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Development of Calcium-Enriched Gluten-Free Cookies

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ABSTRACT

The increased production of boneless bangus is directly proportional to the volume of discarded spines. Bangus bone meal a by-product of bangus deboning was subjected to laboratory analysis to determine its quality and was used as a functional ingredient in the development of gluten-free cookies for people who are gluten sensitive. The study was laid out in Completely Randomized Design with five treatments in three replications. The experimental samples were subjected to different laboratory procedures using standard methods. Bangus bone meal as functional ingredient for gluten-free cookies affects the physical properties, increased the nutritional status and significant difference ($p<0.05$) was observed in the flavor and texture sensory attributes as well as consumers' general acceptability. Bangus bone meal aerobic plate count decreased after three months of storage to <100 cfu/g. However *Escherichia coli* and *Staphyloccus aureus* were detected but remain stable during storage at low temperature. *Shigella* and *Salmonella* species were absence in the product. The addition of 30 grams bangus bone meal has fortified the nutritional composition of gluten-free cookies.

Keywords: bangus bone meal, gluten-free cookies, fortification

INTRODUCTION

Cookies are a form of confectionery product dried to a low moisture content which ensures that they are free from microbial spoilage and confer a long shelf life (Wade, 1988; Okaka, 2009). Cookies are consumed extensively all over the world as a snack food on a large scale in developing countries where protein and caloric malnutrition are prevalent (Chinma and Gernah, 2007). With the increased advocacy on the consumption of functional foods by World nutrition bodies due to different health problems related with food consumption such as celiac disease (life-long intolerance to wheat gluten, characterized by inflammation of the proximal small intestine) (Chinma *et al*., 2012). This poses a challenge to food researchers to develop food products containing functional ingredients in order to meet the nutritional requirements of individual with special dietary needs.

Gluten-free foods are dietary foods consisting of one or more ingredients which do not contain wheat (*Triticum*) species and the gluten level does not exceed 20 mg/kg in total (CODEX Standard, 2008). Gluten-free food products possess poor protein structure forming ability which causes the decrease of sensory quality (Torbica *et al*., 2012; Jambrec *et al*., 2012) and with low mineral and vitamin content and based often on pure starches (Ergin and Herken, 2012). Further,

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gluten-free products are frequently of inferior nutritive value than that of conventional products (Hathan and Prassana, 2011). Therefore, there is the need to enhance the nutritional quality of gluten free food products such as cookies by the addition of locally available abundant functional ingredients.

In the Philippines, milkfish deboning is a lucrative enterprise in every region, volume of boneless milkfish can be produced everyday to meet the increasing demand of the commodity. With the increased production of boneless milkfish is directly proportional to the volume of discarded spines and increased volume of processing wastes since not all body parts were utilized. Large amount of flesh with pin bones embedded are generated during deboning which are generally considered as discarded materials. With the abundance of these materials, they are made into milkfish bone meal as rich in protein, calcium, potassium and other nutrients that can be used as a potential renewable food material for human consumption.

This study was undertaken to develop cookies using gluten-free composite flour blends added with milkfish bone meal and evaluate the cookies physical properties, nutritional composition, bacteriological quality, sensory quality and consumer overall acceptability.

MATERIALS AND METHODS

The study was laid out in Completely Randomized Design (CRD) with five treatments replicated three times. The experimental samples were formulated at Cebu Technological University- Main Campus food laboratory right after the ingredients were purchased from the local markets of Cebu City. Proper food handling practices were observed during the conduct of the study.

The wheat (*Triticum aestivum* L.) flour, rice (*Oryza sativa*) flour, potato (*Solanum tuberosum*) flour, cassava (*Manihot esculenta*) starch, foxtail millet (*Setaria italica*) flour and corn (*Zea mays*) meal, butter, sugar, eggs, guar gum, baking powder, flavoring, and salt were brought from commercial market in Cebu City. Milkfish bone meal was processed at Cebu Technological University - Main Campus food laboratory. Bangus bone meal was a value-added food product from boneless bangus.

