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Antioxidant Purple Corn Protein Concentrate from Germinated Andean Purple Corn Seeds

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Abstract: Ecuador Andean purple corn (*Zea mays* L.) was subjected to a germination process at 15–40 °C for 24–168 incubation hours. Purple corn protein concentrates (PCPCs) were obtained by alkaline extraction at pH 8.0 and pH 10.0, followed by an isoelectric precipitation process at pH 4.0, pH 5.0 and pH 6.0. Proteins and phenolic content of PCPCs was calculated. PCPC antioxidant properties were determined by the ferric-reducing antioxidant power (FRAP) in vitro method and by the 2,2-azinobis, 3-ethyl-benzothiazoline-6-sulfonic acid, (ABTS) in vitro method. Andean purple corn seeds were able to germinate under the germination conditions tested in this study. The higher percentage of germination was of 63.33% at 168 h/25 °C. The PCPCs protein profile was characterized for the presence of six bands with molecular weights of 14.50 kDa, 20.12 kDa, 25.18 kDa, 41.85 kDa, 59.59 kDa, and 65.87 kDa. Germinated PCPC presented a high TPC content with ranges of 605.71–1820.00 mg gallic acid equivalents (GAE)/g PCPC dry weight (DW), germinated PCPC/72 h/25 °C presented a higher value of 1820.00 mg GAE/g PCPC, DW. All germinated PCPCs samples assayed presented strong antioxidant activity when measured by the ABTS and FRAP methods. Germinated PCPC/144 h/35 °C presented high antioxidant activity by ABTS with 804.35 µmol Trolox equivalents (TE)/g PCPC DW and germinated PCPC/144 h/30 °C presented a high value by the FRAP method, 987.83 µmol TE/g PCPC DW.

Keywords: Andean purple corn; *Zea mays*; purple corn protein concentrate; germinated; antioxidant activity

1. Introduction

Corn (*Zea mays* L) stands at third place in importance of cereal crops after wheat and rice with 1147 million tons of fresh weight of seeds produced in 193 million hectares during 2018 [1]. In Ecuador, corn is one of the main crops in the Andean region. In 2018, more than 365,334 hectares were harvested with a total production of 1,324,147 tons. This represents an average yield of 3.62 t/ha [1]. Corn belongs to the Family Poaceae and Genus Zea [2,3]. There are several types of corn grains with different colors such as yellow, blue, brown, green, and purple. Purple corn, also called purple maize, is a native crop of the Andean region in South America cultivated in Peru, Ecuador, Bolivia, and Argentina [4,5]. Maize is a cereal crop widely spread throughout the world from latitude 580 N to 400 S, from sea

level up to more than 3000 m of altitude and in areas with an annual rainfall between 250 mm and 5000 mm [6]. The countries with major corn production in the world are the USA with 30%, China 15%, EU 14%, Brazil 4%, and India 3% [6]. Corn is used for animal and human food for their chemical composition and a high nutritive value. Corn grains have a high starch content (72%), 7–13% protein content, 2.5% of fiber, and 4.8% of oil content. Corn grains have an important content of vitamins and minerals [7–11].

Germination is a biological process that allows plants to use seeds to conserve species. With this process, a series of biological mechanisms are initiated that allow the growth of an embryo. The embryos begin germination with the entry of water and end with the elongation of the embryo axis-terminal. During this complex process, different biological compounds of the seeds undergo catabolism and synthesis processes [12–14]. In the early stages, many of the phytocompounds are used for embryo growth. The synthesis of new phytocompounds used for different purposes including protection mechanisms is activated. In addition, during germination, the seeds reduce some components that are considered anti-nutrients (phytic acid and lectins) [15–18].

At the biotechnological level, sprouts are used to generate germplasm banks. For many years, sprouts have attracted the interest of the food industry for the nutritional value of their components and their biological properties such as the antioxidant activity [19]. Antioxidant compounds are used in the food industry to preserve processed foods against lipid oxidation [20–22]. Recent studies have demonstrated their inhibitory effects against certain diseases such as cancer [23,24]. Therefore, there is a considerable interest in the search for natural compounds with antioxidant properties [25–27]. Many natural extracts obtained from plants containing mainly polyphenols, flavonoids, carotenes, and anthocyanins have been described in the scientific literature for their high antioxidant activity [28,29].

Sprouts can be a natural alternative to obtain compounds with antioxidant capacity (polyphenols and proteins). Different sprouts have been described with biological activities, lentils (*Lens culinaris*), soy (*Glycine max*), amaranth (*Amaranthus caudatus*), quinoa (*Chenopodium quinoa* Willd), and beans (*Phaseolus vulgaris*) [30–35]. Piñuel et al., 2019 described protein isolates of sprouts and hydrolysates obtained from quinoa seeds (*Chenopodium quinoa* Willd) with high antioxidant capacity [36]. The objective of this work was to determine the germinations conditions of Andean purple corn seeds to produce purple corn protein concentrate (PCPC) and to evaluate their antioxidant activity using the 2,2-azinobis, 3-ethyl-benzothiazoline-6-sulfonic acid, (ABTS) and ferric-reducing antioxidant power (FRAP) methods.

2. Material and Methods

2.1. Chemicals Reactive

Folin–Ciocalteu reagent, gallic acid standard, 2, 20-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 6-Hydroxy-2,5,7,8-tetramethylchroman-2 -carboxylic acid (Trolox standard) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Plant Material

Andean purple corn seeds (*Zea mays* L., INIAP-199 bunch of grapes) were collected from 40 corn plants of the crop grown by the Bolivar State University (Guaranda), and the National Institute of Agricultural Research (INIAP), (Quito). The crop was grown in the city of Guaranda, Bolivar in Ecuador at an altitude of 2800 m, south latitude 01°34′15′′ and west longitude 79°0′02′′. The average annual temperature of the site is 13 °C with 75% humidity. The harvest was carried out by hand once the seeds reached physiological and commercial maturity. The seeds were manually shelled and dried in a drying rack in the open air until reaching a humidity of 14%. Then, they were stored in plastic containers.

2.3. Proximal Analysis of Purple Corn Flour

The chemical composition of purple corn flour was analyzed according to standard protocols. Fat was analyzed according to AOAC 2003.06:2012, moisture AOAC 925.10:2012 [37], fiber INEN 522:2013, ash INEN 520:2013 [38], and protein with the Dumas method.

2.4. Germination of Purple Corn Seeds

Andean purple corn seeds were germinated according to the methodology described by Paucar-Menacho et al. (2016) [39]. One hundred seeds were submerged for disinfection in 0.1% sodium hypochlorite solution (1:5 *p/v*) for 30 min at room temperature and then washed with distilled water. The seeds were then submerged in distilled water (1:5 *p/v*) at room temperature for 24 h. Subsequently, the hydrated seeds were introduced to the BINDER KBF 240 (LabReCo, Horsham, PA, USA) constant climate chamber on wet filter paper, with a water circulation system to always keep the seeds moist. Germination was carried out in the dark at the following temperatures with durations of 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h. Three replications were made for each germination condition. The percentage of germination was calculated to each treatment of germination. The percentage of germination = (% normal seeds + % anormal seeds + % dead seeds) = 100%. Normal seeds were evaluated as good ridicule, primary roots and secondary roots well grow; hypocotyl well grow; plumule with good development, with leaves well grow and a healthy cotyledon. Abnormal seeds were evaluated as damaged primary root and absence of secondary roots and cotyledons and leaves deformed, necrotic, or damaged by infections.

