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Effect of Processing Techniques on Bioactive Constituents and Functional Properties of Tamarillo (Solanum betaceum)

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ABSTRACT

Background: Tamarillo (*Solanum betaceum*) is a small but fast-growing, underexploited and seasonal fruit providing significant amounts of micronutrients and bioactive compounds which may produce a range of possible effects. The present study was conducted to determine the effects of processing techniques on the functional properties and bioactive compounds present in Tamarillo. **Methods:** The procured fruits were cleaned and subjected to two different processing methods; steam cooking directly under liquid boiling at 100 ℃ for 5 minutes and the fruits which were frozen below -45 °C for 42 hours and then lyophilized. Fruit samples were extracted using methanol by ultrasonication. Moreover, functional groups were identification and quantification of phytonutrients by Fourier Transform- Infrared Spectroscopy and UV-Spectrophotometer. **Results:** The spectra obtained by FTIR allowed identification of functional groups and secondary metabolites in Tamarillo. Fourier Transferred Infrared Spectroscopy revealed the presence of OH stretching alcoholic/phenolic - (3400-3200 cm-1), C=C stretching aromatic - (1650-1600 cm-1), C-H bending aromatic - (700-420 cm-1) compounds in their respective peaks. The study revealed that total phenolic content of 56.4 and 88.5 mg GAE/g, flavonoids 45.8 and 28 mg QE/g, glycosides 10.6 and 14.5 mg/g and carotenoids content was 0.36 and 0.46 mg/g respectively for both freeze-dried and steamed cooked Tamarillo fruit. Regarding processing of bioactive compounds, the significant differences (P=0.1) were observed. **Conclusion:** Both the freeze drying and steam cooking effectively preserve its bioactive properties. The functional group and polyphenols present in the fruit confirms the presence of antioxidants which protects cells from free radical damage. Finally, the underexploited fruit may be utilized for its nutritional benefits and nutraceutical potential in treating degenerative diseases.

Introduction

Plants serve as abundant sources of bioactive compounds, including polyphenols and carotenoids, with significant applications in the food, pharmaceutical and cosmetic industries. Various extraction techniques have been developed to efficiently isolate these valuable natural products

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for commercial use. Phytonutrients in fruits play a crucial role in mitigating the incidence of chronic diseases. Furthermore, home cooking often involves thermal or heat processing methods such as boiling, steaming, frying and freeze or microwave drying. The impact of these methods on the retention of bioactive compounds remains a subject of ongoing investigation. Some studies have documented a reduction in bioactive content following thermal treatment of fruits and vegetables. Heat processing such as steaming can enhance food digestibility, extractability and the bioavailability of essential nutrients by softening the food matrix. However, the overall effect of heat processing is significantly influenced by processing parameters and the inherent structure of the food matrix. Steaming is generally considered a safer method for preserving the bioavailability of vital nutrients in plant foods. A study by Mehmood *et al*. showed that antioxidant content in fruits and vegetables was higher in steaming compared to microwaving or boiling (Mehmood and Zeb, 2020).

Drying emerges as a preeminent method for preserving fruits, vegetables and herbs characterized by a significant reduction in volume and weight, consequently minimizing packaging, storage and transportation costs. Furthermore, this process induces modifications in flavour and texture, yielding a novel category of products such as snacks that can serve as healthier alternatives to commercially available confectionery. Notably, water removal effectively inhibits the proliferation of microorganisms and suppresses detrimental chemical reactions, thereby extending shelf life (Calín-Sánchez *et al.*, 2020). Food dehydration, by definition, constituted a process involving the removal of water from food through the circulation of hot air. This process effectively deactivates enzymes and inhibits bacterial growth. Dried foods exhibit desirable attributes including enhanced taste, nutritional value, reduced weight and ease of preparation, storage and utilization. Drying exerts a minimal impact on the overall nutritional composition of food. While vitamin A is generally well-preserved during the drying process, its light sensitivity necessitates the storage of vitamin A-rich foods in dark environments. Yellow, orange and red-coloured fruits and vegetables such as Tamarillo are notable sources of β-carotene. Conversely, vitamin C is susceptible to degradation upon exposure to heat. However, pre-treating foods with citrus juices such as lemon, orange or pineapple can effectively enhance vitamin C retention. Dried fruits and vegetables, owing to their high fibre and carbohydrate content and low-fat content represent highly nutritious dietary choices. Freeze-drying also recognized as lyophilization or cryodesiccation, constitutes a dehydration technique commonly employed for preserving perishable materials and facilitating convenient transportation. This process involves freezing the material followed by a reduction in surrounding pressure, enabling the frozen water within the material to undergo sublimation, directly transitioning from the solid phase to the gaseous phase.