Chemical and bacteriological analyses

Analytical methods such as ash and moisture were determined by AOAC (1990), total fat (Solvent extraction method), protein (Kjeldahl method, *N x 6.25*), carbohydrate was determined by calculation the total percentage of moisture, ash, crude fat, fiber and crude protein is subtracted to 100% and the difference now could be the percent carbohydrate, food energy value was calculated by using physiological energy factors expressed in kilocalories (kcal), calcium, potassium, sodium and iron were determined by flame atomic absorption spectrophotometry (AAS), pH (electrometric method), Aw (water activity meter) and crude gluten using gravimetric method. The aerobic plate count was determined following Bacteriological Analytical Manual (2001).

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Determination of physical properties

Physical measurements in terms of width (mm) and thickness (mm) of samples were measured using vernier caliper with 20 micron accuracy measurement. Digital weighing scale was used to determine the weight (g) of cookie. Spread ratio was calculated by dividing the average value of diameter by the average thickness value of cookies (Ayo *et al*., 2010).

Cookie formulation Preparation of treatments

All ingredients were weighed using digital weighing scale and mixed in an electric mixer to form dough. The creaming method was observed during the preparation of dough. Shortening was added and rubbed in until uniform. The egg was added one at the time and the dough was thoroughly kneaded for four minutes. The cookie dough was placed in cookie press, pressed in greased baking sheets and baked in an oven at 340^{o}F for 30 minutes. The cookies were cooled on a wire racks at room temperature for 30 minutes, packed and sealed in a polyethylene food grade bags and were subjected to evaluation after 24 hours storage.

The formulation used in the preparation of cookies is shown in Table 1.

Table 1. Formulation of cookies

Ingredients	T_0	T_1	T_2	T_3	T_4
Milkfish bone meal	-	10g	20g	30g	40g
Wheat flour	360g	-	-	-	-
GF composite flour	-	345g	345g	345g	345g
Butter	225g	225g	225g	225g	225g
Sugar	200g	200g	200g	200g	200g
Eggs	110g	110g	110g	110g	110g
Guar gum	-	15g	15g	15g	15g
Flavoring	10g	10g	10g	10g	10g
Salt	2g	2g	2g	2g	2g
Baking powder	6g	-	-	-	-

Sensory evaluation of cookies

The sensory evaluation of cookies were carried out by 10 laboratory and 50 consumer panelists using the descriptive test and 9-point hedonic rating scale (1= disliked extremely while 9 = liked extremely). The data were analyzed using the ANOVA in completely randomized design.

RESULTS AND DISCUSSION

After processing, bangus bone meal samples were subjected to laboratory analysis and the result of the physico-chemical composition is presented in Table 2.

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Table 2. Physico-chemical composition of bangus bone meal

Parameters	Results
Moisture	51.9 %
Protein	32.6 %
Total Fat	7.60 %
Ash	5.44 %
pH	5.04
Carbohydrates	2.46 %
Food Energy Value	209 kcal/100g
Calcium (as Ca)	17800 mg/kg
Potassium (as K)	1573 mg/kg
Sodium (as Na)	499 mg/kg
Iron (as Fe)	11.04 ppm

The physico-chemical composition of bangus bone meal was relatively high in moisture content (51.9%), protein content (32.6%), ash (5.44%), calcium (17800 mg/kg), and potassium (1573 mg/kg) with a food energy value of 209 kcal/100g. Bone meal is a good source of protein, calcium and potassium. The carbohydrates content (2.46%) of the sample is low and the product is a low-acid food with a pH (5.04). The iron (11.04 ppm) of the product is within the RDA iron ranges from 10 mg to 15 mg. The sodium content (499 mg/kg) of the product is within the standard for special dietary food with *low sodium* content which is not more than 120 mg/100g of the final product as normally consumed (CODEX STAN 53-1981).

