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Valorization of Betalain Pigments Extracted from *Phytolacca americana* L. Berries as Natural Colorant in Cheese Formulation

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Abstract: In response to consumer demand for more sustainable and health-conscious products, the food sector is increasingly shifting towards the use of natural additives. Pokeweed (*Phytolacca americana* L.) is a medicinal plant that contains valuable biologically active compounds, including betacyanins, which serve as its red pigments, along with phenolic acids, flavonoids, polyphenolic compounds, and others. *Phytolacca americana* (*P. americana*) is a plant renowned for its bioactive compounds, which exhibit anti-inflammatory, anti-mutagenic, antioxidant, anticancer, and antibacterial properties. This study investigates the potential of betalain pigments extracted from the berries of *P. americana* as a natural colorant for cheese formulation. The impact of these pigments on the color attributes, sensory qualities, and physicochemical and phytochemical composition of the cheeses was systematically evaluated. The *Phytolacca americana* (PA) powder demonstrated significant levels of total polyphenols (111.95 ± 1.60 mg GAE/g dw) and antioxidant activity (21.67 ± 0.19 µmol TE/g dw). The incorporation of PA powder increased the physicochemical and phytochemical contents and antioxidant activity in the final product (4.40 ± 0.22 µmol TE/g dw for CPAP1 and 6.11 ± 0.22 µmol TE/g dw for CPAP2). The sensory study revealed that the PA-supplemented cheeses were acceptable. The enhanced cheeses present a distinctive color profile, attracting health-conscious consumers looking for innovative dairy products. The study concludes that PA powder can effectively enhance cheese, producing a phytochemical-enriched product that appeals to health-conscious consumers.

Keywords: *Phytolacca americana*; antioxidant activity; pigments; food ingredients; functional cheese

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1. Introduction

Traditional medicine has long utilized plants and food products to treat a wide range of illnesses due to their rich pharmacological properties. Over the past few decades, there has been a growing interest in identifying naturally occurring antioxidants derived from plants, driven by their potential applications in the food, cosmetics, and pharmaceutical industries. These bioactive compounds, including polyphenols, flavonoids, and other secondary metabolites, not only provide health benefits such as reducing oxidative stress

and preventing degenerative diseases but also serve as sustainable alternatives to synthetic additives. The increasing consumer demand for natural, safe, and eco-friendly ingredients further underscores the importance of exploring plant-based antioxidants for diverse applications [1].

P. americana, often known as pokeweed or *Phytolaccaceae*, is a perennial plant native to North America; it is an invasive alien species that is also abundant in the Mediterranean and Black Sea regions [2]. This large herbaceous plant can grow up to three meters tall [3]. It was brought to Europe, Africa, and Asia after being native to America and Mexico. This plant blooms from June to July, and its fruits ripen from August to October. Due to the species' ability to adapt to various environmental factors and climatic circumstances, a rich genetic diversity has developed as a result of ongoing adaptation [4]. Known by various names in Romania, including American pokeweed, pokeweed, poke sallet, and dragon berries, this plant is a typical example of an innovative floral element.

Phytolacca americana L., commonly referred to as pokeweed, is a plant valued for its unique phytochemical composition, and particularly, for betalains, which are water-soluble pigments divided into two major subclasses: betacyanins (reddish violet) and betaxanthins (yellow to orange) [5,6]. These pigments are responsible for the characteristic purple coloration of the plant's fruits, flowers, and stems and are distinct from anthocyanins in both structure and functionality. As natural pigments, betalains are widely recognized for their applications as colorants in food and beverages, providing vibrant pink, red, and violet hues while offering additional antioxidant, anti-inflammatory, and anticancer benefits [7–9].

The potential of *P. americana* berries to serve as a sustainable source of betalains has attracted increasing interest. Historically, the berries' juice has been used by Native Americans as a natural dye, highlighting its enduring value as a colorant [10]. Recent studies suggest that these pigments can be effectively harnessed for their coloring and functional properties, particularly in food matrices that benefit from enhanced visual appeal and bioactive content [8,11]. With the global trend towards natural additives in food products, betalains provide an opportunity to replace synthetic dyes while simultaneously enhancing the nutritional quality of functional foods.

Cheese, a staple dairy product consumed globally, offers a versatile platform for incorporating natural bioactive compounds. Although cheese is rich in proteins, fats, and vitamins, it generally lacks significant quantities of antioxidants or phenolic compounds, which can be introduced through plant-derived ingredients [12,13]. Recent advancements in dairy technology have demonstrated the feasibility of fortifying cheese with plant extracts to improve its sensory, nutritional, and functional properties [14–16].

This study focuses on utilizing the betalain-rich extract from *Phytolacca americana* berries as a natural colorant for semi-hard cheese. The main objectives are to evaluate the antioxidant activity and phytochemical composition of the extract, to assess the stability of betalains during cheese production, and to determine their impact on the color, texture, sensory attributes, and nutritional properties of the final product. By exploring the integration of these natural pigments into cheese formulations, this research highlights a novel approach to producing visually appealing, health-enhancing dairy products that align with consumer demand for sustainable and functional foods.

2. Materials and Methods

2.1. Materials

Raw milk originates from Holstein cows raised on the farm of the Rediu Iasi Research Station, which is part of the Iasi University of Life Sciences. A total quantity of 300 L of milk was used in the present study, 100 L of milk for each experimental batch.

DPPH (2,2-diphenyl1-picrylhydrazyl), gallic acid solution, sodium acetate solution, potassium chloride solution, ethanol, methanol, citric acid, sodium carbonate, Folin-Ciocalteu reagent, sodium hydroxide, aluminum chloride, and sodium carbonate were purchased from Sigma Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany).

2.2. Preparation of Samples

In this research, *P. americana* fruits, as shown in Figure 1, were collected in November 2023 from the experimental plots managed by the Faculty of Agriculture, Department of Medicinal and Aromatic Plants, in Iași, Romania. The fruits were harvested at full maturity, identified by their deep purple to black coloration, which correlates with optimal betalain content [17]. After harvesting, the fruits were washed with distilled water to remove impurities and dried in an oven (Stericell 111, MMM Medcenter, München, Germany) at 40 °C for 48 h until reaching a moisture content of 9.60%. Finally, the dried fruits were ground into a fine powder using an electrically powered grinder (Bosch MC812M844, Stuttgard, Germany) to prepare them for subsequent analysis and application.

Figure 1. (**A**) *P. americana* plant, (**B**) *P. americana* fruits, and (**C**) *P. americana* powder.

2.3. Extraction of Bioactive Compounds from Phytolacca americana (PA) Powder

The ultrasound-assisted extraction method was employed to isolate bioactive compounds from PA powder. This procedure was based on a slightly modified version of the method described by Šeremet et al. [18]. Specifically, 1.0 g of PA powder was mixed with 9 mL of a 70% ethanol solution acidified with 1 mL of citric acid (ratio 9:1, v/v). The mixture was subjected to ultrasound treatment in an ultrasonic bath (Elmasonic S 180 H, Elma, Germany) for 35 min at 40 °C and a frequency of 37 kHz. The resulting supernatant was subsequently collected and centrifuged at 6000 rpm for 10 min at 4 °C. The PA powder was then analyzed to determine the total content of betalains, flavonoids, and polyphenols.

