Global Journal of Advanced Engineering Technologies and Sciences

PROXIMATE CONTENT AND SENSORY ACCEPTABILITY OF CARROT AND SWEET POTATO – FILLED CHOCOLATE BITES INCORPORATED WITH *LACTOBACILLUS CASEI* AND *LACTOBACILLUS PLANTARUM*

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ABSTRACT

In this study, producing carrot and sweet potato-filled chocolate bites incorporated with probiotics including L. plantarum and L. casei was investigated for three weeks. The proximate content and sensory acceptability were determined. Results showed that the developed carrot and sweet potato-filled chocolate bites are high in ash, fat and moisture while they recorded low protein and carbohydrate contents after conducting proximate analysis. On the other hand, sensory analysis showed that carrot and sweet potato - filled chocolate bites during the study were generally acceptable. All sensory parameters showed no significant difference with each other. It can therefore be concluded that the carrot and sweet potato-filled chocolate bites are generally acceptable based on its proximate content and sensory acceptability.

Keywords: Lactobacillus plantarum, Lactobacillus casei, vegetable-filled chocolate

I. INTRODUCTION

There is an increasing trend for functional foods that improve the health and well-being of consumers. Foods that are incorporated with probiotics are one of the functional foods that are trending nowadays. Probiotic can be placed in a widespread variety of food substances. The WHO (World Health Organization) and the FAO (Food and Agriculture Organization) of the United Nations considered probiotic as live microorganisms, which, when administered in adequate amounts will provide a health benefit on the host. Most probiotics are similar to microorganisms naturally found in the human gastrointestinal (GI) tract [8].

Lactic acid bacteria (LAB) are the most common type of probiotic and these bacteria are generally found in dairy produce, creating lactic acid as one of its main fermentation products. These bacteria mostly grow best in moderate temperature neither too hot nor too cold and may be classified as homo-fermentive or hetero-fermentive. *Lactobacillus casei* is a kind of lactic acid bacteria, a bacterium present particularly in hard cheeses and in cheeses made from raw milk [9]. *Lactobacillus plantarum* on the other hand is a prevalent lactic acid bacteria found in fermented foods as well. Application of *Lactobacillus plantarum* and its probiotic properties partakes superbly over the past several years [14].

But probiotic cultures can also be incorporated in fruit and vegetable as substrate for it contains nutrients that support probiotics. Vegetable like carrots, comprise of approximately 88% water, 7% sugar, 1% protein, 1% fibre, 1% ash, and 0.2% fat. Sugars are sustainable food for the probiotics thus carrots can be a potential substrate for probiotic. Free sugars in carrot include sucrose, glucose, xylose and fructose [7]. On the other hand, sweet potato was also used in this study which comprises approximately 54.83% moisture, protein ranging from 0.46% to 2.93%, dietary fiber ranging from 0.49% to 4.71%, lipid ranging from 0.06% to 0.48% and ash ranging from 0.31% to 1.06%. It contains essential mineral nutrients such as Ca, P, Mg, K, S, Fe, Cu, Zn, Mn, Al and B. Sweet potato is also important source of vitamin A, thiamine, riboflavin, niacin, ascorbic acid and many other functional compounds [17]. This indicates that sweet potato can also be a potential substrate for probiotics.

Moreover, chocolates are typically considered comfort food and used as a dessert. Chocolates also often presaged as one of the main reasons in incurring diabetes because of its sugar content but nowadays, studies have shown that chocolates can be a healthy food with the thorough study of the chocolate itself and combining nutrients that can benefit its aficionados.

It would exert a beneficial impact on human health when lactic acid bacteria are incorporated in food due to its numerous health benefits. Chocolate bars with lactic acid bacteria are one application that has been successful. Research shows that chocolate bars with lactic acid is one application that has proven successful. Therefore, the aim of the study is to determine the proximate content and sensory acceptability of the developed carrot and sweet potato filled – chocolate bite incorporated with lactic acid bacteria.

