Journal of Food Technology Research
2020 Vol. 7, No. 2, pp. 178-188.
ISSN(e): 2312-3796
ISSN(p): 2312-6426
DOI: 10.18488/journal.58.2020.72.178.188
© 2020 Conscientia Beam. All Rights Reserved.

STORAGE EFFECTS ON MICROBIOLOGICAL AND SOME ANTIOXIDANT POTENTIALS OF PARTIALLY SUBSTITUTED BREAD PRODUCED FROM WHEAT FLOUR AND FRESH COCONUT MEAT

Akinyede, A. I.[1+]
Akande, M. O.[2]
Babatuyi, C. Y.[3]
Oguntuase, S. O.[4]

[1,2,3,4]*Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria.*
[1]*Email: aiakinyede@futa.edu.ng*
[2]*Email: mathewakakande7@gmail.com*
[3]*Email: cybabatuyi@futa.edu.ng*
[4]*Email: oguntuasesamueloladeji@gmail.com*

(+ Corresponding author)

Article History
Received: 16 June 2020
Revised: 20 July 2020
Accepted: 24 August 2020
Published: 11 September 2020

Keywords
Fresh coconut meat
Bread
Sensory characteristics
Antioxidants
Microbial load
Substitution.

ABSTRACT

Bread is a major wheat product consumed in every household because of its easy handling. Fresh coconut meat which is readily available in all rural area in Nigeria may boost both the economy and provide health benefits to those in the rural area. Therefore, the purpose of this study is to evaluate the effect of partial substitution of wheat flour with fresh coconut meat. Wheat flour was partially (90 – 100 %) substituted with 0 –10% fresh coconut meat in the ratio 100 : 0 (WF), 98 : 2 (WFCM1), 96 : 4 (WFCM2), 94 : 6 (WFCM3), 92 : 8 (WFCM4), 90 : 10 (WFCM5). The study evaluated the effects of substitution on proximate composition, sensory characteristics, storage time on some antioxidant potential and microbial stability. Results showed that the overall acceptability of the bread baked from WFCM5 blend was preferred over WF. On the day of production, WFCM5 had the highest phenolic content and total flavonoids but as the days there were reduction in the antioxidant contents of all the bread samples. The microbial load of Nutrient agar, MacConkey agar and Potato dextrose agar increased as the days increased. The bread from the blends may serve as nutritious and potential accepted bread products. The present study confirms effective substitution of wheat flour with fresh coconut meat. The substituted bread showed improved overall acceptability, some antioxidants potentials and microbial stability which may provide health benefits to those in the rural area in Nigeria.

Contribution/Originality: This study documents the possibility of producing bread from partial substitution of wheat flour with coconut meat and also provides information on its antioxidant potentials and microbial stability for possible health benefits to those in the rural area in Nigeria.

1. INTRODUCTION

Bread is a major wheat based food product commonly eaten by consumers all over the world for its convenience and ready to eat especially Nigeria over the years. It is being served as food or snacks in most homes, restaurants, and hotels and it is served across all age groups (Bolarinwa, Aruna, & Raji, 2017; Oluwajoba, Malomo, Ogunmoyela, O., & Odeyemi, 2012). Bread is rich both in macronutrients and micronutrients which makes it essential for human health (Oluwajoba et al., 2012). Wheat is mainly considered as the baker's choice cereal for production of bread because of its high gluten content which is important in dough formation (Olapade & Oluwole, 2013). Inclusion of agricultural produce to wheat can improve its nutritional status and functionality thereby providing value added income to the country (Odunlade et al., 2017).

© 2020 Conscientia Beam. All Rights Reserved.

Coconut is a mature fruit of the *Cocos nucifera* palm. *Cocos nucifera* belongs to the large Palmaceae family of palm trees. Coco palm grows well under tropical climates and around the world (Nnorom, Nnadozie, Ugwa, & Obike, 2013). The term 'coconut' refers to the entire coconut palm, the seed, or the fruit. There are over sixty species under genus *Cocos* but coconut is the only acceptable specie in this genus (Omotosho & Odeyemi, 2012). Coconut as whole is important to human race especially its fruit which provides relevant food constituent (Solangi & Iqbal, 2011). The health and nutritional benefits which can be obtained when coconuts are consumed are captivating and distinctive. Coconuts are used as a revitalizing drink and as an additive of confectionary, ice, biscuits, cakes and bread (Solangi & Iqbal, 2011). Coconut oil is been referred to as a physiologically functional food because of the unique role it plays in the diet based on its health and nutritional benefits which has been recognized in many parts of the world over centuries (Oseni, Fernando, Coorey, Gold, & Jayasena, 2017). Apart from coconut water and extracted coconut oil, the coconut has a number of other culinary uses. The fleshy part of the seed (coconut meat) can be used fresh or dried in cooking. Coconut-pulp flour is coconut flour made from by-product of coconut milk based food products (Erminawati, Sidik, & Zulfakar, 2018). The flour serves as a good source of dietary fiber which may enhance human digestion. Researches have shown that coconut is rich in amino acids, vitamins (ascorbic acid, folic acid, and pyridoxine), minerals (potassium, nitrogen, calcium, phosphorus, iron, sodium, chloride, and bicarbonate), sugars, fats, and nitrogenous substances (Nnorom et al., 2013).

Today, the awareness of consuming healthy food in daily life is a major concern of consumers, people are conscious of the health implication of any food that is being consumed and this has led to consumers demand for a healthier choice of food product. Past researches have been carried out to assess the possibilities of incorporating natural ingredients to improve the nutritive value of wheat bread which includes coconut flour, coconut pulp flour, leafy vegetable powders industrial onion waste powder, Pumpkin Flour (Erminawati et al., 2018; Gunathilake, Yalegama, & Kumara, 2009; Odunlade et al., 2017; Prokopov et al., 2018; Wahyono, Dewi1, Oktavia, Jamilah, & Kang, 2019). Considering the search for food products which may be nutritious and of health benefit at a low cost for the rural area, however there is dearth of information on the use of fresh coconut meat in the production of bread. Therefore, the present study was aimed to produce bread from fresh coconut meat and wheat flour (WF) and assess the nutritional, microbiological, antioxidant potential and sensory attributes of bread baked with wheat flour/ fresh coconut meat.

