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Potential of Food By-Products

Biochemical, Phytochemical, and Antioxidant Characterization of Sweet Orange (*Citrus sinensis*) Processing By-Products from Côte d'Ivoire

William Kwithony Disseka¹ , Martin Luther King N'gbo² , Ikpé Aristide Didier Kouamé¹
 Alahassane Sidibé¹, Bosson Jean-Aimé Assanvo¹ , Meuwiah Betty Faulet-Ahonzo¹

¹ Faculty of Biochemistry and Food Technology, Department of Food Science and Technology, Laboratory of Biocatalysis and Bioprocessing, Nangui Abrogoua University of Abidjan, Abidjan, Côte d'Ivoire. wdisseka@gmail.com / ikpe71@gmail.com / marwahamdi50@yahoo.fr / alahassanesidibe@gmail.com / assanvo26@gmail.com / bettyfaulet625@gmail.com

² Faculty of Agriculture, University of San Pedro, 01BP 1800 San Pedro 01, Côte d'Ivoire. luthereking2@gmail.com

ABSTRACT

Background: Sweet orange (*Citrus sinensis*), is a widely cultivated fruit valued globally for its high ascorbic acid content. In Côte d'Ivoire, the fruit is predominantly processed for juice production within both informal and industrial sectors. However, processing by-products—namely the peel, pulp membrane, and seeds—constitute a major fraction of the total fruit mass and are routinely discarded, representing a significant loss of valuable bioresources.

Aims: This study aimed to evaluate the valorization potential of these agricultural by-products by establishing their biochemical and phytochemical composition profiles.

Material and Methods: Processing by-products of *C. sinensis* were obtained from Abidjan, Côte d'Ivoire. Physical yield and ascorbic acid content (via DCPIP titration) were evaluated on fresh matrices, whereas oven-dried specimens (65 °C, 48 h) were pulverized for compositional analysis. Proximate parameters (moisture, ash, crude fiber, fat, protein, sugars, and Atwater caloric values) were determined following standard AOAC protocols. Phytochemical screening included spectrophotometric quantification of total phenolics, flavonoids, and condensed tannins, alongside DPPH radical-scavenging assays. Data were analyzed through one-way ANOVA with Duncan's multiple range test and principal component analysis (PCA).

Results: High moisture levels (57.28 – 76.31%) indicated high perishability of the raw by-products. On a dry matter basis, proximate concentrations varied significantly across tissues: total carbohydrates ranged from 73.4 ± 0.06 % to 93.16 ± 0.47%, crude fat from 0.2 ± 0.02 to 7.17 ± 0.04%, and crude protein from 0.98 ± 0.01 to 8.26 ± 0.03%. Additionally, ash and crude fiber contents ranged from 2.98 ± 0.13 to 6.64 ± 0.11% and 2.31 ± 0.04% to 14.16 ± 0.03% respectively, confirming high energetic value. Ascorbic acid in fresh samples ranged between 7.49 ± 1.18 and 150.36 ± 3.6 mg 100g⁻¹ fresh mass. Total phenolic contents spanned 0.39 ± 0.02 to 1.07 ± 0.00 g 100g⁻¹ DM, flavonoids ranged from 5.31 ± 0.13 to 88.57 ± 0.11 mg 100g⁻¹ DM, and condensed tannins ranged from 136.64 ± 5.02 and 194.73 ± 7.8 mg 100g⁻¹ DM.

Conclusions: Given their substantial nutritional and bioactive profiles, *C. sinensis* processing by-products represent promising, low-cost candidates for human food fortification, animal feed formulations, and cosmeceutical applications.

Keywords: *Citrus Sinensis*; Citrus By-Products; Waste Valorization; Bioactive Compounds; Antioxidant Capacity; Functional Foods.

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Corresponding author: Kouamé Ikpé Aristide Didier

E-mail: ikpe71@gmail.com

Tel. +225 (0708782507)

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1 INTRODUCTION

The word "*citrus*" is etymologically derived from the Latin "*acrumen*", which in antiquity designated trees bearing acidic fruit belonging to the family Rutaceae. This botanical family is subdivided into several genera, the most extensively cultivated of which worldwide are *Poncirus*, *Fortunella* and *Citrus* (M'hiri, 2015). Citrus fruits are among the most widely consumed globally, consumed either fresh or in processed juice form, owing to their considerable nutritional

value and distinctive organoleptic properties (Rafiq *et al.*, 2018). Global citrus production was estimated at 124,246,000 metric tonnes, of which 66,974,100 tonnes were attributable to the sweet orange (*Citrus sinensis*), which constitutes the most extensively cultivated (Food and Agriculture Organization [FAO], 2016).

In Côte d'Ivoire, citrus production has demonstrated a marked upward trajectory, increasing from an estimated

42,500 tonnes in 2013 to 66,400 tonnes in 2016 (FAO, 2016). Several species and varieties are cultivated within the country, namely mandarin (*Citrus reticulata*), pomelo (*Citrus paradisi*), lemon (*Citrus limon*), and sweet orange (*Citrus sinensis*) (FAO, 2016). The sweet orange is a fruit of considerable commercial and cultural significance to the Ivorian population, remaining generally available throughout the year, albeit subject to seasonal production variations. Sweet orange constitutes the most widely produced citrus fruit in Côte d'Ivoire with an estimated output of 43,600 tonnes, representing approximately 70% of total national citrus production in 2016 (FAO, 2016). This production, which is principally oriented towards juice extraction, represents a substantial and important source of income for agricultural communities. The industrial processing of orange juice enhances the commercial value of the raw fruit, reduces post-harvest losses, generates employment opportunities across the agri-food value chain, and provides consumers with a diversified range of processed products. Furthermore, this sector stimulates the development of agro-industrial activities and generates substantial quantities of by-products that can be valorized into high-value functional ingredients, thereby contributing to a circular bioeconomy approach.