2.5. Purple Corn Protein-Phenolic Concentrate (PCPC) from Germinated Seeds

Germinated and non-germinated Andean purple corn seeds were ground in a Perten 120 laboratory mill (Perten Instruments, Hägersten, Sweden) until a flour (<500 mm) was obtained. Purple corn flour defatted (10 g) was resuspended in 100 mL of Milli-Q water. The pH of the solution was adjusted to pH 8.0 and pH 10.0 with 2.0 M NaOH. The solution obtained was shaken for 60 min and centrifuged at 10,000× *g* for 60 min at 4 °C. The precipitate obtained was separated and discarded (fiber, ash, and carbohydrates). The pH of the supernatant was adjusted to pH 4.0, pH 5.0 and pH 6.0 with 1 NHCl. The supernatant solutions were centrifuged at 10,000× *g* for 30 min at 4 °C. The pH of the precipitate obtained was adjusted to pH 7.0 using a 0.5 M NaOH solution. PCPC samples produced were dried by a lyophilization technique and frozen and stored at −80 °C [40]. PCPC protein content was calculated by the Dumas method using a macro elemental analyzer (Elemental Vario Macro Cube, Langenselbold, Germany). The PCPC protein percentage was determined with the equation % of protein = 6.25 × % N, where 6.25 is the conversion factor and N is the percentage of nitrogen determined by the instrument [41].

2.6. PCPC Electrophoresis Analysis

PCPC proteins profile was characterized by the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method using a mini-protean cell electrophoresis system (Bio-Rad, Hercules, CA, USA). The gels were prepared with a concentration of 12.00% polyacrylamide. The tinting of gels of polyacrylamide was made using a Coomassie Brilliant Blue G-250 solution for 24 h with shacked up. PCPC protein molecular weight was calculated with the help of Gel Documentation Imagen systems (Analytic Jena Tower, Jena Germany). A marker of standard with molecular weights of 10 kDa–200 kDa (Bio-Rad, Hercules, CA, USA) was used [42].

2.7. PCPC Quantification of Total Phenolic Content (TPC)

The samples for TPC analysis were extracted in all PCPCs samples. The TPC was extracted using methanol (70%) from lyophilized PCPC and stirred for 5 min, followed by an ultrasound technique for 10 min. The extracts were centrifuged, filtered, and calibrated. An aliquot of the solution was

separated and mixed with distilled water, Folin Ciocalteau reagent, and with sodium carbonate (20%). The samples absorbance was measured at 765 nm. TPC quantification was made with a standard calibration curve of gallic acid (GA). The standard curve obtained was (y = 0.0021x + 0.0033, R^2 0.9982). TPC results were expressed as mg gallic acid equivalents GAE/g of PCPC, DW [43].

2.8. Assay of Antioxidant Activity by ABTS Method

Germinated PCPCs (200 µL) were mixed with 3800 µL of ABTS solution (composed of 7 mM ABTS solution with 2.45 mM potassium persulfate solution in a 1:1 ratio) and then diluted with phosphate buffer until obtaining an absorbance of 1.1 ± 0.01 a 743 nm. For the determination of the concentrations, a calibration curve was performed with the Trolox standard solution (200 µmol to 1000 µmol). The curve obtained was (y = 0.012x + 0.2089, R^2 = 0.9901). The data were expressed as µmol of trolox equivalents TE/g PCPC, DW [44].

2.9. Assay of Antioxidant Activity by the Ferric-Reducing Antioxidant Power (FRAP) Method

Germinated PCPCs (1 mL) were mixed with 2.5 mL of pH 6.6 buffer solution. 5 mL of 1% potassium ferrocyanide solution was added. The sample was incubated in a water bath at 50 °C for 20 min. Then, 2.5 mL of 10% trichloroacetic acid solution, 2.5 mL of distilled water and 0.5 mL of 1% ferric chloride were added. Samples and standards were homogenized in a vortex and rested for 30 min in the dark. Finally, the absorbance of the solutions was measured at 700 nm.

For the determination of the concentrations, a calibration curve was performed with the Trolox standard (200 µmol to 1000 µmol). The curve obtained was (y = 0.0016x + 0.1324, R^2 = 0.9985). The data were expressed as µmol of trolox equivalents TE/g of PCPC, DW [45].

2.10. Statistical Analysis

Results were presented as mean ± standard deviation (n = 3). Statistical differences of the samples were evaluated with one-way ANOVA analysis ($p < 0.05$) followed by the Tukey test. The statistical differences were presented with a different letter.

3. Results and Discussion

3.1. Purple Corn Flour Proximal Analysis

Proximal analysis of purple corn flour showed that protein content was 8.58 ± 0.07%, fat content 5.73 ± 0.10%, ash content 0.02 ± 0.00%, moisture content 11.70 ± 0.05%, fiber content 2.91 ± 0.26%, and carbohydrates content 71.06 ± 0.16%. Their composition depends on the environmental conditions of the cultivars, temperature, variety, and type of seeds (yellow, white, black, blue, and purple seeds). Trehan et al. (2018) reported the chemical composition of three varieties of white, yellow, and purple corn flours. They reported a fat content for yellow corn flour (2.85–5.23%), white corn flour (2.03–4.95%), and purple corn flour (1.70–4.61%). They reported protein content of yellow, white, and purple corn flour (8.44–8.70%), (8.73–9.54%), and (9.53–9.88%), respectively [46]. Mansilla et al. (2020) reported different genotypes of purple corn cultivars from Argentina with protein contents between 9.48% to 11.50% and fat contents between 6.72% and 8.21% [47]. The results of protein content of purple corn flour reported in this study were lower than those reported by the other researchers, while the fat content was higher.

3.2. Germination (%) of Purple Corn Seeds

Andean purple corn (100 seeds) were put in the germination process in the incubator for 24 h, 48 h, 72 h, 96 h, 120 h, 144 h, and 168 h at 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C. Table 1 showed the germination percentage obtained at different conditions. Germination percentages were obtained with values between 0.00 ± 0.00 and 63.33 ± 7.23. The highest germination percentages were obtained at the temperature of 25 °C with germination values between 42.00% and 63.33%.

The seeds incubated at 15 °C for 24h to 96 h did not showed germination. At 120 h, they presented a 6.00 ± 0.58% of germination. These values increased with a germination time of 168 h, presenting the highest percentage with a value of 63.33%. The increase in % of germination was proportional to the increase in the incubation time of the seeds to germinate. As the germination temperature increases, the germination percentage decreases. The germinated rates obtained at 40 °C varied between 9.33% and 26.00%. These were the lowest values of germination obtained.

The results obtained present significant differences at $p < 0.05$ when compared to incubation temperature with time of incubation of germination of the Andean purple corn seeds. Govender, Aveling, and Kritzinger (2007) reported percentages of germination of yellow and white varieties from northern KwaZulu-Natal and southern Mozambique with values of 18.70 to 100% depending on the variety. Germination process was made at 25 °C for 7–11 days [8].