Tamarillo (*Solanum betaceum*), an underexploited fruit native to South America, is also cultivated in the Nilgiris District of Tamil Nadu, India. This egg-shaped, juicy fruit, characterized by a sour taste and containing numerous flat seeds, possesses a bitter peel. Commonly referred to as "tree tomato," it is utilized in the preparation of chutneys, sauces, pickles, jams and salads. The fruit is rich in essential nutrients, particularly fiber, vitamins and minerals and it contains significant levels of bioactive substances, including polyphenols and antioxidants, making it a valuable food for managing degenerative disorders. The phytonutrients and antioxidants present in the fruit exhibit anti-cancer properties by effectively scavenging free radicals. Regular consumption of Tamarillo fruit has been associated with reduced blood sugar levels, weight loss, prevention of kidney stones, and improved immunity. This research aims to investigate the impact of processing techniques used especially as freeze-drying and steam-cooking on the phytonutrient constituents and functional characters of Tamarillo. The research aimed to determine how these processing methods affect the presence and preservation of bioactive

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compounds including phenolic, flavonoids, glycosides and carotenoids that contribute to antioxidant and nutraceutical potential (Suganya and Kalpana, 2022). Fruit processing involves techniques such as freezing, drying, and cooking that help preserve fruits beyond their natural harvesting season. Tamarillo is a seasonal and underexploited fruit that is not always available fresh throughout the year. By applying processing methods like freeze-drying or cooking, the shelf life of the fruit can be extended, allowing it to be consumed even during off-seasons. This not only makes the fruit available year-round but also improves its usability in various forms such as powders, preserved foods, extracts, or functional food ingredients, thus increasing its application in food and nutraceutical industries. The present study was conducted to determine the effects of processing techniques on the functional properties and bioactive compounds present in Tamarillo.

Materials and Methods

Procurement of fruits

Ripe Tamarillo fruits were harvested from Gudalur in the Nilgiris district of Tamil Nadu. After collecting, the fruits were cleaned and stored at ambient temperature ranges from 20-25 °C. They were then scaled, and cut into uniform pieces. Whole Tamarillo plant was authenticated by Botanical Survey of India, TamilNadu agriculture University, Coimbatore and the study obtained Institutional Human Ethical Clearance (AUW/IHE/FSN-22-23/XMT-8).

Sample preparation with freeze dried fruit

Foods can be preserved for extended periods through freezing or lyophilization, both of which effectively remove water from food samples. In this study, the freeze-drying technique was selected for drying the Tamarillo fruit samples. A total of 250 grams of fresh fruit, including the peel, was pre frozen for 12 hours and lyophilized at a temperature ranging from -45 °C to -50 °C for 42 hours under a vacuum pressure of 0.010 m to 0.012 m Torr. The resulting dried fruit samples were then ground into a powder kept in airtight container and stored in a frozen state for future use (Ahmed *et al.*, 2013).

Sample preparation by steaming

Steaming involves cooking food over boiling water without the addition of fats (Vintilă, 2016). In this process, water was added to steamer and a perforated stand was placed inside the steamer. The water level was maintained below the stand to ensure effective steaming. The fruit was positioned on the perforated section of the stand, and the steamer was covered with a lid while cooking over medium-high heat. The fruit was cooked by the hot steam produced from the boiling water, with the samples prepared under atmospheric pressure. A total of 50 grams of fruit was steamed for 5 minutes at a temperature of 100 °C.

Identification of functional group using FTIR Spectroscopy

FTIR analysis was carried out using the extracted material at a wavelength of 3600 nm and a mid-infrared spectrum at a wavelength of 4000-600 nm. The functional group was determined using FTIR spectroscopy for the freeze-dried materials. One gram of the sample was collected, combined with 50 milliliters of 80% ethanol, stored in a shaking incubator and filtered through Whatman filter paper No. 40. The filtrate was collected in sterile bottles, centrifuged at 2500 rpm for 15 minutes and kept refrigerated at 5 °C until use (Sheybani *et al.*, 2023).

