PLANT SCIENCE TODAY
Vol XX(1): 1-XX
https://doi.org/10.14719/pst.13361

eISSN 2348-1900
OPEN ACCESS

Research Article | Biotechnology
2026, Volume XX, Issue X, pp. 1-XX

Evaluation of the nutritional and biological value of two orange varieties (*Citrus sinensis* L. and *Citrus clementina* Hort. ex Tanaka): A comparative study

Lahouaria Labga[1], Hayet Mehida[2], Samira Meziani[1]*, Khadidja Chiali Charif[1], Kheira Rabbah[1], Yamina Belkessam[1], Ouafia Liamani[2], Fatima Zohra Chenni[1] & Noureddine Menadi[1]

[1]Laboratory of Biotoxicology, Department of Biology, Faculty of Natural and Life Sciences, Djillali Liabes University, P.O. Box 89–22000, Sidi Bel Abbes, Algeria

[2]Environmental Information Synthesis Laboratory, Institute of Agricultural Sciences, Djilali Liabes University, P.O. Box 89–22000, Sidi Bel Abbes, Algeria

***Correspondence:** meziani_samira@yahoo.fr

Abstract

Citrus by-products, particularly peels, are rich sources of phenolic compounds and other bioactive constituents with significant biological properties. The present study aimed to evaluate and compare the chemical composition, bioactive compounds and antioxidant activities of the juice and peel fractions (flavedo and albedo) of *Citrus sinensis* (L.) Osbeck and *Citrus clementina* Yu. Tanaka. Mature fruits were harvested on 19 January 2022 from an orchard in the Misserghin region (Oran, Algeria). The mineral composition and physicochemical characteristics were determined by X-ray fluorescence (XRF). Total phenolic content, flavonoids and hydrolysable and condensed tannins were assessed in ethanolic extracts of flavedo and albedo. Antioxidant activity was determined by the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay, ferric reducing antioxidant power (FRAP) assay and β-carotene bleaching assay. The mineral analysis showed that the highest concentration of potassium (K^+) was found in clementine flavedo, whereas the highest sodium (Na^+) content was found in orange albedo (3.5 mg/mL). The quantitative phytochemical analysis showed that the ethanolic extracts of orange flavedo and clementine albedo were rich in total phenolic compounds (13.711 ± 0.678 and 18.218 ± 0.511 mg gallic acid equivalents per gram (GAE/g), respectively) and flavonoids (4.49 ± 1.133 and 4.23 ± 1.133 mg catechin equivalents per gram (CE/g) respectively). Orange and clementine flavedo showed the highest levels of hydrolysable tannins (0.245 ± 0.026 and 0.323 ± 0.157 mg catechinic acid equivalents per gram (CAE/g) respectively) and condensed tannins (318.52 ± 12.1 and 214.5 ± 3.54 mg CE/g respectively). The highest antioxidant activity, measured by the DPPH assay, was observed at 50 µg/mL, with half maximal inhibitory concentration (IC_{50}) value of 11.6 µg/mL. The orange albedo extract also showed the highest ferric reducing power among all the tested extracts. The β-carotene contents were similar among the different citrus fractions, with values of 0.238 ± 0.009, 0.128 ± 0.004, 0.110 ± 0.001 and 0.100 ± 0.0007 mg/100 g dry matter. Significant increases ($p \leq 0.05$) in β-carotene content were observed in the peel fractions of orange and clementine. The results showed that the peels of *C. sinensis* and *C. clementina* are good sources of phenolic compounds, tannins, flavonoids, minerals and antioxidant constituents. These results demonstrate the potential of citrus processing by-products as low-cost natural sources of functional ingredients for food, nutraceutical and pharmaceutical applications and thus contribute to the sustainable valorisation of agricultural waste.

Keywords: albedo; antioxidant activity; chemical composition; citrus; flavedo; peel; phénolic compounds

Received: 25 December 2025. **Accepted:** 28 September 2026. **Available online:** Version 1.0: 25 November 2026.

Cite this article: Lahouaria L, Hayet M, Samira M, Khadidja CC, Kheira R, Yamina B, Ouafia L, Fatima ZC, Noureddine M. Evaluation of the nutritional and biological value of two orange varieties (*Citrus Sinensis* L. and *Citrus clementina* Hort. ex Tanaka): A comparative study. Plant Science Today. 2026;XX(X):1-XX. https://doi.org/10.14719/pst.13361

Introduction

Nutrition is now perceived as a public health factor and fruits and vegetables are highly recommended. The arguments underlying the health benefits of fruits and vegetables include a significant contribution of micronutrients necessary for the proper functioning of the body and a protective effect against major chronic diseases (1, 2). Citrus fruits are native to Southeast Asia. These are trees belonging to the family Rutaceae, comprising either 156 or 16 species, depending on whether the authors have taken hybrids into account (3).

The chemical composition of orange peel is subject to variation under the influence of various factors, including variety. Moreover, within one variety, the content of various compounds depends on climatic and environmental factors. The latter provides a reservoir rich in nutritional ingredients (water, proteins, sugars and minerals) and functional ingredients such as fibre, carotenoids, vitamin C and phenolic compounds (4, 5). Citrus peels are rich in phenolic compounds, mainly flavonoids and phenolic acids. The flavonoids in citrus peel are characterised by their antioxidant, therapeutic, antiviral, antifungal and antibacterial activities (6–9).

Orange peel is rich in nutritional ingredients (water, proteins, sugars and minerals) and functional ingredients such as fibre, carotenoids, vitamin C and phenolic compounds. However, the most widespread industrial recovery pathway remains the extraction of essential oils, which can be used as an alternative to synthetic fungicides (10–12), in the formulation of dietary foods and through the direct incorporation of the peel into the production chain of confectionery products (6). Citrus co-products can also be used as livestock feed (13). Citrus peels are also used in the production of biofuels (ethanol) and biogas (14–16), as well as in the manufacture of biodegradable plastics (17) and as corrosion inhibitors for metals and alloys (18, 19). Citrus essential oils are obtained mainly from the peel of the fruit (flavedo).

It is in this context that our work aims to valorise orange peel waste by studying and comparing the chemical composition, natural compounds and antioxidant activity of the juice and peels of the two varieties of *Citrus* (*Citrus sinensis* (L.) Osbeck and *Citrus clementina* Yu. Tanaka).