Figure 1 showed the registered pictures of the Andean purple corn germinated obtained at different temperatures and different germination times. Figure 1a,b showed the seeds germinated at 20 °C and 25 °C during 72 h, 96 h, 120 h, 144 h, and 168 h of incubation. All the seeds showed clear signs of germination with lifting of the cotyledons and elongation of the radicles. Figure 1c showed the purple corn seeds germinated at 30 °C, 35 °C, and 40 °C for 168 h. In the pictures, a clear germination process is observed, and the embryos showed elongation of the radicles. Germinated kernels at 30 °C and 35 °C have clear leaves and roots. The germinated purple corn at 40 °C obtained at the same time showed a delay in germination.

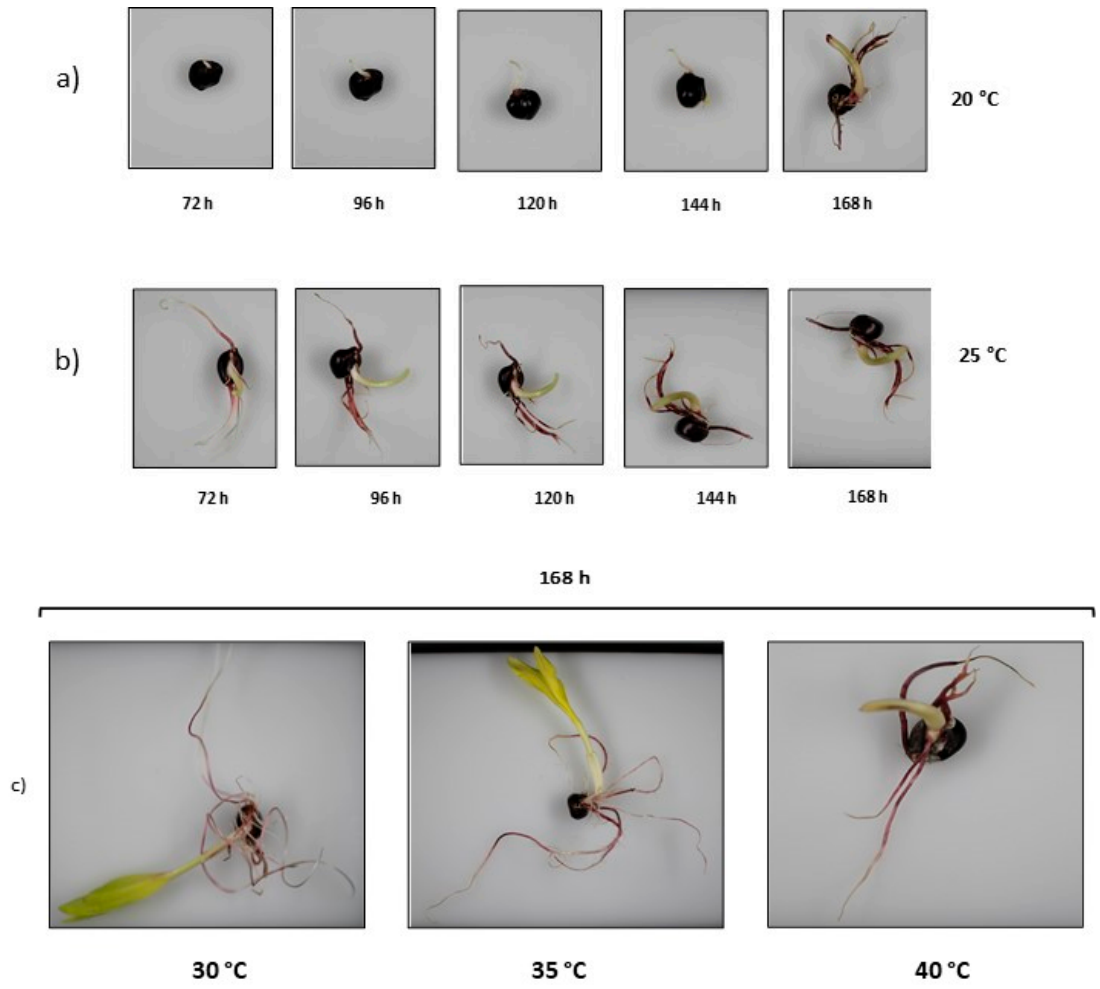

Figure 1. Pictures of germination of Andean purple corn. (**a**) Germination of purple corn seeds at 20 °C for 72, 96, 120, 144, and 168 h; (**b**) germination of purple corn seeds at 25 °C for 72, 96, 120, 144, and 168 h; (**c**) germination of purple corn seeds at 30 °C, 35 °C, and 40 °C for 168 h.

Table 1. Percentage germination at different time and temperature combinations of Andean purple corn seeds.

	% of Germination of Andean Purple Corn Seeds					
Time (h)	**15 °C**	**20 °C**	**25 °C**	**30 °C**	**35 °C**	**40 °C**
24	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 0.58 ^{a}	9.00 ± 3.56 ^{a}	20.67 ± 4.04 ^{a}	9.33 ± 2.08 ^{a}
48	0.00 ± 0.00	3.33 ± 0.71 ^{a}	42.00 ± 7.55 ^{b}	33.00 ± 3.54 ^{b}	36.67 ± 4.73 ^{b}	22.67 ± 2.08 ^{b}
72	0.00 ± 0.00	24.00 ± 6.24 ^{b}	52.67 ± 8.74 ^{c}	37.00 ± 3.54 ^{b}	38.00 ± 5.29 ^{b}	23.33 ± 2.52 ^{b}
96	0.00 ± 0.00	38.67 ± 4.62 ^{c}	54.00 ± 8.19 ^{c}	41.00 ± 3.54 ^{c}	38.67 ± 5.69^{b}	23.33 ± 2.52 ^{b}
120	6.00 ± 0.58 ^{a}	46.67 ± 7.43 ^{d}	58.67 ± 8.39 ^{d}	41.00 ± 3.54 ^{c}	38.67 ± 5.69 ^{b}	23.33 ± 2.52 ^{b}
144	6.00 ± 0.58 ^{a}	53.33 ± 4.51 ^{e}	60.00 ± 8.66 ^{d}	41.00 ± 3.54 ^{c}	40.00 ± 4.58 ^{c}	26.00 ± 2.00 ^{c}
168	6.00 ± 0.58 ^{a}	56.00 ± 4.58 ^{e}	63.33 ± 7.23 ^{e}	41.00 ± 3.54 ^{c}	40.00 ± 4.58 ^{c}	26.00 ± 2.00 ^{c}

Results were expressed as mean ± standard deviation (n = 3) and were evaluated by one-way Anova and Turkey test ($p < 0.05$). Statistical differences were indicated with different letters. Temperatures groups were compared with times groups.

