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RESEARCH ARTICLE



Influence of fermentation on the quality of Fijian *Theobroma cacao* beans over two harvest seasons

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ABSTRACT

Theobroma cacao beans are cultivated in Fiji by smallholder farms in low volumes, mainly for export. Cocoa beans are often processed under ambient conditions by farmers and cocoa processors. Postharvest processing includes natural fermentation from six to ten days in wooden boxes, followed by sun – drying in the open for up to 14 days. The impact of fermentation conditions on key quality parameters of Fijian cocoa beans, such as temperature profile, pH, and total extractable polyphenol content (TPC) are presented in this study. The quality of fermentation was assessed using a standard method, such as a cut test followed by fermentation index (FI) measurement. A temperature increase to 40°C and variations in the pH of the bean mass were evident during natural fermentation. TPC in the cocoa beans was sensitive to temperature and pH. Fermentation was variable due to weather conditions between harvest seasons. The dry season had the best conditions for fermentation as the peak temperature of the bean mass was 40°C and the FI was high (1.39 ± 0.04). There is a need to improve fermentation during the rainy season. This can be achieved by providing training to Fijian cocoa farmers on using better fermentation techniques.

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Cocoa; Fiji; postharvest; polyphenols; fermentation

Introduction

Theobroma cacao trees have been cultivated in the Fiji Islands since the late 1880s. The tropical climate in Fiji is ideal for producing and processing cocoa. Postharvest processing includes fermentation and drying under ambient conditions. In Fiji, cocoa beans are fermented in wooden boxes. Freshly harvested cacao pods are split open with wooden sticks or with the blunt end of a machete by farmers and farm workers to prevent damage to the beans. These freshly extracted cocoa beans are packed into wooden boxes with perforations and covered with banana leaves and a wooden lid. Fermentation is commonly conducted in an open shed in Fiji and lasts for six to ten days.

Fermentation is an essential processing step for developing flavour and aroma precursors during the downstream processing of cocoa beans for chocolate production. The

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biochemical reactions during fermentation change the flavour and aroma profile of cacao beans. Fresh cacao beans are astringent and bitter in flavour. This bitterness is caused by alkaloids, such as caffeine and theobromine, as well as polyphenolic compounds (Stark and Hofmann 2005; Camu et al. 2007). Biochemical reactions during fermentation cause the oxidation of these compounds, which reduces bitterness.

In the Fiji Islands, fermentation depends on the natural microbial flora and ambient conditions. Fermentation is initially anaerobic for 24–48 hours (Schwan and Wheals 2004). Airflow is limited by the pulp when pulp – covered cocoa beans are packed into the fermentation box. The sugar – rich pulp and limited airflow are ideal for the growth of yeast species (*Saccharomyces*, *Candida*, and *Pichia* sp.) and heterofermentative *Lactobacillus plantarum*. Enzymes secreted by yeasts and LAB depolymerise pectin and hydrolyse sucrose into ethanol (EtOH) and CO₂ (De Vuyst and Weckx 2016).

Reduction in pulp volume caused by dripping within 48 hours of fermentation improves air circulation in the box. Air circulation shifts the fermentation process from anaerobic to aerobic phase. Farm workers manually mix cocoa beans to introduce air into the fermentation mass for the next six to eight days. Acidification of cocoa beans occurs in the aerobic phase by acetic acid bacteria (AAB), which oxidises ethanol (EtOH) to volatile acetic acid (De Vuyst and Weckx 2016; Sandhya et al. 2016). Oxidation increases the temperature of cocoa bean mass to between 50–55 °C around the fourth day of fermentation (Afoakwa et al. 2011; De Vuyst and Weckx 2016). Heat and acidification disintegrate the cell membrane and initiate biochemical reactions inside the beans (Schwan and Wheals 2004; Lima et al. 2011). Acidification also affects polyphenol oxidases (PPO), which oxidises polyphenols to tannins and quinones. These compounds impart a desirable dark color to fermented cocoa beans. Acidification and temperature increase are two critical factors that terminate incipient germination. These two key elements require constant monitoring and control for high – quality fermented cocoa beans.

