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# Bioactive Ingredients of Custard Apple (*Annona cherimola* Mill.) by-Products as an Industrial Interest for the Development of Products with High Added Value †

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**Abstract:** Custard apple (*Annona cherimola* Mill.) is a tropical fruit source of bioactive compounds whose main producer worldwide is Andalusia, Spain. Because of its processing, the food industry generates large amounts of by-products, such as peels and seeds. These by-products are rich in phenolic compounds with high antioxidant, anti-inflammatory and anti-aging powers. The objective of this work is to evaluate, by different *in vitro* methods, the antioxidant and anti-inflammatory potential of cherimoya by-products rich in phenolic compounds for the development of cosmeceuticals. In addition, the major phenolic compounds present in the custard apple peel and seed samples were characterized by HPLC-ESI-QTOF-MS. The results showed that both the peel and seed of custard apple have a strong potential against oxidative stress and inflammation. Its phytochemical profile due to the presence of phenolic compounds (catechin, epicatechin, rutin, quinic acid, vanillic acid, etc.) makes both industrial by-products attractive bioactive ingredients for the manufacture of functional food and cosmeceuticals.

**Keywords:** custard apple; by-products; antioxidant; HPLC-ESI-QTOF-MS and phenolic compounds



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## 1. Introduction

Many fruits and vegetables are closely related to the prevention of serious health problems. This correlation is mainly due to the presence of phytochemicals with antioxidant and anti-aging properties that help reduce diseases related to oxidative stress and the aging of the body [1].

In recent years, tropical fruits have acquired great value worldwide thanks to their sensory characteristics and nutritional values. Among tropical fruits, the cherimoya stands out. The custard apple, *Annona cherimola* Mill., is a tropical fruit widely known for its exquisite flavor and usefulness in ancient medicine. Spain, specifically Andalusia, is one of the main producers of cherimoya worldwide thanks to its tropical climate, which is fundamental for its cultivation. The pulp of this fruit is mainly rich in sugars, vitamins, amino acids and phenolic compounds such as procyanidins [2,3]. However, recent studies have established that the non-edible parts of the cherimoya, such as the peel, seeds and leaves, are potential sources of phenolic acids, flavonoids and phytosterols, among others [4–6]. Due to the presence of these phenolic compounds, custard apple by-products can exert an antioxidant, anti-inflammatory and anti-aging effect, being an interesting option to inhibit the negative effects on the organism because of oxidative stress [2,7]. The use of cherimoya by-products could be a good option for the development of pharmaceuticals and high-value-added products while reducing the large amounts of waste generated by their industrial processing, thus reducing the negative impact on the environment.

Recently, interest in skincare has increased due to the harmful effects of exposure to ultraviolet (UV) radiation from the sun. The factors that favor the appearance of signs of aging on the skin can be both extrinsic factors (UV radiation, particles in suspension, irritating substances, etc.) and intrinsic factors (genetic factors, oxidative stress, expression of enzymes that degrade the cellular matrix, etc.) [8]. As far as we know, phenolic compounds have a potent antioxidant capacity and numerous health benefits, but few studies have focused on their therapeutic potential on human skin. Some studies point out the efficacy of phenolic compounds in the prevention of different skin disorders thanks to their antioxidant activity as a protector against UV radiation and their anti-inflammatory and antimicrobial properties [8,9]. Therefore, this study identifies custard apple peel and seeds as potential sources of bioactive ingredients beneficial to skin health and of great interest for their application in the cosmetic industry.

The main objective of this study is to evaluate the therapeutic potential of the peel and seeds of cherimoya grown in Andalusia. For this purpose, an identification of the phenolic composition was carried out by HPLC-ESI-qTOF-MS. Different in vitro assays were performed to evaluate the phenolic profile of the samples. First, the phenolic content was determined by the Folin–Ciocalteu method. Then, the antioxidant capacity of the samples was evaluated by different assays with different mechanisms: single electron transfer reactions (SET) and hydrogen atom transfer reactions (HAT). As methods with SET reactions, the ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) methods were carried out, and as methods with HAT reactions, the oxygen radical absorbance capacity (ORAC) method was carried out. In addition, the radical oxygen species (ROS) uptake capacity was also evaluated. Finally, the ability of the samples to inhibit different enzymes related to skin aging, such as acetylcholinesterase, hyaluronidase, collagenase, elastase, tyrosinase and xanthine oxidase, was determined.

## 2. Materials and Methods

### 2.1. Extraction of Custard Apple Agro-industrial By-products

Custard apple peel and seed were weighed and dried at 80 °C for 9 h in an oven. Once the custard apple by-products were dried, they were ground to minimize particle size.

