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Functional Chemical Properties And Antioxidant Capacity In Cocoa Paste After Vacuum Roasting Of Cocoa Beans

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Abstract

Cocoa beans roasting at a high temperature affect the content of functional compounds and the antioxidant capacity of the cocoa paste or liquor to be obtained. These products are the basis for the production of chocolates. The properties favorable to health in this product are affected. The objective was to evaluate the influence of vacuum roasting on the functional chemical properties and antioxidant capacity of cocoa paste. There were thirteen treatments and one control. The variables were roasting temperature and pressure (normal pressure and vacuum atmosphere). The results showed the influence of vacuum roasting of cocoa beans on the functional chemical properties and antioxidant capacity of pastes. The temperatures of 90°C, and 100°C, and the pressure of vacuum of 0.4 bar less affected the antioxidant capacities expressed as IC_{50} values with DPPH and ABTS radicals,

treatment T13 (100°C and 0.4 bar) produced activities of 84.88 ± 0.87 ug/mL and 48.19 ± 0.34 ug/mL, respectively. The optimization analysis of the roasting process presented 13 optimal solutions, highlighting the treatment at 90 °C and 0.8 bar for its greater desirability 0.816. The contents of phenols, flavonoids, and anthocyanins influenced the antioxidant capacity of cocoa pastes. The component analysis (PCA), PC1, and PC2 explained this direct correlation in 99%, highlighting the influence of phenols, flavonoids, and anthocyanins. Future research on vacuum roasting should determine what happens to specific compounds such as theobromine, catechins, and other phenols.

Keywords: *Phenols, flavonoids, anthocyanins, DPPH, ABTS.*

Introduction

Although it is native to Amazonia, cacao (Theobroma cacao L.) is usually thought to have been domesti[1]cated in Mesoamerica since it was the only place where cultivation evidence was present at the time of European conquest (Clement, De Cristo-Araújo, Coppens D'Eeckenbrugge, Alves Pereira, & Picanço-Rodrigues, 2010). The grain is known throughout the world for being the raw material of chocolate (Nunes et al., 2018). The

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cocoa grains phenols represent the most abundant antioxidants. Responsible for numerous biological properties such as antioxidant, antiproliferative, antiapoptotic, anti-inflammatory, and anti-cancer (Cinar et al., 2021). Cocoa is a natural source of epicatechin, catechin, and procyanidins. Has a high potential antioxidant, compared to other foods such as tea or red wine (do Carmo Brito, Campos Chisté, da Silva Pena, Abreu Gloria, & Santos Lopes, 2017; Ellam & Williamson, 2013).

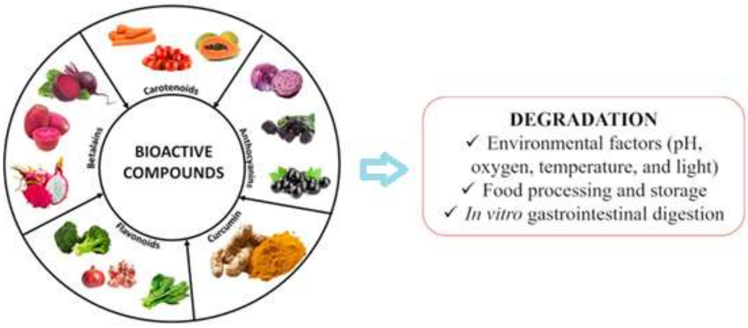

Figure 1 Bioactive compounds and causes of degradation, Modified from (Luiza Koop et al., 2022).

Oleic, stearic, and palmitic fatty acids can be found in cocoa beans; their typical concentrations are, respectively, 34.48 1.49, 31.81 1.51, and 30.01 0.89%. The main bioactives were theobromine (9.79-12.95 mg/g), catechin (3.90-18.22 mg/g), and epicatechin (6.15-13.09 mg/g). Hy1, Hy2, Hy3, Hy4, Hy5, and Hy6 hybrid cultivars also had the highest levels of polyphenols, flavonoids, and flavonols, which translated into the highest overall antioxidant capacity (Ramos-Escudero et al., 2021). During roasting, flavor precursors that develop during fermentation interact to produce the desired chocolate flavor in commercial cocoa products. In the Maillard reaction, amino acids and sugars react, and the products are responsible for the flavor (Cinar et al., 2021; Hinneh et al., 2019). Roasting cocoa results in the development of flavor, but it can damage some of the plant's phytochemical compounds. A temperature of 130°C for 30 minutes does not seem to affect the concentration of epicatechin, procyanidin B2, and theobromine in the sample (Lemarcq et al., 2020). Unroasted cocoa beans had 57 volatiles, but roasted beans produced 71 volatiles. (Marseglia, Musci, Rinaldi, Palla, & Caligiani, 2020). However, it has been noted that roasting lowers the beans' polyphenol concentration. Proanthocyanidins (PAC) dimer-pentamer content, total phenolics, and (-)-epicatechin content were all decreased. On the other hand, roasting at 150°C or above raised the concentrations of catechin, PAC hexamers, and heptamers. These substances have more PL inhibitory power. We discovered that roasting at 170 degrees C time-dependently increased PL inhibitory action, which is consistent with these changes in PAC composition and the preceding findings (Stanley et al., 2018). Therefore, it is necessary to consider the appropriate conditions of the roasting process (time, temperature, humidity, and airflow), as well as the fineness of the beans (Żyżelewicz et al., 2016).

To date, bibliographic research reports do not show the existence of the vacuum roasting process for cocoa beans, and it is known that the high temperatures used in traditional roasting produce losses in the phytochemical compounds of the grain, so, it is reasonable to consider that roasting under vacuum conditions would allow moisture to evaporate easily and that the phytochemical components would not be affected or would be less affected by roasting at lower temperatures than traditional ones. In this context, the objective of the research was to evaluate the influence of vacuum roasting on the functional chemical properties and antioxidant capacity of cocoa paste.