2.4. Total Betalain Content

The diluted extract was put into a reading tube, and the absorbance values were recorded at λ = 538 nm for betacyanins and λ = 480 nm for betaxanthins with an Analytik Jena (Analytik Jena-Specord 210 Plus, Analytik Jena GmbH+Co. KG, Jena, Germany) UV-Vis spectrophotometer [19]. The results were expressed as milligrams per gram of dry weight (DW). The content of betalains was calculated using Formula (1):

$$Betalains\ (\mathrm{mg/g\ dw}) = \frac{A \times MW \times DF}{\varepsilon \times 1 \times m} \quad (1)$$

where *A*—the absorption at 538 and 480 nm for betacyanins and betaxanthins, respectively; *MW*—molecular weight; l—cuvette pathlength; *DF*—the dilution factor; *m*—the amount of sample; and ε—molar extinction coefficients. For betacyanins ε = 60,000 L/mol cm in H_2O; (*MW* = 550 g/mol) and betaxanthins ε = 48,000 L/mol cm in H_2O (*MW* = 308 g/mol) were applied. The total betalain content (in mg per g of sample) was determined by summing the values for betacyanin and betaxanthin.

2.5. Total Flavonoid Content

The content of total flavonoids of PA powder extract was determined using the aluminum chloride technique [20]. Briefly, 1.25 mL of deionized water was used to dilute 250 µL of the plant extracts (1 mg/mL), and 0.075 mL of 5% $NaNO_2$ solution was then added. Following a 5 min dark period, 0.15 mL of a 10% $AlCl_3$ solution was added. Following a 6 min duration, the reaction mixture was supplemented with 0.5 mL of 1M NaOH and 0.775 mL of deionized water. A UV-Vis spectrophotometer (Analytik Jena-Specord 210 Plus, Analytik Jena GmbH+Co. KG, Jena, Germany) was utilized to measure the absorbance at λ = 510 nm. The standard utilized for catechin was 20–100 mg/L, and the flavonoid concentration was reported as mg CE/g DW, or catechin equivalent per gram of dry weight.

2.6. Total Polyphenolic Content

Bolea and Vizireanu [21] describe the Folin–Ciocâlteu method, which was used to analyze the total phenolic contents. In summary, 7.9 mL of deionized water, 0.5 mL of Folin–Ciocalteu reagent (0.25 mol/L), and 100 µL of extract (1 mg/mL) were combined. After 10 min, 1.5 mL of 20% Na_2CO_3 solution was added, and the mixture was then left in the dark for 1 h. At λ = 765 nm, the absorbance was finally measured in comparison to a blank using a UV-VIS spectrophotometer (Analytik Jena-Specord 210 Plus, Analytik Jena GmbH+Co. KG, Jena, Germany). The total phenolic content was expressed as gallic acid equivalent per gram of dry weight (mg GAE/g DW), with Gallic acid serving as the standard (50–250 mg/L) [22].

2.7. DPPH Radical Scavenging Activity

With a few minor adjustments, the DPPH assay was calculated using the colorimetric technique as reported by Shahinuzzaman et al. [22] and Postolache et al. [23] and the results were represented as µmol of Trolox equivalents per gram of dry weight (µmol TE/g DW). In brief, 3.9 mL of a diluted (1:10) DPPH/methanol solution was combined with 0.1 mL of extracts (1 mg/mL). After that, the mixture was left in the dark at room temperature for 90 min. At λ = 515 nm, the mixture's absorbance was measured by a UV-VIS spectrophotometer (Analytik Jena-Specord 210 Plus, Analytik Jena GmbH+Co. KG, Jena, Germany). Equation (2) was utilized to determine the percentage of inhibition for the radical scavenging activity, with Trolox serving as the standard dosage of 0.125 mg/mL.

$$\text{DPPH scavenging activity}(\%) = [(\text{Abs Control} - \text{Abs Sample or Trolox})/\text{Abs Control}] \times 100 \quad (2)$$

where Abs Control is the value of the DPPH solution only; Abs Sample or Trolox is the value of the DPPH solution mixed either with extracts or Trolox solution.

2.8. Preparation of Semi-Hard Cheese Enhanced with PA Powder

For the two types of semi-hard cheese, 200 L of full-fat milk was utilized (Figure 2). The method used for cheese production was the same as mentioned by Rațu et al. [24], with the mention that the milk was split into three batches subsequent to the incorporation of the selected lactic cultures: 100 L of milk was used to make semi-hard cheese (CC); 100 L of

milk was mixed with 1% PA powder (CPAP1); and an additional of 2% PA powder was used to make semi-hard cheese with PA powder (CPAP2).

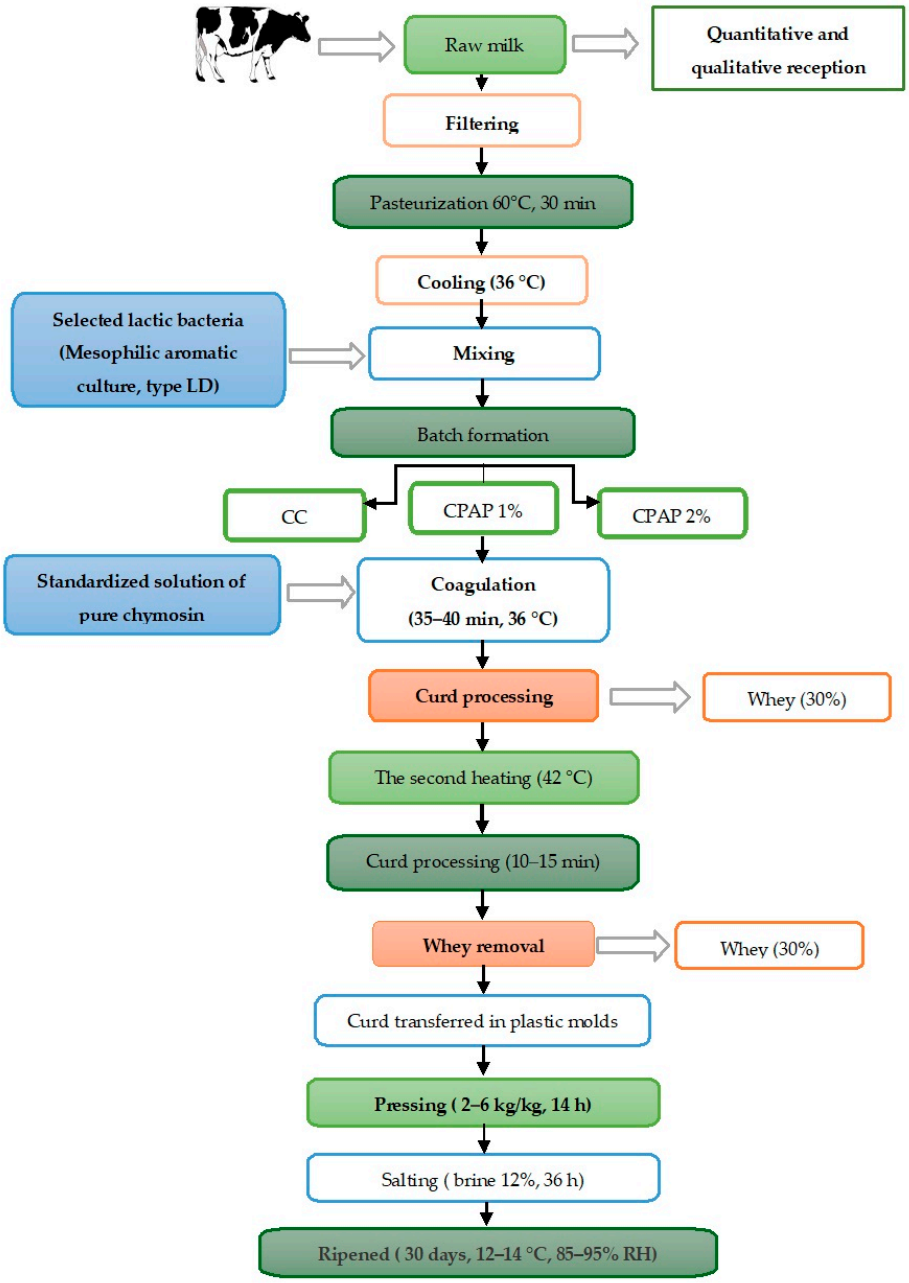

Figure 2. Technological flow of cheese manufacturing.