The current cocoa bean fermentation practices in Fiji do not monitor temperature change. Cocoa processors use a cut test during bean rotation to monitor the colour change of cocoa beans from purple to either pale or dark brown colour. A high cut test score of more than 90% demonstrates successful fermentation and better browning of cocoa nibs (Niemenak et al. 2014). While this technique is widely practiced as a quick and convenient method for monitoring fermentation, there is a high risk of human error involved. Some challenges faced by the cocoa sector in Fiji are poor record keeping of postharvest conditions and fermentation quality indicators. There is also limited postharvest training provided to Fijian cocoa farmers, as they are based in remote locations.

The cocoa sector in Fiji is not using any new postharvest processing techniques or technology. There is a need to introduce new and better ways of fermenting cocoa beans and monitoring the fermentation quality. This would provide cocoa farmers with the skills required to produce premium – quality cocoa beans for export. However, before new postharvest technology and techniques can be introduced to the Fiji cocoa sector, it is important to study the effects of the current traditional fermentation and drying techniques on the quality of Fijian cocoa beans. There is a lack of scientific literature on the impact of traditional fermentation techniques on quality indicators for Fijian cocoa beans. There are projects active in the Pacific region that introduce new fermentation techniques but the findings are not published. This study presents findings on the influence of natural fermentation on some key quality parameters of Fijian *Theobroma cacao* beans over two harvest seasons.

Materials and methods

Fresh and fermented *Theobroma cacao* beans (*var.* Forastero) were sourced from the Fiji Ministry of Agriculture's Naduruloulou research station near Suva. The research station was established in 1975 mainly for tree crops, such as cocoa trees. Fresh cocoa beans and the fermentation box were sourced from this Naduruloulou station. Cocoa beans used for this study were harvested and processed towards the end of the dry season late April to July in 2018, and the wet season from November 2018 to January 2019.

Quality analyses, such as fermentation index, pH, total extractable polyphenol content (TPC), and moisture content were conducted at the food science lab at Massey University, New Zealand. Fermentation quality during field trials was assessed on wet beans at the end of seven days of natural fermentation using a cut test at the University of the South Pacific's (USP) Institute of Applied Science (IAS) in Suva, Fiji Islands. Fermented cocoa beans analysed for fermentation index, pH, and TPC were freeze – dried at IAS – USP before analysis.

Fermentation method

Freshly harvested wet cocoa beans (30 kg) were fermented in a wooden box under ambient conditions for seven days. Three separate batches of wet cocoa beans (30 kg per batch) were fermented for each harvest season, between 2018 and 2019. One wooden box was used to ferment three batches of wet beans weekly for three weeks during harvest. The fermentation box could hold 45 kg of wet beans. Fermentation was carried out in a shed at the Naduruloulou research station by the agriculture officers. Details on the fermentation box dimensions are provided in [Table 1](#). Wet beans were covered with banana leaves and the boxes were closed using wooden lids. The slats in the wooden box had a spacing of 0.5 cm to allow the pulp to drip and for aeration of the cocoa mass.

During the fermentation process, the temperature of the bean mass and ambient air was recorded using an alcohol thermometer. Temperature measurements were recorded from the center of the box, in the middle of the bean mass during bean rotation. The cocoa beans were mixed every two days with hands for aeration. A cut test was conducted during aeration to monitor the fermentation process. Ambient air conditions were monitored using an alcohol thermometer outside the fermentation shed in an open area. Fermented cocoa beans were sun – dried in the open for even days at an average temperature condition of 28–30°C.

Cut test

A cut test procedure adapted from Afoakwa et al. (2011) was used to determine fermentation quality. About 300 defect – free cocoa beans from a batch of 30 kg were selected for

Table 1. Dimension of fermentation box used for fermenting *Theobroma cacao* beans.

Dimensions	Measurements	Unit
Width	0.45	m
Length	0.53	m
Depth	0.30	m
Thickness of timber	0.03	m

a visual inspection of colour change after fermentation. The beans were cut lengthwise to expose the maximum cut surface. These colour profiles were:

- (1) Brown for fully fermented beans.
- (2) Partly brown or partly violet – purple for under – fermented beans.
- (3) Fully purple for unfermented beans.
- (4) Slaty or grey for spoilt beans.

