

DEVELOPMENT OF CITRUS-BASED FUNCTIONAL NUTRITION PRODUCTS THROUGH BIOACTIVE METABOLITE AND ANTIOXIDANT CHARACTERIZATION

¹Pham Ngoc Ha

¹HUS High School for Gifted Students, Hanoi, Vietnam, pngocha2209@gmail.com

ABSTRACT

The major sources of functional compounds are the various types of citrus fruits, which consist of flavonoids, phenolic acids, carotenoids, organic acids, and vitamin C; however, functionality greatly vary from one citrus type to another. The objective of research was to research the nutritional, physicochemical, bioactive compound, and antioxidant attributes of pomelo, Cam sành, calamansi, kumquat, and mixed citrus fruits and to use these functional ingredients for the development of functional nutrition products using citrus fruits. Nutritional composition physicochemical properties, phenolic and flavonoid contents, vitamin C, individual bioactive metabolites, and antioxidant activities constitute the analytical. Nutritional and physicochemical analyses were performed after standard extraction of the samples. High-Performance Liquid Chromatography (HPLC) and Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS) used for detection of the bioactive compounds. The antioxidant capacity was determined by means of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), 2,2'-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS), and Ferric Reducing Antioxidant Power (FRAP) tests. Significant differences samples determined through analysis of variance (ANOVA) using Statistical Package for the Social Sciences (SPSS) through Tukey's test ($p < 0.05$) while strong correlations between selected metabolites and antioxidant activities are established using Pearson correlation. The mixture of citrus fruits possessed the highest known DPPH scavenging activity (67.0%) and ABTS scavenging activity (71.0%). Ascorbic acid showed the highest antioxidant correlations ($r = 0.88$ for DPPH and $r = 0.85$ for ABTS). Hence, scientific research proves the connection between citrus bioactivity composition and production of functional foods derived from citrus.

KEYWORDS: Citrus Fruits, Functional Nutrition, Bioactive Metabolites, Antioxidant Activity, Nutrition Products

1. INTRODUCTION

The fruits such as orange, lemon, lime, grapefruit, along with various species of Citrus family became popular due to their nutritive value and phytochemicals [1]. While traditionally used as fruits or juice, citrus fruits are now under examination as potentially useful sources for the development of functional nutrition products aimed at delivering additional physiological benefits in addition to providing basic nutritional values [2]. This prospect is due to the presence of bioactive compounds such as flavonoids, phenolic acids, carotenoids, limonoids, and other secondary metabolites [3]. Functional nutrition focuses on the use of nutritional substances from foods that promote good health and support physiological processes. Importance of metabolites from the citrus fruit is due to the fact that their antioxidant, anti-inflammatory, antimicrobial, and metabolic regulation abilities [4]. Key metabolites from citrus flavonoids such as hesperidin and polymethoxylated flavones are gaining much attention because of the biological activity they exhibit [5]. The antioxidant properties of citrus fruits attributed to vitamin C and phenols due to the mechanism through which scavenging occurs [6]. Nevertheless, the levels and availability of these components are highly dependent on many factors like type of citrus fruit, cultivar, and location of origin, maturity, processing, and storage [7].

Moreover, processing of citrus fruits leads to the generation of a large amount of citrus peel, pulp, seed, and other byproducts that even more phytochemicals than the edible portion of the fruit [8]. The valorization of citrus byproducts is hence an area of immense interest when it comes to developing sustainable nutrition enriched ingredients. Bioactivity analysis and antioxidant capability are hence important steps in the development of scientifically validated functional nutrition products from citrus fruits [9]. It achieved using advanced techniques of analysis, such as chromatography and spectrophotometry, with other techniques [10]. The analysis of the metabolites along with antioxidants provides an overview of the chemical composition of the compounds in the citrus fruits [11]. The biological activity of the citrus fruits is determined by the bioactive metabolites present in them, their amounts, and biological activity, which varies, all these bioactive metabolites, flavonoids to be considered as one of the most extensively [12].

The key flavanones contained in citrus fruits are hesperidin, naringenin, and eriocitrin. These components can be found primarily in citrus peels, albedo, and membranes [13]. In addition, these components are actively studied in relation to their antioxidant and anti-inflammatory properties, as well as the influence on cardiovascular and metabolic health [14]. Polymethoxyed flavones like nobiletin and tangeretin are also of high interest due to their

abundance in citrus peels. In addition, citrus fruits are considered one of the main sources of vitamin C, which is involved in numerous physiological processes [15]. Vitamin C acts as a reducing agent and helps cells resist oxidative stress. In this regard, vitamin C, flavonoids, and other phytochemicals are complementary antioxidant properties [16]. It is more appropriate to evaluate the effect citrus extract as a system of phytochemicals rather than to evaluate the effect of individual chemicals [17]. Metabolic techniques aid this analysis by offering a detailed analysis of metabolites and the detection of those which function as chemical signatures for specific citrus fruits or processes [18]. There are limitations in research due to the fact that it involves selected species of citrus fruits and fractions and in vitro antioxidant tests. Factors not accurately reflect the bioavailability of bioactive metabolites. Research focused on an extensive evaluation of the nutritional composition, physicochemical characteristics, and biological activities such as antioxidants, flavonoids, phenolic acids, organic acids, and vitamin C present in selected citrus based nutrition and their most effective mixture. The purpose of research to be determines the most effective bioactive compounds for producing a functional nutrition product from citrus fruits.

The background and introduction citrus based functional nutrition products are presented in Section 1. The existing research and research gap on citrus nutrition products are in Section 2. Section 3 focuses on the citrus sample selection, preparation, analysis, and statistical analysis. Section 4 includes analyzes the results on nutritional content and antioxidant activity of selected citrus fruit and develops relationships between metabolites and antioxidants. Section 5 presents the conclusion, limitations and future of citrus-based functional nutrition products.

2. Related Work

Assessing the bioactivity profiling of citrus, new methods of extraction, microbial fermentation, and nutritional fortification of foods for citrus based functional nutrition product. Nevertheless, this scientific field is restricted by the absence of nutrient losses during food processing, and methods. Existing research tends to focus on the integration of these aspects.

2.1 Bioactive Characterization and Functional Development of Citrus Products

Bioactive components and antioxidant ability were analyzed in [19]. Tests such as DPPH, ABTS, FRAP, biochemical tests, and gene expression were performed. Dark red fruits possessed excellent antioxidant abilities, and a correlation observed ($p < 0.01$), limited by numerical comparison. The impact of rootstock and developmental stage on 'Orah' functional compounds was investigated in [20]. Flavonoids, phenolic acids, and antioxidant properties quantified using HPLC-UV. Differences significant; however, this quantitative comparison and hydrolysis and fermentation improved the overall utilization of whole citrus, as developed in [21]. Efficient enzymes and lactic acid bacteria are used for optimization. The phenols increased by 39%, flavonoids by 49%, carotenoids by 21%, and antioxidants increased by 279%, while fermentation caused nutrient loss. Green solvents were tested to extract the carotenoids from orange peels in [22]. The method used here was the C8: Pro deep eutectic solvent extraction method. Extraction of the carotenoids achieved 46.01 $\mu\text{g/g}$, and antioxidant capacity of 2438.8 micromoles of $\mu\text{M TE/mL}$. Sustainability assessment remained limited. Fermented navel orange juice was examined for its functionality in [23]. *Lactiplantibacillus paraplantarum* M23 fermentation was performed for seven days. The antioxidant capacity by 255% DPPH and 347% FRAP. Significant increases were observed in functionalities. However, cellular and sensory validation was not well explored.

