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PRODUCTION AND QUALITY CHARACTERIZATION OF PULP FROM COCOA BEANS FROM TRINIDAD: EFFECTS OF VARYING LEVELS OF PULP ON VALUE-ADDED CARBONATED COCOA BEVERAGES

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ABSTRACT: The cocoa industry in Trinidad and Tobago has experienced a steady decline in production of cocoa over the past 50 years. There is a need to add value to cocoa by developing downstream products such as cocoa beverages. While the cocoa pulp (mucilage), covering the seed coat (testa), has a crucial role in fermentation, if present in excess, it may also hinder the same process. The objectives of this investigation were therefore to develop an acceptable cocoa beverage using surplus cocoa pulp and (1) determine the difference in physicochemical (nutritional) properties between cocoa mucilage (pulp) and liquor made from cocoa nibs and (2) investigate the effects of adding varying amounts of mucilage (10 and 15 g per litre) with a standard quantity of liquor on the sensory acceptability and microbiological quality of carbonated cocoa beverages. Cocoa pods and dried and fermented beans were collected over a period of three months in 2012. The cocoa pulp and seed coat mucilage were extracted and freeze dried. The cocoa beans were made into liquor. Crude protein, % ether extract (crude fat), pH and micro nutrients, Cd, Cu, Na, K, Mn, Zn, Mg and Fe in the cocoa liquor and pulp were analysed. Two carbonated beverages were produced with varying levels of pulp (10 and 15g, respectively) and evaluated for sensory acceptance using a nine-point hedonic scale. Microbial analyses for total aerobic counts, yeast and molds, *Escherichia coli*, in the cocoa pod pulp and dried and fermented beans were conducted. Colour, pH and total soluble solids were measured on both the cocoa pulp and beverage. The data were analyzed using SPSS 16.There were significant differences (P <0.01) between cocoa liquor and cocoa pulp in crude protein and crude fat with cocoa liquor being significantly higher in both. Micro-nutrients of trace minerals showed a significant difference (P <0.005) between the cocoa liquor and pulp in six of the nine samples examined. Cocoa liquor had significantly higher ppm levels (wet wt) for trace minerals than cocoa pulp respectively for Fe (P<0.05: 11.97 vs 2.42), Zn (P<0.0001:25.19 vs 3.29), Na (P<0.01:81.46 vs 49.03), K (P<0.05: 2206.8 vs 853.10) and Mg (P<0.0001: 816.02 vs 153.67), but was lower for Mn (P<0.05: 0.66 vs 0.96). The pH of cocoa pulp was significantly lower (P <0.001) than that of the cocoa liquor. There was no significant difference in sensory qualities between the two cocoa carbonated beverages, which were both "liked slightly" in terms of acceptability. Microbial growth of 10^{-3} CFU/g was only observed on dried and fermented beans. A carbonated beverage with 10 grams of cocoa pulp per litre is recommended for commercialization in the food industry.

Keywords: Cocoa mucilage, cocoa pulp, cocoa liquor, value-addition, sensory acceptability, beverage, nutritional properties.

Introduction

Trinidad and Tobago (T&T) is the birthplace of the Trinitario cacao[4]. The latter resulted due to cross-breeding (hybridisation) between Criollo cacao, introduced to the island by the Spanish circa 1525 (Shephard, 1932), with Forastero cacao, which was introduced from Venezuela in 1757 (Bekele, 2004). The T&T cocoa industry is based on the production of Trinitario cocoa, known for its outstanding flavour characteristics. Cocoa dominated the local economy between 1866 and 1920, but has experienced a steady decline in cocoa production over the past 50 years. It was reported that 914.8 tonnes of cocoa were produced in T&T in 2005, but this dropped to 408.43 tonnes by 2011 (Maurice, 2012). There have been various factors which contributed to this decline, including environmental, disease and pest and socio-economic ones (Bekele, 2004). Industrialization in T&T has shifted interest from agriculture to oil-based production and downstream industries. By the 1970's, the shortage of labour for agriculture had become a serious liability for the cocoa industry. Over the last four decades, cocoa production, exports, acreage under cultivation and farmer participation in T&T have been declining steadily.

Despite increasing industrial and manufacturing activity in T&T, there has been little local development of the primary product, cocoa beans, into secondary and tertiary products. Thus, government agencies and non-governmental organizations are using several incentive programmes, which can help develop research, production and value addition initiatives in the cocoa industry.

One of the main pillars to support the revitalization of cocoa production in T&T is the island's designation by the International Cocoa Organisation (ICCO) as a 100% (exclusive) producer of superior quality, fine or flavour cocoa. Trinidad Selected Hybrids (TSH) and remnant Imperial College Selections (Johnson et al., 2004) comprise the planting material used on local estates, and this ensures the production of superior Trinitario fine or flavour cocoa beans. Trinidad and Tobago is one of five Caribbean islands that produce and export exclusively fine or flavour cocoa as determined by the ICCO.

Both the sensory and physical characteristics of the Trinitario bean contribute to the superior quality. Some sensory characteristics of the liquors produced from local cocoa include fruity (brown raisin), molasses, caramel, winey, nutty, cocoa and aromatic notes (Bekele, 2004). Some characters/flavour notes have also been deemed excellent at the *Salon du Chocolat*, an international chocolate competition held in France annually. The notes that were awarded prizes at this event include spicy in 2011, fruity and floral in 2010, sweetness and fruity in 2009 (Trinidad Guardian, 2011).

