



RESEARCH ARTICLE



Extraction of bioactive compounds from Peruvian purple corn cob (*Zea Mays* L.) by dynamic high pressure

Jhoseline Stayce Guillén Sánchez^{1,2,*} ; Cinthia Baú Betim Cazarin³ ; Miriam Regina Canesin³ ; Felix Reyes Reyes⁴ ; Amadeu Hoshi Iglesias⁵ ; Marcelo Cristianini¹ 

¹ Faculty of Food Engineering, Department of Food Technology, State University of Campinas, UNICAMP, P.O.Box. 6121, 13083-862, Campinas, SP, Brazil.

² Formative Research and Teaching Research Program, Research Vice-rectorate, César Vallejo University, Av. Larco 1770, Trujillo, La Libertad, Perú.

³ Faculty of Food Engineering, Food and Nutrition Department, University of Campinas, UNICAMP, 13083-862, Campinas, SP, Brazil.

⁴ Faculty of Food Engineering, Department of Food Science, University of Campinas, UNICAMP, Rua Monteiro Lobato, 80, 13083-862, Campinas, SP, Brazil.

⁵ ApexScience Laboratory, Marechal Rondon Avenue, 2148, Jd. Chapadão, 13063-001, Campinas, SP, Brazil.

* Corresponding author: jhoselineguillen@gmail.com (J. S. Guillén Sánchez).

Received: 11 February 2023. Accepted: 16 August 2023. Published: 18 September 2023.

Abstract

The objective of this research was to evaluate the effects of dynamic high pressure at different processing temperatures, on the extraction of bioactive compounds from Peruvian purple corn cob. The purple corn cobs were ground and sieved to then be mixed with an acidified 20% ethanol-water solution (pH 2) in a 1:30 (w/v) ratio. The hydroalcoholic extracts were subjected to maceration and high dynamic pressure processes varying temperature (25 °C to 60 °C) and pressure (0.01 MPa - 60 MPa), and the bioactive compounds content and antioxidant activity were evaluated. The results showed that dynamic high pressure at 60 MPa / 45 °C was capable of obtaining extracts with bioactive compound contents and high antioxidant activity corresponding to those obtained using the conventional process of 2.5 hours / 65 °C. Seven different anthocyanins were identified by liquid chromatography in the extracts obtained by the dynamic high pressure, mainly cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside, and their respective malonyl derivatives. The dynamic high pressure increased the extraction of anthocyanins by more than 108% and obtained them in one step, thus appearing as a new alternative, eco-friendly method for the extraction of bioactive compounds from plant tissues.

Keywords: Purple corn cob; Dynamic high pressure; Bioactive compounds; Antioxidant activity; Liquid chromatography.

DOI: <https://doi.org/10.17268/sci.agropecu.2023.032>

Cite this article:

Guillén Sánchez, J. S., Betim Cazarin, C. B., Canesin, M. R., Reyes Reyes, F., Iglesias, A. H., & Cristianini, M. (2023). Extraction of bioactive compounds from Peruvian purple corn cob (*Zea Mays* L.) by dynamic high pressure. *Scientia Agropecuaria*, 14(3), 367-373.

1. Introduction

Purple corn cob is a by-product of purple corn processing that is used as fuel and for animal feed. The cob has a high content of bioactive compounds, including phenolic compounds considered as antioxidant, anti-inflammatory and anti-carcinogenic substances, for which its use has high potential (Aguilar, 2022; Hee Yeon et al., 2023). The extraction of phenolic compounds from purple corn is carried out through an aqueous, alcoholic or hydroalcoholic maceration applying different times and temperatures that can range from 30 minutes to 4 days and from room temperature to 90 °C, respectively (Rafael & Castro, 2023; Aguilar, 2022).

The solvent used also plays an important role since it must have an affinity with the analyte, low viscosity and low toxicity; additionally, the use and disposal of large volumes of solvents cause environmental damage. It has been verified that long extraction times can increase the viscosity of the solvent because the concentration gradient decreases and that high temperatures can degrade thermolabile compounds such as anthocyanins (Rafael & Castro, 2023).

Currently, there are emerging technologies for the extraction of bioactive compounds from food that allow the extraction temperature and time to be significantly reduced, avoiding the degradation of these compounds and considered as eco-friendly

technologies. Among them are ohmic heating, ultrasound, microwaves, high hydrostatic pressure and high dynamic pressure (Eslami et al., 2023). Halalah et al. (2023) used high dynamic pressure to process bilberry juice with added polysaccharides (galactoglucomannans and glucuronoxylans) at 80 MPa and different numbers of steps (1, 3 and 5), to evaluate the anthocyanin content. It was found that the number of steps decreases the anthocyanin content ($p < 0.05$); in the first step, the content was approximately 1000 mg/mL, while in third and fifth step decreased between 10% - 20%. On the other hand, Zhang et al. (2023) showed that high dynamic pressure processing of burdock root (148 MPa / 63 °C / 1 hour) allows extracting polysaccharides composed mainly of fructose, glucose and arabinose with antioxidant power. The mean inhibitory capacity of free radicals of the processed sample was 3.93 mg Trolox/mL, suggesting its use as an ecological and efficient extraction process of the polysaccharide. In a study carried out by Szczepańska et al. (2022) it was verified that the application of high dynamic pressure (DHP) in carrot juice (150 MPa, 5 steps and temperature less than 35°C) decreases the viscosity of the juice, increases the content of carotenoids by 151% (Z -β-carotene) and prolongs its shelf life by three days when stored at 4°C. Liu et al. (2022) also carried out a study on the application of high dynamic pressure in carrot juice combined with peach (250 MPa, 1 step and 25 °C), concluding that pressures higher than 200 MPa retain a greater amount of carotenoids and polyphenols in compared to juice not processed by DHP. Pirozzi et al. (2022) applied high dynamic pressure to agro-industrial residues such as tomato pomace in order to isolate cellulose for biorefinery. Cellulose was isolated using sequential chemical hydrolysis in combination with mechanical pretreatment via DHP. The results showed that the DHP pretreatment (80 MPa and 10 passes) promoted a slight increase in cellulose extraction yield (more than 9%) correlated with the decrease in lignin content and a significant increase in acid extraction yield. phenolics and carotenoids (77.9%). These results suggest that the DHP treatment caused structural changes in the cellular compartments and the disruption of the cell membranes of the sample, releasing intracellular substances such as bioactive compounds. Scientific evidence shows that high dynamic pressure is a non-thermal technology widely used to, partially or totally, replace conventional extraction processes, preserving the sensory and nutritional characteristics of the fluid product. The pressure originated within the system causes physical