2.2 Citrus Bioactive Compounds, Antioxidants, and Functional Applications

The role of boron in protecting from the effects of copper-induced oxidative stress was examined in [24]. Copper was administered to citrus seedlings along with boron for 24 weeks. Copper raised malondialdehyde levels by 312.4% in leaves and 585.4% in roots, with limited citrus data. Examining poncirin and isosakuranetin on gut microbial ecology and metabolism was conducted in [25]. 16S rRNA and LC-MS metabolomics were utilized for statistical testing. Principal Component Analysis (PC) led to an increase in *Bacteroides* by 2.4 times and acetic acid in feces by 1.8 times. Mouse results not translated into clinical use. The experiment determined the extraction conditions of bioactive components of the by-products of orange, as investigated in [26]. The techniques used were Soxhlet and maceration, using ethanol or methanol as solvents. There was an extraction efficiency of 46.32%, with phenolics extracted at 5.57 mg GAE/g. Limited optimization across industrial applications remained. Interstocks used to assess their effect on citrus fruit quality, as explored in [27]. Three interstock treatments were assessed using a control. Citric acid increased by 2.90 mg/g FW, whereas the amino acids, flavonoids, and phenolic compounds increased significantly. Sensory validation limited. Citrus lumia juice for its nutraceutical properties in [28], and bioactive components detected using HPLC-DAD. The total amount of phenolics and flavonoids was found to be 38.8 and 25.96 mg/100 mL, respectively, including 28 polyphenols, and clinical trials inconclusive.

2.3 Citrus-Based Functional Product Development and Bioactive Evaluation

Five navel orange cultivars assessed based on quality and phytochemical attributes in [29]. 14 attributes and three main components were established using multivariate analysis. Variance explained by PCA was 93.125% ($p < 0.05$). Nevertheless, validation of processing is insufficient the production of functional nutrition products. The use of citrus pomaces for polyphenol-fiber enrichment of functional yogurt was explored in [30]. HPLC profiling and antioxidant activity analysis were carried out. The orange pomace exhibited 87% scavenging capacity for DPPH and 77% inhibition of carcinoma. Limited long-term and clinical validation remained.

Assessing antioxidant activity, anti-inflammatory activity, enzyme inhibitory activity, and biofilm inhibition ability of citrus monofloral honey was developed in [31]. Polyphenols and vitamin C were quantified. The inhibition of tyrosinase activity was highly significant, whereas that of cholinesterase activity was 19.11%. The findings restricted to in vitro experiments only. The recovery of essential oil from citrus peel through an innovative ultrasound-assisted hydrodistillation machine was analyzed in [32]. The extraction conditions optimized through GC/MS and GC/FID analysis. Limonene was 81.32%, whereas isolation time was reduced by 83.3%, larger scale. Mixed microbial fermentation was used to improve the quality of citrus juices, as conducted in [33]. The microbes involved were *Hanseniaspora pseudoguilliermondii* B4 and *Bifidobacterium lactis* 20435. There was a reduction in acidity by 96.05%. DPPH and ABTS increased by 34.47% and 52.63%, respectively, but Long-term stability remained limited.

2.4 Integration of Dried Citrus Metabolites into Functional Products

Authenticity parameters for lemon juice were investigated in [34]. Three liquid chromatography techniques assessed using samples. The quantitative limits for coumarins, psoralens, and polymethoxyflavones determined, but limit country-to-country variation. Citrus peel drying methodologies compared for maximizing efficiency, as developed in [35]. The three methodologies, Minimum Inhibitory Concentration Determination (MICD), compared based on their drying kinetics and quality assessments. Infrared Detector (IRD) at 70°C proved to be the optimal methodology, but MICD led to deterioration, and broader overall validation remained limited. To overcome this limitation, research aimed to integrate all processes such as drying process optimization, metabolite profiling, and product development into a single system. HPLC/LC-MS/MS, DPPH, ABTS, FRAP, correlation analysis, and storage stability tests the most effective citrus fractions for the final product.

2.5 Research Gap

To characterization of citrus, bioactive metabolites, and antioxidant properties for analyze the citrus food nutrition product. The existing research reveals an improvement in bioactive compound utilization due to the fermentation process, but there is a loss of nutrients [21]. The boron compounds its nutritional function but rather on oxidative stress [24]. The metabolomics research findings were not applied to humans directly [25]. The proposed research overcomes these limitations through the integration of nutritional value, physicochemical properties, and antioxidants with the help of HPLC, LC-MS/MS, DPPH, ABTS, and FRAP to understand the relationship between metabolites and antioxidants.

3. Materials and Method

Determine the nutritional, physicochemical, bioactive metabolites, and antioxidant properties of some selected citrus fruits for use in functional nutrition products. The workflow of the materials and methods for the citrus-based functional nutrition products is illustrated in Figure 1. The fruits of pomelo, Cam Sánh, calamansi, kumquat, and mixed citrus fruits at a mature stage are collected, washed, dried, and converted to fine powder. The bioactive compounds extracted by the ultrasonic-assisted extraction method. HPLC coupled with tandem mass spectrometry is used to identify bioactive such as flavonoids and organic acids. The antioxidant activities are determined through ABTS, FRAP, and DPPH methods using SPSS.

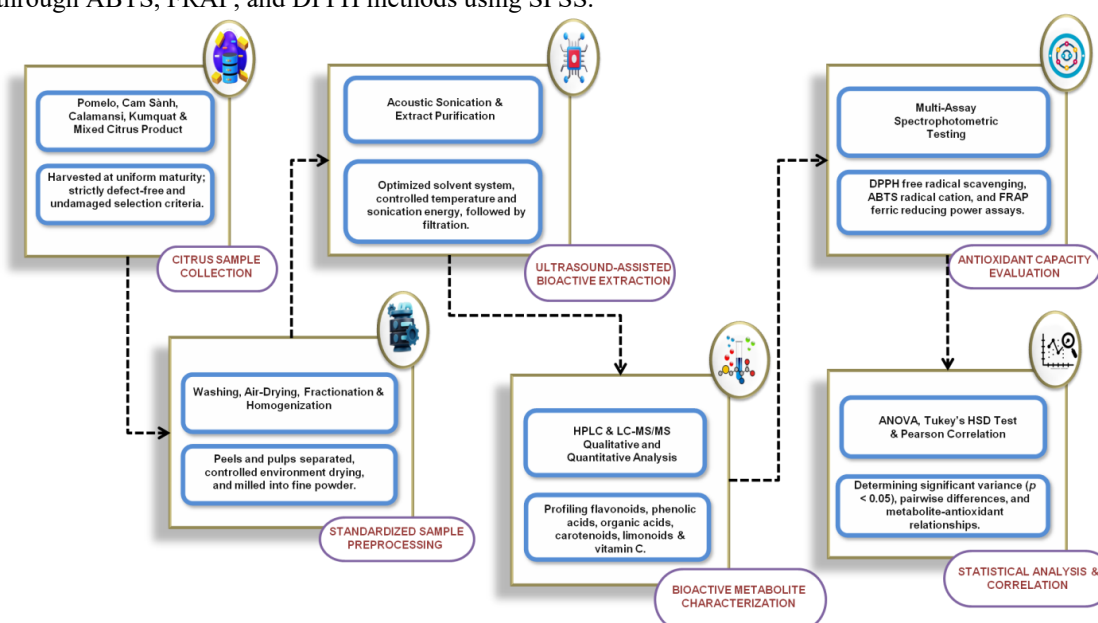


Figure 1: The workflow of the Citrus-Based Functional Nutrition Products

3.1 Citrus Sample Collection and Preparation

Collection of samples is intended for the identification of nutritional content, metabolites and antioxidants in the citrus fruit. Total number of fruits for each cultivar is 30 in good and healthy condition. Five healthy fruits pomelo, Cam Sánh, calamansi, kumquat, and mixed citrus fruits. Healthy mature fruits without any physical damage are collected from trusted sources to reduce variations due to fruit quality and handling. The selected citrus fruits

components are collected in adequate amounts for biochemical and chemical analysis of DPPH, ABTS, FRAP, as presented in Table 1. The harvested pomelo, Cam Sành, calamansi, kumquat, and citrus fruits carefully transported from their source to the laboratory to prevent any deterioration of the fruits. Upon reaching the laboratory, the fruits carefully inspected, and only those healthy and no mechanical damages or disease symptoms are considered for further analysis. Those fruits which same level of maturity in terms of size, color, and firmness are chosen. The selected fruits are carefully washed using fresh water to eliminate or contaminants present on the fruit surface. Afterwards, the fruits dried under controlled conditions. After the separation of the fruits into the respective fractions, namely, peel and pulp, each fraction cut into smaller pieces and homogenized.