II. MATERIALS AND METHODS

MICROBIAL CULTURE AND GROWTH CONDITIONS

Pure cultures of *Lactobacillus casei USTCMS 1050* and *Lactobacillus plantarum* were obtained from the University Of Santo Tomas Collection Of Microorganisms at the Thomas Aquinas Research Complex, Graduate School. The acquired microorganisms served as the mother culture. Three test tubes containing MRS broth for each microorganism were prepared. They served as the sub-culture. The sub-cultures were placed in an incubator for 24-48 hours at $37^{O}C$. After the sub-cultures were prepared, disposable petri plates and MRS Agar were prepared. A loop full amount of *L. casei* and *L. plantarum* that were obtained from the previous sub-cultures were streaked on the MRS Agar blanks prepared in petri plates using multiple streaking. The MRS Agar was then placed inverted in an incubator for 24-48 hours at $37^{O}C$ [16].

Enumeration of Lactic Acid Bacteria in Carrot and Sweet Potato-Filled Chocolate Bites

For the MRS broth culture containing *L. casei* and *L. plantarum,* conventional plate method was used to determine its initial count. One (1) McFarland Barium Sulfate Standard was used to compare the turbidity of the previously washed culture with de-ionized water. First, MRS Broth and MRS Agar were prepared. Then, for the chocolate samples containing the microorganism, 11 grams of chocolate sample was weighed and it was homogenized with a previously prepared and sterilized 99 ml MRS broth using a blender. Serial dilution was conducted up to 10^5 using a micropipette. A 0.1 ml of cell suspension in the 10^5 dilution was obtained and was placed in each triplicate plate for each microorganism on the prepared disposable petri plates using pour plate method. After the agars on the plates were solidified, the plates were placed inverted in an incubator for 24-48 hours at $37^{O}C$ [16].

Preparation of Carrot and Sweet Potato-filled Chocolate Bites

The milk chocolate and unsweetened chocolate were purchased from all about Baking, Quezon Ave, Quezon City, Metro Manila. The carrots were purchased from a local supermarket. The packaging material (Laminated Foil) was purchased at RM Boxes, Sta. Mesa, Manila.

The carrot fillings were prepared by removing the non-edible portion and it was immersed in an acetic acid-water bath (1:4) solution to remove dirt and extraneous materials from the skin of the carrots. Then it was sliced thinly and was blanched for 4-5 minutes at 100^oC. After blanching, the carrots were drained and were submerged into an ice bath. After the carrots were cooled, it was drained and was grinded with a food processor until the sizes of the carrots are fine and even. The sweet potato filling was prepared by removing the non-edible portion and was boiled for 15 – 30mins. to remove the extraneous matters and dirt. It also helps soften the sweet potato. Then after boiling, the boiled sweet potatoes were mashed until good enough for mixing with the melted chocolate. The chocolate coating was prepared by melting and tempering first the milk chocolate at 30-31.1^oC. Moulders were filled with 4.0 grams of milk chocolate and the chocolate was evenly distributed within it. The chocolate was cooled immediately with an ice bath. The chocolate filling was prepared by melting and tempering 200 grams of dark chocolate at 32^oC. Two hundred grams of the prepared carrots and another 200 grams of prepared sweet potato were mixed with the dark chocolate until all the carrots were evenly distributed. Then 6.5 grams of the chocolate filling was placed inside the prepared chocolate coating. A 100 μL (0.1 ml) culture of *L. casei* or *L. plantarum* was injected at the middle of the chocolate using a micropipette. The chocolate bites were sealed with 2.5 grams of milk chocolate.

The chocolate bites were removed from molders and was packaged using a laminated foil.

