INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 21, No. 4, p. 81-94, 2022
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

# Mineral and fat analysis of *Theobroma cacao* nibs incorporated in locally produced Banana bread

**Ronel T. Pacubat***

*Cagayan State University-Lasam Campus, Philippines*

Article published on October 15, 2022

**Key words:** Nibs, Elemental analysis, Lipids, X-ray fluorescence, Inductive coupled plasma-optical emission spectroscopy (ICP-OES), Atomic absorption microscopy (AAS), Gas chromatography (GC), High performance Liquid chromatography (HPLC).

## Abstract

The current study aims to determine the elemental and lipid components of cocoa nibs added in locally-produced banana bread through established methods in food chemistry. X-ray Fluorescence (XRF) Spectroscopy revealed that significant amount of Potassium (40.83%), Calcium (33.11%), Iron (33.46%), Phosphorous (6.17%), Silicon (4.55%), Sulfur (1.80%) is present while Copper (0.32%), Cadmium (0.49%), Manganese (0.84%), Titanium (0.72%), Nickel (0.43%), and Zinc (0.28%) are in trace amounts from the fifty grams (50 g) of cocoa nibs. Aside from traces of Cadmium, no other heavy metals were detected. Cocoa nibs incorporated in locally produced banana bread resulted in addition of Iron (2.88mg), Potassium (179.86mg), Zinc (0.79mg), Calcium (33.20mg) per 100 grams of the banana bread as revealed by inductive coupled plasma-optical emission spectroscopy (ICP-OES) and atomic absorption spectroscopy (AAS). Furthermore, lipid components of the banana bread with cocoa nibs include 19.64% crude fat comprising 13.08% saturated fat, 0% transaturated fat, 29.70mg cholesterol, 1,599 international units (IU) or 0.95mg of vitamin A using AOAC official methods, gas chromatography (GC) and high performance liquid chromatography (HPLC). Hence, Cocoa Nibs is not just a good aroma and flavoring enhancer but also a nutritional enhancer for the locally-produced banana bread.

***Corresponding Author:** Ronel T. Pacubat ✉ ronelpacubatcsu@gmail.com

## Introduction

Value-adding and incorporation in locally-produced food is becoming a major trend in the food industry. Such ventures do not only add up to aesthetic value of the food but also adds up to organoleptic and rheological and nutritional properties of the food being developed. This trend has been seen from the local, regional, national and international platforms aiming for great taste and food innovation.

Banana bread is locally produced bread by the community bakers of province of Cagayan and the rest of the Philippines. Before the coming of colonizers, the country is baking banana bread as one of their food. At the moment, many bakers and distributors innovated the banana bread by incorporating raisins, chocolates, mints and other aroma and flavoring enhancer. Other food business establishments create cake chiffouns, ice cream and ice candies even made from banana bread.

It is important to note that no extensive studies on the elemental and nutritional level of the innovations on banana bread have been made by the previous innovators. Interestingly, cocoa nibs can also be added or incorporated in the locally-produced banana bread. Pacubat and Garcia (2020) mentioned that is an effective aroma and flavoring enhancer. Results shows that the bread samples have significant carbohydrate and fat content thus pushing the researchers to dig more relevant knowledge about mineral and lipid components. Further, there is a minimal colony forming unit in terms of *E. coli* and total viable bacterial count showing clean and safe production of the bread. The sensory evaluation of the bread shows a promising result because the evaluators agreed on using the cocoa nibs as an effective flavoring and aroma enhancer for the locally produced banana bread.

Cacao food products can be a good source of nutrition for the community to address food shortage and malnutrition. Cacao food products and by-products include cacao table a, butter, essential oils, powder and the most popular of them all is chocolate.

After fermentation, cocoa beans turn into nibs. This cocoa nibs is the major precursor of for chocolate production.

As a continuation of the study, the current researchers aims to determine elemental components of the cocoa nibs which comprises minerals, risks assessments such as heavy metals that might be a possible contaminant of the food, and its nutritive effect when added to locally produced banana bread through established methods in food analysis and chemistry. Generally, the study aimed to determine the elemental and lipid composition of cocoa nibs added in the locally produced banana bread. Specifically, it sought to evaluate the amount of these elements present in the cocoa nibs, determine the presence of possible heavy metals from the nibs, and determine the incremental nutritive value of cocoa nibs added in banana bread.

## Materials and methods

*Raw Material Collection and Preparation*

Five kilograms (5kg) of all-purpose flour, two (2) trays of eggs, two kilograms (2kg) of baking powder, one (1) box of baking soda, ten (10) cans of evaporated milk, three kilograms (3kg) of brown sugar, one kilogram (1kg) of salt, and twenty pieces (20 pcs) DariCreme Buttermilk, were purchased at Mall of the Valley, Tuguegarao City. The two kilograms (2kg) of cacao nibs was purchased from the local cacao farmer in Lasam while the ten kilograms (10kg) of ripe banana Lakatan variety was purchased in the public market of Lasam.

In the preparation of the banana bread added with cacao nibs, the oven was pre-heated to $325^0$ F. The loaf pan line was greased at the bottom with a small piece of parchment paper. One cup brown sugar was creamed with one piece butter in a large mixing bowl until light fluffy. Two eggs were added one at a time, mixing after each addition. Four ripe mashed banana was added and one tablespoon milk was stirred for combination. The two cups were mixed with flour, one teaspoon baking powder, one teaspoon baking soda, and a pinch of salt in a bowl.