Table 1: Sample Citrus-Based Fruit Collection for Functional Nutrition Products

Citrus Fruit	Scientific Name	Sample Collection Criteria
Pomelo	<i>Citrus maxima</i>	Mature, fresh, undamaged fruits
Cam Sành	<i>Citrus reticulata</i> × <i>C. sinensis</i>	Mature, healthy, uniform fruits
Calamansi	<i>Citrus</i> × <i>microcarpa</i>	Mature, fresh, disease-free fruits
Kumquat	<i>Citrus japonica</i>	Mature, firm, undamaged fruits
Mixed citrus fruits [Pomelo, Cam Sành, Calamansi, Kumquat]	Pomelo + Cam Sành + Calamansi + Kumquat	Standardized mixture prepared from selected citrus fruits

3.2 Standardized Citrus Sample Processing

The collected pomelo, Cam Sành, calamansi, kumquat, and mixed citrus fruits is undergone processing that is standard for getting the same level of homogeneity of samples among different citrus varieties and portions of the fruits. Fresh and mature fruits of pomelo (*Citrus maxima*), Cam Sành (*Citrus reticulata* × *C. sinensis*), calamansi (*Citrus* × *microcarpa*), and kumquat (*Citrus japonica*) harvested at uniform commercial maturity. A total of 30 healthy, undamaged fruits per individual cultivar collected to ensure sufficient biological representation. Maturity criteria based on standardized size, vibrant surface color, firmness, and total soluble solids.

A standardized mixed-citrus fruit sample prepared by combining equal weight proportions (1:1:1:1 w/w) of the four individual citrus fruits (incorporating their respective pulps and peels in equal ratios). The collected individual fruits and the mixed-citrus sample were transported to the laboratory under controlled temperature conditions (4°C), washed thoroughly with distilled water, and air-dried at room temperature. The peels and pulps were separated, cut into uniform thin slices, and dried in a hot-air oven at 5°C for 48 hours until a constant moisture level below 8.0% achieved. The dried fractions milled into a fine powder (passing through a 60-mesh sieve) using an analytical grinder, packed in vacuum-sealed polyethylene bags, and stored at 20°C until further extraction and analysis.

3.3 Ultrasonic-Assisted Bioactive Compound Extraction

The homogenization of the citrus fruits has done, ultrasonic assisted extraction is employed to isolate the bioactive compounds from the sample through the ideal solvent system. The sample and solvent are mixed, and the mixture by using the right combination of temperature and extraction time and ultrasonic energy. The extracts obtained can now be subjected to filtration/centrifugation to eliminate the solid particles. Extraction of bioactive compounds is done via UAE. In brief, 1.0 g of each sample of citrus powders (both individual and mixed citrus samples) suspended in 70% aqueous ethanol as extraction solvent (1:20 w/v sample-to-solvent ratio). The suspension subjected to ultrasonic bath with a constant frequency of 40 kHz and ultrasonic power of 250 W, kept at 45°C for 30 minutes. After extraction, the suspension centrifuged at 4,000 ×g for 15 minutes at 4°C, and then filtrated. The resulting clear extract collected, evaporated by reduced pressure, and stored at –20°C for further analysis.

3.4 Nutritional and Physicochemical Characterization

Nutritional and physiochemical characteristics of the chosen citrus fruits will be determined. The moisture, ash, protein, fat, carbohydrate, and dietary fiber levels will be determined in accordance with the standardized methods of analysis. In addition, physiochemical characteristics like pH, titratable acidity, and total soluble solids will be evaluated. Determination of vitamin C content will be done to assess nutritional qualities of each citrus fruit.

3.5 Phytochemical Characterization

These citrus specimens will be analyzed for the major phytochemical compounds contained therein. Phenol content and flavonoids content will be estimated using the relevant spectrophotometric tests. Carotenoids content and other pertinent phytochemical compounds will also be assessed using the relevant analysis techniques. The purpose of all this is to assess the overall phytochemical composition of these samples.

3.6 Characterization of Bioactive Metabolites

The citric extracts of pomelo, Cam Sành, calamansi, kumquat, and mixed citric fruits tested to discover and quantify the bioactive compounds that are predominant in the extracts. These bioactive compounds are separated and identified via HPLC and LC-MS/MS analysis methods. The metabolite profiles of each extract were then compared with the aim of finding out which compounds sufficient abundance to contribute to antioxidant activity.

- **High-Performance Liquid Chromatography (HPLC)**

HPLC is used to isolate, characterize, and quantify some selected bioactive compounds in extracts of pomelo, Cam Sánh, calamansi, kumquat, and mixed citrus fruits. Research involved profiling of flavonoids, phenolic acids, organic acids, vitamin C, and other important metabolites. The parameters for HPLC are optimized for characterization and quantification of the components. The outcomes from the HPLC method are compared various citrus fruits to assess any differences in the bioactive profile and fruits possessing desirable functionality. Flavonoids (hesperidin, naringin, eriocitrin), organic acids, and vitamin C (ascorbic acid) identified and quantified by HPLC analysis with DAD and reverse phase C18 column (column size: 250 × 4.6 mm), particle size: 5μ). A mobile phase containing 0.1% formic acid in water (solvent A) and acetonitrile (solvent B) used under gradient elution conditions with a flow rate of 1.0 mL/min. Flavonoids detected at 280 nm, while vitamin C detected at 254 nm.

- **Liquid Chromatography-Tandem Mass Spectrometry Method (LC-MS/MS)**

LC-MS/MS method is used for the identification and characterization of the active compounds present in the selected citrus samples. The method facilitated reliable detection of the chemicals by their chromatographic retention time and mass spectra, which included flavonoids, phenolic acids, limonoids, terpenoids, and others. The metabolite profiles of pomelo, Cam Sánh, calamansi, kumquat, and mixed citrus fruits are analyzed to identify characteristic metabolites and their relative amounts.

Characterization of the phenolic acids, limonoids, terpenoids, and carotenoids was achieved by means of Liquid LC-MS/MS utilizing the ESI source working in both positive and negative ionization modes. The quantification achieved through the use of calibration curves made up of authentic analytical standards. The method validation, including the LOD, LOQ, recovery (from 92 to 105%), and intra-/inter-day precision, demonstrated the reliability of the analytical methods used (table 2).