Moisture Analysis

Moisture content was determined by heating the chocolate bites to a constant weight at 105ºC for two (2) hours and measuring the weight lost due to evaporation of water. The Oven Drying Method (AOAC 14.004) was used. The dry matter was calculated as the difference of moisture content from 100. Approximately 5 g of sample was weighed in a pre-dried aluminum dish and it was placed in the drying oven at 105ºC for 1 hour and 30 minutes. The dish with the dried sample was cooled in a dessicator and was weighed.

Protein Analysis

The amount of protein in the chocolate bites was determined and performed by Qualibet Testing Services Corporation using the Kjeldahl Method wherein approximately 2-5 g of sample was weighed and placed in a Kjeldahl flask. Few pieces of glass boilers were placed on the flask to prevent spillage when boiling occurs then 6.75 g of anhydrous potassium sulfate, 0.75 g copper sulfite pentahydrate, 20 ml conc. Sulfuric acid and 10 ml of 35% hydrogen peroxide were added and cooled. The mixture was allowed to stay overnight in the fume hood. The digestion tube was placed in digesting thermo reactor for 20 mins. at 420^{O}C or until the digest was clear. The digest was then transferred to a 100 ml volumetric flask diluting up to its mark. An aliquot volume of 20 ml was transferred in a distilling flask. A 50 ml of 35% NaOH was added. The distilling flask was placed in a steam distilling unit. A collecting Erlenmeyer flask containing 25 ml of 4% boric acid and 2 drops of methyl red indicator was placed in position in the steam distilling unit. The distilling unit was programmed to have an SP of 140^{O}C, 10 ml of H_2O, distillation time of 2 mins. and steam of 100%. The distillation cycle was started and 100 ml of distillate was collected. Three drops of methyl red indicator was added from the distillate and was titrated with 0.1 N HCl to its first clear red endpoint.

The protein content is calculated using the formula:

$$\%Nitrogen = \frac{(ml\ std\ HCl)(N\ HCl)(1.4007)}{Wt.of\ Sample}$$

$$\%Protein = \%Nitrogen\ x\ Factor$$

Determination of Percent Ash

Percent ash was determined using the general method of dry ashing (AOAC 923.01). First, approximately 5-10 grams of sample was weighed in a previously dried crucible. The sample was charred, and then it was placed inside a muffle furnace to ignite at about 550ºC until the ash is white. The crucible with the ash was then transferred in a dessicator and it was allowed to cool. The amount of ash present was determined gravimetrically.

The ash content is calculated as follows:

$$\%\ Ash\ (Dry\ Basis) = \frac{wt\ after\ ashing - tared\ wt\ of\ crucible}{original\ sample\ wt\ x\ dry\ matter\ coefficient}\ x100$$

Wherein,

Dry matter coefficient = %solids / 100

Fat Analysis

Percent fat was determined using the Soxhlet Extraction Method in Cocoa Products (AOAC Official Method 963.15). Approximately 10-20 grams of sample was placed in a beaker. A 30 mL of water and 20mL of hydrochloric acid was subsequently added into the beaker, and then the solution was heated in a steam bath for 30 minutes with frequent stirring. Five grams of filter aid was then added to the solution, and it was allowed to cool by adding 50mL of ice-cold water. A heavy piece of linen was fitted in a Buchner funnel and it was moistened with water. A gentle suction was applied and a suspension of 3 grams filter aid in 30 mL water was poured over it. Then the hydrolyzed mixture of the sample was filtered using the gentle suction, and the beaker was rinsed thrice using iced cold water. The filtrate was also rinsed thrice and was sucked dry. The filter-cake was then transferred to the original beaker using a filter paper. The funnel was then rinsed with petroleum ether and it was placed in a steam bath to allow the ether to evaporate. The filter-cake was broken down using a glass rod and it was allowed to remain in the bath for the contents to dry completely, then it was transferred in the drying oven and heated for one hour at 100±2ºC. Then 15 grams of powdered anhydrous sodium sulphate was added and thoroughly mixed into the sample. The mixture was then transferred into the fat extraction thimble of the Soxhlet apparatus, and the beaker was washed with petroleum ether. The fat was extracted with at least 300mL of petroleum ether that is being circulated. The extracted fat was transferred in a tared dish and the petroleum ether was allowed to evaporate in a steam bath. Then the weight of the extracted fat was determined gravimetrically.