3.3. PCPCs Protein Profile

Once germinated, the Andean purple corn seeds were used to obtain flour. The flour was used to obtain protein-phenolic concentrates by alkaline extraction (pH 8.0 and pH 10.0) followed by isoelectric precipitation (pH 4.0, pH 5.0, and pH 6.0). Once the PCPCs were obtained, their protein profile was analyzed using the SDS-APGE technique. Figure 2 showed the PCPC protein profile. Figure 2a showed the PCPC profile of non-germinated seeds (control). Six bands with molecular weights of 14.50 kDa, 20.12 kDa, 25.18 kDa, 41.85 kDa, 59.59 kDa, and 65.87 kDa were observed in the gel of polyacrylamide. These bands correspond to albumins, globulins, and glutelin. The prolamins in corn are named zeins and represent around 60.00% of the protein in the corn seeds (fresh weight). Zeins can be divided into four subfamilies: α (19 and 22 kDa), γ (50, 27, and 16 kDa), β (15 kDa), and δ (18 and 10 kDa) [48–50]. The protein profile of non-germinated PCPCs produced at pH 8.0 and pH 10.0 at different precipitation pHs (pH 4.0, pH 5.0, and pH 6.0) was the same (Figure 2a). PCPCs (pH 8.0 and 10 alkaline and pH 4.0 isoelectric precipitation) obtained from seeds germinated at 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C for 72 h presented the same bands with identical molecular weights to those identified in the non-germinated PCPC with values of 14.50 kDa, 20.12 kDa, 25.18 kDa, 41.85 kDa, 59.59 kDa, and 65.87 kDa (Figure 2b,c).

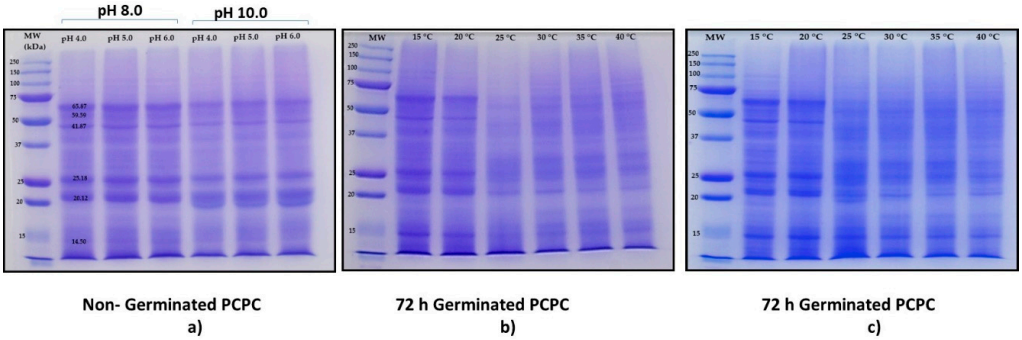

Figure 2. Analysis of protein profile of purple corn protein phenolic concentrates (PCPC) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). (**a**) Non-germinated PCPC obtained at (pH 8.0 and pH 10 alkaline extraction) and different pHs of precipitation; (**b**) germinated PCPC at (pH 8.0 alkaline and pH 4.0 of precipitation) for 72 h; (**c**) germinated PCPC at (pH 10 and pH 4.0 of precipitation) for 72 h.

3.4. PCPCs Protein Content Quantification

The protein content present in PCPCs obtained from germinated seeds at 72 h, 120 h, and 168 h incubated at 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C was quantified using the Dumas method. Table 2 summarizes the results obtained from the protein quantification analysis. Protein concentration in PCPCs ranges between 9.38% (8.0–6.0 pH (alkaline-Ip)) to 33.56% (10.0–4.0 pH (alkaline-Ip)) these

values correspond to PCPC germinated at 25 °C. The highest value was obtained after 168h of incubation. The PCPCs obtained at the two extraction pHs (pH 8.0 and pH 10.0) present similar protein percentage values. Small differences were observed in PCPCs from sprouts 72 h/25 °C, 120 h/20 °C, and 120 h/25 °C. The highest values obtained were PCPC 168 h/25 °C with values of 29.06%, 30.00%, and 33.56%; PCPC 168 h/30 °C presented values of 23.75%, 27.53%, and 29.38%; PCPC 168 h/35 °C presented percentages of 23.80%, 27.60%, and 29.40%. All PCPCs obtained from germinated corn seeds at different times and different temperatures presented protein percentage values higher than the percentage of corn flour from non-germinated corn seeds (8.53%). Statistical analysis indicates significant differences when the time of germination was compared to the pHs alkaline and the isoelectric precipitation at $p < 0.05$.

Table 2. PCPC protein content in germinated Andean purple corn.

pH (Alkaline-Ip)	72 h	120 h	168 h
% Protein-germinated 15 °C			
8.0–4.0	25.16 ± 0.57 ^{b}	21.81 ± 0.18 ^{a}	24.00 ± 0.53 ^{c}
8.0–5.0	24.09 ± 0.22 ^{a}	21.97 ± 0.22 ^{a}	23.06 ± 0.44 ^{b}
8.0–6.0	24.00 ± 0.09 ^{a}	21.94 ± 0.09 ^{a}	21.06 ± 0.45 ^{a}
10.0–4.0	27.16 ± 0.13 ^{c}	24.88 ± 0.00 ^{c}	25.31 ± 0.62 ^{d}
10.0–5.0	26.34 ± 0.04 ^{c}	24.34 ± 0.14 ^{c}	23.91 ± 0.49 ^{b}
10.0–6.0	24.34 ± 0.75 ^{a}	22.00 ± 0.35 ^{b}	23.06 ± 0.32 ^{b}
% Protein-germinated 20 °C			
8.0–4.0	23.16 ± 0.04 ^{b}	18.63 ± 0.18 ^{b}	25.72 ± 0.04 ^{c}
8.0–5.0	22.56 ± 0.00 ^{b}	23.44 ± 0.09 ^{d}	16.84 ± 0.04 ^{a}
8.0–6.0	20.31 ± 0.09 ^{a}	16.50 ± 0.00 ^{a}	22.08 ± 0.02 ^{b}
10.0–4.0	23.50 ± 0.00 ^{b}	27.06 ± 0.17 ^{e}	28.19 ± 0.00 ^{d}
10.0–5.0	22.97 ± 0.13 ^{b}	24.22 ± 0.13 ^{d}	24.48 ± 0.07 ^{c}
10.0–6.0	20.09 ± 0.05 ^{a}	21.88 ± 0.08 ^{c}	22.53 ± 0.04 ^{b}
% Protein-germinated 25 °C			
8.0–4.0	17.78 ± 0.13 ^{c}	10.69 ± 0.18 ^{a}	22.16 ± 3.14 ^{b}
8.0–5.0	18.69 ± 0.09 ^{d}	9.44 ± 0.18 ^{a}	19.09 ± 0.13 ^{a}
8.0–6.0	18.25 ± 0.18 ^{d}	9.38 ± 0.09 ^{a}	19.75 ± 0.35 ^{a}
10.0–4.0	14.88 ± 0.17 ^{b}	27.50 ± 0.09 ^{d}	33.56 ± 1.94 ^{d}
10.0–5.0	13.19 ± 0.04 ^{a}	16.88 ± 0.08 ^{c}	29.06 ± 1.33 ^{c}
10.0–6.0	13.00 ± 0.18 ^{a}	12.47 ± 0.04 ^{b}	30.00 ± 0.44 ^{c}
% Protein-germinated 30 °C			
8.0–4.0	26.56 ± 0.09 ^{d}	26.44 ± 0.18 ^{d}	23.75 ± 0.09 ^{c}
8.0–5.0	25.19 ± 0.22 ^{c}	24.38 ± 0.00 ^{c}	21.00 ± 0.09 ^{b}
8.0–6.0	22.66 ± 0.09 ^{a}	21.44 ± 0.27 ^{a}	18.38 ± 0.00 ^{a}
10.0–4.0	26.25 ± 0.00 ^{d}	27.28 ± 0.13 ^{d}	29.38 ± 0.09 ^{e}
10.0–5.0	26.75 ± 0.18 ^{d}	26.50 ± 0.00 ^{d}	27.53 ± 014 ^{d}
10.0–6.0	24.19 ± 0.00 ^{b}	23.71 ± 0.14 ^{b}	19.31 ± 0.00 ^{a}
% Protein-germinated 35 °C			
8.0–4.0	26.60 ± 0.00 ^{d}	26.50 ± 0.18 ^{c}	23.80 ± 0.09 ^{c}
8.0–5.0	25.20 ± 0.18 ^{c}	24.34 ± 0.04 ^{b}	21.06 ± 0.19 ^{b}
8.0–6.0	22.60 ± 0.04 ^{a}	21.44 ± 0.00 ^{a}	18.40 ± 0.20 ^{a}
10.0–4.0	26.25 ± 0.09 ^{d}	27.25 ± 0.35 ^{c}	29.40 ± 0.00 ^{e}
10.0–5.0	25.53 ± 0.18 ^{c}	26.56 ± 0.27 ^{c}	27.60 ± 0.19 ^{d}
10.0–6.0	24.19 ± 0.00 ^{b}	23.75 ± 0.00 ^{b}	19.35 ± 0.09 ^{a}
% of Protein-germinated 40 °C			
8.0–4.0	21.94 ± 0.88 ^{b}	22.75 ± 0.09 ^{a}	24.53 ± 0.49 ^{c}
8.0–5.0	20.63 ± 0.09 ^{b}	22.22 ± 0.13 ^{a}	19.94 ± 1.06 ^{b}
8.0–6.0	13.47 ± 2.25 ^{a}	22.31 ± 0.09 ^{a}	15.60 ± 2.96 ^{a}
10.0–4.0	28.56 ± 0.35 ^{d}	26.38 ± 0.35 ^{b}	21.34 ± 0.04 ^{b}
10.0–5.0	27.03 ± 1.64 ^{d}	23.66 ± 0.75 ^{a}	21.91 ± 0.04 ^{b}
10.0–6.0	24.13 ± 1.77 ^{c}	22.34 ± 2.87 ^{a}	14.06 ± 0.27 ^{a}