Table 2: Characterization of Bioactive Metabolites in Selected Citrus Samples

Metabolite	Compounds	Technique	Characterization
Flavonoids	Hesperidin, naringin, eriocitrin	HPLC, LC-MS/MS	Identification and quantification
Phenolic acids	Gallic, caffeic, ferulic acids	HPLC, LC-MS/MS	Phenolic profiling
Organic acids	Citric, malic, succinic acids	HPLC	Organic acid profiling
Carotenoids	β-Carotene, lutein, β-cryptoxanthin	HPLC, LC-MS/MS	Carotenoid characterization
Limonoids	Limonin, nomilin	LC-MS/MS	Identification and quantification
Terpenoids	Limonene and related compounds	LC-MS/MS	Terpenoid profiling
Vitamin C	Ascorbic acid	HPLC	Quantification
Other metabolites	Selected citrus metabolites	LC-MS/MS	metabolite profiling

3.7 Antioxidant Characterization for Antioxidant Evaluation

Pomelo, Cam Sánh, calamansi, kumquat, and mixed citrus fruit extracts tested for their antioxidant potential using multiple analyses. Each analysis evaluates antioxidant potential through a unique chemical process that gives a wider evaluation than one analysis. Using DPPH, ABTS, and FRAP together provides complementary information on radical-scavenging and reducing capacities. The exact quantity of citrus fruit (μg/mL) used to extract that has dried and dissolved in a solvent for conducting antioxidant experiments. The optical density measured by the spectrophotometer at a particular wavelength for measuring the reaction of the extract.

3.7.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH assay is performed to test the ability of the extract to scavenge free radicals. It involved the preparation of 4.0 mL of a methanolic solution containing 0.1 mM of DPPH and 0.1 mL of the citrus extract. This mixture incubated for 30 minutes at room temperature in the dark and its absorbance recorded at 517 nm using a blank of methanol. The antioxidants act as donors of hydrogen atoms or electrons, which leads to the reduction of DPPH radicals and consequently reduces absorbance, expressed in Equation (1).

$$\text{DPPH scavenging activity (\%)} = \frac{B_d - B_t}{B_d} \times 100 \quad (1)$$

B_d represents the absorbance of the control, and B_t refers to the absorbance of the citrus. The DPPH test measures the free radical scavenging capacity of the citrus samples, as reported in Table 3. High DPPH scavenging activity implies high radical neutralizing capacity of the samples. The mixed citrus fruits fraction achieves a higher 67.0% DPPH scavenging activity.

Table 3: DPPH Radical-Scavenging Activity of Selected Citrus Fruits

Citrus fruit	DPPH concentration (μg/mL)	Absorbance	DPPH scavenging activity (%)
Pomelo	100	0.420	58.0

Cam Sảnh	100	0.395	60.5
Calamansi	100	0.350	65.0
Kumquat	100	0.375	62.5
Mixed citrus fruits	100	0.330	67.0

3.7.2 2, 2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) Assay

The ABTS test measures the efficiency of the citrus extract's ability to scavenge ABTS radical cation. ABTS radical scavenging activity test is done using ABTS reagent stock solution (7 mM) that prepared using potassium persulfate (2.45 mM). The working solution then diluted with ethanol to give an absorbance value of 0.70 ± 0.02 at 734 nm. After addition of 0.1 mL of extract in 3.9 mL of ABTS working solution and 6 minute incubation in darkness, the absorbance at 734 nm was determined. Antioxidants react with the ABTS radicals in the solution to produce a reduction in blue-green ABTS radicals, which results in a decrease in absorbance, expressed in Equation (2).

$$\text{ABTS scavenging activity (\%)} = \frac{B_d - B_t}{B_d} \times 100 \quad (2)$$

B_d denotes the absorbance of the fruit control, and B_t refers to the absorbance of the extract citrus sample. Citrus antioxidants act to degrade the blue-green $\text{ABTS}^{\bullet+}$ radical cation, which causes a reduction in absorbance. A higher percentage of inhibition is associated with high ABTS scavenging ability under the given assay conditions, as computed in Table 4. The mixed citrus fruits are superior, with 71.0% ABTS scavenging activity.

Table 4: ABTS Radical-Scavenging Activity of Selected Citrus Fruits

Citrus fruit	Extract concentration ($\mu\text{g/mL}$)	Absorbance	ABTS scavenging activity (%)
Pomelo	100	0.365	63.5
Cam Sảnh	100	0.340	66.0
Calamansi	100	0.305	69.5
Kumquat	100	0.325	67.5
Mixed citrus fruits	100	0.290	71.0

3.7.3 Ferric Reducing Antioxidant Power (FRAP) Assay

The method of FRAP measures the reducing power of citrus extracts based on their reduction capability for ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}). The subsequent formation of color is measured spectroscopically and is an indicator of the electron donation potential of the antioxidants in the sample is discussed in Table 5. The mixed citrus fruits show a high 54.6 FRAP value ($\mu\text{mol Fe}^{2+}$ equivalents/g). FRAP of samples was assayed by adding 0.1 mL of extract to 3.0 mL of freshly made FRAP reagent consisting of acetate buffer, TPTZ solution, and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. The reaction incubated for 10 min at 37°C . The results were expressed in terms of $\mu\text{mol Fe}^{2+}$ equivalents/g of dry weight by comparison with the ferrous sulfate standard curve. The experiments were carried out in triplicates using three different biological samples ($n = 3$).

Table 5: Ferric Reducing Antioxidant Power (FRAP) of Selected Citrus Fruits

Citrus fruit	Extract concentration ($\mu\text{g/mL}$)	Absorbance at 593 nm	FRAP value ($\mu\text{mol Fe}^{2+}$ equivalents/g)
Pomelo	100	0.385	42.6
Cam Sảnh	100	0.410	45.3
Calamansi	100	0.465	51.8
Kumquat	100	0.435	48.2
Mixed citrus fruits	100	0.490	54.6

The antioxidants in the citrus juices convert Fe^{3+} -TPTZ to the blue complex of Fe^{2+} -TPTZ, whose concentration is determined spectrophotometrically at about 593 nm. The greater the FRAP value, the higher the ferric-reducing antioxidant power.

3.8 Statistical Analysis

The statistical analysis conducted to investigate any significant differences and correlations between the selected samples of citrus fruits using SPSS 29.0.1.1. ANOVA was employed to examine nutritional composition, content of bioactive metabolites, and antioxidant activity of pomelo, Cam Sảnh, calamansi, kumquat, and mixed citrus fruits. Tukey's post hoc test indicated which groups significantly different values, all p-values <0.05 . Pearson correlation analysis employed to the correlation between individual bioactive metabolites and antioxidant activity, thus helping identify metabolites responsible for functional antioxidant activities. Results are interpreted to select promising citrus materials for product development.

3.8.1 Analysis of Variance (ANOVA) for Citrus-Based Fruit Nutritional Composition

ANOVA whether there significant difference for the nutritional factors of pomelo, Cam Sảnh, calamansi, kumquat, and mixed citrus fruits. ANOVA is a statistical test used to compare means of more than two groups. It tests differences in moisture content, protein content, fat content, ash content, fibre content, carbohydrate content,

and vitamin C. Nutritional analysis was carried out again in replicates using the fruit samples, as computed in Equation (3). Three independent biological replicates were analyzed for each citrus sample, and each analytical measurement was performed in triplicate

$$Z_{ji} = \mu + \tau_j + \epsilon_{ji} \quad (3)$$

Z_{ji} represents the nutritional content observed for the i^{th} replication of the j^{th} citrus sample. μ denotes the mean nutritional content for all the citruses, including pomelo, Cam Sành, calamansi, kumquat, and mixed citrus. τ_j indicates the factor effect on nutritional content due to the j^{th} citrus sample. ϵ_{ji} refers to the error due to experimentation for the i^{th} replication of the j^{th} citrus sample. j represents the category of the citrus sample: pomelo, Cam Sành, calamansi, kumquat, or mixed citrus. i stands for the number of replications in the j^{th} citrus sample.