2. MATERIALS AND METHODS

2.1. Collection of Samples

Coconut fruits were purchased from a local market in Akure, Ondo State, Nigeria and transported to the laboratory for processing. Commercial high quality wheat flour Mama Gold (Crown flour mill) and other bread ingredients were purchased from Oba market in Akure, Ondo state. All the chemicals were of analytical grade.

2.2. Processing of Fresh Coconut Meat (FCM)

Mature coconut fruits were washed under running tap water, the nuts were cracked in order to free the kernel (meat), the hard coat of the meat was scrapped off and the remaining part were shredded and stored at 4 °C before use.

2.3. Baking Process

Six blend formulations Table 1 were baked using the straight dough method as described by Chuahan, Zillman, and Eskin (1992). The Baking formula was 90 – 100 % wheat flour, 0 –10% fresh coconut meat (FCM) as follows WF- 100% Wheat flour, $WFCM_1$ - 98%Wheat flour: 2 % Fresh coconut meat, $WFCM_2$ -96%Wheat flour: 4 % Fresh coconut meat, $WFCM_3$ -94%Wheat flour: 6 % Fresh coconut meat, $WFCM_4$ - 92%Wheat flour: 8 % Fresh coconut meat, $WFCM_5$ -90%Wheat flour: 10 % Fresh coconut meat. All ingredients were mixed in a bread maker

© 2020 Conscientia Beam. All Rights Reserved.

(Phillips) for 5 minutes. The dough was fermented in bowls, covered with wet clean Muslin cloth for 55 minutes at warm temperature, punched, scaled to 250 g dough pieces, proofed in a proofing cabinet at 30°C for 90 minutes at 85% relative humidity and baked at 250°C for 30 minutes. The baked bread samples were de-panned, cooled at ambient temperature and packed in density polyethylene bags prior to analysis.

Table-1. Recipe formulation for the Production of wheat Flour /Fresh coconut meat Bread.

Samples (g)	Wheat Flour (g)	Fresh coconut meat (g)	Sugar (g)	Butter (g)	Yeast(g)	Salt (g)	Improver (g)	Water mls
WF	500	-	60	10	3	8	0.03	300
$WFCM_1$	490	10	60	10	3	8	0.03	295.3
$WFCM_2$	480	20	60	10	3	8	0.03	290.6
$WFCM_3$	470	30	60	10	3	8	0.03	285.9
$WFCM_4$	460	40	60	10	3	8	0.03	281.2
$WFCM_5$	450	50	60	10	3	8	0.03	276.5

Note: WF -100% wheat flour, $WFCM_1$ - 98%Wheat flour: 2 % Fresh coconut meat, $WFCM_2$ -96%Wheat flour: 4 % Fresh coconut meat, $WFCM_3$ - 94%Wheat flour: 6 % Fresh coconut meat, $WFCM_4$ - 92%Wheat flour: 8 % Fresh coconut meat, $WFCM_5$ -90%Wheat flour: 10 % Fresh coconut meat.

2.4. Volume and Weight of Wheat/ Fresh Coconut Meat Loaves

Bread loaf volume was determined using the rapeseed displacement method (AOAC., 2012) with minor modification. A container (box) was filled with millet and the volume of the millet was measured using a measuring cylinder (V_1).The bread sample was placed into the container and the volume of the millet with the bread sample was measured and noted as V_2. The analysis was performed with triplicate bread samples.

$$\text{Loaf volume} = V_1 - V_2 \text{ ml}$$

Loaf weight was determined using the AACC (2000). The weight of bread loaves was measured in gram units using normal weighing methods. Each loaf was weighed one hour after the cooling process. Specific volume (ml/g) was determined by dividing the loaf volume by the loaf weight.

2.5. Sensory Characteristics of Wheat/ Fresh Coconut Meat Bread

All sensory analyses were conducted in a well illuminated sensory laboratory. Sensory evaluation of the coded bread samples with random three digits was carried out using 9-point Hedonic scale where 9 represents extremely like and 1 represents extremely dislike by a thirty member semi - trained panelist (familiar with bread) consisting of both students and staff of the Department of Food science and Technology, Federal University of Technology, Akure. The attributes evaluated were appearance of the crust and crumb, taste, texture, aroma and overall acceptability. Panelists made their responses on score sheets and the sensory scores were analyzed statistically.

2.6. Nutritional Quality of Wheat/ Fresh Coconut Meat Bread

Determination of the proximate composition (moisture, crude fat, crude fiber, crude protein and total ash) of the bread samples were assayed using the standard methods described by AOAC, (2012).Carbohydrate content was calculated by difference as follow:

Carbohydrate (%) = 100 - (% Moisture + % Crude Fat + % Total ash + % Crude fiber + % Crude protein).

2.7. Microbiological Analysis of Wheat/ Fresh Coconut Meat Bread

Total mesophilic (total viable bacterial counts) and fungi counts (yeast and mold counts) were carried out on the bread samples to determine the microbial load of the samples by standard microbiological techniques as described by Fawole and Oso (2007). Bread samples were prepared by mashing and mixing in peptone water. Each sample was serially diluted to 10^{-3} and 0.1 ml aliquot of each bread sample was pour-plated using nutrient agar (NA), MacConkey agar (MCA), and potato dextrose agar (PDA) for the enumeration of aerobic viable bacteria, coliforms, and fungi respectively. All the agar plates inverted after being solidified before NA and MCA plates were

© 2020 Conscientia Beam. All Rights Reserved.

incubated at 37°C for 24 hours and PDA plates at room temperature (25°C) for 3–5 days. The colonies were counted and expressed as colony forming units per gram (cfu/g) and spore forming units of samples using Stuart scientific colony counter. Colonies observed were sub-cultured repeatedly to obtain pure cultures.