phenomena such as cavitation, shear and turbulence that are responsible for the extraction and preservation of the bioactive compounds of the product (Lima & Rosenthal, 2022). Despite this, in recent years there is little scientific literature that shows its use in the extraction of bioactive compounds in by-products such as purple corn cob, unlike its use in the extraction of polysaccharides. For example, Zhang et al. (2023), He et al. (2022), and Zhao et al. (2020) used DHP to optimize yields in the extraction of polysaccharides from burdock root, *Polygonatum kingianum Coll. et Hemsl.* and barley, respectively, reaching values of 29%, 32% and 10%, for each plant material.

In this context, the objective of this study was to extract the bioactive compounds from the purple corn cob by high dynamic pressure at different processing temperatures, as an alternative to the conventional extraction technique and show its potential for use in the Development of beneficial food products for health.

2. Methodology

Conditioning of the sample

The purple corn was acquired from a popular market located in the city of Chimbote, Ancash region, Peru. The purple corn cob was dried in an oven with air circulation at 50 °C for 24 hours, cut and ground to reduce the particle size, and sieved through a 60-mesh sieve for 5 minutes. The purple corn cob powder was placed in low-density polyethylene (LDPE) / ethylene vinyl alcohol (EVOH) bags and sealed using a vacuum sealer (Baseline P 100 / 300 w, MULTIVAC, Germany). The samples were stored at 4 °C until used.

Physical characteristics and composition of the purple corn cob powder

The particle size was determined using a particle analyzer (SIROCCO 2000, MALVER INSTRUMENTS, ENGLAND). The moisture and ash contents were determined by gravimetric methods (AOAC, 2005); the crude protein content was determined using the Kjeldahl method (IAL, 2008) with a conversion factor of 6.25; and the lipid content was determined using the Bligh & Dyer (1959) method.

Conventional extraction (CE)

The corn cob extracts were prepared using a powder: solvent ratio of 1:30 (w / v), and the solvent used was a 20% (v / v) ethanol-water solution acidified to pH 2 with 1 N HCl. The mixture was placed in flexible LDPE / EVOH / activated carbon packages, sealed using a vacuum sealer and heated in a thermostatic bath (MOD 116, FANEM ®, Brazil) for 2.5 hours at temperatures of 25 °C, 45 °C and 65

°C. The extracts were filtered through Whatman filter paper No. 1 under vacuum, placed in amber bottles, covered with aluminum foil and stored at 4 °C.

Extraction by dynamic high pressure (DHP)

The DHP processes were carried out using a continuous homogenizer (Panda Plus, GEA Niro Soavi, Italy) with a maximum flow rate of 9 L / h. The corn cob powder: solvent mixtures were prepared in a 1:30 (w / v) ratio, placed in 400 mL beakers, covered with aluminum foil, and subjected to high dynamic pressure varying the pressures (20 MPa, 40 MPa and 60 MPa / 1 step) and inlet temperatures (25 °C, 45 °C and 65 °C). At the exit from the homogenizer, the samples were collected in other beakers, covered with aluminum foil and cooled in a water/ice bath. The extracts were centrifuged at 1000 x g for 20 minutes at 25 °C, placed in amber bottles, covered with aluminum foil and stored at 4 °C. The **Figure 1** shows the scheme of the purple corn cob extraction process.

Color Analysis

The color of the extracts was measured using a digital colorimeter (HUNTERLAB, UltraScan PRO, USA) based on the CIELAB color space system (L *, a * and b *) (Montes et al., 2005). The cylindrical coordinates Cab * (chroma) and hab (Hue angle) were calculated from the parameters L *, a * and b *, which define the intensity and tone of the samples according to **equations 1** and **2** respectively.

$$Cab * = [(a^*)^2 + (b^*)^2]^{0.5} \tag{Eq.1}$$

$$Hab = \arctan (b^*a^*) \tag{Eq.2}$$

Total monomeric anthocyanin content (TAC)

The TAC was determined using the AOAC differential pH method (2005). Aliquots of the extract were diluted in amber tubes with 0.025 M potassium chloride (pH 1) and 0.4 M sodium acetate (pH 4.5) buffer solutions, and the absorbance of the solutions determined in the visible region at 520 nm and 700 nm using a spectrophotometer (UV-VIS DU® 800, Beckman Coulter, USA).

