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Effect of roasting on flavonoids, phenolics, and antioxidant activity of industrial-pulped and fermented cocoa beans

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ABSTRACT

The extent to which roasting of depulped cocoa beans at varied roasting intensities influences the total polyphenolic content, total flavonoid content, and DPPH scavenging capacity of cocoa liquor remains underexplored. This study investigated the effect of mechanical depulping and roasting intensities on these parameters in Ghanaian cocoa beans. A 3 × 3 full factorial design was employed, with depulping levels (0 %, 50 %, and 100 %) and roasting conditions (110 °C for 60 min, 120 °C for 30 min, and 135 °C for 10 min) as the principal factors. Cocoa beans were depulped mechanically, fermented for six days, dried at 55 °C to a moisture content of 7, 8 %, roasted, deshelled, and milled into cocoa liquor. The results showed that roasting intensity and mechanical depulping significantly influenced the studied parameters. Cocoa liquor obtained from mechanically depulped beans exhibited a higher total polyphenol content but lower flavonoid content and antioxidant capacity. Moderate roasting (120 °C for 30 min) and low-temperature, long-time (110 °C for 60 min) roasting processes preserved more flavonoids and antioxidant activity than high-temperature, short-time (135 °C for 10 min) roasting. These findings highlight the importance of optimizing depulping and roasting conditions to balance the retention of bioactive compounds and antioxidant properties in cocoa liquor.

1. Introduction

Cocoa beans (*Theobroma cacao* L.) are a vital agricultural commodity, prized for their role in chocolate production and their rich content of bioactive compounds, including polyphenols and flavonoids, which contribute to their antioxidant properties (Aprotosoaie et al., 2016; Afoakwa, 2010). However, fresh cocoa beans are characterized by an unpleasant astringency and bitterness, necessitating postharvest treatments such as fermentation, drying, and roasting to develop the desirable flavor and aroma profiles associated with chocolate (Waseem et al., 2024; Afoakwa et al., 2015, 2011). Among these treatments, pulp pre-conditioning, fermentation, and roasting are critical steps that influence the physicochemical and sensory properties of cocoa beans, including their polyphenolic and flavonoid content, as well as their antioxidant capacity (Suleman et al., 2025; Kongor et al., 2016; Afoakwa et al., 2013a).

Pulp pre-conditioning, which involves reducing the pulp content of cocoa beans prior to fermentation, has been shown to affect the biochemical processes during fermentation. Methods such as pod storage, mechanical depulping, and bean spreading are commonly employed (Kongor et al., 2016; Afoakwa et al., 2013a;). Mechanical depulping, in particular, has gained attention for its ability to modulate fermentation dynamics by reducing the pulp-to-bean ratio, thereby influencing the production of flavor precursors and the retention of bioactive compounds (Afoakwa et al., 2015b; Amanquah et al., 2013). However, the extent to which mechanical depulping affects the polyphenolic and flavonoid content of cocoa beans, especially in combination with varying roasting conditions, remains underexplored.

Roasting is another critical postharvest process that significantly impacts the chemical composition of cocoa beans. During roasting, the

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thermal degradation of polyphenols and the formation of Maillard reaction products influence the antioxidant capacity and flavor profile of cocoa (Akonor et al., 2023; Oracz & Nebesny, 2016; Arlorio et al., 2008). While high-temperature, short-time (HTST) roasting has been reported to better preserve polyphenolic content, low-temperature, long-time (LTLT) roasting often maximizes antioxidant capacity (Suleman et al., 2025; Ioannone et al., 2015; Suazo et al., 2014). These findings highlight the complex interplay between roasting conditions and the retention of bioactive compounds, which may vary depending on the degree of pulp removal prior to fermentation.

Despite the growing body of research on the effects of postharvest treatments on cocoa quality, the combined impact of mechanical depulping and roasting intensity on the polyphenolic content, flavonoid content, and antioxidant capacity of cocoa liquor derived from Ghanaian cocoa beans has not been fully elucidated. This study therefore aims to investigate the influence of mechanical depulping and varying roasting conditions on these parameters, with a focus on optimizing antioxidant capacity while balancing the retention of polyphenols and flavonoids. By addressing this gap, the findings of this study will contribute to a better understanding of the factors influencing cocoa quality and provide insights for improving postharvest processing techniques.

2. Materials and methods

2.1. Raw materials

The cocoa pods used in this study were uniformly ripe, with an average length of 20–25 cm, diameter of 10–15 cm, and a characteristic yellow-orange color. The pods were harvested from mixed hybrid varieties (Amelonado and Amazonica) grown in Obosomase, Eastern Region of Ghana. Traditional methods of harvesting cocoa were employed for the harvesting of uniformly ripe cocoa pods, under ambient temperature (28–30 °C) during the day. The harvested cocoa pods were moved instantly to a fermentary.