Sample extraction with ultrasonic techniques

Five grams of processed samples were weighed and combined with 25 milliliters of concentrated methanol. The mixed materials were maintained in an ultrasonic homogenizer at 50–60 °C for 15-20 minutes after being vortexed for 10 minutes. Ultrasound waves as opposed to microwave (electromagnetic wave), are high-frequency sound waves that are audible to humans. An elastic medium such as liquid solvents, soft plant tissue, etc., is significantly impacted by sound waves. When sound waves pass through the medium, the shape of the medium changes and when sound waves are not there, the medium returns to its

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original shape. thus High frequency ultrasonic waves exert a piston-like effect on the medium (Panja, 2018). Cavitation bubbles are created inside the medium throughout the process; when they collapse, a significant amount of energy in these tiny bubbles are released, and localized hot and cold spots are produced. The cavitation effect is the mechanism used in phytochemical extraction.

Estimation of bioactive compounds

Phenolic compounds: The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) in Tamarillo fruit samples. The process involved mixing 5 grams of the sample with 25 ml of 98% methanol, the mixture was then vortexed and ultrasonicated for 60 minutes. The extracts were filtered, centrifuged, and then 1 ml of the extracted sample solution was added to a Folin-Ciocalteu reagent. After 5 minutes at ambient temperature of 20-25 ℃, 1.5 ml of 20% sodium carbonate solution was added, and the mixture was stored in the dark for two hours at room temperature. A UV spectrophotometer assessed the sample absorbance at 765 nm against a control, and the results were extrapolated from a Gallic acid standard curve, expressed as milligrams of Gallic Acid Equivalent (GAE) per 100 grams of dry weight (Diep *et al.*, 2020).

Flavonoids: A 10 ml of sample extracts were shaken vigorously with 4 ml of distilled water and allowed to keep for 5 minutes. Various aliquots of Quercetin working standard (100 g/ml in methanol) were prepared in separate test tubes, adjusting the total volume to 4 ml with distilled water including a blank sample as control. Then 0.3 mL of 5% sodium nitrate and 0.3 ml of 10% aluminum chloride were added to each tube, followed by shaking incubator at 37 °C for 10 minutes. Subsequently, 2 ml of 1 M sodium hydroxide and 10 ml of distilled water were added. The test tubes were shaken thoroughly, the mixture was filtered, and the absorbance of the reaction mixture was measured at 510 nm using a colorimeter against the blank. The total flavonoid content was quantified as milligrams of quercetin equivalent based on a standard curve (Rohilla and Mahanta, 2022).

Cardiac glycosides: A 10% of extract was combined with 10 ml of freshly prepared Baljet's reagent. After one hour, the mixture was diluted with 20 ml of distilled water, and absorbance was measured at 495 nm using a spectrophotometer. A standard curve was created using different concentrations of securidaside. The total glycoside content from triplicate measurements was expressed as milligrams of securidaside per gram of Tamarillo sample extracts (Dibulo *et al.*, 2017).

Carotenoids: A sequential extraction was performed on a 5 g Tamarillo sample. This involved three successive extractions with 10 ml of hexane (containing 0.1% butylated hydroxytoluene, BHT) followed by three extractions with 10 ml of ethyl acetate (containing 0.1% BHT). After each extraction, the suspension was ultrasonicated for 15 minutes at moderate temperature in a dark environment and then centrifuged at 3000 rpm for 10-15 minutes. The resulting suspension was filtered through a 0.45-micrometer syringe filter. The collected filtrate was evaporated to dryness under vacuum and re-dissolved in methanol. The absorption of the mixture was measured at 425 nm, and the total carotenoids content was calculated using the standard curve. The results are expressed in terms of β-carotene as µg per 100 g of the Tamarillo sample (Giuffrida *et al.*, 2018).

Data analysis

Paired sample *t-test* was applied to identify the statistical difference between the effects of drying and cooking on the bioactive compounds of tamarillo fruits. Sigmaplot 14.5 software was used to analyze the statistical values.

Results

Identification of functional groups using FTIR spectroscopy

Functional groups were identified by FTIR Spectroscopy and the results were presented in **Table 1** and **Figures 1** and **2**.

