

## Nutritional composition and antioxidant properties of calamansi (*Citrus microcarpa*) peels in different drying processes

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**Abstract.** Abal KET, Choresca Jr. CH, Fagutao FF, Anuevo G, Apines-Amar MJS, Catienza FA, Pedroso FL. 2024. Nutritional composition and antioxidant properties of calamansi (*Citrus microcarpa*) peels in different drying processes. *Nusantara Bioscience* 16: 292-296. Calamansi (*Citrus microcarpa* Bunge) is abundantly grown in the Philippines and is primarily utilized for its juice, resulting in peels as a waste product. This study aimed to evaluate the effects of different drying techniques (sun-drying, dehydration, and oven-drying) on the nutritional composition and antioxidant properties of calamansi peels. The dried samples were analyzed for nutritional content such as protein, fiber, fat, ash, carbohydrates, and moisture. Antioxidant properties were also measured through a DPPH scavenging activity assay, including total phenolic content and total flavonoid content. The results showed that Calamansi Dehydrated (CDD) had a significantly higher protein and fiber content than oven-dried and sundried, and calamansi oven-dried (COD) peels had the highest carbohydrate, fat, moisture, and ash content. Calamansi Sundried (CSD) has a significantly highest value of 21.04±2.44% scavenging activity, followed by CDD (20.79±5.43% scavenging activity), and then COD (8.91±1.89% scavenging activity) in DPPH assay. For Total Phenolic Content (TPC), CDD has a significantly higher value of 192.6±21.99 mg gallic acid equivalent (GAE) L<sup>-1</sup> dry sample, followed by CSD and COD, with 145.7±6.54 mg GAE L<sup>-1</sup> dry sample and 70.7±8.87 mg GAE L<sup>-1</sup> dry sample, respectively. CSD had the highest value in Total Flavonoid Content (TFC) of 163.3±1.90 mg catechin equivalent (CE L<sup>-1</sup> dry sample) mg catechin Equivalent (CE) L<sup>-1</sup> dry sample, followed by COD (121.0±6.87 mg CE L<sup>-1</sup> sample), and CDD (41.9±6.25 mg CE L<sup>-1</sup> sample). The findings suggest sun-drying and dehydration as drying techniques resulted in higher antioxidant properties and economic viability.

**Keywords:** Antioxidant properties, calamansi, *Citrus microcarpa*, drying techniques, nutritional composition

**Abbreviation:** CDD: Calamansi Dehydrated, CE: Catechin Equivalent, COD: Calamansi Oven-Dried, CSD: Calamansi Sundried, GAE: Gallic Acid Equivalent, DPPH: 2,2-diphenyl-1-picrylhydrazyl, TFC: Total Flavonoid Content, TPC: Total Phenolic Content

### INTRODUCTION

Fruit and vegetable consumption increases due to its significant benefits on human health (Angelino et al. 2019). In 2017, global fruit production reached 124.73 Million Metric Tons (MMT), bananas produced 114.08 MMT, apples yielded 84.63 MMT, grapes 74.49 MMT, the sum of 45.22 MMT for tropical fruits mangoes, mangosteens, and guavas, and pineapples 25.43 MMT (Sagar et al. 2018). Fruits and vegetables are the most consumed commodities, resulting in 42% of food waste (Ganesh et al. 2022).

Citrus fruits are widely grown and utilized for refreshing flavors, health benefits, and affordable prices (Tonogbanua et al. 2018). Citruses are mainly used to make fresh juice or citrus-flavored drinks, leading to a significant amount of waste from peels, pulp, and seeds yearly (Maqbool et al. 2023). Large amounts of waste were dumped in landfills or burned, which caused degraded soil quality and polluted and deoxygenated water. To address this concern, wastes are valorized into animal feed, essential oil extraction,

production of biofuel, and biodegradable packaging films (Chavan et al. 2018). An average of 60% of processed citrus fruits discarded as waste contain valuable components like polyphenols and essential oils, making them nutritionally important and suitable for pharmaceutical applications (Kesbiç et al. 2022). These wastes contain bioactive compounds, such as phenolic acid and flavonoids, which affects various health benefits, including antioxidant, anti-inflammatory, and antibacterial properties (Wang et al. 2016).