The physical attributes of the beans produced locally and sold must meet certain criteria such as there must be no more than 1% defective or broken beans and at least 90-95 beans per 100g of cocoa. The moisture content of the bean must be no more than 7 – 8 %. Since T&T's cocoa usually meets these criteria, local farmers and the Cocoa and

[4] The word *cacao* refers to the tree and its parts, and the word *cocoa* to the products of manufacture.

Coffee Industry Board of T&T, which markets cocoa on their behalf, are able to negotiate favourable (premium) cocoa prices per tonne with international buyers. Currently, mainly large international chocolate manufacturers purchase and transform these cocoa beans into high value, secondary and tertiary products.

Health and Nutritional Benefits of Cocoa

Nutritional research into cocoa has shown the benefits of cocoa and dark chocolate as it relates to lifestyle diseases such as cardiovascular disease (CVD) and type II diabetes (Borchers et al., 2000; Cesar, 2005; Mellor et al., 2010; Scotsman, 2012; Badrie et al., 2013; Jegtvig 2013). This information can be leveraged in the marketing of fine or flavour cocoa, which is used to produce dark chocolates with 65 to 85 and even 99% cocoa solids. It has and will continue to stimulate demand for fine or flavour cocoa. The production of fine or flavour cocoa products locally and regionally can therefore contribute significantly to revenue generation.

The cacao tree's fruits (pods) and seeds (beans) contain high levels of polyphenols (Lee et al., 2003). The antioxidant phenolic phytochemicals in cocoa are used as chemo-preventative agents against oxidative stress damage, which can cause cancer, heart disease, hypertension and other illness. The concentration of polyphenols in cocoa products is dependent on several factors, an example of which is the processing of cocoa and the concentration of cocoa solids in cocoa products like chocolate (WHO, 1996).

Dark chocolate with 60 - 70% of cocoa solids has qualities, which help to improve glucose metabolism by increasing insulin sensitivity and it also decreases blood pressure (Lee et al., 2003). Daily intakes of only 6.3 g of dark chocolate can reduce BP and increase vasodilative Nitrous Oxide (NO), and research suggests that cocoa products can be reasonably incorporated into a dietary approach to lower CVD risk (Taubert, 2007). Further research suggests a potential value in developing flavanol-rich, low-energy cocoa foods, beverages, and supplements, which can be used daily to obtain the aforementioned benefits (Grassi, 2008). Dark chocolate and cocoa powder have been shown to contain high levels of the monomeric flavanols (-)-epicatechin, and (+)- catechin (Lee et al., 2003).

With regard to the nutritional value of cocoa, the USDA (in 2012) indicated that per 100 grams of cocoa powder there are 6.67 grams of protein, 4 grams of fat, and 83.73 grams of carbohydrates. Cocoa powder also has a pH which ranges from 5.4 - 5.8 and may vary according to processing. High levels of the macro-nutrients magnesium, potassium, phosphorous and sodium are present in cocoa powder, and the USDA expressed these as 83, 712, 315, 504 mg /100g, respectively. Micro-nutrients zinc and iron are expressed as 1.46 and 1.19 mg /100g, respectively (USDA 2012).

Promoting Sustainability of the Cocoa Industry Through Diversification and Value-Addition

In the Caribbean, one of the main strategies of promoting sustainability within the cocoa industry is the involvement of the islands' entrepreneurs in the development of local

value added production (Sukha, 2004). Jamaica, Grenada and T&T have participated in such value added production by producing drinking chocolates and gourmet dark chocolates for local markets. The awards bestowed on chocolates from T&T at the *Salon du Chocolate* in Paris have inspired and motivated local entrepreneurs to diversify into dark chocolate (eating and drinking) production and to continue to deliver the Caribbean fine or flavour cocoa mark to both local and foreign buyers and chocolate manufacturers.

Several researchers and manufacturers worldwide have diversified the use of cocoa by developing by-products such as cocoa butter, which has been used for making chocolate, pharmaceuticals and cosmetics, as well as wines, juices, gums and jellies (Figueira et al., 1993; Sukha, 2004; Dias et al, 2007). The white mucilage covering the beans within cocoa pods is known as cocoa pulp. This mucilage/pulp can also be used to add value to the cocoa industry as it is normally is in excess of what is required for proper fermentation of cocoa, a post-harvest process that is critical for proper flavour development. Surplus pulp often drains out of the fermenting mass in the "sweatings".

The pulp is normally sweet and helps develop the colour, aroma, and flavour during fermentation by introducing different microbes such as yeast and bacteria (Nielsen et al., 2007). The flavour is developed in a manner similar to beer and wines, as ethanol, lactic acid and acetic acid are by-products of the yeast and bacteria (*Saccharomyces cereviciae* and *Hanseniaspora guillermondii)* fermentation as well as heat, which reaches 50°C thus helping to kill the bean. This process has a role, along with proper drying of the beans, in producing flavour and aroma precursors.

Cocoa juice contained nutrients such as sugar, minerals fat and protein (Anvoh et al., 2009). The cocoa juice has a soluble solids of 12.5° Brix. The fat and protein content of the cocoa juice is 3.12% and 3 - 5%, respectively. The pH of cocoa pulp has been found to be lower than that of cocoa liquor; 3.78. This is due to the acetic and lactic acid levels in the mucilage (Anvoh, 2009).

