

Nutribun with Intensified Micronutrients

from Cardava Banana (Musa acuminata x balbisiana var cardava) for COVID-19

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Abstract

The 25%, 35%, and 45% cardava banana flour were mixed with all-purpose flour, added with full-cream milk, eggs, garlic paste, sugar, and a mixture of fish and seagrasses powder, and baked in a dehydrator oven with 1500C in 20 minutes. Nine experts were panels, arranged in a Latin Square William Design using a Nine-point Hedonic scale as a survey instrument. It was the 25% content, chosen as the best treatment and subjected to laboratory analysis. A 62.5-gram serving size has 913.5mg of Vitamin C, 40,000mg of Potassium, and 38.9mg of Iron, which was ten times, eight times, and twice higher than the individual daily requirement respectively. The Colors, Aroma, Mouthfeel, Texture, Sweetness, and General acceptability of the treatments were significantly different from each other, except for the Flavors which were non-significant. The ingredients utilized strongly contributed to the flavors and with relatively the same degree of effects on each sample.

Keywords: Food science and nutrition, intensified micronutrients, mothers, nutribun, unripe cardava banana, vitamin C, potassium, iron

1. INTRODUCTION

A 62.5 g nutribun from an unripe cardava banana (*Musa acuminate x balbisiana var cardava*) has high Vitamin C, Potassium, and Iron which are ten times, eight times, and twice higher than the individual's daily value requirement respectively. This was authentically revealed in this study when unripe cardava banana had been processed into flour and utilized as the main ingredient in nutribun- a commercial bread developed and used for feeding during the covid-

19 pandemic in one of the coastal communities in Sagay City, Negros Occidental, Philippines.

On March 12, 2020, the World Health Organization declared a pandemic due to the global spread of coronavirus disease (COVID-19), and the world has paid a high toll of pandemic losses in human lives, and increased poverty, (Ciotti, M., et al, 2020) [4]. Patients with SARS-CoV-2 infection may present symptoms like cough, fever, and shortness of breath, as well as gastrointestinal symptoms such as diarrhea, vomiting, abdominal pain, and severe respiratory symptoms, (Milani, G.P. et al, 2021[31]; Ciotti, M., et al, 2020[4]; Shabir Ahmad Lone & Aijaz Ahmad, (2020) [38]. World Health Organization advised the public to have a higher daily intake of Vitamin C and other significant Vitamins and trace minerals as daily supplements for health resistance, (WHO, 2020) [47].

Vitamin C has multiple pharmacological characteristics, antiviral, antioxidant, anti-inflammatory, and immunomodulatory effects, which make it a potential therapeutic option in the management of COVID-19, (Gavrielatou, E. et al, 2022[16]; Pisoschi, Aurelia Magdalena, et al, 2022[38]; Hui, L. L., et al, 2022[24]. In the same situation, iron is important to promote mental health, its deficiency can lead to health problems like stress, anxiety, and cognitive decline, (McCann, S., Amado, M. P., Moore, S., 2020[30]; Davison, K., Gondara, L., Kaplan, B., 2017[7]; Kim, J., Resnick, M.W., 2014[27]).

Nutribun is a type of bread commercially designed at an affordable price. This was innovated from a nutribun in the year 1970 which was documented in the Philippine Bibliographic datasheet control number PN-AAH-944 and sub-classification (695) SB00000-G732[34]. This entitles, "Nutribun Connection: The Role of Anthropologists in the Planning of Philippine nutrition project," which was presented at the 40th Annual Meeting of the Society for Applied Anthropology by author Jansen, W.H. and corporate authors AID /ASIA /USAID / Philippines last 1980 in Denver, USA. This was made from wheat flour, powdered milk, eggs, and sugar. It has 500 calories and 17 grams of protein, and significant amounts of vitamin B complex, vitamin A, calcium, and iron. This nutribun was the basis of the present nutribun in the market and with a soft and lumpy and flat texture.

Recently, there was an enhanced nutribun developed by the Department of Science and Technology (DOST), Philippines, this was made from squash or pumpkin 165 serving contains 504 calories, 17.8 grams of protein, 6.08 milligrams of iron, and 244 micrograms of vitamin A. Department of Science and Technology (DOST) had introduced this in all areas of the Philippines but not yet fully utilized all over the nation and was not yet visible in the market. This study was looking for a Nutribun, a type of bread that can be eaten as a snack or meal which can answer or resistance to the present COVID-19 and other undernutrition and malnutrition situations. The main ingredient of this nutribun was the unripe matured cardava banana (*Musa acuminate x balbisiana var cardava*), ABB Group of Saba, and a member of the Musaceae family, (Tanaid, R.A., Lauzon, R., 2018 [41]). Processed into flour using the oven-dried method (using a dehydration system). This type of ingredient had been chosen because of its availability in the place.