2.8. Determination of Total Phenol Content (TPC)

Antioxidant activity through TPC was determined according to the method described by Meda, Lamien, Romito, Millogo, and Nacoulma (2005) with minor modification. The bread samples were oven dried at 50ºC for 5 hours and then blended to powdery form. 1 g of each sample was weighed into designated sample bottles and 10ml of distilled water was added to all bottle and left for 24 hours, after which the mixture was shaken together and the mixture were separated into an aqueous solution and residue. About 100 µl of extracted aqueous solution was added to 500 µl of 10% Folin- Ciocalteu reagent. Samples with the reagent were left for 5 min and then 400 µl 7.5% sodium carbonate (w/v) was added. The sample was then incubated at 45ºC for 40 minutes. The absorbance was measured at 765 nm using a spectrophotometer after 2h. Calibration curve of gallic acid was plotted to evaluate the activity capacity of the samples. Result was expressed as milligram of gallic acid equivalents per 100 gram of fresh sample (mg GA/100 g).

2.9. Determination of Total Flavonoids Content (TFC)

Total flavonoid content was determined by a colourimetric method as described by Gorinstein et al. (2007). A 500 µl of the bread extract was diluted with 400 µl of distilled water. Then 50 µl of 9.82 g/ 100 ml CH_3COOK solution was added to the mixture, and after 6 min 50 µl of 10 % $AlCl_3$ solution was added. The mixture was allowed to stand for 5 min and next 500 µl of methanol was added and the total was made up to 2.5 µl with distilled water. The solution was mixed well and the absorbance was measured immediately against the blank at 415 nm using a spectrophotometer Jasco UV-530. The results were expressed as mg of quercetin equivalents.

2.10. Statistical Analysis

Data was generated in triplicate and subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) V. 17.0. Means were separated using Duncan's multiple range test (DMRT) at 95% confidence level.

3. RESULTS AND DISCUSSION

3.1. Physical Properties of Bread Produced From Wheat/ Fresh Coconut Meat

Physical properties of bread samples from wheat/ fresh coconut meat are shown in Table 2. Average heights of the breads were 4.85 – 6.80 cm with whole wheat significantly higher (p<0.05) and 10% fresh coconut meat enrichment shown the least value. Also, average weight as observed in this study indicates that 100% wheat flour had highest value (265 g) and decreased as the fresh coconut substitution increased.

Table-2. Physical properties of wheat/ fresh coconut meat bread.

Samples	Height(cm)	Weight(g)	Loaf volume (ml)	Specific volume (ml/g)
WF	$6.80±0.01^a$	$265.08±0.06^a$	$1320.01±0.01^a$	$4.98±0.01^a$
$WFCM_1$	$6.00±0.01^b$	$264.01±0.02^b$	$1300.04±0.02^b$	$4.92±0.01^b$
$WFCM_2$	$6.00±0.01^b$	$263.02±0.02^c$	$1270.01±0.01^d$	$4.81±0.01^d$
$WFCM_3$	$5.52±0.01^c$	$262.02±0.02^d$	$1240.05±0.03^a$	$4.73±0.01^e$
$WFCM_4$	$5.30±0.02^d$	$260.02±0.01^e$	$1270.03±0.02^c$	$4.88±0.01^c$
$WFCM_5$	$4.51±0.01^e$	$260.03±0.02^e$	$1250.04±0.02^e$	$4.81±0.01^d$

Note: Values are means ± standard error of mean. Values with different superscripts within the same column are significantly different (P≤0.05). WF -100% wheat flour, $WFCM_1$ - 98%Wheat flour: 2 % Fresh coconut meat, $WFCM_2$ -96%Wheat flour: 4 % Fresh coconut meat, $WFCM_3$ - 94%Wheat flour: 6 % Fresh coconut meat, $WFCM_4$ -92%Wheat flour: 8 % Fresh coconut meat, $WFCM_5$ -90%Wheat flour: 10 % Fresh coconut meat.

© 2020 Conscientia Beam. All Rights Reserved.

The loaf volume ranged between 1240 and 1320ml with WF significantly higher ($p<0.05$) than others. Loaf volume is being defined as the space occupied by the bread loaf and the main factor contributing to the loaf volume and crumb structure of bread is its ability to retain gas retention (Rouillé, Bonny, Della Valle, Devaux, & Renou, 2005). The decrease in the loaf volume as the fresh coconut meat increases may arise as a result of the interaction between gluten and fiber which may weaken the gluten formation and result in a lower bread volume (Ariffin, Baharom, Kaur, & Murad, 2015). Loaf volume is important in bread production since dense bread is not been desired by consumers. The specific volume of whole wheat bread which is the control sample (4.98 ml/ g) was significantly higher ($p<0.05$) than other bread samples. The specific volume reduced as FCM addition to the bread formulation increased, this is in agreement with report of wheat/coconut pulp flour (Erminawati et al., 2018) and this was most likely because FCM may have reduced the interaction between gluten and fiber and inhibited free expansion during the fermentation process (Ariffin et al., 2015).