Materials and Methods

Material

Our work focused on comparing the physicochemical and biological properties of the peels of two citrus varieties (*C. sinensis* and *C. clementina*). The fruits were harvested at maturity on 19 January 2022 from the Abde Kadri (Maddeh Ibrahim) farm in the Misserghin region of Oran.

Preparation of extracts

The extraction of the two citrus varieties was carried out using maceration with an organic solvent, followed by evaporation according to the standard method (20). A 10 g sample of powder was used in 100 mL of solvent with increasing polarity (80 % ethanol + 20 % water). The mixture was placed in a magnetic stirrer at room temperature for 24 hr. Once maceration was complete, filtration was carried out using Whatman No. 1 paper and the filtrate represented the ethanol fraction. The solvent was then evaporated using a rotary evaporator at an ambient temperature of 40 °C.

Determination of physico-chemicals analysis of peel

Ash content

The principle is to incinerate the biological material in a porcelain stove at a temperature of 900 °C, or the results represent the average of four trials using the following formula (21):

$TC (\%) = (M_1 – M_2 / P) \times 100$

With:

TC (%) is the ash content (in percentage);

M_1 is the mass (in grams) of the capsule and the drymatter before incineration;

M_2 is the weight (in grams) of a capsule with ashes

P is the test mass (in grams).

Determination of minerals by flame spectrophotometry

A 0.5 g sample of the powder was taken and 6 mL of 37 % chloric acid and 2 mL of 65 % or 69 % nitric acid were added to 25 mL of distilled water. The beaker was covered with a watch glass and placed on a hot plate. The temperature was raised to the boiling point and the mixture was heated for 120 min. The mixture was then allowed to cool, filtered and made up to 100 mL. The extract was then sealed for analysis (22).

X-ray fluorescence measurements determination

X-ray microfluorescence spectrometry

X-ray fluorescence (XRF) microscopy was used to identify and determine most of the chemical elements present in the samples, using equipment available at the Research Technology Platform in Algeria. Fluorescence spectrometry was used to analyse the composition of trace elements in citrus fruits, which are rich in minerals and other substances. This measurement involved the evaporation, carbonisation and pressing of samples, with element concentration determined and estimated directly from linear calibration curves obtained without matrix correction. Each sample was placed individually in the excitation zone of the fluorescence spectrometer and the corresponding fluorescence spectra were recorded. These spectra are graphs representing the intensity of light emitted as a function of wavelength.

Proteins determination by the Bradford method

Protein determination was carried out using a colourimetric test. A volume of 100 µL of the extract was added to 5 mL of Coomassie Brilliant Blue reagent in each tube. The mixture was gently mixed using a vortex mixer, avoiding the formation of foam. After a contact period of 2 min to 1 hr in the dark, absorbance was measured at 595 nm, with some modifications (23, 24). Protein concentrations were determined by reference to a calibration curve obtained with bovine serum albumin (BSA) under the same reaction conditions.

Determination of crude fibre

The determination of crude fibre was carried out (25). A 1 g sample was ground using a Moulinex grinder to obtain a fine powder and then placed in a glass vessel. A volume of 150 mL of 1.25 % sulphuric acid and 3–5 drops of N-octanol as an antifoaming agent were added. The sample was dried in a drying oven at 105 °C for 1 hr and cooled in a desiccator before the dry weight was determined. The ash content was determined after incineration for 4 hr at 550 °C in a high-temperature oven, followed by cooling for 1 hr in a desiccator and weighing the residue. The crude fibre content was calculated as follows:

$Fibres = [(F_1 - F_2) F_0)] \times 100$

Where:

F_0: dry sample weight

F_1: weight of the creuset with residues

F_2: weight of the crater with residues near the ashes

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Determination of total of phenolic compounds

Total polyphenol dosage

The method previously described makes it possible to estimate the total amount of phenolic compounds present in the two extracts: orange peel and mandarin peel, using a spectrophotometer (26). The method is based on the use of the Folin–Ciocalteu reagent. After stirring, 200 µL of each extract was added to 800 µL of sodium carbonate (Na_2CO_3) solution, followed by 1 mL of Folin–Ciocalteu reagent in a haemolysis tube. The tubes were incubated for 30 min. The polyphenol content was measured in micrograms of gallic acid equivalents per milligram of dry extract (mg GAE/mg DM) using a UV spectrophotometer at an absorbance wavelength of 765 nm. The measurement was carried out simultaneously under the same experimental conditions using gallic acid.

Determination of flavonoids

According to previous results, the determination of flavonoids is based on the formation of chromogenic complexes with aluminium chloride ($AlCl_3$) (27). A volume of 400 µL of the extract was added to 2 mL of distilled water and 120 µL of sodium nitrite ($NaNO_2$) in a haemolysis tube. After 5 min, 120 µL of $AlCl_3$ was added to the tube. After 6 min, 800 µL of 4 % sodium hydroxide (NaOH) was added. The volume was adjusted to 5 mL using water and the mixture was then incubated for 15 min in the dark. Absorbance was measured at a wavelength of 510 nm using a spectrophotometer. The concentration of flavonoids was determined using a calibration curve obtained with quercetin.

Total tannins

Condensed tannins

The condensed tannin content was determined (28). The extracts (0.1 mL) were placed in tubes and 3 mL of 4 % weight per volume (w/v) vanillin in methanol solution was added. After vigorous stirring, 1.5 mL of concentrated hydrochloric acid (HCl) was immediately added and the mixture was mixed thoroughly. Absorbance was measured at 500 nm after 20 min of incubation. The calibration curve was prepared under the same conditions using the standard and the results were expressed as milligrams of catechin equivalents per gram of dry matter (mg CE/g DM).

Hydrolysable tannins

The method previously described was used to evaluate hydrolysable tannins through a reaction with ferric chloride ($FeCl_3$) (29). Mixing the tannin extract with the iron chloride reagent produces a blue-black colour in the presence of gallic tannins, whereas a greenish-brown colour is observed in the presence of catechic tannins. A volume of 1 mL of the extract was added to 3.5 mL of a solution of 0.01 M $FeCl_3$ in 0.001 M HCl volume per volume (v/v). The mixture was stirred vigorously and the optical density was measured at 660 nm using a spectrophotometer. The amount of hydrolysable tannins in the extracts was measured using a gallic acid calibration curve under the same experimental conditions as those used for the tested samples.