Table 3. Pathogen and aerobic plate counts of milkfish bone meal with months of storage

Storage Periods	Aerobic Plate Count cfu/g	*E.coli* MPN/g	*Staph. aureus* cfu/g	*Salmonella* in 25 g	*Shigella* in 25 g
0 month	<2,500	<1.8	<10	Absent	absent
1 month	<100	<1.8	<10	Absent	absent
2 months	<100	<1.8	<10	Absent	Absent
3 months	<100	<1.8	<10	Absent	Absent

After three (3) months of storage the product aerobic plate count decreases and stable at < 100 cfu/g. This bacterial load was within the BFAD microbial standard for processed product.

Escherichia coli and *Staphylococcus aureus* counts of the samples remain stable during storage with a count of <1.8 MPN/g of *E. coli* and <10 cfu/g of *Staph. aureus*. However, the *Salmonella* and *Shigella* species were not detected; these pathogens were affected by the heat treatment applied to the product. The product underwent thermal processing and stored at freezing point below 0 ^{o}C for three months, which also affects the stability of the count.

The results obtained from the proximate analysis, food energy value determination and crude gluten % of the wheat flour and gluten-free composite flour is shown in Table 4.

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Table 4. Proximate composition, food energy value and crude gluten results for wheat flour and gluten-free composite flour

Parameters	Flour samples	
	Wheat Flour (WF)	Gluten-Free Composite Flour (GFCF)
Moisture %	13.0	12.1
Ash %	0.60	0.38
Total Fat %	1.00	0.65
Protein %	8.00	4.61
Carbohydrates %	64.0	82.3
Food Energy Value kcal/100g	297.00	353.463
Crude Gluten %	16	0

Wheat flour had higher moisture content, fat content and protein content compared with gluten-free composite flour. However, gluten-free composite flour had higher carbohydrates content and food energy value which is influenced by the different combination of cereals and root crops. The whole wheat flour had higher protein content (8%), this data supports the presence of crude gluten (16%) which is not present in gluten-free composite flour. The presence of gluten in wheat flour is evident since gluten is the protein from wheat flour. The proximate analysis results of wheat flour were not different from the obtained literature. The moisture contents of wheat flour and gluten free flour blend were below the standard moisture content of wheat flour 15.5% m/m max (CODEX STAN 152-1985). The low moisture limits in flour should be required for certain destinations in relation to the climate, duration of transport and storage.

The effect on the incorporation of milkfish bone meal to the physico-chemical composition of gluten-free cookies is shown in Table 5.

Table 5. Physico-chemical composition of gluten-free cookies with and without milkfish bone meal

Parameters	Gluten-free Cookie without bangus bone meal	Gluten-free Cookie with 30g bangus bone meal
Ash	1.02 %	1.24 %
Moisture	0.33 %	2.44 %
Total Fat	25.9 %	25.0 %
Protein	3.90 %	6.00 %
Carbohydrates	68.9 %	65.3 %
Food Energy Value	524 kcal/100g	510 kcal/100
Calcium (as Ca)	338 mg/kg	1533 mg/kg
Potassium (as K)	719 mg/kg	837 mg/kg
Sodium (as Na)	3112 mg/kg	3037 mg/kg
Iron (as Fe)	<10.7 ppm	<10.7 ppm
pH	5.96	5.68

The moisture contents of gluten-free cookie (0.33%) is lower than the gluten-free cookie with 30g bone meal (2.44%). The increase of moisture content has been associated with the addition of bone meal. The higher the moisture content has been associated with short shelf life

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as they encourage microbial proliferation that lead to spoilage (Ndife *et al.*, 2011).

There was an evident increase of the protein content (6.00%) in cookies with the addition of 30 grams bone meal compared to cookies without bone meal. The result support the above data that bone meal is a good source of protein and can enriched the protein level of food.