The quantitative and qualitative reception of milk represented the first stage in the technological process for making cheese. Following the filtration process, the milk was pasteurized for 30 min at 60 °C in a vat. Once the milk had cooled to 35–36 °C, the cultures (mesophilic aromatic culture, type LD-FD-DVS FLORA DANICA by CHR HANSEN) were introduced. The manufacturing sheet states that throughout the inoculation procedure, 50 U of culture were utilized for every 500 L of milk. The milk was allowed to rest for 30 min. Making batches and coagulating the milk at 35 to 36 °C was the next stage. CHY-MAX M liquid (25 IMCU/L milk) was used for this process, as it contains an enzyme that specifically cleaves kappa-casein, resulting in an excellent coagulum [25]. Thirty percent of the whey is removed once the curds are partially processed, and the clotting process takes 35 to 40 min. The second heating phase, which is carried out at 42 °C, entails digesting

the coagulum for 10 to 15 min (or until the produced grain is dehydrated). The purpose of letting it rest is to extract the 30% whey. The curds are pressed (2 kg/kg) for 14 h in a plastic mold that holds 2 kg of curd. The cheese molds are flipped every 10 min for the first 8 h. The maturing stage (30 days at 12–14 °C and RH 85–95%) and the salting stage follow (in 12% brine).

2.9. Physico-Chemical and Phytochemical Evaluation of the Supplemented Semi-Hard Cheese

The samples' pH, fat, ash, total solids, moisture content, and total protein were measured using the techniques recommended by the Association of Official Analytical Chemists [26,27]. Using the methods previously outlined in Sections above, the total batalains, flavonoids, phenolic content, and antioxidant activity of semi-hard cheese supplemented with PA powder were evaluated.

2.10. Color Analysis

The color of the control and supplemented semi-hard cheese were examined using a MINOLTA Chroma Meter CR-410 (Konica Minolta, Osaka, Japan). The parameters L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness) refer to the sample color parameters. Three different replicates of each sample were available.

Other parameters, such as the hue angle (hue angle = 180 + arctan (b*/a*) for quadrant II (−a*, +b*)), which describes the visual color appearance, the Chroma ($\sqrt{(a^*)^2 + (b^*)^2}$), which describes color intensity, and ΔE (ΔE = $\sqrt{(L^* - L_0)^2 + (a^* - a_0)^2 + (b^* - b_0)^2}$, the total color difference, were also determined [28].

2.11. Sensorial Analysis

Twenty untrained tasters evaluated the sensory quality of samples of enriched cheese. The study's main goal and the required protocols for managing personal data were explained to the panelists. Nine attributes, including appearance, aftertaste, section appearance, odor, aroma, texture, color, taste, and overall acceptability, were given to the panelists to rate. Sensory attributes were evaluated by students and academic staff from the Food Technology Department at Iasi University for Life Science. All of the panelists were aware of the qualities of semi-hard cheese. The sensory panel was shown samples from each formulation that had been coded with random numbers. The analysis was completed in compliance with the specifications of ISO 8589 [29]. The temperature in the booths was controlled at about 25 °C, and the sensory evaluation area was kept in an appropriately air-conditioned environment. The untrained tasters graded each attribute using a seven-point hedonic scale (1 being extremely low and 7 being extremely high).

2.12. Texture Analysis of Semi Hard Cheese

A Mark 10 ESM 300 texturometer (Johnson Scale Co., Inc., 36 Stiles Lane, Pine Brook, NJ, USA) equipped with a 7i-50 series digital dynamometer (with a measurement resolution of 0.05 N) was employed to assess the texture of the samples. The compression tests were conducted using a cylindrical probe, specifically, type TA4 (Johnson Scale Co., Inc., 36 Stiles Lane, Pine Brook, NJ, USA), which had a diameter of 38.1 mm and a height of 20 mm. The parallelipipedic warp samples, which had dimensions of 20 mm × 15 mm × 15 mm, were utilized to examine texture. Every texture test was run five times.

2.13. Microbiological Analysis of Milk and Cheese

For each laboratory sample, three replicates of every analytical test pertaining to the microbiological load of semi-hard cheese were carried out in a sterile environment. Ten grams of cheese and 90 mL of buffered peptone water (Bio-Rad, Marnes-la-Coquette,

France) were blended for five minutes at 250 rpm in a lab mixer (Seward, West Sussex, UK) in preparation for the microbiological tests.

According to Najgebauer-Lejko et al. [30], the bacteria, yeast, and molds were separated from the serial dilutions using the spread and pour plate techniques. These dilutions were created by combining 1 mL of the prior dilution with 9 mL of buffered peptone water in test tubes. Following inoculation with 1 mL of sample, the microbiological media utilized for plating included the selective chromogenic agar Rapid *E. coli* 2 (RE) and Rapid Staph (RS; Bio-Rad, Marnes-la-Coquette, France), as well as the non-selective plate count agar (PCA) supplemented with 1 g/L skimmed milk powder and potato dextrose agar (PDA; Scharlau, Barcelona, Spain). Following a 72 h incubation period at 30 °C, total aerobic bacteria were counted in compliance with ISO 7218 [27]. During five days of cultivation on PDA plates at 28 °C, the number of yeast and molds was counted. Following a 24 h incubation period at 37 °C, colonies of Escherichia coli and other coliforms were assessed on RE media, according to the ISO 16140 [31].

Additionally, all samples were cultivated on RS medium, which ensures that coagulase-positive staphylococci (*Staphylococcus aureus*) may be detected and counted in 24 h at 37 °C. After incubation, the automatic colony counter Scan 1200 (Interscience, Saint-Nom-la-Bretèche, France) was used to count the microorganisms in the cheese samples. The results were reported as logarithmic colony forming units per gram (log CFU g^{-1}).

2.14. Statistical Analysis

The results of each analysis were statistically evaluated using Minitab 19 software. A one-way ANOVA and Tukey test were used to assess the differences between the samples. All experimental data were expressed as mean (n = 5) ± standard deviation (SD). Principal components analysis (PCA) was performed on the descriptive sensory data utilizing XLSTAT (Trial Version 2024, Addinsoft, Paris, France).

3. Results and Discussion

3.1. Phytochemical Characterization

Table 1 presents total betalains, flavonoids, and polyphenol contents of ethanol/water extract. Also, the scavenging activity values and DPPH scavenging inhibition of the plant extract are exhibited. The PA powder presented an average of total betalains of 1.06 ± 0.04 mg/g dw, a total flavonoids of 33.74 ± 0.29 mg CE/g dw, and a total polyphenols of 111.95 ± 1.60 mg GAE/g dw

Table 1. Phytochemical characterization and color properties of PA powder.

Phytochemicals	Value
Total betalains (mg/g dw)	1.06 ± 0.04
Total flavonoids (mg CE/g dw)	33.74 ± 0.29
Total polyphenols (mg GAE/g dw)	111.95 ± 1.60
DPPH (µmol TE/g dw)	21.67 ± 0.19
Inhibition (DPPH) %	76.39 ± 0.85
L*	22.72 ± 0.25
a*	7.01 ± 0.12
b*	2.13 ± 0.11
Chroma	7.32 ± 0.14
Hue angle	0.30 ± 0.01

L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness).

The antioxidant activity of the PA powder was determined by measuring the radical-scavenging activity on DPPH showing an antioxidant activity of 21.67 ± 0.19 µmol TE/g dw with a % of inhibition (DPPH) of 76.39 ± 0.85.