Cocoa trace elements can have both positive and negative biochemical effects on humans. Generally, trace minerals are generally needed in quantities of only a few milligrams (mg) or micrograms (mcg) per day. The World Health Organization (WHO, 1996) reported on the nutritional significance, requirements for health, and safe range of daily intake for nineteen trace elements in three categories. These include essential elements such as copper (2 mg/day) and zinc (15 mg/day), probable essential elements such as manganese (5 mg/day) and potentially toxic elements, such as cadmium and mercury. An example of such trace elements and their positive functions are zinc in antioxidant properties and magnesium, which is involved in muscle function, iron (15 mg/day) in red blood count (RBC) formation and copper, which is essential for RBC formation and connective tissue formation.

Objective of This Study

Cocoa pulp juice or beverages are not produced and utilized locally as they are in countries such as Ghana, Brazil, Costa Rica and Malaysia (Anvoh et al., 2009) despite the fact that surplus pulp is a waste product and may even be considered a nuisance in

the local cocoa industry since the TSH planting material typically produces much cocoa pulp. This fact motivated the authors to develop a carbonated cocoa pulp beverage for local consumption to diversify and add value to the cocoa industry as well as to serve as a healthy alternative to low-nutrient sodas for consumption by the populace. Therefore, determining the nutrient content of the pulp of Trinidad Selected Hybrids and developing a consumer-acceptable beverage, which contains a percentage of cocoa liquor and pulp, were undertaken during this pilot study.

Materials and Methods

Cocoa pulp extraction- Cocoa pods from trees of Trinidad Selected Hybrids (TSH) (Maharaj et al., 2011) were collected on the 21st of June, July and August, 2012 (over a 3-month period) from the Montserrat Cocoa Farmers Society Limited (MCFSL) Cooperative, Gran Couva. Figure 1 shows the steps in extraction of cocoa pulp. The cocoa pods were cracked and beans were separated into three bags and stored in a large *Ziplock* bag, then placed in the freezer (-18°C) for 24 hours. Approximately 180 - 190 grams of mucilage and seed coat were extracted from each bag to get a total weight ranging from 360 to 400 grams. The pulp was weighed then placed in the freezer for 24 hours. An Armfield I-Vacuum Freeze Drier (CPS 11962.1921, Armfield Company Limited, United Kingdom) was then used to freeze-dry the cocoa pulp. The freeze dried pulp was later ground into a powder using a coffee grinder (#40 sieve size) for further analysis.

Production of Cocoa Liquor

Approximately 330 g large, plump and undamaged beans were roasted at 145° C for 30 minutes. The beans were then cracked using a cocoa breaker. A pneumatic air-flow winnower built by Commodity Processing Systems Ltd., Colchester, Essex, England, was used to separate the cracked beans into seed nibs and the seed coats. A Proctor Silex E160BY Fresh Grind coffee grinder (Hamilton Beach Brands Inc., Virginia, United States of America) was used to further grind the cocoa nibs via nine one-second pulses and one final two-second pulse. The ground nibs were then gradually placed into a mill (Pascall Engineering Co Ltd, Gatwick Road Crawley Sussex, England, Machine No: 23615) or into a mechanical mortar and pestle and made into cocoa liquor over a period of 1 ½ hours. (Refer to Figure 2.) The liquor was then placed in covered cups and dated. The liquor and pulp were stored in a freezer at -04° C.

Processing of Cocoa Carbonated Drinks

Figure 3 shows the processing steps in the production of cocoa carbonated drinks. Two cocoa carbonated beverages were processed using two levels of cocoa pulp (10 g and 15 g). Using the 5-Log reduction performance standard, the mixtures were heated to 71.1° C for 15 seconds and then bottled as recommended by the U.S. Food and Drug Administration in 2011. The mixture was then filtered using a Whatman #54 filter paper. Carbon dioxide (CO_2) was added using a Soda stream (1017512018, SodaStream USA), and each drink received five 2 g CO_2 pulses (a total of 10 g of CO_2). Each carbonated cocoa beverage contained 4 g of cocoa liquor per litre and 1 g of vanilla per

litre. The total soluble solids of each drink ranged between 13 to 15 °Brix and the pH was between 4.5-4.6.

Analyses

pH

The pH of the cocoa pulp, cocoa liquor and cocoa carbonated beverages was measured electronically on a pH meter: Hach SensION™+ Wastewater pH Meter YO-59004-03Loveland, Colorado.

Total Soluble Solids

The total soluble solids as °Brix was measured on the cocoa pulp, cocoa liquor and cocoa carbonated beverages using an ATAGO Co, LTD pocket (PAL-1, Atago Co Ltd, Minato-ku, Tokyo, Japan)

Compositional Analyses

The cocoa beans were analysed for crude protein, % ether extract (crude fat) and nine trace minerals. Crude protein was measured with the Kjeldahl procedure, where a conversion factor of 6.25% was used in order to convert the nitrogen content to crude protein, ether extract by the high temperature solvent extraction and ash analysis at 600° C for 6 hours using the method of AOAC (1990). The nutrients cadmium (Cd), copper (Cu), sodium (Na), potassium (K), manganese (Mn), zinc (Zn), magnesium (Mg) iron (Fe) and Calcium (Ca) were measured by an atomic absorption spectrophotometer, AAnalyst™ 700 PerkinElmer, Inc. 940 Winter Street Waltham, MA 02451, USA.