Notwithstanding these economic opportunities, the accumulation of by-products generated during orange processing — most notably peels, seeds, and pulp residues — represents a significant environmental burden, attributable to their elevated water and organic matter contents. Uncontrolled disposal of these materials promotes microbial fermentation, the generation of malodorous compounds, the emission of greenhouse gases, and the proliferation of insect vectors and other pest organisms. The valorization of these by-products, which collectively represent approximately half of the fruit's total weight (M'hiri, 2015), therefore helps to mitigate environmental impacts while generating additional economic value. In accordance with the existing scientific literature, sweet orange by-products have been demonstrated to contain a broad spectrum of bioactive compounds, including ascorbic acid (vitamin C), phenolic acids, flavonoids, condensed tannins, carotenoids, essential oils, pectin, and dietary fiber. These constituents are associated with a wide range of antioxidants, antimicrobial, and anti-inflammatory properties, as well as broader health benefits (Mahato et al., 2018; Ousmer and Tahri, 2017; Rafiq et al., 2018). It should be noted, however, that the biochemical composition and bioactive potential of agricultural products are highly dependent upon biological and environmental determinants, including cultivar type, geographical origin, and post-harvest handling conditions (Hirri et al. 2015). Notably, Castro et al. (2020) demonstrated that orange processing by-products can be successfully transformed into flour appropriate for incorporation into food industry

formulations, including the manufacture of biscuits and other bakery products.

Valorization of by-products derived from the consumption of locally produced oranges, a thorough understanding of their compositional characteristics and functional potentialities is indispensable.

In order to facilitate the comprehensive valorization of by-products derived from the consumption and processing of locally produced oranges in Côte d'Ivoire, a thorough understanding of their compositional characteristics and functional potentialities is indispensable. Such knowledge would furthermore provide the scientific community with baseline data on the biochemical composition and nutritional potential of waste streams generated from orange cultivated under Ivorian agronomic and climatic conditions — data that are currently absent from the literature.

Against this background, the central research question guiding this investigation was formulated as follows: "What are the food utilization potentialities of by-products derived from the juice extraction of sweet orange (*Citrus sinensis*) cultivated in Côte d'Ivoire?". The general objective of this study was therefore to systematically identify and evaluate the potential applications of sweet orange juice extraction by-products for valorization purposes. More specifically, the study sought to determine the physicochemical composition of these by-products, as well as their phytochemical profiles and antioxidant activity, with a view to informing their sustainable and value-added utilization within food and agro-industrial systems.

2 MATERIALS AND METHODS

2.1 Plant Material

The plant material employed in this study comprised sweet orange (*Citrus sinensis*) fruits cultivated in Côte d'Ivoire. The oranges were procured from vendors at the central fruit market of the commune of Abobo, Abidjan, Côte d'Ivoire. All fruits selected for this study had attained commercial maturity at the time of procurement.

2.2 Sample Preparation

Three batches of oranges were independently purchased from three distinct vendors operating within the aforementioned market, thereby ensuring sample representativeness. Each batch was subjected to careful manual sorting to exclude visually defective, damaged, or immature fruits. The first, second, and third batches were designated for the first, second and third experimental replicates of each analytical test, respectively. All samples were purchased at approximately 07:00 and transported directly and without delay to the Biocatalysis and Bioprocesses

Laboratory of Nangui Abrogoua University, Abidjan, for immediate processing and subsequent analysis.

2.3 Extraction of Orange Juice By-Products

By-product fractions were obtained according to the following standardized procedure. From each batch, 50 oranges were sorted, rinsed thoroughly with tap water, and manually peeled with a stainless-steel knife to recover the peel fraction. The peeled fruits were subsequently sectioned transversely utilizing the same instrument. The resulting slices were resulting applying a citrus press to extract the juice and to isolate the pulp membrane and seed fractions. Three distinct by-product fractions were thus obtained: peel, pulp membrane, and seeds

2.4 Preparation of Dried Powder Samples

To prepare the powdered samples used required for physicochemical and phytochemical analyses, the fresh by-product fractions obtained from orange juice extraction — peel, pulp membrane, and seeds — were dried in a thermostatically controlled oven (Memmert, Germany) at 65°C for 48 hours. The dried materials were finely ground to a fine powder employing a Moulinex-type laboratory mill (Germany) and stored in hermetically sealed opaque polyethylene containers, protected from light and ambient humidity until further analysis. It should be noted that moisture content and vitamin C contents determinations were performed on fresh, undried samples to avoid heat-induced degradation of these parameters.

2.5 Determination of Mass and Yield

Following the sorting and washing stages, the individual masses of 30 good-quality oranges — randomly selected from each batch — were recorded employing an analytical balance. Upon completion of all by-product extraction steps, the masses of the three recovered by-product fractions (seeds, peel, and pulp membrane) obtained from each batch of 30 oranges were determined. The percentage by-product yield was subsequently calculated through the following mathematical expression:

$$R(\%) = \frac{M_{sp}}{M_o} \times 100$$

Where M_o denotes the total mass of the whole fruit and M_{sp} denotes the recovered mass of by-product fraction.

2.6 Proximate Composition Analysis

The proximate composition of all samples was determined in accordance with the standard methods of the [Association of Official Analytical Chemists \[AOAC\] \(1990\)](#) as detailed below.

Moisture and Dry Matter Content: Moisture content was determined gravimetrically by drying samples in a

thermostatically controlled oven at 105°C during 24 h to constant weight. Dry Matter content was subsequently calculated as: Dry Matter (%) = 100 – Moisture (%).

Ash Content: Ash content was determined by incineration of 5 g of dried sample in a muffle furnace at 550°C for 12 h. The residue allowed to cool to ambient temperature in a desiccator and subsequently weighed. Results were expressed as a percentage of dry matter.

Crude Fiber Content: Two grams of dried and finely ground sample were subjected to sequential acid–base digestion employing 50 mL of sulfuric acid solution (0.25 N) followed by 50 mL of sodium hydroxide solution (0.31 N). The insoluble residue obtained was recovered by filtration, rinsed with hot distilled water, and dried in an oven at 105°C for 8 h. The dried residue was subsequently incinerated in a muffle furnace at 550°C for 3 hours, and the difference in mass between the dried and incinerated residues was utilized to calculate crude fiber content.

Crude Fat Content: Crude fat determined by Soxhlet extraction of 10 g of dried and ground sample employing hexane as the extraction solvent over a period of 7 hours at 110°C. Results were expressed as a percentage of dry matter.

Crude Protein Content: This fraction was determined using the Kjeldahl method, with nitrogen content converted to protein content using a conversion factor of 6.25 ($N \times 6.25$). Results were expressed as a percentage of dry matter.

Total and Reducing Sugar Contents: Ethanol-soluble sugars were extracted according to the procedure described by [Martinez-Herrera *et al.* \(2006\)](#). Total sugars content was subsequently determined by the phenol–sulphuric acid colorimetric method as described by [Dubois *et al.* \(1956\)](#), whilst reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method described by [Bernfeld \(1955\)](#).