3.2. Sensory Evaluation of the Wheat/ Fresh Coconut Meat Bread

The mean sensory scores for each quality attribute evaluated (appearance, aroma, taste, texture, and general acceptability) of the bread samples prepared from the wheat flour/fresh coconut meat blends were presented in Table 3. The sensory evaluation of the bread produced showed that overall acceptability of the bread $WFCM_4$ and $WFCM_5$ was significantly acceptable ($p<0.05$) than the WF (control). The statistical analysis of the data showed that there were no significant differences among the wheat/fresh coconut meat blends at ($P\leq0.05$ with the exception of the WF and $WFCM_5$ for appearance and taste but significant differences were observed in aroma and texture. The scores also indicated that bread baked from $WFCM_4$ and $WFCM_5$ was more acceptable than that from other blends.

Table-3. Sensory evaluation of wheat/ fresh coconut meat bread.

Samples	Appearance	Aroma	Taste	Texture	Overall Acceptability
WF	8.10 ± 0.27^{a}	7.00 ± 0.42^{ab}	7.50 ± 0.40^{a}	7.10 ± 0.48^{ab}	7.50 ± 0.31^{ab}
$WFCM_1$	6.60 ± 0.31^{b}	7.30 ± 0.30^{ab}	7.40 ± 0.42^{a}	7.00 ± 0.33^{ab}	7.20 ± 0.36^{ab}
$WFCM_2$	6.80 ± 0.34^{b}	6.60 ± 0.33^{b}	6.90 ± 0.28^{a}	6.50 ± 0.43^{b}	6.90 ± 0.28^{ab}
$WFCM_3$	6.30 ± 0.21^{b}	6.40 ± 0.31^{b}	6.70 ± 0.33^{a}	6.00 ± 0.52^{ab}	6.50 ± 0.31^{b}
$WFCM_4$	6.80 ± 0.51^{b}	7.30 ± 0.33^{ab}	7.80 ± 0.36^{a}	6.70 ± 0.39^{ab}	7.60 ± 0.31^{a}
$WFCM_5$	7.40 ± 0.37^{ab}	7.80 ± 0.33^{a}	7.80 ± 0.29^{a}	7.70 ± 0.30^{a}	7.80 ± 0.38^{a}

Note: Values are means ± standard error of mean. Values with different superscripts within the same column are significantly different ($P\leq0.05$). WF -100% wheat flour, $WFCM_1$ - 98%Wheat flour: 2 % Fresh coconut meat, $WFCM_2$ -96%Wheat flour: 4 % Fresh coconut meat, $WFCM_3$ - 94%Wheat flour: 6 % Fresh coconut meat, $WFCM_4$ - 92%Wheat flour: 8 % Fresh coconut meat, $WFCM_5$ -90%Wheat flour: 10 % Fresh coconut meat.

3.3. Proximate Composition of Wheat/ Fresh Coconut Meat Bread

The results of proximate analysis of the bread samples were presented in Table 4. The 100% wheat flour bread (WF) had 30.22% moisture content, 11.23% crude protein, 18.89% crude fat, 0.05% crude fiber, 0.53 % total ash and 39.18% carbohydrate. This result compares favorably with values obtained for wheat bread from other researchers (Igbabul, Grace, & Julius, 2014; Nwosu, Owuamanam, Omeire, & Eke, 2014; Odunlade et al., 2017). Bread enriched with fresh coconut meat were 30.65 – 33.45% moisture content,11.36– 12.07 % crude protein, 22.35 – 28.90 % crude fat, 0.2 – 0.81 % crude fiber, 0.93 -1.22% ash and 26.19 – 31.79% carbohydrate. Significant difference at $P< 0.05$ was observed in the moisture content, crude protein and crude fiber, crude fat and carbohydrate.

Moisture is a critical factor in the keeping quality of bread and high moisture can have an adverse effect on storage stability (Madukwe, Obizoba, & Chukwuka, 2013). The bread sample $WFCM_1$ having the highest moisture content may therefore have reduced shelf life in comparison with other samples. The ash content of $WFCM_5$ was the highest (1.30%) while WF had the least ash content of (0.5 %). The low ash content of the wheat flour could be as a result of the fact that white wheat flours have very little amount of minerals. The crude fiber content of the bread samples also increased as the substitution of fresh coconut meat was increased. Sample WF had the least crude fiber content (0.05%) while sample $WFCM_5$ had the highest crude fiber content (0.81%).

© 2020 Conscientia Beam. All Rights Reserved.

Table-4. Proximate Composition of the Wheat/ Fresh Coconut meat bread (g/100g).

Parameters	Moisture content	Crude Ash	Crude Fiber	Crude Protein	Crude Fat	Carbohydrate
WF	30.22 ± 0.01^{f}	0.53 ± 0.03^{a}	0.05 ± 0.01^{f}	11.23 ± 0.02^{e}	18.89 ± 0.02^{f}	39.18 ± 0.04^{a}
$WFCM_1$	33.45 ± 0.02^{a}	0.93 ± 0.02^{a}	0.20 ± 0.01^{e}	11.36 ± 0.02^{d}	22.35 ± 0.02^{e}	31.79 ± 0.01^{b}
$WFCM_2$	32.63 ± 0.02^{b}	1.05 ± 0.03^{a}	0.24 ± 0.01^{d}	11.56 ± 0.01^{c}	23.86 ± 0.03^{d}	30.73 ± 0.02^{c}
$WFCM_3$	32.01 ± 0.01^{d}	1.25 ± 0.02^{a}	0.41 ± 0.01^{c}	11.93 ± 0.02^{b}	24.24 ± 0.02^{c}	28.32 ± 0.02^{d}
$WFCM_4$	32.06 ± 0.01^{c}	1.24 ± 0.02^{a}	0.55 ± 0.02^{b}	11.97 ± 0.01^{b}	26.12 ± 0.02^{b}	27.66 ± 0.01^{e}
$WFCM_5$	30.64 ± 0.01^{e}	1.22 ± 0.01^{a}	0.81 ± 0.01^{a}	12.07 ± 0.02^{a}	28.90 ± 0.01^{a}	26.19 ± 0.01^{f}

Note: Values are means ± standard error of mean. Values with different superscripts within the same column are significantly different ($P \leq 0.05$). WF -100% wheat flour, $WFCM_1$ - 98%Wheat flour: 2 % Fresh coconut meat, $WFCM_2$ -96%Wheat flour: 4 % Fresh coconut meat, $WFCM_3$ -94%Wheat flour: 6 % Fresh coconut meat, $WFCM_4$ -92%Wheat flour: 8 % Fresh coconut meat, $WFCM_5$ -90%Wheat flour: 10 % Fresh coconut meat.