The wet ingredients were mixed and stirred. The batter was poured into the prepared loaf pan. 15 grams of cacao nibs were incorporated on top of the batter. The batter was baked for 45 minutes to 1 hour, or until a toothpick inserted in the center comes and clean. The bread was set aside to cool on a wire rack for a few minutes before removing the bread from the pan and allowing it to cool completely. Lastly, the bread was sealed with a cling wrap to maintain moisture to avoid microbial contamination.

*Cocoa Nibs Preparation*

Three glass containers with 50 grams each of cocoa nibs (UL-F8 variety) obtained from the business center of Cagayan State University -Lasam Campus were packaged and delivered in Nanotech Analytical Services and Training Corp. (NASAT Corp), Muntinlupa City for the mineral and heavy metal analysis of cocoa nibs.

*Mineral and Heavy Metal Detection Using X-Ray Fluorescence Spectroscopy*

The three, fifty grams of cocoa nibs were subjected for X-ray fluorescence spectroscopy. The voltage was run at 50 kilo-Volts (kV) and has a measuring time of 500 seconds. The applied current is 4 micro-Amperes and the collimator of the spectroscopy is 7 millimeters. The identification of elements, concentration by percentage weight (%) were determined from the spectroscopy with the principle of using x-ray emitted by the excited electrons going down to the ground state level and was adsorb by detector of the machine. The elemental graph were also provided by this device.

*Mineral Analysis Using Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) and Atomic Absorption Spectroscopy (AAS)*

Three, 100 grams of banana bread incorporated with cocoa nibs were packaged and delivered in SGS Philippines, Incorporated in Makati City. TB read samples were prepared for dry ashing using a furnace pre-heated and maintained at 500-600°C. The liquid components are evaporated and other organic compounds are evaporated in the presence of oxygen and nitrogen gas. Afterwards, the dried samples were subjected to acid-digestion utilizing a combination of nitric-perchloric acids $HNO_3$–$HClO_4$ (ratio 2:1). The samples were filtered and the filtrate was subjected for inductive coupled plasma-optical emission spectroscopy for potassium, zinc, calcium and sodium and atomic absorption spectroscopy for iron.

*Lipid Analysis of Bread Samples*

The same set of samples from the ICP-OES and AAS were used to determine the amount of crude fat, trans fatty acid, cholesterol and vitamin A. Crude and Trans Fatty acid were determined using Association of Official Agricultural Chemists (AOAC) methods. Fifty grams of the sample were dissolved in 100mL of 1 M petroleum ether. Twenty five grams (25 g) of each samples of banana bread were grounded in a centrifugal mill with a 2mm screen or cutter type (Wiley) mill with a 1mm screen. A solvent resistant marker was labeled for the filter bags in the analysis. The samples were weighed and recorded-the weight of each empty filter bag (W1) and zero the balance. The samples were placed 0.95 – 1.00g in up to 23 of the bags and were recorded for the weight (W2) of each by avoiding the placement of the sample in the upper 4mm of the bag. Inclusion of at least one empty bag in the run was used to determine the blank bag correction (C1 ).

Using a heat sealer, each filter bag were completely sealed closed within 4mm of the top to encapsulate the sample. Extraction of fat from samples is done by placing all bags into a 250-ml container. Addition of enough petroleum ether is done to cover bags and soaked for 10 minutes. Placements of up to 3 bags on each of eight Bag Suspender Trays (maximum of 24 bags) were done and the trays were stacked on the center post of the Bag Suspender with each level rotated 120 degrees in relation to the tray below it. The empty $9^{th}$ tray was placed on top.

Verification of the hot water supply is done on with the drain hose and was securely positioned in the drain. Vessel Lid was open and the Bag Suspender with bags was inserted into the Vessel and place the Bag Suspender Weight on top of the empty $9^{th}$ tray to

keep the Bag Suspender submerged. After the solution has been exhausted, the exhaust valve was closed and Vessel Lid was opened. 1900mL of 50-90°C was added to rinse water. After the Crude Fiber rinsing processes were completed, Vessel Lid was opened and samples were removed. Excess water from the bags was gently press out. Bags were place in a 250ml beaker and acetone was added to cover the bags and soaked for 3-5 minutes.

The filter bags were removed from the acetone and were place on a wire screen to air-dry. Completely dry in an oven at 102 ± 2°C. The filter bags was removed from the oven and immediately placed directly into a collapsible desiccant pouch and flattened to remove any air. The filter bags were cooled to ambient temperature and were weighed. The entire filter bag/samples were ashed in a pre-weighed crucible for 2 hours at 600 ± 15°C, and were cooled in a conventional desiccator. The ash was weigh to calculate loss of weight of organic matter (W3 ) for the determination of crude fat and trans fatty acid.

On the other hand, saturated fat and cholesterol level were determined using gas chromatography. These lipids were extracted by the procedures similar to the Folch method. Chloroform/methanol (2:1, v/v) containing 0.005% butylated hydroxytoluene (as antioxidant) was added (usually 5mL solvent added to 50–100 µl sample) and mixed vigorously for 1 min then left at 4°C overnight. OnemL of 0.9% NaCl was added and mixed again. The chloroform phase containing lipids was collected. The remains were extracted with another 2mL chloroform. The chloroform was pooled and dried under nitrogen and subjected to methylation. To monitor the recovery rate, the fatty acid C23:0 was added to the samples (usually 1 µg added to 2mg tissue sample) as an internal standard.