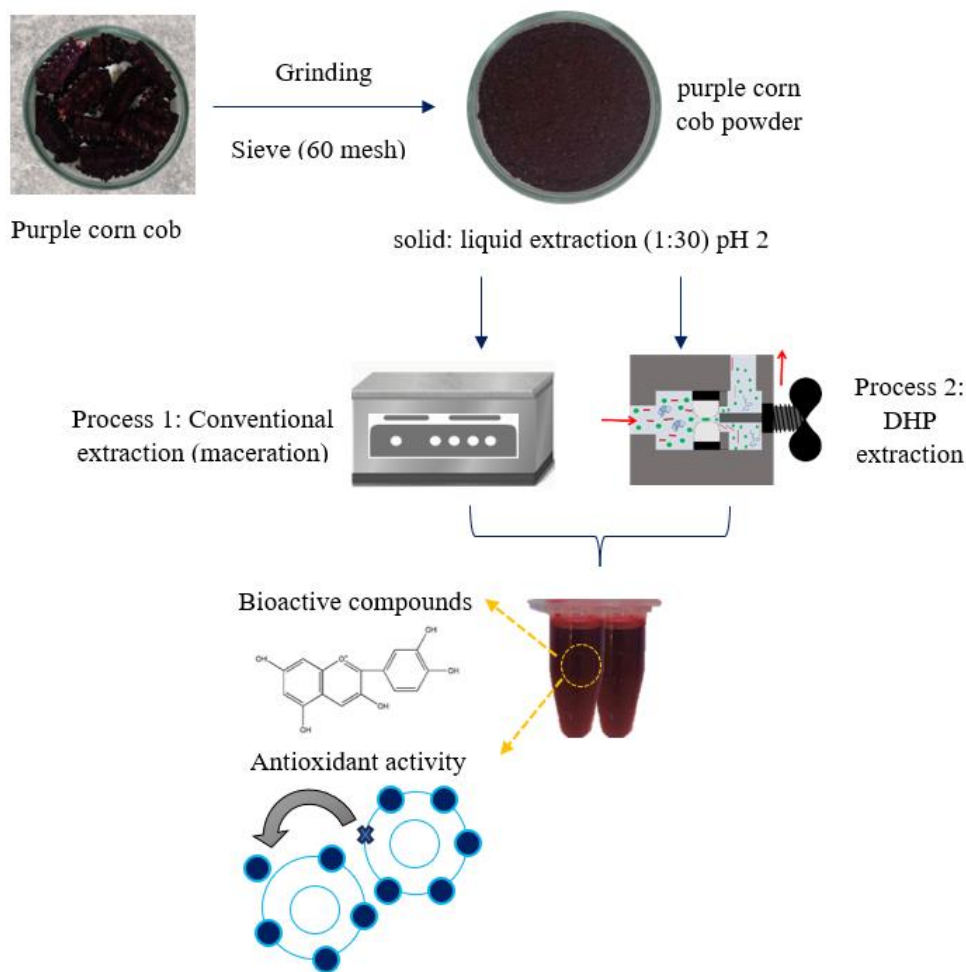


Figure 1. Scheme of the process for obtaining purple corn cob extracts.

The anthocyanin content was expressed as cyanidin 3-O-glucoside according to the following **equation 3**. Where 'ε' is the molar absorptivity, 'l' is the length of the passage through the cell, 'A' is the absorbance, 'MW' is the molecular weight of the reference standard and 'DF' is the dilution factor. The equation was converted to express the results in milligrams of cyanidin 3 glucoside per gram of dry weight or mg Cy3G/g dry weight.

$$\text{Anthocyanin (mg Cyanidin 3 Glucoside/L)} = A \times MW \times FD \times 1000 / \epsilon \times l \quad (\text{Eq.3})$$

Total phenolic compound (TPC) content

The TPC content was determined using the Folin-Ciocalteu colorimetric method according to **Singleton & Rossi (1965)**, with some modifications. 0.5 mL aliquots were taken and transferred to amber tubes, 2.5 mL of the Folin-Ciocalteu solution added, and the volume completed (10 mL) with the extraction solution used in the extraction (20% ethanol-water / pH 2). The samples were left to rest in the dark for 5 minutes, 2 mL of a 7.5% sodium carbonate solution added, and the tubes left in the dark for a further 2 hours. The absorbance was measured at 760 nm using a spectrophotometer (DU® 800 UV/Visible Spectrophotometer, Beckman Coulter, USA). The phenolic compound content was the concentration read multiplied by the corresponding dilution factor and the results were expressed in milligrams of gallic acid (GAE) per gram of dry weight or mg GAE / g dry weight.

In vitro study of the antioxidant activity of purple corn cob extracts processed by dynamic high pressure

Oxygen Radical absorbance capacity - ORAC

The ORAC procedure was carried out according to **Ou et al. (2013)** in 75 mM phosphate buffer (pH 7.4), with a final reaction mixture of 200 µL. Twenty-five microliters of the antioxidant solutions plus 150 µL of a 11.12×10^{-2} µM fluorescein solution were placed in the microplate wells, the microplate preincubated for 10 min at 37 °C and twenty-five microliters of AAPH (2, 2'-azobis (2-methylpropionamidine) dihydrochloride) then added rapidly to each well using a multichannel pipet. The readings were carried out using a microplate reader (Synergy HT, Biotek® Instruments Inc., USA) and the fluorescence recorded every minute for 120 min, the microplate being automatically shaken before each reading. The results were expressed in µmol of Trolox (TE) per gram of dry weight.

Ferric Reducing Antioxidant Power assay - FRAP

In this case the antioxidant activity was determined according to **Benzie & Strain (1996)**. The FRAP

reagent was prepared daily by mixing 2.5 mL acetate buffer (0.3 M pH 3.6), 2.5 mL of a 10 mM TPTZ solution (2,4,6-Tris (2-pyridyl) -s-triazine) and 2.5 mL of a 20 mM aqueous solution of ferric chloride, and maintained at 37 °C. The test solutions were prepared by mixing a 90 µL aliquot of each extract dilution, 270 µL distilled water and 2.7 mL of FRAP reagent, homogenized using a tube shaker and maintained in a water bath at 37 °C.

The absorbance was measured at 595 nm after 30 min using a microplate reader (Synergy HT, Biotek® Instruments Inc., USA) and the results expressed in µmol of Trolox (TE) per gram of dry weight.

Identification of the anthocyanins in the purple corn cob extracts by Ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS)

An ultra-performance liquid chromatography system (Waters Xevo I-Class, Milford, USA) coupled to a tandem mass spectrometry detector (Waters Xevo TQD, Milford, USA) with electrospray source ionization (ESI) in the negative mode was used to identify the anthocyanins in the purple corn cob extracts. The chromatographic separation was carried out using an ACQUITY UPLC® HSS T3 column (100 mm x 2.1 mm x 1.8 µm, Waters, Milford, USA) with a flow rate of 0.6 mL/min. The mobile phase was composed of solvent A (0.1% TFA in water) and solvent B (acetonitrile). The gradient started at 10% B, rose to 35% over 5 min, and then increased to 100% B in 7 min. The MassLynx software (Waters Co. Milford, USA) version 4.1 was used for instrument control and data processing.