Each of the colour profiles was tallied and added together from a sample of 300 cocoa beans. The sum for each colour profile was converted to a percentage and expressed as the cut test score at the end of fermentation. The beans were discarded after the cut test.

Measurement of fermentation index

Fermentation index (FI) is a colorimetric assessment of anthocyanin pigments in cocoa beans after fermentation. These pigments are oxidised during fermentation. FI for cocoa beans during fermentation was measured using an Ultraviolet (UV) – Visible spectrophotometer (Thermo Fisher Scientific™). Samples of cocoa beans ($n = 50$) from three separate batches over two harvest seasons were tested and the results were reported as an average for the harvest season. The FI procedure was adapted from Misnawi (2009).

A standard extraction protocol was used to extract pigments from fermented and dried cocoa beans (0.5 g). Extraction was conducted using 50 mL of methanol and hydrochloric acid (HCl) solutions (97:3 v – v). Methanolic Cocoa extracts (CE) were homogenised for one minute in a vortex mixture and refrigerated at 8 °C for 19 hours, followed by vacuum filtration. The filtrate volume was adjusted to 50 mL with methanol and HCl solution. Absorbance was recorded at 420 and 530 nm using a UV – Vis Spectrophotometer. The FI for cocoa beans was calculated using the following formula:

$$FI = \frac{\text{Absorbance value at } 420 \text{ nm}}{\text{Absorbance value at } 560 \text{ nm}} \quad (1)$$

Analysis of total polyphenol content

The method for total polyphenol content (TPC) was adapted from Carrillo et al. (2014) using the Folin – Ciocalteu (FC) technique, which is a colorimetric technique for detecting TPC and is dependent on the pH of the solution. FC reagent reacts with polyphenolic compounds in cocoa extracts (CE) to form a blue complex. This blue complex is detected and quantified under blue light. An alkaline pH of the solution is essential for maximum retention of chromophores from CE. Therefore, the FC reagent is mixed with sodium carbonate to ensure that turbidity is limited, and the redox reaction is facilitated.

Extracts from fermented and sun – dried cocoa beans (200 mg) were analysed for TPC using a UV – Vis spectrophotometer (Thermo Fisher Scientific™) and gallic acid as the analytical standard. Gallic acid and the general reagents used for TPC analyses are listed in Table 2. These chemicals were obtained from Sigma Aldrich™.

Table 2. List of standards and chemicals used for chemical analyses.

General Chemicals	CAS No.	Supplier
Ammonia	1336–21–6	Sigma – Aldrich
Ethanol	64–17–5	Sigma – Aldrich
HCl	7647–01–0	Sigma – Aldrich
n – Hexane	110–54–3	Sigma – Aldrich
Methanol	67–56–1	Sigma – Aldrich
Sodium carbonate	497–19–8	Sigma – Aldrich
Folin Ciocalteu Reagent	12111–13–6	Sigma – Aldrich
Gallic Acid	5995–86–8	Sigma – Aldrich
Deionised water		Food Chemistry Lab

Preparation of standard solutions and cocoa extracts

Gallic acid standards were prepared in ethanol for analysing TPC in cocoa extracts (CE) at a concentration of 10 g L^{-1} . Serial dilutions of the Gallic acid solutions were prepared at concentrations of 0.05, 0.1, 0.15, 0.25, 0.5, 1, 1.5, 2, 5, and 7 g L^{-1} for standard calibration curves.

Serial extraction of cocoa beans in methanol was conducted by using 200 mg of defatted cocoa beans. Dried cocoa beans (200 mg) were ground and defatted using 2 mL of n – hexane. The mixture was centrifuged at 1565 g for 10 min. The supernatant was decanted, and the procedure was repeated thrice. The defatted samples were serially extracted using one and a half milliliter of methanol (80% v – v) followed by centrifuging for 10 min at 391 g at 4°C . The supernatant was filtered using a $0.45 \mu\text{m}$ nylon filter into 2 mL vials for further analysis. A 1:20 dilution of filtered extracts with FC reagent was used for all analyses. All samples were analysed in three replicates and an average value of the results is presented.