Lastly, Vitamin A (Retinol) was extracted and quantified using high performance liquid chromatography (HPLC). Ten grams of the sample was added to a 1mL butyl-hydroxytoluene in ethanol. Then, 5mL of of hexane was added and shaken using a 100mL beaker. The resulting solution was centrifuged for 30 minutes in 1000 revolution per minute (RPM). 95% ethanols were added to the sample and were subjected for HPLC.

*Statistical Analysis and Graphical Representations*

The means of mineral, heavy metals and lipid composition of the three bread samples with cocoa nibs were determined. Furthermore, the standard deviation of each sample were also calculated and compared with the existing amounts of minerals and lipids of conventional banana bread. Graphical representations is shown to have comparison from the conventional banana bread and the banana bread with cocoa nibs.

**Results and discussion**

*Mineral and Heavy Metal Analysis of Cocoa Nibs*

Table 1 shows the minerals and heavy metals determined by x-ray fluorescence spectroscopy analysis. It can be gleaned that Potassium (K) has the highest concentration (40.83%) in the fifty grams of cocoa nibs. This is in contrast with the study of Katz *et. al* (2010) noting that cocoa dark chocolate has low potassium content. Interestingly, the XRF analysis revealed that it has high content of potassium in cocoa nibs. Potassium (K) is a very important macro-nutrient. In the body, this mineral acts as the major ion that participates in the intracellular fluid in the balance of acidity and alkalinity, balance of osmosis in cells, nerve impulses, contraction of the muscles and sodium and potassium pump of the intracellular matrix of the cell during electron transport chain and synapses, cardiovascular disease prevention, calcium absorption for the bones and many more (IPI, 2013). An adult should aim for 3,500-4,700mg per day and cocoa nibs can suffice the recommended intake of potassium. Deficiency of potassium leads to hypokalemia.

Calcium (Ca) is relatively high as well having a concentration of 33.1096%. This macro-nutrient is also an important mineral for humans. Calcium functions in contraction of muscles, oocyte formation in females during fertility, strong bones and teeth, platelet clotting in the blood, heartbeat regulation and

balance of fluids in and out of the cells (Piste *et.al,* 2012). Osteoporosis is a result of long term calcium deficiency thereby increasing fractures and malformations. Hence, cocoa nibs can also be a good source of calcium.

Iron (Fe) is also evident in the cocoa nibs having a percentage concentration by weight of 10.46% per 50 grams. Iron is an important mineral for blood. It is very important to note as well that an adult has 70% total iron content in the blood's hemoglobin necessary for the binding of oxygen that is vital for life (Abbaspour *et al.*, 2014). Anemia is the leading cause disease when there is a deficiency of iron. Recommended energy and nutrient intake (RENI) shows that 60mg of iron per day is necessary for the body. This can also be obtained from cocoa nibs.

Phosphorous (P) is also relatively high in the cocoa nibs. Based on the table, phosphorous is 6.16% by weight. In the human body, phosphorous becomes inorganic phosphate which is very much essential for cells and bones (Takeda *et al.,* 2004). The inorganic form of phosphate in the serum of the blood is maintained and regulated by the sufficient amount of phosphorous intake. The major energy compound of the body which is adenosine triphosphate (ATP) needs this inorganic phosphate. The regulator of organic phosphate in the body is the kidney. It has been known that phosphorous deficiency leads to hypophosphatemia which includes symptoms of irregular bone formation and fatigue. Furthermore, increase calcium kidney stones may occur to patients having low levels of phosphorous (Carassco *et al.,* 2004).

Silicon (Si) is also present in the cocoa nibs. The percentage concentration is 4.55% per fifty grams. This micro-nutrient is necessary for the development and growth of calcification of Silicon is necessary for the growth and bone calcification and as a biological cross- linking agent of connective-tissue-based membrane structures. This element is considered to have beneficial effects on several human disorders, including osteoporosis, ageing of skin, hair and nails or atherosclerosis. It is important to note that ages 19-49 should consume 0.8 grams per day of food containing phosphorous. Hence, a cocoa nib is an excellent source of this micro-nutrient. Further, this micro-nutrient is found to promote healthy bone and connective tissue and has been related with mental health (Nielsen, 2014). The healthy connective tissue promoted by silicon was observed from athe binding of hydroxyl groups of polyols and thus affecting the formation and usage of collagen.

Sulfur (S) is present as well in the cocoa nibs in small amounts, 1.80% by weight. This mineral is very much essential for the growth of bones and its calcification. Moreover, it is necessary as cellular agent of cross-linking element for connective tissues (Hewlings and Kalman, 2019). This element is considered to have beneficial effects on several human disorders, including osteoporosis, ageing of skin, hair and nails or atherosclerosis. Zinc (Zn) is also present in low amounts (0.28% by weight). This macro-nutrient has an important role in the body. Bhowmik *et al.* (2010) explained that adequate levels of zinc should be a critical component of food to avoid illness of children, develop good growth and posture. This mineral plays an important role in the health of integumentary organs such skin, nail and hair and bones as well as connective tissues. Furthermore, it is a key regulatory co-factor of enzymes in the renewal of cells and formation of DNA.