Available Carbohydrate Content: Available carbohydrate content was estimated by difference in accordance with the [FAO \(2002\)](#) methodology, applying the following formula:

Carbohydrates (% DM) = 100 - [% Protein + % Fat + % Fiber + % Ash]

Energy Value: The gross energy value was determined employing the [Atwater and Rosa \(1899\)](#) conversion factors as follows:

Energy Value (kcal/100 g DM) = $[4 \times (\% \text{ Protein}) + 9 \times (\% \text{ Fat}) + 4 \times (\% \text{ Carbohydrates})]$

2.7 Determination of Vitamin C Content

Vitamin C (ascorbic acid) content was determined according to the titrimetric method described by [Pongracz](#)

(1971). Ten grams of fresh sample was homogenized in 20 mL of a metaphosphoric acid (3% w/v) / acetic acid (8% v/v) extraction solution. The homogenate was centrifuged (Refrigerated Centrifuge TGL-16M, China) at 4,000 rpm for 20 minutes, and the resulting supernatant was collected and transferred to a volumetric flask. One milliliter of the supernatant was titrated against a standardized solution of 2,6-dichlorophenolindophenol (DCPIP). The end-point of titration was indicated by the persistence of a pale pink coloration for a minimum of 15 seconds, marked the end of the dosage. A standard ascorbic acid solution (1 mg/mL) was titrated under identical conditions for calibration purposes). The ascorbic acid content was calculated through the following equation:

$$\text{Vitamine C } \left(\frac{\text{mg}}{100\text{g}} \right) = \frac{(C_{\text{DCPIP}} \times V_{\text{eq}}) \times 5 \times 100}{m_e}$$

Where: V_{eq} denotes the volume (mL) of DCPIP solution required to titrate 1 mL of the sample supernatant; m_e denotes the mass (g) of the fresh sample; and C_{DCPIP} denotes the DCPIP concentration (0.5 g/L).

2.8 Determination of Phenolic Compounds Content

2.8.1 Total Phenolic Compounds (TPC) Extraction

Phenolic compounds were extracted utilizing 70% (v/v) methanol in accordance with the procedure described by Singleton *et al.* (1999). One gram of dried and powdered sample was homogenized in 10 mL of 70% (v/v) methanol. The resulting mixture was centrifuged at 1000 rpm for 10 min, and the pellet was recovered in a further 10 mL of 70% methanol and centrifuged again under identical conditions. All supernatants were pooled in a 50 mL volumetric flask and adjusted to volume with distilled water. The resulting solution constituted the phenolic extract employed for the determination of phenolic compounds.

2.8.2 Total Phenolic Content (TPC)

Total phenolic content (TPC) was determined using the Folin–Ciocalteu colorimetric method as described by Singleton *et al.* (1999). A volume of 1 mL of methanolic extract was combined with 1 mL of Folin-ciocalteu reagent and thoroughly homogenized. Further 3 minutes, 1 mL of aqueous sodium carbonate solution (20%, w/v) was incorporated and the volume was adjusted to 10 mL with distilled water. The mixture was incubated in the dark for 30 minutes and absorbance was measured spectrophotometrically (UV / VIS spectrophotometer, China) at 725 nm against a reagent blank. A calibration curve was constructed employing gallic acid standard solutions ranging from 0 to 1 mg/mL. Results were expressed as milligrams of

gallic acid equivalent per 100 g of dry matter (mg GAE/100 g DM).

2.8.3 Total Flavonoid Content (TFC)

Total Flavonoid Content (TFC) was determined according to the aluminium chloride colorimetric method described by Meda *et al.* (2005). To 0.5 mL of methanolic extract the following reagents were incorporated sequentially: 0.5 mL of distilled water, 0.5 mL of aluminum chloride (10% w/v), 0.5 mL of sodium acetate (1 M) and 2 mL of distilled water. The mixture was incubated in the dark for 20 minutes, and absorbance was measured spectrophotometrically at 415 nm against a reagent blank. A calibration curve was constructed using quercetin standard solutions ranging from 0 to 0.1 mg/mL. Results were expressed as milligrams of quercetin equivalent per 100 g of dry matter (mg QE/100 g DM).

2.8.4 Condensed Tannin Content

The determination of tannins was carried out according to the method described by Broadhurst *et al.* (1978). One milliliter of methanolic extract was combined with 5 mL of vanillin reagent, prepared by dissolving 50 g of vanillin and 4 mL of hydrochloric acid in 100 mL of distilled water. The mixture was incubated in the dark for 20 minutes, and absorbance was recorded spectrophotometrically at 500 nm against a reagent blank. A calibration curve was performed employing tannic acid standard solution ranging from 0 to 2 mg/mL. Results were expressed as milligrams of tannic acid equivalent per 100 g of dry matter (mg TAE/100 g DM).

2.9 Determination of Antioxidant Activity by DPPH Radical Scavenging Assay

Antioxidant activity was determined utilizing the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, adapted from the procedure described by Choi *et al.* (2002) with minor modifications. One gram of powdered sample was homogenized in 20 mL of 70% (v/v) methanol to constitute the methanol extract. Two milliliters of methanolic extract prepared at various concentrations were combined with 1 mL of freshly prepared DPPH (0.3 mM) was incubated in the dark for 30 minutes at ambient temperature. Absorbance was subsequently measured at 517 nm using a UV/VIS spectrophotometer (China). A control tube containing 1 mL of DPPH solution and 2 mL of pure methanol was prepared and read under identical conditions. Antioxidant activity (AA), expressed as the percentage inhibition of the DPPH radical, was calculated through the following formula:

$$AA(\%) = \frac{DO_c - (DO_e - DO_b) \times 100}{DO_c}$$

Where DO_c denotes the absorbance of the control tube (1 mL DPPH + 2 mL methanol); DO_e denotes the absorbance of the test tube (1 mL of DPPH + 2 mL extract at varying concentrations); DO_b denotes the absorbance of the blank tube (1 mL of methanol + 2 mL of extract at varying concentrations)

2.10 Statistical Analysis

All analytical determinations were performed in triplicate, and numerical values are expressed as the arithmetic means $\pm$ standard deviation (SD). Inter-group differences were assessed by one-way analysis of variance (ANOVA), followed by Duncan's post hoc multiple range test for pairwise mean comparisons, utilizing STATISTICA software (version 7.1). In addition, principal component analysis (PCA) was performed employing the same software to classify the three orange by-product fractions (seeds, peel, and pulp membrane) according to their biochemical and phytochemical compositions and antioxidant activity profiles, and to identify the major sources of compositional variability among the fractions.