Procedure for analysing the TPC using UV – Vis spectrophotometer

Gallic acid standards and CE were analysed on a UV – Vis spectrophotometer using blanks and control. In a 2 mL cuvette, $20 \mu\text{l}$ of the sample was pipetted with 1.58 mL of deionised water and $100 \mu\text{l}$ of Folin – Ciocalteu reagent. The solutions were mixed and allowed to rest for five minutes after which $300 \mu\text{L}$ of 20% sodium carbonate solution. The solution was mixed with a pipette and left at 20°C for two hours in the dark. The absorbance of each solution was recorded at 765 nm against the blank. The standard curve was used to calculate TPC in cocoa samples.

Statistical analysis

Statistical analysis of experimental data and drying model was performed using Minitab (Minitab 18, USA) and MS Excel®. All data were reported as the mean (μ) \pm standard deviation (σ) from replicates used in the analysis. Experimental data was compared for differences in means using one – way ANOVA with a general linear method (GLM). Means were regarded as statistically significantly different if the probability that they were equal was $<5\%$.

Results and discussions

The fermentation quality of cocoa beans in the Fiji Islands is often assessed visually by farmers using a cut test. The farmers also estimate the moisture content by pressing

the cocoa beans between their fingers to detect wetness from the beans. Cocoa processors in Fiji also use the cut test but they have portable moisture meters for moisture analysis. The cut test used by farmers and cocoa processors to determine fermentation quality are subject to bias and variability caused by human error. The key factor that controls fermentation quality is the temperature during the fermentation process.

In Fiji, cocoa fermentation is a spontaneous process that involves naturally present microbes. Yeast and bacterial fermentation of cocoa beans produce heat, which increases the temperature of the bean mass to 54°C (Reyes De Corcuera et al. 2020). Temperature is a critical factor in the fermentation process as it controls the biochemical reactions that develop flavour and aroma precursors. Cocoa beans dried between rainy and dry seasons showed a slight difference in temperature on the fourth day of fermentation but this difference was not significant ($P > 0.05$). Fermentation conditions during the rainy season were cooler because of the slightly lower ambient air temperature when compared to the dry season conditions (Figure 1). Under rainy conditions, the temperature of the cocoa bean mass only increased to $31.7 \pm 0.6^\circ\text{C}$ on the fourth day of fermentation. In comparison, cocoa beans fermented during the dry season experienced an increase in temperature to $40 \pm 0.3^\circ\text{C}$ (Figure 2).

The low temperature during fermentation under rainy conditions could mean that the acidification did not occur as the temperature conditions were not favourable for acetic acid bacteria (AAB). Microbial enzymes catalyze biochemical reactions and also cause acidification. These processes have a strong influence on the quality of cocoa beans (Niemek et al. 2014). While yeasts and lactic acid bacteria produce ethanol, AAB produces acetic acid from ethanol through an exothermic pathway (De Vuyst and Weckx 2016).