Sensory Analysis

Two treatments of cocoa carbonated drinks were subjected to Hedonic testing. The sensory attributes were analysed for appearance, colour, taste, flavour, consistency and overall acceptability of the prototypes. Sensory evaluation was conducted at the *Spirit of Chocolate, 'Fête de la cocoa'*, November 2 -3, 2012 at the JFK Auditorium, the University of the West Indies, St. Augustine. The required sample size calculated with a 5% level of significance was 174 respondents ($n = \frac{Z\propto/_2\, \hat{p}\, \hat{q}}{d^2}$) where *n* is the number of individuals needed, *Z* relates the Confidence Interval, *P* is the population to favour the characteristics, Q is the population who do not favour the characteristics and d^2 is the margin of error. The selection of the consumer volunteer panelists was convenient and systematic. Panelists were asked to fill out a hedonic sensory questionnaire sheet. The Hedonic rating scale was according to Peryam and Pilgrim (1957).

Microbial Analysis

The microorganisms were analysed using potato dextrose agar (PDA) for yeasts and molds, plate count agar (PCA) for aerobic mesophiles and eosin methylene blue agar (EMBA) for *Escherichia coli* in cocoa pods and fermented and dried beans, dried pulp and cocoa liquor and the cocoa carbonated beverages (10 and 15 grams of cocoa pulp, respectively). *Staphylococcus aureus* was tested using Mannitol Salt Agar (MSA) on

cocoa pods and dried and fermented cocoa. MSA and EMB plates were incubated at 35° C for 48 hrs. PCA and PDA plates were incubated at 28° C for 24 hours.

Statistical Analysis

The physical, chemical and sensory data were analyzed using SPSS 16 for Windows. Various statistical tests were used to generate frequencies, calculate means, to compare and find associations using the Chi-square test. The Student's 't' test and ANOVA F test were used to determine differences in means. The SPSS software was also used to generate descriptive statistics, which included statistical tables, bar graphs and pie charts. All tests were conducted at the 5% level of significance ($P \leq 0.05$).

Results and Discussion

Pulp and liquor samples collected each month (June, July and August) were significantly different in % crude protein. For each month, the cocoa liquor contained significantly higher ($P < 0.0001$) % crude protein than cocoa pulp (dry wt basis) (Table 1).

In Table 2, data are presented for nine samples, collected over three months. The crude protein of cocoa liquor was 12.75g/100g (dry wt)/1.29g (wet wt) and for cocoa pulp was 5.16g /100 g (dry wt)/1.74 g (wet wt). Table 3 shows the mean scores for the % ether extract (crude fat, dry wt basis) were significantly different (P<0.001) for cocoa liquor and cocoa pulp over the three months, with higher crude fat % for cocoa liquor (dry wt basis) compared to cocoa pulp (dry wt basis). Table 4 shows a higher ether extract (dry wt basis) for cocoa liquor (59.14% versus cocoa pulp 21.82%). Table 5 shows that Fe, K, Na, Mg, Zn and Mn were significantly different in concentrations between cocoa liquor and pulp. Cocoa liquor had significantly higher levels (ppm, wet wt) for trace minerals than cocoa pulp respectively for Fe (P<0.05: 11.97 vs 2.42), Zn (P<0.0001:25.19 vs 3.29), Na (P<0.01:81.46 vs 49.03), K (P<0.05: 2206.8 vs 853.10) and Mg (P<0.0001: 816.02 vs 153.67), but was lower for Mn (P<0.05: 0.66 vs 0.96).The micronutrients for cocoa juice as measured by Anvoh et al. (2009) in mg/L (ppm) were compared to cocoa pulp for Ca (171.5 vs 162.0), Mg (82.5 vs 153.7), Na (30.5 vs 49.0) and K (950 vs 853).

Table 6 shows that the pH of cocoa liquour varied within the range of 5.1 -5.2. The pH of cocoa pulp samples was significantly (P<0.001) lower (3.6-3.9) when compared to the pH of cocoa liquour. Cocoa juice was reported as having a pH of 3.75 (Anvoh et al., 2009). Cocoa juice was found to be very acidic with a titratable acidity at 170±6.28 meq/L. Citric acid was the most predominant organic acid, with an average value of 9.14±0.64 mg/L. Malic and acetic acids contents were 3.6±0.5 mg/L and 2.28±0.7 mg/L, respectively (Anvoh et al. 2009).

Table 7 shows that the total soluble solids of cocoa pulp increased with maturity over the three months period (12.1-15.9° Brix). Cocoa juice had a reported total soluble solids value of 16.2° Brix. Table 8 reported no significant differences among the average mean sensory hedonic scores (5.62-6.72) and in individual sensory attribute between the two levels of cocoa pulp carbonated beverage. The sensory analysis of the two carbonated

cocoa pulp beverages revealed no significant differences for any sensory characteristic. This showed that the different pulp contents did not significantly affect the overall sensory characteristics.

The paired T test indicated significant ($P<0.001$) overall preference for the beverage with 15 grams of cocoa pulp (7 - moderate preference compared to the 10 gram addition of cocoa pulp (6 –liked slightly). Comparisons of the two beverages showed that appearance was favoured slightly more in sample with 10 g cocoa pulp with 49 persons choosing "like moderately" versus 44 persons choosing "like moderately" for sample 1 (15 g pulp). The colour of sample 2 also had slightly greater appeal as persons chose "liked moderately" 47 times versus 41 times for sample 1. The taste of sample 2 was also favoured. Flavour and consistency were observed to be "liked moderately" in the case of sample 1. Acceptability of both drinks was "liked moderately".