Sample WF had the least fat content (18.48%) which may be because it was produced from 100% wheat flour whose fat content was lower than that of fresh coconut meat and fat has shown to impart tenderness, moistness, lubricity, flavor, color and anti-bread staling qualities in the baked products (Pareyt, Finnie, Putseys, & Delcour, 2011). The crude protein contents of the bread showed that $WFCM_5$ had the highest crude protein content of (12.08%) while WF had (11.02%). On the other hand, the carbohydrate content of WF (31.79%) was the highest amongst the bread samples. From the obtained result bread may be a source of high energy food and the crude fiber may improve the digestive system of the consumers.

3.4. Microbiological Analysis of Wheat/ Fresh Coconut Meat Bread

The microbiological analysis of bread samples were presented in Table 5.

Table-5. Microbial analysis of the wheat/ fresh coconut meat bread.

DAYS	BLENDS	NA (CFU/G)	MCA (CFU/G)	PDA (SFU/G)
ONE	WF	0	0	0
	$WFCM_1$	0	0	0
	$WFCM_2$	0	0	0
	$WFCM_3$	0	0	0
	$WFCM_4$	0	0	0
	$WFCM_5$	0	0	0
THREE	WF	0	0	0
	$WFCM_1$	$1 X 10^{3}$	0	0
	$WFCM_2$	$1 X 10^{3}$	0	0
	$WFCM_3$	$1 X 10^{3}$	0	0
	$WFCM_4$	$1 X 10^{3}$	0	0
	$WFCM_5$	$1 X 10^{3}$	0	0
FIVE	WF	$2 X 10^{3}$	$1 X 10^{3}$	0
	$WFCM_1$	$3 X 10^{3}$	0	0
	$WFCM_2$	$3 X 10^{3}$	0	0
	$WFCM_3$	$1 X 10^{3}$	$2 X 10^{3}$	0
	$WFCM_4$	$5 X 10^{3}$	$1 X 10^{3}$	0
	$WFCM_5$	$4 X 10^{3}$	0	0
SEVEN	WF	$2 X 10^{3}$	$1 X 10^{3}$	$2 X 10^{3}$
	$WFCM_1$	$3 X 10^{3}$	$1 X 10^{3}$	$2 X 10^{3}$
	$WFCM_2$	$4 X 10^{3}$	$1 X 10^{3}$	$1 X 10^{3}$
	$WFCM_3$	$2 X 10^{3}$	$2 X 10^{3}$	$1 X 10^{3}$
	$WFCM_4$	$9 X 10^{3}$	$3 X 10^{3}$	$3 X 10^{3}$
	$WFCM_5$	$6 X 10^{3}$	$1 X 10^{3}$	$2 X 10^{3}$

Note: Legend: Nutrient Agar = NA (Mesophilic Bacterial count) ,MacConkey Agar = MCA (Coliform count), Potato Dextrose Agar = PDA (Fungal count), Sfu/g = Spore forming unit per gram, Cfu/g = Colony forming unit per gram, WF -100% wheat flour, $WFCM_1$ - 98%Wheat flour: 2 % Fresh coconut meat, $WFCM_2$ -96%Wheat flour: 4 % Fresh coconut meat, $WFCM_3$ -94%Wheat flour: 6 % Fresh coconut meat, $WFCM_4$ -92%Wheat flour: 8 % Fresh coconut meat, $WFCM_5$ -90%Wheat flour: 10 % Fresh coconut meat.

Bacteria and fungi were not detected in the bread samples produced. These are within the limit set by the Standard Organization of Nigeria, which states that the counts of aerobic bacteria must not exceed 100 cfu/g and coliform growth must not be detected in bread samples. This shows that such bread is safe for consumption as there

© 2020 Conscientia Beam. All Rights Reserved.

was no fecal contamination. Similar result was reported for wheat and potato flour blends (Ijah, Auta, Aduloju, & Aransiola, 2014).

There were steady increase in the microbial load of all the bread samples in all the media used. All the bread samples had the same microbial load of 1×10^3 cfu/g noticeable on day 3 before steady increase from day 5 to day 7 starting from 1.0×10^3 cfu/g to 9.0×10^3 cfu/g on Nutrient agar (NA), while on MacConkey agar (MCA), the microbial load was also noticeable on day 5 with steady increase of 1.0×10^3 cfu/g to 3.0×10^3 cfu/g and on Potato dextrose agar PDA), the microbial load was noticeable on day 7, ranged from 1×10^3 cfu/g to 9.0×10^3 cfu/g.

The pattern of coliform and fungi growth on the bread samples were similar especially on MCA and PDA media. The bacteria population (such as Bacillus spp. and Coliforms) could be as a result of ropy spore- formers which has been reported to survive baking temperature, humid environment (packaged bread) and storage temperature, apart from post-contamination (Bailey & Von Holy, 1993; Debonne, Van Bockstaele, De Leyn, Devlieghere, & Eeckhout, 2018; Rumeus & Turtoi, 2013). The fungi growth on day 7 could be as a result of breakdown of fungi population during long storage could be due to release of organic compounds such as benzene and acetone known to cause headaches, dizziness and nausea in an infected person (Le Lay et al., 2016; Nirmala, Sevvel, & Saarutharshan, 2016; Saranraj & Sivasakthivelan, 2015). This could also be influenced by both intrinsic and extrinsic factors such temperature, oxygen, moisture and inert water.

