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EFFECTS OF MATURITY STAGES AND FERMENTATION OF COCOA BEANS ON TOTAL PHENOLIC CONTENTS AND ANTIOXIDANT CAPACITIES IN RAW COCOA POWDER

Dang Thi Kim Yen, Nguyen Vu Hong Ha✉

International University, Vietnam National University Ho Chi Minh City

✉ To whom correspondence should be addressed. E-mail: nvhha@hcmiu.edu.vn

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SUMMARY

Consumption of cocoa (*Theobroma cacao* L.) and cocoa products is associated with numerous health benefits due to their high levels of polyphenols and antioxidant capacities. In this study, changes of total phenolic contents (TPC) and antioxidant capacities (AC) of raw cocoa powder at four maturity stages, under different fermentation methods and fermentation duration of cocoa beans were investigated. The TPC and AC were measured using Folin–Ciocalteu and Trolox equivalent antioxidant capacity (TEAC)/ABTS assay, respectively. In term of maturity stages, the powder of unfermented cocoa beans harvested from the stage one contained significantly lower levels of TPC (6.39 ± 0.02 g CE/100 g DM) and AC (26.82 ± 0.13 mol TE/100 g DM) than those from the beans harvested from the stage two, three and four. For all maturity stages studied, fermenting cocoa beans increased antioxidant capacities of the raw cocoa powder. Cocoa fermentation using the commercial enzyme Pectinex® Ultra SP-L resulted in lower TPC but higher AC in comparison to those treated without enzymes, however prolonged fermentation time in cocoa beans significantly reduced TPC and AC in the powder. Strong correlations between TPC and AC of fermented samples with (R = 0.923) and without enzyme supplement (R = 0.942) were obtained. Two-way anova analyses showed that changes of TPC and AC of cocoa beans were dependent on maturity stages, fermentation methods and fermentation duration. As a conclusion, fermentation of cocoa beans harvested at the maturity stage two and three was found to be optimum for the high levels of AC and TPC attainment; fermented beans with commercial enzyme could be utilized to reduce labor cost by shortening the fermentation duration.

Keywords: *antioxidant capacities, cocoa beans, maturity, fermentation, raw cocoa powder, total phenolic contents*

INTRODUCTION

Cocoa beans and cocoa products have attracted increasing attention due to their notable polyphenol contents compared to other polyphenol-rich foods such as tea, red wine, acai, blue berry and cranberry (Lee *et al.*, 2003; Cooper *et al.*, 2008; Crozier *et al.*, 2011). It has been reported that many factors such as cultivars (Kim, Shin, 2015), growing regions (Andre *et al*., 2009), fermentation (Cho *et al*., 2011) and drying (Shofian *et al*., 2011) influence on antioxidant capacities and total phenolic contents of plant foods, particularly maturity stages (Tlili et al., 2011). The highest antioxidant capacities were obtained at the late ripening stages of blackberries, pepper fruits and watermelons (Navarro *et al*., 2006; Acosta-Montoya *et al*., 2010; Tlili *et al*., 2011) or insignificantly different during fruit development and ripening of sea buckthorns, apples, and pomegranate fruits (Gao *et al.*, 2000; Duda-Chodak *et al*., 2011; Fawole, Opara, 2013). However, for cocoa fruits, there has been no study reporting about the optimal stage of maturity for harvesting the fruit in term of antioxidant capacities. Currently, cocoa fruit harvesting has been based on experiences of local collectors (Dand, 2010). Harvesting cocoa fruits at an appropriate maturity stage is a critical step directly affecting flavor characteristics of cocoa beans (Dand, 2010). Cocoa beans collected from un-ripe fruits have low sugar content whilst over-ripe ones are easy to be germinated and infected by microorganisms (Dand, 2010). Besides, immature or over-ripe fruits may contain less bioactive compounds that are expected to be found abundantly in the beans (Chatt, 1953; Pereira-Caro *et al*., 2012).

