cosmetics

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From waste to beauty: Evaluation of the utilization of by-products of watermelon (*Citrullus lanatus*) and eggshells of hen (*Gallus gallus domesticus*) as ingredients for cosmetic products.

Juliana Gutiérrez González [1], Sarita Tobón Bedoya [1] and Juan Martín Delgado Naranjo [1,*]

[1] ICIF Research Group, Faculty of Science and Biotechnology, CES University, Medellin 050021, Colombia; gutierrezg.juliana@uces.edu.co, tobon.sarita@uces.edu.co

* Correspondence: jmdelgado@uces.edu.co

Abstract: The current cosmetic industry is seeking new, environmentally friendly, and cost-effective raw materials. Therefore, by-products from the agro-food industry become an interesting opportunity, as some of them possess bioactive compounds with potential cosmetic use. This study investigated the composition of watermelon waste and eggshell waste for the extraction and potential use of mineral antioxidants and collagen. The extraction of antioxidants from three fractions of watermelon waste (green part, white part, and seed) and eggshell was evaluated. For the watermelon fractions, methanol extraction was used, obtaining antioxidant extracts with inhibition percentages of 68.50%, 56.93%, and 82.04% for ABTS and 48.3%, 17.7%, and 68.5% for DPPH, respectively. On the other hand, a mineral composition analysis was performed on the eggshell using ICP-MS, finding calcium and magnesium as its primary constituents. Additionally, the collagen content and type present in the egg membrane were determined, resulting in a content of 500 mg/100 g, mainly represented by type X collagen, with a size of 45 KD. These results recognize the technical potential of some components present in these waste materials; however, it is necessary to delve deeper into the technical-economic feasibility, regulatory framework, and scalability of the process for effective incorporation of these matrices into the global cosmetic industry.

Keywords: Watermelon, eggshell, collagen, antioxidant, food waste and cosmetic.

Citation: To be added by editorial staff during production.

Academic Editor: Firstname Lastname

Received: date
Revised: date
Accepted: date
Published: date

1. Introduction

Currently, the generation of solid waste and its management represents one of the most urgent challenges worldwide. It is known that over 2.01 billion tons of solid waste are collected globally each year, with approximately one third of that amount not receiving adequate management, resulting in devastating consequences for the environment, public health, and the economy (1). It is estimated that, on average, each person generates 0.74 kilograms of waste per day, and it is predicted that this value will increase by 70% due to population growth, changes in eating habits, and global economic development (2). According to data from the Food and Agriculture Organization (FAO), it has been estimated that 3.9 billion tons of food are produced globally each year, of which nearly one third becomes waste and, in Latin America and the Caribbean, 127 million tons of food are lost annually. 30% of that waste comes from food processing and 28% from final consumers(3). By 2022, those wastes were responsible for 10% of global greenhouse gas emissions, contributing to climate change, which has alerted government entities (4).

In Colombia, according to data from the National Planning Department (DNP, its acronym in Spanish), approximately 9.76 million tons of food are lost annually, equivalent to 34% of the total production. The fruit and vegetable production chain accounts for 62% of annual productive losses, equivalent to 6 million tons, which could feed 8 million people per year(5). This issue is of national interest due to the high production figures in this industry. According to the National Agricultural Survey (ENA, its acronym in Spanish) conducted in 2019, the total production of the agricultural industry was 63,247,863 tons, of which 66.7% corresponded to agro-industrial products, 10.6% to fruits, 10.1% to tubers and plantains, 7% to cereals, 4.8% to vegetables and legumes, and 0.7% to scattered fruit trees (6). Within the agro-industry sector, the poultry industry in Colombia produced 16,864 million eggs in 2023, ranking as the fourth-largest egg producer worldwide, and it is expected that this figure will continue to increase in the coming years (7). On the other hand, this industry plays a fundamental role in the Colombian economy, highlighting that in 2021, the value of the agricultural sector in Colombia exceeded 87.4 billion Colombian pesos, representing an increase of around 18% compared to the previous year, and for the first quarter of 2024, this figure continues to grow, with a 1.8% increase compared to the previous year, as reported by the National Administrative Department of Statistics (DANE, its acronym in Spanish) (7).

In response to the problem associated with wastes generated by the food industry, the scientific community and the economic sector are exploring innovative solutions to utilize the waste generated by the food industry and give them a second life, with significant benefits. These by-products can reduce pollution levels and offer significant advantages in various areas, such as the energy sector, agricultural industry, and cosmetic industry, which, this last one is our area of interest (8). It has been demonstrated that their bioactive components can provide antioxidant functions, hydrating and anti-aging properties, as is the case with watermelon *(Citrullus lanatus)* and eggshell *(Gallus gallus domesticus),* which are our study matrices; similarly, it is recognized that, these wastes are of little interest to certain industries, presenting a lower cost, which can be considered another advantage when using these by-products.

Watermelon (*Citrullus lanatus*) has a high content of antioxidants due to the presence of fatty acids and polyphenolic compounds. These antioxidants have the ability to combat free radicals, remineralize skin, prevent the appearance of dark spots, acne, and expression lines, and are capable of eliminating toxins from the skin due to their exfoliating properties (9). On the other hand, eggshell (*Gallus gallus domesticus*) is considered a potential valuable by-product due to its components, such as its high content of calcium carbonate and proteins located in its interstitial membrane, specifically collagen, which represents various benefits to the skin, such as its ability to retain skin moisture, and is the main support of our skin fibers, providing resistance, firmness, and elasticity (10).

