Abstract

The thesis describes the phytochemical profiling and valorisation of three varieties of tamarillos (purple, yellow and red) obtained from North-East India, utilizing the pulp and the waste portion of the fruit, mainly peel and seed, for the development of functional foods. According to literature reports, tamarillo varieties are loaded with a diverse range of bioactive compounds and each type has its definite characteristics. In countries like Brazil, Australia, New Zealand, Peru, Ecuador etc., these fruits are getting popular and numerous products are available in the market. Although all three varieties of tamarillo are available in India, only a few or limited studies have been reported on the profiling and valorization of tamarillo. Thus, depending upon the perceived gap, this research focuses on profiling bioactive compounds present in the varieties of tamarillo using different novel extraction methods and determining the effect of various processing techniques on the bioactive compounds using different optimization designs. The research work also evaluated the development of nutrient-enriched functional foods using the tamarillo pulp, peel, and seeds, and their phytochemical composition.

The current research work is divided into five primary objectives. The first objective evaluated the three varieties' nutritional, biochemical profile, and antioxidant activities. Results revealed that these three tamarillos were rich sources of fibre, vitamin C, and several bioactive compounds. Yellow tamarillo had maximum carotenoids (0.63 mg βCE/100g), and purple tamarillo was rich in total anthocyanins (0.35 C3G/100g). Moreover, both the carotenoids and anthocyanins were found in purple and red tamarillos. RP-HPLC results confirmed that purple tamarillo was rich in anthocyanins and carotenoids, with anthocyanins being the dominant phytochemical; yellow tamarillo was enriched with only carotenoids, whereas both anthocyanins and carotenoids were found with carotenoids as a predominating compound in red tamarillo. Further, three different processing techniques, viz. conventional, ultrasonication (US) (5, 10, and 15 min) and high-pressure homogenization (HPH) (500, 750, and 1000 bar) were applied to tamarillo juice. Results showed that the US at 10 min and HPH at 750 bar enhanced the level of bioactive compounds. In HPLC, six phenolic acids, namely, gallic, chlorogenic, caffeic, p-coumaric, rosmarinic, and ferulic acids, and three carotenoids and anthocyanins were detected in the tamarillo fruit.

In the second objective, optimization of the conditions for the extraction of bioactive compounds was performed using ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SCFE) techniques. This objective was divided into two sections. In the first section, ultrasound-assisted extraction of freeze-dried yellow and red tamarillo pulps was carried out. Initially, four different solvents were used for the extraction of bioactive compounds, and acetone extracted the maximum total phenolic content (TPC), with a value of 15.96 mg GAE/g of the sample. Box-Behnken design with three independent variables of extraction time, amplitude, and solvent concentration was followed, and three dependent variables of TPC, total flavonoids and DPPH radical scavenging activity were employed as the responses for the optimization of the extraction conditions. For yellow and red tamarillo, results revealed that ultrasound amplitude of 46 and 43%, and solvent concentration of 78 and 73%, respectively, gave a maximum yield of dependent variables at 12 min extraction time. These conditions were selected as the optimized conditions for UAE.

In the second section, two extraction techniques viz. SCFE and UAE were studied for the extraction of the phytochemicals from purple tamarillo. In this study, freeze-dried tamarillo powder was used, and optimization of extraction conditions for maximum recovery of phenolics and anthocyanins was targeted. For both techniques, the range of the independent variables was decided based on the complete extraction of phenolic content from the sample. In SCFE, the range taken for extraction time was 30-60 min, temperature was 40-60 °C, and pressure was 150-180 bar. In the case of UAE, extraction time of 10-30 min, temperature of 40-60 °C, and amplitude of 20-60% were taken as the independent variables. Optimization of the extraction conditions was done using a three-factor Box-Behnken design for both the extraction techniques and optimized extraction conditions were selected by maximizing the values of the dependent variables, namely, total phenolic content and total monomeric anthocyanins content. The optimized extraction conditions for SCFE were an extraction time of 49.42 min at a temperature of 49 °C and pressure of 176 bar; at these conditions, the yield was 16 mg GAE/g of TPC and 0.62 mg C3G/g of anthocyanins. On the other hand, the optimized extraction conditions of UAE was an extraction time of 19 min, temperature of 51 °C, and amplitude of 50% with a yield of 20.87 mg GAE/g of TPC and 0.71 mg C3G/g of anthocyanins. Results clearly indicated that UAE was better in extracting bioactive compounds from purple tamarillo than UAE. Through HPLC of phenolic acids, four