Hydrolysable tannins were expressed by the relationship:

T % = OD (M.V/E moles P)

T %: percentage of hydrolysable tannins

OD: Optical density

E: 2169 of gallic acid

M: 300

V: Volume of extract used

P: Sampling weight

Determination of total antioxidant activity

DPPH radical trapping test in shells

This method is based on measuring the ability of antioxidants to scavenge the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical. The effect of each extract on DPPH was measured using the standard procedure (30). A volume of 100 µL of each extract at different concentrations was added to 1 mL of freshly prepared DPPH methanolic solution (0.025 g/L). For the negative control, 100 µL of methanol was mixed with 1 mL of a methanolic DPPH solution at the same concentration as that used for the samples. After incubation for 30 min in the dark at room temperature, absorbance was measured at 517 nm using a spectrophotometer. The reference standard (ascorbic acid) was also analysed using the same procedure.

The inhibition percentage is given according to the following formula:

Anti-radical activity (AE) % = $((A_0 - A_1)/A_0) \times 100$

AE: absorbance in the presence of the inhibitor (sample).

Ferric reducing antioxidant power assay

The method used to assess the reducing power of iron (Fe^{3+}) in the extracts (31). The concentration of the extract ranged from 0.007–2.5 mg/mL. A volume of 1 mL of the extract was mixed with 2.5 mL of 0.2 M phosphate buffer solution (pH 6.6) and 2.5 mL of a 1 % potassium ferricyanide ($K_3Fe(CN)_6$) solution. The mixture was incubated at 50 °C for 20 min, after which 2.5 mL of 10 % trichloroacetic acid was added to stop the reaction. The tubes were centrifuged at 3000 rpm for 10 min. A volume of 2.5 mL of the resulting supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of a 0.1 % aqueous solution of ferric chloride ($FeCl_3$). Absorbance was measured at a wavelength of 700 nm against a blank. Ascorbic acid was used as the positive control and its absorbance was measured in the same way.

B-carotene bleaching test

The four orange peel extracts were evaluated for their antioxidant activity (32). Linoleic acid is oxidised and produces peroxide radicals. A solution of 2 mg of β-carotene in 1 mL of chloroform was prepared. A flask containing 2 mg of linoleic acid and 200 mg of Tween 40 was used to prepare the resulting solution. Once the chloroform had evaporated, 100 mL of oxygen-saturated distilled water was added with vigorous shaking. A volume of 2.5 mL of this solution was placed in tubes, to which 350 µL of each extract or butylated hydroxytoluene (BHT) (2 mg/L) was added. The

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negative control consisted of a tube containing 2.5 mL of the control solution and 350 µL of ethanol.

The absorption is immediately measured at 470 nm, other readings are made at different time intervals (2 hr, 4 hr, 6 hr, 12 hr and 48 hr) and all measurements are made in triplicate. The relative antioxidant activity after 48 hr is calculated according to the following ratio:

AAR = (Abs Echantion-Abs BHT) × 100

AAR: relative antioxidant activity

Abs sample: sample absorption after 48 hr

Abs BHT: absorption of BHT after 48 hr

Determination of vitamin C dosage in juice shells

Vitamin C determination was performed using the modified method, using 2,6-dichlorophenolindophenol (DCPIP) (33). A volume of 2 mL of orange peel extract was added to 23 mL of a 5 % solution of metaphosphoric acid (HPO_3). A volume of 5 mL was then taken and 5 mL of a 20 % solution of hydrochloric acid in HPO_3 was added. The resulting solution was then filtered and 2 mL of the filtrate was mixed with 5 mL of a buffer solution at pH 7.1 and 1 mL of 2,6-dichlorophenolindophenol (DCPIP). Absorbance was measured at 530 nm. A standard curve was prepared using ascorbic acid at concentrations of 0, 5, 10, 15 and 20 µg/L (R^2 = 0.9995). Vitamin C content was expressed in gram per 100 g of orange peel powder.

Statistical analysis

Each test was conducted using 3 replicates and the results are presented as the mean ± standard deviation. To compare means at a significance level of 5 % ($p \leq 0.05$), the data were analysed using Student's *t*-test and two-way analysis of variance (ANOVA), followed by Sidak's multiple-comparison test. The Pearson correlation coefficient (*r*) was used to assess correlations between the variables. Additionally, the polyphenol contents and antioxidant activities of *C. sinensis* and *C. clementina* were compared. GraphPad Prism 8.0.2 was used to analyse the data.

Results

Plants produce a variety of organic compounds, both primary and secondary (34). The roles of primary compounds (sugars and proteins) are associated with the physiological processes of plants, ensuring their growth and development (35).

Ash content and minerals analysis

The ashes rate tells us about the nutritional quality of the sample to be analysed. For the ashes rate always indicates a higher value in *C. clementina* (C_1, C_2: 3 %, 3 %) followed respectively (T_1, T_2: 3 %, 2 %).

The minerals are essential elements for the proper functioning of the body; citrus peel contains variable concentrations. Concerning the results of two minerals (Na^+ and K^+), the results obtained relating to the potassium (K^+) and sodium (Na^+) content (Fig. 1).

The results show that the K^+ concentration of the *C. sinensis* extract is as follows: in T_2 (3.5 mg/mL) and T_1 (3 mg/mL), the same for Na^+: T_2 (2.5 mg/mL), T_1 (1 mg/mL), whereas for *C. clementina* an extract was: C_1 (5.5 mg/mL) and C_2 (1.5 mg/mL); and for Na^+ C_1 (2 mg/mL) and much higher than C_2 (1, 5 mg/mL) so *C. clementina* is rich in K^+ than *C. sinensis*. The ash content provides information about the nutritional quality of the sample being analysed. The ash content was consistently higher in *C. clementina* (C_1 and C_2: 3 % and 3 %), followed by the values for T_1 and T_2 (3 % and 2 %), respectively.

Minerals are essential elements for the proper functioning of the body and citrus peel contains them in variable concentrations. The results obtained for the two minerals (Na^+ and K^+), representing the Na^+ and K^+ contents (Fig. 1).

The results show that the K^+ concentrations in the *C. sinensis* extracts were 3.5 mg/mL in T_2 and 3 mg/mL in T_1. The corresponding Na^+ concentrations were 2.5 mg/mL in T_2 and 1 mg/mL in T1. For *C. clementina*, the K^+ concentrations were 5.5 mg/mL in C_1 and 1.5 mg/mL in C_2, whereas the Na^+ concentrations were 2 mg/mL in C_1 and 1.5 mg/mL in C_2. Thus, *C. clementina* showed a higher K^+ concentration than *C. sinensis*.