The PA powder's color changes were 22.72 ± 0.25 for the L* value, 7.01 ± 0.12 for the a* value, and 2.13 ± 0.11 for the b* value. The hue angle and Chroma color parametric yielded values of 0.30 ± 0.01 and 7.32 ± 0.14, respectively. Regarding the color features, it can be observed that the PA exhibited a luminosity (L*) parameter that was closer to the value associated with white, an a* parameter that was closer to red, and a b* parameter that was closer to yellow. The color parameters are associated with the primary bioactive compound in the powder. As can be observed, the high a* value suggests that the sample predominate color is reddish, which indicates the presence of the betalains, as cited by Gengatharan et al. [32]. The Chroma displayed a similar pattern to the parameter a*, suggesting that the red hue had the most influence on determining the color of the PA powder. The hue angle was positioned in the first quadrant of the color solid and signifies the degree of redness of the sample. Based on the values of a* and b*, which showed a yellow and red trend typical of betalain pigments, the data was plotted in the first quadrant (+a*, +b*).

The extract from *P. americana* showed outstanding antioxidant qualities and a notable concentration of polyphenols. *P. americana* can be used as a natural ingredient in food products because of its notable coloring ability and antioxidative proprieties.

Our results are consistent with those of Nabavi et al. [33], who reported a total phenolic content of 102.11 ± 6.37 mg GAE/g of extract powder, a total flavonoid content of 24.7 ± 1.24 mg quercetin equivalent/g of extract powder, and an IC50 for DPPH radical-scavenging activity of 62.0 ± 2.1 µg/mL [34]. The strong activity of *P. americana* indicates that the donation of hydrogen may serve as a potential mechanism for the antioxidant activity of this plant. In another study, the values of the 50% inhibition concentration (IC50) of fruit extracts of *P. americana* for the DPPH radical were 2.19 ± 0.45 µg/mL, demonstrating a high DPPH scavenging capacity [34]. Phenolic compounds have been documented to possess antioxidant properties, enabling them to prevent oxidation by giving electrons to free radicals through an effective reduction mechanism [35].

A preliminary investigation conducted on *P. americana* fruit pigments discovered prebetanin (betanin 6′-O-sulphate) as the main betacyanin found, along with betanin and betanidin 5–O-[(5′′–O–E-feruloyl)–2–O–β–d-apiofuranosyl] β–D-glucopyranoside. An additional investigation on cell cultures obtained from the stem explants of *P. americana* has verified the existence of an additional pigment, lampranthin II (betanidin 5-O-(6′-O-E-feruloyl)-β-d-glucopyranoside) [6]. The observed varying results for the phytochemical content of the PA powder can be attributed to the variations in phytochemical variability in the raw material, environment, and extraction conditions.

3.2. Characterization of Bioactive Potential of Supplemented Cheeses

Different percentages of PA powder, ranging from 1% (semi-hard cheese with 1% PA powder CPAP1) to 2% (semi-hard cheese with 2% PA powder CPAP2), were added to highlight the added value of semi-hard cheese and the bioactive potential was analyzed (Table 2). The stability of the bioactive compounds and the phytochemical characterization were tracked over the course of 21 days of storage. The results demonstrate that adding PA powder to cheeses improves their quality, as evidenced by the higher betalain and polyphenol contents. These substances help to produce cheese products with higher levels of antioxidant activity. The results of this investigation suggest that PA powder may be used as a substitute for artificial colorants and antioxidants. The results demonstrated that the addition of PA powder to semi-hard cheeses considerably increased the samples' total

betalains, total polyphenols, and total flavonoids levels. As the powder level increased from 1% to 2%, the quantities of phytochemicals and antioxidant activity were significantly elevated ($p < 0.05$). Furthermore, a direct and positive association between the concentration of powder and antioxidant activity was discovered; this means that raising the powder content to 2% also resulted in a substantial increase in antioxidant activity ($p < 0.05$). The PA powder's phenolic components and specific betalains are probably what led to the rise in antioxidant activity. The flavonoid content in CPAP1 (1.01 ± 0.01 mg CE/g dw) and CPAP2 (1.23 ± 0.09 mg CE/g dw) was higher than in the control. Similarly, the total polyphenol content increases from 6.02 ± 0.02 mg GAE/g dw (CPAP1) to 6.25 ± 0.22 mg GAE/g dw (CPAP2), and betalain levels rise from 0.76 ± 0.04 mg/g dw to 0.90 ± 0.06 mg/g dw These incremental differences could be attributed to the solubility and interaction effects within the cheese matrix, which are known to alter during cheese processing and storage [36]. Research on the phenolic profile of vegetable matrix-enriched cheeses, such as those supplemented with PA powder, reveals significant interactions between phenolic compounds and dairy proteins, particularly caseins. Caseins, known for their proline-rich structure and flexible secondary configuration, enhance hydrogen bonding with phenolic compounds, resulting in a reduced bioavailability of phenolics in the final product [37,38]. This phenomenon may explain why the increase in PA concentration from 1% to 2% did not yield a proportionally higher phytochemical content or antioxidant activity, as shown in Table 2. It appears that the matrix interaction creates a saturation effect, limiting the effective utilization of additional phenolic compounds within the cheese.

Table 2. Phytochemical characteristics and antioxidant activity for cheese samples.

Parameters	Storage Time (Days)	CC	CPAP1	CPAP2
Total betalains (mg/g dw)	0	-	0.76 ± 0.04^{bA}	0.90 ± 0.06^{aA}
	21	-	0.73 ± 0.03^{bA}	0.87 ± 0.03^{aA}
Total flavonoids (mg CE/g dw)	0	0.49 ± 0.02^{cA}	1.01 ± 0.02^{bA}	1.23 ± 0.09^{aA}
	21	0.31 ± 0.02^{cB}	0.72 ± 0.01^{bB}	1.19 ± 0.11^{aB}
Total polyphenols (mg GAE/g dw)	0	3.48 ± 0.05^{cA}	6.02 ± 0.02^{bA}	6.25 ± 0.22^{aA}
	21	3.04 ± 0.04^{cB}	5.61 ± 0.02^{bB}	5.98 ± 0.20^{aB}
DPPH (µmol TE/g dw)	0	2.37 ± 0.06^{cA}	4.40 ± 0.22^{bA}	6.11 ± 0.22^{aA}
	21	2.17 ± 0.05^{cB}	4.00 ± 0.18^{bB}	5.89 ± 0.26^{aB}

Mean values of each sample accompanied by distinct superscript lowercase letters within the same row are substantially different ($p < 0.05$). Mean values of the same sample over time accompanied by distinct superscript uppercase letters in the same column are substantially different ($p < 0.05$).

The samples' TA, TP, and TF contents was reduced ($p < 0.05$) after 21 days of storage. Storage studies indicate a slight decline in phytochemical content and antioxidant activity in both CPAP1 and CPAP2 over time. This decrease could be attributed to the degradation of bioactive compounds or further interactions with dairy components. Nevertheless, the phytochemical analysis of the enhanced cheese was still higher than that of the control.

On the first day of storage, the cheeses' TP ranged from 6.02 ± 0.02 to 6.25 ± 0.22 mg GAE/g dw of fortified cheese, and on the last day of storage, it dropped to 5.61 ± 0.02 to 5.98 ± 0.20 mg GAE/g dw of fortified cheese. Additionally, the CPAP1 and CPAP2 activity remained higher than in the control after 21 days, although the DPPH scavenging activity decreased in all samples. The Tukey test indicated that the differences between the enhanced cheeses were statistically significant ($p < 0.05$). Similar studies showed a comparable decline in shelf life. According to the authors, bioactivity is lost as a result of extract deterioration during storage. In their research on cheeses enhanced with pumpkin powder, natural plant extracts, and green tea extracts, respectively, Khorshidian et al. [39] and Olszewska et al. [40] have both reported comparable outcomes. In response to the consumer demand

for more functional and nutritious diets, food manufacturers have recently considered incorporating naturally occurring antioxidants that are generated from plant by-products. Particularly with regard to dairy products, cheeses are increasingly being enriched with a variety of unconventional ingredients, such as physiologically active compounds, and a nutritionally balanced profile [41]. The most impressive antioxidants present in fruits and vegetables are phenols, as research results demonstrate. According to Kaptan and Sivri [42], dairy products enhanced with rosemary, basil, and thyme showed increased antioxidant activity and phenol content compared to the control cheese. Furthermore, all samples displayed a decrease in antioxidant activity and phenol concentration during the course of the storage period.

