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Under-Utilization of Composite Flour and Wheat Flour Cookies: Effect on Some Biochemical Parameters in Wistar Rat Model

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Abstract

Food sources provide food for the survival of humans and animals. It becomes necessary to look at the nutritional composition of rice-based composite flour and wheat flour cookies and their effect on some biochemical indices. Rice (RF), water yam (WF), soya beans (SF), sweet potato (SPF), and coconut flour (CF) were processed and blended in the ratio of 40:30:15:10:5 to obtain rice-based composite flour. The wheat flour cookies and rice-based cookies were fed to 21 albino rats grouped into three experimental groups for 21days. Group 1 (control) fed on 100% animal feed, Group 2 fed on 50% rice-based composite flour cookies and 50% animal feed, and Group 3 fed on 50% wheat flour cookies and 50% animal feed. At the end of the 21days feeding period, the blood samples of the albino rats were collected through the cardiac puncture into treated sample bottles for biochemical analysis. The results showed that that triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) cholesterol. Serum protein, electrolytes (sodium, potassium, and chloride), creatinine, and blood glucose were increased significantly ($P<0.05$) in the group fed with rice-based composite flour cookies when compared with wheat flour cookies group. The wheat flour group had a significant increase ($P<0.05$) in the bodyweight compared to the control group and the rice-based composite flour group. Therefore, it is imperative to incorporate these new sources of food into our diet to complement good health and help fight against metabolic diseases.

Keywords: Rice, water yam, soya beans, sweet potato, coconut flour.

INTRODUCTION

Malnutrition refers to an imbalance in a person's dietary intake of energy (WHO, 2020). There are three broad groups of malnutrition: Under-nutrition, over nutrition, and overweight, obesity, and even diet-related diseases. Combating malnutrition is one of the greatest challenges we face globally. Between 45-67% of deaths among children less than 5 years of age are linked to under-nutrition. Malnutrition is linked to Poverty as one of the underlying

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causes of health cries with people living in low-income countries and it could contribute to the slow economic growth and productivity (WHO, 2020).

Food security could as a matter of fact improve nutrition and promote sustainable agriculture by United Nations general Assembly proclaimed in 2016-2025 (WHO, 2020). Malnutrition can be prevented in developing countries by improving agricultural practices, encouraging food diversification, consumption of locally grown crops rich in nutrients (Foley *et al*., 2011).

There is need to practice cereal-based food with legume to supplement for the production of quality products that are rich in nutrient. Therefore, this study will produce composite flour that will be richer in nutrient up to 100% wheat product containing essential amino acid such as lysine. Though, cereals are very rich in methionine and cysteine which are deficient in legumes (Dhingra &Jood, 2000; FAO, 2004). Moreover, the high minerals and vitamin contents of these food based crops are responsible for the increased nutritive quality of the supplemented products (Hotz and Gibson, 2007; Uwaegbute and Anyika, 2008). In particular, the functional properties of the composite flour are suitable for the production of bakery products (Hood and Jood, 2005; Akubor, 2008).

Mixing different composite flours are quite different from the ready-mixed flours familiar to millers and bakers. Whereas ready-mixed flours contain all the non-perishable constituents of the recipe for a certain baked product, composite flours are only a mixture of different vegetable flours rich in starch or protein. (Ubbor and Akobundu, 2009). The mixture of composite flours are flours from tubers rich in starch (e.g. cassava, yam, sweet potato) and/or protein-rich flours (e.g. soy, peanut) and/or cereals (e.g. maize, rice, millet, buckwheat), with or without wheat flour" (Suresh *et al.,* 2014). Recently in Nigeria, the consumption of ready-to-eat baked products is continually growing and there has been an increase in reliance on imported wheat (Ampofo, 2009). The goal of earlier research with composite flours was to save the largest possible percentage of wheat flour in the production of certain baked products. They are nutritive snacks produced from unpalatable dough that is transformed into the appetizing product through the application of heat in the oven (Olaoye *et al.,* 2007). Moreover, staple crops are grown in Nigeria other than wheat such as rice, sweet potato, water yam, and cereals that can be used for bakery foods.