Fermentation is one of the vital steps significantly impacting to the development of flavor precursors in cocoa beans (Wollgast, Anklam, 2000). After removed from the fruits, fresh cocoa beans are spontaneously fermented by successive activities of yeasts, lactic acid, acetic bacteria and fungi (Schwan, Wheals, 2004). The microbes performing ferrmentation come from knives used to open cocoa pods, workers' hands and unclean containers from previous batches (Schwan, Wheals, 2004). Unlike brewery, wine or cider fermentation, the use of commercial starter culture in cocoa fermentation has not been developed (Schwan, Wheals, 2004; Lefeber *et al*., 2012) due to limits of cost, logistics and culture maintenance (Holzapfel, 2002). Furthermore, utilizing starter culture may not always lead to expected results since pod removal and fermentation of cocoa still take place in smallholders where contamination can occur any time (Schwan, Wheals, 2004). Meanwhile, the addition of commercial enzymes in particular pectinase, an enzyme used to hydrolyze pectin, can increase the efficiencies of pulp extraction, shorten time of fermentation and improve cocoa quality such as high ratio of fermented beans and high cup testing scores (Almeida *et al*., 2003; Binh *et al*., 2012). Different fermentation methods require different fermentation duration but the maximum time is often 7 days (Schwan, Wheals, 2004; Prabhakaran Nair, 2010). Prolonged fermentation would cause off-flavoring of cocoa beans due to growing of unwanted microorganisms such as bacilli and filamentous fungi (Schwan, Wheals, 2004).

Cocoa is classified as a high-value crop with the economic value of US$4 billion yearly (Ackar *et al*., 2013; Andrzejuk, 2014). Due to increasing demand from worldwide consumption, cocoa farms in growing countries have been supported and funded to improve production rates (BACP, 2010; Fountain, Hütz-Adams, 2015). This requires local smallholders to have good farming practices including harvesting and fermentation experience to produce high-quality cocoa beans. For those reasons, this study was therefore conducted to evaluate impacts of maturity stages and fermentation with and without enzyme on antioxidant properties of cocoa beans grown in Vietnam. The results obtained from the current work could be necessary for the farmers, producers, scientists to optimize antioxidant levels in the cocoa products.

MATERIALS AND METHODS

Sample preparation

Cocoa flowers (TD3 genotype) were labelled and after approximately 3 months cocoa fruits were harvested from a cocoa farm in April 2014 in Ben Tre province, Vietnam. There were two plots; each of them contained about 200 kg of fresh cocoa beans and introduced to two different experiments. The process of transforming fresh cocoa beans to cocoa powder includes removing cocoa beans from the fruits before carrying out fermentation in a standard wooden box. After each chosen fermentation time, cocoa beans were subjected to drying, roasting and winnowing. Cocoa nibs were collected and ground to produce cocoa liquor. Cocoa butter was extracted out of the liquor by pressing, left the pressed cakes that were allowed to cool at 20^{o}C before pulverized into raw cocoa powder. The whole processing was carried out at a local cocoa company (Lam Anh Cocoa Company, Ltd.).

The experiment one: cocoa fruits were collected at four different maturity stages. At the stage 1, the fruits had purplish red color. Those in stage 2 had red-orange streaks appeared along the grooves of the fruits. In the stage 3 and stage 4, the red-orange color took up 50% and 100% of the fruits, respectively. Each maturity stage was divided into two parts. One part was fermented in 5 days (the fermentation time currently applied by Vietnamese farmer), meanwhile another part was not fermented. Next processing steps followed the same procedure described above.

The experiment two: Cocoa fruits at stage 3, the stage Vietnamese farmers prefer to harvest, were collected. The cocoa beans were subjected into fermentation with and without enzyme pectinase (Pectinex® Ultra SP-L, Novozymes, Denmark) (5 ml/10 kg cocoa mass); each treatment was lasted for 3, 5 and 7 days. After each given fermentation time, the beans were took out and sun-dried until the moisture content was lower 10%. Next processing steps followed the same procedure described above.