Material and methods

Chemicals

Hydrochloric acid (Germany Merck) 36.5 percent; gallic acid ($C_7H_6O_5$) (USA Sigma) at 98.1 percent; Follin-Ciocalteau phenol reagent 2N, (Sigma Aldrich); 96 percent pure ethanol; 1,1-Diphenyl-1-picri-hydrayl DPPH (Sigma Aldrich, USA); 2,2-azinobis(2-methylpropionamidine) (AAPH, 97 percent); distilled water H_2O.

Sample

Cocoa beans of the native hybrid were provided by the Alto Huallaga Cooperative. Geographic coordinates were: -9.142827, -76.019125.

Sample analysis

Physicochemical analysis

Humidity method 931.04. Protein method 970.22. Fat method 963.15, ashes method 972.15, crude fiber method 930.20, pH, method 970.21, Titratable acidity, method 942.15 **(Latimer, 2016)**. The carbohydrate content was calculated by difference, Subtracting from 100 % the contents of moisture, protein, fat, ash, and crude fiber percentage.

Determination of total polyphenol content

Total phenols were determined by spectrophotometry (UV Vis GENESYS™ 10S, Thermo Scientific™, Waltham, EE. UU.) using the reagent Folin-Ciocalteu, expressed as gallic acid equivalents (Franková et al., 2022).

Anthocyanins, differential pH method

According to a cyanidin-3-glucoside basis, the difference in the pigments' absorbance at 520 nm is proportional to the pigment content. Because they absorb at pH 4.5 and 1.0, degraded anthocyanins in polymeric form are resistant to color change regardless of pH and are not counted in the assays **(Lee, Durst, Wrolstad, & Collaborators:, 2019)**.

DPPH radical scavenging activity

A method based on inhibition of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The UV-VIS spectrophotometer (UV-Vis GENESYS™ 10S, Thermo Scientific™, Waltham, EE. UU.). At 510 nm was used (Brand-Williams, Cuvelier, & Berset, 1995); (Franková et al., 2022).

ABTS radical scavenging activity

In the $ABTS^{\bullet+}$ radical scavenging assay (an electron transfer-based assay), the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$). An antioxidant turned the dark blue pigment into colorless ABTS (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993). The UV-VIS spectrophotometer (UV-Vis GENESYS™ 10S, Thermo Scientific™, Waltham, EE. UU.) (Dasgupta & Klein, 2014).

Treatments

The proposed treatments consider the variation of the roasting temperature and the use of vacuum during this operation. Table 1 shows that treatment T1 is the control, and T2 to T14 were the treatments considered in the roasting of cocoa used in the investigation. The parameters were temperature in degrees centigrade and vacuum pressure in bars.

Table 1 Treatments considered during the roasting of dry fermented cocoa grains.

Treatments	**Temperature (ºC)**	**Vacuum pressure (Bar)**
T1 (dry grain as a control)	-	-
T2 (roasting)	110	0
T3 (roasting)	100	0
T4 (roasting)	90	0
T5 (roasting)	100	0.8
T6 (roasting)	110	0.8
T7 (roasting)	110	0.4
T8 (roasting)	100	0.4
T9 (roasting)	90	0.8
T10 (roasting)	90	0.4
T11 (roasting)	100	0.4
T12 (roasting)	100	0.4
T13 (roasting)	100	0.4
T14 (roasting)	100	0.4

Treatments T2, ..., T14, were created using a 3^2 level factorial response surface design, which studied the effects of 2 factors in 13 runs. The design was executed in a single block, using the Design Expert 13.0.12.0 program. The processes in the production of chocolates, which involve most of the chemical reactions for the development of the chocolate flavor, are the fermentation, drying, and roasting of the cocoa bean and the conching of the chocolate (Barisic et al., 2019). At the level of cocoa processing, roasting is one of the basic technological operations that affect the quality of chocolate since the moisture content is reduced (from 7% to 1-5%). Transformation reactions of bioactive compounds were observed, such as phenols by oxidation, condensation, and complexation. Roasting facilitates the evaporation of undesirable compounds such as acetic acid. Also, it is a fundamental operation in the formation of the chocolate flavor because during this stage the Maillard reactions take place (Santander Muñoz, Rodríguez Cortina, Vaillant, & Escobar Parra, 2020; Żyżelewicz et al., 2018**)**. Roasting conditions affect the formation of biogenic amines 2-phenylethylamine followed by tyramine, tryptamine, serotonin, and dopamine (Oracz & Nebesny, 2014).

Statistical analysis

For each dependent variable response, mean values and standard deviations were calculated. To compare the statistical differences between the roasting conditions, analysis of variance (ANOVA) and Tukey's test were used, p values less than 0.05 were considered significant, for these statistical analyses, the STATGRAPHICS 19.1.2 (64 bit) program was used, Copyright © 1982-2020. To optimize the vacuum roasting process, the 3^2 factorial response surface design was used, with 4 central points, Design Expert 13.0.12.0 program was used. The Unscrambler©2016 CAMO version 10.4 software was used to perform the correlation analysis (Esbensen, Swarbrick, Westad, Whitcomb, & Anderson, 2018).

Table 2 Build Information to perform factorial analysis with response surface

File Version	13.0.12.0	Design Expert	
Study Type	Response Surface	Subtype	Randomized
Design Type	3 Level Factorial	Runs	13
Design Model	Quadratic	Blocks	No Blocks
Build Time (ms)	1.0000		

Results and discussion

Characteristics of dry fermented cocoa beans

Table 4 lists the physicochemical characteristics and antioxidant capacity of grains that have undergone dry fermentation. There was a high concentration of crude fat. According to reports, cocoa beans and cotyledons contain a significant amount of fat (18.65–49.48 g/100 g), proteins, carbohydrates, and phenols. Phenols including catechin, epicatechin, and derivatives from epicatechin, as well as methylxanthines like theobromine, were identified. Theobromine was the major bioactive compound detected in different samples (Hernández-Hernández, Fernández-Cabanás, Rodríguez-Gutiérrez, Fernández-Prior, & Morales-Sillero, 2022). Fat represents approximately half of the cocoa bean's weight and in a prediction investigation of fat content, it was reported that the content ranged between 51.3 and 68.0% and the calibration and prediction R^2 values were 0.93–0.98 and 0.92–0.97, respectively, according to the different PLS regression models used (Caporaso, Whitworth, & Fisk, 2021), our fat content result is within the reported range (52.11 ± 0.07 %).