The samples were analyzed in the MS scan mode, and the compound masses extracted according to **Pascual-Teresa et al. (2002)** using the following operating conditions: capillary voltage 3.5 kV, source temperature 150° C, de-solvation temperature 550 °C, cone gas flow 20 L/h and de-solvation gas flow 900 L/h.

Statistical Analysis

For the statistical analysis, the measurements obtained were evaluated using the statistical program IBM SPSS v. 22 (SPSS Inc., Chicago, USA), and analysis of variance (ANOVA) and Tukey's test methods were used to compare means. A level of significance $p < 0.05$ was established.

3. Results and discussion

Characterization of the purple corn cob powder

The average diameter of the particles was expressed as D [4,3] (Brouckere average diameter),

which indicates the central point around which the frequency of the volume of the distribution rotates (Tonon et al., 2009). The moisture, ash, protein and carbohydrate were $12.02 \pm 0.04 \%$, $3.41 \pm 0.03 \%$, $5.21 \pm 0.06 \%$ and $79.33 \pm 0.03 \%$, respectively. According to Strack & Wray (1989) some pigments such as simple anthocyanins, can be found esterified to one or several carbohydrates, or acylated anthocyanins, which can be found esterified to carbohydrates and an acyl radical.

Effect of DHP on the TAC of purple corn cob extracts

Figure 2 shows the effects of DHP on the TAC values (mg Cy3G / g dry weight) in the purple corn cob extracts processed at three temperatures, as compared to the contents determined using CE and in the non-pressurized extracts obtained at the same temperatures. Parameters such as the solvent and the solid: solvent ratio were kept constant.

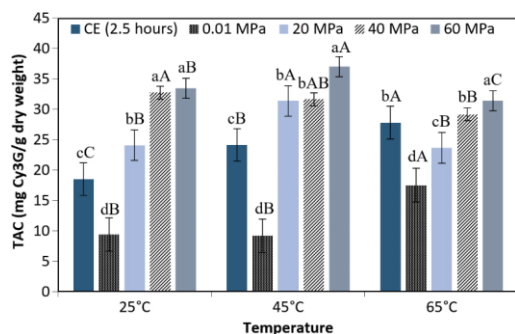


Figure 2. TAC of the extracts obtained by different processing methods and at different temperatures. CE for 2.5 hours, non-pressurized at 0.01 MPa for 3 minutes and DHP (20 MPa, 40 MPa and 60 MPa). The bars are means \pm standard deviation ($n = 3$). Means with the same lower-case letters in the same column indicate that there is no significant difference ($p > 0.05$, Tukey test) between the extracts subjected to different processing methods considering the same temperature. Different uppercase letters mean there is significant difference ($p < 0.05$, Tukey test) for different temperatures considering the same processing method.

Figure 2 shows the DHP process at 25 °C, 45 °C and 65 °C for three minutes, demonstrates that the increment in DHP from 20 MPa to 60 MPa significantly increased ($p < 0.05$) the value for TAC in the extracts by 39%, 18%, and 33%, respectively. It was also observed that the extracts obtained by the process at 20 MPa / 25 °C presented 32% more TAC than the extracts obtained by CE at 25 °C for 2.5 hours ($p < 0.05$). The DHP process at 40 MPa / 25 °C could produce an extract with a high value for TAC corresponding to approximately 14% more TAC than that obtained in the extract produced by CE at 65 °C for 2.5 hours. According to Lima & Rosenthal (2022), these results are those expected in processes involving dynamic pressure, due to the shear exerted by DHP on the plant cells, causing a

reduction in particle size and consequently the extraction of metabolites. However, for DHP processes, increases in temperature only produced favorable responses for increases from 25 °C to 45 °C. Anthocyanins have been reported to be easily degradable at increasing temperatures from 70 °C to 100 °C (Rossi et al., 2017). Therefore, it is inferred that temperatures higher than 45 °C accompanied by the mechanical action caused by dynamic high pressure contribute to the decrease in the anthocyanin content of the extracts.

Effects of DHP on the TPC of purple corn cob extracts

Figure 3 shows the effects of DHP, respectively, on TPC (mg GAE / g dry weight), in purple corn cob extracts processed at three temperatures, as compared to the contents found in extracts obtained using CE and in non-pressurized extracts obtained at the same temperatures. Parameters such as the solvent and solid: solvent ratios were maintained constant.

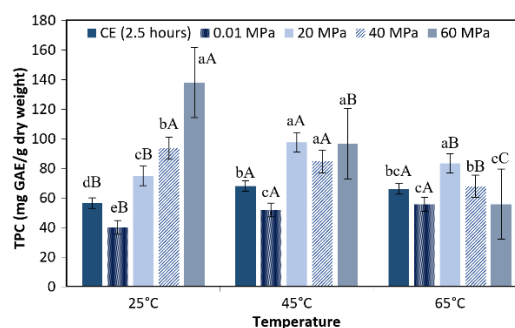


Figure 3. TPC of the extracts obtained by different processing methods at different temperatures. CE for 2.5 hours, non-pressurized at 0.01 MPa for 3 minutes and DHP (20 MPa, 40 MPa and 60 MPa). The bars are means \pm standard deviation ($n = 3$). Means with the same lower-case letters in the same column indicate that there is no significant difference ($p > 0.05$, Tukey test) between the extracts subjected to different processing methods, considering the same temperature. Different uppercase letters mean there is a significant difference ($p < 0.05$, Tukey test) between the different temperatures, considering the same process, example 60 MPa at 25 °C, 45 °C and 65 °C ($p < 0.05$), 40 MPa at 25 °C and 65 °C ($p < 0.05$), 20 MPa at 45 °C and 65 °C ($p < 0.05$), and CE at 25 °C and 45 °C ($p < 0.05$).

The dynamic processes at 25 °C, an increase in DHP from 20 MPa to 60 MPa resulted in a significant increase of 84% in the TPC contents of the extracts. However, at 65 °C there was a decrease in the TPC contents of 33% ($p > 0.05$) which may have been because of the heat produced by the rapid depressurization during the process at 60 MPa or an additional increase of ~ 10 °C in the temperature (Kaneiwa et al., 2013). Zapata et al. (2015) reported that another possible cause of the decrease in TPC could be the storage conditions, which could affect the stability of the phenolic compounds due to

chemical degradation and losses caused by volatilization (González et al., 2010). These compounds may have been degraded and taken part in the formation of isomers and low molecular-weight compounds (Clifford, 2000).