3.5. Some Antioxidant Potentials of the Breads

Kitts, Chen, and Jing (2012) reported that the antioxidant activity of white bread is usually attributed to the presence of Maillard reaction products that are known to possess free-radical scavenging activities.

Table-6. Total Phenol content of bread incorporated with coconut blend.

Day	Blend	Total Phenol (mg.GAE/100g)
ONE	WF	35.6 ± 1.1^{e}
	$WFCM_1$	48.8 ± 1.0^{d}
	$WFCM_2$	48.8 ± 0.8^{d}
	$WFCM_3$	52.7 ± 1.0^{c}
	$WFCM_4$	60.3 ± 1.0^{b}
	$WFCM_5$	63.7 ± 1.1^{a}
THREE	WF	35.6 ± 2.2^{d}
	$WFCM_1$	38.6 ± 1.3^{d}
	$WFCM_2$	40.2 ± 2.5^{c}
	$WFCM_3$	47.7 ± 1.0^{b}
	$WFCM_4$	48.7 ± 1.0^{b}
	$WFCM_5$	57.2 ± 1.4^{a}
FIVE	WF	33.2 ± 2.2^{c}
	$WFCM_1$	37.8 ± 1.3^{c}
	$WFCM_2$	37.1 ± 2.5^{c}
	$WFCM_3$	40.7 ± 1.0^{b}
	$WFCM_4$	42.7 ± 1.0^{ab}
	$WFCM_5$	43.7 ± 1.5^{a}
SEVEN	WF	32.4 ± 1.8^{c}
	$WFCM_1$	34.7 ± 2.2^{b}
	$WFCM_2$	35.5 ± 1.3^{b}
	$WFCM_3$	37.1 ± 2.8^{ab}
	$WFCM_4$	39.2 ± 0.8^{a}
	$WFCM_5$	39.3 ± 2.4^{a}

Note: Values represent mean ± standard deviation (n = 3). Values with different superscripts within the same column are significantly different ($P \leq 0.05$). WF -100% wheat flour, $WFCM_1$ - 98%Wheat flour: 2 % Fresh coconut meat, $WFCM_2$ - 96%Wheat flour: 4 % Fresh coconut meat, $WFCM_3$ -94%Wheat flour: 6 % Fresh coconut meat, $WFCM_4$ -92%Wheat flour: 8 % Fresh coconut meat, $WFCM_5$ -90%Wheat flour: 10 % Fresh coconut meat.

The phenolic contents of the six bread samples were expressed as mg gallic acid per gram of dry weight Table 6. The total phenolic content of the bread samples (35.6 to 63.7mgGAE/g) significantly ($p < 0.05$) increased as the

© 2020 Conscientia Beam. All Rights Reserved.

level of inclusion of fresh coconut meat increased. Among the bread samples, the bread ($WFCM_5$) had the highest phenolic content (63.7±1.1 mg/g GAE) while WF had the lowest phenolic content (35.6±1.1 mg/g GAE) and the differences between all samples were statistically significant. The results for phenol content were higher than the total phenol in pumpkin enriched bread which ranged from 4.16 mg GAE /g to 16.16 mg GAE /g (Wahyono et al., 2019) but lower to that observed in wheat bread supplemented with leafy powders which ranged from 87.16 mg GAE /g to 105.5 mg GAE /g (Odunlade et al., 2017). The inclusion of fresh coconut meat in bread caused improvement in the phenolic properties of the bread which is an indicator of antioxidant potential of the bread.

On the third day of production, a decrease in the phenolic components of the bread was observed with only sample WF retaining its phenolic properties. $WFCM_3$ and $WFCM_3$ experienced a significant drop from 52.7±1.0 (mg/g GAE) and 60.3±1.0 (mg/g GAE) to 47.7±1.0 (mg/g GAE) and 40.2±2.5 (mg/g GAE) respectively. On the fifth and seventh day of production there was a significant drop in the phenolic content of the breads except for the bread sample with (WF) and ($WFCM_2$) which retain its phenol contents.

Table-7. Total Flavonoid content of bread incorporated with coconut blend

Day	Blend	Total Phenol (mg.GAE/100g)
ONE	WF	4.9 ± 0.10^{f}
	$WFCM_1$	5.2 ± 0.08^{e}
	$WFCM_2$	5.4 ± 0.18^{d}
	$WFCM_3$	5.7 ± 0.09^{c}
	$WFCM_4$	5.8 ± 0.08^{b}
	$WFCM_5$	6.0 ± 0.10^{a}
THREE	WF	4.6 ± 0.11^{d}
	$WFCM_1$	5.1 ± 0.10^{c}
	$WFCM_2$	5.3 ± 0.21^{b}
	$WFCM_3$	5.3 ± 0.08^{b}
	$WFCM_4$	5.8 ± 0.18^{a}
	$WFCM_5$	5.8 ± 0.08^{a}
FIVE	WF	3.9 ± 0.11^{e}
	$WFCM_1$	4.2 ± 0.10^{d}
	$WFCM_2$	4.4 ± 0.21^{c}
	$WFCM_3$	4.8 ± 0.08^{b}
	$WFCM_4$	4.8 ± 0.16^{b}
	$WFCM_5$	5.1 ± 0.15^{a}
SEVEN	WF	3.6 ± 0.33^{d}
	$WFCM_1$	3.7 ± 0.21^{c}
	$WFCM_2$	3.7 ± 0.31^{c}
	$WFCM_3$	4.2 ± 0.92^{b}
	$WFCM_4$	4.4 ± 0.33^{a}
	$WFCM_5$	4.4 ± 0.45^{a}

Note: Values represent mean ± standard deviation (n = 3). Values with different superscripts within the same column are significantly different (P≤0.05). WF -100% wheat flour, $WFCM_1$ - 98%Wheat flour: 2 % Fresh coconut meat, $WFCM_2$ - 96%Wheat flour: 4 % Fresh coconut meat, $WFCM_3$ -94%Wheat flour: 6 % Fresh coconut meat, $WFCM_4$ -92%Wheat flour: 8 % Fresh coconut meat, $WFCM_5$ - 90%Wheat flour: 10 % Fresh coconut meat.