Freeze-dried and steam cooked Tamarillo fruit

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samples had more or less unique spectroscopic signatures. The FTIR spectra of the following functional groups can be used to confirm the presence of phenolic substances in the extracts of Tamarillo fruit samples: OH alcoholic/phenolic (3400-3200 cm-1), C=C aromatic (1650-1600cm-1), C-H aromatic (700-420cm-1). The peak values and their functional groups are depicted in **Table 1** and **Figures 1** and **2**. Analysis of freeze-dried and steam-cooked Tamarillo fruit samples reveals distinct spectroscopic signatures, with FTIR Spectroscopy spectra, confirming the presence of phenolic substances in Tamarillo fruit samples. Key functional groups identified include OH alcoholic/phenolic groups (3400-3200 cm-1), C=C aromatic groups (1650-1600 cm-1), and C-H aromatic groups (700-420 cm-1). The FTIR spectra of freeze-dried and steam-cooked Tamarillo fruit samples exhibit similar bands but with varying magnitudes, reflecting differences in composition.

Table 1. FTIR peak values and functional groupings of Tamarillo.

Peak values (cm^{-1})	Functional group
3695.61	Phenols
2970.38	Alkenes
1743.65	Aliphatic acid / polysaccharides
1450.47	Aromatic / polysacahraides
1381.03	Nitro compound / cutin / waxes
1273.02	Ether / polysaccharides and phenolics
1049.28	Esters / polysaccharides
879.54	Carboxylic acids

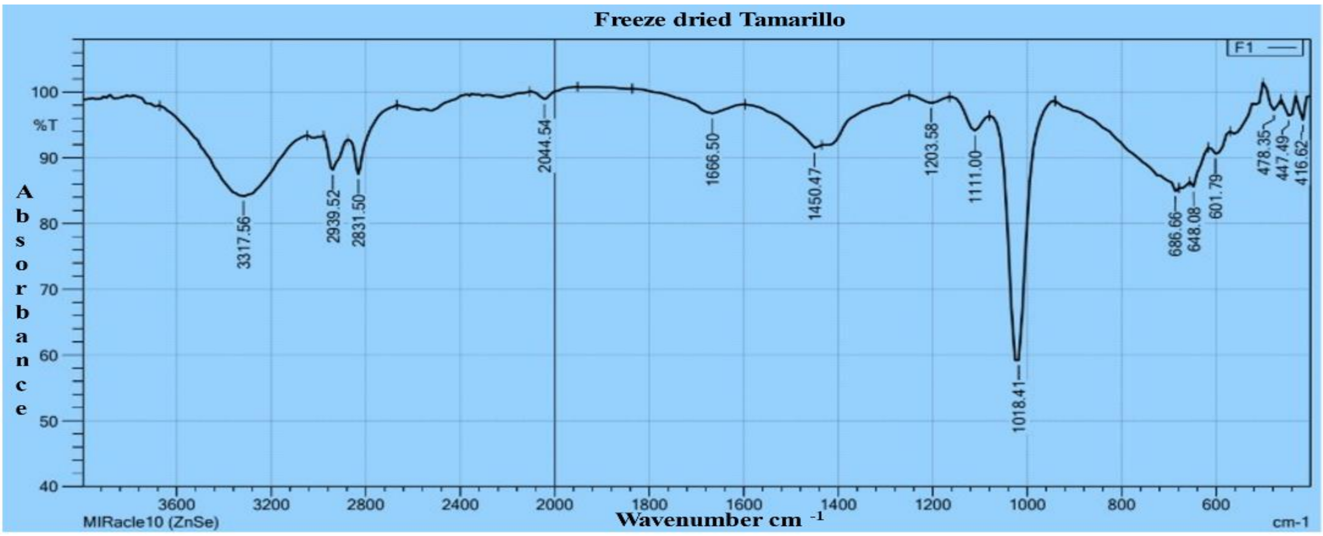

Figure 1. The spectra characteristic bands of Freeze-dried Tamarillo fruit.