Traces of nickel (Ni), manganese (Mn), copper (Cu), titanium (Ti) and cadmium (Cd) is detected as well in the cocoa nibs; 0.44%, 0.84%, 0.32%, 0.72% and 0.48% respectively. Traces of nickel are very much essential for the proper regulation and function of human body for lipid metabolism. However, high amount of this element can cause toxicity for the body. Manganese is also important cofactors of enzymes. It is an essential micro-nutrient for enzymes such as arginase, glutamine synthase, pyruvate carboxylase and superoxide dismutase (Chen *et al.*, 2018). Without this manganese, these enzymes will not function well for development and growth, digestion, reproduction and antioxidant defense mechanisms of the body.

Copper is an essential co-factor of enzymes. Aside from this importance, the most frequent clinical manifestations of copper deficiency are anemia, neutropenia, and bone abnormalities (National Reserach Council, 2000). Titanium has no biological importance in the body. It is very inert type of mineral and do not participate in metabolism. Further, no reports and studies revealed that low or high amounts of this mineral are toxic for the human body (Tibau *et al.,* 2017).

On the other hand, Cadmium is a mineral that poses serious health risk in humans and animals. It is important to note that cadmium is a heavy metal that occurs as a natural constituent in earth's crust along with Copper, Lead, Nickel and Zinc. This metal is absorb by the cacao tree mistakenly and will concentrate mainly in cocoa powder (Oliva *et al.,* 2020). Food Administration Authority and the World Health Organization (1993) mentioned that an intake limit of 0.2mg cadmium/kg body weight was set based on the critical renal cadmium concentration of between 100 and 200 µg/g wet weight that corresponds to a urinary threshold limit of 5–10 µg/g creatinine. Fortunately, the 15 grams of cocoa nibs incorporated in the locally produced banana bread is at very low to trace amount of cadmium (0.48% of 15 grams is 0.072 grams, a level below the safe limit). Other than Cadmium, no other heavy metal was detected by XRF in the cocoa nibs.

**Table 1.** XRF Analysis on the Concentration of Minerals and Heavy Metals (% weight) present in 50 grams Cocoa nibs.

| Element | Concentration | Unit | Line | Intensity | Judgment |
|---|---|---|---|---|---|
| Cu | 0.3166 | Wt% | Ka | 15012.01 | --- |
| Cd | 0.4892 | Wt% | Ka | 12648.19 | --- |
| K | 40.8269 | Wt% | Ka | 435721.17 | --- |
| Ca | 33.1096 | Wt% | Ka | 256787.75 | --- |
| Fe | 10.4580 | Wt% | Ka | 375580.60 | --- |
| P | 6.1684 | Wt% | Ka | 13114.53 | --- |
| Si | 4.5535 | Wt% | Ka | 3356.30 | --- |
| S | 1.8042 | Wt% | Ka | 9909.15 | --- |
| Mn | 0.8406 | Wt% | Ka | 24887.61 | --- |
| Ti | 0.7196 | Wt% | Ka | 9353.86 | --- |
| Ni | 0.4346 | Wt% | Ka | 18506.69 | --- |
| Zn | 0.2787 | Wt% | Ka | 15613.94 | --- |

Fig. 1 represents the graphical representation of the relative peak/intensity of elements detected by XRF analysis in 50 grams of cocoa nibs. The y-axis of the graph refers to amount of the element while the x-axis refers to x-ray plot at the k alpha line emitted by the secondary electrons of the element. It can be gleaned on the graph that Calcium and Iron has the highest concentration detected on the intensity of 256, 487.75 and 375, 580.60 respectively. On the other hand, other minerals are detected below iron and calcium Hence, this graph confirms the table discussed above.

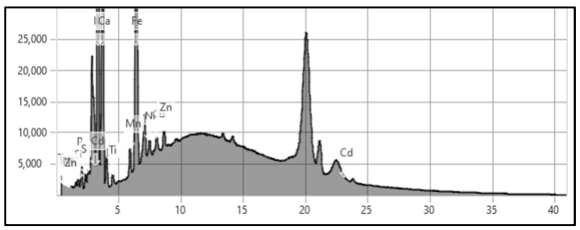



**Fig. 1.** Concentration (% weight) v.s. X-ray Plot of Elements Present in Cocoa Nibs.

*Comparison on the essential micro-nutrients of Banana Bread with Cocoa Nibs and Conventional Banana bread*

Table 2 represents the presence of minerals present in the banana bread with and without 15 grams of cocoa nibs. It can be gleaned on the table that Iron (Fe), Potassium (K), Calcium (Ca), and Sodium (Na) is present for both bread samples but Zinc (Zn) is absent for the conventional banana bread, as detected by inductive coupled plasma-optical emission spectroscopy (ICP-OES) and atomic absorption spectroscopy (AAS). It is important to note that these elements are necessary for body metabolism as described above.