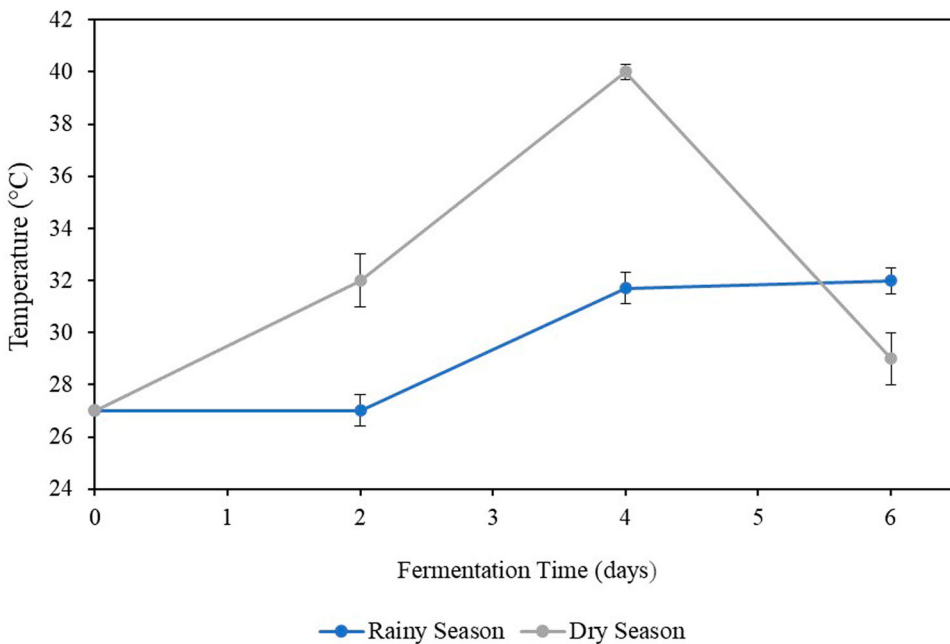


Figure 1. Average temperature profile of ambient conditions during fermentation of cocoa beans in rainy and dry season. Error bars shown on the graph are standard deviations of the mean values.

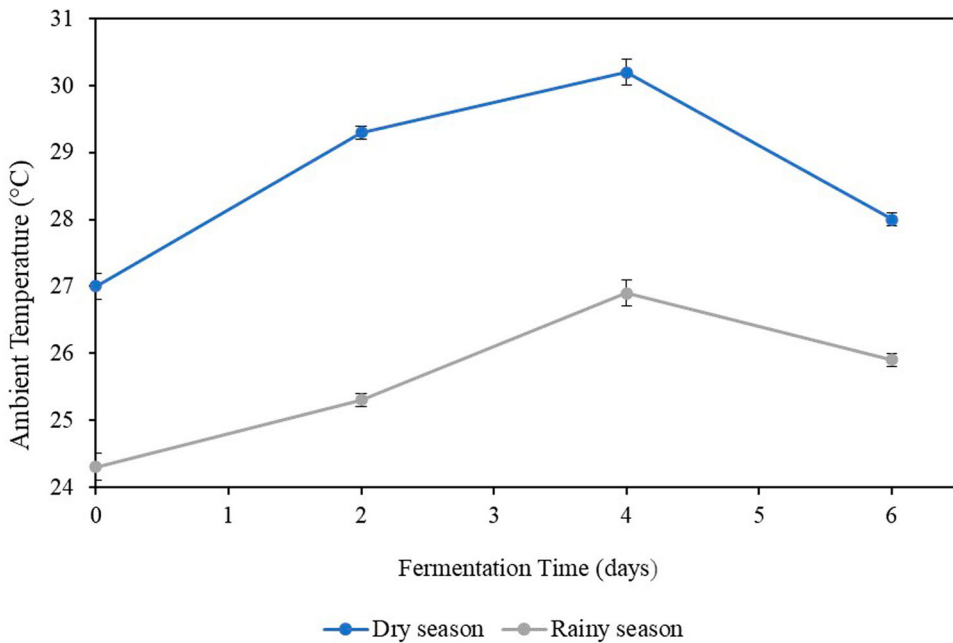


Figure 2. Average temperature profiles of cocoa beans during fermentation under rainy season and dry season. The error bars are the standard deviation of the mean values.

This reaction produces heat and increases the temperature of the cocoa bean mass. The delay in acidification increases the risk of contamination with pathogenic bacillus and mycotoxic fungi (Schwan and Wheals 2004; Lima et al. 2011). These pathogenic bacteria and microbes increase the risk of spoilage (Schwan and Wheals 2004). The batch of cocoa beans fermented during the rainy season had a 30% loss of product from spoilage while losses were lower during the dry season (Figure 3).