Table 8 (a) shows consumer preference for both drinks. Ninety-six persons stated their preference for drink (10 g cocoa pulp) and this was significantly more ($P < 0.0001$) than those who preferred carbonated beverage (15 g cocoa pulp). Table 9 shows that for the fermented beans, there were 7.0×10^4 CFU/g of yeasts and molds and 1.2×10^5 total aerobic microbes. These observations showed yeast and bacteria were only present in fermented beans at 10^3, which was supported by the findings of Nielsen et al. (2007).The microbial tests on both the carbonated beverages revealed no growth at 10^3, which indicate little cross contamination between the cocoa pulp and liquor in the conversion to carbonated drinks.

Discussion

Soluble solids, pH, brix, % crude protein, % ether extract by ether extractions and micro-nutrients: copper, zinc, iron and manganese and macro-nutrients: sodium, potassium, calcium and magnesium and cadmium of cocoa pulp from cocoa samples, collected in June, July and August, 2012, were measured in this study and compared to the values obtained for cocoa liquor. Furthermore, the content of micronutrients, determined for cocoa pulp, differed from that of the cocoa juice observed by Anvoh et al. (2009). This may be due to different methods of extraction and the fact that pulp as well as the seed wall was used in this experiment. Contents of Ca, Mg, Na and K were higher for cocoa pulp (Table 5) compared to the values reported for cocoa juice as determined by Anvoh et al. (2009). The values for Ca, Mg, Na and K (ppm), respectively, for cocoa juice were 171.5±34.01 ppm, 82.5±0.81, 30.5±3.77 and 950 ± 16.32 .

Based on the findings of this study, mean crude protein content in cocoa liquor samples was 12.75 g/100 g. This is almost twice the value reported by the USDA (2012) of 6.67 g/100g. The ether content (crude fat) of the liquor sample was 59.1%, which falls just above the range according to Encyclopedia Britannica (2012) of 52.5%. However, the ether content for the cocoa pulp obtained in this study was 21%.

USDA (2012) stated that the levels of the micro-nutrients Mg, K, P and Na in cocoa powder are 83, 712, 315, and 504 mg /100 g, respectively (Note: 1ppm = 0.1 mg/100 g) The findings for cocoa liquor in this experiment is different from that of the USDA (2012)

for cocoa powder. In cocoa liquor (dry wt), the values (mg/100 g) were lower for Mg 241.1, K 652.6 and Na 24.9 (Table 6) than for cocoa powder. USDA (2012) reported the pH level of the liquor to be 5.3 which were close to the values (pH 5.1-5.2) shown for cocoa liquor (Table 6).

The cadmium content (mg/kg) of cocoa liquor and cocoa pulp (wet wt basis) were 0.51 and 0.16 ppm, respectively, and dry wt basis of 1.50 and 1.61. These levels are well within the *provisional tolerable weekly intake* (PTWI) of Cd of 60-70 micrograms /kg wet weight (WHO 1992). Cocoa liquor had significantly higher ppm levels (wet wt) for trace minerals than cocoa pulp respectively for Fe ($P<0.05$: 11.97 vs 2.42), Zn ($P<0.0001$:25.19 vs 3.29), Na ($P<0.01$:81.46 vs 49.03), K ($P<0.05$: 2206.8 vs 853.10) and Mg ($P<0.0001$: 816.02 vs 153.67), but was lower for Mn ($P<0.05$: 0.66 vs 0.96).

Both % crude protein (dry wt) and crude fat content g/100g in cocoa pulp and liquor varied significantly ($P < 0.0001$) with cocoa liquor having higher mean scores of 12.1 and 59, respectively. Each month, the difference between the two samples was significant at $P < 0.05$ for both % crude protein and % ether extract (crude fat), and cocoa liquor contained greater % crude protein and % ether extract throughout the three months. pH values for the two samples differed significantly ($P = 0.001$). Cocoa pulp had a mean score for pH of 3.7 and cocoa liquor 5.7.

Following the processing and extraction of cocoa pulp to be freeze dried, there was no observed microbial growth, therefore, contamination was limited by processing (drying). The cocoa liquor also showed no microbial growth at 10^3, therefore, processing has limited the cross contamination of the yeast and molds during conversion from cocoa beans to cocoa liquor.

Conclusion

The overall aims and objectives of this study were to determine the difference between cocoa pulp and liquor over three months by using nutritional, physical and chemical testing and also to develop and test the physical and sensory properties and acceptability of a combined cocoa pulp and liquor soda made from beans collected each month from June to August, 2012.

From the analysis of the various tests, it can be concluded that a link was established between the results of this research project and those of authors such as Anvoh et al. (2009) and the USDA (2012). Due to the type of tests carried out, there was also a difference in some of the results obtained here relative to those obtained by the aforementioned authors.

The results demonstrate that cocoa pulp is nutritious, comparable to cocoa liquor, as indicated by the micro-nutrient content of potassium. The latter is known to be high in cocoa liquor and chocolate, but this experiment showed levels in pulp exceeding those of cocoa liquor.