The results of the total flavonoids in the bread samples are shown in Table 7. The total flavonoids in the bread samples (4.9 to 6.0 mgQUE/100g) significantly ($p < 0.05$) increased as the level of inclusion of fresh coconut meat increased. The content of total flavonoids in bread with 10% coconut meat substitution ($WFCM_5$) was higher (6.0 mgQUE/100g) when compared to breads in all other bread samples and the (WF) which had the lowest phenolic content (4.9 mgQUE/100g) and the differences between all samples were statistically significant. The results for flavonoid in the bread samples content were higher than those obtained for buckwheat, Amaranth and Quinoa bread (Chlopicka, Pasko, Gorinstein, Jedryas, & Zagrodzki, 2012) onion powder on bread (Prokopov et al., 2018). From the day three to seven, decrease in flavonoid of all the bread samples as the storage days increased was observed. By the seventh day there were significant differences among the wheat/fresh coconut meat blends at (P≤0.05) with the

© 2020 Conscientia Beam. All Rights Reserved.

exception of the $WFCM_4$ and $WFCM_5$. The data obtained from this work may support the possible application of fresh coconut meat to the formulation of bread with increased health-enhancement.

4. CONCLUSION

The study revealed that bread samples with improved nutritional value and overall acceptable sensory attributes could be produced from wheat flour/fresh coconut meat at10% enrichment. The ropy- spore forming bacteria and fungi on the bread may have led to its spoilage on the 7^{th} day after production. The total phenol and flavonoid contents of the fresh coconut meat substitution were higher than wheat flour which may suggest its antioxidant potentials thereby contributing benefits to human health as well as contribution to the bread aroma.

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
Acknowledgement: All authors contributed equally to the conception and design of the study.

REFERENCES

AACC. (2000). *Approved methods of the American Association of Cereal Chemists* (10th ed.). St. Paul, Minn: AACC.

AOAC. (2012). *Official methods of food analysis* (19th ed.). Washington, DC, USA: Association of Official Analytical Chemists.

Ariffin, F., Baharom, M. A., Kaur, B., & Murad, M. (2015). The physicochemical properties and sensory evaluation of bread made with a composite flour from wheat and tempoyak (fermented durian). *American Journal of Applied Sciences, 12*(11), 775-784.

Bailey, C., & Von Holy, A. (1993). Bacillus spore contamination associated with commercial bread manufacture. *Food Microbiology, 10*(4), 287-294. Available at: https://doi.org/10.1006/fmic.1993.1033.

Bolarinwa, I. F., Aruna, T. E., & Raji, A. O. (2017). Nutritive value and acceptability of bread fortified with moringa seed powder. *Journal of the Saudi Society of Agricultural Sciences, 18*(2), 195-200. Available at: .

Chlopicka, J., Pasko, P., Gorinstein, S., Jedryas, A., & Zagrodzki, P. (2012). Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. *LWT-Food Science and Technology, 46*(2), 548-555. Available at: https://doi.org/10.1016/j.lwt.2011.11.009.

Chuahan, G. S., Zillman, R., & Eskin, N. M. (1992). Dough mixing and breadmaking properties of quinoa-wheat flour blends. *International Journal of Food Science & Technology, 27*(6), 701-705. Available at: https://doi.org/10.1111/j.1365-2621.1992.tb01241.x.

Debonne, E., Van Bockstaele, F., De Leyn, I., Devlieghere, F., & Eeckhout, M. (2018). Validation of in-vitro antifungal activity of thyme essential oil on Aspergillus niger and Penicillium paneum through application in par-baked wheat and sourdough bread. *LWT, 87*, 368-378. Available at: https://doi.org/10.1016/j.lwt.2017.09.007.

Erminawati, W., Sidik, R. L., & Zulfakar, H. (2018). *Formulation and characterization of bread using coconut pulp flour and wheat flour composite with addition of xanthan-gum International Symposium on Food and Agro-biodiversity (ISFA)*. Paper presented at the IOP Conf. Series: Earth and Environmental Science 102 (2018) 012010. Available at: https://doi.org/10.1088/1755-1315/102/1/012010.

Fawole, M. O., & Oso, B. A. (2007). Laboratory manual of microbiology (pp. 11-24). Ibadan: Spectrum Books Ltd.

Gorinstein, S., Vargas, O. J. M., Jaramillo, N. O., Salas, I. A., Ayala, A. L. M., Arancibia-Avila, P., . . . Trakhtenberg, S. (2007). The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. *European Food Research and Technology, 225*(3-4), 321-328. Available at: https://doi.org/10.1007/s00217-006-0417-7.

Gunathilake, K., Yalegama, C., & Kumara, A. (2009). Use of coconut flour as a source of protein and dietary fibre in wheat bread. *Asian Journal of Food and Agro-Industry, 2*(3), 382-391.

Igbabul, B., Grace, N., & Julius, A. (2014). Quality evaluation of composite bread produced from wheat, maize and orange fleshed sweet potato flours. *American Journal of Food Science and Technology, 2*(4), 109-115. Available at: https://doi.org/10.12691/ajfst-2-4-1.

© 2020 Conscientia Beam. All Rights Reserved.