Sample extraction

Raw cocoa powder (1 g) was defatted twice with 10 ml of n-Hexane (Prolabo, France) at 200 rpm, 30˚C for 10 mins. The pellet was obtained by centrifugation at 4000g, 4˚C for 5 mins in a refrigerated centrifuge (Z326K, Germany). To evaporate n-hexane, the defatted powder was placed

in a fume hood overnight and was stored at -20˚C until analysis commenced.

Extraction and analysis of total phenolic contents

TPC extraction was followed the procedure of Othman and his colleagues (2010) with some modifications. In details, the defatted samples were extracted twice with 10 ml of 50% ethanol (Prolabo, France) (pH 2) at 200 rpm, 37˚C for 60 mins. Supernatants were taken from centrifuging at 4000g, 4˚C for 15 mins. Extracts were combined and filled up to 25 ml. Total phenolic contents were measured according to the method described by Velioglu and his teammates (1998). Catechin (Sigma-Aldrich, MO) was used as standard (0 - 0.125 mg Catechin equivalent (CE)) and results were expressed as mg CE/100 g dry matter (DM).

Measurement of antioxidant capacity

The antioxidant capacity of the ethanolic extract was evaluated based on the method of Re and his colleagues (1999) with some modifications. The ABTS powder (Sigma-Aldrich, MO) was dissolved in phosphate buffered saline pH 7 (Sigma-Aldrich, MO) to 7 mM and then mixed with 2.45 mM potassium persulfate (Merck, Germany). The solution was incubated at room temperature for 16 h to generate ABTS radical cation ($ABTS^{\bullet+}$). The $ABTS^{\bullet+}$ solution was diluted with extracting solvent to the absorbance of 0.72 ± 2 at 734 nm. Next, 100 µl of the extracts was added to 900 µl of diluted $ABTS^{\bullet+}$ solution. The reaction was monitored over 6 mins. Solutions of trolox concentrations (Sigma-Aldrich, MO) were used as standards (0 – 200 µM trolox equivalents (TE)).

Statistical analysis

All the chemical analyses were performed with independent replicates (n = 3). Values were expressed as mean ± standard error (SE). Analyses of significant differences and Pearson correlation were conducted using Minitab software version 16 (Minitab Inc. Pennsylvania, USA).

RESULTS AND DISCUSSION

Effects of maturity stages on total polyphenol contents and antioxidant capacities of cocoa beans

Extracting cocoa polyphenols was carried out with ethanol 50% (pH 2) at 37˚C, within 60 minutes. Preliminary screenings of this study indicated that 50% ethanol extracted the highest phenolic antioxidants as compared to concentrations of 10 to 70% (data not shown). Two-way ANOVA was used to elucidate whether combined effects between maturation and fermentation of cocoa beans on total phenolic contents and antioxidant capacities in raw cocoa powder. Both statistical analyses of the phenolic contents and antioxidant capacities presented that there were significant interactions ($P \leq 0.001$) between maturity stages and fermentation (Table 1). Therefore, the changes of TPC and AC at different maturity stages were strongly dependent on the fermentation treatments ($P < 0.001$). The results in this study showed that phenolic values in unfermented beans increased with the increase of maturity stages (Table 1). The values ranged from 6.39 ± 0.02 to 8.04 ± 0.24 g CE/100 g DM (Table 1). Pereira-Caro and his colleagues (2012) observed the same trend with the appearance of individual phenolic compounds (N-phenylpropernoyl-L-amino acids, flavonols, catechins, epicatechins and anthocyanins). The authors noted that the polyphenol accumulation, both minor and major cocoa phenolics, increased consistently with the cocoa bean development (Pereira-Caro *et al*., 2012). It means that TPC increased by increasing maturity stages of the cocoa fruits. It has been found that the broadened plastid structure is the sign of polyphenol synthesis in immature cocoa cells and the polyphenol synthesis is accelerated by the cocoa bean development (Martini *et al.*, 2008; Elwers *et al.,* 2012). However, they remained unchanged in ripe cotyledon tissues (Elwers *et al*., 2012). These observations was in line with the current results regarding insignificant differences in TPC of unfermented cocoa power from stage 2 to 4 (Table 1).