Incorporating PA powder increased the betalains, flavonoids, polyphenols, and in vitro antioxidant activity of supplemented cheeses. In a study conducted by Rashidinejad et al. [43], the impact of adding green tea catechins on the phenolic content and antioxidant capabilities of low-fat cheese was examined. The total phenolic content and antioxidant activity of all cheeses, including the control samples, increased during the ripening process. The elevated total phenolic content values can be attributed to the existence of milk-derived substances and diminished analytical precision, as well as the absence of the specificity of the Folin–Ciocâlteau reagent employed for total phenolic content analysis. This reagent reacts not only with phenols but also with other reducing compounds like carotenoids, amino acids, sugars, and vitamin C [44].

Another study examined the impact of varying quantities of grape extract on the process of cheese making. The antioxidant activity and total phenolic content of grape extract were enhanced. However, the grape extract caused an increase in brittleness and a decrease in firmness [45]. Alonso et al. [46] found a direct relationship between the antioxidant activity and the total polyphenolic content of samples using a positive correlation. The study yielded successful results when cheese was fortified with red and white grape pomace.

The results presented in this study support the findings reported by Pasini Deolindo et al. [47] about the use of jaboticaba (*Myrciaria* sp.) skin flour (5 g/kg of cheese) in the production of Petit Suisse cheese. Specifically, the study observed a decrease in DPPH values after a storage period of 28 days. Polyphenols undergo oxidation during food storage due to many factors such as light, temperature, pH, enzymes, and relative humidity. Consequently, a reduction in phenolics is anticipated in preserved foodstuffs. However, Ramos et al. [48] found that fermented milk produced with a lyophilized extract of clove (*Syzygium aromaticum*) and green mate (*Ilex paraguariensis*) showed an increase in total phenolic content after 14 days of storage. The authors suggest that this result may be attributed to the solubilization of reducing substances in the dairy matrix.

3.3. Color Evaluation

The color attributes (L*, a*, b*) of the supplemented cheese samples were measured and recorded in Table 3 at the initial moment and after 21 days of storage at 4 °C. As anticipated, PA powder offers a promising opportunity to improve the texture, flavor, and nutritional value of whey cheese. Given its strong correlation with desirability and overall food quality, color evaluation plays a significant role in assessing the quality of food products.

Table 3. Colorimetric parameters of semi-hard cheese enriched with PA powder during cold storage for 21 days.

Samples	Storage Time (Day)	L*	a*	b*	Chroma	Hue Angle	ΔE
CC	0	84.18 ± 0.41^{aA}	-1.61 ± 0.02^{aA}	23.41 ± 0.21^{aA}	23.47 ± 0.20^{aA}	178.49 ± 0.01^{aA}	-
	21	80.51 ± 0.38^{bA}	-1.37 ± 0.01^{bA}	19.92 ± 0.12^{bA}	19.97 ± 0.11^{bA}	178.50 ± 0.02^{aA}	-
CPAP1	0	50.77 ± 0.53^{aB}	3.27 ± 0.02^{aC}	9.21 ± 0.30^{aB}	9.78 ± 0.28^{aB}	1.23 ± 0.01^{aB}	36.63 ± 0.22^{aB}
	21	46.82 ± 0.49^{bB}	7.51 ± 0.03^{bC}	5.91 ± 0.22^{bB}	9.77 ± 0.12^{aB}	1.22 ± 0.02^{aB}	37.55 ± 0.30^{aB}
CPAP2	0	41.68 ± 0.42^{aC}	1.98 ± 0.13^{aB}	7.33 ± 0.28^{aC}	7.59 ± 0.23^{aC}	1.31 ± 0.02^{aB}	45.58 ± 0.32^{aA}
	21	38.21 ± 0.33^{bC}	5.76 ± 0.20^{bB}	3.49 ± 0.23^{bC}	6.73 ± 0.20^{aC}	0.54 ± 0.02^{aB}	45.94 ± 0.33^{aA}

L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness). The color parameter's temporal change is emphasized using lowercase letters. The color variations among the samples at the same moment of time are indicated by capitalized letters. Values that do not share a lower/uppercase letter are significantly different ($p < 0.05$).

The evaluation of the color characteristics demonstrated that the incorporation of PA had a noteworthy effect on the color of the cheeses. More precisely, the experimental cheeses exhibited the highest levels of redness (a*) and the lowest levels of lightness (L*) and yellowness (b*).

The Chroma of the color, which indicates its intensity and saturation, reached its highest value in the control cheese. The total color change parameter, or ΔE, had a range of 36.63 ± 0.22 to 45.94 ± 0.33 for the analyzed samples. When the hue angles were below 10°, the value of the hue angle was directly related to the color received and indicated the level of redness in the enriched sample [28].

The most significant factor that can influence a consumer's acceptance of food products is color [49]. The optimal method for incorporating betalain extracts into cheese was the one that accomplished the necessary color intensity without compromising the cheese's flavor and texture. The resulting cheese samples were then subjected to a panel of sensory evaluations to ascertain the effect on overall consumer perception and the color stability. The inclusion of PA powder had a significant effect on the brightness of cheeses, and a statistically significant rise was noted in terms of redness. The observed changes in the chromatic coordinates a* and b* throughout ripening were solely caused by the ageing process, likely due to changes in pigment concentration and oxidation.

Over the of 21 days in storage, the CPAP2 sample's ΔE ranged from 45.58 to 45.94. During storage, the inclusion of PA powder caused a significant ($p < 0.05$) rise in ΔE. In CPAP2 cheese, the color's Chroma—which conveys its intensity and saturation—was at its peak. The hue angle value showed the redness of both enriched samples and was proportionate to the received color because it was less than 10°. In general, an angle of 0° or 360° represents a red hue, whereas an angle of 90°, 180°, or 270° represents a yellow, green, or blue hue [50]. Cheeses' lightness was significantly affected by the addition of PA powder, and their redness increased statistically significantly. In a comparable manner, other researchers saw a decrease in L* values after incorporating plant extracts into their investigations [51,52].

The red value (a*) steadily rose when PA powder was added to the supplemented cheese. As the amount of PA powder increased, the other color features for the enhanced cheese's white L* and yellow b* gradually decreased in comparison to control samples.

The findings of Antonić et al. [53], in their review on the use of grape pomace in the development of food formulations, reported that was a notable reduction in the brightness of colors in formulations that were supplemented with grape pomace. This effect was more pronounced in samples with higher concentrations of this plant matrix. As expected, the incorporation of PA led to significant changes in both the exterior and interior color indexes.

The experimental cheeses had a prominent dark purple color, as indicated by a substantial rise in redness, followed by a decrease in brightness and yellowness.

3.4. Chemical Composition and Storage Stability of Semi-Hard Cheese Supplemented with PA

The chemical composition of the semi-hard cheese that was manufactured is detailed in Table 4. The examination of the collected media values reveals differences between CC and the cheese containing PA (CPAP1 and CPAP2) across nearly all examined variables. Cheese's chemical composition improved after PA powder was added, as compared to the control. The content of moisture, fat, protein, ash, and dry matter changes significantly ($p < 0.05$) as a result of the incorporation. Furthermore, adding PA powder to cheese causes its moisture and fat content to drop while its protein, ash, and total solids levels rise in direct proportion to the amount of powder added.

Table 4. Chemical composition and storage stability of supplemented semi-hard cheese.