X-ray fluorescence measurements results

The XRF spectra allowed us to identify and measure mineral elements in peel samples from two citrus varieties (*C. sinensis* and *C. clementina*). Table 1 and Fig. 2 illustrate the absorption spectrum, identifying more than ten elements. This measurement provides vital information on their nutritional value, potential for valorisation and potential for environmental contamination. The elements detected in the highest proportions in the peel samples included essential macroelements (calcium (Ca), potassium (K), sulphur (S) and phosphorus (P)), important trace elements (iron (Fe) and zinc (Zn)) and rare metals (rhodium (Rh) and palladium (Pd)). The intensity of the lines in the X-ray fluorescence (XRF) spectra is associated with the intensity of the emitted radiation, meaning that the radiation is more intense for the higher lines. These lines are attributed to bromine and Ca in both samples. Analysis using XRF software allows the elemental mass percentages of the elements present in the samples to be calculated.

In *C. clementina* (C), the K content was substantially higher, suggesting that the clementine variety may absorb nutrients more effectively. This might encourage improved tolerance to drought and water stress and could affect the fruit's texture and flavour. In *C. sinensis* (T), Ca was more prevalent, contributing to the structural stability of the peel. This may suggest improved post-harvest preservation and mechanical resistance. The higher Ca content may provide *C. sinensis* peels with greater firmness than clementine peels, possibly reflecting the influence of macroelements on peel properties.

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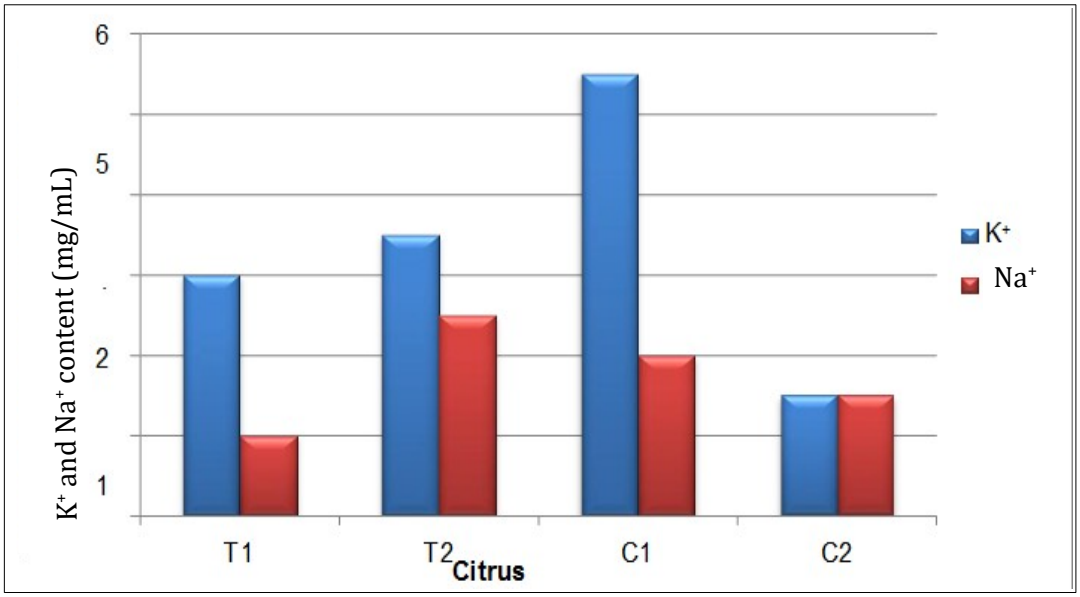

Fig. 1. Minerals content on (sodium (Na^+) and potassium (K^+)) in mg/mL of two *Citrus* varieties. T_1: orange flavedos, T_2: Orange albedos, C_1: *Clementina flavidos*, C_2: *Clementina albedos*.

The higher K content in *C. clementina* indicates potentially more efficient nutrient absorption by the clementine variety. It may promote better resistance to water stress and drought and could influence the taste and texture of the fruit. Calcium, which is more abundant in *C. sinensis*, plays a key role in the structural stability of the peel and may indicate better mechanical resistance and post-harvest preservation.

In light of these results, potassium and calcium appear to be the predominant constituents of the chemical composition of the two citrus varieties, with both varieties being rich in these mineral elements.

Total polyphenols and citrus extract flavonoids dosage result

Phenolic metabolites are secondary compounds known for their biological properties. These substances possess a high antioxidant capacity that may be beneficial to health. The polyphenol concentrations of the extracts of the two *Citrus* varieties are shown in Fig. 3 and are expressed as mg gallic acid equivalents (GAE)/g of citrus extract. The results indicate that the *C. sinensis* extracts contained polyphenol concentrations of 13.711 ± 0.678 mg GAE/g in T_2 and 12.702 ± 5.524 mg GAE/g in T_1, while the *C. clementina* extracts had polyphenol concentrations of 9.789 ± 1.017 mg GAE/g in C_2 and 18.218 ± 0.511 mg GAE/g in C_1.

Flavonoids are one of the most common and diverse groups of natural compounds. The results of the determination of flavonoid content in the various extracts are illustrated in Fig. 3. The content is expressed as mg catechin equivalents (CE)/g of *Citrus* extract. According to the experiment, the flavonoid concentration of the *C. sinensis* extract was 4.49 ± 1.133 mg CE/g in T_1, which was higher than that in T_2 (2.193 ± 1.315 mg CE/g). In addition, the flavonoid concentrations of the *C. clementina* extracts were 3.84 ± 2.08 mg CE/g in C_1 and 4.23 ± 1.133 mg CE/g in C_2, which were relatively similar. Thus, the flavonoid contents of *C. clementina* and *C. sinensis* were relatively similar.

Table 1. Comparative analysis of major and trace elements analysis in the whole peels of two *Citrus* varieties (*Citrus sinensis* and *Citrus clementina*)

Element	C (%)	T (%)	Observations
Phosphorus (P)	0.09	0.05	More abundant in CM, essential for cellular metabolism.
	0.10	0.07	Slightly higher in CM, important for protein formation.
Potassium (K)	3.98	2.52	More present in CM, essential for osmotic regulation and stress resistance.
Calcium (Ca)	6.40	7.41	Higher in TM, important for cell wall rigidity
Iron (Fe)	0.03	0.02	Essential trace element, slightly more abundant in CM.
Zinc (Zn)	0.01	0.00	Detected only in CM, essential for enzymatic activity and disease resistance.
Rhodium (Rh)	4.96	4.39	Rare metal, probable environmental contamination.
Palladium (Pd)	4.59	4.18	Rare metal, presence should be investigated for anthropogenic origin.