Results were expressed as mean ± standard deviation ($n = 3$) and were evaluated by one-way Anova and Turkey test ($p < 0.05$). Statistical differences were indicated with different letters.

Corn seed has albumins protein that represent 8.00% of protein, DW. Albumins proteins are soluble in a water solution. Globulins protein represent 9.00% of protein soluble in salts and glutelin proteins represent 40.00% of protein, DW, which are soluble in alkaline solutions [51]. PCPC presented albumins, glutelin and globulin proteins. Normal purple corn seeds were used in this study to get germinated PCPCs. The protein contents reported in this work are in line with values reported in the literature for different zein protein concentrates. Corn protein concentrate are used for industrial applications such as adhesives, biodegradables plastics, coating (edible, moisture-resistant) for food products, cosmetic powders, dietary fibers, textile fibers, microencapsulated pesticides, microspheres, long acting matrix tablet formulations, nutrient delivery system for ruminants, high potency sweeteners, hair fixative, and other industrial purposes [52–62].

3.5. TPC Content of Germinated PCPCs

TPC content was determined in germinated Andean purple seeds PCPCs for 24 h, 48 h, 72 h, 96 h, 122 h, 144 h, and 168 h at 15 °C, 20 °C, 25 °C, 30 °C, 35 °C, and 40 °C. Table 3 showed the TPC value in germinated PCPCs. Geminated PCPC had a high TPC content with a range of 350.95 to 1820 mg GAE/g PCPC, DW, germinated PCPC/72 h/25 °C had a high value of 1820.00 mg GAE/g PCPC, DW. PCPC/72 h/25 °C had a high TPC value in the two groups (pH 8.0 and pH 10) with 1820.00, 1803.00, and 1746.00 mg GE/g PCPC, DW for the group obtained at pH 8.0 of extraction and 1550.00, 1736.00, and 1426.00 mg GAE/g PCPC, DW for the group pH 10. PCPC/168 h/35 °C presented a TPC value of 989.05–1605.71 mg GAE/g PCPC, DW. The TPC values obtained at longer germination times and higher temperature had higher values of TPC content. The pH alkaline (pH 8.0 and pH 10) affect the TPC content. Significant differences were observed at $p < 0.05$ when were compared pH vs. germination time.

Mansilla et al. (2020) described purple corn growth in Argentina with TPC values between 438.00 to 1933.00 mg GAE/100g, DW [47]. Trehan et al. (2018) described the TPC content of three varieties of yellow, white, and purple seeds corn—yellow corn (1170.00–1640.00 mg GAE/g Sample), white corn (903.00–1332.00 mg GAE/g Sample), and purple corn (1223.00–1843.00 mg GAE/g Sample) [46]. De la Parra et al. (2007) have described TPC content of five different varieties of corn seeds yellow, white, red, blue, and high carotenoid variety with values of 260.70–320.10 mg GAE/100 g DW [63]. Mora-Rochin et al. (2010) reported a TPC content of white corn flour (167.40 mg GAE/100 g, DW), blue corn (142.10 mg GAE/100 g DW), red corn (140.70 mg GAE/100 g DW), and yellow corn (137.70 mg GAE/100 g DW) [64]. PCPC concentrates from germinated kernels increased the TPC content when compared to the TPC content reported for purple corn flour.

The isoelectric precipitation pH (pH 4.0, pH 5.0, and pH 6.0) used to precipitate the proteins allows the isolation of a considerable amount of phenolic components. PCPCs continue to maintain a purple coloration which indicated their presence in protein concentrates. Total phenolic can be separated from PCPCs with repeated methanol extractions. Six consecutive extraction processes allowed the isolation of 100.00% of the phenolic components present in the PCPCs from germinated seeds. All PCPCs tested in this work with alkaline extraction and isoelectric precipitation had a high TPC content. Future work on HPLC-MS-MS should be carried out to identify the phenolic components present in the PCPCs and to determine whether there are differences in the phenolic depending on the pH extraction and the pH precipitation.

Piñuel et al. (2019) described red bean protein concentrate (RBPC) from *Phaseolus vulgaris* with high antioxidant activity (ABTS and FRAP) and capacity to inhibit lipid peroxidation in the zebrafish model. They reported that RBPC had a high TPC content with values of 135.57–521.66 mg GAE/g per sample. RBPC showed antioxidant activity by ABTS with values of 81.55–257.12 µmol TE/g of sample dependent of pH assayed and a FRAP value of 45.16–95.80 dependent of pH assayed. They reported that RBPC antioxidant activity could be due to the presence of polyphenols in RBPC [20].

Table 3. Total phenolic content (TPC) of germinated PCPCs.