Food insecurity has been on the increase due to lots of reasons, including dependence on imported food such as wheat which is very costly (Nord *et al.,* 2009). People experiencing food insecurity are either experiencing hunger or are at risk of experiencing hunger. It is a well-established fact that lack of appropriate minimal nutrition leads to chronic illness, anxiety and depression, and behavior problems, and other negative health effects, with low-income households being particularly vulnerable to experiencing long-term effects of food insecurity (Wilensky and Satcher, 2009; Morton*, et al*., 2018).

However, in Nigeria, there seems to be a lack of food diversification as most locally grown crops are underutilized as evident in the over-dependence on wheat, which is expensive to import, and in turn has a negative consequence on the economy (Olaoye and Ade-Omowaye, 2011; Muktar, 2011).

Studies on composite flours and their products (such as cookies) prepared from potatoes, cassava, plantain, and soybeans have received wide discussion (Olaoye *et al.,* 2006; Ohimain, 2014). This lack of empirical studies on rice-based flour as a substitute for wheat-based flour for cookies preparation has created a gap in the literature, which has necessitated this study.

MATERIALS AND METHODS

Procurement of raw materials

Rice, sweet potato, coconut, and soybean was purchased from Watt Market in Calabar, Cross River State Nigeria. Wheat flour as well as additives like sugar, baking fat, and flavor among others was also purchased in Watt Market, Calabar.

Methods

Preparation of soybean, sweet potato, coconut and water-yam flours

Soybean flour was prepared according to the method described by Ndife *et al.,* (2011); while the Sweet Potato flour was prepared using the method described by Etudaiye *et al.,* (2008). Coconut flour was prepared following the method of Okafor & Usman (2013), while using Udensi et al (2008) method, water-yam flour was prepared.

Preparation of Flour Blends: Rice flour (RF), Sweet potato flour (SPF), water-yam flour (CF), and Soybean Flour (SF) was blended using the method described by Eneche (1999)

Animal study

Twenty-one (21) albino Wistar rats within the age of 6-7 weeks old weighing between 80-100grams were used for this study. The Wistar rats were purchased from the animal house of the Department of Biochemistry, University of Calabar. They were housed in wooden cages and fed with the finished product. The animals were allowed to acclimatize for 7days before the commencement of the feeding period which were kept for 30 days. The rats were sorted by their weight and divided randomly into three groups of seven rats each.

Blood sample collection

The end of the feeding period of 30days, the weights of the animals was measured, and then the animals were anesthetized with chloroform in a fume chamber and sacrificed by cervical dislocation method. Blood samples were collected from Ocular veins into Non-EDTA tubes using sterile needles and syringes. Serum collected was separated by centrifugation at 2500rpm for 15minutes and then used for total cholesterol, Triglyceride, High-density lipoproteins, and low-density lipoproteins determination.

Table 1: Experimental Design

Experimental Group	Number Animals	Percentage of feed
Group 1	7	Animal feed (100%)
Group 2	7	50% Composite flour cookies and 50% and feed
Group 3	7	50% Wheat flour cookies and 50% animal feed.

Blood glucose determination

The blood sample was collected after overnight fasting from the rats by the ocular method. The concentration of glucose in the blood sample was determined using the one-touch glucometer and test strips. A drop of whole blood was placed on a strip connected to the glucometer. The glucometer automatically displayed the concentration of the blood glucose, expressed in mg/dl (American Diabetes Association, 2003).

Food, water intake, and the body weight of rats: Food and water intake were monitored daily and determined by weighing serving dishes before and after meals. The body weight of the rats was monitored every three (3) days using a weighing balance.