The banana bread with 15 grams incorporated cocoa nibs as toppings has higher amount than the conventionally produced banana bread. Iron (Fe) is higher with mean amount of 2.88mg and standard

deviation of 0.02 per 100mg of the bread sample. The banana bread without cocoa nibs has a very low amount of this element having mean amount of 0.0014mg and standard deviation (SD) of 0.18 per 100mg of the sample. This is tantamount to the results given by XRF analysis implying that Iron is high in cocoa nibs. Potassium (K) is also high in banana bread with cocoa nibs having mean amount of 179.86mg with SD of 0.19 per 100mg of sample compared with banana bread without cocoa nibs, 134mg/100mg of the sample. Again, this result is in congruent with the XRF analysis of cocoa nibs. Zinc (Zn) is also high in the innovated product but it is not detected in the conventional banana bread. Calcium (Ca) and Sodium (Na) is also high in the product than the conventional one with mean amount of 33.20mg, SD= 0.88 and 455.63mg, SD=0.66 per 100mg of the sample respectively. Inductive Coupled Plasma coupled with Optical Emission Spectroscopy (ICP-OES) and Atomic Absorption Spectroscopy (AAS) are analytical devices that have great accuracy in measuring elements in samples. Inductively coupled plasma optical emission spectroscopy (ICP-OES) is the technique of choice for many different applications, including those in the environmental, metallurgical, geological, petrochemical, pharmaceutical, materials, and food safety arenas. It can be applied to varying sample types such as aqueous and organic liquids and solids. On the other hand, Atomic absorption spectrometry (AAS) is an easy, high-throughput, and inexpensive technology used primarily to analyse compounds in solution. As such, AAS is used in food and beverage, water, clinical, and pharmaceutical analysis. It is also used in mining operations, such as to determine the percentage of precious metal in rocks (Thermo Fischer, 2010).

**Table 2.** Amount of Micro-nutrient Present in Bread Samples.

| Element | Banana Bread w/ Cocoa Nibs | Banana Bread w/out Cocoa Nibs | Detection |
|---|---|---|---|
| Iron (Fe) | 2.88(+-0.02)mg/100mg | 0.0014(+-0.18)mg/100mg | AAS |
| Potassium (K) | 179.86(+-0.19)mg/100mg | 1.34(+-0.7)mg/100mg | ICP-OES |
| Zinc (Zn) | 0.79(+-0.1)mg/100mg | --/100mg | ICP-OES |
| Calcium (Ca) | 33.20(+-0.88)mg/100mg | 0.01(+-0.09)mg/100mg | ICP-OES |
| Sodium (Na) | 455.63(+-0.66)mg/100mg | 302 (+- 0.22)mg/100mg | ICP-OES |

*Cmparison on the amount of lipids of Banana Bread with Cocoa Nibs and Conventional Banana bread*

Table 3 represents the presence and amount of lipid of banana bread with and without cocoa nibs from AOAC official methods, gas chromatography (GC) and high performance liquid chromatography (GC). It can be gleaned on the table that the crude fat for each sample contains almost similar amounts, 19.64% and 10.40% per 100 g respectively. These amounts are considered to be low as compared with other food staples and products in the market. Under the crude fat component, very low amount of saturated fat is detected for both bread samples having 13.08 and 12.09%. Low amounts of saturated fat are necessary for body metabolism. Recommended energy and nutrient intake (RENI, 2013) indicates that Filipino ages 19-49 should take 14.4 grams per day or lower of food containing saturated fat to avoid artherosclerosis and other cardiovascular diseases. Both bread samples does not contain trans fatty acid using the AOAC official methods. Iqbal (2010) in his review paper mentioned that trans-fatty acid consumption is directly link with cardiovascular diseases and diabetes milletus. Trans-fatty acid is considered to be a low density lipoprotein. Hence, the consumption of banana bread poses no threat for ingesting trans-fatty acid. Very low amount of Cholesterol is also present for both bread samples, 29.70mg and 28.9mg per 100 gram of samples respectively detected and quantified by GC. Like saturated fatty acid, low amount of consumption of this lipid is also beneficial for healthy diet. American Heart Association (2012) indicates that a human should not exceed 300mg per day to avoid cardiovascular diseases. Thus, both bread samples has ideal amounts of cholesterol. Vitamin A or retinol, otherwise known as retinoic acid, is also detected and quantified in both bread samples by HPLC.

It can be gleaned on the table that vitamin A is higher in the innovated product than the conventional bread, 1, 599 International Units (IU) than 492.8 IU per 100 gram of the bread sample. This difference is attributed to the addition of cocoa nibs. Vitamin A is necessary for proper eye function, immune system, heart and lungs (National Institute of Health, 2005). Hence, cocoa nibs is an excellent source of vitamin A.

**Table 3.** Lipid Component of Banana Bread With and Without Cocoa Nibs.

| Lipid Component | Banana Bread w/ Cocoa Nibs | Banana Bread w/out Cocoa Nibs | Detection |
|---|---|---|---|
| Crude Fat | 19.64%/100 g | 19.40%/100 g | AOAC Official |
| -Saturated | 13.08% | 12.09% | Methods |
| Fat | 0.00 g/100g | 0.00 g/100g | GC |
| -Trans Fat | 29.70mg/100 g | 28.9mg/100g | AOAC Official |
| Cholesterol | 1,599 IU/100 g | 492.8 IU/100 g | Methods |
| Vitamin A | | | GC |
| | | | HPLC |

## Conclusions and recommendations

Value-adding and incorporation in locally-produced food is becoming a major trend in the food industry. The study aimed to determine the mineral, heavy metal and lipid components of the cocoa nibs of UL-F8 variety incorporated as flavoring and aroma enhancer of the locally produced banana bread. There have been no extensive studies for the cocoa nibs, a fermented product of cocoa beans, about its mineral and heavy metal composition. Detection and quantification was done using analytical tools in food chemistry such as x-ray fluorescence, atomic absorption spectroscopy and inductive coupled plasma-optical emission spectroscopy for mineral and heavy metal analysis, and AOAC methods, gas chromatography and high performance liquid chromatography for lipids. These analytical tools are of great significance for the analysis of functional food.