phenolic acids and three anthocyanins were identified in the sample extract. Residues after sample extract were dried and further evaluated using scanning electron microscopy to determine extent of disintegration of cellular structure. It was noticed that UAE caused more rupture and cellular disintegration in contrast to SCFE. The obtained extract possessed very high antioxidant properties and can be utilized as a natural colorant by food industries.

The third objective involved the development of carotenoid-enriched puree using yellow tamarillo. This objective was divided into two sections. The first section dealt with the study of the effect of addition of extra virgin olive oil (EVOO) and thermal treatment at 100-200 °C of yellow tamarillo puree. In this study, samples without EVOO and with 5% EVOO were taken and thermally treated at five different temperatures (100, 121, 150, 175, and 200°C) for a treatment time of 2, 4, and 6 min. The effect of EVOO and thermal treatment was analyzed by evaluating the TPC, TFC, DPPH radical scavenging activity, total carotenoid content, and colour parameters of the samples. Results revealed that puree treated without oil showed a higher loss of phytochemicals than those treated with oil. Addition of oil increased TCC level in the puree. TCC in sample treated without oil for 6 min was 0.87 mg βCE/100 g, which increased to 1.06 mg βCE/100 g in sample treated with oil. Moreover, the colour values of the puree with oil had increased with treatment temperature and time.

The second section deals with the study of the effect of HPH on tamarillo puree. For this, puree samples were subjected to three different pressures (500, 700, and 1000 bar) and 1, 2, and 3 passes. Results indicated that the application of HPH led to the reduction in particle size of puree, to a size <1000 nm. The reduction in particle size was further confirmed by light microscopy test. On the other hand, TPC, TCC, DPPH radical scavenging activity, and colour parameters of each treated sample were analysed. At 1000 bar-1 pass, the TPC and TCC was 6.19 mg GAE/g and 0.79 mg βCE/100 g, respectively; therefore a selection of 1000 bar pressure and 1 pass was selected for further processing. HPLC analysis of phenolic acids and carotenoids was done; results showed that the major carotenoids present in the tamarillo puree were β-carotene and β-cryptoxanthin. Further, the thermal death time (D value) of four different microorganisms, namely *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus* was determined at four different temperatures (65, 75, 85, and 95°C). At 95 °C, the D values of all the inoculated microorganisms were

found to be less in comparison to values at 65, 75, and 85 °C. The HPH-treated bottled puree was given thermal treatment to cause 5 log reductions in microbial cells. Storage study of the bottled puree was conducted for 120 days at 25 ± 5 °C, and different phytochemicals and total microbial count was assessed at an interval of 30 days. Puree showed a reduction in the phytochemicals content with time but was safe for consumption in terms of microbial load up to 120 days.

The fourth objective involved development of foam mat dried powders of red tamarillo using different foaming agents. Foaming agents, namely egg albumin, whey protein concentrate, soy protein concentrate, and gelatin were used at 5 and 10% concentrations. Different tests like foam stability, foam expansion, and foam density were evaluated. Results revealed that foam prepared using whey protein concentrate at 10 % showed a maximum foam expansion of 52.25 %, whereas egg albumin at 5 % yielded minimum foam expansion of 22 %. While drying, initially, three different temperatures of 50, 60, and 70 °C were taken for the study, out of which 50 °C was selected for further studies based on maximum retention of TPC (433.91 mg GAE/100 g) and TCC (1.82 mg βCE/100 g). After foaming, the foam was spread on a tray with a thickness of 3 mm and dried at 50 °C until constant weight was reached. Five different drying models were applied, out of which the Logarithmic model was recommended as the best because of its high R^2 value (0.97-0.99) and low RMSE value (0.01-0.03). Increasing the concentration of foaming agents had a significant impact on the powders' physicochemical and phytochemical properties. The TPC and TCC of foam mat dried powder prepared using 10 % whey protein concentrate was 769 mg GAE/100g and 1.71 mg βCE/100g, respectively. RP-HPLC and SEM results also showed that whey protein concentrate at 10% was found to be best for developing foam mat dried powder. Four phenolic acids, namely gallic, chlorogenic, caffeic, and p-coumaric acid, with a concentration of 890, 403, 84, and 297 µg/g, respectively were identified. Two carotenoids, namely β-carotene and β-cryptoxanthin, were identified and quantified in all the samples using RP-HPLC. A storage study of 120 days at three different temperatures (-18, 6, and 25°C) was performed. Results showed that degradation of phytochemicals present in the foam mat-dried tamarillo powder occurred with an increase in storage temperature.