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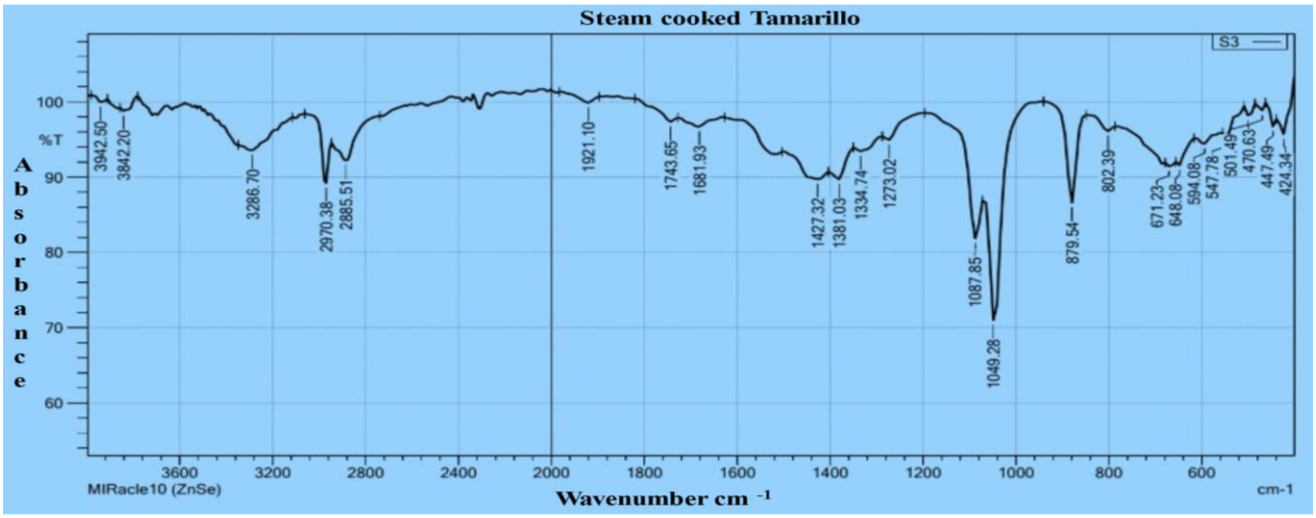

Figure 2. The spectra characteristic bands of Steam cooked Tamarillo fruit.

Bioactive compounds of Tamarillo fruit

Quantification of phenolic compounds in Tamarillo fruit was conducted by Folin-Ciocalteu method (Kamiloglu *et al.*, 2016), and the results are shown in **Figure 3** and their statistical results are depicted in **Table 2**. Polyphenols in plants can act as antioxidants and protect cells from free radical damage, as their ability to scavenge free radicals helps maintain homeostasis.

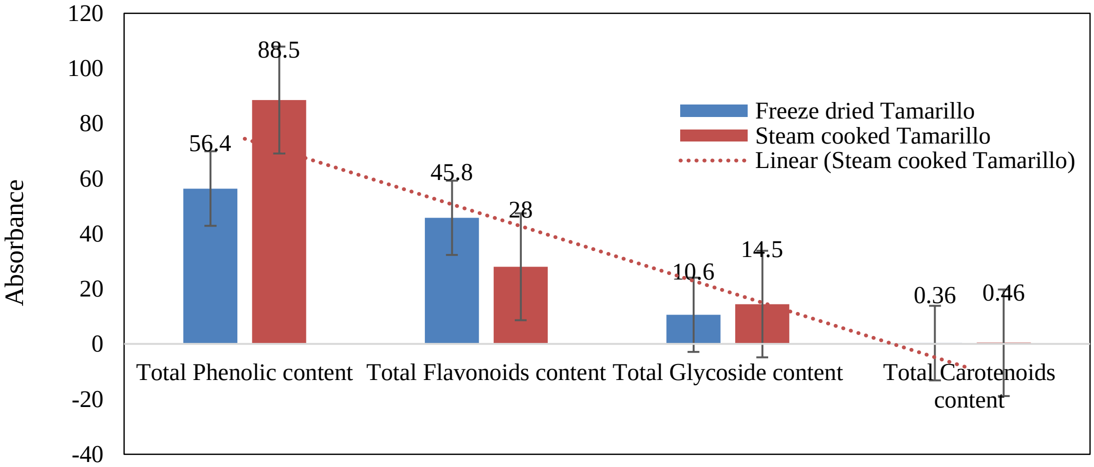

Figure 3. Bioactive compounds of freeze dried and steam cooked Tamarillo fruit.

Table 2. Statistical data for bioactive compounds of freeze dried and steam-cooked Tamarillo.