Cocoa nibs of UL-F8 variety has high amounts of potassium and calcium detected by XRF analysis. These two elements are very important macro-nutrient. Iron is also evidently high in cocoa nibs. Iron is an important mineral for blood. Silicon (Si) is in low amounts by weight. The present study suggests that this element has a biological role in the human body. Sulfur (S) is present as well in the cocoa nibs in small amounts. This mineral is very much essential for the growth of bones and its calcification.

Traces of nickel (Ni), manganese (Mn), copper (Cu), titanium (Ti) and cadmium (Cd) is detected as well in the cocoa nibs. Iron (Fe), Potassium (K), Calcium (Ca), and Sodium (Na) is present for both bread samples but Zinc (Zn) is absent for the conventional banana bread, as detected by inductive coupled plasma-optical emission spectroscopy (ICP-OES) and atomic absorption spectroscopy (AAS). It is important to note that these elements are necessary for body metabolism. Lipid analysis detects the presence of crude fat for each samples containing almost in similar amounts. These amounts are considered to be low as compared with other food staples and products in the market. Very low amount of saturated fat is detected for both bread samples and no trans-fat is detected. Very low amount of Cholesterol is also present for both bread samples. Vitamin A is higher in banana bread with incorporated cocoa nibs than the conventionally produced banana bread. As recommendation of this study, value-adding using cocoa nibs can also be done to other food products to be an aroma, flavoring and nutrient enhancer. On the other hand, Cadmium should be totally removed from cacao food products for it poses health and economic risks. Food biosensors and metal adsorption should be done before or after the fermentation of cocoa beans into cocoa nibs.

## Acknowledgement

The researchers would like to express their sincerest gratitude to the Research and Development of Cagayan State University, Product Development Training Center, Director Gilbert Magulod Jr., Ph.D., CEO Ricardo B. Casauay, Phd and the NASAT Incorporation together with SGS Philippines.

## References

**Abbaspour N, Hurrell R, Kelishadi R.** 2014**.** Review on iron and its importance for human health. Journal of research in medical sciences: The official Journal of Isfahan University of Medical Sciences **19,** 164-174.

**Akinyele IO, Shokunbi OS.** 2015. Comparative analysis of dry and wet digestion methods for the determination of trace and heavy metals in food. Food Chemistry **173,** 682-684.

**Bakkali K, Martos NR, Souhail B, Ballesteros E.** 2009. Characterization of trace metals in vegetables by graphite furnace atomic absorption spectrometry after closed vessel microwave digestion. Food Chem **116,** 590-594.

**Bhowmik K. Sampath Kumar P.** 2010. A potential medicinal importance of zinc in human health and chronic disease. Department of Pharmaceutical Sciences, Coimbatore Medical College, Coimbatore, Tamilnadu, India.

**Chen P, Bornhorst J, Aschner M.** 2018. Manganese metabolism in humans. Front Biosci (Landmark Ed) **23,** 1655-1679.

**Fontana Jr AJ, Campbell CS.** 2004. Water activity (Chapter 3 In: Nollet L (ed.) Handbook of Food Analysis, 2nd edn. Marcel Dekker, Inc., New York p. 39-54.

**Food and Nutrition Research Institute (FNRI).** 2002. Recommended energy and nutrient intake and List of PDRI.

**Hewlings, Susan and Douglas Kalman.** 2019. Sulfur in Human Health. Department of Nutrition, Central Michigan University USA.

**Iqbal MP.** Trans fatty acids- A risk factor for cardiovascular disease. Pak J Med Sci **30,** 194-197.

**Katz DL, Doughty K, Ali A.** 2011. Cocoa and chocolate in human health and disease. Antioxidants & redox signaling **15,** 2779-2811.

**Kuboyama K, Sasaki N, Nakagome Y, Kataoka M.** 2005. Wet Digestion. Anal Chem **360,** 184-191.

**Nielsen, Forest.** 2014. Update on the possible nutritional importance of silicon. Journal of Trace Elements in Medicine and Biology **28**.

**Oliva, Manuel. Karol Rubio. Melissa Epquin. Gladys Marlo and Santos Leiv.** 2017. Cadmium Uptake in Native Cacao Trees in Agricultural Lands of Bagua, Peru.

**Piste Pravina, Didwagh Sayaji and Mokashi Avinash.** 2012. Calcium and its Role in Human Body P.G .Department of Chemistry, Y. C. Institute of Science, Satara, Maharashtra, India.

**Takeda E, Yamamoto H, Nashiki K, Sato T, Arai H, Taketani Y.** 2004. Inorganic phosphate homeostasis and the role of dietary phosphorus. J Cell Mol Med **8,** 191-200.

**Tibau, Anita V. Blanche D. Grube. Braulio J. Velez. Victor M. Vega. Joachim Mutter.** 2017. Titanium exposure and human health. Center for Environmental and Toxicological Research, University of Puerto Rico, Newport Beach CA.