Cut test

The cut test is a highly recommended quality control tool for the fermentation of cocoa beans (Shamsuddin and Dimick 1986). This procedure is used by farmers and cocoa processors to monitor colour changes inside the cocoa beans during fermentation. Well-fermented cocoa beans should ideally change colour from purple to either brown or pale colour (Niemenak et al. 2014). Figure 4 shows the different colour profiles used to categorise fermented cocoa beans. The natural purple colour of the cotyledons is from the presence of polyphenols (Elwers et al. 2010). These polyphenols are released from the cells during acidification after 96 hours of fermentation, which causes the cell walls to lyse (Lopez and Dimick 1995). The polyphenols are oxidised to dark-coloured compounds that impart a brown colour to fully fermented cocoa beans. Oxidation of polyphenols also reduces astringency and bitterness in cocoa beans.

The cut test results for cocoa beans fermented for seven days during the rainy season and dry seasons in Suva are presented in Table 3. The cut test score for cocoa beans fermented during the rainy season was lower when compared to cocoa beans from the dry

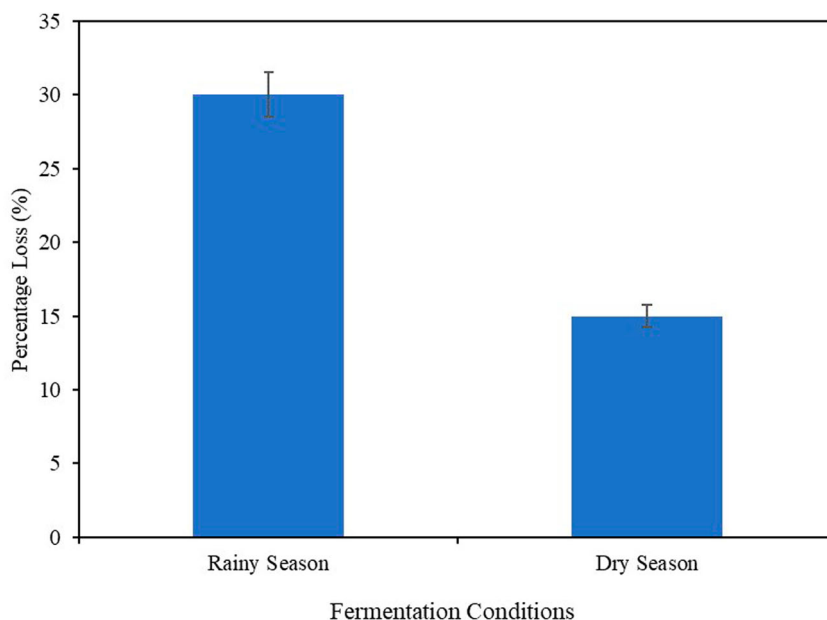


Figure 3. Percentage loss in cocoa beans during fermentation conditions at two harvest seasons. The percentage loss is an average of three batches that were fermented in one harvest season. Error bars show the percentage error of the mean values at 5%.

Table 3. Cut test score of cocoa beans from different harvest seasons.

Harvest Period	Percentage of Fermented beans (%)	Percentage of Unfermented beans (%)
Rainy Season	63 ^a	37
Dry Season	96 ^b	4

season. The cooler temperature conditions during the rainy season may have caused a significantly lower cut test score of 63% for cocoa beans when compared to cocoa beans fermented during the dry season, which was 96% ($P < 0.05$). While a cut test score of 60% and above indicates good quality fermentation, a higher score is an indicator of better browning from oxidation reactions (Niemenak et al. 2014). The fermentation conditions during the dry season were better for the fermentation of cocoa beans when compared to the cocoa beans harvested during the rainy season.