Generally, the main purpose of the study was to produce cardava banana flour, to be utilized and produced nutribun and used in a feeding program in one of the communities in Sagay City, Negros Occidental which considered with iron anemia deficiency during COVID-19. Furthermore, this study aimed to determine the following: microbiological content; toxicity; shelf-life; physical and chemical content such as moisture, macronutrients like Carbohydrates, Protein, Fats, and Fibre, and micronutrients like Vitamin C, Potassium, Magnesium, and Sodium as NaCl, Iron, and Phenolic Acid; and acceptability using sensory and organoleptic evaluation such as Color, Aroma, Flavor, Mouthfeel, Sweetness, Texture and General Acceptability. This product development was conducted at the Food Technology Research and Development Center of Northern Negros State College of Science and Technology, Sagay City, Negros Occidental, Philippines, and the acceptability assessment was conducted in one of the Sitios of the Barangay of Sagay City, Negros Occidental, Philippines.

2. MATERIALS AND METHODS

Materials

The raw material used in the development and production of cardava banana flour was the cardava banana (*Musa acuminate x balbisiana var cardava*). The nutribun was done using the following ingredients: Cardava banana flour, all-purpose flour, full cream milk, fresh eggs, fish and seagrasses powder, garlic paste, sugar, and yeast.

Functions of Ingredients in the Composition of Cardava Banana Nutribun Bread The cardava banana was the main ingredient in the development of this cardava banana nutribun bread. This material was available in the community and this food contains high macro and micronutrients. The all-purpose flour was added due to observation that after undergoing several prototypes of using the cardava banana flour, the grains of the bread was dull and with rough texture, so it was then tried to mix this cardava banana flour with allpurpose flour to enhance the texture of the bread from dull to finer and clearer texture. The yeast leavens the dough and gives a light sponge-like texture, it provides flavor, and aroma and contributes to the nutritional value of cardava banana nutribun; it enhances the characteristics of flavor and other yeast-leavened products. The sugars were metabolized when yeast produces carbon dioxide and produced a spectrum of intermediate metabolites, which were the precursors of cardava banana nutribuns' flavor. The full cream milk performs many functions in this cardava banana nutribun, this serves as a source of liquid to hydrate the dry ingredients, adds flavor, supports browning, and softens texture. Eggs, aside from its nutrient content, egg yolk emulsifies, thickens, and binds the dough, contributes to browning because of the Maillard reaction when baking, promotes clear grain texture, shortens gluten, and softens the cardava banana nutribun texture. Egg whites help binds the dough because it has a character that promotes mixture strength. A mixture of garlic pastes and sugar, aside from its chemical content, made the bread enhance its flavor, aroma, and mouthfeel. The mixture of fish and seagrasses powder quantifies bread with protein and iodine.

Methods

Preparation

In the acquisition of cardava banana raw materials, it was done at around 6:00 O'clock in the morning, in the early month of August, when there was still morning dew, the unripe Cardava banana was acquired; peeled, and sliced with a kitchen knife; laid, and oven-dried using the dehydrator for 20 minutes or reaching the Maillard reaction. Grounded, powdered, blended, and refined using the food processor, blender and stored at room temperature.

Experimental Design and Treatment

This study was using the 3x3 factorial design of experiments (DoE), (Granato, D., de Ara, M., de Ou, E., (2013) [18] where 9 samples were developed, a Placket-Burman design, (Vanaja, K. & R.H. Shobha Rani (2007) [45] which made into 9 replications. Complete Randomized Design and Optimization were also used by determining the low and high levels samples by 9 requested expert panels' tastes' test using the Latin Square William Design, (Wang, B.S., Wang, Xiao-Jin, Gong, Li-Kun 2009)[46] and 9-Point Hedonic Scale, (Yeung, H., Homwongpanich, K., Michniuk, E., Rovai, D., Migliore, M., Lamert, A., Lahne, J., (2021) [48]; da Silva, A., da Silva, R., Ferreira, M., Minim, V., da Costa, T., Perez, R., (2013) [6]. Until three treatments were chosen as the most accepted samples as final and highly optimized treatments.

Treatments' Composition

Nine treatments were made as the results of the 3x3 factorial experimental design using 3 factors in 3 levels. Factor A was the A_1 which was composed of 45% (112.5 g) cardava banana flour, 55% (137.5 g) all-purpose flour, and 1% (3.15 g) yeast; A_2 was composed of 35% (87.5) cardava banana flour, 65% (162.5g) all-purpose flour, and 1% (3.15 g) yeast; A_3 was 25% (62.5 g) cardava banana flour 75% (187.5) all-purpose flour and 1% (3.15 g) yeast. Factor B which was the 3 levels was B_1 , the 125 grams full cream milk and 2 pieces (90g) of eggs; B_2 was a 62.5 grams mixture of garlic paste and sugar; and factor B_3 was the 3 grams of fish and seagrasses powder.