The carbohydrate content (68.9%) and food energy values (524 kcal/100g) of gluten-free cookies was higher in gluten-free cookie compared to the other sample. The food energy values is directly proportional to the total amount of carbohydrates, protein and fat content in the food.

There was a significant increase of the calcium content (1533 mg/kg) of gluten-free cookie with 30 grams milkfish bone meal compared to samples without bone meal with contain 338 mg/kg calcium. This data could be attributed by the addition of bone meal which is high in calcium. Calcium is a mineral needed in the development of strong bones and teeth. Scientific findings revealed the role of micronutrient is important for better health.

Table 6. Bacteriological quality and water activity (Aw) of gluten-free cookies with 30 grams milkfish bone meal stored after 24 hours and 30 days

Days of Storage	Aerobic Plate Count/g	Aw
1 day old	< 1.0×10^2	0.405
30 days old	< 1.0×10^2	0.621

After baking the cookies were cooled, packed and stored at room temperature. Samples were subjected to estimated aerobic plate count per gram and Aw after 24 hours and 30 days storage. The aerobic plate count of the product is within the acceptable limit (5.0×10^4) for bakery products (USDA 2000). The limited bacterial count of cookies is attributed to the less water activity which does not support bacterial growth and cookies underwent thermal processing.

The results obtained from the physical measurements of cookies using wheat flour and gluten free composite flour with varying levels of milkfish bone meal are shown in Figures 1 and 2.

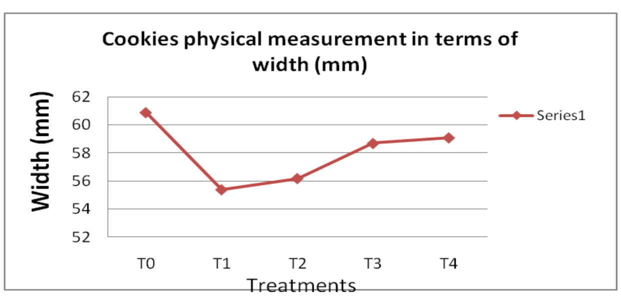

Figure 1. Physical measurements of cookies in terms of width (mm) as affected by varying levels of milkfish bone meal compared with the control

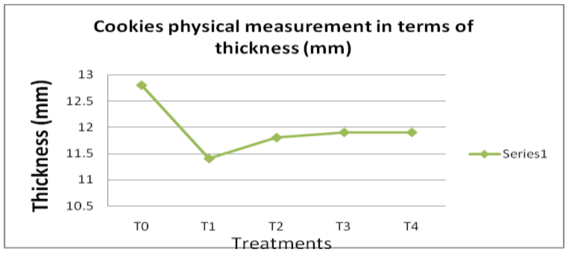

Figure 2. Physical measurements of cookies in terms of thickness (mm) as affected by varying levels of milkfish bone meal compared with the control

The increased in the width (mm) and thickness (mm) of wheat cookies (T_0) is influenced by the addition of sodium bicarbonate and the presence of gluten which helps develop structure in the cookie. The width (mm) of other treatments increases as the varying level of bone meal increased. The higher the level of bone meal influenced the increased of the width measurement of cookies.

The weight of the experimental cookies was between 11.8-12.6 g which was directly affected by the loss of water during baking correlated with the optimum cooking time.