The *Citrus* genus originated in Southeast Asia and has evolved into numerous varieties and hybrids through natural or artificial crossbreeding, including oranges, lemons, grapefruits, limes, and mandarins that have been extensively researched and are commercially cultivated (Zhong and Nicolosi 2020). Calamansi (*Citrus microcarpa* Bunge), also known as calamondin, is a natural hybrid of mandarin and oval kumquat (*Citrus reticulata* Blanco and *Citrus japonica* Thunb.) (Lim 2012). The climate and fertile soil of the Philippines allow for a year-round culture of citrus fruits

(Idquival et al. 2023). This species is widely cultivated as an alternative to lime due to its tolerance to pests and diseases and is commercially produced in syrups, juices, tea, concentrates, and purees. Additionally, calamansi is used for cough and a natural anti-inflammatory medication (Alinejhad et al. 2016). It is one of the staple fruit juices in the Philippines (Quijano et al. 2021). Calamansi peels have been tested for their efficiency in lowering the blood glucose level of albino rats *Rattus albus*. It also contains coumarin derivatives as an effective anticoagulant and can be utilized as an herbal remedy for managing blood glucose (Rocha et al. 2020).

Calamansi is included in the major fruit crops in the Philippines that are cultivated and exported (Mapalo and Rosillo-Magno 2018). From April to June 2023, calamansi production reached an estimated 14.24 thousand metric tons, showing a 4.0% increase from 13.69 thousand MT in the same quarter in 2022. Zamboanga Peninsula emerges as the leading producer, contributing 3.00 thousand metric tons, accounting for 21.1% of the total calamansi production in that quarter (PSA 2023). The Philippines is a significant exporter of calamansi juices in Japan, South Korea, Hong Kong, the USA, and Canada (Rodeo 2016).

The calamansi fruit peel, a byproduct of juicing, contains 1% flavonoids, 7.14% pectin, 0.51% limonin, 5.98% reducing sugar, and 4.25% essential oils (Zou et al. 2016). The calamansi peel is rich in pectin and essential oils, contributing to the fruit's overall flavor and nutritional profile. Calamansi peels from different regions were extracted using dichloromethane and hexane and analyzed for volatiles, aromatic profiles, and phenolic acids using gas chromatography-mass spectroscopy (Cheong et al. 2012). The results showed 79 identified compounds comprised over 98% of the volatiles. Malaysian calamansi peel had the highest level of a specific compound, methyl N-methylanthranilate. Principal component and canonical discriminant analyses were used to distinguish the peels from different regions. Additionally, ultra-fast liquid chromatography identified caffeic, p-coumaric, ferulic, and sinapic acids in the peels. The Philippines calamansi peel had the highest total phenolic acids, with p-coumaric acid being the most common free phenolic acid and ferulic acid being the primary bound phenolic acid.

Citrus byproducts contain a diverse selection of biologically active components, including essential oils, pectins, carotenoids, and limonoids, which can be extracted and utilized in various industries, such as food, feed, pharmaceuticals, and cosmetics (Panwar et al. 2021). Utilizing calamansi peel wastes can maximize the potential of the byproduct while addressing fruit waste disposal challenges in developing countries. Hence, this study aims to characterize the nutritional profile, specifically biomolecules carbohydrates, protein, lipid, ash, and fiber content of calamansi peels in different drying methods. It also aims to determine the antioxidant properties using DPPH scavenging activity assay, Total Phenolic Content (TPC), and Total Flavonoid Content (TFC) of calamansi peel waste as preliminary analyses in utilization of the calamansi byproduct.

## MATERIALS AND METHODS

### Collection and drying of samples

This study collected samples of unripe calamansi peels from a calamansi juice processing industry in Siay, Zamboanga Sibugay, Philippines. The collected samples were weighed, minced using a food processor (Kaisa Villa Electric Food Processor 2L), and stored at -4°C until the drying process began. Frozen samples were air-thawed first and then subjected to different drying methods. The codes were CSD-Calamansi Sundried, COD-Calamansi Oven-dried, and CDD-Calamansi Dehydrated. The study adopted a sun drying method by Farahmandfar et al. (2020), with some modifications, using a wooden frame and cloth drying rack placed in an open area and fresh calamansi peels were sun-dried around 15-37°C for 48 hours until they reached a 10% moisture content. After that, samples were processed into a fine powder using a 45-micron stainless sieve for further analysis.