T: *Citrus sinensis*, C: *Citrus clementina.*

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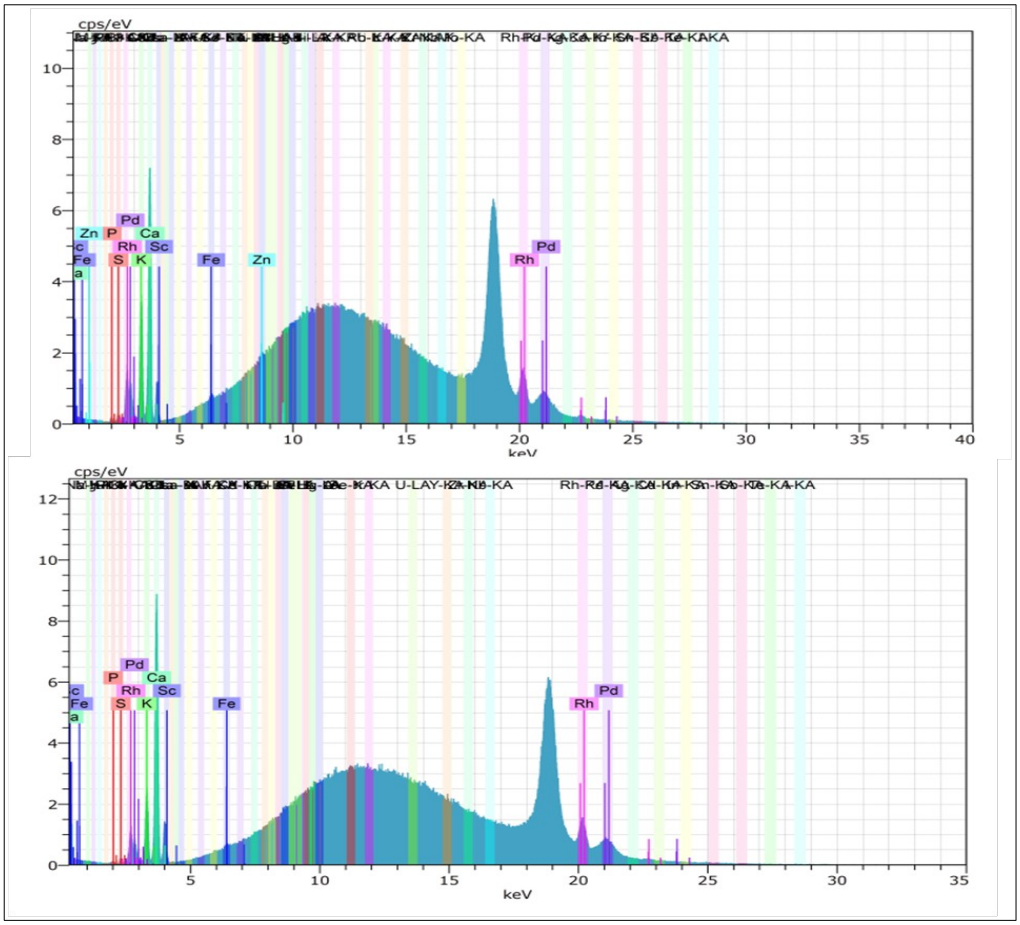

Fig. 2. Results of X-ray fluorescence (XRF) analysis in the whole peels of two *Citrus* varieties (*Citrus sinensis* and *Citrus clementina*).

T: *Citrus sinensis*, C: *Citrus clementina.*

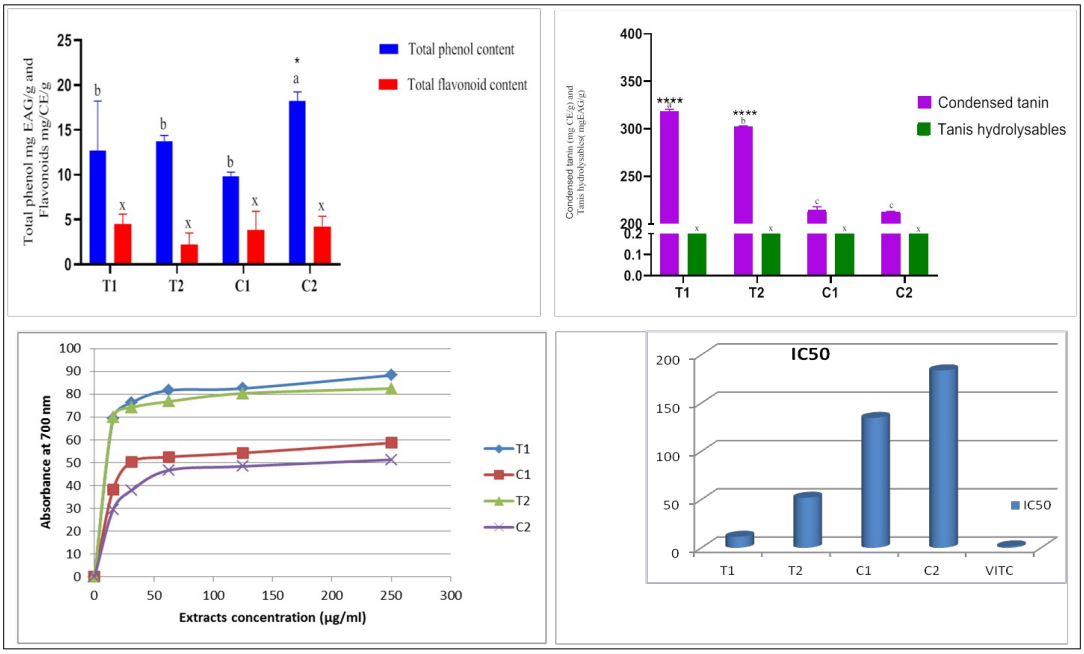

Fig. 3. Concentration of polyphenols (mg EAG/g), flavonoids (mg CE/g), tannins condensed (mg EAG/g) and evaluation on antioxidant activity by DPPH test follow by representation the inhibition concentrations (IC_{50} Test) of extracts of the two varieties of *Citrus* compared to ascorbic acid. T_1: orange flavedos, T_2: Orange albedos; C_1: *Clementina flavidos*, C_2: *Clementina albedos*, significant at $p < 0.05$, $p < 0.0001$ extremely significant ****, $p < 0.05$ Significant *

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Tannins are naturally occurring secondary plant metabolites with diverse chemical structures. They are widely synthesised by higher plants and are generally classified into hydrolysable and condensed tannins. The hydrolysable tannin contents are presented in Fig. 3 and are expressed as mg gallic acid equivalents (GAE)/g of *Citrus* extract. The findings indicate that the hydrolysable tannin contents of the *C. sinensis* extracts were similar: 0.245 ± 0.026 mg GAE/g in T_1 and 0.238 ± 0.070 mg GAE/g in T_2. The same was observed for the *C. clementina* extracts, with values of 0.32 ± 0.133 mg GAE/g in C_1 and 0.323 ± 0.157 mg GAE/g in C_2. The hydrolysable tannin content of *C. sinensis* peel (0.483 ± 0.096 mg GAE/g) was lower than that of *C. clementina* peel (0.643 ± 0.29 mg GAE/g).