It was determined in cocoa beans that the main fatty acids were oleic, stearic, and palmitic (respective averages of 34.48 ± 1.49, 31.81 ± 1.51, and 30.01 ± 0.89%). Theobromine (9.79–12.95 mg/g), catechin (3.90–18.22 mg/g), and epicatechin (6.15–13.09 mg/g) represented the major bioactive. Also, hybrid cultivars (Hy1, Hy2, Hy3, Hy4, Hy5, and Hy6) provided the highest content in polyphenols, flavonoids, and flavonols, also resulting in the highest total antioxidant capacity (Ramos-Escudero et al., 2021), our results in total phenols were higher than those reported, and possibly the antioxidant activity is greater.

The concentration ranges of phenols depend on the type of matrix, treatment, and country, as well as their relationship with the main bioactive compounds present in cocoa that are associated with their possible antioxidant biological potential and health-related benefits (Gil, Uribe, Gallego, Bedoya, & Arango-Varela, 2021), groups of phenols have in cocoa beans: catechins or flavan-3-ols, anthocyanins, and proanthocyanidins also contain methylxanthine alkaloids, theobromine, and caffeine, that contribute to the bitterness and have several pharmacological effects (Carrillo, Londoño-Londoño, & Gil, 2014).

Table 4 Physicochemical characteristics and antioxidant capacity of dry fermented grains

Component	Contents
Humidity (%)	7.08 ± 0.01
Crude fat (%)	52.11 ± 0.07

Crude protein (%)	16.63 ± 0.11
Total ash (%)	3.40 ± 0.01
Crude fiber (%)	24.42 ± 0.11
Carbohydrates (%)	20.43 ± 0.11
Fat energy (Kcal)	75.72 ± 0.21
Protein-energy (Kcal)	11.20 ± 0.25
Carbohydrate energy (Kcal)	13.20 ± 0.04
Total energy (Kcal)	618.48 ± 0.04
Acidity Titratable as acetic acid (%)	1.43 ± 0.07
pH	4.97 ± 0.03
Total phenols (g GAE/100 g)	3.98 ± 0.02
Total flavonoids (mg EC/100 g)	0.66 ± 0.01
Total anthocyanins (mg/100 g)	14.70 ± 1.34
DPPH - IC_{50} (ug/mL)	77.21 ± 0.65
ABTS - IC_{50} (ug/mL)	35.04 ± 1.34

As expressed by various authors, the drying of cocoa beans after fermentation ends when the grain reaches 6-8% moisture. Low moisture content prevents mold growth and makes the beans more stable for transport and storage (Barisic et al., 2019), resulting in moisture content being 7.08 ± 0.01%, an intermediate value in the reported range.

The pH value found in dry fermented beans is within the range reported in other cocoa fermentation studies. The acidity increased after fermentation (Chagas Junior, Ferreira, Gloria, Martins, & Lopes, 2021), our pH result was 4.97 ± 0.03, and the acidity titratable as acetic acid (%) was 1.43 ± 0.07, high value. In conclusion, we can indicate that the dry fermented grains presented good quality physicochemical characteristics.

Characteristics of cocoa pastes

Cocoa nibs were ground in a ball mill to obtain a cocoa paste until reaching a particle size of fewer than 40 microns is part of the grinding operation, which is considered essential for the right flavor and texture (Afoakwa, Paterson, Fowler, & Ryan, 2008).

The physicochemical characteristics and antioxidant capacity of cocoa pastes are in Table 5. This table shows the comparative results of the dry fermented cocoa beans (T1, as a control) and the thirteen roasting treatments (T2, ..., T14), depending on the moisture content, titratable acidity, pH, phenols, flavonoids, anthocyanins, antioxidant capacities such as DPPH and ABTS.

The humidity in the pasta samples is well below the humidity of the dry grain (7.08±0.01%) because of normal atmospheric pressure and vacuum pressure roasting of the grains, husking, and operations before obtaining cocoa paste. These variations in the moisture content in the paste are statistically different, are two similar blocks, one formed by treatments T2, T3, T4, T6, and T7, with the lowest contents, varying between 2.35±0.09% to 2.86±0.11%, and the other block made up of treatments T2, T4, T5, T6, T7, T8, T12, T14, with contents between 2.69±0.18% to 3.20±0.14%, treatment T11 reached the highest moisture content with 3.94±0.2%.. In general terms, vacuum roasting cocoa beans produces comparable results to non-vacuum roasting. The obtained humidity results were higher than those reported in an investigation in Mexico humidity varied between 1.64 to 1.97%. The differences in humidity between the pasta samples could be mainly due to the process, date of preparation or the type of packaging (Sol Sánchez, Naranjo González, Córdova Avalos, Ávalos de la Cruz, & Zaldívar Cruz, 2016**)**.

The acetic acid in the dry cocoa bean (T1) is statistically higher than in most cocoa paste due to the effect of roasting and other operations before obtaining the paste (Barisic et al., 2019). During roasting, the acidity decreases due to a reduction in the concentrations of volatile acids, not affecting non-volatile acids, such as oxalic, citric, tartaric, succinic, and lactic acids (Gutiérrez, 2017). In the titratable acidity results, the cocoa pastes samples T12 and T14, whose dry fermented beans were vacuum roasted, presented the lowest acidity contents, with values of 0.95±0.03% and 1.11±0.04% acetic acid, statistically equal values ($p>0.05$). The results obtained were like those obtained in the Colombian research on the physicochemical characterization of cocoa liquor from six varieties, which reported an average of 1.25 ± 0.07% (Erazo Solórzano et al., 2021), but higher than those reported in a Mexican investigation, where the content of acetic acid varied between 0.34 to 0.62% (López-Báez, 2018).