In contrast to the results obtained from unfermented beans, TPC of fermented samples were reduced when the beans were more ripen, from 8.50 ± 0.27 to 7.19 ± 0.28 g CE/100g DM (Table 1). The values measured in the stage 1, 2 and 3 were insignificantly different; however significantly reduced when fruits become fully ripe (stage 4). In both unfermented and fermented treatments, changes in antioxidant capacities of cocoa beans by maturity stages followed the same trend as observed from the phenolic values. However, antioxidant capacities of the fermented cocoa beans were significantly higher than those of the unfermented one (Table 1). Similar phenomenon between unfermented and fermented beans have been found in soybeans (Chang *et al.,* 2009; Dajanta *et al*.,

2013), red beans (Chou *et al*., 2008), and legumes (pigeon pea, bambara groundnut, african yam bean, kidney bean) (Oboh *et al*., 2009; Ademiluyi, Oboh, 2011). Fermentation process allows biochemical changes of cocoa polyphenols; meanwhile those of unfermented beans do not have such changes (Jati, 2009). In addition, the higher antioxidant capacities of the fermented beans as compared to the unfermented ones may be results of the microbial hydrolysis of polyphenols, leading to more bioactive compounds released. During fermentation, microbial proliferation causes rapid increases in heat, diffusion of acetic acid, lactic acid and ethanol into cotyledons (Afoakwa *et al*., 2008). As a result, cocoa cells lose its integrity and their cell walls are de-compartmentalized, allowing reactions between enzymes and substrates (Afoakwa *et al*., 2008; Albertini *et al*., 2015).

Table 1. TPC and AC of unfermented and fermented cocoa beans at different maturity stages. *Significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.* Data are expressed as mean ± standard error (n = 3). Mean values within treatment followed by different uppercase letters are significantly different at $p < 0.05$. Mean values within the same maturity stage followed by different lowercase letters are significantly different at $p < 0.05$.

Treatments	Total Polyphenol contents (g CE/100g DM)	Antioxidant Capacity (mol TE/100g DM)
Unfermented cocoa beans		
Stage 1	6.39 ± 0.02^{By}	26.82 ± 0.13^{By}
Stage 2	7.73 ± 0.41^{Ax}	30.57 ± 0.68^{Ay}
Stage 3	7.92 ± 0.13^{Ax}	30.86 ± 1.41^{Ay}
Stage 4	8.04 ± 0.24^{Ax}	31.63 ± 0.41^{Ay}
Fermented cocoa beans		
Stage 1	8.50 ± 0.27^{Ax}	45.12 ± 0.98^{Ax}
Stage 2	8.58 ± 0.16^{Ax}	45.18 ± 0.51^{Ax}
Stage 3	7.69 ± 0.16^{ABx}	44.37 ± 0.88^{ABx}
Stage 4	7.19 ± 0.28^{Bx}	41.80 ± 1.38^{Bx}
Two-way Anova analysis		
Maturity stages	**	*
Treatments	***	***
Maturity stages ×Treatments	***	***

Effects of fermentation methods on total polyphenol contents and antioxidant capacities of cocoa beans

Data of two-way ANOVA analysis showed that both fermentation treatments and fermentation duration had significant and synergistic effects on the total phenolic contents ($P \leq 0.001$) and antioxidant capacities ($P \leq 0.01$). This meant that changes of TPC and AC in cocoa samples at different fermentation duration depended on the treatment methods. The obtained results from fermentation with and without enzyme addition showed downward trends of TPC (Table 2). The decreases of polyphenols in the two fermentation treatments could be due to their diffusion out of cotyledons (Camu *et al*., 2008). During fermentation, microbial proliferation causes a rapid increase in heat, diffusion of acetic acid, lactic acid and ethanol into cotyledons (Afoakwa *et al*., 2008). Thereafter, fermentation liberates polyphenols out of cotyledons, causing polyphenol diffusion into fermentation sweating (Wollgast, Anklam, 2000), the liquid then drains away via the utilization of pulp carbohydrates by yeast (Wollgast, Anklam, 2000; Schwan, Wheals, 2004). Moreover, polyphenol compounds could also be decreased by oxidation and polymerization reactions (Afoakwa *et al*., 2012; Suazo *et al*., 2014). As oxygen penetrates into cocoa beans, polyphenols are oxidized by enzyme polyphenol oxidases into o-quinones (Afoakwa *et al*., 2012). The latter react with proteins, peptides and then polymerize with other polyphenols to form polymers such as condensed tannins (Schwan, Wheals, 2004; Camu *et*