For condensed tannins, Fig. 3 shows the results expressed as mg catechin equivalents (CE)/g of *Citrus* extract. The amount of condensed tannins in the *C. sinensis* extracts was 318.5 ± 2.121 mg CE/g in T_1, which was higher than the value reported for T_2 (303.5 ± 0.707 mg CE/g). In the *C. clementina* extracts, the value for C_1 (214.5 ± 3.535 mg CE/g) was higher than that for C_2 (212.5 ± 0.707 mg CE/g). Thus, the amount of condensed tannins in *C. sinensis* was higher than that in *C. clementina*.

Results of antioxidant activity

DPPH test

The graph represents the antioxidant activity of the samples from the two *Citrus* varieties, as determined by the DPPH test. The results are presented as regression curves in Fig. 3, showing that the percentage inhibition of DPPH radicals increased with increasing extract concentration.

According to the curves, the percentage inhibition by ascorbic acid was higher than that of the other samples. The curves for T_1 and T_2 were almost similar, as were those for C_1 and C_2. Overall, the curve for *C. sinensis* showed greater inhibition than that for *C. clementina*. When comparing the IC_{50} values of the extracts of the different *Citrus* varieties with that of ascorbic acid, the IC_{50} values of the citrus extracts were higher than that of ascorbic acid (1.345 mg/mL), which was used as a reference antioxidant. The IC_{50} value of C_2 (183.785 mg/mL) was higher than that of C_1 (134.479 mg/mL). Similarly, the IC_{50} value of T_2 (51.752 mg/mL) was higher than that of T_1 (11.306 mg/mL). In conclusion, the DPPH results showed a pattern opposite to that of the IC_{50} values; that is, the IC_{50} values for *C. clementina* were higher than those for *C. sinensis*. Antiradical activity can be affected by many factors, such as solvent polarity, the extraction procedure and variation between the species used (36).

Frap test

This approach evaluates the ability of compounds in the extracts to reduce ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). It is a quick, simple and reproducible method (37). In this study, tests were conducted using the ferric reducing antioxidant power (FRAP) method on two citrus varieties, namely *C. clementina* and *C. sinensis*. The results obtained were used to prepare the graph shown in Fig. 4.

The iron-reducing ability is linked to an increase in sample concentration (38). Based on the results shown in Fig. 4, the reducing capacity of the *Citrus* extracts was dose-dependent, increasing with increasing extract concentration. The results indicate that the iron-reducing capacity of the extracts was significantly lower than that of ascorbic acid. T_2 showed a significantly greater Fe-reducing capacity than T_1, whereas C_1 showed a lower reducing capacity than C_2, indicating greater reducing power in the *C. sinensis* extracts than in the *C. clementina* extracts. The direct reduction of Fe^{3+} by the peel and stem extracts of the two citrus species was measured.

β-carotene

Carotenoids are pigments that colour both the exterior and interior of citrus fruits. Cultivation conditions, regions of origin and fruit ripeness affect the composition and concentration of carotenoids. The β-carotene content increased over time, reaching 0.6 mg/L (Fig. 5).

The concentration of β-carotene in *C. sinensis* was therefore much higher than that in *C. clementina* in terms of the measured antioxidant activity. These results differ slightly from the other findings. This difference may be due to the nature of the solvent used or the variety.

Results of vitamin C in the juice of the citrus extract

The histogram of vitamin C content, expressed as mg ascorbic acid equivalents (EC)/g of *Citrus* extract showed in Fig. 6. The results show that the vitamin C content of the *C. clementina* juice extract was 19 ± 4.242 mg (EC)/g. This value was higher than that found in *C. sinensis*, which was 12.4 ± 0.565 mg (EC)/g.

Discussion

The chemical composition of orange peels is subject to variation under the influence of various factors, including variety. In addition, within the same variety, the content of various compounds depends on climatic and environmental factors. The latter constitute a rich source of nutritional ingredients (water, proteins, sugars and minerals) and functional ingredients such as fibre, carotenoids, vitamin C and phenolic compounds (4). A diet rich in carotenoids can reduce the risk of cancer, muscle degeneration, skin damage induced by sunburn and cardiovascular diseases (39, 40).

The extraction of chemical compounds from orange peels has attracted considerable scientific interest in their use as natural antioxidants and preservatives, mainly in food but also in the pharmaceutical and cosmetic industries (4). It is in this context that our work aims to study the nutritional and biological aspects of orange peels and to evaluate the antioxidant activity of the juice and peels of the two *Citrus* varieties (*C. sinensis* and *C. clementina*).

The ash contents found in the seeds ranged from 2.00–3.00 %. The ash content indicates the mineral content of the sample, suggesting that these peels are important sources of micronutrients. In a study of the chemical composition of *C. sinensis* seeds, found an ash content ranging from 1.59–3.09 % (41).