The following treatments' compositions were made by combining factor A and factor B. The first combination was A1+B1 + B2 + B3 (45% (112.5g) cardava banana flour, 55% (137.5) all-purpose flour, 1% (3.15 g) yeast,125g full cream milk and 2 pcs (90g) eggs, 15 g mixture of garlic paste and sugar, 3 g mixture of fish & seagrasses powder); the second was A1+B1+B2 (45 % (112.5 g) cardava banana flour, 55% (137.5 g) all-purpose flour, 1% (3.15 g) yeast, 125g full cream milk and 2 pcs (90g) eggs,15 g mixture of garlic paste and sugar); the third was A1+B1 (50% (125g) cardava banana flour, 50% (125g) allpurpose flour, 1% (3.15 g) yeast, 125g full cream milk and 2 pcs (90g) eggs). In the fourth sample was the A2+B1+ B2 + B3 (35 % (87.5g) cardava banana flour, 65% (162.5g) allpurpose flour, 3.15 g yeast, 125g full cream milk and 2 pcs (90g) eggs,15 g mixture of garlic paste and sugar, and 3 g mixture of fish and seagrasses powder); the fifth was A2+B1+ B2 (35 % (87.5 g) cardava banana flour, 65% (162.5 g) all-purpose flour, 3.15 g yeast, 125g full cream milk and 2 pcs (90g) pcs eggs, 15 g mixture of garlic paste and sugar); the sixth was A2+ B1 (35 %(87.5 g) cardava banana flour, 65% (162.5 g) all-purpose flour, 3.15 g yeast, 125g full cream milk and 2 pcs (90g) eggs). In the seventh sample was the A3+B1+B2+B3 (25 % (62.5 g) cardava banana flour, 75% (187.5g) all-purpose flour, 3.15 g yeast, 125g full cream milk and 2 pcs (90 g) pcs eggs, 15 g mixture of garlic paste and sugar, and 3 g mixture of fish & seagrasses powder); the eighth was A3+B1+B2 (25 % (62.5 g) cardava banana flour, 75% (187.5 g) all-purpose flour, 3.15 g yeast, 125g full cream milk and 2 pcs (90 g) eggs, mixture of garlic paste and sugar). In the ninth sample was the A3+B1 (25 % (62.5 g) cardava banana flour, 75% (187.5 g) all-purpose flour, 3.15 g yeast, 125g full cream milk, and 2 pcs (90 g) eggs. The tenth one was the control sample which was made of bread flour, milk, sugar, eggs, and yeast commercially available in the market.

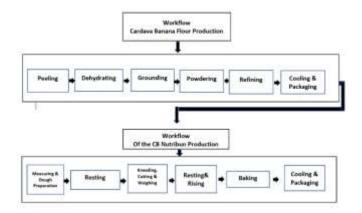


Figure 1: Work Flow of the Production of Cardava Banana Nutribun Bread

There was two product development in the production of the cardava banana nutribun, flour, and nutribun bread. In flour production, unripe matured cardava bananas were weighed, peeled, sliced, oven-dried, grounded, powdered, blended, and stored at room temperature. Cardava banana nutribun bread production was done through measuring for dough preparation, resting the dough, kneading, cutting weighing, re-resting for rising the dough, baking, cooling and storing, and packaging.

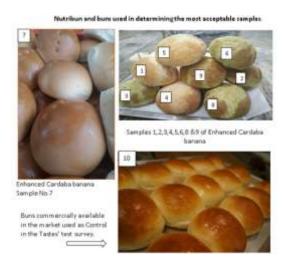


Figure 2: Nine Cardava Banana Nutribun Bread Samples or Treatments and the Control Bun Bread Used in the Study

Description and Determination of the Acceptability Level of Cardava Banana Nutribun Bread

Cardava banana nutribun bread weighed 62.5 g, circular in volume, light brown to Maillard reaction, dull white and organic texture of approximately 7.5 cm in diameter and thickness of 6cm, and the cost per piece is \$0.09. Nine-point Hedonic scale was used in the determination of the acceptability using organoleptic evaluation such as the Color, Aroma, Flavor, Mouthfeel, Sweetness, Texture, and General acceptability. This ranges from a scale of 0 - 1.99 which was described as disliked extremely, 2 - 2.99 as disliked very much, 3 - 3.99 as disliked moderately, 4 - 4.99 as disliked slightly, and 5 - 5.99 as neither liked nor disliked, 6 - 6.99 as liked slightly, 7 - 7.99 as liked moderately, 8 - 8.99 as liked very much, and 9 - 9.99 as liked extremely.

The expert panels were 9 expert bread bakers, 3 adult people (40 years and above) with 15 years and above experience as bread bakers, 3 young adults (29 to 39 years old) bread bakers with 8 to 14 years of experience in baking, and 3 young bakers (18 to 28 years old) 3 to 7 years experienced in bread baking. Expert panels that determined the low and high-level samples were notified through text messages and formal letters 2 weeks prior to the conduct of the taste test. The taste test area was prepared with enough lights that enhanced the appetizing appearance, color, and texture of the bread.