The cadmium content (mg/kg) of cocoa liquor and cocoa pulp (wet wt basis) were 0.51 and 0.16 ppm, respectively, and dry wt basis of 1.50 and 1.61 (Table 5). The World Health Organisation (WHO, 1992) has established a provisional tolerable weekly intake

(PTWI) of 7 µg/kg body weight. This PTWI value corresponds to a daily tolerable intake level of 70 µg of cadmium for the average 70-kg man and 60 µg of cadmium per day for the average 60-kg woman. The cocoa nibs from some areas of Trinidad and Tobago were found to be unlikely to meet food safety standards for cadmium in chocolates and other cocoa products, if >50% cocoa solids are used in such products (Ramatahal, 2013). Cadmiun concentrations in the beans were found to be weakly positively correlated with the concentration of total Cd in the soil in Peru (Crozier, 2012). Cocoa liquor was found to be a better source of crude fat (% ether extract) and crude protein compared to cocoa pulp.

The potential for the commercialization of new cocoa beverage products was clearly demonstrated by this preliminary research as, generally, people liked the carbonated drinks produced. This was evident from the observed mean sensory scores of 6. However, they preferred less pulp (10 g) in the carbonated drink as indicated by the overall sensory evaluation and general preference stated.

The processing of the drink was shown to have reduced the levels of microbial growth. This result indicates that the process of producing the drink was safe and limits microbial contamination. Therefore, the production of a carbonated cocoa beverage, made from cocoa pulp with added cocoa liquor, may have a positive impact on revenue generation in the cocoa industry. It involves the conversion of a waste product (cocoa pulp) into a nutritious, marketable and valuable commodity that was generally accepted by the sample population surveyed during this pilot study.

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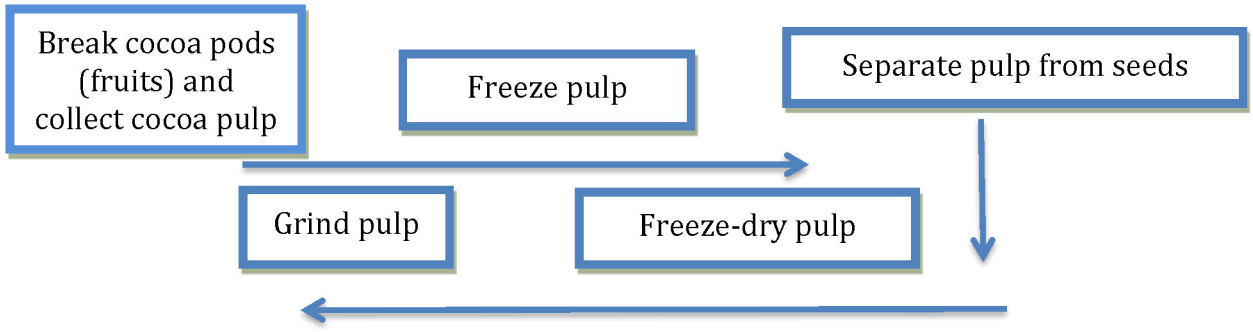

Figure 1. Extraction of cocoa pulp during this study.

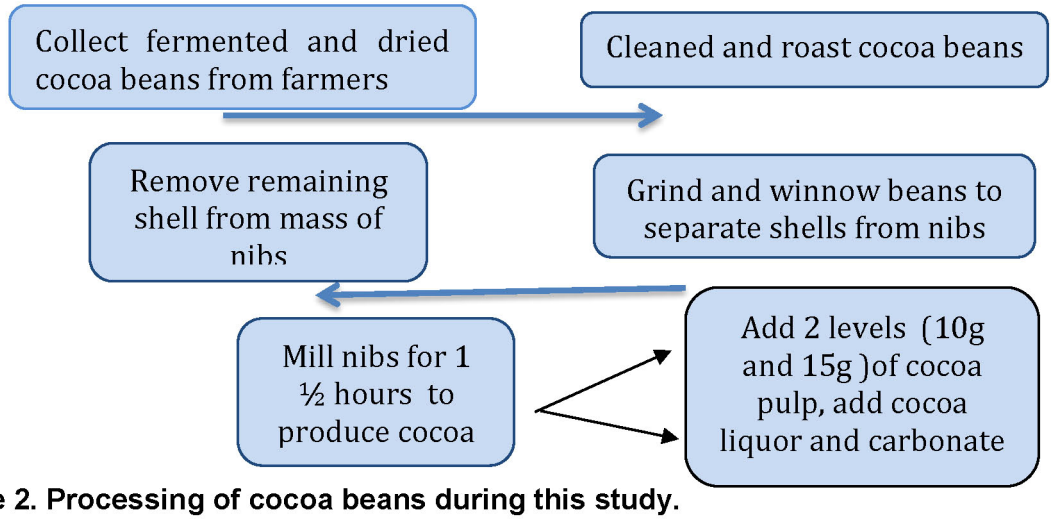

Figure 2. Processing of cocoa beans during this study.