The last objective dealt with the development of carotenoids-enriched mayonnaise and carotenoids-enriched nanoemulsion from the waste portion (seed and peel) of yellow tamarillo that was generated while fulfilling objective 3. This objective was further divided into two sections; the former section deals with the extraction of carotenoids from tamarillo peel. For this, EVOO was used as an extraction solvent, and optimization of extraction conditions was done using two different extraction techniques, viz. ultrasound-assisted extraction (UAE) and high shear disperser (HSD). A three-factor central composite design in RSM was employed to determine the best-optimized conditions. At the optimized values of extraction time of 5.50 min, temperature of 49 °C and speed of 15000 rpm HSD yielded TCC with a value of 3.81 mg βCE/100 g, however in UAE, the optimized extraction conditions yielded TCC of 2.01 mg βCE/100 g at an extraction time of 8 min, temperature of 50 °C and amplitude of 76%. Further, RP-HPLC analysis of the extracted samples was studied and results of greater extraction of phenolic aids and carotenoids were observed in HSD samples. Carotenoids extracted using HSD in EVOO were used in the processing of mayonnaise. A nine-scale Hedonic scale was used for evaluating the sensory attributes of carotenoids-enriched mayonnaise. Reduction in the carotenoids content was observed during *in-vit*ro digestion of mayonnaise and lower release of the carotenoids occurred in the gastric phase in comparison to the intestinal phase. The carotenoids-enriched mayonnaise will be a new functional food product for the Indian as well as global market.

The latter section deals with the utilization of tamarillo seed oil for the development of nanoemulsion incorporated with carotenoids-enriched tamarillo peel powder. The seed oil was extracted by Soxhlet extraction method. The fatty acid profile of the tamarillo seeds oil was evaluated using GC-MS, and results showed that linoleic acid, palmitic acid and stearic acid were present in the oil. Further, HSD optimized conditions of extraction time of 5.50 min, temperature of 49 °C, and a speed of 15000 rpm were used for the extraction of carotenoids from tamarillo peel. A three-factor Box-Behnken design of independent variables of oil concentration, surfactant concentration, and run time was used to fabricate the carotenoids-loaded nanoemulsion. The optimized values of oil concentration of 6.17 %, surfactant concentration of 2.74%, and time of 21 min delivered the nanoemulsion with a particle size of 199 nm, polydispersity index of 0.27, and encapsulation efficiency of 91%. The zeta potential value of the optimized nanoemulsion was found to be -32 mV. Further, the optimized carotenoids-loaded

nanoemulsion was further evaluated to determine the pH, ionic strength, and stability at different temperatures (4, 25, and 55°C). The particle size was below 300 nm at pH values ranging from 2-7. Particle size was 221 nm at lower ionic concentrations and 290 nm at higher ionic concentration. The particle size of carotenoids-loaded NE was noticed to increase at higher temperature, and on 14^{th} day, the size increased to 290, 351, and 490 nm on storage at 4, 25, and 55°C, respectively. TEM analysis indicated that the optimized nanoemulsion particles were between 218 and 220 nm in size. During *in-vitro* digestion, changes in particle size after each digestion phase and changes in the carotenoid composition of the nanoemulsion were studied, and the release of total carotenoids from the nanoemulsion was 57% during intestinal digestion.

Keywords: Tamarillo, Phenolics, Anthocyanin, Carotenoids Valorization, HPLC, Bioaccessibility, product development