Table 1 below was the arrangement of the panels' participants and treatments of cardava banana nutribun bread composition using the optimized Latin Square William Design. Nine treatments for every panel participant were arranged for a simultaneous taste test. The sensory and organoleptic evaluation of cardava banana nutribun was composed of internal and external parts. The internal evaluation such as Appearance (grain), Crumb color, Flavor, Aroma, Mouthfeel, and Crumb texture; external sensory evaluation – Bread volume, Break and shred, Symmetry (uniformity of shapes), Crust color, and Crust surface. This study used this method as standard criteria for determining the high and low-level samples.

Table 1: Treatment Replication of Cardava Banana Nutribun Bread Using Optimization and Latin Square William Design and Arrangement of Samples for Panel Tastes' Test Ranking and Identification of High and Low-Level Samples of Cardava Banana Nutribun Bread

Panel	Treatment								
	10	2	.3		50	36	.7		9
1	A		c	D		F	G	*	
2		c	D			6	н	33	A
3	C	D			G	н	t	A	
4	D	E		6	*	1	A		c
5	t		6	*	1	A		c	B
		G	м		A		c		
7	6	н	1	A	8	c	0	ŧ	
В	н		A		c	D	t		G
9	1	A		c	D	t		G	

The three most accepted samples chosen by panels were A₃+B1+B2+B3, A2+B1+B2+B3, and A1+B2+B3 as ranked one, two, and three. These were chosen as high-level samples and

underwent laboratory tests to ensure food safety and were subjected to acceptability through taste tests. The fourth one was the sample or treatment A3+B1+B2, followed by A2+B1+B2, then sixth in rank was A1+B1+B2, seventh was the control sample, eighth was A3+B1, ninth was A2+B1 and 10th was A1+B1.

Analysis of Cardava Banana Nutribun Bread Determination of Microbiological Content

Microbiological testing of food is important to ensure that products manufactured are safe for human consumption. This includes Aerobic Plate Count (CFU/g) using the pour plate method, Escherichia coli (CFU/g) with the use of multiple tube fermentation technique, Salmonella using 3 M Petrifilm, Staphylococcus aureus (CFU/g) using the compact dry media method, Listeria, using the reveal 2.0 for listeria, Yeasts (GFU/g) using the pour plate method (72 hours) and Molds (CFU/g) using the pour plate method (72 hours).

Determination of Toxic Substance

The chemistry of the mixture may create toxicity to the human body and every human body has an average daily intake which is why a balanced diet was created. It is important to determine the number of nutrients per serving that an individual has to undertake. So, with this determination of the toxicity level of cardava banana nutribun bread and the level of microbiological content was considered, then food safety was assured for sensory evaluation and consumers' tastes test. In this study, toxicity was assessed by measuring the % of mortality by preparing four samples or treatment trials using 250 g, 500g, 750g, and 1,000g content.

Physical and Chemical Content Analysis

Food is made - up closely of edible biochemical which are mainly derived from living sources such as plants and animals, (Potter, N. & Hotchkiss, J., 2012) [37]. In this study, Cardava banana Nutribun bread was analyzed through the following physical properties: *food moisture*, the moisture was determined by measuring the dough to the desired size and deducting the baked bread after baking at 150°C for 20 minutes reaching Maillard Reaction, another process was by using the food analysis equipment and the same result was obtained. The cardava banana nutribun bread samples one, two, and three, the % moistures were 21, 22.21, and 21.41%. Those were determined by deducting their weight before and after baking. Another one is *heat temperature*, the heat and Time factors were the 150°C in 20 Minutes reaching Maillard Reaction. This study used the drying method principle in the determination of moisture.

$$M_{\text{INITIAL}} - M_{\text{DRIED}}$$
%Moisture = ----- X 100
 M_{INITIAL}

Here, $M_{\rm INITIAL}$ and $M_{\rm DRIED}$ are the mass of the sample before and after drying, respectively. The basic principle of this technique is that water has a lower boiling point than the other major components within foods, for instance, lipids, proteins, carbohydrates, and minerals. Sometimes a related parameter, known as the *total solids*, is reported as a measure of the moisture content. The total solids content is a measure of the amount of material remaining after all the water has been evaporated.

The chemical content determined in this study was considered from the iron anemia deficiencies which the following macro and micronutrients were determined: Fibre, Carbohydrates, Calories, Fat, Protein, Vitamin C, Magnesium, Sodium as NaCl, Iron, Potassium, and Phenolic acid. The Negros Prawn Analytical Laboratory adapting the Official Methods of Analysis of the Association of Analytical Chemists and the ASEAN Manual of Food Analysis Regional Center of ASEAN Network of Food Data System, Institute of Nutrition, Mahidol University, Thailand, 2011 were used as standards in this assessment.

Determination of Food Product Shelf-life

Shelf life is referred to as the time, under defined storage conditions, during which food remains safe, retains desired sensory, chemical, physical, and biological characteristics as well as complies with any label declaration, (Manzocco, L., Nicoli, M.C., (2016) [29]. The Shelf life of a product can be defined as a group of microbiological, chemical, and sensory tests performed on food products that determine at what point in time is the product no longer safe to consume and when the product losses its peak quality markers. Food moisture and heat temperature applied were also determinants of food product shelf-life. So % moisture and the heat temperature applied were determined then, upon production and undergoing laboratory tests, the shelf life of the products was determined by storing them at 18 0 C- 22 0 C room temperature. In this study, two product shelf-life was determined, the cardava banana flour and the cardava banana nutribun bread. Before cardava banana nutribun bread has been processed, the shelf-life of cardava banana flour had been assessed and it took one year before it was decomposed.