Thus, the present work aims to evaluate the potential transformation and use of these two food wastes in supplies for cosmetic industry based on the high value molecules available in those wastes. With this development, it is expected to propose alternatives for the utilization of food wastes to promote a circular economy and foster the sustainability of consumer goods production. Finally, this research aspires to take an important step towards responsible cosmetic production and the sustainable management of natural resources.

2. Materials and Methods

2.1 Extraction, Obtention and Quantification of Collagen and its Derivatives Present in Hen Eggshell membrane.

2.1.1 Pretreatment of hen eggshells.

The extraction and concentration of collagen was carried out from hen eggshells, mainly from commercial eggs categorized as double AA by their size (60-62 g), which were collected from daily consumption and then stored in refrigeration until further use. These were carried to the Bioprocesses laboratory of the University of Antioquia, in Medellin, Colombia and initially underwent a sanitization process, where they were washed with abundant water to eliminate organic residues such as soluble non-collagen compound, lipid, pigment and off-flavor. Afterwards, they were disinfected by immersion in a 5% (V/v) sodium hypochlorite solution for 10 minutes. Once disinfected, the eggshells were rinsed again to remove excess hypochlorite and finally left in a ditch to dry at room temperature for 2 hours.

2.1.2 Separation of Interstitial Membranes from Eggshells.

Three methods of membrane separation from eggshell were evaluated, taking three samples of 40 g of previously disinfected shells. Sample 1 underwent a grinding process, sample 2 was fractured into small pieces, and sample 3 was manually fragmented into larger pieces. Additionally, a separation procedure reported by Chávez et al. (2021) (11) was carried out with some modifications. A 0.5 M EDTA solution was used in a 4:1 relation with each of the three previously fractured samples, after which the samples were subjected to agitation (100 RPM) for 24 hours in a MaxQ400 orbital shaker (Thermo Scientific, FL, USA). After this time, samples were centrifuged for 5 minutes and the loose membranes were collected, while the membranes that remained adhered to their shell were manually separated. To evaluate the manual detachment of the membranes, a quantity of shells was submerged in distilled water for 1 hour to facilitate their separation. Subsequently, the membranes were manually detached from the shells and subjected to a natural drying process at 25°C for 1 hour.

2.1.3 Obtention of Collagen from hen eggshells membrane.

- **Basic Hydrolysis Extraction of Collagen.**

To remove the non-collagen proteins, a basic hydrolysis method established by Serrano et al. (2011) (12) was used un eggshell membranes, with some modifications. Briefly, an alkaline solution 0.3M was prepared using distilled water sodium hydroxide, and 10 g of eggshell membranes were placed in a in a 1:10 sample:solution ratio. Subsequently, the samples were gently agitated (100 RPM) for 8 hours at a temperature of 30°C in a MaxQ400 orbital shaker (Thermo Scientific, FL, USA), then samples were left to repose for 1 more hour. Finally, the supernatant was discarded, and the membranes were washed several times with distilled water until a pH of approximately 7.0 was achieved in the washing water.

- **Solubilization of Collagen in Acidic Medium.**

A 0.5 M acetic acid solution was prepared for the solubilization of collagen. The membranes treated with alkaline solution were poured into the acetic acid solution and left in gentle agitation (100 RPM) at a temperature of 12°C for 48 hours (about 2 days) in a Maxq400 orbital shaker (Thermo Scientific, FL, USA). The sample: solution relation was maintained constant (1:10). Later, liquid phase was filtered with Whatman filter paper No. 42 and stored at 4°C.

- **Tangential Concentration**

To concentrate the present collagen in the sample, a volume of supernatant (soluble collagen) was summited a tangential filtration process. Previously, the hoses and membranes that would come into contact with the collagen were cleaned and sterilized using a sodium hydroxide 1.0 M and sodium hypochlorite solution 5000 ppm, respectively. Tangential filtration was carried out in 5 cycles to obtain the concentrated collagen in 1/5 of initial volume, separating it for subsequent quantification. At the end of the process, the parts of the system were cleaned with 10% ethanol for its reuse. Subsequently, the concentrated and solubilized collagen in acidic medium was subjected to precipitation with NaCl (10% M/v) and the pH was adjusted with NaOH and stored at 4°C in a 15 mL falcon tube sealed.

- **Recovery of Crystallized Collagen**

The collagen concentrate from eggshell membranes was filtered under vacuum in a Kitasato funnel assembled with a 47 mm diameter and 0.22 μm pore size cellulose acetate membrane (Sartorius, Göttingen, Germany), trapping the collagen solids, which were then dried for 8 hours at 45°C in a convective drying oven (Binder FD-115, Tuttlingen, Germany).

2.1.4 Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

In this study, Method 3015A of Acid-Assisted Microwave Digestion was used to prepare eggshell samples (13). Briefly, 10 g of sample were subjected to a digestion process, releasing the components of interest, including calcium, potassium, and magnesium. Subsequently, an inductively coupled plasma mass spectrometry (ICP-MS) was used to determine the elemental composition of the sample. The analyses were performed at the Faculty of Engineering of the University of Antioquia.

