Evaluation of the Microbial Load and Proximate Compositions of High-Fiber Composite Flours Made From Wheat, Tiger Nut Fiber and Cassava Blends

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ABSTRACT

The study aimed to evaluate the microbial load and proximate compositions of high-fiber composite flours made from wheat, tiger nut fiber and cassava blends. High fiber inclusion (up to 20%) was achieved presenting an excellent option for formulating healthier foods. The microbial count for the total viable count (TVC), total coliform count (TCC) and total fungi count (TFC) were determined. TVC had the ranges of 0.40 x 10^2 CFU/g to 13.30 x 10^8 CFU/g (0 to 192 days of storage), Also TCC were not detected at day 0 but varied to 12.22 x 10^8 CFU/g after 192 days of storage. In the same way, TFC had the ranges of 3.50 x 10^2 CFU/g to 20.24 x 10^8 CFU/g (0 to 192 days of storage). After 144 days, the microbial load of the high-fiber composite flours peaked at levels of x10^8 indicating unsafe limits, below this storage level, acceptable safety levels were compared to Kenya Bureau of Standards (KEBS) ranges of TVC (≤ 1 x 10^5 CFU/g, TCC (< 1 x 10^2 CFU/g) and TFC (≤ 1 x 10^4 CFU/g). The proximate compositions had moisture range of 4.65 – 5.89%, protein (8.50 – 13.84%), fat (1.37 – 1.77%), crude fiber (2.10 – 3.50%), ash (0.26 – 0.57%) and carbohydrates (77.50 – 80.65%), the dry matter content ranged from 94.11 – 95.35% and energy range of 368.08 – 380.24 Kcal. The tiger nut fiber supplementation was achieved with partial replacement up to 20% presenting an excellent option for formulating healthier food alternatives

Keywords: Composite flours, high-fiber flours, microbial load, proximate composition, healthier alternatives

INTRODUCTION

Composite flour had been reported as an innovative flour that attracted much attention in research as well as food product development (Hasmadi *et al.,* 2022). Composite flour had better nutritional value concerning elements of minerals, vitamins, fibers, and proteins than flour milled from any specific cereal alone. Shanti *et al.* (2019) reported that the composite flour mixture could provide a balanced nutrient. Tiger nut fiber is a by-product (residue) of the production of tiger nut milk. A lot of attention had been given recently to the utilization of by-products from food processing industries with high nutritional content due to their high-value products (Alvarez-Jubete *et al.*, 2020). In recent decades, there has been an increasing shift towards the consumption of high-fiber foods as opposed to carbohydrate-dense diets. The consumption of dietary fiber is associated with improved digestion, enhanced nutrient absorption, and better blood sugar control, especially for individuals with metabolic disorders such as type 2 diabetes. The blending of flours from various sources, such as wheat, tiger nut fiber, and cassava, offers a unique opportunity to develop noodle products with enhanced nutritional benefits. To ensure improved shelf keeping of these composite flours, care must be given to the good manufacturing practices

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(GMPs) and improved reduction in the moisture content (less than 12 %) of composite flours. The aim of this study is to evaluate the microbial load of high-fiber composite flours made from wheat, tiger nut fiber and cassava blends.

MATERIALS AND METHODS

Wheat and yellow tiger nuts healthy seeds were purchased from a Relief market in Owerri, Imo state of Nigeria while the cassava tubers were sourced from National Root Crops Research Institute, Umudike, Abia State, Nigeria for uniformity and proper identification.

Processing of wheat seeds, tiger nut and cassava tubers into flour

The processing method outlined by Jaya *et al.* (2021) was adopted in processing the tiger nut fiber with slight modification on the fiber obtaining method. The tiger nut was sorted to remove stones, debris, and spoiled nuts. It was then washed under a running water. After that, the tiger nuts were thoroughly washed again with 0.1 % sodium hydroxide solution to eliminate microorganisms, remaining dirt and impurities. The washed tiger nut was grated and sieved using muslin sac to separate the milk and the fiber, the final fiber obtained was sparged with hot water to separate more extractives and the dried in an oven at 60°C for 12 hours to reduce moisture content. After drying, it was grounded into fine powder using a mill.