3 RESULTS AND DISCUSSIONS

3.1 By-product Yield Performance

The mean mass of individual orange fruits was 243.27 ± 19.62 g, with a mean total by-product yield of $50.99 \pm 4.77\%$ (Table 1). The mean masses of the seeds, peel, and pulp membrane fractions were 4.44 ± 1.88 g, 48.49 ± 12.03 g, and 71.20 ± 8.15 g, respectively, yielding mean fractional by-product yields of $1.84 \pm 0.82\%$, $19.81 \pm 3.96\%$, and $29.34 \pm 3.43\%$ for seeds, peel, and pulp membrane, respectively. The total by-product yield obtained in present study (50.99%) is in close agreement with the findings of M'hiri (2015), who demonstrated that by-products generated during citrus juice extraction account for approximately half of the total fruit mass, thereby confirming the substantial by-product burden associated with orange juice processing and underscoring the importance of their systematic valorization.

Table 1. Mass and Yield of Orange By-Products

Samples	Parameters	
	Mass (g)	Yield (%)
Oranges	243.27 ± 19.62	100
Seeds	4.44 ± 1.88	1.84 ± 0.82
Peel	48.49 ± 12.03	19.81 ± 3.96
Pulp membrane	71.20 ± 8.15	29.34 ± 3.43
Total by-products	124.13 ± 15.75	50.99 ± 4.77

3.2 Biochemical Composition and Energy Value

The results of the biochemical characterization and energy value determination of the three orange (*Citrus sinensis*) by-product fractions are presented in Table 2

Moisture Content

The moisture content of the fresh by-product samples ranged from 57.28 ± 2.24 (seeds) to $76.31 \pm 1.89\%$ (pulp membrane), with a statistically significant difference observed among the three fractions at the 5% significance threshold ($p \leq 0.05$). The highest moisture content was recorded in the pulp membrane (76.31%), whilst the lowest was recorded in the seeds (57.28%). The peel fraction exhibited an intermediate moisture content of $63.10 \pm 1.54\%$. The elevated moisture contents recorded across all by-product fractions ($57.28 - 76.31\%$) are consistent with and corroborate the highly perishable nature of these materials, as previously described by Ousmer and Tahri (2017). Indeed, high moisture content in food matrices are well established as a primary driver of microbial spoilage, promoting the proliferation of deteriorative microorganisms that adversely affect both the nutritional quality and commercial viability of the product (Dossou et al., 2007). In accordance with the guidelines of the *Centre Technique de Coopération Agricole et Rurale* [CTA] (2008), drying constitutes an effective and widely applicable strategy for the preservation of perishable food materials. The substantially reduced moisture contents recorded in the dried powder samples ($3.02 \pm 0.16 - 7.86 \pm 0.15\%$) — all of which fall below the critical threshold of 12%

Table 2. Biochemical Composition and Energy Value of Orange By-Products

Biochemical parameters	Seeds	Peel	Pulp membrane
Moisture (RF) (%)	57.28 ± 2.24^a	63.10 ± 1.54^b	76.31 ± 1.89^c
Humidity (DM) (%)	3.02 ± 0.16^a	5.87 ± 0.48^b	7.86 ± 0.15^c
Ash (% DM)	2.98 ± 0.13^a	6.64 ± 0.11^b	3.35 ± 0.47^a
Fiber (% DM)	5.88 ± 0.02^b	14.16 ± 0.03^c	2.31 ± 0.04^a
Fat (% DM)	7.17 ± 0.04^c	2.78 ± 0.09^b	0.2 ± 0.02^a
Protein (% DM)	8.26 ± 0.03^c	3.02 ± 0.04^b	0.98 ± 0.01^a
Total sugars ($g \cdot 100g^{-1}$ DM)	0.38 ± 0.03^a	1.15 ± 0.05^b	1.86 ± 0.02^c
Reducing sugars ($mg \cdot 100 g^{-1}$ DM)	56.9 ± 2.20^a	181.47 ± 10.96^b	242.62 ± 9.66^c
Assimilable carbohydrates (% DM)	75.70 ± 0.13^b	73.4 ± 0.06^a	93.16 ± 0.47^c
Energy value ($Kcal \cdot 100 g^{-1}$ DM)	400.36 ± 0.61^c	330.71 ± 0.82^a	378.32 ± 1.79^b

Note: The values obtained in the table are the mean $\pm$ standard deviation of 3 trials. The different letters on the same line indicate that the values of the physicochemical parameters of the three (03) orange by-products are not statistically identical ($p < 0.05$).

— confirm that the drying treatment confers extended shelf life upon the by-product fractions (Adebowale *et al.*, 2008). Furthermore, the lower moisture content obtained at the level in the seed fraction may be attributed to the inherently hydrophobic nature of lipid-rich matrices. Analogous observations have been reported for sesame seeds by Disseka (2019).

Ash Content

Statistically significant differences ($p \leq 0.05$) in ash content were observed among the three by-product fractions. The highest ash content was recorded in the peel fraction ($6.64 \pm 0.11\%$), whilst no statistically significant difference was observed between the ash contents of the pulp membrane ($3.35 \pm 0.47\%$) and the seeds ($2.98 \pm 0.13\%$) ($p > 0.05$). Ash content is recognized as an indicator of the total mineral content of a food material (Dewey and Brown, 2003). Dietary minerals are essential and indispensable to numerous physiological functions, including skeletal mineralization, dental integrity, muscle contraction, and enzymatic catalysis (Toukara *et al.*, 2017). The ash content recorded for the peel in the present study exceeded values previously reported for mandarin (*Citrus reticulata*) peel (4.68%) by Ghanem *et al.* (2012) and for lemon (*Citrus limon*) peel (4.06%) by Magda *et al.* (2008), suggesting that *C. sinensis* peel cultivated under Ivorian agronomic conditions may constitute a particularly mineral-rich by-product fraction.