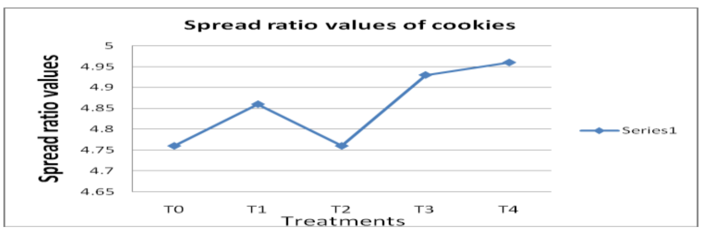

Figure 3. Spread ratio of gluten free cookies as affected by the addition of varying levels of milkfish bone meal compared with cookies using wheat flour

The spread ratio of gluten-free cookie is between 4.76-4.96 this indicates that the presence of guar gum providing good cohesions of the net work of the protein and carbohydrates. Protein gluten in flour will form a web in cookie dough when heated. During baking, the gluten goes through an apparent glass transition, thereby, gaining mobility that allows it to interact and form a web. The study of Noor *et al*. (2012) and Miller *et al*. (1997) revealed that the formation of continuous gluten web increases the viscosity and stops the flow of cookie dough. Ayo *et al*., (2010) poor cohesion could allow the outflow of some ingredients such as sugar that could melt at the high temperature of baking hence increasing the spreadability of the material.

Table 7. Descriptive sensory result of the experimental samples

Treatments	Color	Odor	Flavor	Texture
T_0	3.32	3.57	3.22	3.20
T_1	2.80	3.60	3.26	3.08
T_2	3.04	3.53	3.18	3.12
T_3	3.44	3.70	3.58	3.48
T_4	3.47	3.71	3.59	3.49

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Sensory panelists described subjectively the sensory properties of the wheat flour cookies and gluten-free cookies with bone meal as brownish appearance and moderately pleasant odor. The browning of the cookie could also occur due to the caramelization of the sugar during the baking process. Moreover, the darkening of the color of the cookie dough might be due to Maillard browning reactions between reducing sugars and proteins. However, treatments sensory qualities differ in the flavor and texture attributes. The findings support the idea that the increasing percentage of milkfish bone meal influences the flavor and texture properties of cookies. The baking conditions such as temperature, baking time, cookie composition, amounts of water absorbed during dough mixing, amounts of water evaporated during baking, and the addition of bone meal, all contributed to the texture, color, flavor and appearance of the final product.

Results of the consumer acceptability on the sensory evaluation of gluten-free cookie samples containing different levels of milkfish bone meal and the wheat flour cookie is shown in Figure 4.

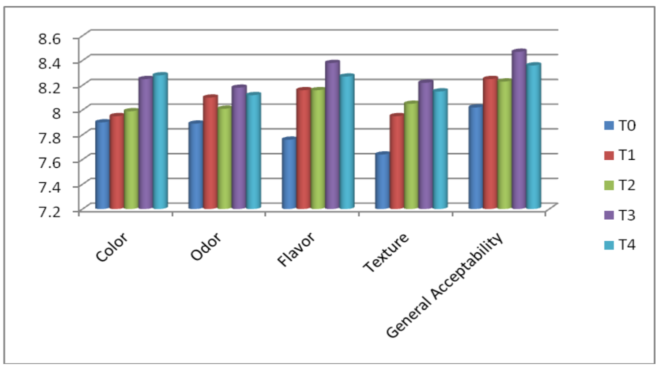

Figure 4. Preference and acceptability scores of cookies

Sensory evaluation of the wheat flour cookies and gluten-free cookies with different levels of milkfish bone meal revealed that there was a significant mean difference ($p<0.05$) in the flavor, texture and general acceptability. The addition of different levels of milkfish bone meal affects the texture and flavor attributes. As reported by Ergin (2012) that corn flour was observed to increase the hardness values when used with other flours. However, there was no significant difference in color and odor attributes in cookies made from gluten-free flour and wheat flour. This implies that gluten-free cookie with bone meal is comparable in color and odor characteristics with wheat flour cookie. Based on the data, T_3 had the highest level of acceptance rating compared to other treatments.

CONCLUSION AND RECOMMENDATION

Cookies can be developed using 345g (38.03%) gluten-free composite flour and the addition of 30g milkfish bone meal affects the physical properties, nutritional status and sensory characteristics of the cookies. A quality bone meal can be used as functional ingredient-fortification in food products, hence it is recommended.

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