	TPC (mg GAE/g PCPC, DW)						
pH	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168 h**
TPC germinated at 15 °C							
8.0–4.0	910.48 ± 0.02 ^{b}	834.29 ± 0.02 ^{b}	1248.57 ± 0.11 ^{d}	1170.00 ± 0.11 ^{b}	900.95 ± 0.04 ^{a}	1205.71 ± 0.03 ^{c}	1289.05 ± 0.03 ^{d}
8.0–5.0	1029.52 ± 0.01 ^{c}	734.29 ± 0.01 ^{a}	1158.10 ± 0.03 ^{c}	1167.62 ± 0.04 ^{b}	943.81 ± 0.03 ^{b}	755.71 ± 0.01 ^{a}	1112.86 ± 0.03 ^{b}
8.0–6.0	710.48 ± 0.01 ^{a}	712.86 ± 0.01 ^{a}	812.86 ± 0.01 ^{a}	898.57 ± 0.02 ^{a}	1143.81 ± 0.02 ^{d}	1243.81 ± 0.03 ^{d}	886.67 ± 0.05 ^{a}
10.0–4.0	948.57 ± 0.01 ^{b}	1460.48 ± 0.01 ^{e}	1243.81 ± 0.03 ^{d}	1379.52 ± 0.04 ^{c}	1172.38 ± 0.01 ^{e}	1329.52 ± 0.03 ^{e}	1381.90 ± 0.02 ^{e}
10.0–5.0	1177.14 ± 0.02 ^{d}	1074.76 ± 0.01 ^{d}	1134.29 ± 0.06 ^{c}	1370.00 ± 0.02 ^{c}	1165.24 ± 0.02 ^{e}	1458.10 ± 0.05 ^{f}	1574.76 ± 0.05 ^{f}
10.0–6.0	1058.10 ± 0.01 ^{c}	984.29 ± 0.01 ^{c}	936.67 ± 0.03 ^{b}	1400.95 ± 0.04 ^{d}	998.57 ± 0.06 ^{c}	962.86 ± 0.02 ^{b}	1196.19 ± 0.04 ^{c}
TPC germinated at 20 °C							
8.0–4.0	540.32 ± 0.01 ^{b}	613.33 ± 0.00 ^{b}	1248.57 ± 0.11 ^{d}	1170.00 ± 0.11 ^{b}	900.95 ± 0.04 ^{a}	1205.71 ± 0.03 ^{c}	1289.05 ± 0.03 ^{d}
8.0–5.0	621.27 ± 0.01 ^{c}	699.05 ± 0.01 ^{c}	1158.10 ± 0.03 ^{c}	1167.62 ± 0.04 ^{b}	943.81 ± 0.03 ^{b}	755.71 ± 0.01 ^{a}	1112.86 ± 0.03 ^{b}
8.0–6.0	416.51 ± 0.01 ^{a}	554.60 ± 0.01 ^{a}	812.86 ± 0.01 ^{a}	898.57 ± 0.02 ^{a}	1143.81 ± 0.02 ^{c}	1243.81 ± 0.03 ^{c}	886.67 ± 0.05 ^{a}
10.0–4.0	694.29 ± 0.01 ^{d}	741.90 ± 0.01 ^{d}	1243.81 ± 0.03 ^{d}	1379.52 ± 0.04 ^{c}	1172.38 ± 0.01 ^{c}	1329.52 ± 0.03 ^{d}	1381.90 ± 0.02 ^{e}
10.0–5.0	656.19 ± 0.01 ^{d}	591.11 ± 0.01 ^{a}	1134.29 ± 0.06 ^{c}	1370.00 ± 0.02 ^{c}	1165.24 ± 0.02 ^{c}	1458.10 ± 0.05 ^{e}	1574.76 ± 0.05 ^{f}
10.0–6.0	614.92 ± 0.01 ^{c}	762.54 ± 0.01 ^{d}	936.67 ± 0.03 ^{b}	1400.95 ± 0.04 ^{d}	998.57 ± 0.06 ^{c}	962.86 ± 0.02 ^{b}	1196.19 ± 0.04 ^{c}
TPC germinated at 25 °C							
8.0–4.0	453.33 ± 0.03 ^{b}	536.67 ± 0.02 ^{c}	1820.00 ± 0.01 ^{d}	1491.43 ± 0.03 ^{e}	1100.95 ± 0.09 ^{d}	1258.10 ± 0.01 ^{c}	1529.52 ± 0.02 ^{f}
8.0–5.0	350.95 ± 0.04 ^{a}	522.38 ± 0.03 ^{c}	1803.33 ± 0.05 ^{d}	1217.62 ± 0.09 ^{b}	920.00 ± 0.05 ^{b}	1081.90 ± 0.01 ^{a}	1250.95 ± 0.02 ^{b}
8.0–6.0	1577.14 ± 0.04 ^{f}	508.10 ± 0.03 ^{b}	1746.19 ± 0.06 ^{c}	1212.86 ± 0.17 ^{b}	781.90 ± 0.04 ^{a}	1136.67 ± 0.01 ^{b}	1155.71 ± 0.03 ^{a}
10.0–4.0	660.48 ± 0.01 ^{d}	493.81 ± 0.02 ^{b}	1550.95 ± 0.10 ^{b}	1398.57 ± 0.05 ^{d}	1541.43 ± 0.04 ^{f}	1620.00 ± 0.00 ^{e}	1315.24 ± 0.01 ^{c}
10.0–5.0	617.62 ± 0.05 ^{c}	531.90 ± 0.02 ^{c}	1736.67 ± 0.08 ^{c}	1284.29 ± 0.09 ^{c}	1265.24 ± 0.01 ^{e}	1362.86 ± 0.06 ^{d}	1436.67 ± 0.03 ^{e}
10.0–6.0	1172.38 ± 0.04 ^{e}	384.29 ± 0.02 ^{a}	1429.52 ± 0.03 ^{a}	1003.33 ± 0.03 ^{a}	972.38 ± 0.03 ^{c}	1284.29 ± 0.03 ^{c}	1355.71 ± 0.02 ^{d}
TPC germinated at 30 °C							
8.0–4.0	1143.81 ± 0.02 ^{b}	993.81 ± 0.04 ^{b}	1217.62 ± 0.02 ^{c}	1222.38 ± 0.05 ^{b}	1324.76 ± 0.03 ^{c}	1277.14 ± 0.01 ^{b}	1265.24 ± 0.06 ^{b}
8.0–5.0	1179.52 ± 0.04 ^{c}	1150.95 ± 0.03 ^{e}	1229.52 ± 0.18 ^{c}	1441.43 ± 0.02 ^{e}	1196.19 ± 0.02 ^{b}	1258.10 ± 0.01 ^{b}	1343.81 ± 0.03 ^{c}
8.0–6.0	1179.52 ± 0.04 ^{c}	920.00 ± 0.01 ^{a}	927.14 ± 0.02 ^{a}	1003.33 ± 0.01 ^{a}	1015.24 ± 0.02 ^{a}	950.95 ± 0.01 ^{a}	979.52 ± 0.04 ^{a}
10.0–4.0	1486.67 ± 0.03 ^{c}	1155.71 ± 0.03 ^{d}	1284.29 ± 0.05 ^{c}	1391.43 ± 0.07 ^{d}	1472.38 ± 0.02 ^{e}	1386.67 ± 0.01 ^{d}	1389.05 ± 0.07 ^{d}
10.0–5.0	1158.10 ± 0.01 ^{b}	1008.10 ± 0.05 ^{b}	1346.19 ± 0.06 ^{d}	1481.90 ± 0.02 ^{e}	1408.10 ± 0.05 ^{d}	1358.10 ± 0.01 ^{c}	1631.90 ± 0.03 ^{e}
10.0–6.0	1022.38 ± 0.03 ^{a}	1103.33 ± 0.01 ^{c}	1191.43 ± 0.06 ^{b}	1300.95 ± 0.01 ^{c}	1329.52 ± 0.02 ^{c}	1365.24 ± 0.01 ^{c}	1393.81 ± 0.03 ^{d}