2.2. Experimental design

A 3 × 3 full factorial design was deployed for this research. The main factors to be studied were mechanically depulped beans (0:100, 50:50, 100:0) and roasting intensity (110 °C for 60 min, 120 °C for 30 min, 135 °C for 10 min). The polyphenol content, flavonoid content and antioxidant capacities of the cocoa liquor were then studied.

2.3. Sample preparation

The cocoa pods were transported immediately after harvest and upon arrival at the fermentary, the pods were broken. The cocoa beans were taken out of the pod by the aid of the hand. The cocoa beans that were assigned to depulping were depulped accordingly with the aid of a mesh. The cocoa beans were rubbed in the mesh to separate the pulp from the beans.

A 5 kg batch of cocoa beans, consisting of varying ratios of depulped and whole beans: 0:100 (0 % depulping), 50:50 (50 % depulping), and 100:0 (100 % depulping). Five kilograms of the varied ratios were independently fermented on and covered with banana leaves employing the heap fermentation technique. Fermentation proceeded with a regular interval (2 days) of turnings for six (6) days.

After fermentation, the cocoa beans were placed into sterile polythene bags and were taken to the lab for drying. The cocoa beans were placed on drying trays, well-spaced, and oven-dried at 55 °C for 4 days to achieve 7–8 % moisture content using an air oven (Universal Oven UF75, Memmert GmbH + Co. KG, Germany). The dried beans were bagged and sealed airtight and stored in a dark room free from strong odour at ambient temperatures (25–28 °C) until ready for use.

About 300*g* of sampled cocoa beans were placed on drying trays, well-spaced and were roasted in an air oven at temperatures 110 °C, 120 °C and 135 °C for 60, 30 and 10 min, respectively. The temperature of the oven for each of the roasting treatments under study was adjusted at that specified temperature of roasting for not <30 min to maintain a state of equilibrium.

The beans were moved onto another tray and were permitted to cool to ambient temperature after roasting. The cooled roasted cocoa beans were then packed in airtight black bags and were labelled with respect to the degree of depulping and roasting conditions. The samples were kept at room temperature in an odour-free dim room until ready for use. This protocol was repeated for all the various depulping rates.

Deshelling of the roasted cocoa beans was done manually with the aid of a knife and the nibs were milled into a fine liquor of a specified particle size using Aura 2 in 1 Blender (Russell Hobbs, China). The resultant cocoa liquor was stored in a well-labelled black airtight polythene sample bag until ready for use.

2.4. Analytical methods

2.4.1. Polyphenol extraction

The phenols were obtained by extracting thrice with 80 % acetone in water as described by Natsume et al. (2000). Acetone was selected for its superior solubility of polyphenols in cocoa matrices, though ethanol or methanol could also be suitable alternatives (Maldonado & Figueroa, 2023; Osei Tutu et al., 2023). A 5*g* of the cocoa liquor sample was weighed into the centrifugation tube after adding 25 ml of acetone to it, the solution was then vortexed and centrifuged at 1500 × g for 10 min. The liquid portion was carefully and slowly decanted into a 50 mL volumetric flask. This protocol for the extraction was repeated twice. The liquid from the second and third extraction was decanted into the same 50 mL volumetric flask.

2.4.2. Quantification of phenolic content

The total polyphenol content was determined using the Folin–Ciocalteu method outlined by Singleton and Rossi (1965). The measurement of the absorbance of the solution was spectrophotometrically determined at 750 nm using Gallic acid as the reference sample.

A 0.5 mL 10 % Folin Ciocalteu reagent was added to 0.1 mL of the extract. The mixture was then permitted to be in a steady position for 5 min, after which 0.5 mL 20 % Sodium bicarbonate (Na_2CO_3) was added to it. The volume was adjusted to 5 mL by adding more deionized water. Incubation of the mixture at 45 °C for 30 min in a water bath was done after mixing the mixture for 10 ss. Absorbance was then read at 750 nm using a spectrophotometer (UV-1800 240 V IVDD, Shimadzu USA Manufacturing Inc., USA).

The total polyphenolic content was reported in milligrams of gallic acid equivalents per gram of the sample. Linear standard curves were derived for the standard solution in the concentration range of 5–200mgL^{-1}. While the Folin-Ciocalteu method provides a reliable estimate of total polyphenol content, its lack of specificity for phenolic compounds necessitates complementary techniques for a detailed profile. High-Performance Liquid Chromatography (HPLC) is recommended for future studies to identify individual polyphenols and understand their specific contributions to antioxidant capacity.

2.4.3. Quantification of total flavonoids

Quantification of the total flavonoid content of the sample was done by employing the aluminium chloride calorimetric assay as outlined by Atanassova et al. (2011). Prior to the transfer of 0.3 ml 5 % $NaNO_2$, one (1) millilitre of the extract aliquot or catechin standard solution (20, 40, 60, 80 and 100 mg/l) was transferred into a 10 ml volumetric flask holding 4 ml of deionized water. 0.3 ml 10 % $AlCl_3$ was transferred into the flask holding the mixture after five (5) min. Prior to topping up the volume to 10 ml with deionized water, an addition of two (2) millilitres of 2 ml 1 M NaOH to the mixture was done on the 6th min. After thoroughly mixing the solution, the absorbance was read spectrophotometrically (UV-1800 240 V IVDD, Shimadzu USA Manufacturing Inc.,

USA) at 510nm. The total flavonoid content of the samples was reported as mg catechin (CE)/ 100 g cocoa liquor. The analysis of the samples was done in triplicate.