Percent (%) fat was determined as:

$$Total\ Fat\ \%\ by\ Mass = \frac{10000\ x\ w}{W\ x\ (100 - M)}$$

Wherein,
w= weight in grams of fat
W= weight in grams of prepared sample for the test
M= moisture, percent by weight, in the prepared sample.

Sensory Analysis

Sensory evaluation was conducted using 9-point Hedonic Rating Scale. A total of 15 semi-trained panellists composed of food science students evaluated the samples. Sensory parameters evaluated include: appearance, aroma, texture, flavour and general acceptability. Data analysis was performed using student T-test to determine significant difference between samples containing *L. casei* and *L. plantarum.*

Statistical Analysis

Bacterial counts were expressed as mean. Bacterial counts were the average of triplicate experiments. The significant differences between the microbial counts were calculated using the paired - difference t-test. One-way Analysis of Variance was also used to determine the significant difference of microbial counts from week 0 to week 3. Paired-difference t-test was used in determining the significant difference of *L. casei* and *L. plantarum* in its physico-chemical analyses of carrot and sweet potato-filled chocolate bites while one way Analysis of Variance was also used to determine the significant difference of the physico-chemical analysis of carrot and sweet potato - filled chocolate bites from week 0 to week 2.

III. RESULTS & DISCUSSION

The proximate analysis is a parameter measured in determining the nutritional content of a product. In the study, the products developed were carrot and sweet potato-filled chocolate bites incorporated with *L. casei* and *L. plantarum.* The obtained data for the proximate content of the said developed product are seen in Table 1.

Table 1. *Proximate Content of Carrot and Sweet Potato – Filled Chocolate Bites*

Parameters	**Carrots**		**Sweet Potato**	
	L. casei	***L. plantarum***	***L. casei***	***L. plantarum***
Ash (%)	3.46	2.91	2.41	2.49
Fat (%)	37.34	33.05	37.79	35.47
Protein (%)	5.52	5.70	4.21	4.14
Moisture Content (%)	20.00	20.17	15.58	15.15
Carbohydrates (%)	33.67	38.17	40.02	42.75

To further interpret the data, [11] signifies fat as extracted lipids that are solid in room temperatures. The fat content of the carrot filled chocolate bites containing *L. casei* and *L. plantarum* were 33.04 and 37.34 percent accordingly. While the fat content of the sweet potato filled chocolate bites containing *L. casei* and *L. plantarum* were 35.47 and, 37.79 percent correspondingly. The fat content of sweet potato filled chocolate bites containing *L. casei* and *L. plantarum* were higher than carrot. Chocolate are relatively high in fat [4], the difference in fat content may be due to the substrates used. In contrary with the results, carrots contain about 0.2 grams of fat per a hundred grams while sweet potato contains 0.1 grams of fat per hundred grams [15]. The refrigeration of the chocolate has helped it be able to maintain its quality. According to [1], the standard fat content of typical chocolate product is at 33% and based on the results obtained, the fat content of the products stated in table 1 are accepted. Sweet potato–filled chocolate bites obtained a higher fat content compared to carrot – filled chocolate bites.

Protein is a major structural component of natural foods. Free amino acids are growth factors for fermentative microorganisms. Release of amino acids is common among lactic acid bacteria. In general, it suffices to provide the necessary amino acids for growth [6]. The protein content of the carrot filled chocolate bites containing *L. casei* and *L. plantarum* were 5.52 and 5.70 grams per 100 grams of sample singly. The results of sweet potato-filled chocolate bites were 4.14 and 4.21 grams per 100 grams of sample. In relation [5], the protein content of sweet potato is relatively low. Thus, the protein content of the sweet potato filled chocolate bites is lower than the carrot filled chocolate bites. The standard values for the protein of chocolate as stated by [1] is at 7 grams per 100 gram sample therefore it can be noted that the values obtained for both carrot and sweet potato filled chocolate bites in terms of protein content are acceptable but relatively low. This may be due to the processes made in the chocolate bites that contributed to slightly low protein content.