2.1.5 Determination of Protein Content of Eggshell Membranes

The protein content was determined using the AOAC 981.10 method, which is based on the quantification of total nitrogen and its subsequent conversion to collagen using the protein factor 6.25 (14). To perform the method, 2 mg of dry membrane were weighed, which was then subjected to acid digestion with sulfuric acid and a catalyst, followed by distillation to collect the generated ammonia, and finally, the ammonia was valued with hydrochloric acid.

2.1.6 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS- PAGE)

The SDS-PAGE electrophoresis was performed following the method established by Laemmli in 1970 (15). Briefly, the crystallized collagen was dissolved in Laemmli's loading buffer (BIO-RAD, CA, USA), the mixture was incubated at 95°C for 5 minutes, and then cooled to 25°C. The SDS-PAGE separating gel was prepared at a concentration of 10% and

the stacking gel at 5%, with the proportions described in the reference method. The running buffer was prepared with 30.3 g/L of Tris base and 144 g/L of glycine. The electrophoresis was performed in a Mini-PROTEAN Tetra Vertical chamber (BIO-RAD, CA, USA) at 200 V for 90 minutes. Once the electrophoresis was finished, the gels were stained with a solution of R-250 brilliant blue Coomassie for 1 hour and decolorized repeatedly until the bands were observed. As a molecular weight marker, Precision Plus Protein (BIO-RAD, CA, USA) with a scale of 250-10 KD was used. As a positive control of the assay, a sample of commercial gelatin, whose main protein component is bovine collagen, was evaluated.

2.1.7 Hydroxyproline Measurement

To determine collagen content, it was necessary to establish the hydroxyproline amino acid content following the method described by Neuman et al. (1950) (16) with some modifications proposed by Quintero et al. (2017) (17). 50 mg of dry eggshell membrane were hydrolyzed with 1 mL of 6N HCl in sealed tubes, at 120°C for 3 hours in a dry block (HB120-S, Dlab, China), and then the pH of the sample was neutralized with 6.0 N NaOH and adjusted to a volume of 3 mL with deionized water. The hydrolysis was performed in triplicate. Subsequently, 1 mL of each hydrolyzed replicate was pipetted into 10 mL tubes and 1 mL of $CuSO_4$ 0.01 M, NaOH 2.5M, and H_2O_2 6% were added in strict order. Each tube was then shaken for 5 minutes and heated to 80°C for 10 minutes to eliminate excess H_2O_2, shaking strongly and releasing the tube cap occasionally to avoid overpressure. Then, the tubes were cooled in an ice bath and 4 mL of H_2SO_4 and 2 mL of 5% dimethyl-aminobenzaldehyde in n-propanol were added, with constant agitation. Finally, the samples were heated for 20 minutes at 70°C and left to cool to 25°C for spectrophotometric analysis at 560 nm. The procedure was repeated to perform a calibration curve using known amounts of a hydroxyproline standard in aqueous solution from 0 to 20 mg/mL. The collagen content was determined according to the model described by Serrano Jennifer (2011) which uses the average content factor of hydroxyproline in collagen of 7.46.

From this, the amount of collagen present in the sample was determined using equation 1.

$$Collagen\ concentration\ (mg)/100g = \frac{mg\ of\ hydroxyproline}{100\ g\ of\ membrane}\ x\ 7.46 \quad (1)$$

2.2 Obtention, Determining, and Quantifying Extracts of Antioxidants from Watermelon By-products.

2.2.1 Sample Preparation

Watermelons (*Citrullus lanatus*) were purchased from the Central Mayorista de Antioquia in Medellín, Colombia. Once in the laboratory, they were subjected to an initial washing process to remove any unwanted residue using distilled water. After that, the watermelons were cut into parts, and the seed, white rind, and green rind were obtained, which correspond to the watermelon byproducts. After separating them, they undergo a cleaning process to remove any unwanted residue using distilled water and a strainer (for the seeds).

2.2.2 Moisture Content Determination

To determine the moisture content, equation 2 and an analytical balance ATX-224 (Shimadzu, Kyoto, Japan) were used.

$$\%\ Moisture\ Content\ = \frac{Wet\ Sample\ (g) - Dry\ Sample(g)}{Wet\ sample\ (g)}\ x\ 100 \quad (2)$$

2.2.3 Total Antioxidant Extraction

50 g of each type of sample were dried at 60°C for 36 hours before performing the extraction procedures. After that, each sample was ground for 10 minutes in a food processor (Nutribullet, FL, USA) to achieve a homogeneous particle size. To extract the antioxidants, present in each fraction of study, the method described by Neglo et al. (18) was used with some modifications. Approximately 15g of each sample were weighed and transferred to 500 mL Flasks, followed by the addition of 200 mL of reagent-grade methanol (Panreac, Barcelona, Spain). Then, the samples were placed in an orbital shaker Maxq400 (Thermo Scientific, MA, USA) for 24 hours at 25°C. The result of this agitation was then subjected to a rotary evaporation process at 60°C for approximately 15 minutes to remove most of the methanol. Finally, the volume sample were adjusted with methanol in a volumetric flask of 200 mL.

The final samples were then brought to volume with methanol and stored in Falcon tubes for later use.