Acceptability through Sensory and Organoleptic Evaluation

The Sensory evaluation through organoleptic properties such as Color, Aroma, Flavor, Sweetness, Texture, Mouthfeel, and General Acceptability was determined by the use of the Nine-point Hedonic scale which is presented in Table 1. The participants for the taste test were the 50 mothers of one of the places in Sagay City, Negros Occidental, Philippines. Systematic sampling was used to determine the 50 participants out of 500 mothers, grouping them into ten members with the mothers holding the numbers one, three, five, seven, and nine were the chosen participants for the taste test. Statistical tools like Mean, ANOVA, and Standard Deviations were used in the determination of data.

3. RESULTS

After six months of surveys, and prototypes were made, the sample composition was designed and experimented with carefully. It was then subjected to analytical laboratory assessment and the following results are shown below:

Table 2. Microbiological Laboratory Test Results of the Three Treatments of Cardava Banana Nutribun Bread

Parameters		Result				
	Treatments					
	1	2	3			
Aerobic Plate Count (CFU/g)	4 × 10*	4 × 10 ⁴	4 × 10 ¹			
Escherichia Coli (CFU/g)	Not Detected	Not Detected	Not Detected			
Listeria	Negative	Negative	Negative			
Staphylococcus Aureus (CFU/g)	Not Detected at 10^1	Not Detected at 10 ¹	Not Detected at 10 ³			
Salmonella (25g Compact Dry Media)	Not Detected at 10 ¹	Not Detected at 10 ¹	Not Detected at 10			
Yeast and Molds	1 × 10 ³	1 ×1 01	1 x 10 ¹			

Ensuring food safety, the microbiological assessment through laboratory testing was undergone, and Table 2 were the results. These results imply food safety and within the safe limits of the Hazard Analysis and Critical Control Point (HACCP) of the Food and Drugs Administration (FDA).

Table 3: Toxicity Assessment Results of Cardava Banana Nutribun Bread

Sample 3		ric.		
	Trial 1	Trial 2	Trial 3	
Mean				
250	0	0	0	0
500	o	0	0	0
750	0	0	0	0
1000	0	0	0	0
LC50	Omg/L			

After the microbiological testing, a toxicity assessment was then conducted. It was done using four samples trial 250, 500, 750, and 1000 mg content. Results in Table 3 imply that cardava banana nutribun bread is safe to eat and can be subjected to acceptability using organoleptic properties.

Table 4: Shelf-life Assessment of Cardava Banana Nutribun Bread

Cales	Appearance Arona/Testure	\$12, 626, 655-953 (S4255);	
		Color: Epprension: Anima-Testum	Caler Appearance Arena Tecture
1			
2			
4			

The shelf-life assessment of cardava banana nutribun bread of samples 1, 2, and 3 was placed on a store shelf together with other pieces of bread. As shown in Table 4, in the first 24 hours, there was no deterioration of Appearance, Color, Aroma, Flavor, Sweetness, and Texture. On day 2, or after 48 hours, the parameters were still the same. On day 3, samples 1,2, and 3 had deterioration in flavor and texture. It was then taken out from the shelf and transferred into a chilling compartment in the fridge, and after 72; 120, and 168 hours there was deterioration in flavor and texture. In this study, the shelf life of samples 1,2, and 3 last until 72 hours or 3 days.

Cardava banana nutribun bread shelf-life lasts up to three days or 72 hours. Upon production, this bread shall be subjected to further consumption. Other ways like storing at the chilling can be advisable but deterioration in flavor and texture was detected.

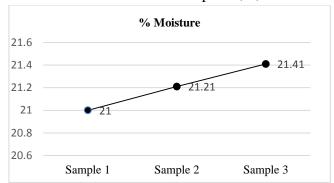
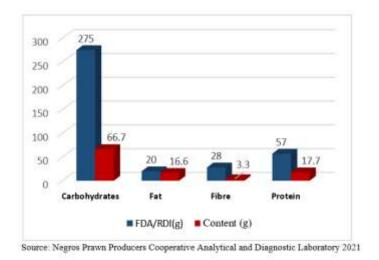


Table 5: % Moisture Content of Samples 1, 2, and 3 of Nutribun

The results of the assessment of the moisture content of samples one, two, and three were 21, 21.21 and 21.41 respectively as shown in Table 5. This means that, when the content of unripe cardava banana flour is increased, the % moisture is decreased.