Table 3. *Cont.*

	TPC (mg GAE/g PCPC, DW)						
pH	**24 h**	**48 h**	**72 h**	**96 h**	**120 h**	**144 h**	**168 h**
TPC germinated at 35 °C							
8.0–4.0	1500.95 ± 0.03 ^{b}	1170.00 ± 0.01 ^{c}	1355.71 ± 0.01 ^{c}	1396.19 ± 0.04 ^{d}	1289.05 ± 0.05 ^{b}	1424.76 ± 0.04 ^{e}	1448.57 ± 0.03 ^{c}
8.0–5.0	1715.24 ± 0.06 ^{e}	979.52 ± 0.02 ^{a}	1227.14 ± 0.02 ^{b}	1298.57 ± 0.04 ^{c}	1467.62 ± 0.06 ^{c}	1210.48 ± 0.04 ^{b}	1436.67 ± 0.05 ^{c}
8.0–6.0	1131.90 ± 0.03 ^{a}	1074.76 ± 0.02 ^{b}	1010.48 ± 0.02 ^{a}	936.67 ± 0.03 ^{a}	889.05 ± 0.03 ^{a}	984.29 ± 0.04 ^{a}	989.05 ± 0.03 ^{a}
10.0–4.0	1708.10 ± 0.02 ^{e}	1484.29 ± 0.02 ^{d}	1455.71 ± 0.02 ^{d}	1070.00 ± 0.00 ^{b}	1446.19 ± 0.02 ^{c}	1272.38 ± 0.02 ^{c}	1605.71 ± 0.05 ^{d}
10.0–5.0	1620.00 ± 0.00 ^{d}	1648.57 ± 0.01 ^{e}	1872.38 ± 0.01 ^{f}	1446.19 ± 0.04 ^{e}	1534.29 ± 0.05 ^{d}	1289.05 ± 0.05 ^{c}	1605.71 ± 0.02 ^{d}
10.0–6.0	1593.81 ± 0.03 ^{c}	1667.62 ± 0.02 ^{e}	1767.62 ± 0.02 ^{e}	1510.48 ± 0.01 ^{f}	1446.19 ± 0.03 ^{c}	1327.14 ± 0.04 ^{d}	1174.76 ± 0.03 ^{b}
TPC germinated at 40 °C							
8.0–4.0	1150.95 ± 0.04 ^{c}	1120.00 ± 0.06 ^{b}	605.71 ± 0.02 ^{a}	774.76 ± 0.04 ^{c}	917.62 ± 0.03 ^{b}	917.62 ± 0.03 ^{b}	917.62 ± 0.03 ^{b}
8.0–5.0	896.19 ± 0.04 ^{b}	912.86 ± 0.04 ^{a}	743.81 ± 0.04 ^{c}	703.33 ± 0.03 ^{b}	955.71 ± 0.05 ^{c}	955.71 ± 0.05 ^{c}	955.71 ± 0.02 ^{c}
8.0–6.0	755.71 ± 0.06 ^{a}	1110.48 ± 0.07 ^{b}	691.43 ± 0.06 ^{b}	674.76 ± 0.06 ^{a}	824.76 ± 0.04 ^{a}	824.76 ± 0.04 ^{a}	824.76 ± 0.08 ^{a}
10.0–4.0	1298.57 ± 0.04 ^{e}	1424.76 ± 0.09 ^{d}	1077.14 ± 0.03 ^{d}	850.95 ± 0.03 ^{d}	974.76 ± 0.02 ^{c}	974.76 ± 0.02 ^{c}	974.76 ± 0.01 ^{c}
10.0–5.0	1236.67 ± 0.04 ^{d}	1177.14 ± 0.01 ^{c}	1079.52 ± 0.02 ^{d}	953.33 ± 0.06 ^{e}	1279.52 ± 0.05 ^{e}	1279.52 ± 0.05 ^{e}	1279.52 ± 0.01 ^{e}
10.0–6.0	1327.14 ± 0.00 ^{f}	1053.33 ± 0.03 ^{c}	1070.00 ± 0.03 ^{d}	924.76 ± 0.05 ^{e}	1117.62 ± 0.03 ^{d}	1117.62 ± 0.03 ^{d}	1117.62 ± 0.03 ^{d}

Results were expressed as mean ± standard deviation ($n = 3$) and were evaluated by one-way Anova and Turkey test ($p < 0.05$). Statistical differences were indicated with different letters.

In the light of the above discussion, we consider that in the protein concentrates obtained from plants, the presence of phenolic in the samples and their possible influence on the biological activities that are evaluated must be considered. For this reason, we decided to name them purple corn protein phenolic concentrates (PCPC).

3.6. Germinated PCPCs Antioxidant Activity by ABTS and FRAP Methods

Germinated PCPCs obtained at pH 10.0 of alkaline extraction and pH 4.0 of precipitation, 48–168 h and 20–40 °C incubation temperatures were used to evaluate the antioxidant activity by ABTS and FRAP methods. Table 4 showed the PCPCs antioxidant activity values by the ABTS method. PCPCs antioxidant activity values varied between 175.37 µmol to 804.35 µmol TE/g PCPC, DW range. PCPC/35 °C presented higher values between 196.42 µmol to 804.35 µmol TE/g PCPC, DW. The highest value was registered for PCPC/144 h/35 °C. In general, the high values of antioxidant activities were related to high temperature and time of germination. When compared the groups of temperatures vs. times of germination, statistical differences were observed. Trehan et al. (2018) reported antioxidant activity of yellow, white, and purple corn flours by ABTS and DPPH method. They reported values of 3.81–4.92 µmol TE/mg of yellow corn flour, 3.88–4.53 µmol TE/mg of white corn flour, and 4.18–4.83 µmol TE/mg of purple corn flour. They reported antioxidant activity of purple corn flour using the DPPH method with values of 0.77–0.84 µmol TE/mg of purple corn flour. These authors reported antioxidant activities of muffins made with yellow, white, and purple corn flours but with lower values than flours [46].

Table 4. PCPC antioxidant activity by the 2,2-azinobis, 3-ethyl-benzothiazoline-6-sulfonic acid, (ABTS) method.