3.8.2 Tukey's Test for the Individual Citrus-Based Fruit Comparison

Tukey's test is used to determine significant differences between citrus-based fruits after conducting an ANOVA. A post-hoc statistical test used to make multiple comparisons of pairs of means. It is used to compare parameters of nutritional value, physicochemical properties, bioactive metabolites, and antioxidants of pomelo, Cam Sành, calamansi, kumquat, and mixed citrus fruits. Repetitive experimental measures of each property identify differences in means, confidence intervals, and p-values for pairwise comparison of significantly different citrus-based fruits, as expressed in Equation (4).

$$\text{HSD} = r \times \sqrt{\frac{\text{MSE}}{m}} \quad (4)$$

HSD represents Tukey's Honestly Significant Difference test used to there is a significant difference between the means of two citrus samples. r refers to the studentized range critical value that depends on the number of citrus groups and degrees of freedom of error. $\sqrt{\frac{\text{MSE}}{m}}$ stands for the Mean Square Error is calculated using ANOVA, and it shows within-group variability. m indicates the number of replicates for each citrus sample mean.

3.8.3 Pearson Correlation for Evaluating Metabolite–Antioxidant Relationships

Pearson correlation is used to identify correlations among metabolites and antioxidant activities of pomelo, Cam Sành, calamansi, kumquat, and mixed citrus fruits. Pearson correlation is an analytical tool used to determine the strength of the relationship between two variables. It helps determine whether there is relationship between the levels of metabolites and DPPH, ABTS, and FRAP activities. Quantitative measures of metabolites and antioxidants. Correlation coefficients and p-values help to detect significant positive and negative correlations; this process is expressed in Equation (5).

$$r = \frac{\sum (Y_j - \bar{Y})(X_j - \bar{X})}{\sqrt{\sum_j^m (Y_j - \bar{Y})^2 + \sum_j^m (X_j - \bar{X})^2}} \quad (5)$$

r represents the correlation coefficient, which measures the strength and direction of the relationship between a bioactive metabolite and antioxidant capacity. X_j refers to the concentration of the selected bioactive metabolite in the j^{th} citrus sample. Y_j denotes the antioxidant capacity measured in terms of DPPH, ABTS, or FRAP corresponding to the j^{th} observation. $\bar{X}$ indicates the average concentration of the selected bioactive metabolite. $\bar{Y}$ stands for the average antioxidant capacity. $\sum_j^m$ represents the replicate j number for the overall observations, withmpaired observations for the correlation calculation.

3.9 Selection of Bioactive Citrus Fraction

The optimal citrus material shall be chosen considering a number of criteria including bioactive metabolites content, total phenolics and flavonoids concentration, vitamin C levels, and antioxidant and nutritional qualities. Data received in respect of each sample of the citrus material will be compared in order to determine the optimal material which will then be taken into account when creating functional nutrition products.

3.10 Nutrition Product Development and Product Characterization

The chosen citrus fraction will be included into an appropriate functional nutrition food product. The process of formulation will involve determining the right amounts of the chosen citrus fraction along with other components of the product. Then, prototype formulations will be made using standardized processing conditions. The formulation will be optimized with regard to nutritional properties, physical and chemical properties, bioactivity and antioxidant property of the product.

Characterization of the developed functional nutrition product will be carried out to assess its nutritional content, physiochemical, phytochemical, and antioxidant attributes. The nutritional composition, pH value, titratable acidity, total soluble solids, and vitamin C content will be determined. Total phenolic and flavonoids content, bioactive compounds, and antioxidants will also be assessed. Sensory attributes may also be assessed for the product acceptability.

The stability of the developed nutritional product in terms of storage is going to be examined throughout the storage time period. The variations in the levels of active metabolites, vitamin C, phenols, flavonoids, antioxidant capacity, pH value, titratable acidity, and sensory characteristics will be analyzed after set periods of time. The results obtained from such analysis will help us to determine stability and acceptability of the product during storage.

4 RESULTS

The results showed unique nutritional, physicochemical, bioactive metabolite, and antioxidant properties of pomelo, Cam Sảnh, calamansi, kumquat, and mixed citrus fruit. There were highly significant differences in the physicochemical and antioxidant characteristics the tested citrus fruits ($p < 0.05$). The mixed citrus fruits showed the highest antioxidant properties, with values of 67.0% and 71.0% for scavenging radicals in DPPH and ABTS tests, respectively, and 54.6 $\mu\text{mol Fe}^{2+}$ equivalents/g in the FRAP test. Pearson correlation test further confirmed that the high antioxidant activities of the tested samples were significantly related to some of their bioactive constituents, especially ascorbic acid ($r = 0.88$ for DPPH) and eriocitrin.

4.6 ANOVA Result for Nutritional Composition of Selected Citrus Samples

ANOVA to examine the nutritional composition of pomelo, Cam Sảnh, calamansi, kumquat, and the mixed citrus fruits analyzed in Table 6 and Figure 2. Differences in the values of moisture, protein, fat, ash, dietary fibre, carbohydrate, and vitamin C were found, with all p-values less than 0.05. The value of F for vitamin C is the highest at 12.56, which implies that there is a great difference in the nutritional composition of the citrus fruits. Tukey's post hoc analysis was performed to detect significant differences between of samples.

Table 6: ANOVA Results for Citrus-Based Fruit Nutritional Composition

Nutritional Parameter	F-value	p-value	Significance	Tukey's Test
Moisture (%)	8.42	0.004	Significant	$p < 0.05$
Protein (%)	5.76	0.012		
Fat (%)	4.38	0.026		
Ash (%)	3.91	0.038		
Dietary fibre (%)	7.25	0.006		
Carbohydrate (%)	6.84	0.008		
Vitamin C (mg/100 g)	12.56	<0.001		

Note: F-value is the ratio of the between-group variability and within-group variability. If the p-value is < 0.05 , then the pomelo, Cam Sảnh, calamansi, kumquat, and mixed citrus fruits show a statistically significant difference. This test is conducted using Tukey's post-hoc test. The asterisks (*, **, ***) denote the level of statistical significance according to p-value ($p < 0.05$, $p < 0.01$, $p < 0.001$).

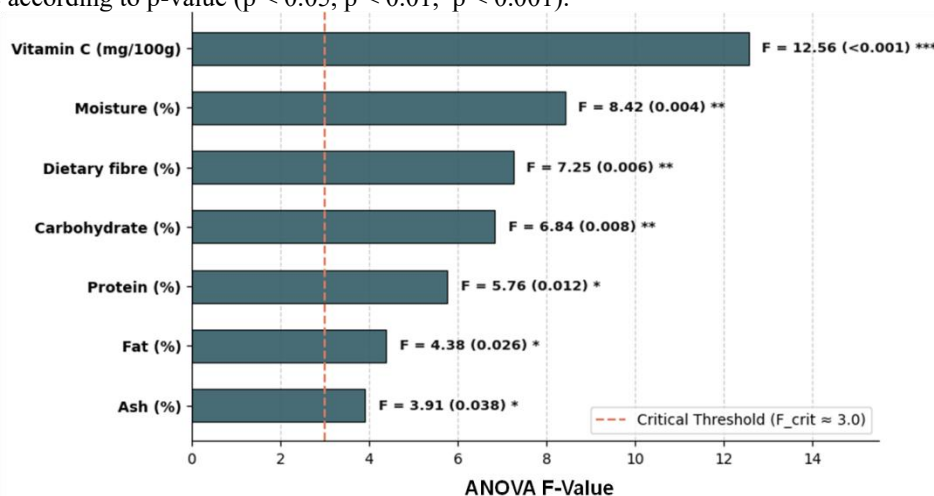


Figure 2: The Result of ANOVA for the Nutritional Composition of Citrus-Based Products

Physicochemical characteristics of the chosen citrus fruits displayed a wide variation between different cultivars due to differences in organic acid contents and maturity. The properties of physicochemical properties presented in Table 7. Calamansi had the lowest pH value (2.45) and highest titratable acidity (4.60%) while kumquat had the highest soluble solids content (13.50° Brix). Low moisture contents and water activities recorded for all powder samples, which ensured proper storage potential.