Fermentation index

Another technique to analyse the quality of cocoa beans is by testing for the fermentation index (FI) (Shamsuddin and Dimick 1986; Takrama et al. 2006). This laboratory – based test measures the intensity of brown colour development in methanolic cocoa extracts (CE). Ideally, fully fermented and dried cocoa beans should have an FI of more than one. The average FI values for fully fermented and freeze – dried cocoa beans of the Forastero varieties during rainy and dry seasons are presented in Table 4. While cooler



Figure 4. A sample of cocoa beans from day seven of fermentation used for cut test. A copy of a standard colour profile is given as a reference to the colour profile of the beans.

conditions during the rainy season were unfavourable for the fermentation process, there is evidence of further oxidation of anthocyanins and polyphenols, which may have slightly increased the FI to one. This value is significantly lower than the FI of cocoa beans fermented during the dry harvest season ($P > 0.05$), as shown in Table 4. Fermentation conditions for cocoa beans during the dry season were better for the oxidation of polyphenolic compounds. The results indicate that the tropical conditions in Fiji are favourable for fermentation as the FI values for both rainy and dry harvest seasons were comparable to studies by Romero-Cortes et al. (2013) and Racine et al. (2019).

In addition to FI, another effective technique to monitor fermentation quality is to measure the pH of cocoa beans. Microbial enzymes and secondary metabolites during fermentation causes changes in the cocoa bean pH. Acidification is one example where acetic acid accumulation in the cotyledon changes the cocoa bean pH, which

Table 4. Fermentation index of fermented Fijian *Theobroma cacao* beans (var. Forastero) after seven days of fermentation.

Harvest Season	Batch No.	Fermentation Time (h)	Fermentation Index
Rainy season	1	168	1.04 ^a ± 0.01
Dry season	2	168	1.39 ^b ± 0.02

affects the activity of endogenous enzymes. These enzymes affect the development of flavour and aroma precursors. The ideal pH for fermented and dried cocoa beans should be five or between 4.75 and 5.19, as this creates a better aroma profile during downstream processing (Biehl et al. 1982; Ofofu-Ansah et al. 2013).

During fermentation, there was a notable decrease in the average pH of cocoa beans after 96 hours for all harvest seasons (Table 5). This decline in pH indicates that there are acids produced by lactic acid bacteria and acetic acid bacteria. The production of acetic acid increases the bean temperature, which devitalises the embryo. This is a critical factor in terminating incipient germination and affects the quality of fermented cocoa beans (Biehl et al. 1982). Acidification is critical to the production of chocolate flavour and aroma profile in cocoa beans during downstream processing as the low pH activates endogenous enzymes, which metabolise proteins, polyphenols, lipids, and polysaccharides in the cotyledon (Biehl et al. 1982; Schwan and Wheals 2004; De Vuyst and Weckx 2016).

Total polyphenol content in cocoa beans

Theobroma cacao beans are naturally high in polyphenol content, which is about 12–18% of dry bean weight (Elwers et al. 2010). The polyphenol content in cacao is dependent on cultivar, maturity, harvest, geographic origin, and processing techniques (Wollgast and Anklam 2000). Other factors, such as genetic diversity and growth conditions, such as light intensity, temperature, relative humidity, fertilisers, soil, and stress also influence polyphenol content (Gil 2012). In fresh cacao beans, polyphenolic compounds remain inside the parenchyma. During fermentation, the cell membrane disintegrates, which causes the polyphenols to leach out with the cellular fluid (De Vuyst and Weckx 2016). Oxidation of polyphenols by polyphenol oxidase (PPO) darkens the colour of cocoa beans, which is a good indicator of high – quality fermentation.

A notable decline in the total extractable polyphenol content (TPC) in cocoa beans fermented during the dry harvest season was observed. The TPC declined from 68 mg

Table 5. pH and titratable acidity during fermentation process of *Theobroma cacao* beans from wet and dry harvest seasons.

Fermentation Time (h)	pH	Total titratable acidity
0	4.39 ^a ± 0.89	–
96	3.70 ^b ± 0.34	0.14 ^a ± 0.02
168	5.61 ^a ± 0.31	0.37 ^b ± 0.04