Component (%)	Product	Storage Period (Day)	
		0	21
Moisture	CC	38.2 ± 0.05^{aA}	37.95 ± 0.12^{aB}
	CPAP1	35.44 ± 0.07^{bA}	35.16 ± 0.15^{bB}
	CPAP2	33.83 ± 0.35^{aA}	33.49 ± 0.08^{cB}
TS (total solids)	CC	61.85 ± 0.08^{cB}	62.05 ± 0.09^{cA}
	CPAP1	64.59 ± 0.06^{bB}	64.86 ± 0.15^{bA}
	CPAP2	66.19 ± 0.30^{aB}	66.52 ± 0.07^{aA}
Fat	CC	33.81 ± 0.07^{aA}	33.95 ± 0.08^{aA}
	CPAP1	33.62 ± 0.05^{bB}	33.83 ± 0.07^{aA}
	CPAP2	33.29 ± 0.04^{cB}	33.57 ± 0.08^{bA}
Total protein	CC	24.7 ± 0.07^{cA}	24.77 ± 0.11^{cA}
	CPAP1	24.94 ± 0.08^{bB}	25.07 ± 0.08^{bA}
	CPAP2	25.18 ± 0.04^{aB}	25.41 ± 0.14^{aA}
Ash	CC	4.31 ± 0.12^{bA}	4.34 ± 0.03^{bA}
	CPAP1	5.87 ± 0.09^{aA}	5.91 ± 0.02^{aA}
	CPAP2	5.94 ± 0.07^{aA}	5.97 ± 0.03^{aA}
pH	CC	5.31 ± 0.02^{aA}	5.11 ± 0.04^{aB}
	CPAP1	5.2 ± 0.04^{bA}	5.04 ± 0.04^{aB}
	CPAP2	5.18 ± 0.04^{bA}	4.97 ± 0.04^{bB}

There is a significant difference ($p < 0.05$) between any two means of each sample, within the same column, that has a distinct superscript lower letter; There is a significant difference ($p < 0.05$) between any two means of the same sample over time, within the same row, that has a distinct superscript capital letter.

Regarding chemical composition, the addition of natural functional components, such as those derived from fruits, vegetables, grains, and other foods, to cheese will result in a slight modification of its nutritional profile [52,54]. The addition of PA to semi-hard cheese resulted in a substantial enhancement of its chemical composition in comparison to the control. Finally, the inclusion of 1% and 2% powder in cheese resulted in an increase in the levels of fat, total protein, ash, and crude fiber.

As expected, the product's pH decreased statistically significantly ($p < 0.05$) when the powder concentration increased from 1% (CPAP1) to 2% (CPAP2). When compared to CC, the pH value decreased by around 3%, reaching 5.18 ± 0.04. The pH value for the control was measured to be 5.31 ± 0.02, while for CPAP1, it was 5.20 ± 0.04. With an average value of $66.19 \pm 0.30\%$ for the CPAP2 product, the addition of PA powder increased the dry matter content. There were no detectable changes in the moisture content. The dry matter content of the cheese was affected by the ripening period, when CC cheese lost more humidity than both the CPAP1 and CPAP2 products, respectively. There are two types of water in cheese: bound and free. Although it is inaccessible for biological processes, bound water is in charge of hydrating hydrophilic molecules and dissolving solutes.

The amount of total solid and total protein in the whey cheese increases significantly when a larger percentage of PA powder is added ($p < 0.05$). Significant differences ($p < 0.05$)

were seen in the total solids between the control and the enriched samples (CPAP1 and CPAP2). After 21 days of storage, the total protein content in the control, CPAP1, and CPAP2 cheeses was 24.77 ± 0.11%, 25.07 ± 0.08%, and 25.41 ± 0.14%, respectively. Furthermore, it is clear that as the concentrations of PA powder increased in tandem, the protein content of the prepared cheese gradually increased as well. The findings show that while the addition of powder reduced moisture, the addition of cheese increased the amount of ash. The cheese that had the highest ash content was CPAP2 (5.97 ± 0.03%), and the cheese with the lowest ash content was CC (4.34 ± 0.03%).

Scientists employed powdered ingredients in the formulation process to enhance the quality of cheese samples. The utilization of grape pomace enhances the nutritional value of semi-hard cheese. The grape pomace powder had minimal impact on the physicochemical parameters of the cheese samples [55]. According to Granato et al. [54], it is necessary to assess the chemical stability of a food that contains bioactive chemicals in order to confirm the effectiveness of this product. The total titratable acidity dropped and the pH increased as a result of refrigerated storage for 28 days.

3.5. Results of Sensorial Analysis

The sensorial analysis was performed following the different sensorial characteristics such as appearance, aftertaste, section appearance, odor, aroma, texture, color, taste, and overall acceptability. The cheese samples were subjected to the sensory analysis and the results are presented in Figure 3. According to the data presented in Figure 3, the panelists assigned product scores ranging from six to seven ("like very much" and "extremely like") for each feature. Figure 4 presents the principal components analysis of the sensory attributes of the control and enhanced semi-hard cheeses.

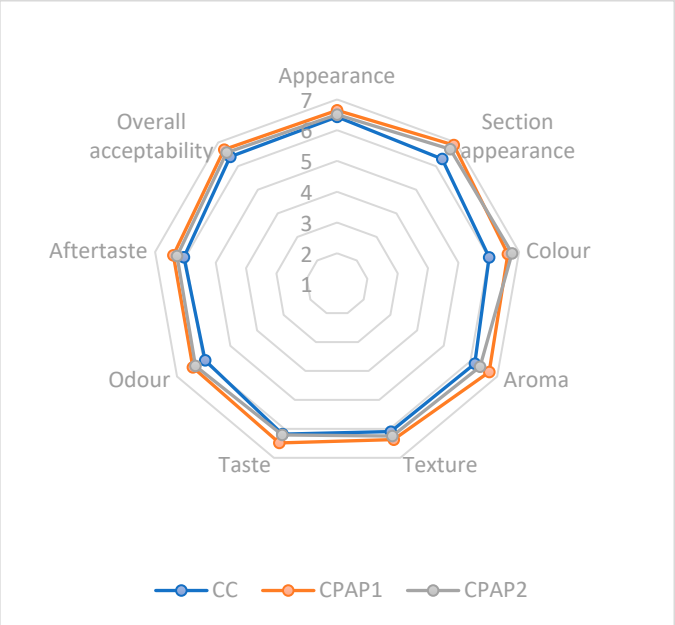

Figure 3. Spider diagrams corresponding to the descriptive sensory analysis of the control and enhanced semi-hard cheeses (control (CC), cheese with 1% PA (CPAP1), and cheese with 2% PA (CPAP2)).

The attributes that were assessed consistently showed an influence from including PA. The PA addition resulted in an improvement in odor and scent strength, taste perception, aftertaste sensation, appearance, section appearance, and color while exerting a detrimental impact on texture qualities. The taste of the cheese was not substantially altered by the addition of 2% PA powder. The CPAP1 and CPAP2 samples first tasted light and fresh and had a drier, granular look. Similarly, there was no appreciable variation in the cheeses'

odor descriptor scores. Overall, the odor scores remained above the acceptable threshold, where a score of greater than six was achieved. The CPAP1 cheese with 1% PA powder exhibited superior color intensity compared to the control. In general, the CPAP1 cheese that was fortified with 1% of PA powder demonstrated positive sensory assessments, as evidenced by its superior overall acceptance score.