Statistical analysis
Data are presented as mean ± SEM. Data were analyzed using a one-way analysis of variance (ANOVA) with SPSS (version 20) window statistical software program Student "t" test was used for pair-wise comparison and differences were considered significant at $p<0.05$.

RESULTS
Recent investigation on the under-utilization of composite flour and wheat flour cookies and their effect on some biochemical parameters in the experimental animals are presented in the table below. Serum lipid profile (total cholesterol, triglyceride (Tg), HDL, LDL, and VLDL-cholesterol), serum protein (total protein, albumin, and globulin), serum electrolytes, urea, creatinine, glucose, body weight changes, feed, and water intake were also investigated.

The results (**Table: 2**) were observed in the total cholesterol, triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL) levels of rats fed with cookies made from 100% wheat flour, 50% commercial rat feed: 50% rice-based composite flour cookies and 100% commercial rat feed (control). Total cholesterol level showed a significant difference at $p<0.05$. Rice-based composite flour cookies (1.17±0.24) showed a lower total cholesterol value when compared to wheat flour cookies (1.18±0.05) and the control group (1.56±0.01). There was a significant decrease at $p<0.05$ in Triglyceride (TG) level in group 2 fed with rice-based composite flour cookies (1.04±0.03) and wheat cookies (0.91±0.01) when compared with control (1.17±0.01) respectively. However, there was a significant increase in TG level in the group fed with Rice-based composite flour cookies (1.04±0.03), when compared with the group fed with wheat cookies (0.91±0.01). Similarly, high-density lipoprotein cholesterol level (HDL-c) showed a significant decrease at $p<0.05$ in the groups fed with rice-based composite flour cookies (0.38±0.01) and wheat cookies (0.32±0.01) when compared with the control group (0.41±0.00) respectively. Likewise, TG level in the group with wheat cookies (0.32±0.01) decreased significantly at $p<0.05$ when compared with the group fed with rice-based composite flour cookies (0.38±0.01). More so, low-density lipoprotein cholesterol level (LDL-c) showed a significant decrease at $p<0.05$ in the groups fed with wheat cookies (0.49±0.01) and Rice-based composite flour cookies (0.60±0.01) when compared with control (0.60±0.01) respectively. Similarly, LDL-c level in the group fed with wheat cookies (0.49±0.01) decreased significantly at $p<0.05$ when compared with the group fed with rice-based composite flour cookies (0.60±0.01). The result also indicates that very-low-density lipoprotein cholesterol level (VLDL-c) was significantly higher in the control group (0.53±0.01) when compared with the group fed rice-based composite flour cookies (0.46±0.01) and wheat cookies (0.42±0.01) respectively. While no significant difference ($p<0.05$) was observed in the atherogenic index of plasma.

Table 2: Effect of some formulated cookies on the lipid profile of Wistar rats

	TC (mmol/L)	TG (mmol/L)	HDL-c (mmol/L)	LDL-c (mmol/L)	VLDL-c (mmol/L)	Atherogenic index of plasma
Group 1 (Control)	1.56 ± 0.01	1.17 ±0.01	0.41 ±0.00	0.61 ±0.01	0.53 ±0.01	0.45 ±0.01
Group 2 (Rice-based composite Flour cookies)	1.17 ±0.24 ×	1.04 ±0.03×	0.38 ±0.01×	0.60 ±0.01×	0.46 ±0.01×	0.44 ±0.01
Group 3 (Wheat flour cookies)	1.18 ±0.05 ×, a	0.91 ±0.01×, a	0.32 ±0.01×, a	0.49 ±0.01×,a	0.42 ±0.01×,a	0.45 ±0.01

Values are expresses as mean ± SEM, n = 6
*= significant different from control at p<0.05
a= significant different from Rice-based composite flour cookies at p<0.05

The effect of formulated cookies on glucose and serum proteins as shown below in **Table: 3** Glucose level decreased significantly (p<0.05) in the group fed with rice-based composite flour cookies (4.50±0.09) and wheat cookies (3.62±0.07) when compared with control (5.42±0.07) while total protein level in the groups fed with rice-based composite flour cookies (70.83±0.95) and wheat cookies (61.33±0.88) was significantly lower at p<0.05 when compared with control (74.17± 0.95) respectively. Similarly, the result also indicates that total protein in the group fed with rice-based composite flour cookies (70.83±0.95) significantly increased when compared with the group fed with wheat cookies (61.33±0.88).