Crude Fiber Content

Statistically significant differences in crude fiber content were observed among all three by-product fractions ($p \leq 0.05$). The highest fiber content was recorded in the peel fraction (14.16%), whilst the lowest was recorded in the pulp membrane (2.31%). Dietary fiber plays a pivotal role in the maintenance of intestinal transit and digestive health, and has been extensively associated with a reduced risk of cardiovascular disease, improved glycemic regulation, and the maintenance of a healthy commensal intestinal microbiota (Peter and Tolulope, 2015). The particularly elevated fiber content of the orange peel fraction, as independently confirmed by the United States Department of Agriculture (USDA, 2019) supports its potential application as a dietary fiber-enriching ingredient in functional food formulations, with documented preventive and therapeutic implications for nutritional disorders including constipation, colorectal cancer, type 2 diabetes, and obesity (Nunez). Beyond human nutrition, the incorporation of orange peel by-products into ruminant feed substrates — such as wheat straw — has been reported to enhance digestibility, owing to the fermentable fiber fraction present in citrus peels (Bampidis and Robinson, 2006).

Crude Fat Content

Statistically significant differences in crude fat content were observed among the three fractions ($p \leq 0.05$). The highest lipid content was recorded in the seeds ($7.17 \pm 0.04\%$), while the lowest was recorded in the pulp membrane ($0.2 \pm 0.02\%$). The overall lipid contents of the samples ranged from 0.20 to 7.17% , reflecting the characteristically low-fat content of fruit-derived matrices, consistent with the general nutritional composition of fruits as documented by FAO (2003) and in contrast to oilseeds, which are recognized as major sources of edible, industrial, and pharmaceutical lipid fractions (Ines *et al.*, 2010). The lipid fraction of the seed by-product is of particular interest from a cosmetic and nutraceutical perspective, given that citrus seed oils have been reported to contain appreciable concentrations of vitamin E — a lipid-soluble antioxidant with well-documented beneficial effects on dermal and hair health (Ines *et al.*, 2010).

Crude Protein Content

Statistically significant differences in crude protein content were observed among the three by-product fractions ($p \leq 0.05$). Protein contents ranged from $0.98 \pm 0.01\%$ (pulp membrane) to $8.26 \pm 0.03\%$ (seeds), with the peel recording an intermediate value of $3.02 \pm 0.04\%$. The crude protein content of the seed fraction (8.26%) substantially exceeded the 3.1% reported by Akpata and Akubor (1999) for both shelled and unshelled sweet orange (*Citrus sinensis*) seed flours, suggesting varietal and environmental influences on seed protein accumulation. The appreciable protein content of the seed fraction presents promising valorization potential for incorporation into food formulations targeted at specific dietary populations — most notably individuals adhering to plant-based or vegan dietary regimens, in which animal-derived protein sources are excluded — as a means of meeting recommended protein dietary reference values (12 – 15% of total energy intake). Additionally, the seed fraction could serve as a cost-effective supplementary protein source in animal nutrition formulations.

Available Carbohydrate Content

Statistically significant differences in available carbohydrate content were observed among the three by-product fractions at the 5% significance threshold ($p \leq 0.05$). The highest carbohydrate content was recorded in the pulp membrane fraction ($93.16 \pm 0.47\%$). This value considerably exceeds the carbohydrate contents reported for commonly utilized cereal grain ingredients, including millet (77.89 to 89.19%) and maize (71.87 to 85.64%) (N'guessan *et al.*, 2014). Given that cereals represent the predominant carbohydrate source in conventional livestock feed

formulations, the high carbohydrate content of the orange pulp membrane fraction suggests its considerable potential as an alternative or complementary carbohydrate-rich feed ingredient for ruminant nutrition, serving as a partial substitute for millet and maize in compound feed formulations (Arthington *et al.*, 2002).

Energy Value and Soluble Sugar Contents

Statistically significant differences in energy values were observed among the three by-product fractions at the 5% significance threshold ($p \leq 0.05$). The highest energy value was recorded in the seed fraction (400.36 ± 0.61 Kcal/100g), followed by the pulp membrane (378.32 ± 1.79 Kcal/100g) and the peel (330.71 ± 0.82 Kcal/100g). The comparatively elevated energy value of the seed fraction is attributable to its significantly higher crude lipid and protein contents relative to the other fractions, given the well-established higher caloric density of lipids (9 kcal/g) and protein (4 kcal/g) compared with carbohydrates. The energy values recorded across all three by-product fractions suggest that these materials may be considered energy-dense concentrate feeds for ruminant nutrition, presenting a viable substitute for conventional cereal-based feed ingredients (Arthington *et al.*, 2002). Furthermore, given that the energy requirement for the maintenance of young domestic animal species has been estimated at approximately 120 kcal/kg body weight (Momar, 1993), the energy contributions of these by-product fractions may be considered nutritionally significant in the context of livestock feeding strategies.

Statistically significant differences were additionally observed in the ethanol-soluble sugar contents — both total and reducing — among the three fractions ($p \leq 0.05$). The highest total sugar content was recorded in the pulp membrane (1.86 ± 0.02 g/100 g), which also exhibited the highest reducing sugar content (242.62 ± 9.66 mg/100 g). The lowest reducing sugar contents were recorded in the peel (181.47 mg/100 g) and seeds (56.90 ± 10.96 mg/100 g), respectively.

3.3 Phytochemical Composition

The vitamin C, total phenolic, flavonoid, and condensed tannin contents the three orange (*Citrus sinensis*) by-product fractions — pulp membrane, peel, and seeds — are presented in Table 3.

Vitamin C Content

Statistically significant differences in vitamin C content were observed among the three fractions at the 5% significance threshold ($p \leq 0.05$). The highest vitamin C content was recorded in the peel fraction (150.36 ± 3.6 mg/100g), whilst the lowest was observed at the seeds (7.49 ± 1.8 mg/100g). The pulp membrane exhibited an intermediate

Table 3. Phytochemical Composition of Orange By-Products

Antioxidant parameters	Seeds	Peel	Pulp membrane
Vitamin C (mg/100 g)	7.49 ± 1.8^A	150.36 ± 3.6^C	49.66 ± 3.60^B
Total polyphenols (g EAG/100 g DM)	0.39 ± 0.02^A	1.07 ± 0.00^C	0.72 ± 0.00^B
Flavonoids (mg EQ/100g DM)	5.31 ± 0.13^A	88.57 ± 0.46^C	19.33 ± 1.69^B
Tannins (mg EAT/100g DM)	165.33 ± 4.65^B	136.64 ± 5.02^{AB}	194.73 ± 7.8^C

Note: The values obtained in the table are the mean $\pm$ standard deviation of 3 trials. The different letters on the same line indicate that the phytochemical parameter values of the three (03) orange by-products are not statistically identical ($p < 0.05$).

vitamin C content of 49.66 ± 3.60 mg/100g. The markedly elevated vitamin C content of the peel fraction relative to the pulp membrane and seeds may be attributable to the well-documented strong positive correlation between ascorbic acid and flavonoid content, with a correlation coefficient of 0.99 observed in the present study. Given its particularly high ascorbic acid content, orange peel presents considerable potential as a cost-effective and accessible natural source of vitamin C in food fortification and functional food applications, offering a viable alternative to synthetic vitamin C supplementation within the food industry.