2.2.4 Total Polyphenol Content Determination

To determine the total polyphenol content, the method established by Mehra et al. (19) was used with some modifications. 100µl of each extract, 5 mL of distilled water, and 500µl of Folin-Ciocalteu reagent were mixed in 10 mL volumetric flasks, left to rest for 5 minutes in darkness, and then 1.5 mL of 20% sodium carbonate was added. The flasks were then brought to volume with distilled water and left to incubate for 2 hours in total darkness. After that, 200 µL of each sample were pipetted onto a microplate and analyzed at 680 nm in a UV-VIS Synergy H1M spectrophotometer (Bioteck, VT, USA). On the other hand, a standard curve of gallic acid (Panreac, Barcelona, Spain) in 96% methanol was prepared at concentrations of 20, 40, 60, 80, and 100 mg/mL. The total antioxidant content is expressed as mg Gallic acid/g extract and was determined using equations 3 and 4.

$$[Concentration\ ]_{\ mg\ /\ mL}\ =\ \frac{ABS\ sample - m}{b} \quad (3)$$

Where:

m: pendent

b: intercept with axe y.

$$Gallic\ Acid\ (mg)\ /sample(g) =\ [concentration] \times \frac{200\ ml}{Sample\ weight\ (g)} \quad (4)$$

2.2.5 Spectrophotometric Methods for Determining Total Antioxidant Activity: DPPH and ABTS Radical Scavenging Assays

2.2.5.1 DPPH Radical Scavenging Assay

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity of each of the extracts obtained was determined following the procedure described by Chanda et al. (20) with modifications. The DPPH radical was prepared by dissolving 100 mg of the reagent in 50 mL of methanol and then incubated in darkness at 25°C for 30 minutes. Then, approximately 30 µL of each extract (seed, green rind, and white rind) were added to 270 µL of the DPPH working solution, mixed, and incubated at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm using a UV-VIS Synergy H1M spectrophotometer (Bioteck, VT, USA) and a methanol blank. The experiment was performed in triplicate for all samples.

The DPPH radical scavenging capacity was reported using the Trolox equivalent method, according to the DPPH radical scavenging capacity reported by Frangu et al. (2020) (21) using a Trolox calibration curve of 0 to 1250 µmol/L. From this, the percentage of inhibition (equation 5) and Trolox equivalents (equations 6 and 7) were calculated.

$$Imhibition\ percentaje = \frac{Abs\ standard - Abs\ sample}{Abs\ estandar}\ x\ 100\ , \quad (5)$$

$$Eq\ trolox = \frac{Abs - 3.5589}{0.0023}\ , \quad (6)$$

$$\mu mol\ trolox\ /\ mg\ sample = \frac{Eq\ trolox}{mL}\ x\ \frac{1}{DF}\ x\ \frac{\mu mol\ trolox}{mL}\ x\ 25\ ml\ x\ \frac{1}{w\ (g)}\ x\ \frac{1\ g}{1000\ mg}\ , \quad (7)$$

Where:
DF: dilution factor.

2.2.5.2 ABTS Radical Scavenging Assay

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging capacity of each of the extracts obtained was determined following the procedure described by Re et al. (22) with modifications. The ABTS radical was prepared by dissolving 10 mg of the reagent in 50 mL of methanol and then incubated in darkness at 25°C for 24 hours. Then, approximately 100 µL of each extract (seed, green rind, and white rind), previously diluted 1:10, were added to 100 µL of the ABTS working solution, mixed, and incubated at room temperature for 30 minutes. After incubation, the absorbance was measured at 734 nm using a UV-VIS Synergy H1M spectrophotometer (Bioteck, VT, USA) and a methanol blank. The experiment was performed in triplicate for all samples.

The ABTS radical scavenging capacity was reported using the Trolox equivalent method, following the same methodology used for DPPH and employing a Trolox calibration curve of 0 to 1000 µmol/L. From this, the percentage of inhibition (equation 5) and Trolox equivalents (equations 6 and 7) were calculated.

3. Results

3.1 Results of The Extraction, Obtention, And Quantification of Collagen And Its Derivatives Present in Hen Eggshells Membrane.

3.1.1 ICP-MS Elemental Analysis

The results of the ICP-MS analysis of the Hen eggshells presented in Table 1, reveal considerable concentrations of several mineral elements of interest in the cosmetic industry, such as magnesium, strontium, silicon, sodium, and calcium, among others. According to the results, calcium is the predominant element, representing 98.02% of the total minerals in the shell, followed by magnesium, sodium, strontium, and silicon with 1.63%, 0.14%, 0.12%, and 0.08%, respectively. King'Ori (2011) reported a calcium content in chicken eggshells close to 90% and indicates that it is likely the most bioavailable source of calcium of all. (23)

Table 1. Elemental analysis ICP-MS

Ions detected for ICP – MS method in eggshells samples.						
Sn 189.98	**Tl 190.85**	**As 193.75**	**Hg 194.22**	**Se 196.09**	**Mo 203.84**	**Sb 206.83**
0.00589	N/D	0.00257	N/D	0.00628	0.00002	N/D
Zn 213.85	**Pb 220.35**	**Cu 224.70**	**Cd 226.50**	**Co 228.61**	**Ni 231.60**	**Be 234.86**
0.00897	N/D	0.01349	0.00006	0.00282	0.00040	0.00004
Sr 421.55	**Au 242.79**	**B 249.67**	**Si 251.61**	**Mn 257.61**	**Fe 259.94**	**Li 670.78**
6,24393	N/D	N/D	4.50493	0.00196	0.06342	0.00484
Mg 279.07	**Mg279.07**	**Cr 284.32**	**V 292.40**	**Al 308.21**	**Na 589.59**	**Ba493.40**
42.00556	45.46768	N/D	0.00044	0.02939	7.55254	0.14578
Ca 315.88	**Ag 328.06**	**Ti 334.94**				
5244.25	N/D	N/D				

N/D: No detected.