Table 7: Physicochemical Properties of Selected Citrus Fruits

Citrus Fruit	pH	Titratable Acidity (%)	Soluble Solids (°Brix)	Moisture (%)	Water Activity (a_w)	Color (L^* , a^* , b^*)
Pomelo	3.45 $\pm$ 0.12	1.15 $\pm$ 0.08	9.20 $\pm$ 0.35	7.50 $\pm$ 0.20	0.42 $\pm$ 0.01	68.5, 2.1, 35.4
Cam Sảnh	3.20 $\pm$ 0.10	1.85 $\pm$ 0.12	11.40 $\pm$ 0.40	6.80 $\pm$ 0.15	0.38 $\pm$ 0.02	55.2, 18.4, 48.1
Calamansi	2.45 $\pm$ 0.08	4.60 $\pm$ 0.15	8.10 $\pm$ 0.25	6.20 $\pm$ 0.18	0.35 $\pm$ 0.01	50.8, -4.2, 42.6
Kumquat	3.10 $\pm$ 0.09	2.40 $\pm$ 0.10	13.50 $\pm$ 0.50	7.10 $\pm$ 0.22	0.40 $\pm$ 0.02	60.4, 14.5, 52.3

Mixed citrus fruits	3.05 ± 0.11	2.50 ± 0.14	10.55 ± 0.38	6.90 ± 0.19	0.39 ± 0.01	58.7, 10.1, 44.8
---------------------	-------------	-------------	--------------	-------------	-------------	------------------

Note: Color values are reported as L*, a*, and b* coordinates. The water-activity unit is dimensionless.

4.7 Tukey's Test Result for Nutritional, Bioactive Metabolite, and Antioxidant Characterization

The post-hoc Tukey's comparisons of nutritional attributes between pomelo, Cam Sành, calamansi, kumquat, and mixed citrus are presented in Table 8. The significant difference ($p < 0.05$) in most of the pairwise comparisons in moisture, dietary fibre, carbohydrate, and vitamin C; however, some comparisons, such as Cam Sành and kumquat with regard to some nutritional attributes, did not show any significant difference ($p > 0.05$). Vitamin C showed significant differences in all pairwise comparisons, suggesting variability the citrus Samples.

Table 8: Tukey's Test Results for Individual Citrus-Based Samples Comparison

Nutritional Parameter	Pomelo vs. Cam Sành	Pomelo vs. Calamansi	Pomelo vs. Kumquat	Pomelo vs. Mixed Product	Cam Sành vs. Calamansi	Cam Sành vs. Kumquat	Cam Sành vs. Mixed Product	Calamansi vs. Kumquat	Calamansi vs. Mixed Product	Kumquat vs. Mixed Product
Moisture (%)	0.032*	0.004*	0.018*	0.011*	0.041*	0.215	0.026*	0.028*	0.019*	0.034*
Protein (%)	0.146	0.021*	0.038*	0.016*	0.016*	0.284	0.031*	0.047*	0.024*	0.042*
Fat (%)	0.318	0.027*	0.064	0.045*	0.019*	0.176	0.038*	0.083	0.052	0.071
Ash (%)	0.086	0.012*	0.031*	0.018*	0.044*	0.263	0.029*	0.072	0.035*	0.048*
Dietary Fibre (%)	0.024*	0.006*	0.039*	0.008*	0.018*	0.326	0.021*	0.045*	0.013*	0.037*
Carbohydrate (%)	0.051	0.009*	0.028*	0.014*	0.022*	0.187	0.027*	0.036*	0.018*	0.041*
Vitamin C (mg/100 g)	0.018*	<0.001*	0.006*	<0.001*	0.012*	0.034*	0.008*	0.026*	0.004*	0.019*

Note: p-values shown are those obtained after correction for multiple comparisons using Tukey's test. * indicates the statistical significance at $p < 0.05$.

4.8 Pearson Correlation Result between Citrus Metabolites and Antioxidant Activities

The relationship between bioactive compounds and the antioxidant is reported in Table 9. The analyzed antioxidants revealed a strong positive correlation between ascorbic acid and DPPH, ABTS, and FRAP assays, with correlation coefficients equal to $r = 0.88, 0.85,$ and 0.81 , correspondingly ($p < 0.001$). The positive and statistically significant correlations observed in naringin, gallic acid, caffeic acid, and ferulic acid, but correlation coefficients differed between the tested compounds. As for limonin, a moderately positive correlation revealed concerning DPPH, ABTS, and FRAPs assays, with correlation coefficients being equal to $0.52, 0.48,$ and 0.55 , correspondingly.

Table 9: Pearson Correlation Results for Evaluating Metabolite–Antioxidant Relationships

Control Variable	Hesperidin	Naringin	Eriocitrin	Gallic acid	Caffeic acid	Ferulic acid	Ascorbic acid	Limonin
DPPH – Pearson correlation (r)	0.82**	0.74**	0.86**	0.77**	0.69*	0.65*	0.88**	0.52
DPPH – Sig. (2-tailed), p	0.002	0.005	<0.001	0.003	0.015	0.024	<0.001	0.085
DPPH – N	15	15	15	15	15	15	15	15
ABTS – Pearson correlation (r)	0.79**	0.71**	0.83**	0.75**	0.73**	0.67*	0.85**	0.48
ABTS – Sig. (2-tailed), p	0.003	0.008	<0.001	0.002	0.004	0.018	<0.001	0.112
ABTS – N	15	15	15	15	15	15	15	15

FRAP – Pearson correlation (r)	0.76**	0.69*	0.80**	0.72**	0.68*	0.64*	0.81**	0.55
FRAP – Sig. (2-tailed), p	0.005	0.014	0.001	0.006	0.021	0.030	<0.001	0.065
FRAP – N	15	15	15	15	15	15	15	15

Note: $p < 0.05$ (*), $p < 0.01$ (**). DPPH, ABTS, and FRAP represent antioxidant activity assays. Pearson's correlation coefficient (r) indicates the strength and direction of the linear association between each bioactive compound and the corresponding antioxidant activity measure. N = sample size.

4.9 DISCUSSION

Properties of chosen citrus fruits nutritional, physicochemical, metabolite, and antioxidant play an essential role in the creation of functional nutrition products based on citrus fruits. At that, the above-mentioned researches have certain flaws: research [19] is limited only to comparative analysis of individual bioactive metabolites and antioxidants which does not allow obtaining a full understanding of sample interactions. Besides, few comparative on various cultivars do not make it possible to evaluate thoroughly the bioactivity and antioxidant properties of each particular cultivar [20]. Some other challenges are connected with the lack of validation related to processing conditions and translation of phytochemistry results into practice of functional nutrition products development [29], and there are also some safety evaluations of developed processes and their applications to functional products [32]. **Implications:** The described research successfully resolves the aforementioned issues through a systematic analysis of pomelo, Cam Sành, calamansi, kumquat, and mixed citrus fruits using standardized sample preparation procedure, HPLC, LC-MS/MS, and DPPH, ABTS, FRAP tests and ANOVA, Tukey's test, Pearson correlation analysis.