Variation in the pH of the cotyledons manifests itself during fermentation, beginning with a pH of 6.60. From the first day of fermentation, it decreases slowly until 6.30; then, and from the third and fourth days do it in an accelerated way, reaching a value of approximately 4.75 value increases during the drying up to 5.40, lower than the reported value 5,58 ± 0.31 (Quevedo Guerrero, Ramírez Villalobos, Alfonso Portillo, García Batista, & Tuz Guncay, 2022). High pH values indicate over-fermentation, but values below 5 indicate faulty or deficient fermentation (Portillo et al., 2013). The pH of the dry bean was 4.97 ± 0.03, a higher degree of acidity than that determined in the different paste samples evaluated and obtained by vacuum roasting dry fermented cocoa beans. The pH values of the pastes were statistically similar ($p<0.05$), except for the T13 treatment, the pH values were lower than the reported 5.51 ± 0.02 in a storage investigation (Roda & Lambri, 2019) and 5.75 ± 0.07 in the Colombian (Erazo Solórzano et al., 2021).

The most prevalent antioxidants in the human diet are phenolic chemicals. They have a wide variety of structural elements, with aromatic rings' hydroxyl groups serving as distinguishing features. These substances are categorized as simple phenols, phenolic acids, flavonoids, xanthones, stilbenes, and lignans based on the number of phenol rings and the structural components that connect rings to one another. Each category has unique modes of action linked to a structural specificity that endows the compounds with antioxidant capabilities (Vuolo, Lima, & Maróstica Junior, 2019). In cocoa beans, phenolic compounds contribute to sensory characteristics stability and antimicrobial protection. Cocoa is a rich source of phenolic acids and flavan-3-ols (Mudenuti et al., 2021). Roasting reduces polyphenol content polyphenols, especially flavonoids and catechins; its high temperatures influence the bitter taste that results from the formation of insoluble compounds between flavonoids and proteins, peptides, polysaccharides, and products of the Maillard reaction. Also, roasting promotes the epimerization of (-)-epicatechin to (+)-catechin. Cocoa liquor is the most important intermediate product in the cocoa industry (Gil et al., 2021). The results obtained from phenol contents show that the dry bean presented the highest value of 3.98 ± 0.02 g GAE/100g, cocoa pastes values between 1.12 ± 0.02 to 1.91 ± 0.05 g GAE /100g, values are in the range of reported (1.55 - 6.04%) for samples of cocoa liquors from different countries (Natsume et al., 2000). The results were lower than reported in the cocoa paste from areas in Perú, 2.82 ± 0.03 to 5.33 ± 0.05 g GAE /100g (Quispe, Camasca, & Peláez, 2020), 2.85 g GAE /100g was also reported in a sample of cocoa liquor from Brazil (Gil et al., 2021).

Flavonoids are a group of natural polyphenol substances abundant in vegetables, fruits, grains, and tea that play essential roles in many biological processes, and environmental factors in plants, having antioxidant effects as other bioactivities (e.g., antimicrobial and anti-inflammatory properties) in the human diet, reduce the risk of disease, help in low the level of blood pressure in humans, classified into seven subclasses: flavonols, flavones, isoflavones, anthocyanidins, flavanones, flavanols, and chalcones (Etaware, 2021; Shen et al., 2022), but there are three main groups of flavonoids in cocoa: catechins (flavan-3-ols), anthocyanins, and proanthocyanidins (Molina-García, Llorent-Martínez, & Fernández-de Córdova, 2018). Table 3 shows the flavonoid content of the dry bean was 0.66 ± 0.01 mg CE/100g. Content in the cocoa paste samples varied between 0.24 ± 0.00 to 0.43 ± 0.01 mg CE/100g, showing that the lowest content was the product of more severe roasting conditions (T6 at 110 °C and 0.8 bar of vacuum pressure). In the research carried out by (Urbańska et al., 2021), comparing samples of unroasted cocoa beans and the liquor obtained, the flavonoids such as catechin and epicatechin decreased in cocoa liquor. Likewise, the process temperature increased (Fernández-Romero, Chavez-Quintana, Siche, Castro-Alayo, & Cardenas-Toro, 2020).

Anthocyanins are bioactive substances that belong to flavonoids, water-soluble pigments that give flowers, fruits, vegetables, and some cereal grains their colors. The Greek words anthos (flower) and kianos are the origin of the word anthocyanin (blue) (Fang, 2015). Fruits such as jussara are rich sources of (2.956 mg/100 g dry weight), guajiru (958 mg/100 g dry weight), jambolan (771 mg/100 g dry weight), and acerola (261 mg/ 100 g dry weight) (Luiza Koop et al., 2022). Studies conducted in animals and in vitro demonstrate the processes through which anthocyanins could stop or cure diseases (Guo & Ling, 2015). The results of the content of anthocyanins in the dry bean and cocoa paste show that the temperature of 100 °C and the vacuum pressure of 0.8 bar (T6) was the roasting conditions of the bean that decreased the content of anthocyanins, from 14.70 ± 1.34 mg of cyanidin-3-glucoside/100g in the dry bean up to 5.57 ± 0.91 mg/100g, in the cocoa paste. In the investigation of the effect of roasting on five Colombian cocoa clones, values decreased after roasting to contents between 17 - 99 mg/100g, values higher than the one determined in the dry grain investigated (Zapata Bustamante, Tamayo Tenorio, & Alberto Rojano, 2015). Anthocyanins are a group of flavonoids, natural compounds in fruits and vegetables, which contribute positively to human health (Mattoo et al., 2022). The content of anthocyanins present in the dry cocoa bean pastes was affected by the elevated temperature and high vacuum during bean roasting by the temperature and conching time required to obtain them.