2.4.4. Antioxidant capacity (Free radical scavenging activity) using DPPH assay

A slightly modified protocol as outlined by Brand-Williams et al. (1995) was employed for the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. While effective, the DPPH method predominantly measures lipophilic antioxidant activity, potentially underestimating the contribution of hydrophilic compounds (Yamauchi et al., 2024; Akonor et al., 2022). Complementary assays like ABTS or ORAC are recommended for a comprehensive evaluation. A 3 mL aliquot of the extract or ascorbic acid (standard) was measured and mixed with 1 mL of Tris–HCl buffer. 1 mL of 0.1 mM DPPH/methanol mixture was then added and mixed thoroughly. The mixture was allowed to be in a steady position in the dark at ambient temperature for 20 min. Reading of the Absorbance was done at 517 nm with the aid of a spectrophotometer (UV-1800 240 V IVDD, Shimadzu USA Manufacturing Inc., USA). With a 5 to 20 mgL^{-1} concentration range of the ascorbic acid as the standard, a linear standard curve was derived.

2.5. Statistical analysis

Analysis of variance (ANOVA) of the data was done with the aid of Statgraphic Centurion version 15.2.14 (STSC, Inc., Rockville, MD, USA). With significance level, pegged at 95 % differences between factor levels was assessed using Fisher's least significant difference (LSD). General linear model procedures were conducted using Statgraphic Centurion version 15.2.14 (STSC, Inc., Rockville, MD, USA) to assess the combined influence of mechanically depulped beans and roasting intensities (temperature/ time) on the studied parameters. Models were formed to relate mechanical depulping and roasting conditions on the total flavonoid content, total polyphenol content and antioxidant capacities (scavenging capacity).

3. Results and discussion

3.1. Chemical composition of dried fermented cocoa beans

The initial composition of the cocoa samples, including total polyphenol content, flavonoid content, moisture, DPPH scavenging activity was analyzed prior to roasting. Table 1 summarizes the initial values for these parameters, which serve as a baseline for interpreting changes during roasting. The total phenolic content increased with higher depulping rates, from 288.02 ± 0.27 mg GAE at 0 % to 320.25 ± 0.34 mg GAE at 100 %. A similar trend was observed for total flavonoid content, rising from 2043.21 ± 0.74 mg CE to 2224.81 ± 0.45 mg CE. This increase suggests that reduced pulp enhances the extraction or retention of these bioactive compounds, possibly due to improved oxygen penetration and enzymatic activity during fermentation. The moisture content exhibited a slight increase with higher depulping, from 6.89 ± 0.47 % at 0 % to 7.21 ± 0.19 % at 100 %. This could result from changes in the interaction between the beans and the pulp, affecting water absorption during drying. Conversely, DPPH scavenging activity declined with increasing depulping rates, from 53.25 ± 0.45 mg AAE g^{-1} at 0 % to 47.05 ± 0.34 mg AAE g^{-1} at 100 %.

Table 1
Chemical characteristics of dried fermented cocoa beans of varying depulping rate.

Depulping Rate	Chemical Characteristics			
	Total Phenolic Content (mg GAE)	Total Flavonoid Content (mg CE)	Moisture Content (%)	DPPH Scavenging Activity (mg AAE g^{-1})
0 %	288.02 ± 0.27[a]	2043.21 ± 0.74[a]	6.89 ± 0.47[a]	53.25 ± 0.45[a]
50 %	304.23 ± 0.53[b]	2132 ± 0.34[b]	7.11 ± 0.21[b]	50.15 ± 0.53[b]
100 %	320.25 ± 0.34[c]	2224.81 ± 0.45[c]	7.21 ± 0.19[c]	47.05 ± 0.34[c]

Different letters (a, b, c) indicate significant difference between the depulping rates for each parameter.

Despite the elevated phenolic and flavonoid content, the reduced antioxidant activity suggests the degradation or loss of specific bioactive compounds during processing or potential antagonistic interactions. These initial values were consistent with those reported by Gültekin-Ozgüven et al. (2016) and Oracz and Nebesny (2016).