## Appendices

Mineral and Heavy Metal Analysis of Cocoa Nibs by XRF Spectroscopy

**Nanotech Analytical Services and Training Corp. (NASAT Corp)**
G/F B136 L1 C. Arellano St., Katarungan Village (Daang Hari), Poblacion, Muntinlupa City 1776, Philippines
Tel. No.: (632) 576-8922 * Fax: (632) 576-1599 * Mobile: 0908-8856051
Email: customercare@nasatcorp.com * Website: www.nasatcorp.com

**I. Sample Description:**

| Sample | Description |
|---|---|
| 1 | Sample 1 |

**II. Work Required:**

- Check for focused elements listed below
  - Cadmium
  - Copper
  - Lead
  - Mercury

**Nanotech Analytical Services and Training Corp. (NASAT Corp)**
G/F B136 L1 C. Arellano St., Katarungan Village (Daang Hari), Poblacion, Muntinlupa City 1776, Philippines
Tel. No.: (632) 576-8922 * Fax: (632) 576-1599 * Mobile: 0908-8856051
Email: customercare@nasatcorp.com * Website: www.nasatcorp.com

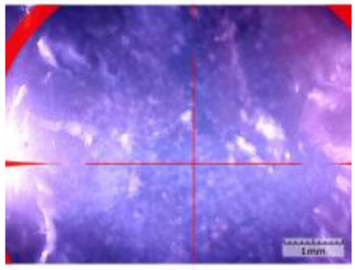
**Measurement Information**

| | | | | |
|---|---|---|---|---|
| Project | Cagayan State Univ – 12– | Date | 12/22/2020 | 9:14 AM |
| Measurement | Measurement 1 | Analysis | Analysis 3 | |
| Workflow | | User | | |

**Measurement Settings**

| | | |
|---|---|---|
| Voltage | 50 | kV |
| Filter | None | |
| Meas Time | 500 | s |
| Current | 4 | μA |
| Collimator | 7 mm | |
| Processing Time | Process 2 | |

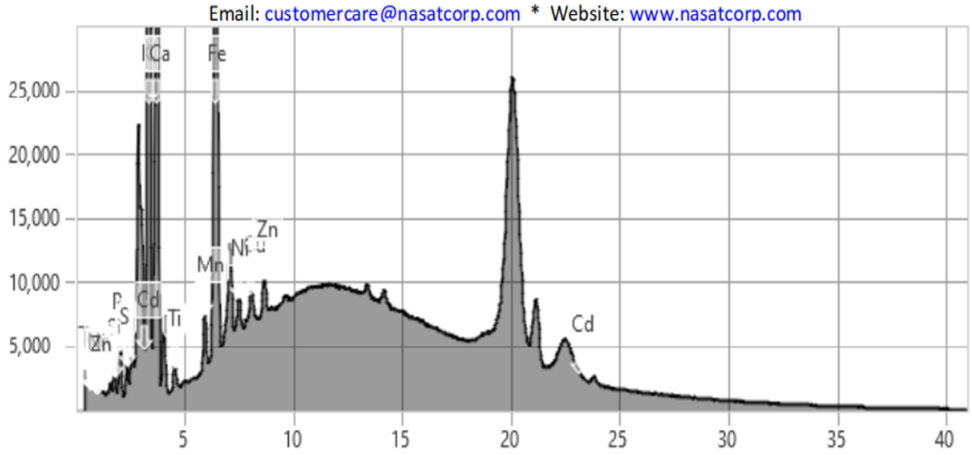
**Sample Image**



**Result**

| Element | Concentration | Unit | Line | Intensity | Judgment |
|---|---|---|---|---|---|
| Cu | 0.3166 | wt% | Kα | 15012.01 | --- |
| Cd | 0.4892 | wt% | Kα | 12648.19 | --- |
| K | 40.8269 | wt% | Kα | 435721.17 | --- |
| Ca | 33.1096 | wt% | Kα | 256487.75 | --- |
| Fe | 10.4580 | wt% | Kα | 375580.60 | --- |
| P | 6.1684 | wt% | Kα | 13114.53 | --- |
| Si | 4.5535 | wt% | Kα | 3356.30 | --- |
| S | 1.8042 | wt% | Kα | 9909.15 | --- |
| Mn | 0.8406 | wt% | Kα | 24887.61 | --- |
| Ti | 0.7196 | wt% | Kα | 9353.86 | --- |
| Ni | 0.4346 | wt% | Kα | 18506.69 | --- |
| Zn | 0.2787 | wt% | Kα | 15613.94 | --- |

**Nanotech Analytical Services and Training Corp. (NASAT Corp)**
G/F B136 L1 C. Arellano St., Katarungan Village (Daang Hari), Poblacion, Muntinlupa City 1776, Philippines
Tel. No.: (632) 576-8922 * Fax: (632) 576-1599 * Mobile: 0908-8856051
Email: customercare@nasatcorp.com * Website: www.nasatcorp.com




**- END OF REPORT -**

| In Settlement of the fallowing | | |
|---|---|---|
| INVOICE NO. | AMOUNT | |
| | | |
| | | |
| | | |
| VATable Sales | | |
| VAT-Exempt Sales | | |
| VAT Zero Rated Sales | | |
| 12% VAT | | |
| Total Amount Payable | | |
| PAYMENT IN FORM OF: | | |
| Cash | | |
| Check | | |
| M.O. | | |
| Total Payment P | | |

10 Bklts (50x3) 1001-1500
BIR Authority to Print No. OCN 9AU0000814131
Date Issued: 07-18-18 Valid Until: 07-17-23
J.M.I. PRINTING SERVICES NON-VAT REG. TIN: 251-792-825-000
B20 L 23 Fb Angelo St. Katarungan Vill. Pob. Muni. City TEL: 8085593
ACCREDITATION NO. 53BMP20140000000021 ACCREDITATION DATE OCTOBER 20,2014