Table 5 Physicochemical characteristics and antioxidant capacity of cocoa pastes

Treatments	Humidity (%)	Titratable acidity (% acetic acid)	pH	Phenols (g GAE/100g)	Flavonoids (mg CE/100 g)	Anthocyanins (mg/100 g)	Antioxidant capacity	
							DPPH (IC_{50} ug/mL)	ABTS (IC_{50} ug/mL)
T1 (dry grain)	7.08 ± 0.01g	1.43 ± 0.07d	4.97 ± 0.03a	3.98 ± 0.02h	0.66 ± 0.01h	14.70 ± 1.34g	77.21 ± 0.65a	35.04 ± 1.34a
T2 (paste)	2.69 ± 0.18ab	1.25 ± 0.04bcd	5.24 ± 0.04c	1.59 ± 0.02cd	0.34 ± 0.00cd	11.51 ± 0.92cdefg	107.14 ± 0.35g	50.07 ± 0.20c
T3 (paste)	2.35 ± 0.09a	1.20 ± 0.06bc	5.20 ± 0.01bc	1.83 ± 0.06efg	0.41 ± 0.02fg	12.85 ± 0.46defg	92.52 ± 0.47d	47.05 ± 0.22b
T4 (paste)	2.85 ± 0.11abc	1.32 ± 0.05cd	5.18 ± 0.02bc	1.59 ± 0.04cd	0.34 ± 0.01cd	10.84 ± 0.58cdefg	101.83 ± 0.26f	51.89 ± 0.18d
T5 (paste)	2.92 ± 0.12bc	1.20 ± 0.05bc	5.19 ± 0.02bc	1.71 ± 0.05de	0.36 ± 0.00cde	10.70 ± 0.83bcdefg	108.05 ± 0.16g	53.37 ± 0.21d
T6 (paste)	2.86 ± 0.11abc	1.21 ± 0.04bc	5.15 ± 0.03b	1.12 ± 0.02a	0.24 ± 0.00ab	5.57 ± 0.91a	108.93 ± 0.51g	69.72 ± 0.72i
T7 (paste)	2.79 ± 0.15abc	1.29 ± 0.04bcd	5.14 ± 0.02b	1.37 ± 0.02b	0.29 ± 0.01b	7.87 ± 0.46abc	112.11 ± 1.02h	58.50 ± 0.37g
T8 (paste)	3.20 ± 0.14bcde	1.19 ± 0.06bc	5.17 ± 0.01bc	1.54 ± 0.04c	0.32 ± 0.01bc	6.61 ± 0.58ab	153.86 ± 0.52i	63.49 ± 0.18h
T9 (paste)	3.64 ± 0.13ef	1.28 ± 0.05bcd	5.18 ± 0.02bc	1.75 ± 0.06def	0.39 ± 0.03efg	10.25 ± 0.96bcdef	95.79 ± 0.58e	55.33 ± 0.21e
T10 (paste)	3.31 ± 0.17de	1.24 ± 0.05bc	5.17 ± 0.02bc	1.91 ± 0.05fg	0.41 ± 0.01fg	13.07 ± 0.46efg	88.68 ± 1.03c	46.99 ± 0.38b
T11 (paste)	3.94 ± 0.24f	1.22 ± 0.07bc	5.18 ± 0.01bc	1.77 ± 0.04ef	0.38 ± 0.01defg	9.36 ± 0.66abcde	94.20 ± 1.26de	55.51 ± 0.13e
T12 (paste)	2.99 ± 0.16bcd	0.95 ± 0.03a	5.16 ± 0.01bc	1.77 ± 0.04ef	0.37 ± 0.01def	8.84 ± 0.64abcd	92.62 ± 0.41d	55.66 ± 0.15ef
T13 (paste)	3.42 ± 0.19def	1.16 ± 0.04bc	5.14 ± 0.01b	1.94 ± 0.08g	0.43 ± 0.01fg	13.52 ± 3.18fg	84.88 ± 0.87b	48.19 ± 0.34b
T14 (paste)	3.09 ± 0.19bcde	1.11 ± 0.04ab	5.18 ± 0.03bc	1.78 ± 0.04efg	0.39 ± 0.02efg	8.10 ± 1.00abc	93.90 ± 0.66de	57.11 ± 0.53fg

The results are expressed as the mean of three repetitions ± SD. Values represented with the same letters are not statistically different according to the Tukey test ($p<0.05$).

Antioxidants slow down the oxidation process in the body as well as in food. Redox equilibrium, which is necessary for normally functioning cells, is maintained by dietary antioxidants. This helps to minimize oxidative stress, which is caused when pro-oxidants (Reactive oxygen and nitrogen species, or ROS/RNS) outweigh antioxidants in favor of the former (Apak, 2019). Oxidative stress is associated with noncommunicable diseases such as cancer (Cömert & Gökmen, 2022; Martinelli et al., 2021). Cocoa beans contain flavonoids, one of the most beneficial subunits of polyphenols. Flavonoids are cardio-protective based on their antioxidant and antiplatelet activities, immune-regulatory effects, and their effects on endothelial cells (Sansone et al., 2015; Żyżelewicz et al., 2018). Cocoa intake increases serum antioxidant capacity, protecting the endothelium from oxidative stress and endogenous ROS (Etaware, 2021). All the bioactive compounds in the dry fermented cocoa beans were present in the pastes obtained from them, but the content of these compounds decreased. Pastes studied showed antioxidant activity as IC50 inhibition values expressed as a function of DPPH and ABTS free radicals. This antioxidant capacity was higher in dry grains, with 77.21 ± 0.65 and 35.04 ± 1.34 ug/mL, respectively. These antioxidant capacities were also higher in the paste obtained with the T13 treatment, with 84.88 ± 0.87 and 48.19 ± 0.34 ug/mL, respectively. In this regard, antioxidant capacity in a sample of cocoa liquor from Brazil was determined, with 7957 ± 589 (μmol TE/100 g dm) DPPH and phenol content of 2.85 g GAE/100g (Gil et al., 2021). In cocoa pastes from Perú from different geographical areas were evaluated, antioxidant capacities of 180.24 ± 3.83, 111.79 ± 2.36, and 165.53 ± 2.94 reported as IC_{50} DPPH (Quispe et al., 2020), lower antioxidant capacities than our results. In 31 samples of cocoa pastes capacity with DPPH and ABTS radicals was determined, and values of 233.85 ± 1.48 μM ET/g to 412.34 ± 2.26 μM ET/g, were found (Tolentino, Camasca, & Peláez, 2019).