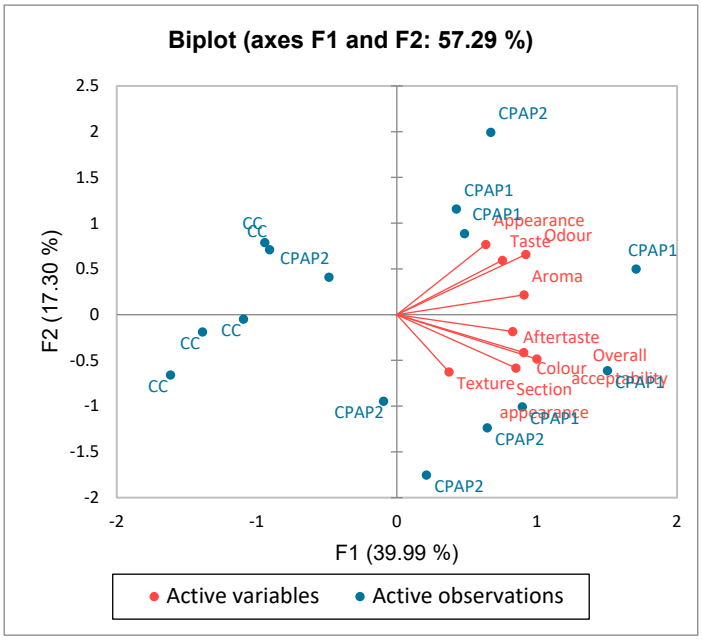

Figure 4. Principal components analysis (PCA) of the sensory attributes of the control and enhanced semi-hard cheeses (control (CC), cheese with 1% PA (CPAP1), and cheese with 2% PA (CPAP2)).

The PCA biplot, illustrated in Figure 4, offers a visual depiction of the locations of the three cheese samples (plain, compared to those treated with PA) according to the sensory qualities delineated in Figure 3. The primary principal component (PC1) explained 39.99% of the variance and comprised the two cheese samples with PA. The second component constituted 17.30% of the total and comprised plain cheese. Consequently, the two axes represented 57.29% of the total variation. The characteristics of appearance, taste, odor, and aroma exhibited a high correlation on the first axis, F1. Similarly, the qualities of sectional appearance, color, texture, aftertaste, and overall rating were also found to have a positive connection in the same quadrant on the first axis, F1. Since all sensory attributes were exclusively linked to the same axis, F1, the plain cheese was classified as neutral.

Figure 5 demonstrates that the addition of PA had a significant effect on the hue of the product. Nevertheless, testers particularly valued this specific attribute, as seen by CPAP1 receiving a higher score compared to CC and CPAP2. According to preliminary findings, cheese can be successfully colored with an intense, natural hue using betalains extract from *Phytolacca americana* L., and the color appears to be stable over time. The cheese with a purple hue due to betalains appears to be well-liked by the panelists. The berries color also varies from bright red to bluish black. Because betalains have certain health-promoting qualities, they can be utilized to improve the flavor and color of semi-hard cheese. The findings suggest that the PA powder, which contains a high concentration of phenolic compounds, does not interfere with the acceptability and sensory characteristics of the semi-hard cheese.

(**a**) (**b**) (**c**)

Figure 5. Images of the control and enhanced semi-hard cheeses. (**a**) Control (CC), (**b**) cheese with 1%PA (CPAP1), and (**c**) cheese with 2%PA (CPAP2).

Consumers' preferences for taste, scent, color, and overall quality were determined through sensory evaluations. The results of the sensory evaluations indicated that the supplemented cheeses maintained their overall sensory desirability while exhibiting distinct acid taste characteristics. The inclusion of PA powder impacted the sensory characteristics of the cheeses. In general, the inclusion of fibers or by-products has an important impact on the sensory characteristics of dairy products [52,56,57]. In this study, the evaluated attributes were consistently influenced by the addition of PA. In detail, the addition of PA increased the odor and aroma intensity, taste perception, aftertaste sensation, appearance, section appearance, and color while negatively influencing texture attributes. For the color intensity, the CPAP cheese with 1% PA powder was better assessed, compared to the control. Overall, the CPAP cheese enriched with 1% of PA powder exhibited favorable sensory evaluations due to having the highest overall acceptability score. Similar results were obtained by Costa et al. [58], who conducted a study where they examined the use of white and red wine grape pomace for enhancing bovine Primosale cheese. Additionally, the findings of Anderson et al. [59] demonstrated that adding Cornelian cherry and conventional blackcurrant to cheeses could increase their bioactive potential and raise their total phenolic content without changing their flavor or overall organoleptic evaluation. Furthermore, even at the highest tested concentrations, this method has not seen the undesirable influence of polyphenols on the lactic acid bacteria, suggesting the potential use of both cornelian cherry and blackcurrant in cheeses.

3.6. Texture Analysis of Cheese

According to studies on texture profiles, the semi-hard cheese has an elastic, supple texture. It has less stiffness than Parmesan but more structure than soft cheeses due to its hardness, which puts it in the midst of the soft and hard cheese spectrum. In keeping with the qualities of semi-hard cheeses, it is especially elastic, making it simple to slice, and has a cohesive texture that prevents it from crumbling [37,38].

The enriched samples were firmer than the control, as Table 5 previously showed, but the addition of PA powder had no perceptible effect on hardness scores. This implied that the panelists could withstand the higher hardness of the reinforced samples. Different authors concluded that perceived differences between items do not always affect their acceptability. According to the research of Lucera et al. [60], cheese samples that had red and white wine grape pomace added had the best nutritional outcomes, followed by broccoli, artichoke, and maize bran. Red and white wine grape pomace added to spreadable cheese, however, could maximize technical choices by increasing antioxidant components without altering the cheese's gastronomic qualities. According to many specialists, a complex interplay between the content of the milk and cheese, production processes, and ripening circumstances affects the texture of cheese [61,62].

Table 5. Textural parameters of the control and supplemented cheeses during storage for 21 days.

Parameter	Product	Storage Period (Day)	
		0	21
Hardness (N)	CC	2.45 ± 0.05^{cB}	2.73 ± 0.03^{bA}
	CPAP1	2.54 ± 0.03^{bB}	2.84 ± 0.04^{aA}
	CPAP2	2.61 ± 0.02^{aB}	2.89 ± 0.04^{aA}
Cohesivity (%)	CC	73.45 ± 0.04^{bA}	71.55 ± 0.03^{Ba}
	CPAP1	73.48 ± 0.04^{abA}	71.57 ± 0.03^{abB}
	CPAP2	73.5 ± 0.03^{aA}	71.60 ± 0.04^{abB}
Gumminess (N)	CC	180.25 ± 3.42^{cB}	195.47 ± 2.85^{cA}
	CPAP1	186.78 ± 2.73^{bB}	203.25 ± 3.03^{bA}
	CPAP2	191.54 ± 1.93^{bA}	206.78 ± 3.39^{aA}
Elasticity (%)	CC	84.85 ± 0.05^{bB}	$86.12b \pm 0.05^{aA}$
	CPAP1	87.15 ± 0.03^{aB}	88.57 ± 0.04^{aA}
	CPAP2	87.33 ± 0.03^{aB}	88.76 ± 0.04^{aA}
Chewiness (mJ)	CC	152.95 ± 2.97^{cB}	168.34 ± 2.54^{cA}
	CPAP1	162.77 ± 2.32^{bB}	180.01 ± 2.72^{bA}
	CPAP2	167.28 ± 1.69^{aB}	183.54 ± 3.0^{aA}

Cohesivity (%): Area2 compression/Area1 compression; Gumminess (N): Hardness (N) × Cohesivity; Elasticity (%): (Distance2)/(Distance1) × 100; Chewiness (mJ): Elasticity (mm) × Gumminess (N). There is a significant difference ($p < 0.05$) between any two means of each sample, within the same column, that has a distinct superscript lower letter. There is a significant difference ($p < 0.05$) between any two means of the same sample over time, within the same row, that has a distinct superscript capital letter.

