EFFECT OF COCOA PROCESSING ON TOTAL PHENOLIC CONTENTS IN COCOA BEANS AND ITS FREE RADICAL SCAVENGING CAPABILITY

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Malaysian Cocoa J. (2021) 13(1): 97-100

ABSTRACT - *The polyphenols in cocoa beans are stored in the pigment cells of the cotyledons. Cocoa polyphenols diffuse from their storage cells during fermentation. Cocoa beans drying is to dry cocoa beans to its moisture contents not more than 7.5% and to stop fermentation processes. Roasting of cocoa beans involve reactions among the cocoa flavour precursors, that are readily available from the fermentation and drying processes; which is essential to develop further the chocolate flavour. This paper is to report the effect of the cocoa processing chains on the extractable Total Phenolic Contents in cocoa and their capability to scavenge free radicals from a 0.06mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.*

Keywords: Cocoa; free radicals scavenging; Total Phenolic Contents

INTRODUCTION

Fermentation, drying and roasting are the vital cocoa processing chains in order to develop the desire cocoa flavours.

In normal practises by the small holder of cocoa farmers, fresh cocoa beans, which are extracted from the fresh harvested cocoa fruits, are subjected to fermentation process for 5-6 days in a wooden fermentation box with turning for aeration at every two days interval. Well fermented cocoa beans are sun dried for another 6-7 days to reduce its moisture contents to less than 7.5% (wt/wt) (Zahouli *et al*., 2010) for storage and to stop fermentation process. Various biochemicals reactions occur during these processes. Nevertheless, it is well understood that these processes are crucial to form the desire cocoa flavours precursors.

Roasting of the fermented cocoa beans is to develop the typical desired cocoa flavours besides removing the undesired compounds with low boiling points such as acetic acid (Oliviero *et al*., 2009). The cocoa roasting process is carried out at high temperatures (120–150°C).

Polyphenols in cocoa beans are stored in the pigment cells of cotyledons. Depending on the number of anthocyanins, those pigment cells, also known as polyphenol-storage cells, are range in colour from white to deep purple. For instances, *Criollo* cocoa beans which are only approximate two third of the polyphenols content of *Forestero* beans have rather whiter in colour compared to deep purple of *Forestero* beans (Voigt and Lieberei, 2015).

Polyphenols have become an intense focus of research interest due to their physiological functions. Polyphenols are formed biogenetically from the shikimate and the acetic pathway. Antioxidant, antimutagenic and anti-tumour are among the health-beneficial effects offered by phenolic compounds (Azizah, *et al*., 2007).

The objectives of this paper are to report the changes of the cocoa beans' extractable total phenolic contents with regards to the cocoa beans processing chains and its free radicals scavenging capability.

MATERIALS AND METHODS

Fresh unfermented cocoa beans and well fermented dried cocoa beans were collected from Malaysian Cocoa Board research centre located at Bagan Datuk, Perak, Malaysia. Roasting processes on the well fermented dried cocoa beans was carried out in an oven at 135ºC for 30 minutes.

Extraction of cocoa phenolics

One gram of cocoa beans was added with 50ml of extraction medium, ground with food processor in low speed for 3 seconds. Extraction treatments were shown in Table 1. The extract was then filtered with filter paper (Whatman no. 4). De-pulping processes were carried out for fresh unfermented cocoa beans and freeze dried prior to extraction. Each treatment was carried out in triplicates.

Table 1 Extraction parameters

Extraction medium	Extraction method	Temperature (°C)	Duration (min)
Aqueous	incubation at 150 rpm stirring rate	40, 60, 80	5, 10, 15
Aqueous	Sonication (sonicator probe with ½" hon at 20KHz)	40, 60, 80	5, 10, 15
Ethanol: water (30:70; 55:45; 80:20)	incubation at 150 rpm stirring rate	40, 60, 80	5, 10, 15
Ethanol: water (30:70; 55:45; 80:20)	Sonication (sonicator probe with ½" hon at 20KHz)	40, 60, 80	5, 10, 15

Total Phenolic Content (TPC) determination

The extract was diluted six folds with distilled water. One millilitre of diluted extract was added with 2.5ml Folin-Ciocalteu reagent and 2.0ml 7.5% sodium bicarbonate. The mixture was vortexed and incubated at 45°C for 15 minutes prior to measure with UV-visible spectrophotometer at 750nm. A standard curve of gallic acid with a series concentration of 0 to 0.5 mg/ml was prepared. Total phenolic content, C in mg GAE/g sample, was calculated based on the equation below (Samuel Yap, 2018):

$$C\ (GAE/g\ sample) = k \times c \times V/M \quad \{1\}$$

where
k = sample dilution factor
c = concentration determined from standard curve (mg/ml)
V = volume of extraction medium (ml)
M = sample weight used for extraction (g)

Free Radicals Scavenging Capability

Free radicals scavenging capability of the extract were determined by drawing 0.5ml sample added with 5.0ml 0.06 mM DPPH solution, mixed well and incubated in dark for 30 minutes prior to measure with UV-Visible spectrophotometer at 520nm. Percentage of free radicals scavenging capability was calculated as the equation below (Samuel Yap, 2018):

$$RSC(\%) = [(Abs(c) - Abs(S)/Abs(c)] \times 100 \quad \{2\}$$

where:
RCS = DPPH radical scavenging capability
Abs(c) = Abs for control
Abs(S) = Abs for sample

RESULTS AND DISCUSSIONS

Extractable Total Phenolic Contents (TPC)

Analysis of Variance showed no significant differences in term of extractable TPC between fresh beans and dried beans. However, extractable TPC was significantly lower from the roasted beans (Table 2).