5 CONCLUSION

The proposed research characterized pomelo, Cam Sành, calamansi, kumquat, and mixed citrus fruits in terms of their nutritional, physicochemical, bioactive, and antioxidant properties. Standardized collection, preparation, processing, and ultrasound-assisted extraction are used to the analysis. The antioxidant tested using FRAP, DPPH, and ABTS assays, which the mixed citrus sample showed the highest values of 54.6%, 67.0%, and 71.0 $\mu\text{mol Fe}^{2+}$ equivalents/g, respectively. ANOVA test results the existence of significant differences between the samples ($p < 0.05$). Tukey's test is used for further analysis of differences between the samples. The Pearson correlation coefficient showed a high correlation between the chosen bioactive compounds and their antioxidant activities (ascorbic acid, $r = 0.88$). The anti-oxidative nature of the mixed citrus fruits prompted their choice for functional nutrition products. **Limitation and Future Scope:** But there is a limitation that research is carried out on certain citrus fruit samples, evaluation parameters, and techniques to determine the antioxidants. Any change in production, ripening, processing, and storage process change the bioactivity profile of these citrus fruits. Further study will confirm these results through more samples and other processes such as metabolomics, sensory, bioavailability, and clinical trials.

REFERENCES

- Pieracci, Y., Pistelli, L., Cecchi, M., Pistelli, L. and De Leo, M., 2022. Phytochemical characterization of citrus-based products supporting their antioxidant effect and sensory quality. *Foods*, 11(11), p.1550. <https://doi.org/10.3390/foods11111550>
- Peng, M., Gao, Z., Liao, Y., Guo, J. and Shan, Y., 2022. Development of citrus-based functional jelly and an investigation of its anti-obesity and antioxidant properties. *Antioxidants*, 11(12), p.2418. <https://doi.org/10.3390/antiox11122418>
- Guo, H., Zheng, Y.J., Wu, D.T., Du, X., Gao, H., Ayyash, M., Zeng, D.G., Li, H.B., Liu, H.Y. and Gan, R.Y., 2023. Quality evaluation of citrus varieties based on phytochemical profiles and nutritional properties. *Frontiers in Nutrition*, 10, p.1165841. <https://doi.org/10.3389/fnut.2023.1165841>
- Li, Q., Yao, J., Zheng, W., Wang, J., Liao, L., Sun, G., Wang, X., Deng, H., Zhang, M., Wang, Z. and Xiong, B., 2023. Hetero-grafting affects flavonoid biosynthesis in sweet orange 'Newhall' (Citrus sinensis) peels: a metabolomics and transcriptomics analysis. *Frontiers in Plant Science*, 14, p.1218426. <https://doi.org/10.3389/fpls.2023.1218426>
- Kumar, D., Ladaniya, M.S., Gurjar, M., Mendke, S., Kumar, S. and Ghosh, D., 2023. Elucidation of flavanones, phenols and antioxidant capacity influenced by drying methods from physiologically dropped underutilized Citrus grandis fruits. *Frontiers in Plant Science*, 14, p.1193635. <https://doi.org/10.3389/fpls.2023.1193635>
- Zeng, J., Chen, C., Chen, M. and Chen, J., 2022. Comparative transcriptomic and metabolomic analyses reveal the delaying effect of naringin on postharvest decay in citrus fruit. *Frontiers in Plant Science*, 13, p.1045857. <https://doi.org/10.3389/fpls.2022.1045857>
- Cao, X., Ru, S., Fang, X., Li, Y., Wang, T. and Lyu, X., 2022. Effects of alcoholic fermentation on the non-volatile and volatile compounds in grapefruit (Citrus paradisi Mac. cv. Cocktail) juice: A combination of UPLC-

- MS/MS and gas chromatography ion mobility spectrometry analysis. *Frontiers in Nutrition*, 9, p.1015924.<https://doi.org/10.3389/fnut.2022.1015924>
8. Cesar, T., Oliveira, M.R., Sandrim, V., Mendes, A., Bruder, R., Oliveira, R., Sivieri, K. and Milenkovic, D., 2025. Citrus flavonoid supplement enhances glycemic and metabolic control in prediabetic patients on metformin: a randomized controlled trial. *Frontiers in Nutrition*, 12, p.1639901.<https://doi.org/10.3389/fnut.2025.1639901>
 9. Stabrauskiene, J., Marksa, M., Ivanauskas, L., Viskelis, P., Viskelis, J. and Bernatoniene, J., 2023. Citrus× paradisi L. fruit waste: The impact of eco-friendly extraction techniques on the phytochemical and antioxidant potential. *Nutrients*, 15(5), p.1276.<https://doi.org/10.3390/nu15051276>
 10. Raji, A.O., Adebayo, O.F. and Sanusi, S.M., 2022. Antioxidant potentials and storage stability of pasteurised mixed fruit juices from pineapple and bitter orange. *Food Bioscience*, 49, p.101937.<https://doi.org/10.1016/j.fbio.2022.101937>
 11. Kumar, D., Ladaniya, M.S., Gurjar, M. and Kumar, S., 2022. Impact of drying methods on natural antioxidants, phenols and flavanones of immature dropped Citrus sinensis L. Osbeck fruits. *Scientific Reports*, 12(1), p.6684.<https://doi.org/10.1038/s41598-022-10661-7>
 12. Hussain, T., Kalhor, D.H. and Yin, Y., 2023. Identification of nutritional composition and antioxidant activities of fruit peels as a potential source of nutraceuticals. *Frontiers in Nutrition*, 9, p.1065698.<https://doi.org/10.3389/fnut.2022.1065698>
 13. Yang, M., Jiang, Z., Wen, M., Wu, Z., Zha, M., Xu, W. and Zhang, L., 2022. Chemical variation of Chenpi (Citrus peels) and corresponding correlated bioactive compounds by LC-MS metabolomics and multibioassay analysis. *Frontiers in Nutrition*, 9, p.825381.<https://doi.org/10.3389/fnut.2022.825381>
 14. Quan, Q., Liu, W., Guo, J., Ye, M. and Zhang, J., 2022. Effect of six lactic acid bacteria strains on physicochemical characteristics, antioxidant activities and sensory properties of fermented orange juices. *Foods*, 11(13), p.1920.<https://doi.org/10.3390/foods11131920>
 15. Pan, T., Kong, L., Zhang, X., Wang, Y., Zhou, J., Fu, Z., Pan, H., She, W. and Yu, Y., 2023. Fruit quality and volatile constituents of a new very early-ripening pummelo (Citrus maxima) cultivar 'Liuyuezao'. *Frontiers in Plant Science*, 13, p.1089009.<https://doi.org/10.3389/fpls.2022.1089009>
 16. Owoyemi, A., Porat, R., Lichter, A., Doron-Faigenboim, A., Jovani, O., Koenigstein, N. and Salzer, Y., 2022. Large-scale, high-throughput phenotyping of the postharvest storage performance of 'rustenburg' navel oranges and the development of shelf-life prediction models. *Foods*, 11(13), p.1840.<https://doi.org/10.3390/foods11131840>
 17. Li, C., Wang, S., Wang, J., Wu, Z., Xu, Y. and Wu, Z., 2022. Ozone treatment promotes physicochemical properties and antioxidant capacity of fresh-cut red pitaya based on phenolic metabolism. *Frontiers in Nutrition*, 9, p.1016607.<https://doi.org/10.3389/fnut.2022.1016607>
 18. Jiao, Y., Zhang, S., Jin, H., Wang, Y., Jia, Y., Zhang, H., Jiang, Y., Liao, W., Chen, L.S. and Guo, J., 2023. Fruit quality assessment based on mineral elements and juice properties in nine citrus cultivars. *Frontiers in Plant Science*, 14, p.1280495.<https://doi.org/10.3389/fpls.2023.1280495>
 19. Yang, R., Yang, Y., Hu, Y., Yin, L., Qu, P., Wang, P., Mu, X., Zhang, S., Xie, P., Cheng, C. and Zhang, J., 2023. Comparison of bioactive compounds and antioxidant activities in differentially pigmented Cerasus humilis fruits. *Molecules*, 28(17), p.6272.<https://doi.org/10.3390/molecules28176272>
 20. Li, S., Yang, L., Wang, M., Chen, Y., Yu, J., Chen, H., Yang, H., Wang, W., Cai, Z. and Hong, L., 2024. Effects of rootstocks and developmental time on the dynamic changes of main functional substances in 'Orah'(Citrus reticulata Blanco) by HPLC coupled with UV detection. *Frontiers in Plant Science*, 15, p.1382768.<https://doi.org/10.3389/fpls.2024.1382768>
 21. Li, Y., Guo, L., Mao, X., Ji, C., Li, W. and Zhou, Z., 2024. Changes in the nutritional, flavor, and phytochemical properties of Citrus reticulata Blanco cv.' Dahongpao' whole fruits during enzymatic hydrolysis and fermentation. *Frontiers in Sustainable Food Systems*, 8, p.1474760.<https://doi.org/10.3389/fsufs.2024.1474760>
 22. Vinas-Ospino, A., Jesus, A.R., Paiva, A., Esteve, M.J., Frígola, A., Blesa, J. and López-Malo, D., 2024. Comparison of green solvents for the revalorization of orange by-products: Carotenoid extraction and in vitro antioxidant activity. *Food Chemistry*, 442, p.138530.<https://doi.org/10.1016/j.foodchem.2024.138530>
 23. Huang, M., Lai, C., Liang, Y., Xiong, Q., Chen, C., Ju, Z., Jiang, Y. and Zhang, J., 2025. Improving the functional components and biological activities of navel orange juice through fermentation with an autochthonous strain, *Lactiplantibacillus paraplantarum* M23. *Food and Bioprocess Technology*, 149, pp.249-260.<https://doi.org/10.1016/j.fbp.2024.11.027>
 24. Chen, X.F., Chen, H.H., Huang, W.L., Huang, W.T., Huang, Z.R., Yang, L.T., Ye, X. and Chen, L.S., 2024. Boron reduced copper excess-induced oxidative damage in Citrus sinensis by modulating reactive oxygen species and methylglyoxal formation and their detoxification systems. *Antioxidants*, 13(3), p.268.<https://doi.org/10.3390/antiox13030268>
 25. Cao, X., Guo, X., Fang, X., Ru, S. and Li, E., 2022. Effects of poncirin, a citrus flavonoid and its aglycone, isosakuranetin, on the gut microbial diversity and metabolomics in mice. *Molecules*, 27(11), p.3641.<https://doi.org/10.3390/molecules27113641>
 26. García-Gómez, N., Pérez-Vásquez, R., Pasquel-Reátegui, J.L., Coronado-Jorge, M.F., Navarro-Ramírez, E., Documet-Petrlik, K.G., Vidaurre-Rojas, P., Sánchez-Dávila, K. and Cárdenas-García, Á., 2026. Citrus waste as a