Likewise, albumin level decreased significantly at p<0.05 in the group fed with Rice-based composite flour cookies (33.50±0.96) and wheat cookies (28.17±0.65) when compared to control (34.83±0.60) respectively. Meanwhile, albumin level in the Rice-based composite flour cookies fed group (33.50±0.96) increased significantly at p<0.05 when compared with the wheat cookies group (28.17±0.65).

Furthermore, globulin level in the control group (39.33±0.71) increased significantly at p<0.05 when compared with rice-based composite flour cookies (37.50±0.43) and wheat cookies (33.33±0.49) respectively. Also, the globulin level in the rice-based composite flour cookies fed group (37.50±0.43) shows a significant increase (p<0.05) when compared with the wheat cookies group (33.33±0.49).

Table 3: Effect of dietary intake of formulated cookies on serum proteins and glucose level of Wistar rats

	Glucose (mmol/L)	Total protein (g/L)	Albumin (g/L)	Globulin (g/L)
Group 1(Control)	5.42 ±0.07	74.17 ± 0.95	34.83 ±0.60	39.33 ±0.71
Group 2 (Rice-based composite Flour cookies)	4.50 ±0.09*	70.83 ±0.95*	33.50 ±0.96*	37.50 ±0.43*
Group 3 (Wheat flour cookies)	3.62 ±0.07*,a	61.33 ±0.88*,a	28.17 ±0.65*,a	33.33 ±0.49*,a

Values are expresses as mean ± SEM, n = 6
* = significant different from control at $p<0.05$
a= significant different from Rice-based composite flour cookies at $p<0.05$

The result in Table 4 shows that sodium ion level significantly decreased at $p<0.05$ in the group fed with wheat cookies (137.67±0.33) when compared with the control (139.33± 0.49). However, there was a significant increase ($p<0.05$) in the rice-based composite flour cookies fed group (139.00±0.37) when compared with wheat cookies (137.67±0.33). Also, potassium ion levels were seen to decrease significantly ($p<0.05$) in both the rice-based composite flour cookies fed group (4.05±0.08) and wheat cookies (3.32±0.048) when compared with the control (4.82±0.08) respectively. Likewise, potassium ion level in the group fed with wheat cookies (3.32±0.048) decreased significantly ($p<0.05$) when compared with the group fed with rice-based composite flour cookies fed group (4.05±0.08).

Furthermore, chloride levels in the groups fed with wheat flour cookies (107.33±0.49) and rice-based composite flour cookies (109.00±0.37) were decreased significantly at $p<0.05$ when compared with the control group (112.17±0.75) respectively. Chloride levels in the group fed with wheat cookies (107.33±0.49) decreased significantly at $p<0.05$ when compared with the group fed with rice-based composite flour cookies (109.00±0.37).

Table 4: Effect of some formulated cookies on the serum electrolytes of Wistar rats

	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^-(mmol/L)
Group 1(Control)	139.33 ± 0.49	4.82 ±0.08	112.17 ±0.75	17.50 ±0.22
Group 2 (Rice-based composite Flour cookies)	139.00 ±0.37	4.05 ±0.08*	109.00 ±0.37*	18.00 ±0.37
Group 3 (Wheat flour Cookies)	137.67 ±0.33*,a	3.32 ±0.048*,a	107.33 ±0.49*,a	20.33 ±0.21*,a

Values are expressed as mean ± SEM, n = 6
*= significant different from control at $p<0.05$
a= significant different from Rice-based composite flour cookies at $p<0.05$