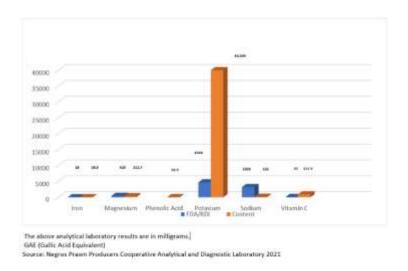
Table 6: Analytical Laboratory Tests Results of Macronutrients in 25% Unripe Cardava Banana Flour of 62.5g g Serving Size Cardava Banana Nutribun Bread with Comparison on the Suggested Daily Value of Food and Drugs Administration (FDA)



11278

Macronutrients are chemical food substances required for individual daily intake in a larger amount of the diet like Carbohydrates, Fats, and Proteins. Results in Table 6, indicated by red color show the content of cardava banana nutribun bread with 25% unripe cardava banana flour. Carbohydrates have 66.7g, the required daily intake is 275g; fat has 16.6 g, and the required daily intake is 20g; Protein has 17.7g and the required daily intake is 57g. Comparative results imply that this 62.5g cardava banana nutribun bread has a significant amount of Carbohydrates, Fats, Protein, and Fibre which can supplement a daily meal.

Table 7: Analytical Laboratory Tests Results of Micronutrients in 25% Unripe Cardava Banana Flour of 62.5g Serving Size Cardava Banana Nutribun Bread and Suggested Daily Value of Food and Drugs Administration (FDA)



Micronutrients are vitamins and minerals needed by the body in small amounts. Their impact on the body's health is critical, and deficiency in any of them can cause severe and even life-threatening conditions. These enable the body to produce enzymes, hormones, and other substances needed for normal growth and development, (WHO, 2022) [40].

Table 7 shows the comparative results of micronutrients or chemical laboratory tests of 25% unripe cardava banana flour in 62.5g serving size of cardava banana nutribun bread and the recommended daily intake (RDA) of the Food and Drugs Administration (FDA). Micronutrients like Iron was 38.9mg and the FDA daily value is 18 mg; Magnesium was 312.7mg and the FDA recommended daily value is 420 mg; Potassium was 40g (40,000mg) and the FDA recommended daily value is 4700mg; Sodium was 150mg and the FDA recommended daily value is 3200 mg, and Vitamin C was 913.5 and FDA recommended daily value is 90mg; Phenolic acid was 62.4mg. These results imply that this cardava banana nutribun has intensified content of Vitamin Vitamin C, Potassium, and Iron which were 10th times, eighth times, and doubled the recommended daily value respectively. Vitamin C is 10th times higher than the daily requirement.

Table 8: Level of Acceptability and Significant Difference of Cardava Banana Nutribun Bread Treatments through Organoleptic Evaluation

Parameter	N					
		1 (45%(BE)	2 (35%CBF)	3 (25%CBF)	4 (Control A)	p-value
Color	50	8.22	8.10	8.26	7.96	0.018
Aroma	50	8.24	8.10	8.08	7.92	0.008
Flavor	50	7.92	8.22	8.24	7.78	0.051
Mouthfeel	50	8.24	7.82	8.04	7.94	0.008
Sweetness	50	7.66	7.78	7.70	7.42	0.024
Texture	50	7.66	7.86	7.86	7.36	0.016
General Acceptability	50	7.86	7.72	7.44	7.32	0.062

The basis of scales and descriptions of the data were the following: for 0 - 1.99, Disliked extremely; for 2 - 2.99, Disliked very much; for 3 - 3.99, Disliked moderately; for 4 - 4.99, Disliked slightly; 5 - 5.99, neither like nor dislike, 6 - 6.99, Liked slightly, 7 - 7.99, Liked moderately, 8 - 8.99; Liked very much, 9 - 9.9, Liked extremely.

Table 8 shows the acceptability level and significant difference between cardava banana nutribun bread treatments and the control bun using organoleptic evaluation and also the significant difference in the parameters. The mean results for Color were 8.22, 8.10, 8.26, 7.96 and the p-value was 0.018; Aroma was 8.10, 8.10, 8.08, 7.92, and the p-value was 0.008; Mouthfeel was 7.92, 7.82, 8.04, 7.94 and p-value was 0.008; Texture was 7.66, 7.96, 7.86, 7.36 and p-value was 0.016; Flavor was 8.24, 8.22, 8.24, 7.78 and p-value was 0.051; Sweetness was 7.64, 7.78, 7.70, 7.42 and p-value was 0.024; General acceptability were 7.86, 7.72, 7.44, 7.32 and p-value was 0.062. All samples were in the Liked category. Nutribun samples 1,2 and 3 were on the Liked Moderately level and the Control bun was on the Liked Slightly level. Each parameter was significantly different from the other

4. DISCUSSION

Determination of Microbiological Content of Cardava Banana Nutribun Bread and its Effects on its Toxicity and Shelf-Life