3.1.2 Total nitrogen determination and total protein quantification.

The analysis of the hen egg membrane yielded the results presented in Table 2. The total nitrogen percentages obtained for samples 1, 2, and 3 were 13.97%, 13.51%, and 14.24%, respectively. By applying the nitrogen-to-protein conversion factor of 6.25, the estimated protein content of the chicken egg membrane is within the range of 84.43% to 89.01%. These results reveal a high protein content in the chicken eggshells membrane, making it a valuable source of proteins. Ponkham et al. (2010) reported a protein content of 88.2% in chicken eggs membranes, which is very close to the value established in this study.

Table 2. Determination of protein content in dried eggshells membrane

Determination of protein content		
Sample	**Total Nitrogen %**	**Protein % (N% × 6.25)**
1	13.97	87.32
2	13.51	84.43

3	14.24	89.01

Given the high protein content of the egg membrane, it is suggested to use it as an ingredient of interest in the formulation of cosmetic products. Proteins are essential for maintaining the health and elasticity of the skin, and their inclusion in cosmetic products can offer a range of benefits, such as hydration, firmness, and skin regeneration. The use of egg membrane in cosmetic products takes advantage of its high protein content to offer skin benefits while providing a natural and sustainable alternative to other synthetic ingredients.

3.1.3 Collagen content in eggshell membranes.

The following calibration curve of hydroxyproline was used for the quantification of collagen (Figure 1).

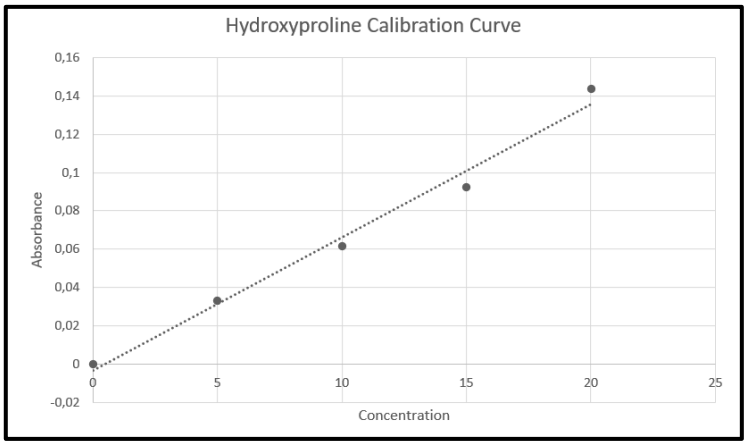

Figure 1. Hydroxyproline Calibration Curve

Based on this curve, the equation of the line (equation 8) was obtained:

$$\boldsymbol{ABS = 0.00492\,[HDP] - 0.00237}\ , \qquad (8)$$

Where:

Abs: Sample absorbance at 560 nm.

HDP: Hydroxyproline concentration mg/mL

The results obtained are presented in Table 3. Additionally, the amount of hydroxyproline present in the sample was determined using equation 1 and considering the data from Table 3. The total collagen content was quantified from the hydroxyproline content using a factor of 7.45.

Table 3. Results Considered in the Measurement of Hydroxyproline and Total Collagen

Parameter	Result	Units
Average membrane hydroxyproline result	17.66 ± 0.39	mg HPL/mL
Sample volume (1 mL sample + 1 mL NaOH)	2.00	mL

Sample weight	49.80	mg
Collagen factor	7.46	Factor
Collagen concentration mg/g	528.97	mg collagen / 100 g dry membrane

As shown in Table 3, it was found that there are 528.97 mg of collagen per 100 g of dry membrane, indicating the presence of collagen in small quantities in the eggshell membrane.

3.1.4 SDS-PAGE Electrophoresis

Figure 2. shows the results of the SDS-PAGE electrophoresis performed on collagen extracted from eggshell membranes. The gel showed evidence of collagen hydrolysis despite using mild extraction conditions, channel 2, showed a blue band near to 45 KD molecular size, channel 3 showed bands near to 75 KD.

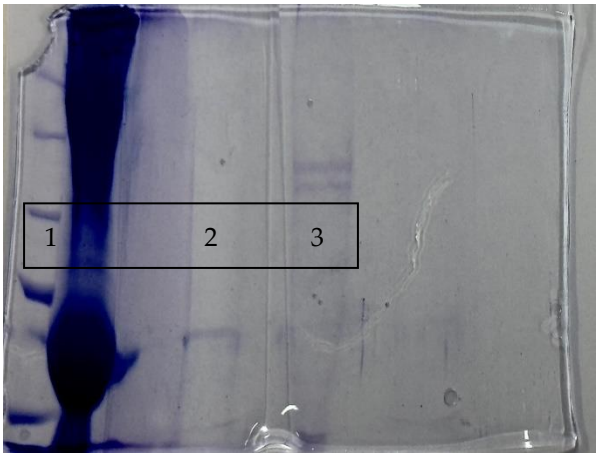

Figure 2. SDS-PAGE Electrophoresis of Hydrolyzed Collagen. Channels: 1) Molecular weight ladder; 2) collagen concentrated of eggshells membrane and, 3) edible gelatin (+control).