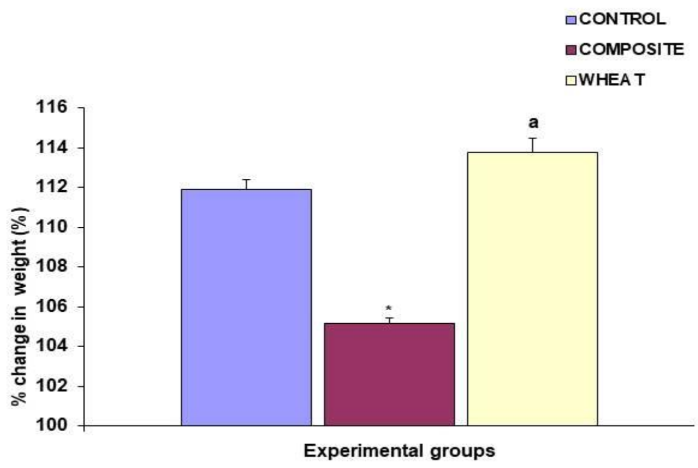

Fig 1: Percentage changes in body weight in the different experimental groups.
*Significantly different from the control at P<0.05.
=Significantly different from wheat at P<0.05;
Values are expressed at Mean±SEM, n=7.

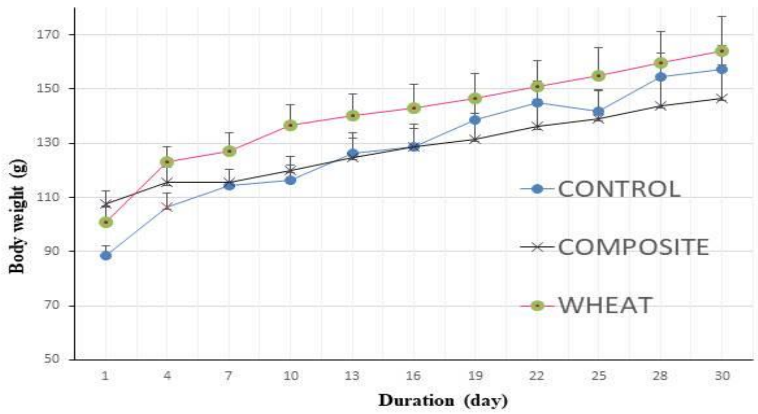

Fig 2 : Moving averages of body weights of the different experimental groups. Values are expressed as mean ± SEM, n = 7.

Bodyweight change

The bodyweight of Wistar rats fed with wheat flour cookies (group 3) significantly increased at $p<0.05$ when compared with the Wistar rats that was fed with animal feed (control group 1) and group 2 (rice-based composite flour cookies) while the bodyweight of Wistar rat in rice-based composite flour cookies (group 2) decreased significantly at $p<0.05$ when compared to the control group 1 and wheat flour cookies group 2.

DISCUSSION

Nutritional composition of both cookies was compared with each other as well their effects on some biochemical parameters in the rat model were investigated. Lipid profile result showed that total cholesterol (TC) content of the control group, the control group had a higher TC value followed by wheat flour cookies and rice-based flour having the least value. (Charles, 2016). A total cholesterol value of below 5.2mmol/L is desirable and reduces the risk of developing heart attacks and other forms of heart disease even in the blood vessels. All the three groups' had an acceptable total cholesterol value and this can be attributed to the high fiber flours used in the cookie production.

In terms of triglyceride content, the control group had a higher value followed by Rice-based flour and wheat flour cookies having the lowest value. An increase in triglycerides value can be linked to excess consumption of alcohol and sweets, obesity, smoking, and a sedentary lifestyle. Normal levels of triglycerides are 1.7mmol/L or 150 mg/dL. Higher level of triglycerides increases the risk for heart disease and metabolic syndrome (Charles, 2016). All the three experimental groups had an acceptable triglyceride values as this can be a attributing factor of the high fiber flours and the small quantity of sugar (150g) used for the cookie production.