ABTS (µmol TE/g PCPC, DW)						
Germinated PCPC-pH 10/pH4.0						
Time (h)	**15 °C**	**20 °C**	**25 °C**	**30 °C**	**35 °C**	**40 °C**
24	365.20 ± 0.01 ^{d}	323.90 ± 0.01 ^{c}	240.45 ± 0.01 ^{b}	497.00 ± 0.05 ^{d}	564.44 ± 0.09 ^{a}	619.18 ± 0.05 ^{f}
48	364.20 ± 0.01 ^{d}	348.62 ± 0.01 ^{c}	242.08 ± 0.09 ^{b}	246.02 ± 0.05 ^{a}	407.71 ± 0.10 ^{d}	546.10 ± 0.02 ^{e}
72	618.48 ± 0.04 ^{c}	531.55 ± 0.02 ^{d}	496.20 ± 0.03 ^{c}	453.34 ± 0.03 ^{c}	506.91 ± 0.06 ^{e}	215.52 ± 0.06 ^{a}
96	603.87 ± 0.03 ^{c}	234.06 ± 0.01 ^{b}	246.06 ± 0.03 ^{b}	417.63 ± 0.03 ^{b}	196.42 ± 0.03 ^{a}	498.61 ± 0.05 ^{d}
120	490.21 ± 0.09 ^{b}	197.87 ± 0.06 ^{a}	570.97 ± 0.07 ^{d}	493.02 ± 0.04 ^{d}	402.75 ± 0.09 ^{c}	404.77 ± 0.07 ^{b}
144	407.21 ± 0.09 ^{a}	562.68 ± 0.02 ^{e}	175.37 ± 0.01 ^{a}	547.58 ± 0.02 ^{e}	804.35 ± 0.08 ^{e}	488.44 ± 0.05 ^{d}
168	490.45 ± 0.09 ^{b}	571.49 ± 0.09 ^{e}	603.60 ± 0.03 ^{e}	278.57 ± 0.06 ^{a}	235.10 ± 0.04 ^{b}	470.05 ± 0.01 ^{c}

Results are expressed as mean ± standard deviation (n = 3) and were evaluated by one-way Anova and Turkey test ($p < 0.05$). Statistical differences were indicated with different letters.

Table 5 showed the results obtained of PCPCs antioxidant activity by the FRAP method. The samples showed values with 100.46 to 984.83 µmol TE/g PCPC, DW. The higher value was observed for PCPC/144 h/30 °C with 984.83 µmol TE/g PCPC, DW. Germinated PCPC at 35 °C presented values between 168.25 µmol to 922.25 µmol TE/g PCPC, DW. In general, higher antioxidant activities were observed for similar behavior at high temperatures and high germination times presenting higher antioxidant activity. Statistical analysis showed significant differences when the groups of temperatures were compared to the incubation times at $p < 0.05$. Coco and Vinson (2019) reported antioxidant activity of raw kernel 8.73–13.40 mg of catechin/g for nine varieties of corn by the FRAP method. Raw kernel was subjected to in vitro simulation digestion. The antioxidant activity was evaluated by the FRAP method. They found a value of 0.76 mg catechin/g of sample [65]. Yang and Zhai (2010) reported antioxidant activity of total anthocyanins content (TAC) isolated from purple corn kernels using the DPPH, FRAP, and TEAC methods (16.20 mmol and 18.70 mmol of $FeSO_4$/g DW).

Table 5. PCPC antioxidant activity by the ferric-reducing antioxidant power (FRAP) method.

FRAP (μmol TE/g PCPC, DW)						
Germinated PCPC pH 10-pH 4.0						
Time (h)	**15 °C**	**20 °C**	**25 °C**	**30 °C**	**35 °C**	**40 °C**
24	257.98 ± 0.01 ^{c}	208.58 ± 0.01 ^{a}	154.86 ± 0.01 ^{a}	155.52 ± 0.93 ^{a}	168.25 ± 3.33 ^{a}	173.61 ± 0.01 ^{a}
48	243.44 ± 0.01 ^{c}	243.40 ± 0.01 ^{b}	165.11 ± 0.02 ^{a}	454.86 ± 0.01 ^{a}	771.68 ± 0.67 ^{c}	611.19 ± 0.09 ^{c}
72	741.49 ± 0.09 ^{d}	770.21 ± 0.21 ^{d}	678.18 ± 0.10 ^{d}	815.04 ± 0.20 ^{c}	922.25 ± 0.52 ^{d}	628.94 ± 0.70 ^{c}
96	807.29 ± 0.09 ^{e}	339.15 ± 0.36 ^{b}	335.36 ± 0.91 ^{c}	750.69 ± 0.44 ^{b}	349.39 ± 0.02 ^{a}	552.42 ± 0.59 ^{b}
120	100.46 ± 0.21 ^{a}	286.71 ± 0.69 ^{c}	772.85 ± 0.29 ^{c}	877.92 ± 0.36 ^{d}	747.02 ± 0.09 ^{c}	452.69 ± 0.06 ^{a}
144	121.00 ± 0.21 ^{b}	751.10 ± 0.49 ^{d}	236.10 ± 1.52 ^{b}	984.83 ± 0.38 ^{e}	363.58 ± 0.51 ^{a}	699.23 ± 3.17 ^{d}
168	120.01 ± 0.21 ^{b}	800.83 ± 0.30 ^{e}	817.12 ± 0.55 ^{e}	762.63 ± 0.13 ^{b}	435.30 ± 0.38 ^{b}	701.44 ± 0.35 ^{e}

Results are expressed as mean ± standard deviation (n = 3) and were evaluated by one-way Anova and Turkey test ($p < 0.05$). Statistical differences were indicated with different letters.

Various researchers have reported a relationship between polyphenol and anthocyanin content of purple corn kernels and their antioxidant activities [4]. López-Martinez et al. (2014) described antioxidant activity of three varieties of purple corn using nitric oxide radical scavenging activity and superoxide radical scavenging activity methods. The ethanol extracts showed a strong antioxidant activity in the following order: generic purple > Oaxaca 332 > Veracruz 42 with 62.00%, 48.00%, and 32.00% of NO scavenging activity, respectively [66].

The protocol used in this study to obtain antioxidant PCPC was validated from a lot of seeds of Andean purple corn of the variety (*Zea mays* L., INIAP-199 bunch of grapes) grown in Guaranda, Ecuador. The proteins and polyphenols content present in seeds can vary with the variety tested and the seeds growing conditions. For this reason, this protocol could be validated with different varieties of corn seeds (purple, white, and yellow corn) grown under different environmental conditions, different irrigation conditions, and different fertilization conditions. The protocol could also be validated on transgenic corn seeds to compare the results.

4. Conclusions

Andean purple seeds can be germinated in a wide range of temperatures (15–40 °C) and times (24–168 h). Alkaline extraction (pH 8.0 and pH 10) followed by the isoelectric precipitation (pH 4.0, pH 5.0, and pH 6.0) is a good method to obtain protein phenolic corn concentrate (PCPC). A higher concentration of protein and TPC can be obtained in PCPC compared with non-germinated corn kernel flours. The germination process of Andean purple corn kernels can be used to obtain PCPC with biological activities, such as antioxidant activity, that can increase the nutritional value and quality of this food product. The germinated purple corn PCPCs have a high content of phenolic components that are responsible for the antioxidant activity. The protocol used to obtain antioxidant PCPCs in this study is a preliminary protocol that must be tested in different varieties of corn seeds grown under different environmental conditions. This protocol needs to consider the influence of the dormancy stages of the seeds.

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