The results obtained for the TPC contents of the extracts were higher than those reported by Saucedá-Galvez et al. (2021) for apple juice obtained by DHP at 300 MPa / 20 °C and Karacam et al. (2015) for strawberry juice obtained by DHP at 60 MPa / room temperature / 2 steps. In the results obtained for the TAC and TPC contents in extracts obtained DHP the process at 60 MPa / 25 °C produced the best results for TPC, while for TAC it was 60 MPa / 45 °C.

Effects of DHP on the color of the purple corn cob extracts










Table 1 shows the color parameters of the purple corn cob extracts obtained DHP at different temperatures and the visual appearances of the

purple corn cob extracts obtained from the different processes.

Table 1 shows that the L* and C* parameters of the extracts were not significantly altered ($p > 0.05$) by increases in the DHP at 25 °C and 65 °C. All the processes presented positive values for a* and negative values for b*, which indicates that the extracts were represented by the colors red and blue, respectively. These color trends resemble those reported by Itthisoponkul et al. (2018) and by Monroy et al. (2016). Regarding the value for h° at a constant processing temperature, an increase in DHP did not significantly alter this parameter ($p > 0.05$). All the processes presented values for h° within the range from 270.18° to 292.09°, values higher than those obtained using non-pressurized and CE processes. Considering the cylindrical chromatic space CIE-L* C* h° system proposed by Padrón et al. (2012) as the reference, the hue angles correspond to a blue-violet color.

Table 1

Colorimetric parameters (L*, a*, b*, C and h°) in the purple corn cob extracts obtained DHP at three temperatures, compared with the parameters obtained through CE (static maceration-2.5 hours) and non-pressurized extract in the same temperatures

Temperature	Process Conditions	Visual appearance	L*	a*	b*	C*	h°
25 °C	0.01 MPa, 3 minutes		23.29±0.25 ^{aA}	-0.21±0.02 ^{cB}	-0.49±0.02 ^{cC}	0.53±0.02 ^{aA}	247.08±1.14 ^{bB}
	60 MPa		23.04±0.01 ^{aB}	0.14±0.03 ^{aA}	-0.35±0.04 ^{aA}	0.38±0.04 ^{bcB}	292.09±4.52 ^{aA}
	CE (2.5 horas)		23.05±0.06 ^{aA}	0.04±0.04 ^{aA}	-0.43±0.01 ^{abA}	0.43±0.02 ^{bA}	274.69±6.38 ^{aA}
45 °C	0.01 MPa, 3 minutes		23.09±0.02 ^{aA}	-0.05±0.01 ^{aA}	-0.39±0.02 ^{ab}	0.48±0.02 ^{aA}	255.45±0.61 ^{aA}
	60 MPa		19.09±0.44 ^{aA}	0.00±0.05 ^{bb}	-0.57±0.06 ^{ab}	0.58±0.06 ^{aA}	270.20±4.49 ^{abA}
	CE (2.5 horas)		23.00±0.01 ^{aA}	-0.03±0.12 ^{ab}	-0.40±0.04 ^{abA}	0.41±0.06 ^{abA}	267.41±18.94 ^{aA}
65 °C	0.01 MPa, 3 minutes		23.07±0.03 ^{ba}	-0.05±0.02 ^{abA}	-0.34±0.01 ^{aA}	0.35±0.01 ^{ab}	262.41±1.96 ^{aA}
	60 MPa		22.82±0.25 ^{ab}	0.03±0.00 ^{ab}	-0.51±0.02 ^{bb}	0.51±0.02 ^{aA}	273.37±0.37 ^{ab}
	CE (2.5 horas)		22.99±0.01 ^{aA}	-0.02±0.01 ^{abB}	-0.42±0.10 ^{aA}	0.42±0.10 ^{aA}	267.61±2.07 ^{aA}

* Colorimetric parameters (L*, a*, b*, C* and h°) of the extracts obtained by different processing and at different temperatures. CE (conventional extraction) for 2.5 hours, 0.01 MPa (non-pressurized) for 3 minutes and DHP. The bars are means ± standard deviation (n = 3). Equal lower-case letters mean that there is no significant difference ($p > 0.05$, Tukey test) between the extracts subjected to different processing considering the same temperature.

In vitro study of the antioxidant activities of the purple corn cob extracts obtained by DHP

Table 2 shows a comparison of the antioxidant activities (AA) of the non-pressurized extracts, and those obtained by DHP and CE under the conditions that showed high bioactive compound contents. The AA values of the extracts were determined using the ORAC and FRAP assays and the results expressed in μM Trolox (TE) / g dry weight in both cases. Of the extracts obtained by CE, those obtained in 2.5 hours/ 65 °C showed the highest AA values according to ORAC. The extract obtained by DHP at 60 MPa/25 °C showed 37% and 123% more AA than that obtained by CE in 2.5 hours/ 65 °C ($p < 0.05$), according to FRAP and ORAC, respectively. Similar results were obtained by Saucedá-Galvez (2021) in apple juice obtained by DHP at 300 MPa/20 °C, the antioxidant activity of the juice increased by 238% for the ferric ion reduction capacity and 286.4% for the absorption of free radicals.

The methodologies used revealed that the AA values of the purple corn cob extracts obtained by DHP were significantly higher than the values in the extracts obtained by CE, results which are consistent considering that the extracts obtained using high-pressure presented higher TPC contents (Jurić et al., 2019). Several studies have shown a direct relationship between the antioxidant activity and the contents of these compounds (Jurić et al., 2019; Saikaew et al., 2018; Wang et al., 2016; Andrés et al., 2016).