Water and other volatile materials are vaporized and organic substances are burned during the process of dry ashing. Ash also provides the measurement of the total amount of minerals in a sample [10]. Ashing was performed once and the results for carrot-filled chocolate bites containing *L. casei* and *L. plantarum* were 3.46 and 2.91 percent respectively. As for the sweet potato - filled chocolate bites containing *L. casei* and *L. plantarum* were 2.41 and 2.49 percent accordingly. The carrot-filled chocolate bites had higher ash content than that of sweet potato-filled chocolate bites. This may be related to the pre-treatment methods prepared in the substrates. Carrots were blanched in the process while sweet potatoes were boiled. [18] stated that blanching does not affect the concentrations of most minerals found in vegetables and their concentrations stay stable after cooking, but with prolonged boiling few water-soluble minerals such as potassium may lost because the mineral leeches out into the boiling water. This phenomenon may have happen to the sweet-potato and hence decreasing its ash content but according to www.nutritiondata.self.com, the standard value for the ash content of a typical chocolate is at 2.3 grams and therefore, based on the ash results obtained, the ash values were generally accepted based on the standards.

The average moisture content obtained for carrot and sweet potato-filled chocolate bites were different in terms of value. The average moisture content of carrot-filled chocolate bites incorporated with *L. casei* and *L. plantarum* were 20.00 and 20.17 percent respectively while the average moisture content of sweet potato-filled chocolate bites incorporated with L. casei and *L. plantarum* were 15.58 and 15.15 percent respectively. It can be seen that carrot-filled chocolate bites had a higher moisture content compared to sweet potato-filled chocolate bite. This can be referred to the high moisture content of carrots compared to sweet potato. The obtained moisture content does not also confer with the standards given by [3] that the standard moisture content of a chocolate must not be greater than 1.2%. Again, it can be referred to the filling of the chocolate as carrots and sweet potato is regarded to have high moisture which contributes to the high moisture content of the chocolate itself.

The last proximate content measured was carbohydrates. Carbohydrates are known to provide energy in our body. Based on the results obtained, the carrot-filled chocolate bites incorporated with *L. casei* and *L. plantarum* have 33.67% and 38.17% of carbohydrates respectively while the sweet potato-filled chocolate bites incorporated with L. casei and L. plantarum have 40.02% and 42.75% of carbohydrates respectively. According to [1] the standard carbohydrate value in typical chocolate is at 57%. The values obtained were relatively low, mostly in carrot–filled chocolate bites because carrots are not considered to be high in carbohydrate. On the other hand, sweet potato-filled chocolate bites have a higher carbohydrate content compared to the carrot-filled chocolate bite. This may be due to the high carbohydrate content of sweet potato mostly with starch therefore it contributed to a high carbohydrate content of the sweet potato filled chocolate bites.

Sensory Acceptability of Carrot and Sweet Potato – Filled Chocolate Bites

Table 2. *Sensory Acceptability of Carrot - Filled Chocolate Bites*

Parameters	**Carrots**					
	L. plantarum			***L. casei***		
	Wk0	**Wk1**	**Wk2**	**Wk0**	**Wk1**	**Wk2**
Appearance^{NS}	7.9	7.7	8.1	7.8	7.8	8.0
Flavor^{NS}	8.1	7.7	7.6	8.1	8.0	7.7
Texture^{NS}	8.1	7.7	7.6	8.1	7.7	7.9