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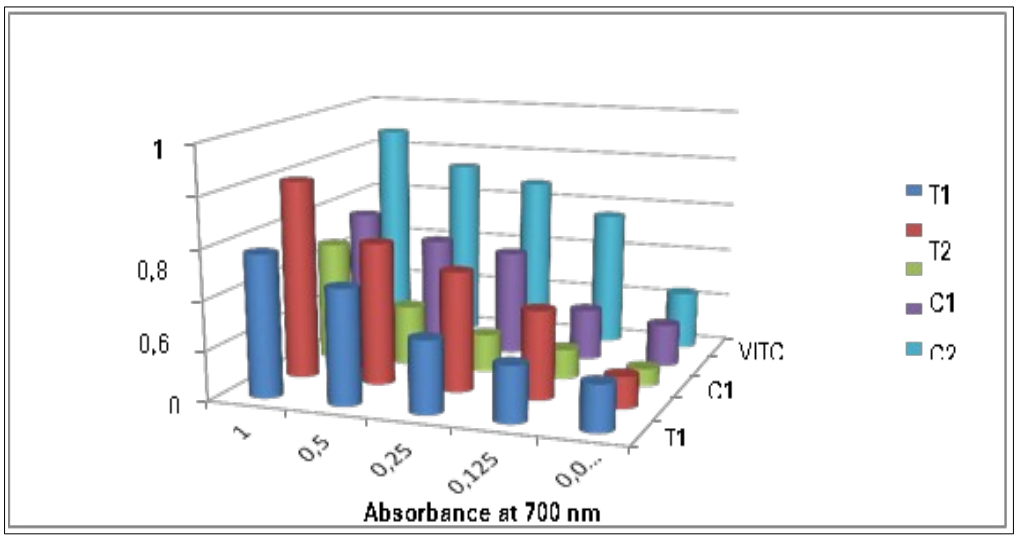

Fig. 4. Antioxidant activity (frap test) of the *Citrus* varieties. T1: Orange flavedos; T2: Orange albedos; C1: *Clementina flavidos*; C2: *Clementina albedos.*

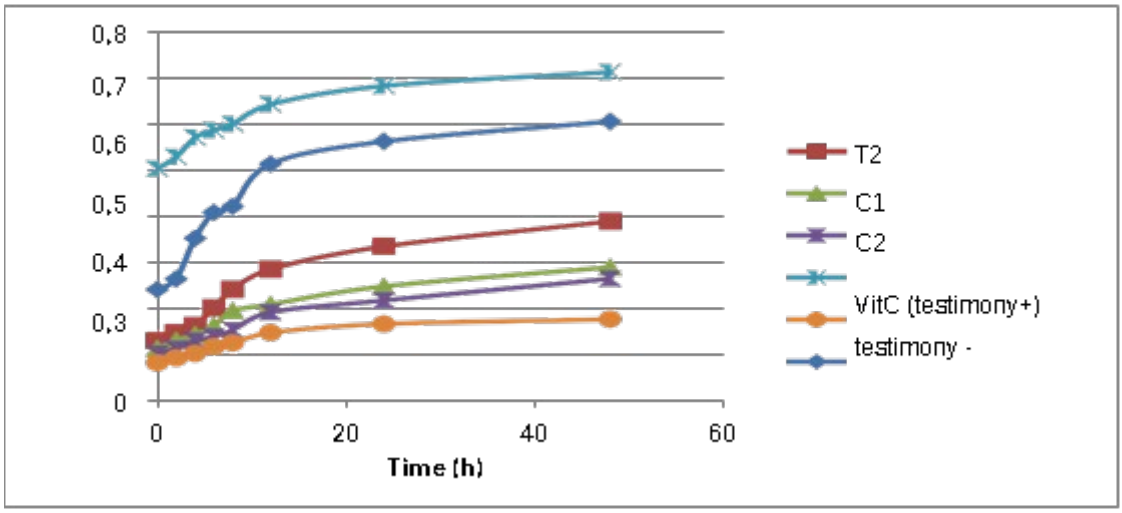

Fig. 5. B-carotene whitening kinetics at 490 nm in the presence of extracts of both varieties of *Citrus* and the positive tester (VITC).T1: orange flavedos; T2: orange albedos; C1: *Clementina flavidos*; C2: *Clementina albedos.*

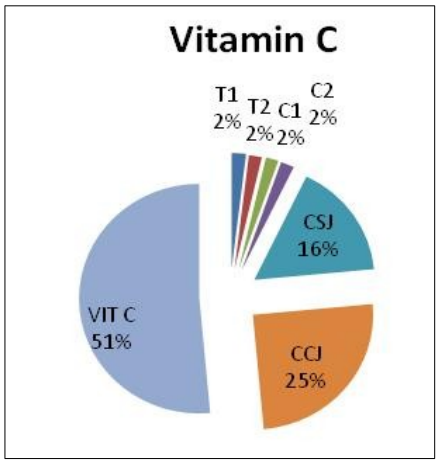

Fig. 6. Vitamin C at 530 nm in the presence of extracts of two *Citrus* fruit varieties.T1: orange flavedos, T2: orange albedos; C1: *Clementina flavidos*; C2: *Clementina albedos*; CSJ: Citrus sinensis juice; CCJ : *Citrus clementina juice.*

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On this basis, *C. clementina* can be considered a potent source of K^+ and Na^+. On the other hand, the low concentrations of K^+ and Na^+ in *C. sinensis* could be due to differences in cultivation conditions, post-harvest handling and storage treatments (42). Regarding mineral composition, maltese oranges are rich in Ca (1201.21 mg/100 g) and K (220.40 mg/100 g), while mandarins are particularly rich in K (641–831 mg/100 g) (43, 44). The results are broadly similar to those found by these studies. Vitamin C has substantial antioxidant properties that play an important role in disease prevention (45). Furthermore, vitamin C affects the absorption of some minerals. It also plays a role in the absorption of other minerals (46).

Dietary fibres are components of plant cells that resist digestion by human digestive enzymes and are recognised as important components of a healthy diet (47). The dietary fibre content of orange peel showed in Fig. 2, indicating that these samples may be relatively poor sources of fibre. Nevertheless, these by-products can be used for food enrichment and animal feed formulation (48). Previous researchers found a fibre content of 4.6 % in orange peel (*C. aurantium*) (48). The lignin content is approximately 7.52 % for oranges and 13.54 % for mandarins (49–51, 40), whereas the cellulose content is higher in oranges than in mandarins and lemons, at 30.53 % and 23.06 % respectively. Regarding total fibre content, oranges and mandarins have the same reported content (11.04 %) (51). Thus, orange peel is reported to contain the most dietary fibre, followed by mandarin and lemon peels. Several studies have shown that citrus peels also contain dietary fibre (6.30–82.69 g/100 g dry basis) (52, 53).

Fruit and vegetable juices are considered good sources of dietary polyphenols. Thus, *C. clementina* is much richer in polyphenols than *C. sinensis*. The phenolic complexes play a vital role in cells and contribute to antioxidant capacity (54). Fruits with higher total phenolic contents may have greater antioxidant capacity.