The presence of specific bacteria and their concentration must be determined, to assess and control safety hazards, and potential for spoilage, and to ensure correct product characteristics for the reason that <u>Food contamination</u> is a severe public health problem resulting in food-borne diseases that affect human beings around the world. In this study, the following were determined through microbiological laboratory testing. First was the *Aerobic Plate Count*, the standard content of samples required for testing is 100 grams, (Guidelines for microbiological examination for ready-to-eat foods by NSW, 2009) [23], and the satisfactory level content of APC in food is <10⁴ in a colony. In this study, the satisfactory level results indicate that this ready-to-eat food has a good microbiological quality. Next was *Escherichia coli*, the content of the sample is 100 grams at 30⁰ C within 48 hours and the satisfaction level based on the guideline is <20 CFU per gram and should not be detected in 25 grams, (Food and Environmental Hygiene Department, Hong Kong 2001)[13], in this

cardava banana nutribun bread, the Escherichia coli has not been detected, this means that this bread was free from Escherichia coli; for *Salmonella*, in this study, the method used was the 3M Petrifilm, and the satisfactory level should not be detected in 25 grams sample, analytical laboratory results show that this bread was free from salmonella; for *Staphylococcus aureus*, in this study, the method used in determining the staphylococcus aureus was Compact Dry Media, and the satisfactory level is <20 CFU per gram, (Guidelines for Food and Environmental Department, Hong Kong 2001)[22], this bread was free from Staphylococcus aureus. The method used in determining the *Listeria* in this study was Reveal 2.0 Listeria and the satisfactory level was absent in 100CFU/gram or not detected in 25 grams, (Food Standards in Australia and New Zealand, 1991) [15], so this bread was free from Listeria; the *Yeasts and Molds*, in this study, the satisfactory level baseline is 10 ³/g using 3M Petrifilm, (DUS 979, 2021) [10], cardava banana nutribun bread was safe from yeasts and molds. Cardava banana nutribun bread samples one, two, and three were safe to eat.

Safety on microbiological content also denotes of none toxic food. Results of the toxicity assessment of this cardava banana nutribun bread imply that this food is within the level of food safety of the FDA (Food and Drug Administration). In the same situation, cardava banana nutribun bread shelf-life was contributed by its microbiological content, moisture content, and the amount of heat or heat temperature during production. Based on observation the shelf-life of cardava banana nutribun bread lasts up to three days or 72 hours. Upon production, this bread shall be disposed of for consumption. Other ways like storing at the chilling compartments can be advisable but deterioration in flavor and texture was detected.

Determination of Macronutrients or Chemical Content of Cardava Banana Nutribun Bread

In this study, Cardava banana nutribun bread has been analyzed through the following chemical properties: the *Carbohydrates*, the Food and Drug Administration (FDA) recommends that people get 275 grams of carbohydrates each day in a 2,000-calorie diet, this includes dietary fiber, total sugars, and added sugars, which were listed on food labels. The Philippine Dietary Reference (PDRI) Intake 2018 [35] recommends average carbohydrates daily intake for adults from 55 to 79%, so 62.5 grams of the Carbohydrate content of a nutribun is enough for carbohydrates daily requirement; on the other hand, Fat like monounsaturated and polyunsaturated fats are healthy fats that insulate human body organs, (Arshad, M.S., et al, 2020[2]; Timilsena, Y.P., Wang, B., Adhikari, R., Adhikari, B., 2017)[42], the saturated Fat, FDA recommends a 20g daily intake so this cardava banana nutribun bread contains good fat and is within the fat daily value requirement; next is the Fibre, is a complex carbohydrate, a type of sugar that is broken down by the body to provide fuel in the form of glucose and cannot be digested by the human body, it is also a part of plant material in the diet that is resistant to enzymatic digestion which includes cellulose, noncellulosic polysaccharides such as hemicellulose, pectic substances, gums, mucilages, and non-carbohydrate component lignin, (Dhingra, D. et al, 2011)[9]; fibre has two types: soluble and insoluble; soluble fiber helps reduce high cholesterol and manage blood sugar, while insoluble fiber helps alleviate constipation; both are important and can be found in plants, meat from fish, and animal foods, (Stephen, A. et al, 2017)[40]; children and adults need at

least 20 to 30 grams of fiber per day for good health; the updated FDA Daily Value recommends 28g daily intake, and since this cardava banana nutribun bread can be considered either functional or comfort food fiber content is enough for fiber daily requirements, so the remaining amount of fiber daily value requirements can be taken from other meals of the day; another is *Protein*, during breakfast, 10 grams or less of Protein from oatmeal, nuts, or berries, 25 g during lunch, 5 grams at snacks, and 40 grams during dinner is an advisable pattern, (Hyman, M., 2018)[25], the FDA daily value for Protein is 50 grams and this this nutribun contains protein intended as a portion of comfort food.