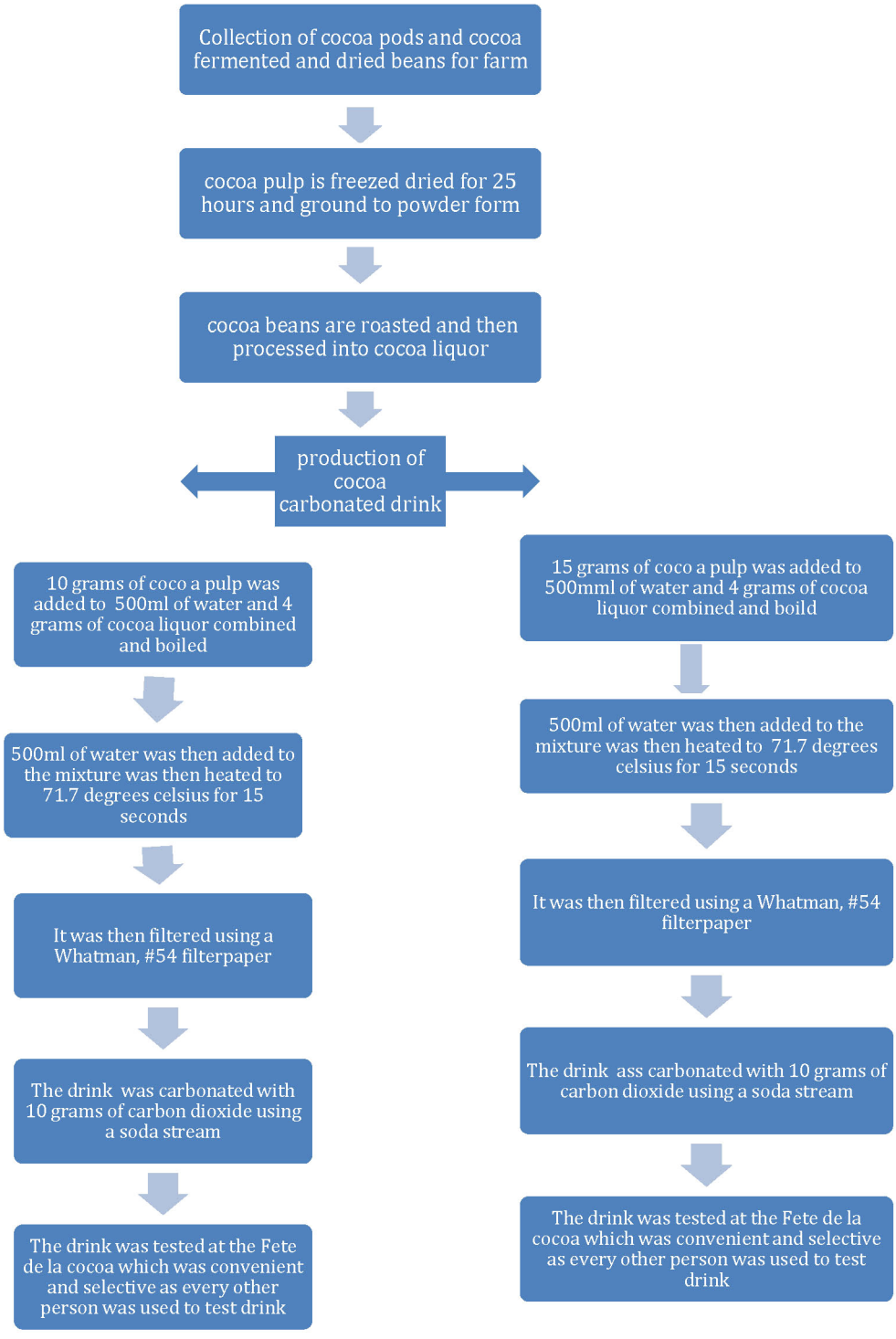

Figure 3. Production of the carbonated cocoa beverages.

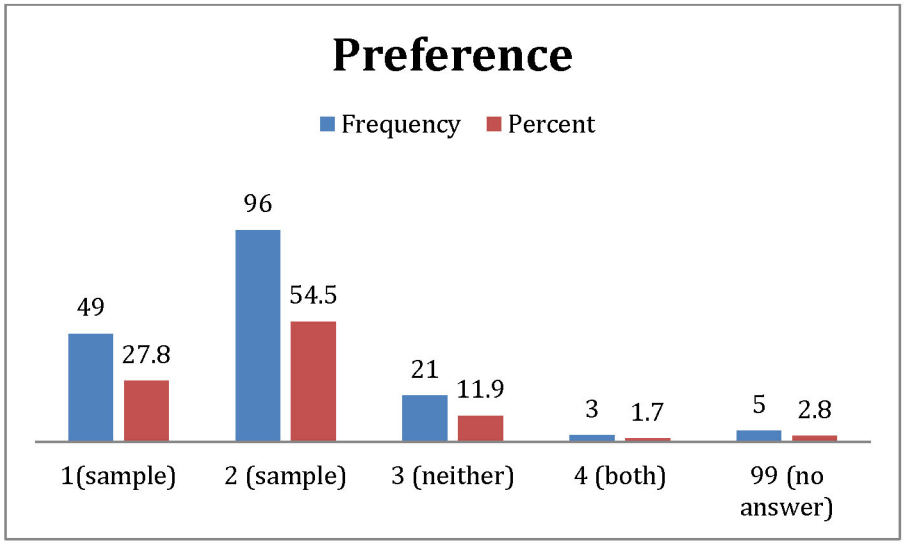

Figure 4. Frequency and preference for the cocoa beverages.

Table 1. Means and standard deviations (SD) and significance in difference of % crude protein each month between cocoa liquor and pulp (dry weight basis).