Moreover, for dehydration, the fresh calamansi peels were loaded onto a food dehydrator (OneTwoFit Food Dehydrator Machine 5 Layers) and dried at 45°C for 48 hours to achieve a moisture content of 10%. After the drying, the samples were powdered and sieved into 45-micron and vacuum sealed for analysis. A laboratory oven (Isotherm® Forced Convection Laboratory Oven) was used for oven drying. Calamansi samples were then arranged in an aluminum tray and were dried at temperatures of 50 °C for an average of 36-40 hours to reach a moisture content of 10%. Then, samples were processed, sieved into fine powder using a 45-micron, and sealed for analysis.

### Proximate analysis of the samples

For proximate analyses, 100 grams of each dried sample were sent to the National Fisheries Research and Development Institute (NFRDI)-Integrated Research Laboratory, Quezon City, to analyze protein, lipid, moisture, and ash. The fiber analysis was performed by a laboratory technician at Mindanao State University at Naawan-Institute of Fisheries Research and Development (MSUN-IFRD). Furthermore, the carbohydrate content was subtracted from the sum of the other components.

Protein content was determined using AOAC 954.01 by digesting the sample with sulfuric acid and a catalyst, then distilling and titrating the resulting ammonia. Lipid content was measured using AOAC 960.39 by extracting lipids with a solvent mixture, evaporating the solvents, and weighing the remaining lipids. Ash content was assessed following AOAC 938.08 by ashing the sample in a furnace and weighing the residue. Crude fiber was analyzed with AOAC 962.09 by treating the sample with acid and alkali, then drying and ashing. Carbohydrates were calculated by subtracting the percentages of protein, fat, moisture, ash, and fiber from 100%. Moisture content was determined by drying the sample and calculating the weight loss.

### Antioxidant analyses of the samples

#### *Ultrasound-assisted extraction*

A method used by Mahmood et al. (2019), with some modifications, was used for the extraction process. Five

grams of the pulverized samples were added with 80% ethanol (100 mL) and placed at a sonicator power of 20 kHz for 15 min at room temperature. After sonication, the mixture was filtered and analyzed for its phytochemical composition and antioxidant activity.

#### Radical scavenging activity (DPPH)

With some modifications, Hossain and Rahman's (2010) protocol was used to analyze radical scavenging activity. About 1.6 mL ethanolic extract of the samples and 2.4 mL of 0.1 mM ethanolic solution of DPPH were mixed thoroughly. The control was prepared by mixing 1.6 mL 80% ethanol and 2.4 mL 0.1 mM ethanolic solution of DPPH. The mixture was left in the dark for 20 minutes at room temperature. At 517 nm, a UV-VIS spectrophotometer measured changes in absorbance. Ascorbic acid as a positive control was prepared but without the sample. The scavenging activity was measured with the formula shown below:

$$\% \text{ Radical Scavenging Activity} = (\text{Control OD} - \text{sample OD}) / \text{control OD} \times 100.$$

#### Total Polyphenol Content (TPC)

For total phenolic content determination, Folin-Ciocalteu reagent in the concentration of 0.50mg/mL in distilled water was used. The 40 - $\mu$ L of the extract was mixed with 200 - $\mu$ L of Folin-Ciocalteu's phenol reagent and 600 - $\mu$ L of 20% sodium carbonate. Then, it was diluted with water to a total volume of 5 mL. The mixture was left to stand for two hours, and the absorbance of the blue-colored solution was measured using a UV-VIS spectrophotometer at 765 nm. For TPC quantification, it was used with linear regression analysis, using gallic acid as the standard reference at concentrations ranging from 0 to 1000 mg L<sup>-1</sup>. The analytical results represented milligrams of Gallic Acid Equivalents (GAE)/ liter (mg GAE L<sup>-1</sup>).