source of high-value compounds: effect of solvent system and extraction time on bioactive compound recovery. *Recycling*, 11(4), p.77. <https://doi.org/10.3390/recycling11040077>

27. Wang, T., Sun, G., He, S., Liao, L., Xiong, B. and Wang, Z., 2025. Effect of Different Interstocks on Fruit Quality, Amino Acids, and Antioxidant Capacity in ‘Yuanxiaochun’ Citrus. *Antioxidants*, 14(10), p.1149. <https://doi.org/10.3390/antiox14101149>

28. Smeriglio, A., La Neve, A., Mangano, M., Imbesi, M., Cornara, L. and Trombetta, D., 2025. Rediscovering *Citrus lumia* ‘Pyriformis’: Phytochemical Profile and Multifunctional Properties of Its Fresh Juice. *Foods*, 14(23), p.3997. <https://doi.org/10.3390/foods14233997>

29. Tao, M., Zhang, P., Wang, T., Zheng, D., Xia, Z., Zhang, X., Li, Y., Zhou, Y. and Peng, X., 2026. Identification of key quality indicators for Zigui navel orange varieties through multivariate analyses. *Food Chemistry: X*, p.103702. <https://doi.org/10.1016/j.fochx.2026.103702>

30. Alamoudi, S.A., Saad, A.M., Alsubhi, N.H., Alrefaei, G.I., Al-Quwaie, D.A., Binothman, N., Aljadani, M., Alharbi, M., Alanazi, H., Babalghith, A.O. and Almuhayawi, M.S., 2022. Upgrading the physicochemical and sensory quality of yogurt by incorporating polyphenol-enriched citrus pomaces with antioxidant, antimicrobial, and antitumor activities. *Frontiers in Nutrition*, 9, p.999581. <https://doi.org/10.3389/fnut.2022.999581>

31. Fratianni, F., Amato, G., d’Acerno, A., Ombra, M.N., De Feo, V., Coppola, R. and Nazzaro, F., 2023. In vitro prospective healthy and nutritional benefits of different Citrus monofloral honeys. *Scientific Reports*, 13(1), p.1088. <https://doi.org/10.1038/s41598-023-27802-1>

32. Abdel Samad, R., El Darra, N., Al Khatib, A., Chacra, H.A., Jammoul, A. and Raafat, K., 2023. Novel dual-function GC/MS aided ultrasound-assisted hydrodistillation for the valorization of *Citrus sinensis* by-products: phytochemical analysis and antibacterial activities. *Scientific Reports*, 13(1), p.12547. <https://doi.org/10.1038/s41598-023-38130-9>

33. Li, M., Qin, J., Zhong, B., Hao, F. and Wu, Z., 2023. Improving acidity and flavors of citrus juice as well as its antioxidant activity by cofermentation with a combination of deacidification bacteria. *Food Bioscience*, 53, p.102592. <https://doi.org/10.1016/j.fbio.2023.102592>

34. Jungen, M., Dragičević, N., Rodriguez-Werner, M., Schmidt, S., Dinis, K., Tsamba, L., Jamin, E., Fiedler, T., Fischbach, N., Steingass, C.B. and Camel, V., 2023. A pragmatic authenticity assessment of lemon (*Citrus limon* [L.] Burm. f.) juices by its profile of coumarins, psoralens, and polymethoxyflavones. *Food Control*, 146, p.109529. <https://doi.org/10.1016/j.foodcont.2022.109529>

35. Zhu, Y., Tian, X., Wang, C., Cai, J., Feng, Z., Zhu, J. and Bai, J., 2025. Multi-index analysis and comprehensive evaluation of different drying techniques for citrus peels based on the entropy weight method. *Agriculture*, 15(23), p.2433. <https://doi.org/10.3390/agriculture15232433>

Abbreviation	Full Term
ABTS	2,2'-Azino-bis(3-Ethylbenzothiazoline-6-Sulfonic Acid)
ANOVA	Analysis of Variance
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
GC/FID	Gas Chromatography–Flame Ionization Detection
GC/MS	GC–Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
HPLC-DAD	HPLC-Diode-Array Detection
HPLC-UV	HPLC-Ultraviolet Detection
HSD	Honestly Significant Difference
IRD	Infrared Detector
LC-MS	Liquid Chromatography–Mass Spectrometry
LC-MS/MS	LC–Tandem Mass Spectrometry
mg GAE/g	Milligrams of Gallic Acid Equivalents per gram
mg/g FW	mg-per gram of Fresh Weight
MICD	Minimum Inhibitory Concentration Determination
MSE	Mean Square Error
PC / PCA	Principal Component / Principal Component Analysis
SPSS	Statistical Package for the Social Sciences
16S rRNA	16S Ribosomal Ribonucleic Acid
µg/g	Micrograms per gram
µM TE/mL	Micromoles of Trolox Equivalents per millilitre
DAD	Diode-Array Detector
ESI	Electrospray Ionization
LOD	Limits of Detection
LOQ	Limits of Quantification