To comprehend the impact of two independent variables or factors such as temperature (°C) and vacuum pressure (bar) conditions under which the roasting of cocoa beans was conducted, a three level 3^2 factorial response surface design of experiments was utilized. Phenols, flavonoids, anthocyanins, and antioxidant capacity as measured by DPPH and ABTS radicals were the dependent variables under investigation. The levels of the factors in the there-level factorial design are typically referred to as "low" and "high" levels and are, correspondingly, coded as (-1) and (+1). In this design the factors are 2, factor A which is the temperature (°C) and factor B which is the vacuum pressure (bar), parameters used in the cocoa bean roasting operation.

Figure 2 represents the response surface and contour graphs constructed to observe the effect of the factor's temperature (°C) and vacuum pressure (bar), on the contents of phenols (g GAE/100g), flavonoids (mg CE/100g), and anthocyanins (mg/100g) after the process of roasting of dry cocoa beans. Figure 2(a) shows a curved response surface and contour lines, where the phenol content increases as the vacuum pressure increases and the roasting temperature of the grains decreases. Figure 2(b) represents the curved response surface and the contour lines, where the flavonoid content increases as the vacuum pressure increases and the temperature decreases, similar behavior to the previous case. Figure 2(c) shows a flat response surface and the contour plot, showing that the anthocyanin content increases slightly at lower temperature and lower vacuum pressure, during the roasting process.

Figure 3 represents the response surface and contour graphs constructed to observe the effect of the factor's temperature (°C) and vacuum pressure (bar), in antioxidant capacity expressed as radicals DPPH (IC_{50} ug/mL), and ABTS (IC_{50} ug /mL) after the process of roasting of dry cocoa beans. When antioxidant capacity is expressed as IC50, lower values are better. Figure 3(a) shows a plan response surface and contour lines, where the antioxidant capacity increases when the vacuum pressure and temperature decrease. Figure 3(b) represents the curved response surface and the contour lines, it is shown that the antioxidant capacity increases when the vacuum pressure and temperature decrease, the effect of temperature being less significant.

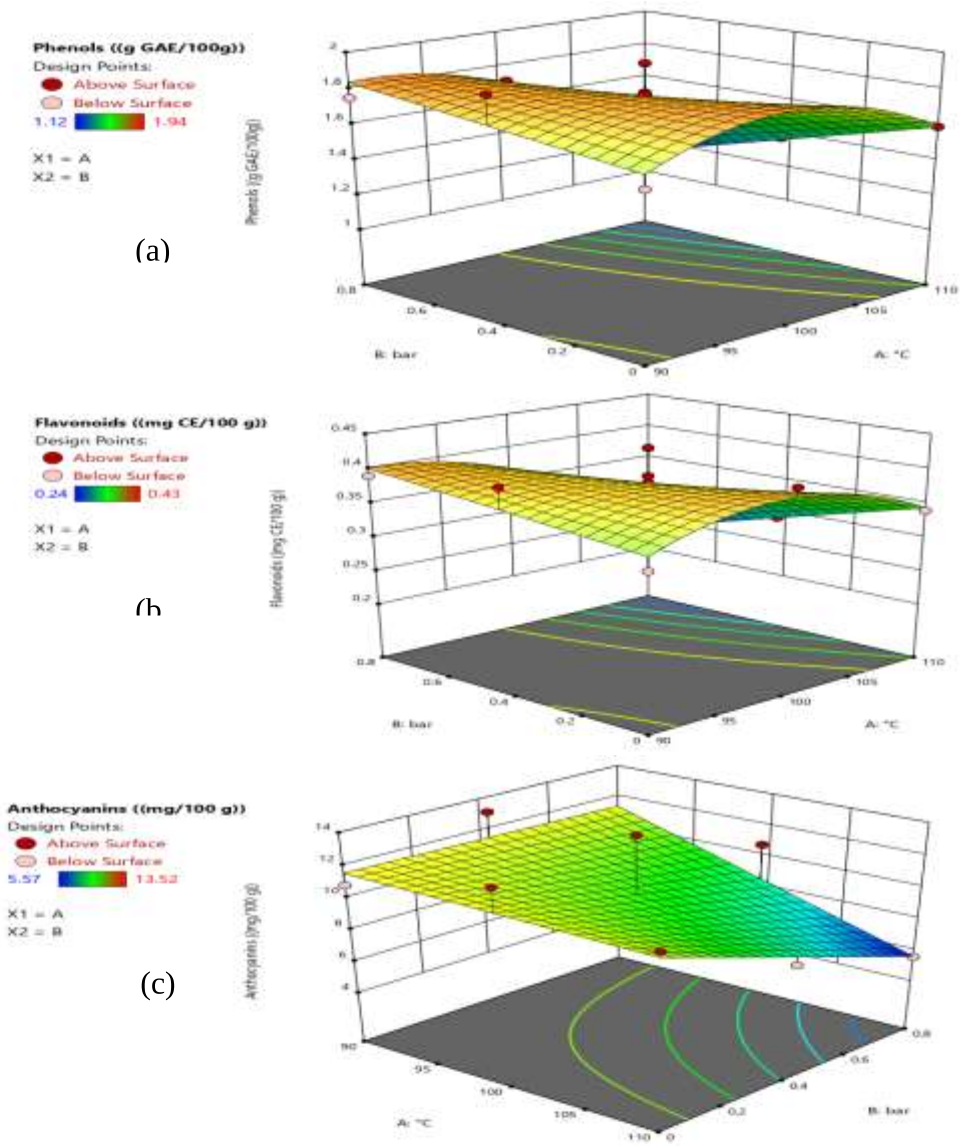

Figure 2 Response surface and contour plots showing the interactive effect on the content of phenols (g GAE/100g), flavonoids (mg CE/100g) and anthocyanins (mg/100g) in cocoa paste.