3.2. Total phenolic compounds

The total polyphenolic contents of the samples were observed to be in the range of 259.35 mg GAE g^{-1} of cocoa liquor to 328.73 mg GAE g^{-1} of cocoa liquor (Fig. 1). Roasting intensity of the cocoa beans had a significant impact ($p < 0.05$) on the total polyphenolic content. The cocoa liquor samples derived from the cocoa beans roasted at 120 °C for 30 min irrespective of the depulping rate were found to contain the highest polyphenolic content (Fig. 1). The polyphenolic content of the cocoa liquor samples obtained from the cocoa beans roasted at 110 °C for 60 min was not statistically different (p > 0.05) from the total polyphenols contents of the samples obtained from the beans roasted at 135 °C for 10 min albeit, the total phenolic content of those roasted at 110 °C for 60 min was slightly higher than the total phenolic content of those roasted at 135 °C for 10 min.

$$\text{Total phenolic content} = 288.27 - 7.28633a + 17.7043b + 0.320003c \quad (1)$$

where:

a = 1 if Roasting intensity=110 °C for 60 min, −1 if Roasting intensity=135 °C for 10 min, 0 otherwise

b = 1 if Roasting intensity=120 °C for 30 min, −1 if Roasting intensity=135 °C for 10 min, 0 otherwise

c= depulping rate

Eq. (1) was derived using a general linear model (GLM) analysis to predict total phenolic content based on roasting intensity and depulping rates. The coefficients were calculated based on experimental data, with roasting intensity and depulping treated as categorical and continuous variables, respectively. Eq. (1) indicated that at 95 % confidence interval, a unit increase in depulping rate resulted in an increase of 0.320003 mg GAE in the total phenolic content of the cocoa liquor sample if all other terms in the equation are held constant. The total polyphenolic content of all the samples were statistically significantly different ($p < 0.05$) except those derived from 100 % depulped beans and roasted at 135 °C for 10 min and 50 % depulped beans roasted at 110 °C for 60 min.

The values are similar to the findings of Oliveira et al. (2011) for roasted nibs of organically cultivated cocoa (301.43 ± 0.55 mg/g) and conventionally cultivated cocoa (289.43 ± 0.75 mg/g). The values were also in accordance with the findings of Ortega et al. (2008) (302.5 mg/g) who characterized polyphenols in varied cocoa samples. However, the values of the total polyphenolic content reported in this work were higher than the values reported for roasted cocoa beans by Oracz and Nebesny (2016). The total phenolic content may be overestimated which could be due to the interaction of the Folin-Ciocalteu reagent with the non-polyphenolic compounds such as irons, manganese and proteins present in the sample (Acheampong et al., 2024; Osei Tutu et al. 2024; Osei Tutu, et al., 2019; Georgé et al., 2005). This might have contributed to the high values observed in this work as compared with those reported in other studies.

Generally, roasting of cocoa beans results in the reduction of polyphenols. However, higher temperature roasting, according to Oracz

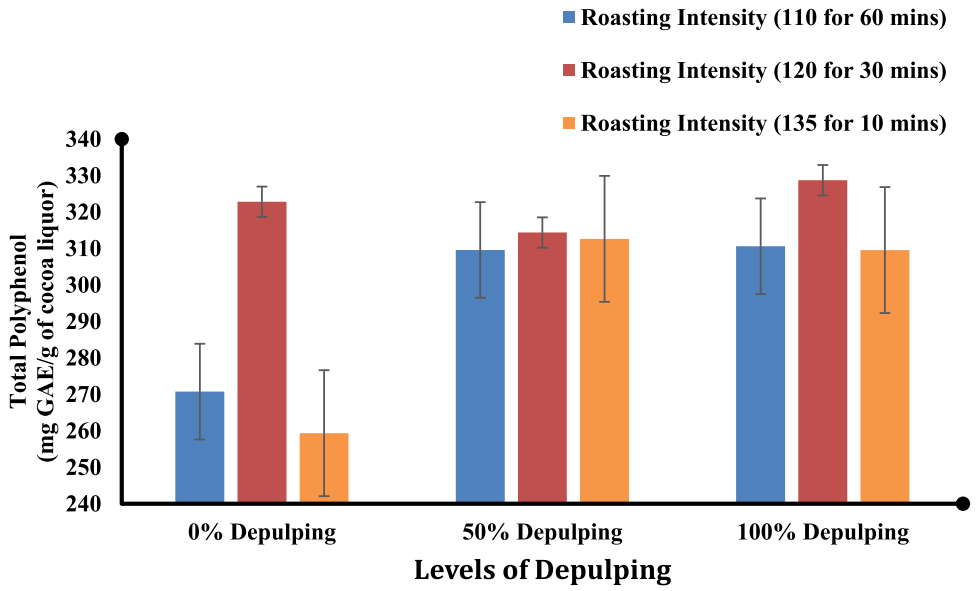

Fig. 1. Effect of depulping and roasting intensities on the total phenolic content of cocoa liquor.

et al. (2015) results in conversion of (-)-epicatechin to (+)-catechin in an epimerization reaction. This might have accounted for the no significant difference between the total phenolic contents of the samples derived from the cocoa beans roasted at 110 °C for 60 min and 135 °C for 10 min. Afoakwa et al. (2015b) and Oracz et al. (2015) indicated that increasing the temperature and/or time of the roasting process result in faster breakdown of the phenolic content of cocoa beans, accounting for less polyphenol residues in the cocoa beans. Afoakwa et al. (2015b) explained that the intense redox activity of the phenolic compounds results in their rapid oxidation to form diphenols and then quinone when they come into contact with high temperature and oxygen. Consequently, the quinones polymerizes with molecules such as proteins, amino acids, sugars and other polyphenols.