al., 2008; Afoakwa *et al*., 2012; Suazo *et al*., 2014). Condensed tannins or proanthocyanidins along with hydrolysable tannins are two groups of tannin compounds (Gessner, Steiner, 2005). High-molecular-weight tannins can further form insoluble complexes with proteins by hydrogen bonding during cocoa fermentation (Redgwell *et al.*, 2003; Ozdal *et al.*, 2013). The newly formed structure no longer has phenolic properties as it could interact with Maillard products during roasting process, leading the formation of Klason lignin, an insoluble material which is remained unaffectedly after acidic treatments (Redgwell *et al.*, 2003). In addition, it has been reported that individual phenolic compounds such as anthocyanins and epicatechins are lost during cocoa fermentation (Payne *et al*., 2010; Afoakwa *et al*., 2014). Anthocyanins under enzymatic reaction with glucosidases are converted to anthocyanidins (Afoakwa *et al*., 2014). The latter are oxidized by polyphenol oxidase into quinones which react with amino acids and proteins to form melanins (Afoakwa *et al*., 2014). In general, these reported studies were in agreement with the current results regarding the decrement of total phenolic contents after day 5 and 7 of cocoa fermentation.

The present work has confirmed reported results of several previous studies. They recorded that polyphenols, tannins, (+)-catechin and (-)-epicatechin and consequently antioxidant capacities significantly reduced during fermentation. The similar was observed for different cocoa bean varieties from different geographical origins phenomenon (Bonvehi, Coll, 1997; Caligiani *et al*., 2007) and fermented using different methods such as heap (Afoakwa *et al.,* 2012), plastic barrels (Di Mattia *et al*., 2013) or wooden box (Albertini *et al.*, 2015). The increased losses of total phenolics by extending fermentation time were observed in the present study (Table 2). Indeed, when compared to the day 3 of fermentation, the concentrations of polyphenols in fermentation without enzyme obtained from the day 5 slightly reduced, only 0.45%; however, greater decreased by the day 7 (17.36%). For enzymatic treatments, the TPC at the day 5 (14.69%) and the day 7 (30.51%) was decreased more rapidly and steadily in comparison with the values recorded in the day 3. The faster rate of phenolic reduction in fermentation with enzyme supplement may be attributed to the present of pectinase enzyme added that was accelerating the pectin hydrolysis. Pectinase enzyme is naturally produced by indigenous yeasts in cocoa fermentation and its function involves in pectin depolymerization of cocoa pulp (De Vuyst, Weckx, 2016). However, adding more the commercial pectinase enzymes to cocoa fermentation could speed up the hydrolysis of pectin and enhances quality of the treated beans (Schwan, Wheals, 2004). Moreover, the pectinase addition to cocoa bean fermentation helps lower the viscosity of cocoa pulp via breakdown of pectin chains, thus preventing under-fermentation (Schwan, Wheals, 2004). Cocoa pulp with high viscosity could obstruct the voice spaces in cocoa mass and diminish aeration for later bacterial proliferation in fermentation (Schwan, Wheals, 2004). Therefore, these current results suggested that the enzyme pectinase supplement could accelerate cocoa fermentation process. Inside the cocoa pods, cocoa pulp covering cocoa beans are free from microbes until they are extracted for fermentation (Schwan, Wheals, 2004). As soon as being extracted, the cocoa pulp gets inoculated with yeasts and bacteria from the surroundings (Jespersen *et al*., 2005; Nielsen *et al.*, 2006). These microbes play vital roles in removal of mucilagous pulp out of the beans and the production of important metabolites such as ethanol, lactic acid, and acetic acid (Schwan, Wheals, 2004). In this study, the pectinex Ultra SP-L enzyme used broke down pectin in cocoa pulp, allowing the formation of void spaces for oxygen penetration and the diffusion of crucial metabolites into the beans (Schwan, Wheals, 2004; Ouattara *et al*., 2010). The oligomers obtained from the degradation process of pectins were then used as substrates for microorganisms and consequently, the fermentation was accelerated (Ouattara *et al*., 2010).