UPLC–MS/MS analysis of the anthocyanins in the purple corn cob extracts obtained by DHP and CE

UPLC-MS/MS was applied to the anthocyanin compositions of the purple corn cob extracts obtained by high-pressure technologies and CE. The compounds were identified by comparing their retention times (Pascual-Teresa et al., 2002). Seven anthocyanins were tentatively identified: cyanidin-3-glucoside, pelargonidin-3-glucoside, peonidin-3-glucoside and their respective malonyl derivatives. The areas of the chromatographic bands of the anthocyanins obtained using the different extraction processes (DHP and CE) (Table 3). Figure 4 shows the mass chromatograms obtained by UPLC-MS/MS for the DHP process. In this study, the chromatographic profiles were like those found by Aoki et al. (2001) in an extract from purple corn seeds, and by Pascual-Teresa et al. (2002) in a commercial extract from purple corn, but different from those reported by Yang et al. (2009) who did not detect the presence of the malonyl derivatives.

Table 3

Areas of the chromatographic bands obtained by technology for the compounds found in the extracts of purple corn

Peak	Compounds	DHP	CE
1	Unknown	199436	120026
2	Cyanidin-3-glucoside	8121862	11242858
3	Pelargonidin-3-glucoside	2062073	2497220
4	Peonidin-3-glucoside	5188770	6995596
5	Cyanidin-3-(6-malonylglucoside)	4825857	7855386
6	Pelargonidin-3-(6-malonylglucoside)	2480925	3792038
7	Peonidin-3-(6-malonylglucoside)	3483688	4622761

Table 2

Comparative of the antioxidant activity as determined by the ORAC and FRAP assays in the conditions of greater efficiency of DHP

Antioxidant Activity (μM TE/g dry weight)			
Process	Variables	FRAP	ORAC
Non-pressurized	0.01 MPa/25 °C/3 minutes	312.51 \pm 11.14 ^e	980.55 \pm 42.32 ^{de}
	0.01 MPa/45 °C/3 minutes	480.87 \pm 41.67 ^{de}	1222.77 \pm 74.50 ^{bcd}
	0.01 MPa/65 °C/3 minutes	753.34 \pm 16.03 ^{abc}	1145.39 \pm 46.63 ^{cd}
DHP	60 MPa/25 °C	929.31 \pm 19.57 ^a	1519.68 \pm 33.12 ^a
	60MPa/45 °C	704.07 \pm 58.89 ^{abcd}	1440.70 \pm 21.93 ^{ab}
	60 MPa/65 °C	753.14 \pm 32.25 ^{abc}	1329.93 \pm 49.42 ^{abc}
CE	2.5 hours/25 °C	591.96 \pm 39.57 ^{cd}	849.31 \pm 22.36 ^{ef}
	2.5 hours/45 °C	509.09 \pm 44.12 ^{de}	999.52 \pm 16.82 ^{ed}
	2.5 hours/65 °C	676.75 \pm 9.95 ^{bcd}	682.29 \pm 21.64 ^f

* The data are presented as the means \pm standard deviation ($n = 3$). Same letters in the column indicate that there are no significant differences among the different process conditions ($p < 0.05$, Tukey test).

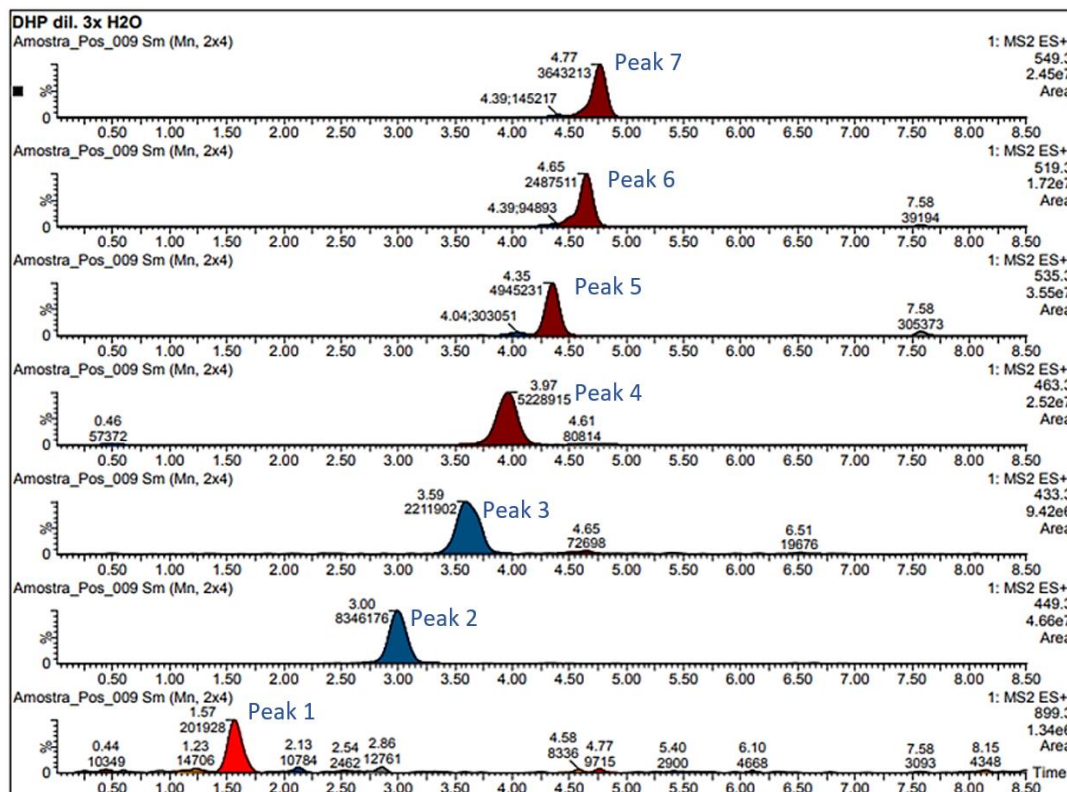


Figure 4. Mass chromatograms obtained by UPLC-MS/MS, highlighting the majoritarian anthocyanins in purple corn cob extract using DHP process (60 MPa /45 °C / 1 step).