Contrary to the indistinct trend of the total polyphenol content of fermented cocoa beans with respect to depulping from samples containing 0 % depulped beans (22.23 mg /g) 50 % depulped beans (19.9 mg/ g) and 100 % depulped beans (21.47 mg/ g) observed by Amanquah et al. (2013), an increasing significant effect ($p < 0.05$) of depulping on the total polyphenolic content of the samples was observed as indicated in Eq. (1). Higher depulping levels above 50 % could lead to under-fermentation as a result of insufficient pulp to cause temperature rise and /or produce the required amounts of ethanol to ensure killing of the beans, resulting in less breakdown of the polyphenolic content, which could have resulted in the high polyphenol content of the depulped samples (Amanquah et al., 2013).

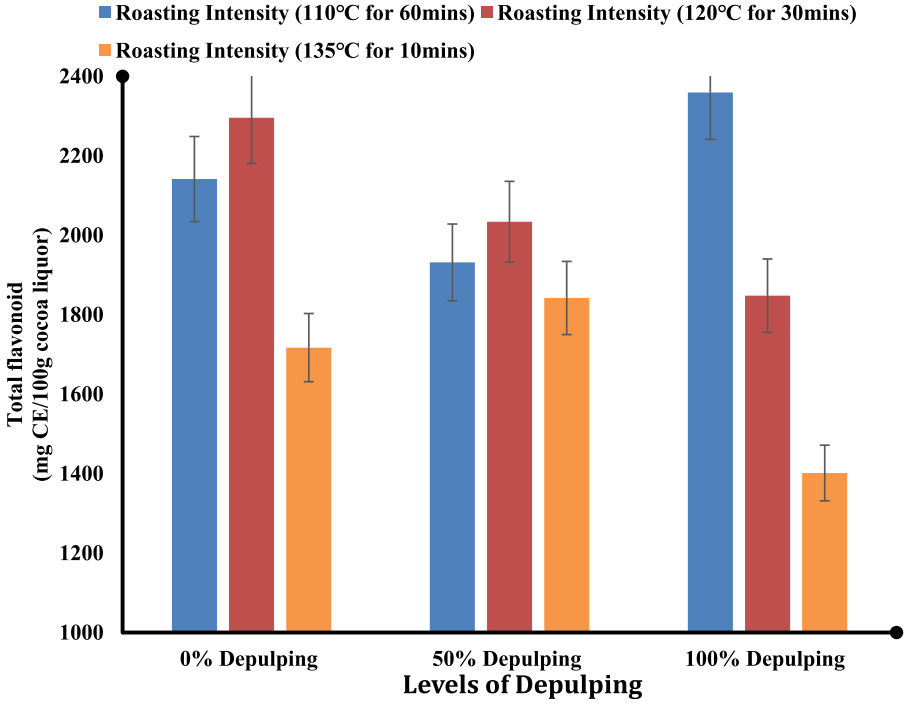

Fig. 2. Effect of depulping and roasting intensities on the flavonoid content of cocoa liquor.

3.3. Total flavonoid content

Although the samples containing 100 % depulped beans, roasted at 135 °C for 10 min recorded the lowest flavonoid content, roasting intensity and depulping had varying effects on the total flavonoid content of the nine cocoa liquor samples (Fig. 2). With the exception of the samples containing 100 % depulped beans, samples roasted at 120 °C for 30 min recorded the highest flavonoid among the samples of the same depulping rate. Samples roasted at 135 °C for 10 min recorded the lowest total flavonoid content at the same depulping rate.

At 95 % confidence interval, the total flavonoid content of all the nine samples were statistically significantly different from each other. The analysis of variance (ANOVA) revealed that all the predictors studied (roasting intensity and depulping) significantly influenced ($p < 0.05$) the total flavonoid content of the cocoa liquor samples.

$$\text{Total Flavonoid Content} = 2043.21 + 191.863a + 106.872b - 1.81872c \quad (2)$$

Where:

a = 1 if Roasting intensity=110 °C for 60 min, −1 if Roasting intensity=135 °C for 10 min, 0 otherwise

b = 1 if Roasting intensity=120 °C for 30 min, −1 if Roasting intensity=135 °C for 10 min, 0 otherwise

c = depulping rate

The equation of the general linear model (GLM) (Eq. (2)) indicated that at 95 % confidence interval, a unit increase in depulping rate resulted in a reduction of 1.81872 mg CE/g in the total flavonoid content of the cocoa liquor sample if all other terms in the equation are held constant. However, as indicated earlier in Eq. (1), a unit increase in depulping rate resulted in an increase of 0.320003 mg GAE in the total phenolic content of the cocoa liquor sample. Vazquez-Araujo et al. (2019) explained that flavonoids, including flavan-3-ols are lost when the cocoa beans are depulped due to oxidative processes causing the reduction in total flavonoid content, however, phenolic acids and non-flavonoid phenolics might be more stable or less affected by depulping. Additionally, the exposure of the bean kernel to air and processing conditions after depulping could lead to the formation or concentration of certain phenolic compounds causing an increase in the total phenolic content.