Cheese's structural qualities are influenced by the presence of fat globules scattered throughout a hydrated protein matrix. Better hardness can be maintained with a robust network that is more resistant to proteolytic processes. Proteases from starter cultures and other microorganisms, as well as endogenous milk enzymes and leftover chymosin, cause proteolysis, which softens cheese. A delicate equilibrium between the hydration and proteolysis of the casein strands takes place during the ripening process, which tends to lessen the cheese's hardness. Nevertheless, the process's loss of moisture causes the concentration of proteins to rise, which has the opposite effect on hardness. Cheese's hardness is influenced by free water, which rises as humidity falls. CPAP2 cheese showed the biggest moisture reduction. Additionally, it displayed less proteolysis than CC, which resulted in a notable drop in hardness, indicating a quicker rate of protein decomposition. Other authors found similar results and came to the conclusion that the amount of free fat in milk and dairy products and other factors such as temperature, pH, and the mixing ratio, as well as the fine structure of these components, significantly affect the binding of casein to the dairy components and have a significant impact on their quality. The use of extra enzyme preparations or cultures of microorganisms that stimulate lipolysis and proteolysis is advised in order to increase the quantity of fat that is available in cheese products [63,64].

Hardness naturally increased as a result of a better balance between the reduction of water content and proteolytic events. The notable variations at one day persisted until 21 days, when their values increased for all three types of cheese. The natural age process of cheese maturation is reflected in the growth of textural qualities, with the exception of cohesiveness. Maintaining a consistent texture profile over the course of the shelf life is essential for both sensory acceptance and marketing success. The cheese's protein matrix changes from having a spongy texture to having a denser structure. The control sample had the lowest hardness value of 2.45 ± 0.05 on the first day of measurements and 2.73 ± 0.03 after 21 days of ripening. Consequently, it is discovered that the addition of PA powder helps to explain the noted rise in cheese paste hardness as determined by its compressive strength. This outcome is most likely due to the fact that PA affected the experimental cheeses' dry matter (from 61.85 in CC to 64.59–66.19 in CPAP1 and CPAP2, respectively) or fat content (from 33.81 in CC to 33.62–33.29 in CPAP1 and CPAP2, respectively). Due to moisture loss and biochemical alterations that take place over time, it has been discovered that the

hardness of the cheese paste drops when it is stored. The impact of *Jujube* polysaccharide and *Lycium barbarum* polysaccharide on the physicochemical characteristics of goat milk cheese, including its texture, rheological characteristics, and microstructure, was examined by Wang et al. [65]. Because a stronger casein network structure was formed, they found that the goat milk cheese with 1% *Jujube* had the best rheological qualities, hardness, and water retention capacity.

When powder was added, the gumminess values were raised in comparison to the control sample. Gumminess increased over the course of the 21-day ripening period, presumably as a result of structural alterations like protein network modifications or moisture redistribution. Textural alterations may result from the action of proteases or the irreversible denaturation of proteins, which may weaken protein links.

In a comparable manner, the CPAP1 and CPAP2 cheeses were chewier and gummy than the CC cheese, suggesting that their more rigid network resulted in stronger internal links. The basic metrics that they rely on can be used to define the textural criteria of chewiness, which is the result of hardness, cohesiveness, and springiness, as well as gumminess, which is the product of hardness and cohesiveness. The increased dry matter content and lower moisture content of the CPAP1 and CPAP2 cheeses in comparison to the CC cheese can be used to explain the rise in textural characteristics. Additionally, this pattern can be attributed to the way the PA powder's protein and fiber combine with the moisture in the cheese to create a stronger three-dimensional structure that raises the cheese's hardness, gumminess, and chewiness. Because PA powder contains a lot of soluble fiber, adding it to curd significantly improves moisture retention. By acting as sponges, these fibers draw in and hold onto water inside the cheese's matrix, increasing its moisture content and suppleness. Customers benefit greatly from this development since it improves the cheese's texture and makes it appealing to customers. Similar results were found by Giroux et al. [66] in their investigation of cheeses enhanced with green tea extract. They claim that the potential of polyphenols to reduce moisture content and alter the structural arrangement of the paracasein matrix is what causes their influence on cheese texture. The qualities of adhesiveness and springiness also showed similar tendencies, increasing in the supplemented samples. Cohesion was unaffected by the fortification.

3.7. Results of Microbiological Analysis of Milk and Cheese

The microbiological study of the semi-hard cheese without PA powder (CC, control sample) and the supplemented semi-hard cheese with PA powder is summarized in Table 6, which shows that the product was safe to eat. These findings show that the product under evaluation has been deemed safe for ingestion by humans. As a result, every sample examined showed that *coagulase-positive staphylococci* and *Escherichia coli* were not present. Conforming to the limits set by European regulation 2073/2005 [67], the samples were analyzed in accordance with ISO16649-1 [68] and ISO6888-1 [69] standards.

All samples showed negative results for coagulase-positive staphylococci (*Staphylococcus aureus*) and *Escherichia coli* from the perspective of microbiological criteria. The presence and number of microbes in foods, including cheeses, are influenced by the pH, salt concentration, type, and amounts of additional powders.

All cheese samples were analyzed for *L. monocytogenes*, and its presence was not identified in any of the three samples examined in the study. Consequently, a minimal degree of contamination during processing and an improvement in the hygienic quality of the samples analyzed is evidenced by the lack of *L. monocytogenes*, *E. coli*, and coagulase-positive staphylococci following the incorporation of PA powder. The hygienic procedures used during the production of cheese and during milking have a direct impact on the starting concentrations of potentially hazardous bacteria in milk. According to our research,

Phytolacca's antibacterial properties might cause a little drop in the overall number of bacteria in the 1% and 2% samples when compared to the control. Solís-Salas et al. [70] also found similar results, demonstrating that the leaves of *P. americana* contain chemicals that have antibacterial properties against medically significant microorganisms. Since their effects are linked to antioxidant chemicals, it thus serves as a source for novel antibiotics.

Table 6. Microbial profiles of semi-hard cheeses.

Microbiological Parameter	Limits	CC	CPAP1	CPAP2
TVC (total viable count) (log CFU g^{-1})	$<10^2$	7.61 ± 0.04^a	7.53 ± 0.03^a	6.21 ± 0.03^b
Listeria monocytogenes	Absent in 25 g	Absent	Absent	Absent
Escherichia coli (log CFU g^{-1})	10^2–10^3	Absent	Absent	Absent
Coagulase positive staphylococci (log CFU g^{-1})	10^2–10^3	Absent	Absent	Absent

There is a significant difference ($p < 0.05$) between any two means of each sample within the same row that has a distinct superscript lower letter.

Our investigation indicated that the antibacterial capabilities of *Phytolacca* can cause a modest decrease in the total viable count in 1% and 2% samples relative to the control. The inclusion of 2% PA powder in cheese enhanced the inhibition of spoilage bacteria, likely due to the elevated concentration of antimicrobial substances in the examined product. Still, the recorded levels remained within the maximum limits established by European Regulation 2073/2005 [67].

4. Conclusions

This study demonstrates that PA powder, rich in bioactive compounds, exhibits remarkable antioxidant activity, making it a promising natural food preservative, colorant, and antioxidant. The addition of PA powder to cheese significantly improved the total phenolic content, the antioxidant potential, and certain nutritional characteristics compared to the control sample. The sensory evaluation revealed that the PA-supplemented cheeses, particularly those prepared with 1% powder, achieved high acceptability among panelists. This highlights the potential for developing novel, healthy, and visually appealing cheese products that align with the increasing consumer demand for functional and clean-label foods. By replacing artificial dyes with natural additives like *P. americana* extract, this research contributes to advancing sustainable food production. However, further optimization of the formulation and storage stability is necessary to fully realize the potential of this supplemented cheese product. Overall, incorporating PA powder into semi-hard cheese formulations offers an effective approach for enhancing antioxidant levels and improving the nutritional profile of dairy products. To support the promise of the candidate plant extracts for various uses, more testing of these extracts' biological activity against a wider microbial panel and toxicity investigations is required.

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Data Availability Statement: Data supporting reported results are available, upon request, from the authors.

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

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