Next, extraction was carried out by a solid-liquid extraction technique. For this purpose, ten grams of ground custard apple by-products were weighed into glass jars, and 100 mL of ethanol and water were added as GRAS solvent in an 80:20 (*v*:*v*) ratio. A magnetic stirrer was introduced into the flasks at 170 rpm at 45 °C for 2 h to ensure mixing, and all supernatants obtained were collected, filtered and concentrated in a rotary evaporator.

### 2.2. HPLC-ESI-qTOF-MS Analysis

Custard apple seed and peel extracts at 5000 mg/L were analysed using high-performance liquid chromatography (ACQUITY UPLC H-Class System; Waters, Mil-ford, MA, USA) coupled to electrospray (ESI) quadrupole time-of-flight mass spectrometry. The separation was performed in an ACQUITY UPLC BEH Shield RP18 Column, 130 Å, 1.7 µm, 2.1 mm × 150 mm at a flow rate of 0.7 mL/min using a volume injection of 10 µL.

The mobile phases were water-acidified with acetic acid 0.5% v/v (A) and acetonitrile (B). All operating parameters set are collected here: source temperature 100 °C; scan duration 0.1 s; resolution 20,000 FWHM; desolvation temperature 500 °C; desolvation gas flow 700 L/h; capillary voltage 2.2 kV; cone voltage 30 V; cone gas flow 50 L/h.

### 2.3. In Vitro Assays for Bioactive Determination of Phenolic Compounds in Custard Apple By-Products

All undermentioned assays performed were carried out on a Synergy H1 Monochromator-Based Multi-Mode Microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA).

#### 2.3.1. Evaluation of In Vitro Antioxidant Potential

The antioxidant properties of custard apple by-product extracts were evaluated by FRAP, TEAC and ORAC assays. Total phenolic content (TPC) was also determined according to the Folin–Ciocalteau method. The FRAP, TEAC and TPC assays are based on the measurement of absorbance, with wavelengths of 593, 734 and 760 nm. On the other hand, the ORAC method is based on the measurement of fluorescence, with excitation and emission wavelengths of 485 and 520 nm, respectively. All measurements were performed in triplicate.

#### 2.3.2. Evaluation of Free Radical and ROS Scavenging Potential

A colorimetric method was used to evaluate superoxide, while a fluorometric method was used to evaluate nitric oxide and HOCl. The results were expressed as the necessary concentration of custard apple by-product extract needed to inhibit ROS/RNS formation by half (IC50).

#### 2.3.3. Evaluation of Enzymatic Inhibition Potential

All tests were carried out in triplicate, and the IC50 was calculated using different concentrations of custard apple by-product extracts.

## 3. Results and Discussion

### *3.1. Characterization of Custard Apple Seed and Peel Extracts by HPLC-ESI-qTOF-MS*

Fifty-five compounds were tentatively identified, some of which were identified for the first time in both custard apple by-products.

The compounds were ordered according to their retention times, together with $m/z$, molecular formula, name and, where appropriate, quantification values.

Both seed and skin extracts showed a diverse phenolic composition. The main compounds identified were organic acids, terpenoids, phytohormones, flavones, glycosylated flavan-3-ols, flavanones, isoflavans and lignans.

Many compounds, such as poncirin (flavanone), miconoside A (flavanone), kaempferol rutinoside, rutin (flavan-3-ol), and chemical and citric acids, among others, had been previously identified. However, some compounds in the skin and seed of cherimoya had never been reported in this species but in other species of the Annonaceae family, such as the glycosidic derivative cleistrioside 5 or some lignan derivatives. Other compounds identified have been found in several plant matrices, such as litsaglutinan A, a phytohormone derived from abscisic acid, or osmanthuside B, a glycosidic phenylethanoid, among others.

### *3.2. Evaluation of Total Phenol Content and Antioxidant Capacity Using TEAC, FRAP and ORAC*

Table 1 shows the phenolic content and antioxidant capacity of each by-product. In the results, slight differences between custard apple seed and peel can be appreciated, especially for the FRAP and ORAC methods, where the seed showed a better TPC value than the peel. In the TEAC test, a greater contrast was observed between the seed and the peel, with the former standing out.

For custard apple seed, the results may be a consequence of a greater presence of phenolic compounds than in the peel. In other plant matrices, a direct relationship between phenolic content and antioxidant activity has been demonstrated [10]. However, we found hardly any studies in the literature on the antioxidant properties of custard apple by-products.