3.2 Results of the Obtention, Extraction, and Determination of Antioxidants Present in Watermelon Rind

3.2.1 Determination of Moisture Percentage

Using Equation 2, it was determined that watermelon rind had a high moisture content, with percentages of 91.16% for the green part, 97.27% for the white part, and 61.14% for the seeds. These values reflect a significant water presence in the samples. However, despite the high moisture levels, no significant interference was observed in the antioxidant extraction process or its measurement. The obtained values match those reported by Alka et al., (2018) and validate the results (24)

It is important to note that, throughout the article, it will be demonstrated that the seed was the sample with the highest antioxidant presence in all evaluations. This finding raises the possibility that the lower water content in the seed, compared to the other watermelon parts, has significantly contributed to this observation, as lower water content allows for a higher proportion of different compounds.

3.2.2 Determination of Total Polyphenol Content

Using Equations 3 and 4, the amount of Gallic Acid per gram of sample was determined, yielding the following results:

Table 4. Determination of total polyphenolic content

Determination of total polyphenolic content		
Sample	**mg/g**	**Average mg/g**
White part	1733.28	1877.32
	1249.09	
	2649.58	
Green part	2484.14	2391.57
	2359.49	
	2331.09	
Seed	1896.29	2578.20
	3260.11	

The amount of total polyphenolic compounds founded amd samples were 1877. 32, 2391.57 and 2578.2 polyphenolic mg/g from white part, green part and seeds, respectively.

3.2.3 Measurement of antioxidant activity through DPPH

The following results were obtained for the Trolox calibration curve:

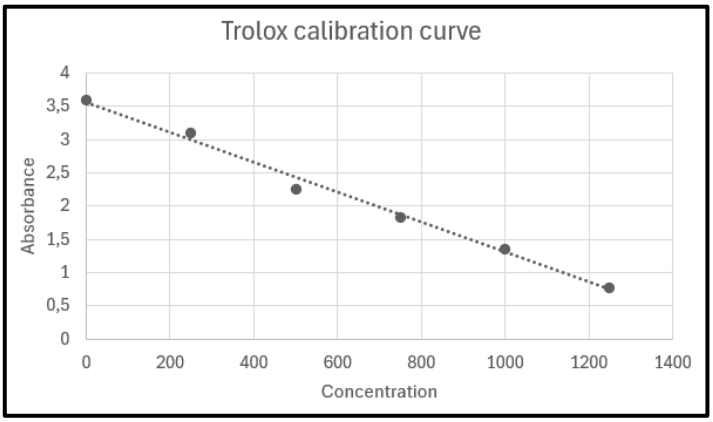

Illustration 3. Trolox Calibration Curve

Based on this curve, the straight-line equation (Equation 9) was obtained:

$$\boldsymbol{Y = 0.00225X + 3,56}\,, \qquad (9)$$

Using this equation, the coefficient of determination was calculated, resulting in a value of 0.992. This value suggests that the regression line fits the observed data almost perfectly in the given context, providing solid evidence that the used regression model is highly reliable.

Furthermore, the Trolox equivalents for each sample were determined, and the inhibition percentage was calculated by replacing the obtained values in the straight-line equation of the Trolox calibration curve and the absorbances obtained from each sample.

The results are described in Table 5:

Table 5. Trolox equivalent results

Trolox equivalent results (μmol trolox / mg sample)					
Sample	**Concentrated sample**	**1:10 dilution**	**1:25 dilution**	**1:50 dilution**	**1:100 dilution**
White part	0.0170	0.0018	0.0007	0.0003	0.0002
Green part	0.0163	0.0017	0.0007	0.0003	0.0002
Seed	0.0485	0.0060	0.0019	0.0009	0.0005

Results showed that seeds had 3.4 times more antioxidant capacity than green part, and 2,85 times more than white part. The diluted extracts in Ethanol, showed lower capacity and it was consisted with the chemical performance of antioxidants compounds. In the same way, the DPPH scavenging capacity evidenced that again, seed have highest antioxidant activity of the test (table 6). Seeds, green part and white part, showed an inhibition of DPPH radical of 68.5%, 43.8% and 17,7%, respectively.