The multiple comparison test revealed that the total flavonoid contents of the cocoa liquor samples derived from the beans roasted at 110 °C for 60 min and 120 °C for 30 min were not significantly different (p > 0.05) from each other. The total flavonoid content of the cocoa liquor samples roasted at 110 °C for 60 min and 120 °C for 30 min were however, significantly higher ($p < 0.05$) than the total flavonoid content of the samples derived from the beans roasted at 135 °C for 10 min.

Flavonoids, according to Gültekin-Ozgüven et al. (2016) constitute about 37 % of the total polyphenols in raw cocoa beans. The high antioxidant capacities of cocoa beans and cocoa products are attributed to the elevated levels of flavan-3-ols, specifically (-)-epicatechin and (+)-catechin, as well as procyanidins (Oracz & Nebesny, 2016). The low total flavonoid values recorded for the samples roasted at 135 °C for 10 mins could be attributed to the combined effect of high temperature and prolonged exposure, leading to degradation of polyphenolic and flavonoid content (Akonor et al., 2023; Afoakwa et al., 2015; Oracz et al., 2015).

From Eq. (2), mechanical depulping had a decreasing effect on the total flavonoid content of the samples. This could be ascribed to the cellular destruction during depulping which exposed the flavonoids in the cocoa beans to atmospheric oxygen which might have increased the susceptibility of the flavonoid content to oxidation and /or increase the leaching of the flavonoid content of beans into fermentation sweatings during fermentation.

From the multiple comparison test, the LTLT and moderate roasting intensities employed in this work had less degradative effect on the flavonoid content of the beans as compared to the HTST employed. This implies that the exposure of the samples to high roasting temperature of 135 °C might have resulted in much degradation of the polyphenolic content and by extension the flavonoid content of the cocoa beans (Osei Tutu et al., 2024; Afoakwa et al., 2015; Oracz et al., 2015). Thermal gradients during roasting may result in uneven heat distribution, leading to over-roasted and raw sections within the cocoa beans, especially at higher temperatures (Rojas et al., 2022). This observation aligns with findings by Suazo et al. (2014) and Rojas et al. (2022), which emphasize the importance of uniform temperature control to minimize quality variations.

3.4. Antioxidant capacity (DPPH scavenging activity)

Generally, the cocoa liquor samples derived from the beans roasted at 135 °C for 10 min irrespective of the depulping rate of the cocoa beans recorded the lowest scavenging activity (Fig. 3). With the exception of the samples containing 100 % depulped cocoa beans, all the samples roasted at 120 °C for 30 min recorded the highest antioxidant capacity with regards to their respective depulping rate (Fig. 3). For the samples derived from the beans roasted at 120 °C for 30 min, a decreasing trend was observed as depulping rate increases; from 60.70 mg AAE g^{-1} for samples containing 0 % depulped beans to 46.23 mg AAE g^{-1} for samples containing 100 % depulped cocoa beans (Fig. 3).

The cocoa liquor samples obtained from the cocoa beans roasted at 110 °C for 60 min had a varying trend with respect to their depulping rate, in that, cocoa liquor containing 50 % depulped cocoa beans recorded the lowest antioxidant capacity among the three depulping treatments, with highest antioxidant capacity observed in the samples containing 100 % depulped cocoa beans. The antioxidant capacity of the nine samples were observed to be statistically significantly different from each other ($p < 0.05$).

A multiple range test revealed that with the exception of the cocoa liquor samples derived from 0 % cocoa beans roasted at 110 °C for 60 min and 100 % cocoa beans roasted at 110 °C for 60 min, all the other samples were found to be statistically significantly different ($p < 0.05$) with respect to their antioxidant capacities.

$$\text{DPPH Scavenging Activity} = 53.2466 + 0.969296a + 4.08707b - 0.0620478c. \quad (3)$$

Where:

a = 1 if Roasting intensity=110 °C for 60 min, −1 if Roasting intensity=135 °C for 10 min, 0 otherwise

b = 1 if Roasting intensity=120 °C for 30 min, −1 if Roasting intensity=135 °C for 10 min, 0 otherwise

c = depulping rate

The analysis of variance (ANOVA) indicated that roasting intensity and depulping had a significant effect ($p < 0.05$) on the antioxidant capacity of the cocoa liquor samples. At 95 % confidence interval, the equation of the general linear model (GLM) depicted that increasing the depulping rate by one percent causes a reduction of 0.062 mg AAE g^{-1} in the antioxidant capacity of the cocoa liquor sample, when all other terms in the equation are held constant.