Samples	June	July	August
	Crude protein mean,% dry wt ± (SD) and wet wt	Crude protein mean,% dry wt ± (SD) and wet wt	% Crude protein mean, % ± SD and wet wt
Pulp	5.9 (0.45) 2.00	4.39 (0.17) 1.48	5.17 (.34) 1.75
Liquor	12.6 (0.35) 1.28	12.5 (0.6) 1.27	13.1 (0.07) 1.43
P-values	0.0001***	0.0001***	0.0001***

***Significant at the 99.99% level
(moisture content of cocoa pulp = 74.73% and cocoa liquour = 9.19%)

Table 2. Crude protein and the difference in cocoa pulp and liquor over the three months (dry weight basis)

Samples	# of samples	Crude protein mean, dry wt mean% ± (SD)	F-value	*P*-value
Pulp	9	5.16 (0.69)	724.57	0.0001***
Liquor	9	12.75 (0.49)		

*** Significant at the 99.99% level

Table 3. Crude fat in cocoa liquor and pulp for each month

Samples: June	% crude fat dry wt mean (SD)	F value	*P*-value
Pulp	29.27 (1.73)	638.8	0.000***
Liquor	60.58 (1.18)		
Samples: July			
Pulp	15.85 (1.80)	256.7	0.001**
Liquor	55.60 (3.05)		
Sample: August			
Pulp	18.27 (4.67)	242.8	0.0001***
Liquor	61.23 (1.01)		

*** Significant at the 99.99% level
**Significant at the 99% level
(moisture content of cocoa pulp = 74.73% and cocoa liquour = 9.19%)

In Table 3, the mean scores afor the % ether extract (crude fat, dry wt basis) were significantly different (P<0.001) for cocoa liquor and cocoa pulp over the three months.

Table 4. Mean Crude Fat (%) Extract between Cocoa Pulp and Cocoa Liquour.

Samples	# of samples	% Crude Fat SD	F-value	*P*-value
Pulp	9	21.82 (6.67)	229.8	0.0001***
Liquor	9	59.14 (3.18)		

***** Significant at the 99.99% level**

Table 5. Differences in trace minerals in cocoa liquour and cocoa pulp.

Trace mineral	Cocoa liquor dry wt means, ppm (wet wt)	Cocoa pulp dry wt means, ppm (wet wt)	*P*-value
Cadmium (Cd)	1.50 (0.51)	1.61 (0.16)	0.875
Iron (Fe)	35.4 (11.97)	23.97 (2.42)	0.018*
Copper (Cu)	23.4 (7.91)	21.67 (2.19)	0.72
Manganese (Mn)	1.94 (0.66)	9.46 (0.96)	0.035*
Zinc (Zn)	74.5 (25.19)	32.49 (3.29)	0.0001***
Sodium (Na)	240.9 (81.46)	484.4 (49.03)	0.003*
Potassium (K)	6526 (2206.8)	8427.0 (853.10)	0.046*
Calcium (Cd)	1363 (460.65)	1600.0 (161.97)	0.29
Magnesium (Mg)	2414 (816.02)	1518.0 (153.67)	0.0001***

*** Significant at the 99.99% level
*Significant at the 95% level

Table 6. pH values of cocoa liquor and cocoa pulp.

Sample	pH (average)
June cocoa liquor	5.2
July cocoa liquor	5.1
August cocoa liquor	5.3
June cocoa pulp	3.6
July cocoa pulp	3.9
August cocoa pulp	3.9
10 gram cocoa pulp beverage	4.6
15 gram cocoa pulp beverage	4.5

Table 7. Total soluble solids of cocoa pulp over a 3 month period and carbonated cocoa pulp beverage.

Sample	Soluble Solids, °Brix
June cocoa pulp	12.1
July cocoa pulp	14.0
August cocoa pulp	15.9
10 g carbonated beverage	15.0
15 g carbonated beverage	13.1

Table 8. Sensory attributes of the cocoa beverages with varying levels of cocoa pulp.

Sensory attributes	Means ±SE	*P*-value
Appearance 15g/L	6.36±0.55	0.889
Appearance 10g/L	6.29±0.13	
Colour 15g/L	6.37±0.55	0.905
Colour1 10g/L	6.31±0.13	
Taste 15g/L	6.23±0.55	0.983
Taste 10g/L	6.31±0.14	
Flavour 15g/L	6.89±0.77	0.502
Flavour 10g/L	6.37±0.14	
Consistency 15g/L	7.25±0.74	0.743
Consistency 10g/L	7.07±0.55	
Acceptability 15g/L	7.19±0.77	0.925
Acceptability 10g/l	7.14±0.55	

9-like extremely; 8-like very much; 7-like extremely; 6-like moderately; 5-neither like nor dislike; 4 –dislike slightly; 3-dislike moderately; 2-dislike very much; 1-dislike extremely

Table 9. Microbial analysis of dried and fermented cocoa beans.

Agar	Dilution: 10^1		Dilution:10^2		Dilution:10^3	
PDA	>300	>300	>300	>300	70	70
PCA	>300	>300	>300	>300	120	120
EMBA	0	0	0	0	0	0
MSA	0	0	0	0	0	0

Potato dextrose agar (PDA) for yeasts and molds 7.0×10^4 cfu/g
Plate count agar (PCA) for total aerobes : 120×10^5 cfu/g
Mannitol Salt agar (MSA) for *Staphylococ*cus
Eosin Methylene Blue Agar (EMBA) for fecal coliforms