The differences between the two citrus fruits were more significant in terms of total phenol content. The *C. sinensis* extract contained polyphenol concentrations of 13.711 ± 0.678 mg GAE/g in T_2 and 12.702 ± 5.524 mg GAE/g in T_1. The polyphenol content was also higher in clementine albedo, while the flavonoid content in the albedo of the *C. sinensis* extract was less than 50 % of the corresponding value in the comparison described. Polyphenols, including phenolic compounds and flavonoids, are secondary metabolites of plants that may protect against the development of diseases such as diabetes, osteoporosis, cardiovascular diseases, cancer and neurodegenerative diseases (55).

Some studies found that hydroethanolic leaf extracts contained 45.54 ± 3.70 mg GAE/g dry matter and 125.28 ± 4.15 mg GAE/g dry matter respectively, in terms of polyphenol content (56, 57). Our results therefore differ from those reported in the studies mentioned above. The phenolic compounds play an essential role in protecting plants from ultraviolet (UV) radiation and pathogenic attack and they contribute to plant pigmentation (58, 59).

The hydroethanolic extract of *C. clementina* had a value of 41.03 ± 1.63 mg GAE/100 g dry matter (60). The values obtained in the present study were much higher than those reported in this study. These results suggest that the extracts contained higher concentrations of polyphenolic compounds. Based on the solvent used, we observed that hydroethanolic extracts contained higher concentrations of polyphenolic compounds than aqueous extracts. Another important point is that the Folin–Ciocalteu reagent is not specific to phenolic compounds because it measures the reducing capacity of the sample through electron transfer and, thus, may also be reduced by many non-phenolic compounds, such as vitamin C, sugars and organic acids (50, 61). This difference probably results from the fact that the Folin–Ciocalteu reagent reacts with a wide range of compounds, giving a higher apparent phenolic content (30). The phenol content of a plant depends on several intrinsic and extrinsic factors (62). The results therefore confirm that polyphenolic compound concentrations vary significantly depending on the solvent and extraction method.

Tannins are secondary compounds with diverse chemical structures that form complexes with proline-rich proteins, thereby inhibiting protein synthesis in cells. They are widely produced in the plant kingdom and are generally divided into hydrolysable and condensed tannins. Condensed tannins are mainly found in seed walls and play an important role in the defence system of seeds exposed to oxidative damage caused by environmental factors such as light, oxygen, free radicals and metal ions (63). The synergistic actions of tannins, flavonoids, alkaloids and saponins are known to inhibit the growth of pathogens (64). The quantification of tannins was carried out using previous method (65). A calibration curve was prepared using catechin. The defence system of seeds against oxidative damage caused by environmental factors such as light, oxygen, free radicals and metal ions is mainly associated with condensed tannins (63).

The free radical-scavenging method (DPPH) is used to evaluate the antioxidant activity of our samples because it is simple, fast and effective, owing to the high stability of free radicals (66). The antioxidant activity is determined by measuring the concentration of extract required to neutralise 50 % of the radicals. The IC_{50} parameter represents the concentration required to achieve 50 % inhibition of DPPH radicals. The results are expressed as IC_{50} values, which are calculated from the curves showing the percentage inhibition (I %) as a function of the concentration of each extract. It should be remembered that the lower the IC_{50} value, the greater the antioxidant activity of the extracts.

The antioxidant activity of the fruits was evaluated by measuring DPPH radical-scavenging activity. As expected, the citrus samples from both varieties with higher total phenolic and flavonoid contents showed greater antioxidant activity, since total phenolic and flavonoid contents are correlated with radical-scavenging

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activity (Fig. 3) (9). The IC_{50} values for *C. clementina* were higher than those for *C. sinensis*. The reducing capacity of a compound can serve as a significant indicator of its potential antioxidant activity (40). Previous work has indicated a direct relationship between antioxidant activity and the reducing power of the components of some plants (67).

In this work, we used the FRAP method to test two citrus varieties, *C. clementina* and *C. sinensis*. The results obtained allowed us to construct the graph shown below. It was observed that the Fe-reducing capacity was proportional to the increase in sample concentration (68–70).

In citrus fruits, carotenoids are the pigments responsible for the external and internal colouration of the fruit. The composition and concentration of carotenoids are influenced by growing conditions, geographical origin and fruit maturity. Lemon contains β-cryptoxanthin, a member of the carotenoid family, which contributes to the yellow and green colouration of lemons (71). The concentration of β-carotene in *C. sinensis* was therefore much higher than that in *C. clementina* in the comparison described. According to the results, β-carotene content increased over time. This finding can be compared with early results, which reported a β-carotene concentration of 0.5 mg/L (72). The concentration of β-carotene in *C. sinensis* was therefore much higher than that in *C. clementina* in terms of the measured antioxidant activity. The colours of the peel and pulp of most ripe oranges are attributable to carotenoids. Some varieties, known as blood oranges, such as the Maltese orange, derive their colour from carotenoids. Carotenoids in a balanced diet can reduce the risk of cancer, muscle degeneration, skin damage caused by sunburn and cardiovascular disease (39, 40).

Vitamin C is an essential micronutrient for humans. It is a potent antioxidant and an immune stimulant that can prevent cellular damage caused by harmful and unstable compounds called free radicals, thereby potentially protecting against environmental oxidative stress (73). The juices analysed had lower vitamin C contents in both varieties of citrus, with values much lower than those reported in the literature.

Conclusion

The fruits of the citrus family (*Citrus sinensis* and *Citrus clementina*) offer a wealth of functional ingredients, including secondary metabolites and antioxidant compounds. Based on the research and results obtained, there were differences in the physicochemical, chemical and biological characteristics of the two varieties, although they belong to the same family, Rutaceae. These results may help assess consumer needs in terms of bioactive products.

Acknowledgements

The authors express their gratitude to the University of Sidi Bel Abbès, Laboratory of Biotoxicology, for financially supporting this research.

Authors' contributions

LL contributed to writing the article. SM and NM contributed to reviewing and correcting the work and interpreting the results. OL and KCC revised the manuscript. KR and YB participated in data collection and the use of computer tools. FZC designed the writing process. HM contributed to the financial support for the article and its revision. All authors have read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

Authors do not have any conflict of interests to declare.

Ethical issues

None

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Additional information

Peer review: Publisher thanks the Sectional Editor and anonymous reviewers for their contribution to the peer review of this work.

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Publisher information: Plant Science Today is published by Horizon e-Publishing Group with support from Empirion Publishers Private Limited, Thiruvananthapuram, India.

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https://doi.org/10.14719/pst.13361