High-density lipoprotein (HDL) cholesterol is considered as a good" cholesterol because it physiological functions is to keep the LDL or "bad" cholesterol from building up in the arteries. The higher the HDL, the better. HDL levels of 60 mg/dL or 1.5mmol/L can help reduce the risk of heart disease. Conversely, HDL levels of 40 mg/dL or 1mmol/L lower for men and below 50mg/dL or 1.293mmol/L for women are considered a high-risk factor for developing heart disease. (Charles, 2016). All the three groups of HDL-C values were very low and undesirable, hence to improve the heart health of consumers, raw ingredients (flour) used for rice-based composite flour cookies should be enriched with high fiber whole grains and legumes to increase the content of HDL-C in the cookies.

Low-density lipoprotein (LDL) cholesterol, physiologically referred to as "bad" cholesterol, is the type that deposit fat substance on the walls of the arteries. White blood cells combine with the LDL cholesterol, forming artery-narrowing plaque, which restricts blood flow. The optimal level of LDL cholesterol for most people is below 100 mg/dL or 2.6mmol/L. (Charles, 2016) (Dashti *et al*, 2011). All the three groups LDL-c values were at the acceptable range.

Very Low-density lipoprotein (LDL) known as bad cholesterol, is produced in the liver and released into the bloodstream to supply blood tissues triglycerides; about half of a VLDL particle is made up of triglycerides. High levels of VLDL-c narrow the artery walls and restrict blood flow. The normal VLDL levels are from 2 to 30mg/dl or 0.1 to 1.7mmol/L. All three the groups' VLDL-c values fall between the normal and healthy levels of VLDL-c. Therefore, rice-based composite flour cookies are healthy and safe for consumers.

The lipid profile result showed acceptable total cholesterol, triglyceride, low-density lipoprotein, and very-low-density lipoprotein cholesterol value, and undesirable high-density lipoprotein cholesterol value. Prolonged consumption of foods with high fiber content may help bring HDL-cholesterol to its normal range. The borderline for HDL-cholesterol is 50-35mg/dl and the high-risk level is less than 35mg/dl. A significant increase of total cholesterol and lowering in HDL-cholesterol is an undesirable biochemical state for the avoidance of atherosclerosis and ischaemic conditions. An increase in the ratio of total cholesterol and HDL-Cholesterol increases the risk of heart disease (Luc & Fruchart, 1991).
The atherogenic indices are indicators of the risk of developing heart disease. With every increase in the values of these indices there is an increased risk of developing cardiovascular disease and vice versa (Martirosyan *et al*., 2007: Brehm *et al.,* 2004; User *et al.,* 2006). Therefore, a low atherogenic index reduces the risk of developing cardiac attacks. All the three experimental groups studied were observed to have lower HDL-c values when compared to the atherogenic index of plasma. Therefore excess consumption of rice-based may increase the risk of developing heart disease but this can be checked by increasing fiber content in flour.

Serum total protein is a biochemical test used for measurement of the total amount of protein in serum which is responsible for the transportation of hormones, lipids, ions, and assists in immune function (Theodore, 2005). The normal values of total proteins in the blood range from 60 to 80g/l, albumin range from 35 to 50g/l, and globulin range from 20 to 35g/l (Pathology Harmony (UK), 2013). All three groups' values were at the normal range for total protein, albumin, and globulin and this indicates that the kidney and the liver were free from diseases related to low or high total protein.

The effect of some of the formulated cookies on the blood glucose level of rats shows that after 30 days of feeding increase in the blood glucose level of the control group as compared to the rice-based composite flour cookie group and wheat flour cookie group. Rice-based composite flour was significantly ($p<0.05$) higher than that of the wheat flour cookies. The "normal" blood glucose level is about 4 – 7 mmol/l. In this study, the rice-based composite flour cookie group was observed to have glucose concentration within the normal blood glucose level but the wheat flour cookie group glucose value was significantly lower than the acceptable range.