Total Phenolic Content (TPC)

Statistically significant differences in total phenolic content were observed among the three by-product fractions at the 5% significance threshold ($p \leq 0.05$). The highest TPC of the peel fraction is consistent with the well-established natural protective function of phenolic compounds, which are characteristically synthesized and accumulated in greater concentrations in the outer integuments and peels of plant tissues, where they serve as primary chemical defense compounds against biotic and abiotic stressors (Ghosh, 2013). The phenolic content recorded for the peel in the present study was higher than that reported by Magda *et al.* (2008) for *Citrus reticulata* peel (780 mg EAG/100g DM), but lower than the value of 2,520 mg EAG/100g DM obtained by Ghanem *et al.* (2012) for *Citrus limon* peel. The TPC of the seed fraction (390.09 mg EAG/100g DM) considerably exceeded values previously reported by Ousmer and Tahri (2017) for aqueous and ethanolic extracts of *Citrus sinensis* and *Citrus aurantium* seeds. Such discrepancies in polyphenol quantification are commonly attributed to differences in extraction methodology and solvent polarity (Dent, 2013), as well as to a range of intrinsic and extrinsic parameters including cultivar type, post-harvest storage conditions, environmental growing conditions, fruit maturity at harvest, and the genetic background of the plant material (Lagha-benamrouche and Madani, 2013). The incorporation

of phenolic-rich by-product fractions into food formulations may be of particular benefit for nutritionally vulnerable population groups — including children — by providing endogenous antioxidant protection against allergenic anti-nutrient activity and lipid oxidation-associated rancidity.

Total Flavonoid Content (TFC)

Statistically significant differences in TFC were similarly observed among the three fractions ($p \leq 0.05$). The highest flavonoid content was recorded in the peel (88.57 ± 0.46 mg QE/100 g DM), whilst the lowest was recorded in the seeds (5.31 ± 0.13 mg QE/100 g DM). The pulp membrane exhibited an intermediate flavonoid content of 19.33 ± 1.69 mg QE/100 g DM. The predominance of in the peel fraction relative to the pulp membrane and seeds could be attributable to the well-established synergistic relationship between flavonoids and vitamin C; flavonoids are recognized to reinforce and stabilize ascorbic acid by inhibiting its oxidative degradation, thereby conferring venotonic and vascular protective effects on the plant tissue (Miller & Rice-Evans, 1997). This distribution pattern of flavonoids across orange fractions is consistent with the observations of Ousmer and Tahri (2017) for *C. sinensis* and *C. aurantium* in Algeria. The flavonoid content recorded for orange peel in the current study was higher than that reported for *C. sinensis* (45.56 mg EQ/100g DM) and substantially lower than the 320.8 mg EQ/100g DM for *C. aurantium* from aqueous extracts by the same authors. This variability in flavonoid composition has been attributed to factors including harvest time, the anatomical part of the plant sampled, and interspecific genetic differences (Al-Anbari & Hasan, 2015). The consumption of food products enriched with orange by-products may therefore contribute to the neutralization of reactive oxygen species, the enhancement of ascorbic acid bioavailability, and the prevention of oxidative stress-associated conditions, including certain forms of skin cancer (Rodrigues & Pintado, 2024).

Condensed Tannin Content

Statistically significant differences in condensed tannin content were observed among the three by-product fractions at the 5% significance threshold ($p \leq 0.05$). The highest tannin content was recorded in the pulp membrane (194.73 ± 7.80 mg TAE/100 g DM), followed by the seeds (165.33 ± 4.65 mg TAE/100 g DM), whilst the lowest tannin content was recorded in the peel fraction (136.64 ± 5.02 mg TAE/100 g DM). The tannin content of the pulp membrane recorded in the present study considerably exceeded the value of 46.3 mg TAE/100 g DM reported for orange peel by Oluremi et al. (2007), underscoring the notable variability in tannin accumulation across different orange fractions and between studies. Condensed tannins present in ruminant diets at low to moderate concentrations have been widely demonstrated to

exert beneficial rather than detrimental nutritional effects. Several studies have established that condensed tannins at appropriate dietary inclusion levels play an important role in enhancing the nutritional value of feed and the quality of animal products, primarily through their capacity to protect dietary protein from excessive ruminal fermentation, thereby increasing the flow of rumen-undegraded protein to the small intestine (Mueller-Harvey et al., 2019; Waghorn, 2008). Beyond animal nutrition, the tannin-rich fractions of the orange by-products characterized in the present study may present prospective applications in both human and animal food systems, attributable to their documented biological activities, including anti-diarrheal, anti-inflammatory, antioxidant, antiparasitic, and antibacterial properties.

3.4 Antioxidant Activity

The antioxidant activity of the methanolic extracts of the three orange by-product fractions — seeds, peel, and pulp membrane — increased proportionally with increasing extract concentration, consistent with a dose-dependent radical scavenging mechanism. The half-maximal inhibitory concentrations (IC_{50}) of each extract against the DPPH free