#### Total flavonoid content

Total Flavonoid Content (TFC) was measured using the aluminum trichloride with catechin as the reference compound. One (1) mL of the calamansi extract was added to 300  $\mu$ L of a 5% NaNO<sub>2</sub> solution and allowed to stand for 6 minutes; then, it was added with 300  $\mu$ L of 10% aluminum trichloride and incubated for 5 minutes. After that, 2 mL of 1M NaOH was added to the solution and adjusted with distilled water to achieve a total volume of 5 mL. For 15 minutes, it was incubated. After the observation of the color of the solution, the absorbance was measured (510 nm). TFC concentration was evaluated using catechin as a standard, ranging from 0 to 500 mg L<sup>-1</sup>. Values for total flavonoid content were expressed as mg CE L<sup>-1</sup> dry sample.

#### Statistical design

Data were analyzed by utilizing one-way Analysis of Variance (ANOVA), and the mean values were ranked and compared using Duncan multiple range tests, employing the software program R Software version 4.2.2 for Windows. Significant differences were considered when  $p < 0.05$ . Values obtained in all analyses were expressed as mean  $\pm$  Standard Error (M  $\pm$  SE).

## RESULTS AND DISCUSSIONS

#### Proximate analyses

The nutritional content of calamansi subjected to different drying techniques is shown in Table 1. Calamansi Oven-Dried (COD) samples showed the highest carbohydrate, fat, moisture, and ash content. Dehydrated calamansi (CDD) samples showed the highest protein and fiber content.

#### Antioxidant properties

The results showed a significant difference in antioxidant activity in three different drying methods (Table 2).

**Table 1.** Proximate composition of calamansi peels subjected to different drying methods

Drying method	Carbohydrates	Crude protein	Crude Fat	Moisture	Ash	Fiber
CSD	58.29 $\pm$ 0.24b	8.26 $\pm$ 0.11b	1.48 $\pm$ 0.10a	14.31 $\pm$ 0.51a	4.83 $\pm$ 0.02b	12.83 $\pm$ 0.46b
COD	63.21 $\pm$ 0.33a	6.89 $\pm$ 0.08c	1.55 $\pm$ 0.12a	12.81 $\pm$ 0.47b	6.14 $\pm$ 0.02a	9.40 $\pm$ 0.21c
CDD	57.34 $\pm$ 1.24b	8.54 $\pm$ 0.09a	1.20 $\pm$ 0.02b	12.40 $\pm$ 0.75b	4.86 $\pm$ 0.03b	15.66 $\pm$ 0.43a

Note: CSD: Calamansi Sundried, CDD: Calamansi Dehydrated, COD: Calamansi Oven-dried. Values are mean  $\pm$  SE of triplicate determinations. Values with different superscripts in the same column significantly differ at  $p < 0.05$

**Table 2.** Antioxidant properties of calamansi peels in different drying methods

Drying method	DPPH (% Scavenging Activity)	TPC (mg GAE/L)	TFC (mg GAE/L)
CSD	21.04 $\pm$ 1.41a	145.7 $\pm$ 6.54a	163.3 $\pm$ 1.90a
CDD	20.79 $\pm$ 3.13a	192.6 $\pm$ 21.99a	41.9 $\pm$ 6.25b
COD	8.92 $\pm$ 1.09b	70.7 $\pm$ 8.87b	121.0 $\pm$ 6.87c

Note: CSD: Calamansi Sundried, CDD: Calamansi Dehydrated, COD: Calamansi Oven-dried. Values are mean  $\pm$  SE of triplicate determinations. Values with different superscripts in the same column significantly differ at  $p < 0.05$