Determination of Micronutrients or Chemical Content of Cardava Banana Nutribun Bread

The micronutrient results were indicated with units of a milligram (mg) because of the lesser amount needed by the individual. In the assessment of *Iron*, the FDA recommends 18mg of Iron daily intake, so a piece of cardava banana nutribun bread has more than double the content of Iron daily value requirement, and eating ½ a piece of nutribun every day answers the Iron Anaemia problem of a person. *Magnesium* is important for many processes in the body, including regulating muscle and nerve function, blood sugar levels, and blood pressure, and making protein, bone, and DNA. FDA, (Grober, U., Schmidt, J., Kisters, K., 2015) [20], recommends a daily intake of 420 milligrams, and 62.5 grams of cardava banana nutribun has 312.7 milligrams, a 1 ½ piece is enough to complete the Magnesium daily intake. The *Potassium*, on the other hand, FDA recommends 4700 milligrams of daily intake of Potassium and this cardava banana nutribun bread contains 40g 0r (40,000 mg) of Potassium, this was eight times higher than the daily requirement of an individual. The Sodium as in NaCl, FDA, recommends NaCl intake of up to 2,300 milligrams, about a teaspoon of table salt. Consuming too much salt can lead to hypertension and this cardava banana nutribun bread has minimal Sodium content which is enough as a functional food. The Vitamin C on the other hand, the recommended daily allowance for vitamin C in the United States for male and female adults is 60 mg. In the United Kingdom and Canada, the recommended daily allowance has been 30 milligrams. In the Philippines, RDA (Recommended Daily Allowance for children is 45 milligrams, for an adult male is 70 milligrams, for a female is 60 mg, for pregnant women is 70 mg and 95 mg for lactating mothers. This cardava banana nutribun bread has 10 times higher than the daily required amount of Vitamin C which is important to a person's daily intake. This Nutribun has also Phenolic Acid content which is enough to resist microbes. Like flavonoids, Phenolic acid is also inflammation-resistant, (Kiokias, S., Oreopoulou, V., 2021) [28].

Level of Acceptability Using Organoleptic Evaluation and Significant Difference between the Parameters

The following were the interpretations of the data in Table 8. The Food *Colors* of samples one, two, and three were liked very much by the audience, and the control was liked moderately. This proves that color plays a key role in food choice by influencing taste thresholds. Colors convey critical information about edibility, flavor identity, and intensity. Browning with the help of brushing egg whites as external coats add a desirable outer appearance to the bun. The *Aroma* of samples one, two, and three were liked very much by the audience, and the control was liked moderately. The smell was the most direct among the five human sensory characteristics. The aroma was the key driver of the human flavor

experience. These were crucial for the synergy of food. This means that the blended mixture of garlic paste was a strong factor that was liked very much by the audience. The *Flavors* of samples one, two, and three were in the liked very much category and the control was in the like moderately. This means that the garlic paste mixed with sugar contributed to the flavor. Mouthfeel is defined as the sensations arising from the interactions of ingested food, mixed with saliva, with the receptors in the mouth that respond to tactile stimuli during mastication, (Smith and Margolskee, 2001) [39]. The mouthfeel of samples one, two, and four, was liked moderately by the audience and Sample 3 was liked very much. This means that combinations of ingredients in sample 3 have sensations that respond to tactile stimuli during mastication. The *Sweetness* of samples one, two, three, and four were in the liked moderately category. This means that all samples observed the right amount of sugar. *The texture* is an important aspect of consumer food acceptance and preference and the fact that specific consumer groups including infants, the elderly, and people with special needs require texturemodified foods, (Food Science, Wood Head Publishing, 2015) [14]. The Textures of samples one, two, three, and four were in the liked moderately category. This means that the four samples have relatively the same texture. The general Acceptability of samples one, two, three, and four were in the liked moderately category. This means that the audience accepted all four samples relatively at the same level.

The Color, Aroma, Mouthfeel, Sweetness, Texture, and General acceptability of the four samples were significantly different from each other, except for the Flavors which were relatively the same. This means that the ingredients utilized which contributed to the flavors, especially the garlic paste and sugar have relatively the same degree of effects on each sample and further investigation will be done to check on its relativity.

5. CONCLUSION

Cardava banana flour has low microbiological content that leads to zero toxicity. Shelf-life of the cardava banana flour last until and beyond 365 days, only when this flour is mixed with eggs and milk and baked into bread, this lasts only until seventy-two hours. For any flour that is combined with milk and eggs, shelf-life of the bread products lasts three to four days.

This cardava banana nutribun bread from unripe cardava banana (*Musa acuminata x balbisiana var cardava*) has intensified the micronutrient content of Vitamin C, which has ten times, Potassium which is eight times higher than the required daily value and doubled the Iron daily requirement of a person. The breakthrough is that this bread has high Vitamin C content which by taking a 1/3 size or 20 grams of cardava banana nutribun, bread can answer the daily requirement of Vitamin C of an individual. Taking ½ size or 31.25 grams of this cardava banana nutribun bread, will answer the 18-gram daily value requirement of Iron. Nutribun has also a significant content of Calories, Fats, Carbohydrates, Proteins, Magnesium, Sodium, Potassium, and Phenolic acid.

Types of ingredients added to each treatment have contributed to the effects in color, aroma, mouthfeel, and general acceptability. Flavor and sweetness have relatively the same. The further appraisal was then conducted to point out the relativity of each treatment. The most

acceptable compositions of intensified nutribun imply that the 21st Century mothers are carried by the new principle of food preference that the majority of the new generation liked extremely the super tasty and bold food.

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7. CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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