Results obtained from the both fermentation treatments showed downward trends of measurable TPC and AC (Table 2). Whereas samples fermented with enzyme resulted in higher phenolic contents, ones without enzyme supplement had higher antioxidant capacities. This indicated that the prediction of AC values in fermented cocoa samples could not solely follow and depend on their phenolic contents but on the synergistic effects of polyphenols with the others and/or other compounds presented in the samples (Shahidi *et al*., 1994). Furthermore, the contribution of phenolic contents to antioxidant capacities is owing to not only their mere presence but also the proper orientation and substitution of their functional groups (Moktan *et al*., 2008). The lower AC values of samples fermented with enzyme addition may be due to the greater reduction of ethanol production leading to the generation of less

bioactive or inactive compounds (De Melo Pereira *et al*., 2012; Zhao, Fleet, 2014; De Vuyst, Weckx, 2016). The presence of pectinase enzyme may cause anerobic phase for yeast fermentation to disappear rapidly, hence causing less ethanol production. Ethanol was produced by yeast through conversion of glucose available in cocoa pulp (De Vuyst, Weckx, 2016). It was reported that the changes in ethanol production during cocoa fermentation could influence the inhibition of certain microbes and the dynamics of microbial successions (De Melo Pereira *et al*., 2012; De Vuyst, Weckx, 2016). In addition, a study of Zhao and Fleet (2014) indicated that the presence of ethanol may be essential for proper activity of glycosidase and polyphenol oxidase enzymes during cocoa fermentation. Therefore, the reduced production of ethanol when fermentation duration prolonged may affect enzymatic hydrolysis in enzymatic fermentation, resulting in less bioactive or active compounds presenting for high antioxidant capacities.

Table 2.TPC and AC of cocoa beans under fermentation with and without enzyme. *Significance: *P ≤ 0.05; **P ≤ 0.01; ***P ≤ 0.001.* Data are expressed as mean ± standard error (n = 3). Mean values within treatment followed by different uppercase letters are significantly different at p < 0.05. Mean values within the same fermentation day followed by different lowercase letters are significantly different at p < 0.05.

Treatments	Total Polyphenol contents (g CE/100g DM)	Antioxidant Capacity (mol TE/100g DM)
Fermentation without enzyme		
Day 3	8.96 ± 0.14^{Ay}	38.90 ± 0.81^{Ax}
Day 5	8.92 ± 0.15^{Ay}	38.62 ± 0.64^{Ax}
Day 7	7.38 ± 0.07^{By}	33.69 ± 0.62^{Bx}
Fermentation with enzyme		
Day 3	11.57 ± 0.22^{Ax}	35.15 ± 0.57^{Ay}
Day 5	9.87 ± 0.08^{Bx}	31.03 ± 0.52^{By}
Day 7	8.04 ± 0.10^{Cx}	25.68 ± 0.41^{Cy}
Two-way Anova analysis		
Treatments	***	***
Duration	***	***
Treatments x Duration	***	**