Samples	t- value	Sig. (2-tailed)	95% confidence interval of the difference	
			Lower	Upper
Freeze dried Ttamarillo Steam-cooked Tamarillo	-0.443	0.688	-34.4	28.2
P-value		0.138		

The results showed that the phenolic content of freeze-dried Tamarillo fruit was 56.4 mg/GAE and steam-cooked Tamarillo fruit extract exhibited 88.5 mg/GAE of phenolic content. The bioactive compounds present in freeze dried and steam cooked Tamarillo fruit was subjected to the paired sample *t-test*. The above elucidated *t-test* and significance values showed no significant difference between freeze dried and steam cooked samples of Tamarillo fruit.

The study found that there was a strong correlation between total phenolic content and phenolics groups present in the fingerprint regions of spectra. Furthermore, it was confirmed that the phenolic content was 56.4 and 88.5 mg GAE/100g, and that there was a positive correlation between the FTIR spectral data and the total phenolic content. Flavonoid content was more in freeze-dried Tamarillo of 45.8mg QE/g compared to steam-cooked Tamarillo fruit which exhibited 28 mg QE/g. Flavonoids have more biochemical characteristics; one of the better characters has its antioxidant capacity. Flavonoids are important for wellbeing due to their role as powerful antioxidant sources in the biological systems of plants and animals.

Carotenoids are generally dispersed in water insoluble, naturally existing pigments of yellow, orange and red. Tamarillo fruit extracts own their carotenoids content of 0.36 mg/g in freeze-dried Tamarillo and 0.46 mg/g in steam cooked Tamarillo fruit, respectively.

Discussion

Specific regions within the spectra indicate contributions from various components including O–H stretching modes of water absorption (3317 cm^{-1}), –C−H stretching in fatty acids (2939 cm-1), –C=O stretching of methyl esterified carbonyl (1666 cm^{-1}), symmetric stretching of carboxylate anion (1450 cm^{-1}), and C=O and C−C stretching of acids (1111 cm^{-1}). The region between 1300 and 800 cm^{-1} shows changes corresponding to the typical fingerprint region of citrus pectin, which aligns with reports of high methoxyl pectin in Tamarillo and low methoxyl pectin in mucilage. The spectra also show similarity to the characteristic peaks of inulin at 3317–2939 cm^{-1} and 1018–686 cm^{-1}. Since phenolic compounds are key contributors to antioxidant activity, the analysis focuses on the bands associated with these constituents. The extracts are rich in phenolic compounds, as indicated by infrared signals at 3317 cm^{-1}, 2939 cm^{-1}, 2831 cm^{-1}, and 2044 cm^{-1}. The antioxidant activities detected in Tamarillo fruit extracts are primarily attributed to these phenolic compounds (Rito *et al.*, 2023).

The analysis focused solely on the bands attributed to phenolic compounds, as they are the primary chemicals responsible for the extract's antioxidant action. With a high nutraceutical and commercial potential for the processed fruit, Tamarillo remains a little known yet it is an intriguing fruit. The majority of research on the antioxidant capacity of Tamarillo was conducted on the fruit itself, focusing on individual processing techniques such as drying and steaming. Therefore, the purpose of this study was to shed light on the fruit's total potential. To ascertain the antioxidant activity of Tamarillo, functional group identification and quantification of bioactive compounds were conducted. Several chemical components found during the screening of the fruits have been described in the functional group identification research. Phenolic acids, a major class of phenolic compounds, have garnered significant attention lately because of their possible health advantages, which include anti-inflammatory, antiviral, anticarcinogenic, antioxidant, and vasodilatory effects. These compounds are present in many fruits and vegetables. Steamed Tamarillo fruit has a higher content of phenolic compounds than freeze-dried fruit. Phenolic content found in Tamarillo fruit by steam cooked helps to retain the nutrient and phytonutrient content view to possess antioxidant and antibacterial activities. Fruits own their quality sources of naturally beneficial and nutritional supplementation due to the phenolic content that are responsible to acts as an antioxidant that protects cells from free radical damage (Diep *et al.*, 2020). The association between functional group is identified by FTIR and quantitative phytochemical analysis confirming Tamarillo fruit provides

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valuable insights (Saeed *et al.*, 2019). Compared to the freeze-dried and steam-cooked Tamarillo samples, steam cooked samples induced higher retention of phenolic content. This means that plants containing phenolic compounds have a variety of beneficial biological effects, such as antioxidant, anti-inflammatory, cardiovascular, and anti-diabetic functions. Other activities of the plant food have a strong cancer inhibiting property (Mutalib *et al.*, 2017). Even though the phenolic content of Tamarillo fruit is the same or less compared to other fruits which owns the strong antioxidant activity than most of the regularly eaten fruits with antioxidant power. Phenolic acids are the main object even as they have an important role in counteracting the activity of oxidation and extremely boosting health and preventing degenerative disorders. It is noted that apart from phenolic content any plant phytonutrients including carotenoids and triterpenoids influences better scavenging actions. Phenolic content is the predominant phytonutrient compound in plants connected to antioxidant action (Baysal *et al.*, 2021).