Aroma^{NS}	8.5	8.1	8.1	8.3	8.1	8.2
Color^{NS}	7.7	7.9	7.6	8.1	7.6	7.7
General^{NS} Acceptability	8.1	7.8	7.7	8.1	7.7	7.7

**ns = no significant difference for all parameters with degrees of freedom (df) = 14 at 5% level of significance using t-test*

Table 3. *Sensory Acceptability of Carrot - Filled Chocolate Bites*

Parameters	**Sweet Potato**					
	L. plantarum			***L. casei***		
	Wk 0	**Wk 1**	**Wk2**	**Wk 0**	**Wk 1**	**Wk 2**
Appearance^{NS}	8.5	8.2	8.6	8.4	8.3	8.2
Flavor^{NS}	7.7	7.6	8.0	7.5	7.6	7.9
Texture^{NS}	8.1	8.2	7.9	7.5	8.3	8.1
Aroma^{NS}	8.5	8.2	7.7	8.2	7.9	8.0
Color^{NS}	8.5	8.5	8.2	8.5	8.5	8.4
General^{NS} Acceptability	8.3	7.9	8.0	7.7	7.9	8.1

**ns = no significant difference for all parameters with degrees of freedom (df) = 14 at 5% level of significance using t-test*

The carrot and sweet potato-filled chocolate bites were considered to be generally accepted. As seen in Table 2 and 3, all the parameters measured have no significant difference with each other and is considered to be comparable. The fermentation process did not affect greatly the flavor of the chocolate as it did not turn to be sour as expected. This was due to the storage temperature used (refrigerated temperature) which inhibited the fermentation process and made the condition favourable for *L. casei* and *L. plantarum*. The microorganisms also carry the same product making it comparable for aroma and color. The color tends to be glossy brown due to the milk chocolate coating. Panellists also commented on the grittiness of the carrot-filled chocolate bites and the mushy texture of the sweet potato – filled chocolate bites. This could be due to the fibers from the carrots and the mashed sweet potato but as stated by [4], there is a phenomenon in chocolate called chocolate's seizing or the chocolate becoming a solid, grainy mass. This happens when a little moisture was added into the chocolate. The fine, dry sugar and cocoa particles glue together to change melted chocolate into a solid, grainy mass. As for the filling, it is made up of dark chocolate incorporated with carrots. The same ingredients were used in making the carrot-filled chocolate bite, thus making the color and aroma comparable. The carrot and sweet potato-filled chocolate bites appear to be glossy brown due to the tempering of milk chocolate. Chocolate might have also undergone tempering before it is formed and packaged. The purpose of tempering is to develop an even, smooth colour and shiny gloss, an ability to contract any mold, stability, and a longer shelf life [2].

IV. CONCLUSION & RECOMMENDATION

The study conducted dwelt on the development of carrot and sweet potato-filled chocolate bites incorporated with *L. casei* and *L. plantarum*. Proximate and sensory analyses were conducted to further evaluate the developed product. It can be concluded that the results in proximate analysis of carrot and sweet potato-filled chocolate bites recorded a high ash, fat, and moisture content and generally greater in the given standards. Meanwhile, the developed chocolate bites recorded low protein and carbohydrate content and not in conformance with the standards. Lastly, the results in sensory analysis showed that carrot and sweet potato - filled chocolate bites were generally acceptable. The indicated sensory parameters showed no significant difference with each other and can be concluded that the carrot and sweet potato-filled chocolate bites are generally acceptable based on its proximate content and sensory acceptability.

For further studies, the researchers recommend to try other types of vegetables or fruits as a substrate for the incorporation of lactic acid bacteria. Another recommendation could be the encapsulation of microbes which can help maintain its population. Consumer acceptability of filled chocolate bites can also be done to further determine whether the chocolate bites incorporated with vegetable fillers and probiotics could be accepted by the large-scale markets in the future. Lastly, developing standards for vegetable filled chocolate products incorporated with lactic acid bacteria.

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