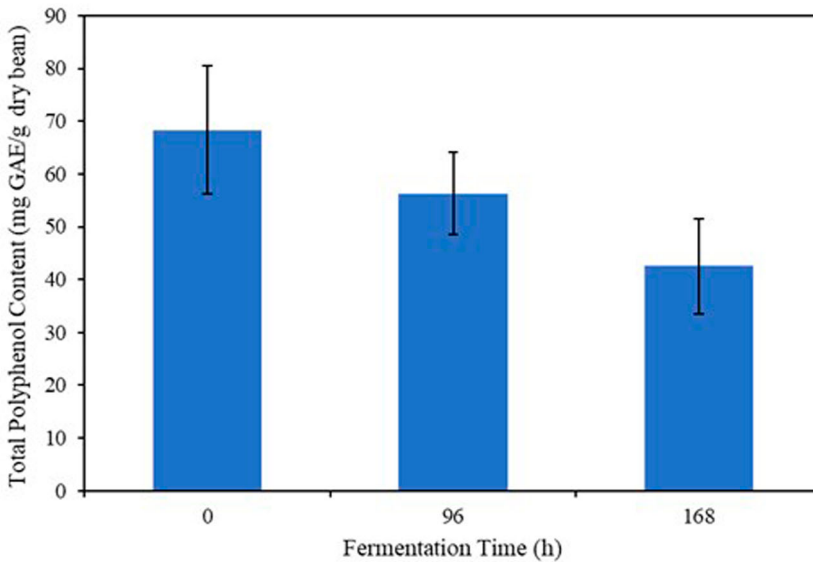


Figure 5. Changes in the total polyphenol content of *Theobroma cacao* beans during fermentation under dry harvest season. The error bars show the standard deviation of the mean values.

GAE – g dry bean at 96 hours of fermentation to 57 mg GAE – g dry bean at the end of fermentation (Figure 5). Cocoa polyphenols are temperature sensitive and the increase in the temperature during acidification at 96 hours of fermentation could have been one factor that affected the TPC (Mizobusti et al. 2010; Abhay et al. 2016; Teh et al. 2016; Menon et al. 2017). The fermentation temperature is also favourable for PPO enzymes that are active between 20–50°C (Mizobusti et al. 2010). Oxidation of polyphenols reduces the astringent and bitter flavour in cocoa beans.

Conclusion

The cocoa sector in Fiji needs to improve the postharvest processing techniques and technology to produce high – quality cocoa beans for export consistently. Currently, the key quality parameters for dried cocoa beans sold to export markets are moisture content and bean count. Bean count is used for grading cocoa beans, and this determines the price of the dried product. Another factor is the absence of odour in dried cocoa beans. This problem arises during the harvest in the rainy season when fermentation and drying are slow and product wastage from mould and insect infestation increases. Some farmers burn biomass for rapid drying during the rainy weather. Smoke from burning biomass taints the cocoa beans, which is rejected by premium quality exporters. Smoke – tainted cocoa beans are sold to bulk grinding markets at a lower price.

The fermentation process under rainy conditions is quite slow because of cool conditions. Rainy weather with cool conditions can lengthen fermentation time and increase the likelihood of insect and larval infestation and product loss. Traditional methods of fermentation are not effective during the rainy season. Therefore, there is a need to

explore other options to control fermentation quality. Some key changes noted during fermentation were:

- (1) Decline in pH after 96 hours of fermentation.
- (2) Reduction in bean mass by 50–60%.
- (3) Darkening of bean colour to brown or reddish brown.
- (4) Increase in cocoa bean temperature.

Fermentation in Fiji is highly dependent on natural conditions and cocoa processors often use bean count and cut tests to monitor the quality of cocoa beans during fermentation and sale of dry beans. Fiji does not have cocoa quality standards and processing guidelines for Fijian farmers. Standard postharvest protocols and training can ensure consistency in the quality of Fijian cocoa beans. While moisture content and bean count are indicators for grading cocoa beans, there are no set guidelines for the bioactive quality, which influences the development of flavour precursors during downstream processing. Better control of fermentation conditions can improve fermentation time and the quality of bioactive constituents. The cut test is a standard practice used during fermentation to monitor the extent of polyphenol oxidation but a more accurate analysis of fermentation quality can be provided by testing for the FI. The study recommends proper training on postharvest handling techniques for Fijian cocoa farmers and processors to improve the postharvest processing conditions and the quality of cocoa beans for premium quality chocolate. Experimentation with different methods of fermentation and drying is recommended for future work in Fiji.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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