4. Conclusions

The present work demonstrated that dynamic pressures greater than 20 MPa and temperatures below 60°C had a positive effect on the extraction of bioactive compounds and a high content of antioxidant activity with the capacity for ferric ion reduction and oxygen radical absorption. The combination of dynamic high pressure and temperature allowed obtaining up to 108% more content of phenolic compounds and 32% more anthocyanin content in the purple corn cob extracts, compared to conventional extraction at 65 °C and 150 minutes. Processing at different dynamic pressures caused visual changes in the color of the extracts, which were statistically significant in chroma and brightness. Although the effects of conventional techniques in the extraction of bioactive compounds from purple corn are known, those of the high dynamic pressure process were not, so now it appears as a potential and promising alternative technology to improve the extraction of phytochemicals that allow the development of new products with healthy characteristics. Based on the results, it is suggested to optimize the extraction processes of bioactive compounds by high dynamic pressure as a function of temperature and solvent concentration.

Acknowledgment

This study was financially supported by the Consejo Nacional de Ciencia, Tecnología e Innovación Tecnológica, (CONCYTEC, Peru) through its executing unit Programa Nacional de Estudios de Investigación Científica y Estudios Avanzados (PROCIENCIA) as part of the project “Disminución de la absorción del cadmio en el cacao peruano mediante la edición genética de sus transportadores empleando la tecnología CRISPR-CAS9” (CONTRACT N° 26-2018-FONDECYT-BM-IADT-AV).

ORCID

- J. S. Guillén Sánchez [ID https://orcid.org/0000-0002-0899-6725](https://orcid.org/0000-0002-0899-6725)
- C. B. B. Cazarín [ID https://orcid.org/0000-0002-9849-2546](https://orcid.org/0000-0002-9849-2546)
- M. R. Canesin [ID https://orcid.org/0000-0002-2892-3814](https://orcid.org/0000-0002-2892-3814)
- F. G. Reyes Reyes [ID https://orcid.org/0000-0003-0126-3817](https://orcid.org/0000-0003-0126-3817)
- A. H. Iglesias [ID https://orcid.org/0000-0003-1119-5627](https://orcid.org/0000-0003-1119-5627)
- M. Cristianini [ID https://orcid.org/0000-0001-7740-0449](https://orcid.org/0000-0001-7740-0449)

References

- Aguilar, Y. (2022). Optimization of baking time and the formulation using Cushuro and purple corn meal in cookies. Tesis, Universidad Nacional de Trujillo, Trujillo.
- Andrés, V., Villanueva, M., & Tenorio, M. (2016). The effect of high-pressure processing on colour, bioactive compounds, and antioxidant activity in smoothies during refrigerated storage. *Food Chemistry*, 192(1), 328-335. <https://doi.org/10.1016/j.foodchem.2015.07.031>
- AOAC. Horwitz, W. (Ed). (2005). Official methods of Analysis of AOAC International, 18th edition, AOAC International, Maryland, USA.
- Aoki, H., Kuze, N., & Kato, Y. (2002). Anthocyanins isolated from purple corn (*Zea Mays L.*). *Foods and Food Ingredients Journal of Japan*, 199, 41-45.