Correlations between antioxidant capacities and total phenolic contents

The correlations between the antioxidant capacities and total phenolic contents were studied using the Pearson's correlation analysis and the results are presented in Figure 1. During maturation, positive correlations obtained from both unfermented and fermented cocoa beans with coefficients were 0.748 and 0.746, respectively (Figure 1A and 1B). These coefficients were comparable to that obtained from a study of Castrejón and his colleagues (2008) in which the correlation between phenolic compounds and antioxidant capacities of highbush blueberries during ripeness was 0.829. In the same way, Hu and Xu (2011) evaluated the antioxidant capacities of different types of waxy corn (JXN8 and XHD85 corn types) during maturity and verified that the content of total phenolics significantly correlated with the ABTS scavenging activity (R = 0.89 and R = 0.79, respectively). For the fermentation experiment, the Pearson correlation analysis showed strong correlation between AC and TPC values of naturally fermented cocoa beans without enzyme (R = 0.942) and with enzyme pectinase (0.923) (Figure 1C and 1D). Likewise, an analysis carried by Duhan and his teammates (2016) found a very strong correlation (R = 0.994) between the TPC and AC measured by ABTS assay of fermented wheat grain during six days of fermentation. Also during 108 hours of fermentation, there were strong and significant correlations between ABTS, ORAC (Oxygen Radical Scavenging Capacity) and total phenolics (0.962 and 0.905, correspondingly) of *Phaseolus vulgaris* L., a legume cultivar (Guzmán-

Uriarte *et al.*, 2013). Due to positive correlations obtained between antioxidant capacities and TPC (Fig 1. A, B, C, D), it could be suggested from the current study that polyphenols were the major compounds contributed to antioxidant capacities of cocoa beans in all treatments.

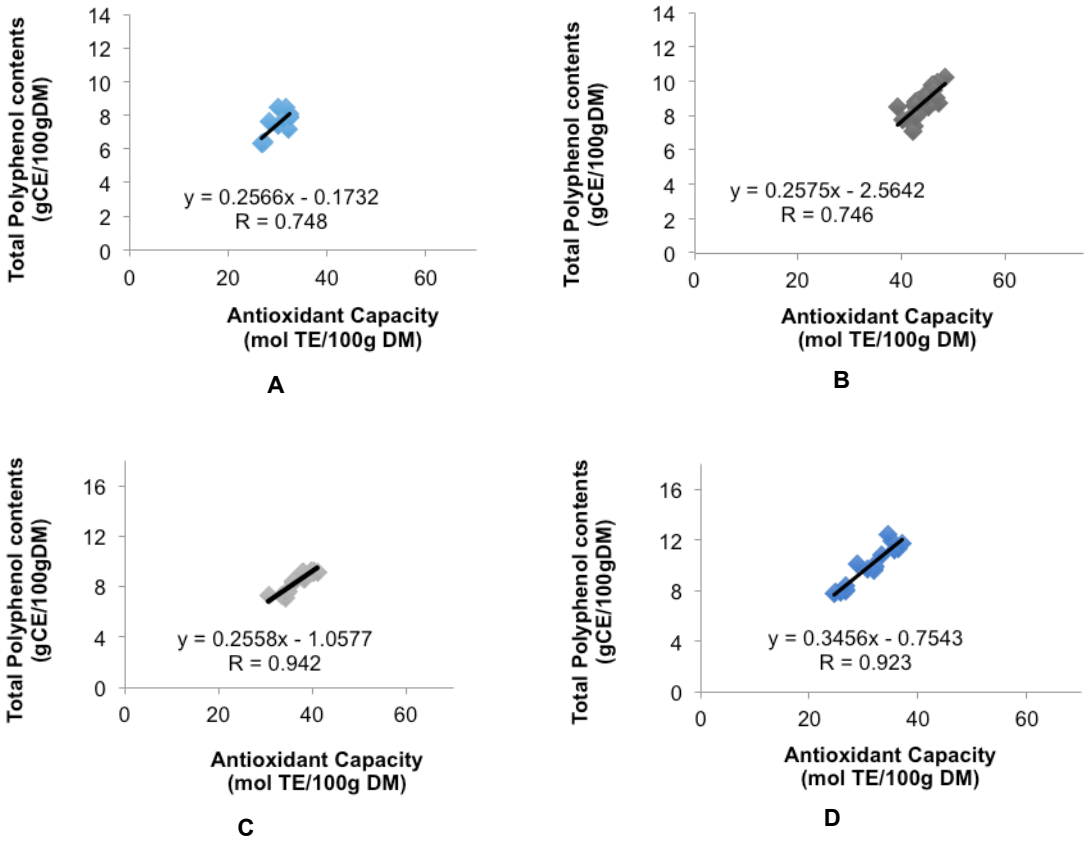

Figure 1. Correlation analyses between antioxidant capacities and total phenolic contents of unfermented cocoa beans (A), fermented cocoa beans (B), cocoa bean fermentation without enzyme (C), and cocoa bean fermentation with enzyme (D).