## Discussion

Sun-dried calamansi peels have comparable results with other methods regarding carbohydrates, crude fat, and ash. However, it has a significantly higher value in moisture. Sun drying is a traditional food preservation method, but it is less efficient in water removal due to reliance on environmental factors, slow evaporation rates, and risk of contamination (Elmsaad et al. 2024). A study by Siri wattananon and Maneerate (2016) showed that sun-drying retained higher amounts of dietary fiber in dried pumpkin, yardlong bean, red cabbage, and guava compared to hot air oven drying and freeze-drying. Moreover, a study by Wahdaningsih et al. (2023) showed that the highest total flavonoid content (22.5%) and total phenol content (37.35%) were found in sun-drying compared to oven-drying with total flavonoid content (20.698%) and total phenol content (36.648%). In contrast, some studies showed that sun-drying yielded the least antioxidant compared to freeze-drying, microwave oven, and oven-drying (Sarkar et al. 2024). The efficiency of sun drying and nutrient retention is greatly influenced by the prevailing environmental conditions, such as temperature, humidity, and sunlight availability (Elmsaad et al. 2024).

In the present study, dehydrated (CDD) samples showed the highest protein and fiber content. Moreover, dehydrated calamansi peels had the highest phenolic content (192.6 mg GAE/L). This is comparable to another study that measured the polyphenol content of lemons (160.57 mg/L), oranges (193.78 mg/L), and sweet limes (232 mg/L) (Ahn et al. 2020). A steady airflow during dehydration further aids in maintaining a low oxidizing environment, which is critical for preserving antioxidant properties (Muñoz-Fariña et al. 2023). High temperatures can lead to the rapid degradation of sensitive antioxidant compounds. Research indicates that retention of antioxidant capacity decreases with increasing dehydration temperature. By maintaining a dynamic flow of air, the oxygen concentration can be diluted or kept below critical levels necessary for oxidation, thus preventing the degradation of sensitive materials (Saini et al. 2014). Proper air circulation is essential to replace moisture-laden air with drier air, which is crucial for maintaining the efficiency of the drying process (Putra and Ajiwiguna 2017).

Calamansi oven-dried samples (COD) showed the highest carbohydrate, fat, moisture, and ash content. Convective oven drying is effective in moderate temperatures, typically between 60°C and 70°C, to remove moisture from food while minimizing nutrient degradation. This controlled temperature ensures that the essential constituents of dried fruits, vegetables, and other food products are maintained effectively (Onwude et al. 2022). However, this study observed oven-dried calamansi peels have significantly lower antioxidant activity than dehydrated and sun-dried peels. The preservation of antioxidants in dried fruits is related to the drying temperature. Kittibunchakul et al. (2023) found that lower temperatures enhance antioxidant activity, with temperatures below 50°C supporting better retention of bioactive compounds. High temperatures can accelerate oxidative reactions in polyphenols, leading to reduced

antioxidant activity (Antony and Farid 2022). Studies show that increased temperatures negatively impact polyphenol content. Pascariu et al. (2014) examined dried grape pomace and found that the highest total polyphenol content occurred in grape pomace dried at 20°C, with degradation increasing as the temperature increased. Sarkar et al. (2024) showed that freeze-dried pomegranate peels contained the highest values of total phenolic content, DPPH radical scavenging activity, vitamin C, and ferric-reducing antioxidant power compared to microwave drying, oven drying at 50°C, and sun drying, and sun drying was being the lowest quality.

Temperatures above 100°C negatively impact nutritional and sensory quality, although high temperatures accelerate drying; however, prolonged exposure results in lower nutrient retention (Turkmen et al. 2020; Jayawardena et al. 2022). This study used the lower temperature in sun-drying and dehydration at 37-40°C, and a higher temperature in oven drying (50°C) was employed. When considering energy efficiency and practicality, traditional sun-drying remains a practical choice. Additionally, dehydration using food dehydrators is a viable option due to its commercial availability and cost-effectiveness.

In conclusion, this study evaluated various drying techniques for calamansi peels: sun drying, dehydration, and oven drying. The oven-drying method effectively retains carbohydrates and fat but reduces antioxidant levels. Dehydration efficiently preserves protein and fiber, enhancing the overall nutritional profile. Additionally, sun drying demonstrates significant retention of flavonoids and higher DPPH scavenging activity, while dehydration preserves the polyphenols of the calamansi peels, highlighting its efficacy in preserving bioactive compounds. Hence, this study suggests that sun drying and dehydration present practical and cost-effective options for drying calamansi peels, particularly for future applications.

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