Table 2 Extractable Total Phenolic Contents (TPC) in mg GAE/g sample from fresh cocoa beans, dried cocoa beans and roasted cocoa beans

Sample	Means value of TPC (mg GAE/ g sample)
Fresh Beans	109.58 ± 56.83[a]
Dry Beans	112.97 ± 0.08[a]
Roasted Beans	35.59 ± 12.74[b]

Note: samples with same letter were not significantly difference at p>0.05.

During fermentation, sugars in the pulp are transformed to acetic acid and lactic acid by environmental microorganisms and, acidification of the cocoa beans occur (Schwan and Wheals, 2004). Proteolytic processes start after 1-3 days of fermentation when the beans are killed by opening of the shell (testa) at the micropyle caused by acidification of the pulp and increased temperature, thereby enabling penetration of the nib by acetic and lactic acids (Voigt and Leiberei, 2015). The extractable TPC results of the study showed no significant differences between fresh cocoa beans and well fermented dried cocoa beans suggested that during fermentation, no significant reaction on polyphenols is taking place. It is believed that during fermentation, polyphenols are just diffused with cell liquids from their storage cells without significant oxidation.

These polyphenols, together with the hydrophilic peptides and hydrophobic free amino acids from the progressive alteration of cocoa protein by *aspartic endoprotease* and *carboxypeptides*, to form cocoa specific flavour precursors (Voigt, 2010). A significant drop of extractable TPC in roasted cocoa beans indicates phenolic tanning or oxidation occurs in roasting process. Cocoa beans are roasted to develop further the chocolate flavour, which should already exist in the form of precursors arising from the correct fermentation and drying of the original beans.

Free radicals scavenging capability

Free radicals scavenging test by DPPH assay showed that no significant differences between the extract from fresh cocoa bean and well fermented dried cocoa beans. However, a significant drop from cocoa roasted beans was observed (Figure 1). These was in line with the results shown above that both were having similar contents of extractable TPC. A significant drop of free radicals scavenging capability in roasted cocoa beans is expected as roasted cocoa beans had less extractable TPC.

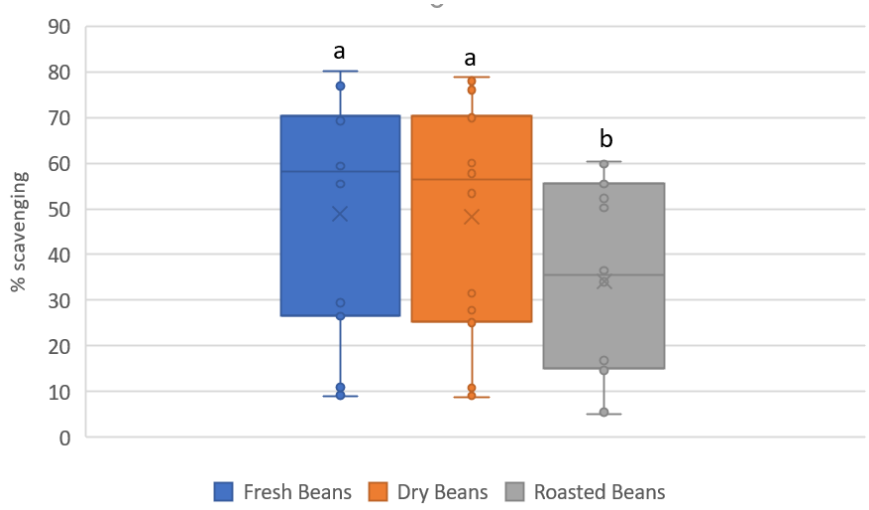

Figure 1 Free radicals scavenging capability of the 0.02g cocoa sample/ml extracts respectively from fresh cocoa beans, dry cocoa beans and roasted cocoa beans.
Note: samples with same letter were not significantly different at p>0.05.

CONCLUSIONS

This study revealed that fresh cocoa beans and well fermented dried cocoa beans gave comparable amount of extractable total phenolic contents (TPC) indicated no substantial reactions on phenolic compounds during the phases of fermentation and drying of cocoa beans. A significant drop of extractable TPC on the roasted cocoa beans suggested that phenolic tanning or polyphenols oxidation occurs in roasting process. A similar profile was observed in its free radicals scavenging capability, whereby, there were no significant differences between extract sample from fresh cocoa beans and dried cocoa beans. A significant lower value was observed from the extract from roasted cocoa beans.

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