TPC, FRAP and ORAC values show that both by-products have a valuable phenolic richness, which could be of interest to the food and pharmacological industry.

**Table 1.** Evaluation of total phenolic content, antioxidant capacity and radical scavenging ability of custard apple by-product extracts.

| Methodology | CAS Extract | CAP Extract |
|---|---|---|
| **TPC (mg GAE/g DE)** | 30.4 ± 0.7 | 28.771 ± 0.008 |
| **FRAP (mmol $Fe^{2+}$/g DE)** | 0.292 ± 0.005 | 0.27 ± 0.01 |
| **TEAC (µmol TE/g DE)** | 171 ± 2 | 130.0 ± 0.4 |
| **ORAC (mmol TE/g DE)** | 0.368 ± 0.005 | 0.324 ± 0.009 |
| **$\cdot O_2^-$ (mg/L) [1]** | N. A. | N. A. |
| **HOCL (mg/L) [1]** | 11 ± 2 | 28 ± 4 |
| **·NO (mg/L) [1]** | 1.5 ± 0.2 | 11.8 ± 0.3 |
| **Collagenase (mg/L) [1]** | 660 ± 20 | 690 ± 30 |
| **Hyaluronidase (mg/L) [1]** | 170 ± 10 | 460 ± 20 |
| **Elastase (mg/L) [3]** | 800 ± 60 | 410 ± 30 |
| **Tyrosinase (mg/L) [1]** | 157.1 * | 120 ± 10 |
| **AChE (mg/L) [2]** | 26 ± 4 | 12 ± 1 |
| **XOD (mg/L) [1]** | 7.2 ± 0.7 | 4.4 ± 0.4 |

Data are means ± standard deviation (n = 3) *. [1] $IC_{50}$, i.e., quantity (mg/L) of custard apple peel and seed extract needed to decrease the amount of the reactive species in the assay by 50%. [2] Percentage of inhibition at 111.11 mg/L (maximum concentration tested). [3] $IC_{25}$, i.e., quantity (mg/L) of custard apple peel and seed extract needed to decrease by the amount of the reactive species in the assay by 25%. * No standard deviation; only one test was carried out in good terms (n = 1).

### *3.3. Evaluation of Free Radical and ROS/RNS Scavenging Potential*

To fully determine the antioxidant profile of custard apple by-product extracts, their ability to scavenge free radicals was evaluated using some reactive oxygen and nitrogen species (ROS and RNS).

Table 1 shows the amount of custard apple by-product necessary to inhibit half of the concentration of the reactive species (IC50). The results show the high anti-radical capacity of the seed. The presence of poncirin in the seed could explain its IC50 values for -NO and HOCl since poncirin has previously been shown to be a natural flavonoid that reduces oxidative damage by inhibiting the effects of different reactive species [11]. Despite this, it was not possible to evaluate the superoxide species, coinciding with other studies previously performed.

### *3.4. Evaluation of Enzymatic Inhibition Capacity*

In the skin, an enzyme imbalance due to an overproduction of oxidative reactions can lead to the degradation of the extracellular matrix (ECM) and different fibers such as collagen, hyaluronic acid and elastin, affecting the integrity of the skin [12]. In addition, an excess of melanin, as a consequence of UV light, can lead to disorders related to skin darkening. The enzyme tyrosinase, involved in melanin biosynthesis, is closely related to these hyperpigmentation phenomena [13].

On the other hand, the enzymes acetylcholinesterase (AChE) and xanthine oxidase (XOD) are involved in neurodegenerative mechanisms, promoting oxidative stress in the brain and nervous system [14,15].

The inhibition of collagenase, hyaluronidase, elastase, tyrosinase, AChE and XOD enzymes could be an interesting strategy for the treatment of various pathologies.

Table 1 shows the results obtained, expressed as IC50 values. The inhibitory effect of custard apple peel against XOD is noteworthy. Other noteworthy values are those of both by-products against tyrosinase and seed extract against hyaluronidase.

These results could reveal that custard apple by-products are an important source of neuroprotective compounds with great potential against the mechanisms involved in skin aging.

## 4. Conclusions

In conclusion, both custard apple seed and peel can be considered sources of interesting bioactive compounds for the food, pharmaceutical and/or cosmetic industries. However, the custard apple seed should be highlighted for its higher phenolic content and greater antioxidant capacity as a free radical scavenger. Both by-products exerted potent activity against the enzyme XOD and hyaluronidase. Therefore, custard apple by-products could be used in the industry for their therapeutic properties and under a circular economy with the objective of not generating waste.

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