- Benzie, F., & Strain, J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry*, 239, 70-76.
- Bligh, E., & Dyer, W. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, 37(8), 911-917.
- Clifford, M. (2000). Review. Anthocyanins – nature, occurrence and dietary burden. *Nutritional Enhancement of Plant-based Food in European Trade*, 80, 1063-1072.
- Eslami, E., Carpentieri, S., Pataro, G., & Ferrari, G. (2023). Comprehensive overview of tomato processing by-product valorization by conventional methods versus emerging technologies. *Foods*, 12(166). <https://doi.org/https://doi.org/10.3390/foods121666>.
- González, M., Lobo, G., & Gonzalez, M. (2010). The effect of extraction temperature, time and number of steps on the antioxidant capacity of methanolic banana peel extracts. *Separation and Purification Technology*, 71, 347-355.
- Halahlah, A., Piironen, V., Mikkonen, K., & Ho, T. (2023). Wood Hemicelluloses as Innovative Wall Materials for Spray-Dried Microencapsulation of Berry Juice: Part 1—Effect of Homogenization Techniques on their Feed Solution Properties. *Food and Bioprocess Technology*, 16(4), 909-929.
- He, X., Yue, J., Qiu, F., Tang, Y., Su, J., Zhao, Y., . . . Sun, J. (2022). Effect of Dynamic High-Pressure Microfluidization Assisted Extraction on the Structure and Activity of Polysaccharides from Polygonatum kingianum Coll. et Hemsf. *Food Science*, 43(19), 95 - 101.
- Hee Yeon, K., Ki Yeon, L., Minju, K., Minji, H., Ponnuvel, D., & Songmun, K. (2023). A Review of the Biological Properties of Purple Corn. *Scientia Pharmaceutica*, 91(1), 6.
- IAL - Instituto Adolfo Lutz (2008). Procedimentos e determinações gerais. In: Zenebon O, Pascuet N, Tiglea, P (eds.), Métodos Físico-Químicos para análise de alimentos, pp.83-158.
- Itthisoponkul, T., Naknan, P., Prompun, S., & Kasemwong, K. (2018). Evaluation of high-pressure treatment for improvement of physicochemical and functional qualities in purple corncobs. *International Food Research Journal*, 25(1), 246-253.
- Jurić, S., Ferrari, G., Velikov, K., & Donsi, F. (2019). High-pressure homogenization treatment to recover bioactive compounds from tomato peels. *Journal of Food Engineering*, 262, 170-180.
- Kaneiwa, M., Augusto, P., & Cristianini, M. (2013). Effect of high-pressure homogenization (HPH) on the physical stability of tomato juice. *Food Research International*, 51(1), 170-179.
- Karacam, C. H., Sahin, S., & Oztop, M. H. (2015). Effect of high-pressure homogenization (microfluidization) on the quality of Ottoman Strawberry (F. Ananassa) juice. *LWT - Food Science and Technology*, 64, 932-937.
- Lima, M., & Rosenthal, A. (2022). High pressure homogenization applied to fruit juices: Effects on microbial inactivation and on maintenance of bioactive components. *Food Sci and Techn International*. <https://doi.org/10.1177/10820132221124196>
- Liu, J., Bi, J., Liu, X., Liu, D., Verkerk, R., Dekker, M., . . . Wu, X. (2022). Modelling and optimization of high-pressure homogenization of not-from-concentrate juice: Achieving better juice quality using sustainable production. *Food Chemistry*, 370, 131058. <https://doi.org/10.1016/j.foodchem.2021.131058>
- Montes, C., Vicario, I., Raymundo, M., Fett, R., & Heredia, F. (2005). Application of tristimulus colourimetry to optimize the extraction of anthocyanin from Jaboticaba (*Myrcia Jaboticaba* Berg.). *Food Research International*, 38, 983-988.
- Monroy, Y., Rodriguez, R., Sartoratto, A., & Cabral, F. (2016). Extraction of bioactive compounds from cob and pericarp of purple corn (*Zea mays* L.) by sequential extraction in fixed bed extractor using supercritical CO₂, ethanol, and water as solvents. *The Journal of supercritical fluids*, 118, 10-19.
- Ou, B., Chang, T., Huang, D., & Prior, R. (2013). Determination of total antioxidant capacity by oxygen radical absorbance capacity (ORAC) using fluorescein as the fluorescence probe: First action 2012.23. *Food composition and additives*, 96(6), 1372-1376. Doi: 10.5740/jaoacint.13-175
- Padrón, C., Padrón, G., Montes, A., & Oropeza, R. (2012). Determinación del color en epicarpio de tomates (*Lycopersicon esculentum* Mill.) con sistema de visión computarizada durante la maduración. *Agronomía Costarricense*, 36(1), 97-111.
- Pascual-Teresa, S., Santos-Buelga, C., & Rivas-Gonzalo, J. (2002). LC-MS Analysis of anthocyanins from purple corn cob. *Journal Science Food Agriculture*, 82, 1003-1006.
- Pirozzi, A., Ferrari, G., & Donsi, F. (2022). Cellulose Isolation from Tomato Pomace Pretreated by. *Foods*, 11(266). <https://doi.org/https://doi.org/10.3390/foods11030266>
- Rafael, E., & Castro, D. (2023). Extracción y cuantificación de Antocianinas de maíz morado (*Zea Mays* L) utilizando dos solventes a diferentes temperaturas y tiempos de extracción. *Revista Latinoamericana de Difusión Científica*, 5(8), 47-80.
- Rossi, E., Moroni, F., Badin, E., Massolo, F., Rodoni, L., Vicente, A., & Lespinard, A. (2017). *Estudio cinético de la degradación térmica de antocianinas en pulpas y jugos de frambuesa*. IX Jornada de biología y tecnología poscosecha. Libro I Congreso Argentino de Biología y Tecnología Poscosecha. IX Jornadas Argentinas de Biología y Tecnología Poscosecha. Paraná: Universidad Nacional de Entre Ríos. UNER.
- Sauceda-Galvez, J., Codina-Torrella, I., Martínez-García, M., Hernández, M., Gervilla, R., & Roig-Sagues, A. (2021). Combined effects of ultra-high pressure homogenization and short-wave ultraviolet radiation on the properties of cloudy apple juice. *LWT*, 136(110286). <https://doi.org/10.1016/j.lwt.2020.110286>
- Saikaew, K., Lertrat, K., Meenune, M., & Tangwongchai, R. (2018). Effect of high-pressure processing on colour, phytochemical contents and antioxidant activities of purple waxy corn (*Zea mays* L. var. ceratina) kernels. *Food Chemistry*, 243, 328-337. <http://dx.doi.org/10.1016/j.foodchem.2017.09.136>
- Singleton, V., & Rossi, J. (1965). Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture*, 16(3), 144-158.
- Strack, D., & Wray, V. (1989) Anthocyanins. In: Harbone H (ed). Methods in Plant Biochemistry, Plant Phenolics. Academic Press, New York, pp. 325-356.
- Szczyptańska, J., Skąpska, S., Połaska, M., & Marszałek, K. (2022). High pressure homogenization with a cooling circulating system: The effect on physicochemical and rheological properties, enzymes, and carotenoid profile of carrot juice. *Food Chemistry*, 370, 131023. <https://doi.org/10.1016/j.foodchem.2021.131023>
- Tonon, R., Brabet, C., & Hubinger, M. (2009). Influência da temperatura do ar de secagem e da concentração de agente carreador sobre as propriedades físicoquímicas do suco de açaí em pó. *Ciência e Tecnologia de alimentos*, 29(2), 444-450.
- Wang, B., Huang, Q., Venkatasamy, C., Chai, H., Gao, H., Cheng, N., Cao, W., Lv, X., & Pan, Z. (2016). Changes in phenolic compounds and their antioxidant capacities in jujube (*Ziziphus jujuba* Miller) during three edible maturity stages. *LWT - Food Science and Technology*, 66, 56-62. <https://doi.org/10.1016/j.lwt.2015.10.005>.
- Yang, S., Chen, Z., Zhai, W., Piao, X., & Piao, X. (2009). Extraction and identification of anthocyanin from purple corn (*Zea mays* L.). *Food Science & Technology*, 44(12), 2485-2592.
- Zapata, I., Sepúlveda-Valencia, U., & Rojano, B. (2015). Effect of Storage Time on the Physicochemical, Probiotic and Antioxidant Properties of Yogurt Flavored with Colombian Berry (*Vaccinium meridionale* Sw). *Información tecnológica*, 26(2), 17-28. <https://dx.doi.org/10.4067/S0718-07642015000200004>
- Zhang, C., Song, J., Liu, Y., Geng, N., Ma, K., Wang, L., & Li, Y. (2023). Optimization of Dynamic High Pressure Microfluidization-Assisted Extraction of Burdock Root Polysaccharide and Its In Vitro Antioxidant Activity. *Science and Technology of Food Industry*, 44(12), 260 - 267.
- Zhao, Y., Zhou, H., Huang, Z., & Zhao, R. (2020). Different aggregation states of barley β -glucan molecules affects their solution behavior: A comparative analysis. *Food Hydrocolloids*. <https://doi.org/10.1016/j.foodhyd.2019.105543>.