Table 6.DPPH radical scavenging capacity

Green Part		
Dilution	**Concentration g/mL**	**Inhibition %**
Concentrated	0.615	43.8%
1/10	0.062	19.1%
1/25	0.025	4.8%
1/50	0.012	4.4%
1/100	0.006	0.6%
White part		
Concentrated	0.600	17.7%
1/10	0.060	12.9%
1/25	0.024	9.1%
1/50	0.012	6.0%
1/100	0.006	5.4%
Seeds		
Concentrated	0.220	68.5%
1/10	0.022	24.2%
1/25	0.009	10.8%
1/50	0.004	4.2%
1/100	0.002	13.1%

3.2.4 Measurement of antioxidant activity through ABTS

Based on Equations 5, 6, and 7, Trolox equivalents and inhibition percentage were determined, resulting in the data shown in Table 7:

Table 7. Measurement of antioxidant activity through ABTS

Measurement of antioxidant activity through ABTS			
Sample	**Trolox Equivalent**	**Inhibition %**	**Average inhibition %**
White part	0,6057	59,84	56,93
White part	0,5671	54,02	
Green part	0,6580	68,97	68,50
Green part	0,6618	68,03	
Seed	2,0168	82,04	82,04

The results indicate that the antioxidant activity measured through the ABTS method shows a similar trend to that observed with the DPPH method. This suggests that the compounds present in the watermelon samples have a consistent antioxidant capacity, regardless of the measurement method used. However, it is important to note that the ABTS method may provide a complementary or even different view of the antioxidant capacity compared to the DPPH method, due to differences in reaction mechanisms and testing conditions.

4. Discussion

4.1 Extraction, obtention, and quantification of collagen and its derivatives present in the hen eggshells membrane.

Calcium, the most abundant mineral in the eggshell, represents a potential ingredient for cosmetic applications. In the skin, for example, calcium ions (Ca^{2+}) serve as a universal signal to modulate cellular functions in keratinocytes. The distribution and dynamics of Ca^{2+} in the skin play an important role in epidermal homeostasis. In the mammalian epidermis, there is a characteristic calcium gradient between the lower and upper layers of the epidermis, with low levels in the basal and spinous layers and progressively increasing levels towards the granular layer, only to decrease again in the cornified layer. Some studies have shown that eggshells can be used as an ingredient in body scrubs, and some authors suggest that the calcium present in the eggshell helps to tone and firm the skin, combating sagging and fine lines. According to a study published in Annals of Dermatology, the epidermal calcium gradient, calcium homeostasis in the endoplasmic reticulum, and calcium influx through TRP, Orai1, or VGCC channels play a crucial role in keratinocyte differentiation, barrier formation, wound healing, and skin barrier homeostasis (25).

The collagen content determined was consistent with other reports published by other researchers, such as Ponkham et al., (2010), who extracted collagen from membranes with yields of around 507 and 495 mg/100 g of dry sample using acetic and citric acid extractions. However, all authors emphasize the difficulty of extracting the molecule without accidentally hydrolyzing it.

The finding of this concentration suggests the possible cosmetic potential of this sample, as the amount of collagen may be small compared to other sources, but its presence is significant, especially considering its origin from an agro-industrial byproduct. The ability to extract this component from the eggshell membrane opens the door to its potential use in various cosmetic products, such as anti-wrinkle creams, rejuvenating serums, or revitalizing masks, formulated with collagen. These products could offer significant benefits for the skin, while promoting more sustainable practices within the cosmetics industry.

As mentioned earlier, the value of this result is even higher due to its origin as a byproduct of the agro-industry. The use of industrial waste in the production of cosmetic products not only reduces waste but also promotes sustainability and a circular economy.

In the other hand, there are around 28 recognized types of collagen presented in different kind of sources. According to several authors, the collagen present in hen eggshell membranes consists mostly of collagen types I, V, and X (26). These three types of collagens can be used in various commercial fields. Collagen is a fibrous protein that connects and supports other bodily tissues, such as skin, bones, tendons, muscles, and cartilage. It has been demonstrated that eggshell membrane collagen has few autoimmune and allergic reactions and high biosecurity (27).

The molecular weight of collagen type I is around 300 KD, type V ranges from 200 KD to 300 KD, and type X has a much lower molecular weight, between 45 KD and 65 KD. The electrophoresis results revealed a band of hydrolyzed collagen near 45 KD, suggesting the detection of collagen type X in the crystallized collagen obtained from eggshell membranes. On the other hand, the gelatin sample shows bands above 75 KD. The band near 45 KD could also correspond to hydrolyzed fragments of collagen type X, as it has been observed that a gentle digestion of collagen type X with pepsin-type proteases releases a non-helical domain that reduces the molecular size from 59 KD to 45 KD (28).

Collagen peptides are widely used in cosmetic products, known for being an anti-aging ingredient. They are often found in serums and moisturizing creams in combination with other anti-aging and/or antioxidant ingredients.

Some novel uses of eggshell membrane collagen(23), (29), which have been recently reported, include:

- In cosmetics and burn surgery, where it improves the appearance of the skin (plastic surgery).
- In orthopedics and dentistry as a treatment aid.
- In cancer treatment to increase muscle mass and hair thickness in patients.
- In sports nutrition to enhance athletes' performance.
- In the food industry as a flavoring agent.

4.2 Extraction, and Determination of Antioxidants Present in Watermelon wastes.

It's worth noting that gallic acid plays a fundamental role in the evaluation of antioxidants due to its purity and stability, making it a commonly used standard in studies of this type. Its use as a standard allows for a precise comparison of antioxidant concentrations between different samples, providing a solid basis for interpreting the results.

In this context, the seed demonstrated the highest concentration of gallic acid per gram of sample, followed by the green part and, finally, the white part. This observation suggests that the seed contains the highest amount of antioxidants compared to the other parts of the watermelon, making it a resource of greater interest in terms of antioxidant potential.

