts. Higher concentration of phenolic acids and anthocyanins were extracted by UAE as compared to SCFE. SEM morphology of sample matrix showed extensive disintegration after UAE process.

8.1.3. Standardization of the bottling of tamarillo puree and study of the *in vitro* bioaccessibility of carotenoids

- The effect of temperature (100-200ºC), time (2-6 min), and EVOO (extra virgin olive oil) on the bioactive properties and colour parameters of tamarillo puree was determined. The increase in heating temperature (up to 175ºC) and time increased the phenolic content, flavonoids content, and antioxidant activity. Carotenoids were found to increase from 0.65 to 1.06 (mg β carotene/ g) in puree added with EVOO at 5% level that was heated at 200ºC for 6 min. In puree added with EVOO, the lightness (L*) and redness (a*) values of puree were found to be reduced, but yellowness (b*), hue angle, and chroma improved with treatment temperature and time. Addition of oil exhibited positive influence on retention of the bioactive compounds in comparison to puree without oil, which can be attributed to their enhanced extractability. The desirable effect of heating temperature and time, and addition of virgin olive oil will be helpful in the canning of oil enriched tamarillo puree.

- High pressure homogenization (HPH) and thermal treatment were applied to process tamarillo puree. Three different pressures (500, 700, and 1000 bar) and passes (1, 2, and 3 passes) were applied to the puree. It was found that increase in pressure and number of passes led to destruction of particles, and particle size below 1000 nm was achieved. Increase in the colour values, polyphenols, carotenoids, and DPPH scavenging activity was observed after HPH application. At 1000 bar pressure and single pass, total polyphenols increased by 14%, total carotenoids by 21%, and DPPH scavenging activity by 30%. HPLC confirmed the presence of four phenolic acids: gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid at a concentration of 112, 254, 63, and 103 µg/g. β-cryptoxanthin and β-carotene were the major carotenoids present in HPH processed tamarillo. A 5 log reduction was achieved after 16 min of thermal

processing, which was determined using *Escherichia coli, Staphylococcus aureus*, *Listeria monocytogenes*, and *Bacillus cereus*. The bioaccessibility of TPC and TCC was found to be 72 and 74% of the content present in freshly bottled tamarillo puree that was given combined HPH and thermal treatment. Storage study revealed a decrease in the concentration of bioactive compounds; however, puree was acceptable up to 4 months of processing.

8.1.4. Development of tamarillo powder using connective drying and study of the drying behaviour and powder properties

- Four foaming agents namely egg albumin, whey protein concentrate, soy protein concentrate and gelatin were used for the study. The bulk density, tapped density, wettability, solubility, hygroscopicity, colour values, and SEM of all foam mat dried powders were evaluated.
- Initial physicochemical results of foam showed that whey protein was a good foaming agent forming a decent foam, but increase in concentration from 5 to 10% played crucial role in refining the quality of foam in terms of food density, foam expansion and foam stability.
- Among all foaming agents, SPC and WPC at 10% concentration was found to give better powder quality, followed by egg albumin and least effective was gelatin. With an increase in foaming agent's concentration, better retention of phytochemicals was seen in the extract from foam mat dried powder.
- The total phenolics, flavonoids, antioxidant activity, and vitamin C were analyzed for all powders, and highest bioactivity in all the experimental results were found in the powder using WPC as the foaming agent. WPC at 10% concentration retained maximum concentration of phenolic acids as observed from HPLC chromatograms. In WPC-10, HPLC analysis showed 890, 403, 85, and 297 μg/g of gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid, respectively, and β-cryptoxanthin and β-carotene was found to be 27.47 and 19.47 (μg/g).
- SEM images revealed that powder prepared by WPC was better in holding the structure even after drying, which was corroborated by foam stability results.

8.1.5. Study of the physical and chemical properties of tamarillo seed oil nanoemulsions incorporated with extracted carotenoids of the fruit

- Tamarillo peel was used for the extraction of carotenoids using high shear disperser (HSD) and ultrasound assisted extraction (UAE) in extra virgin olive oil that was taken as an extraction solvent.
- Rotatable central composite design with three independent variables was applied for the optimization of the extraction of total carotenoids using HSD and UAE. Quadratic model was found to be significant and optimized conditions for HSD were time of 5.50 min, temperature of 49 °C, and speed of 15000 rpm, which gave the maximum carotenoids content of 3.81 mg βCE/100g with desirability of 0.96. The optimized conditions for UAE, on the other hand, were an extraction time of 8 min, temperature of 50 °C, and 76% amplitude, which extracted 2.01 mg βCE/100g of the carotenoids with desirability of 1.00. HSD extract showed an increase of about 15% TPC along with enhanced antioxidant activity. HPLC analysis of HSD-treated carotenoids enriched oil showed the presence of four phenolic acids, viz. gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid, and three carotenoids namely, zeaxanthin (53.65 μg per 100g), β-cryptoxanthin (194.32 μg per 100g), and β-carotene (593.35 μg per 100g). HSD extract enhanced the carotenoids level in mayonnaise and the product was liked for its sensory attributes. Development of carotenoids-loaded nanoemulsion was done using ultrasonication. Tamarillo seed oil was used as the solvent for extraction of carotenoids from tamarillo peel using high shear disperser.

- A Box-Behnken design with three independent variables, viz. oil concentration, emulsifier concentration, and ultrasonication time was used for optimization. In optimization, ultrasonication time and emulsifier concentration played important roles in the development of nanoemulsion. The optimized condition for fabrication of nanoemulsion was 6.17% oil, 2.7% emulsifier, and 21 min of ultrasonication time that yielded particle size of 201 nm and zeta potential of -32 mV. Particle size and carotenoids retention were significantly affected by pH and stability. An increase in particle size from 201 to 578 nm was observed from the initial to intestinal phase, respectively. TEM results showed a particle size of approximately 220 nm of nanoemulsion.

8.2 Future scope

The study findings serve as the foundation for future research that can be undertaken to profile the various other bioactive compounds present in tamarillos from North-East India. Different novel or other processing methods can be employed, which will improve the extraction yield of bioactive compounds present in tamarillo fruit along with nutritional value, while causing minimal degradation of bioactive compounds. In bottled tamarillo puree, a thorough investigation is required to determine the effect of interaction with other ingredients, which were added for the preparation of final bottled puree, on the total phenolic acids and carotenoids present. A cell line study can be done to determine the effect of cellular uptake of bioactive compounds from bottled tamarillo puree. A proper and broad *in-vivo* study is required to determine the accuracy of the bioaccessibility of the carotenoids and interaction of nanoemulsion with food ingredients in the gut.