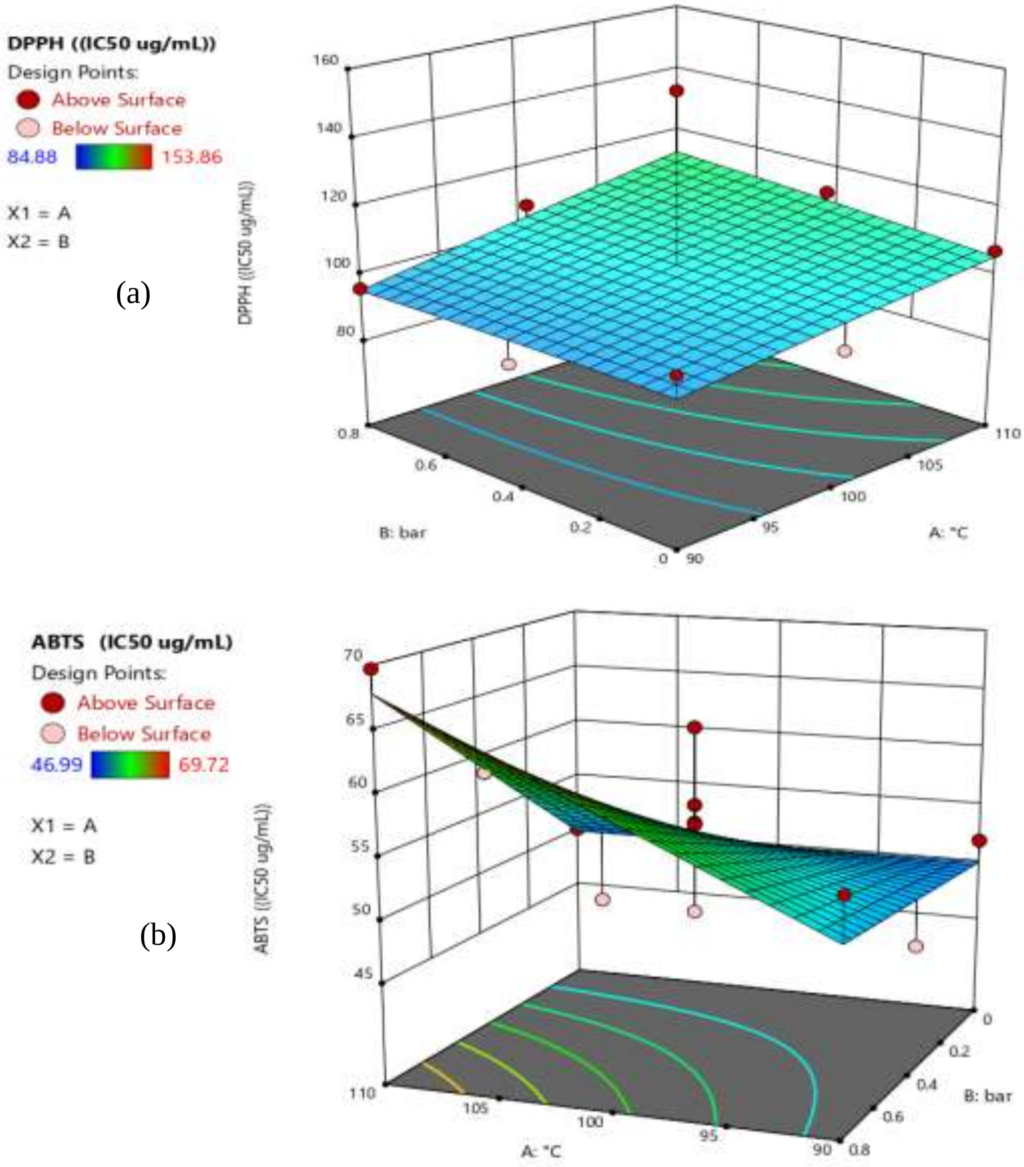

Figure 3 Response surface and contour plots showing the interactive effect in antioxidant capacity expressed as radicals DPPH (IC_{50} ug/mL), and ABTS (IC_{50} ug /mL) in cocoa paste.

The numerical optimization report contains two tables, the first (Table 6) summarizing the criteria constraints used to produce the second table of optimal solutions for the process. The goal is not to maximize the desirability value. The factor configurations that result in the highest desirability scores indicate that there is an island of acceptable outcomes. There may well be multiple islands (local optima) to explore.

Table 6 Constraints considered for factors A, B or independent variables and dependent variables.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Independent variables (factors)						
A:°C	is in range	90 (-1)	110 (+1)	1	1	3
B:bar	is in range	0 (-1)	0.8 (+1)	1	1	3
Dependent variables						
Phenols	maximize	1.12	1.94	1	1	3
Flavonoids	maximize	0.24	0.43	1	1	3
Anthocyanins	maximize	5.57	13.52	1	1	3
DPPH	minimize	84.88	153.86	1	1	3
ABTS	minimize	46.99	69.72	1	1	3

Table 6 shows the solutions proposed to the problem of optimizing the roasting of cocoa beans, under conditions of three temperatures and three vacuum pressures. We see that proposal number 1 presented the best results, considering the effect of temperature in °C, vacuum pressure in bars on the five response variables, phenols, flavonoids, anthocyanins, DPPH and ABTS. This proposal showed a high desirability with a value of 0.861.