The seeds of *Citrullus lanatus* have been used in the past for their oil in the cosmetic and pharmaceutical industries. Due to their high protein and fat content, the seeds were also used to improve infant nutrition. The seed oil contains oleic, palmitic, stearic acids, and linoleic glucosides (30).

Considering the results obtained, the watermelon seed result stand out, demonstrating superior performance in terms of inhibition percentage and Trolox equivalents. These results emphasize the importance of the seed as a rich antioxidant source and support its potential application in the formulation of cosmetic products with antioxidant properties. However, we should not overlook the other parts of the watermelon, as they show lower antioxidant activity compared to the seed but still demonstrate the presence of antioxidants.

The inhibition percentage provides a direct measure of a sample's antioxidant capacity by indicating the degree to which the compounds in the sample can neutralize DPPH free radicals (Table 6). A higher inhibition percentage suggests a greater presence of antioxidants in the sample, reflecting its ability to protect against oxidative damage and stress in the skin.

In this sense, the fact that the watermelon seed demonstrated the highest inhibition percentage implies that the compounds present in the seed have a significant capacity to neutralize free radicals, making it a particularly valuable source of antioxidants. On the other hand, Trolox equivalents provide a way to compare a sample's antioxidant capacity with that of the reference antioxidant Trolox. Trolox equivalents express the amount of Trolox (a common synthetic antioxidant often used as a standard) that would be necessary to produce the same antioxidant effect as the analyzed sample.

Therefore, the fact that the watermelon seed showed a higher number of Trolox equivalents compared to the other watermelon parts further supports its high antioxidant capacity. This observation reinforces the idea that the seed has a rich chemical composition in antioxidants, making it a valuable ingredient for the formulation of cosmetic products designed to combat premature skin aging and other adverse effects of oxidative stress.

It is worth noting that, although the watermelon seed stands out as the part with the highest antioxidant capacity, the other fruit parts still play an important role and can be considered in the formulation of cosmetic products, as the findings emphasize the diversity of antioxidant compounds present in watermelon and their potential application in skin care and cosmetics in general.

Regarding the specific results, it is observed that all samples show a higher inhibition percentage with the ABTS method compared to the DPPH method. This finding suggests that the compounds present in the watermelon samples may have a greater capacity to neutralize ABTS+ radicals compared to DPPH radicals, which may be due to differences in the structure and stability of these radicals.

The seed once again stands out as the sample with the highest inhibition percentage, with a value of 82.04%, followed by the green part with 68.50% and the white part with 56.93%. The consistency in the trend observed in the results of both methods reinforces the idea that the watermelon seed is the part with the highest antioxidant capacity, followed by the green part and the white part.

The Trolox equivalents, used to compare the antioxidant capacity of the samples, follow the same trend observed in the inhibition percentages. This suggests that the highest Trolox equivalent value is associated with the sample that exhibits the highest inhibition percentage, which is consistent with the idea that a higher presence of antioxidants in a sample reflects a greater capacity to neutralize free radicals.

These results provide a solid basis for comparing and discussing the findings with previous research in literature. The consistency in the trends observed in the results of both measurement methods strengthens the validity of the findings and highlights the importance of the watermelon seed as a rich antioxidant source. In the following discussion, we will delve into the comparison of these results with similar studies and analyze their relevance in the context of antioxidant research and their application in cosmetic products.

5. Conclusions

The results obtained during this research are encouraging, as they show the presence of bioactive compounds of interest in the analyzed by-products. Specifically, antioxidants in watermelon; and proteins, particularly collagen, and other valuable compounds such as calcium, which plays a crucial role in regulating skin health, contributing to its protection against conditions like atopic dermatitis and psoriasis (25). These assays were demonstrated and supported through several tests and trials, which suggest the potential of these by-products and their possible use in cosmetic products as sources of valuable bioactive compounds.

However, it is important to highlight that certain difficulties were identified when trying to carry out efficient and effective processes with these by-products, as during the project development, various problems were encountered that affected optimal results. Among these difficulties are the purity of the samples, the procedures to which they were previously subjected, and the time they spent in those conditions, being factors that can significantly influence the quality and quantity of the bioactive compounds present in the by-products. Furthermore, the cost-benefit relationship in the use of these by-products is raised, as despite identifying bioactive compounds in the evaluated by-products, it was concluded that high-cost procedures are required to obtain satisfactory results. This raises questions about the economic viability of using these by-products in the large-scale production of bioactive compounds for commercial applications. Additionally, the magnitude of the benefit derived from the presence of these bioactive compounds in the by-products is questioned. Despite their presence, it may be that their quantity or impact is not significant enough to justify the costs and processing associated with the extraction and purification of these compounds.

In conclusion, although this study shows an interesting potential in the use of by-products as sources of bioactive compounds, it is necessary to address the identified difficulties and carefully evaluate the economic viability and real impact of these compounds in commercial applications before considering their large-scale implementation.

Supplementary Materials: Not applicable

Author Contributions: S.B. developed the experimental design, conducted the experiment along with J.G., curated the data, performed statistical analysis, and wrote the original draft. J.M.D.N. formulated the research goals and aims, supervised, reviewed the manuscript, and helped to edit the final draft. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable

Informed Consent Statement: Not applicable

Acknowledgments: The authors gratefully acknowledge the financial and operative support provided by the Bioprocess Research group of University of Antioquia.

Conflicts of Interest: The authors declare no conflicts of interest.

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