Ijah, U. J. J., Auta, H. S., Aduloju, M. O., & Aransiola, S. A. (2014). Microbial, nutritional and sensory quality of bread produced from wheat and potato flour blends. *International Journal of Food Science Technology, 2014*, 1-6.

Kitts, D. D., Chen, X.-M., & Jing, H. (2012). Demonstration of antioxidant and anti-inflammatory bioactivities from sugar–amino acid Maillard reaction products. *Journal of Agricultural and Food Chemistry, 60*(27), 6718-6727.

Le Lay, C., Coton, E., Le Blay, G., Chobert, J.-M., Haertlé, T., Choiset, Y., . . . Mounier, J. (2016). Identification and quantification of antifungal compounds produced by lactic acid bacteria and propionibacteria. *International Journal of Food Microbiology, 239*, 79-85. Available at: https://doi.org/10.1016/j.ijfoodmicro.2016.06.020.

Madukwe, E., Obizoba, I., & Chukwuka, O. (2013). Nutrient assessment of processed rice (Oryza sativa), soybean (Glycine max Merr) flours/groundnut (Arachis hypogea) paste and sensory attributes of their composites. *International Journal of Scientific and Research Publications, 3*(8), 1-8.

Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry, 91*(3), 571-577. Available at: https://doi.org/10.1016/j.foodchem.2004.10.006.

Nirmala, R., Sevvel, P., & Saarutharshan, S. (2016). Study on fungi associated with spoilage of bread. *International Journal of Advanced Research in Biological Science, 3*(4), 165-167.

Nnorom, I. C., Nnadozie, C., Ugwa, R., & Obike, A. I. (2013). Proximate and trace metal analysis Of Coconut (Cocos nucifera) collected from Southeastern, Nigeria. *ABSU Journal of Environment, Science and Technology (JEST), 3*, 357-361.

Nwosu, J. N., Owuamanam, C., Omeire, G., & Eke, C. (2014). Quality parameters of bread produced from substitution of wheat flour with cassava flour using soybean as an improver. *American Journal of Research Communication, 2*(3), 99-118.

Odunlade, T., Famuwagun, A., Taiwo, K., Gbadamosi, S., Oyedele, D., & Adebooye, O. (2017). Chemical composition and quality characteristics of wheat bread supplemented with leafy vegetable powders. *Journal of Food Quality, 2017*(3), 1-7. Available at: https://doi.org/10.1155/2017/9536716.

Olapade, A., & Oluwole, O. (2013). Bread making potential of composite flour of wheat-acha (Digitaria exilis staph) enriched with cowpea (Vigna unguiculata L. walp) flour. *Nigerian Food Journal, 31*(1), 6-12. Available at: https://doi.org/10.1016/s0189-7241(15)30050-3.

Oluwajoba, S. O., Malomo, O., Ogunmoyela, O. A. B., O., D. O. E., & Odeyemi, A. (2012). Microbiological and nutritional quality of warankashi enriched bread. *Journal of Microbiology, Biotechnology and Food Sciences, 2*(1), 42–68.

Omotosho, I., & Odeyemi, F. (2012). Bio-nutritional constituents of coconut fruit and its possible medicinal applications. *African Journal of Plant Science, 6*(12), 309-313. Available at: https://doi.org/10.5897/ajps11.021.

Oseni, N. T., Fernando, W., Coorey, R., Gold, I., & Jayasena, V. (2017). Effect of extraction techniques on the quality of coconut oil. *African Journal of Food Science, 11*(3), 58-66. Available at: https://doi.org/10.5897/ajfs2016.1493.

Pareyt, B., Finnie, S. M., Putseys, J. A., & Delcour, J. A. (2011). Lipids in bread making: Sources, interactions, and impact on bread quality. *Journal of Cereal Science, 54*(3), 266-279. Available at: https://doi.org/10.1016/j.jcs.2011.08.011.

Prokopov, T., Chonova, V., Slavov, A., Dessev, T., Dimitrov, N., & Petkova, N. (2018). Effects on the quality and health-enhancing properties of industrial onion waste powder on bread. *Journal of Food Science and Technology, 55*(12), 5091-5097. Available at: https://doi.org/10.1007/s13197-018-3448-8.

Rouillé, J., Bonny, J.-M., Della Valle, G., Devaux, M., & Renou, J. (2005). Effect of flour minor components on bubble growth in bread dough during proofing assessed by magnetic resonance imaging. *Journal of Agricultural and Food Chemistry, 53*(10), 3986-3994. Available at: https://doi.org/10.1021/jf047953r.

Rumeus, I., & Turtoi, M. (2013). Influence of sourdough use on rope spoilage of wheat bread. *Journal of Agroalimentary Processes and Technologies, 19*(1), 94-98.

Saranraj, P., & Sivasakthivelan, P. (2015). Microorganisms involved in spoilage of bread and Its control measures. In: Bread and its fortification Nutrition and Health Benefits, Edition: 13 Chapter 7, Publisher (pp. 132-149). New York, USA: CRC Press, Taylor and Francis Group.

© 2020 Conscientia Beam. All Rights Reserved.

Solangi, A. H., & Iqbal, M. Z. (2011). Chemical composition of meat (kernel) and nut water of major coconut (Cocos nucifera L.) cultivars at coastal area of Pakistan. *Pakistan Journal of Botany, 43*(1), 357-363.

Wahyono, A., Dewi1, A. C., Oktavia, S., Jamilah, S., & Kang, W. W. (2019). *Antioxidant activity and total phenolic contents of bread enriched with pumpkin flour*. Paper presented at the In a Wahyono et al 2020 IOP Conf. Ser.: Earth Environ. Sci. 411 012049.

Views and opinions expressed in this article are the views and opinions of the author(s), Journal of Food Technology Research shall not be responsible or answerable for any loss, damage or liability etc. caused in relation to/arising out of the use of the content.

© 2020 Conscientia Beam. All Rights Reserved.