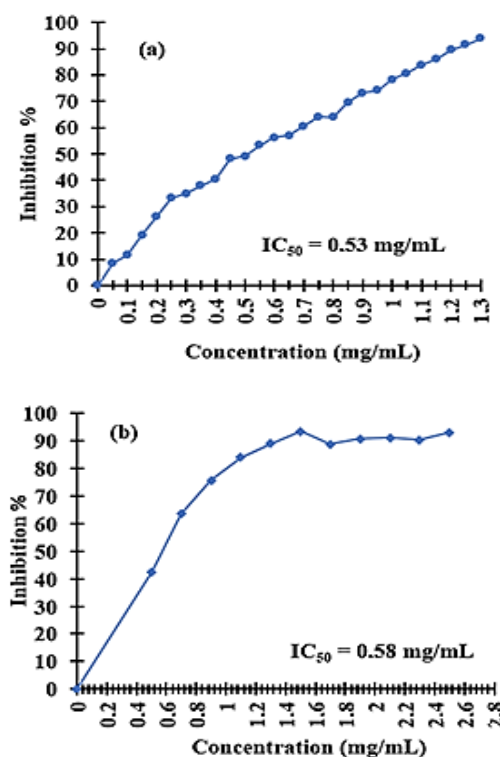


Figure 1. Percentages of DPPH Inhibition as a Function of Sample Concentrations

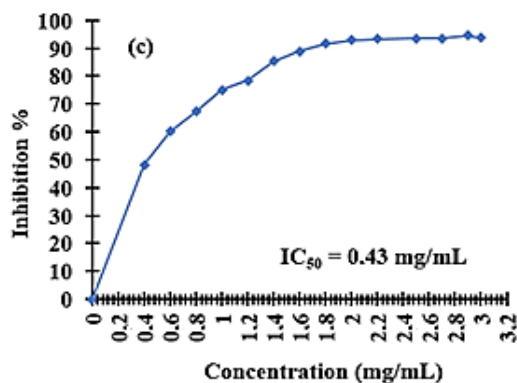


Figure 1. (Continued)

radical were determined graphically by projection onto the corresponding inhibition curves (Figure 1a, 1b and 1c). The lowest IC_{50} value was recorded for the peel extract (0.43 mg/mL), indicating superior radical scavenging capacity, followed by the pulp membrane ($IC_{50} = 0.53$ mg/mL) and the seeds ($IC_{50} = 0.58$ mg/mL).

In accordance with the convention established by Kholkhal (2014), a lower IC_{50} value is indicative of greater antioxidant potency, as it corresponds to the concentration of extract required to neutralize 50% of the available DPPH radicals. Consequently, the peel fraction demonstrated the most potent antioxidant capacity among the three fractions evaluated. This superior performance may be attributed to the strong positive correlations observed between, on the one hand, vitamin C content and antioxidant activity, and on the other, flavonoid content and antioxidant activity — both of which were highest in the peel fraction. The IC_{50} value recorded for the peel extract in the present study was higher than the 0.102 mg/mL reported by Ousmer and Tahri (2017) for *C. sinensis* peel extracts from Algeria, suggesting that differences in solvent, extraction methodology, cultivar, or environmental conditions may influence antioxidant potency. Conversely, the peel IC_{50} recorded herein was lower than the 0.564 mg/mL reported by Syakirah-Zulkifli *et al.* (2012), indicating that the by-products characterized in the present study possess comparatively stronger radical scavenging activity than those examined by the latter authors. The regular consumption of food products derived from these orange by-products may therefore contribute to the attenuation of oxidative stress by inhibiting lipid peroxidation chain reactions and protecting cellular constituents from free radical-mediated damage.

3.5 Principal Component Analysis (PCA)

3.5.1 PCA of biochemical parameters

Principal component analysis of the biochemical composition data of the three orange by-product fractions

yielded three distinct compositional groupings (Figure 2: a-b). The results indicate that crude protein and crude lipid contents were predominantly associated with the seed fraction, whilst the peel fraction was characterized by the highest crude fiber and ash contents. Available carbohydrates and ethanol-soluble sugars were primarily associated with the pulp membrane fraction. The first two principal component axes (F1 and F2) collectively accounted for all variance in the biochemical dataset, as represented by the PCA correlation circle (Figure 2: a-b), confirming the discriminatory power of the biochemical parameters in differentiating among the three by-product fractions.

3.5.2 PCA of Phytochemical Parameters

The first two principal component axes (F1 and F2) collectively accounted for the totality of variance in the phytochemical dataset of the orange by-product fractions (Figure 2: c-d). The F1 axis alone explained 87.26% of the total variability, whilst the F2 axis accounted for the remaining 12.74%. Graphical representation of the phytochemical parameter loadings revealed that the peel fraction was characterized by the highest concentrations of vitamin C, total phenolic compounds, and flavonoids, confirming its status as the phytochemically richest fraction among the by-products examined. In contrast, the highest condensed tannin content was associated with the pulp membrane fraction. Notably, none of the phytochemical variables measured in the present study were positively correlated with the seed fraction, suggesting that the seed's nutritional value resides primarily in its biochemical composition — notably its protein and lipid contents — rather than its phytochemical profile.

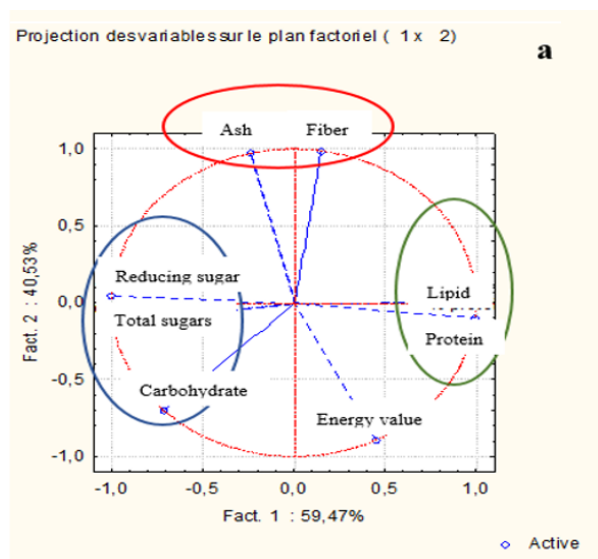
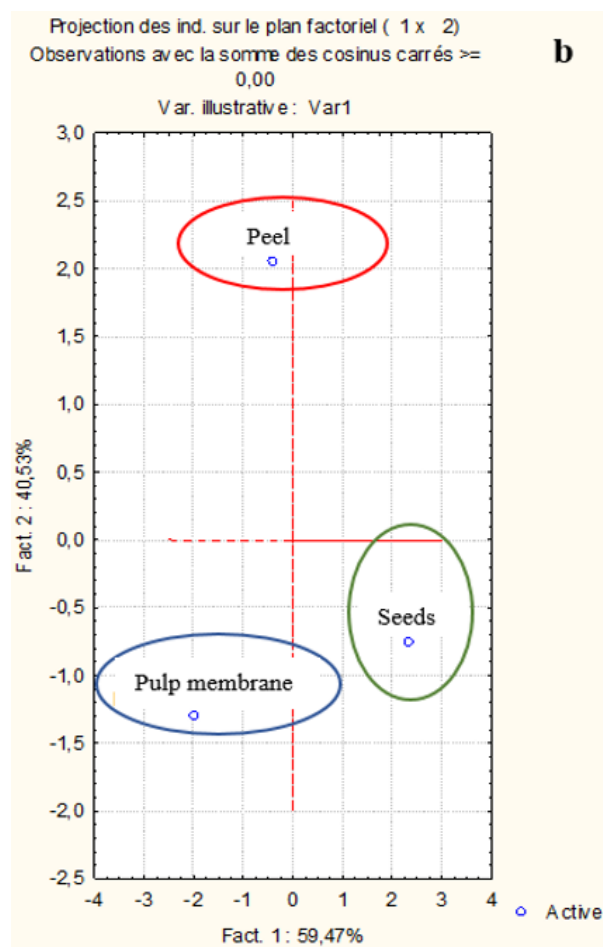