The compounds with antioxidant/scavenging capacity in the extracts of the samples interact with DPPH which is a known stable radical. The potential antioxidant capacity of the extract of the sample is reflected in the intensity of discoloration (Oliveira et al., 2011; Afoakwa et al., 2008; Ramli et al., 2001). The low antioxidant values reported for the samples roasted at 135 °C for 10 min as indicated in Fig. 3 could be attributed to the combined effect of high temperature and prolonged exposure of the samples to high roasting temperature of 135 °C for 10 mins which might have resulted in much degradation of the polyphenolic content and by extension the flavonoid content of the cocoa beans (Afoakwa et al., 2015; Oracz et al., 2015; Afoakwa and Paterson, 2010). With the exception of the samples containing 100 % depulped cocoa beans, all the

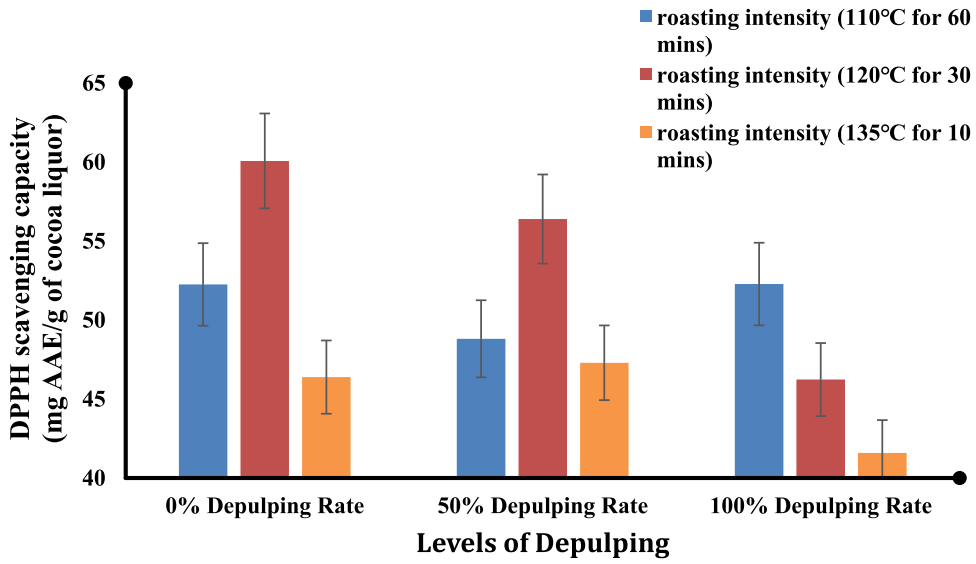

Fig. 3. Effect of depulping and roasting intensities on the antioxidant capacity (DPPH scavenging activity) of cocoa liquor samples.

samples roasted at 120 °C for 30 min recorded the highest antioxidant capacity with regards to their respective depulping rate. This could be ascribed to the earlier findings that indicated that moderate roasting intensity generally had a less degradative effect on the flavonoid content than HTST and LTST employed in this work.

The reduction in the total flavonoid content of the samples as depulping levels increased as indicated earlier might have contributed to the reduction in the DPPH scavenging capacity of the samples. In as much as the flavonoid content of cocoa beans have been widely documented to be the key chemical constituents of cocoa beans responsible for the antioxidant capacity of cocoa beans and cocoa products (Andújar et al., 2012; Oracz & Nebesny, 2016; Othman et al., 2007; Wollgast & Anklam, 2000), the decreasing effect of depulping on the flavonoid content could therefore be responsible for the decline in the antioxidant capacity (scavenging activities) of the cocoa liquor samples.

The decreasing impact of the HTST roasting intensity on the antioxidant capacities of the cocoa liquor samples irrespective of the depulping rate indicated that high temperature short time (HTST) roasting (135 °C for 10 min) resulted in the lowest antioxidant capacity as compared to low temperature long time (LTLT) (110 °C for 60 min). However, roasting at a moderate temperature for moderate duration resulted in the highest antioxidant capacity (Fig. 2). This findings is in accordance with the findings of Ioannone et al. (2015) who observed that although HTST roasting processing maintained the polyphenolic content better than LTLT, LTLT roasting processing generally maximized the antioxidant capacity of the cocoa beans. Conversely, findings of Suazo et al. (2014) indicated that roasting at 130 °C and 150 °C, irrespective of the duration of the roasting process (45, 60, 90 min) employed caused an increase in the antioxidant activity. Suazo et al. (2014) explained that the concentration of polyphenols measured by Folin-Ciocalteu technique does not always have a close relationship with the antioxidant capacity determined with the DPPH assay and therefore the antioxidant assay with respect to the DPPH scavenging assay may be due to other components of the cocoa bean other than the polyphenolic content.