Flavonoids are one of the organic compounds from phenols that are accountable for the diversity of medicinal uses and have been produced by plants to manage microbial contamination. Flavonoids own their capacity to make human defensive biological catalyst system. Previous studies recommended that flavonoids are good in prevention of degenerative disorders and other infectious diseases. It also acts as an antibacterial activity from the fruit extracts, and the nutritional constituent play a main role in protective mechanism of cancer prevention. Hence, the flavonoid content of Tamarillo fruit may include content in chemoprevention (Kainat *et al.*, 2022).

Glycosides perform a variety of main roles in biological activities ,treat congestive heart diseases, and have been commercially accessible in the form of digoxigenin (Badri *et al.*, 2023). Current investigation of potential glycosides has focused on its potent administration in the treatment of cancer. Hence, regular consumption of antioxidant-rich fruits like Tamarillo improves heart functions, and it may prevent cardiac failure and regulate heart functions and also own its antiproliferative action (Newman *et al.*, 2008).

Carotenoids are antioxidant and have the ability to prevent cardiovascular diseases and reduce the formation of cancer cells. Carotenoids content in Tamarillo can reduce the risk of heart diseases and cancer and protect cells from free radical damage. Hence, it may reduce chronic diseases and help prevent inflammation and oxidative stress.

The results of current study detailed the impact of drying on the bioactive compounds in the Tamarillo fruit. Low temperatures are employed throughout the freeze-drying process, which may help preserve heat-sensitive antioxidants and also the fruit cooked by steaming retains. Hence, freezing and steaming technique efficiently retain the overall antioxidants present in Tamarillo. Among the two processing techniques steamed cooked Tamarillo resulted in the highest retention of phenolic compounds. Tamarillo is an underexploited fruit packed with bioactive compounds highlighting its nutraceutical potential. This study evaluated the bioactive compounds after processing including freeze drying and steam cooking and give insights into how different treatments affect bioactive compounds. Application of FTIR and UV Spectrophotometer identifies the functional groups and quantified the fruits phytonutrients accurately. Bioactive content in Tamarillo confirms its antioxidant potential and other functional properties that may contribute to prevent degenerative disorder. Other processing methods like drying, canning and pasteurization can be included in the future study. The study can also address the effect of processing through analyzing its sensory characteristics of color, taste and other consumer acceptability for human utilization and make available in the market. The stability of Tamarillo by long term storage after processing may be studied in the future.

Conclusion

The current study revealed that Tamarillo is a promising source of phenolic antioxidants and carotenoids. According to the findings of the study,

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the functional group analysis of the freeze-dried Tamarillo sample confirmed the presence of secondary metabolites such as phenolic content. The fruits polyphenol content is responsible for its potent antioxidant capacity, which shields cells from damage caused by free radicals. Steaming is a home-based and easy process that helps to rise the bio-accessibility of phytonutrients in the plants. Both the steam-cooked and freeze-dried Tamarillo fruits are suggested for consuming in everyday diets due to their retention of nutrient including fiber, polyphenols, and antioxidants content after processing. Among the phenolic compounds, phenolic acids have attracted considerable interest in the past few years due to their potential health benefits including antioxidant, antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and vasodilatory actions. The bioactive compounds in Tamarillo contributes to antioxidant activity according to FTIR and UV spectroscopy; the phytonutrients and functional characteristics accessed for the Tamarillo fruit exhibited significant levels of health benefits, and a greater chance of becoming well-known fruit. Due to its high phenol content, it may help lower blood pressure, blood sugar levels, and have possible anti-carcinogenic and anti-obesity effects.

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Authors' contributions

Arivazhagan S framed methodology, analysed and interpreted data, prepared the draft manuscript, and did statistical analysis. Kalpana CA conducted supervision and guidance, provided opinion and input, did the reviewing, and made suggestions for writing manuscripts, editing and validating.

Conflict of interest

All authors declared no conflict of interest.

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