Figure 2. Principal Component Analysis (PCA) of Biochemical and Phytochemical Constituents

(a) Distribution of biochemical constituents of samples



(b) Sample Distribution

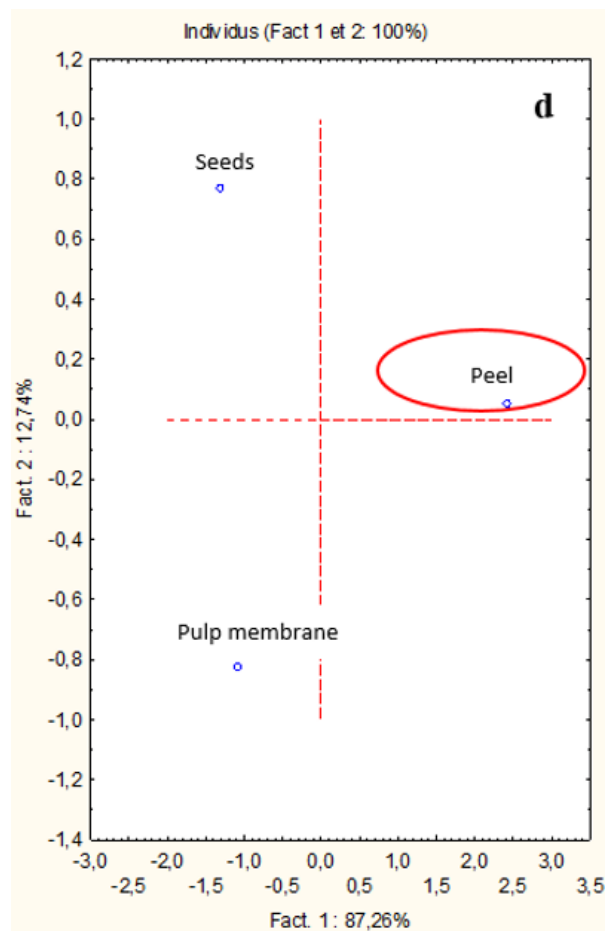
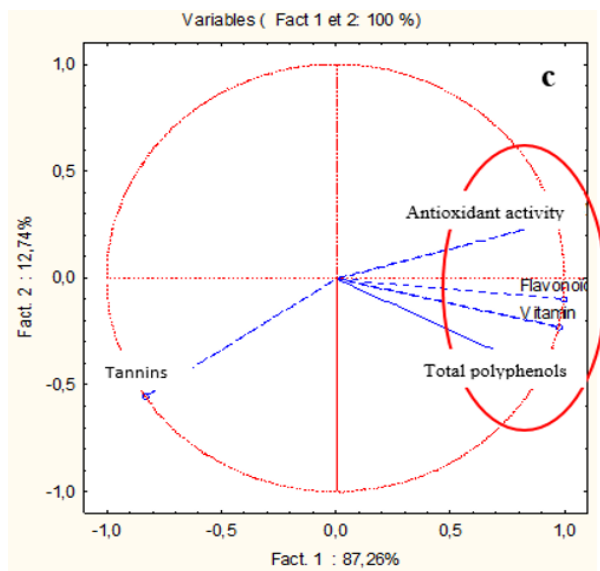


Figure 2. (Continued)

(d) Sample Distribution



(c) Distribution of phytochemical constituents of samples

4 CONCLUSIONS

The present study set out to systematically identify and characterize the physicochemical, biochemical, and phytochemical composition of by-products generated during the extraction of juice from sweet orange (*Citrus sinensis*) cultivated in Côte d'Ivoire, with a view to evaluating their potential for valorization in food and non-food applications. The collective findings of this investigation demonstrate that the three by-product fractions examined — seeds, peel, and pulp membrane — are characterized by elevated moisture contents in their fresh state and collectively constitute valuable and nutritionally diverse sources of macronutrients, including crude lipids, available carbohydrates, crude proteins, and dietary fiber, as well as bioactive phytochemical compounds endowed with significant antioxidant activity.

More specifically, the compositional analysis revealed a clearly differentiated distribution of nutrients and bioactive constituents across the three fractions. The peel fraction was

distinguished by the highest contents of ash, crude fiber, vitamin C, total phenolic compounds, and flavonoids, and demonstrated the most potent antioxidant capacity as assessed by the DPPH radical scavenging assay. The pulp membrane fraction exhibited the highest concentrations of ethanol-soluble sugars — both total and reducing — available carbohydrates, and condensed tannins, rendering it a particularly carbohydrate-dense by-product fraction. The seed fraction was characterized by the highest crude protein and crude lipid contents, thereby conferring upon it the highest gross energy value among the fractions examined.

Collectively, these findings confirm that the by-products derived from sweet orange juice extraction in Côte d'Ivoire represent a largely underexploited reservoir of nutritional and functional value. Their systematic valorization presents significant prospects for application across multiple sectors, including human food fortification and functional food product development, animal nutrition as energy- and protein-rich feed supplements, and non-food industries such as cosmetics — given the vitamin E-rich lipid fraction of the seeds — and pharmaceutical or nutraceutical applications, on the basis of their documented antioxidant, anti-inflammatory, and antimicrobial bioactive profiles.

These results provide a foundational scientific dataset for the valorization of locally produced orange by-products under Ivorian agronomic conditions and contribute to the broader global effort towards reducing food processing waste through the principles of circular bioeconomy. Future research is recommended to investigate the extraction, purification, and stability of individual bioactive compounds from these fractions, as well as their safety profiles, bioavailability, and technological performance in specific food and non-food formulation contexts.

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