NASAT LABS Services To The Nano World

**NANOTECH ANALYTICAL SERVICES AND TRAINING (NASAT) CORP.**
Block 136 Lot 1 C. Arellano St., Katarungan Village, (Daang Hari)
Poblacion, Muntinlupa City, 1776 Philippines
Tel. Nos.: (632) 334-6016 * Fax (632) 807-8392
VAT REG. TIN: 007-279-301-000

Nº 1317

OFFICIAL RECEIPT

Date 11/16/22

Received from Roniel Pacubat

Business Style / Name _____ TIN _____

Address Cagayan State University

THE SUM OF PESOS One Thousand Five Hundred Sixty Eight Pesos Only. (P 1,568.00)

in full / partial payment of 1 Sample XRF Analysis

Total Sales (VAT Inclusive) _____
Less: VAT _____
Total _____
Less: SC/PWD - Discount _____
TOTAL AMOUNT DUE _____

Sr. Citizen TIN
OSCA/PWD ID No.
Signature

By: _____
Authorized Signature

"THIS OFFICIAL RECEIPT SHALL BE VALID FOR FIVE (5) YEARS FROM THE DATE OF ATP."

SGS

PAB ACCREDITED
TESTING LABORATORY
PNS ISO/IE 17025:2005
LA-1996-011F

Manila, 04 January 2021

Page 1 of 2

PHL20-06562

## RESULTS OF ANALYSIS

CLIENT : PACUBAT, RONEL

ADDRESS : CENTRO D2 LASAM, CAGAYAN (3524) .

DATE RECEIVED : 25-November-2020

DATE REPORTED : 04-January-2021

Analysis based on sample(s) submitted by PACUBAT, RONEL, SGS Philippines, Inc. does not guarantee that shipment/delivery corresponds to sample(s) submitted nor does SGS Philippines, Inc. guarantee that sample(s) submitted is a (are) random preparation of the shipment/delivery.

Lab # : PHL20-06562.001

Sample Name : BANANA BREAD ADDED WITH CACAO NIBS

| Analysis Name | Result | Methodology |
|---|---|---|
| Iron (Fe) | 2.88 mg/100 g | Dry Ashing, Acid Digestion and Quantitation by Atomic Absorption Spectrophotometer (AAS) |
| Potassium (K) | 179.86 mg/100 g | Dry Ashing, Acid Digestion, and Quantitation by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) |
| Zinc (Zn) | 0.79 mg/100 g | Dry Ashing, Acid Digestion, and Quantitation by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) |
| Calcium (Ca) | 33.20 mg/100 g | Dry Ashing, Acid Digestion, and Quantitation by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) |
| Sodium (Na) | 455.36 mg/100 g | Dry Ashing, Acid Digestion, and Quantitation by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) |
| Crude Fat | 19.64 % | Based on AOAC Official Methods, 20th Edition, 2016 |
| Trans Fatty Acid | <0.03 g/100 g | Based on AOAC, 19th Edition, 2012 |
| Saturated Fat | 13.08 % | Gas Chromatography (P-LAB-005-INSTRU) |
| Cholesterol | 29.70 mg/100 g | Gas Chromatography (P-LAB-002-INSTRU) |
| Vitamin A | 1,599 IU/100g | High Performance Liquid Chromatography (QLY-LAB-1131) |

Note: 1. "<" : less than means the test result is lower than the Minimum Detection Limit.
2. Minimum Detection Limit: Values after the less than (<) sign.
3. Testing Period: December 02 to 14, 2020.

SGS

## CLIENT CLAIM STUB

**Job Number** : **PHL20-06562**
Client Name : PACUBAT, RONEL T.
Address : CENTRO D2, LASAM, CAGAYAN (3524)

Date : 11/25/2020
BOSS No. : 1001523
Purchase Order No :

Sample : BANANA BREAD ADDED WITH CACAO NIBS
FEES (Pesos) : Php 33,437.60
Report Due On : 12/17/2020 (4:00PM)
Billed By : TONETTE

### REQUESTED LABORATORY ANALYSES

| LAB NO | SAMPLE | CLIENT REFERENCE | ANALYSIS REQUESTED | COST OF ANALYSIS |
|---|---|---|---|---|
| PHL20-06562.001 | BANANA BREAD ADDED WITH CACAO NIBS | | Metals (Dry Ashing/Acid Digestion) by AAS (Dry Ashing, Acid Digestion and Quantitation by Atomic Absorption Spectrophotometer (AAS)) | 600.00 |
| | | | Crude Fat by Acid Hydrolysis Method (Based on AOAC Official Methods, 20th Edition, 2016) | 1,080.00 |
| | | | Metals (Dry Ashing/Acid Digestion) by ICP (Dry Ashing, Acid Digestion, and Quantitation by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES)) | 3,820.00 |
| | | | Sample Preparation (-) | 600.00 |
| | | | Trans Fatty Acid (Based on AOAC, 19th Edition, 2012) | 7,135.00 |
| | | | Cholesterol by GC (Gas Chromatography (P-LAB-002-INSTRU)) | 4,755.00 |
| | | | Saturated Fat by Gas Chromatography (Gas Chromatography (P-LAB-005-INSTRU)) | 7,135.00 |
| | | | Vitamin A by HPLC (High Performance Liquid Chromatography (QLY-LAB-1131)) | 4,730.00 |
| | | | **Sub Total :** | **29,855.00** |
| | | | **Plus VAT :** | **3,582.60** |
| | | | **Total Amount :** | **33,437.60** |