Table 6 Numerical optimization report, showing optimal solutions for the roasting process.

Number	°C	bar	Phenols	Flavonoids	Anthocyanins	DPPH	ABTS	Desirability	
1	**90.000**	**0.800**	**1.836**	**0.401**	**11.356**	**95.356**	**51.673**	**0.816**	**Selected**
2	98.029	0.000	1.834	0.396	11.416	99.974	49.943	0.814	
3	98.105	0.000	1.834	0.396	11.414	100.014	49.943	0.814	
4	97.937	0.000	1.833	0.396	11.417	99.926	49.943	0.814	
5	98.130	0.000	1.834	0.396	11.414	100.027	49.943	0.814	
6	97.787	0.000	1.833	0.396	11.420	99.848	49.944	0.814	
7	98.439	0.000	1.835	0.397	11.407	100.186	49.941	0.814	
8	97.609	0.000	1.832	0.396	11.424	99.755	49.945	0.814	

9	98.562	0.000	1.836	0.397	11.405	100.250	49.941	0.814
10	98.654	0.000	1.836	0.397	11.403	100.297	49.941	0.814
11	101.500	0.000	1.824	0.395	11.347	101.725	49.929	0.803
12	90.000	0.540	1.780	0.387	11.427	95.406	51.121	0.796
13	90.008	0.460	1.763	0.383	11.447	95.428	50.955	0.789

Table 7 shows the coefficient magnitudes and significance for all analyzed responses. It is color coded by significance. Makes it easy to see what terms are common to all the response models. The independent variables phenols, flavonoids and ABTS had significant (**$p < 0.05$**) effects in the numerical optimization process and the anthocyanin and DPPH variables had no effect ($0.05 \leq p < 0.1$ $p \geq 0.1$).

Table 7 coefficient magnitudes and significance for all analyzed responses

Independent variables	Intercept	A	B	AB	A²
Phenols	1.76286	**-0.195**	-0.0716667	**-0.1575**	**-0.207857**
p-values		**0.0046**	0.1905	**0.0333**	**0.0160**
Flavonoids	0.38	**-0.045**	-0.0166667	**-0.0375**	**-0.045**
p-values		**0.0080**	0.2303	**0.0442**	**0.0330**
Anthocyanins	9.93	-1.535	-1.44667	-1.3375	
p-values		0.1104	0.1295	0.2394	
DPPH	102.861	6.98	1.88	1.9575	-0.448095
p-values		0.4297	0.8284	0.8537	0.9697
ABTS	54.8369	4.01333	**4.90167**	4.0525	
p-values		0.0722	**0.0346**	0.1275	

p-value shading: **$p < 0.05$** $0.05 \leq p < 0.1$ $p \geq 0.1$

Figure 4 represents the correlation between the response based on the principal component analysis (PCA), the figure that the components PC1 (91%) and PC2 (8%) explain 99% of the correlations between the response variables. The PCA analysis demonstrates that the antioxidant capacity of cocoa pastes, expressed as DPPH and ABTS radicals, depends on the content of phenols (PE), flavonols (FL), and anthocyanins (An). The moisture variables (Mo), titratable acidity (TA), and pH do not affect the antioxidant capacity of cocoa paste. The PCA analysis was used in the investigation of various works for the analysis of the antioxidant capacity of 31 samples of cocoa paste (Tolentino et al., 2019) and in the "Evaluation of the Physical and Sensory Characteristics of Cocoa Liquor Associated with Sowing Models" **(Moreno-Martínez, Gavanzo-Cárdenas, & Rangel-Silva, 2019)**.

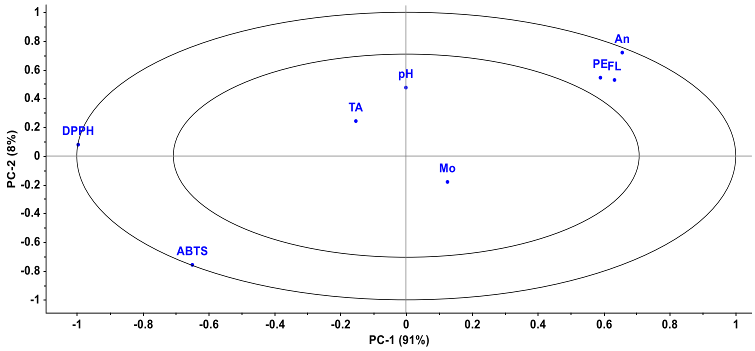

Figure 4 Correlation graph of the PCA, PC1 (91%), and PC2 (8%), which explain the relationship between the variables of antioxidant capacity (DPPH, ABTS), polyphenols (PE), flavonoids (FL) and anthocyanins (An).

Conclusion

Vacuum roasting of cocoa beans influenced the chemical-functional properties and antioxidant capacity of pastes. Temperatures of 90°C, 100°C, and the pressure of vacuum of 0.4 bar less affected the antioxidant capacities expressed as IC_{50} values with DPPH and ABTS radicals, treatment T13 (100°C and 0.4 bar) produced activities of 84.88 ± 0.87 ug/mL and 48.19 ± 0.34 ug/mL, respectively. The contents of phenols, flavonoids, and anthocyanins in dry fermented cocoa beans decreased after they were subjected to roasting and conching to obtain the corresponding cocoa pastes. The optimization analysis of the roasting process presented 13 optimal solutions, highlighting the treatment at 90 °C and 0.8 bar for its greater desirability 0.816. The contents of phenols, flavonoids, and anthocyanins influenced the antioxidant capacity of cocoa pastes. PC1 and PC2 explained this direct correlation in 99%, highlighting the influence of phenols, flavonoids, and anthocyanins and indicating that the variables humidity, titratable acidity, and pH did not influence the antioxidant capacity of cocoa paste. To investigate the effect of vacuum roasting of cocoa on the specific chemical components of the cocoa paste or liquor and on its antioxidant capacity.

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