CONCLUSIONS

It could be concluded from the current work that the maturity stage and fermentation of cocoa beans significantly affected their antioxidant capacities of raw cocoa powder. The values of unfermented beans increased by the ripening; in contrast, that of fermented ones reduced by their maturity. This finding could be a useful reference data for the producers when processing different kinds of cocoa products. Although adding pectinase helps to released more TPC presented as catechin equivalent, the cocoa beans treated with the enzyme had significantly lower antioxidant capacity as compared to those without enzyme. Further researches should be conducted to investigate effects of primary processing of cocoa beans on their principal phenolic compounds such as proanthocyanidins, catechins and anthocyanins to release comprehensive database about antioxidant properties of Vietnamese cocoa beans.

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NGHIÊN CỨU ẢNH HƯỞNG CỦA ĐỘ CHÍN VÀ QUÁ TRÌNH LÊN MEN HẠT CACAO ĐẾN TỔNG HÀM LƯỢNG POLYPHENOL VÀ KHẢ NĂNG CHỐNG OXY HÓA CỦA BỘT CA CAO THÔ

Đặng Thị Kim Yến, Nguyễn Vũ Hồng Hà

Trường Đại học Quốc tế, Đại học Quốc gia, Thành phố Hồ Chí Minh

TÓM TẮT

Việc sử dụng các sản phẩm từ ca cao (*Theobroma cacao* L.) mang lại nhiều lợi ích về sức khỏe do cacao chứa hàm lượng chất polyphenol và khả năng chống oxy hóa (AC) cao. Tuy nhiên, có nhiều yếu tố ảnh hưởng đến các hợp chất chống oxy hóa trong quá trình chế biến hạt ca cao. Trong nghiên cứu này, những ảnh hưởng của độ chín, phương pháp lên men và thời gian lên men hạt cacao đến tổng hàm lượng chất polyphenol (TPC) và khả năng chống oxy hóa của bột ca cao thô đã được nghiên cứu. Tổng số hàm lượng chất polyphenol và khả năng chống oxy hóa được đo lần lượt bằng phương pháp Folin-Ciocalteu và ABTS-TEAC (Trolox equivalent antioxidant capacity). Bột thô từ hạt ca cao không lên men ở độ chín một có hàm lượng polyphenol và khả năng chống oxy hóa thấp hơn bột thu nhận những hạt thu hoạch ở độ chín hai, ba và bốn. Khi tiến hành lên men hạt ca cao ở cả bốn độ chín, khả năng chống oxy hóa của bột thô tăng lên đáng kể. Bột thô thu nhận từ các hạt ca cao được bổ sung enzyme Pectinex® Ultra SP-L có hàm lượng polyphenol cao hơn nhưng khả năng chống oxy hóa thấp hơn so với bột thu nhận từ những hạt lên men không sử dụng enzyme. Thời gian lên men kéo dài dẫn đến sự sụt giảm đáng kể của TPC và AC có trong bột ca cao thô. TPC và AC có mối quan hệ tích cực về mặt thống kê đối với hạt ca cao lên men có enzyme (R = 0,923) và không có enzyme bổ sung (R = 0,942). Phân tích Two-way Anova cho thấy rằng sự thay đổi của AC và TPC có trong bột ca cao thô phụ thuộc vào các giai đoạn trưởng thành của hạt ca cao, phương pháp lên men và thời gian lên men. Kết quả thu nhận từ nghiên cứu cho thấy, quá trình lên men ca cao ở giai đoạn trưởng thành hai và ba có thể mang lại hàm lượng cao chất polyphenol và khả năng chống oxy hóa; việc lên men bổ sung enzyme có thể được sử dụng để giảm bớt chi phí công lao động do thời gian lên men được rút ngắn.

Từ khóa: *Bột ca cao thô, hạt ca cao, khả năng chống oxy hóa, lên men, tổng hàm lượng chất polyphenol*