Electrolytes (salts) are chemical substances in the body that regulate the ions in body fluids and can conduct electricity. Electrolytes also, maintain the balance between the normal functioning of cells and organs.

The potassium content of rice-based composite flour cookies is higher than wheat flour cookies and at an acceptable range. This result can be attributed to the raw ingredients (composite flour) used for cookie preparation. One of the flours supplying a good amount of potassium is Soybeans. Soybeans contain about 1797mg of potassium, other flours with an appreciable amount of potassium are sweet potato flour and coconut flour (USDA Nutrient Database for Standard Reference, 2018). Potassium is primary responsible for the proper functioning of the nerves and muscles, particularly the heartbeat. Potassium normal range is 3.5 - 5.0 (mmol/L). Any abnormality can cause an increase in potassium (hyperkalemia) or a decrease in potassium (hypokalemia) which can seriously affect the nervous system and even increase the chance of arrhythmias (irregular heartbeats). Sodium regulates the transmission of sodium into intracellular and extracellular compartment of individual cells for electrical signals and communication via; the brain, muscles, and nervous system. The movement of sodium in and out of cells is vital in generating electrical signals. If there is an extreme fluctuation in sodium levels this can cause cells to malfunction, which can prove to be fatal. A normal blood sodium level is 135 – 145 mmol/L, where there is excess sodium levels in the blood could lead to' hypernatremia and if there is a decrease in sodium levels in the blood could also lead to 'hyponatremia'. (Standley, 2019). The present study indicates that sodium values fall within the normal acceptable range, as there was no level of significant ($p<0.05$) difference between the control group and the rice-based composite flour cookie group. But a significant decrease of wheat flour cookies in comparison with the control group respectively.

Chloride physiologically functions to maintain the body's fluids like other electrolyte markers. The normal serum chloride range is 98 - 108 mmol/L. In this study, the control group (112.17mmol/L) and the rice-based composite flour cookies group (109 mmol/L) concentration of chloride was above the normal range while the wheat flour cookies group (107.33 mmol/L) falls within the range. Indicating an increase in chloride levels that would lead to a condition called hyperchloremia. However, an elevated level is seen in the case of diarrhea, kidney disease, and sometimes in overactive parathyroid glands. (Stanley, 2019).
Bicarbonate is used to maintain the normal levels of acidity (pH) in the blood and other fluids in the body. The normal serum range for bicarbonate is between 22-30 mmol/L. The present study, indicate that all the three study groups had concentrations (17.50, 18.00, and 20.33mmol/L) lower than the normal value. (Stanley, 2019). To improve electrolyte balance more fluids should be consumed.

The result in **figure 1** showed that the wheat flour cookie group (group 3), gained weight significantly which might be attributed to the use of refined white flour with a high glycemic index causing rapid weight gain. The control group gained weight, slightly lower than the wheat flour cookie group. On the other hand, rice-based composite flour cookies (group 2) maintained slow but steady weight gain. This may be due to fiber source (sweet potato and water yam flour) inclusion in the sample cookies. One of the health benefits of fiber is to increase the weight and size of your stool and soften it. Hence normalizes bowel movement.

Conclusion

The outcome of the study indicates that nutritious cookies could be processed alternatively from different recipes mentioned above. Therefore, replacing wheat flour with rice-based composite flour in the preparation of cookies has several nutritional advantages such as gluten-free, cookies that are rich in protein, minerals, fat, and energy with little or no negative effect on biochemical indices. Industrial production of rice-based composite flour will greatly increase the utilization of locally grown crops (like rice, water yam, sweet potato, etc.) which are under-utilized. Rice-based composite flour can be cheaper as an alternative to wheat flour and also help in the management of protein-energy malnutrition in developing countries.

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