The regression analysis revealed that the association of the concentration of total phenolic contents of the extracts of the samples and the capability to scavenge free radicals was statistically insignificant ($p > 0.05$). Similar observations were made by Afoakwa et al. (2015b), Othman et al. (2007), Suazo et al. (2014) and Leite et al. (2013). Othman et al. (2007) stated that the concentration of reducing substances, such as polyphenols determined with the Folin-Ciocalteu technique, does not always have a close relationship with antioxidant capacity measured with DPPH techniques and that other compounds such as the methylxanthines may contribute to DPPH scavenging capacity of the cocoa liquors. Leite et al. (2013) observed that the extract with the highest total polyphenol content had the second strongest DPPH scavenging activity.

Leite et al. (2013) explained that this phenomenon could be due to the interactions of the flavonoids with each other which might have decreased or increased the scavenging activity and possibly the elevated concentrations of polyphenols. Conversely, Gültekin-Ozgüven et al. (2016) reported a high regression coefficient ($R^2 = 0.7010$) between phenolic extract of cocoa and the scavenging capacity of DPPH by cocoa polyphenols. This report was in agreement with the findings of Arlorio et al. (2008) who reported a regression coefficient of $R^2 = 0.75$ between the polyphenolic extract and the scavenging capacity of DPPH by cocoa polyphenolic extract. Redovniković et al. (2009) also found a very strong regression coefficient ($R^2 = 0.9868$) between the polyphenolic content and the antioxidant capacity (DPPH scavenging capacity) of cocoa liquor samples.

A regression analysis (Table 2) indicated that the linear factor of total flavonoid content had a significant impact on the antioxidant capacity (DPPH scavenging capacity) of the cocoa liquor samples, with a moderately high regression coefficient ($R^2 = 72.4716$ %). This implies that although other factors could have a positive impact on the antioxidant capacity (DPPH scavenging capacity) of the cocoa liquor, the total flavonoid content of the cocoa liquor alone could explain 72.4716 % of

Table 2
Regression and correlation analyses between total polyphenolic content, total flavonoid content and DPPH scavenging capacity of cocoa beans.

Parameter	Analysis	Total Polyphenolic Content	Total Flavonoid Content	DPPH Scavenging Capacity
Total Polyphenolic Content	Regression	1.00	0.0182	0.0309
	Correlation	1.00	0.1348	0.1759
Total Flavonoid Content	Regression	0.0182	1.00	0.7247*
	Correlation	0.1348	1.00	0.8513*
DPPH Scavenging Capacity	Regression	0.0309	0.7247*	1.00
	Correlation	0.1759	0.8513*	1.00

* Significant at $P < 0.05$.

the variability in the antioxidant capacity (DPPH scavenging capacity) of the cocoa liquor, suggesting a high relationship between the total flavonoid content and the antioxidant capacity (DPPH scavenging capacity) of the cocoa liquor samples. Findings of Leite et al. (2013) indicated that antioxidant capacity showed a strong relationship with the monomeric phenolic contents (flavonoids) other than the total polyphenolic content of cocoa liquor and chocolate. The findings of this work therefore indicate a high correlation between antioxidant capacity (DPPH scavenging capacity) and flavonoids other than the polyphenols present in cocoa samples; observations in accordance with the findings of Fonseca et al. (2011) and Leite et al. (2013). This observation might be attributed to the heterogeneous nature of phenolics, where not all phenolics exhibit potent antioxidant activity due to differences in the number and position of hydroxyl groups. In contrast, the significant correlation between total flavonoids content and scavenging ability highlights the key role of flavonoids as powerful antioxidants with more uniform and predictable activity due to their specific chemical structures (Hassanpour & Doroudi, 2023).

4. Conclusion

The findings of this work indicated that mechanical depulping and roasting intensity had significant influences on the total polyphenolic contents, total flavonoid contents and the antioxidant capacity (DPPH scavenging capacity) of the cocoa liquor samples. Increasing depulping levels had significant decreasing effects on the total flavonoid contents and antioxidant capacity (DPPH scavenging capacity) of the samples. Contrarily, increasing depulping levels caused increasing effects on the total polyphenolic contents of cocoa liquor samples. The moderate (120 °C for 30 min) and LTLT (110 °C for 60 min) roasting processes employed caused only minimal changes in the total flavonoid contents. The moderate roasting intensity had the highest mean antioxidant capacity, followed by the samples containing the beans processed using the LTLT roasting intensity. Similar observation was made for the total polyphenolic residuals in the cocoa liquor samples.

Data availability statement

The data associated with this study are included in article/supp. material/referenced in article.

CRediT authorship contribution statement

Bernard Kwabena Asiedu: Writing – original draft, Resources, Methodology, Investigation, Data curation, Conceptualization. **Emmanuel Ohene Afoakwa:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Crossby Osei Tutu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Investigation, Formal analysis. **Rexford Obeng:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Data curation. **Nii Korley Kortei:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis. **Papa Toah Akonor:** Writing – review & editing, Visualization, Validation, Investigation, Formal analysis. **Agnes Simpson Budu:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Conceptualization. **Firibu Kwesi Saalia:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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