The method described by Adebayo *et al.* (2020) was adopted in processing the cassava tuber into flour with a slight modification on the method of drying. The peeled cassava tubers were grated into small particles. The grated cassava mash is soaked for 12 hours and then spray dried in a spray drier. The dried cassava was then milled into flour using a hammer mill.

The processing method described Olatunji *et al.* (2021) was adopted in manufacturing of wheat flour. The wheat grains were sorted, cleaned, and washed with a clean water to get rid of any remaining contaminants. Following an overnight soak in clean water, the cleaned grains were poured through a plastic sieve. The grains were hammer milled and then re-milled after being oven dried for 12 hours at 60 °C

Formulation of the processed flours

Flour blends were prepared using different ratios of wheat, tiger nut fiber, and cassava flour. The following blending ratios were used: WTC1 (Wheat/tiger-nut fiber/cassava): 74.30 g wheat flour, 20 g tiger nut fiber, 5.70 g cassava flour, WTC2: 75 g wheat flour, 20 g tiger nut fiber, 5 g cassava flour, WTC3: 71.86 g wheat flour, 18.14 g tiger nut fiber, 10.00 g cassava flour, WTC4: 100 g wheat flour (control) as presented in Table 1. Each blend was mixed thoroughly using a mechanical mixer for 10 minutes to ensure homogeneity.

	Flour Ratios (g)		
Samples	Wheat	Tiger nut fiber	Cassava
WTC1	74.30	20.00	5.70
WTC2	75.00	20.00	5.00
WTC3	71.86	18.14	10.00
WTC4 (Control)	100.00	0.00	0.00

Table 1: Formulation of high-fiber composite flours made from wheat, tiger nut fiber and cassava blends.

Determination of the microbial count of high-fiber composite flours made from wheat, tiger nut fiber and cassava blends.

The composite flour blends were evaluated for microbial count on the day of production (Day 0) to 192 days post – production storage. Each of the composite flour blends (1g) was rinsed in 99ml of sterile peptone water.

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The resultant homogenate was used to make a dilution of 10^{-1}. From this 10^{-1} dilution, 1ml was passed serially into another tube to make a 10^{-2} dilution of the composite flour based on the method described by Ezeama (2007). The spread plate technique was adopted for plating aliquots of 1ml of the dilutions in duplicate onto different media plates: Tryptone soy agar (TSA) for total bacterial count, Sabouraud-Dextrose agar (SDA) for total fungal count and MacConkey agar (MCA) for total coliform count. All the media were prepared according to the manufacturer's instructions. Tryptone soy agar (TSA) and MacConkey agar (MCA) plates were incubated for 24 hours at 37°C. Sabouraud-Dextrose agar (SDA) was left at room temperature after an initial 4-hour incubation. At the end of the incubation time, the culture plates were observed for the growth of microorganisms, and colonies were counted using a colony counter machine. The number of microbial colonies growing on each plate was determined and recorded as CFU/g of composite flour blends. The range of 25 to 250 colonies (Tinasuewicz *et al*., 1980) were considered optimum for counting.

Determination of proximate composition of composite flour blends

Determination of Moisture Content

The moisture content of the composite flour blends was determined using the method described by Onwuka, (2018). The aluminum dishes were washed and thoroughly dried in an oven and put into a desiccator to cool and then weighed. Exactly 2 g of the samples were weighed into previously weighed moisture dishes. The weight of the dish and the sample inside was taken. The dish with the samples was dried in an oven at 105 ^{0}C for 4 hours. The dried sample was cooled in a desiccator and weighed. The weight was recorded while the sample was returned to the oven for further drying. The drying, cooling and weighing was done repeatedly until a constant weight was obtained. The moisture content of the samples was calculated as follows:

$$\text{Moisture (\%)} = \frac{W2-W3}{W2-W1}\ x\ \frac{100}{1} \quad (1)$$

Where: W_1 = weight of the empty crucible.

W_2 = weight of the crucible + sample before drying

W_3 = final weight of dish + sample after drying to a constant weight

Determination of Crude fiber.

The crude fiber content of each composite samples was determined with the method as described by Onwuka (2018). A 2 gram of each sample was boiled under reflux for 30 min with 200 ml of a solution containing 1.25 g of H_2SO_4 per 100 ml of water. After that the sample was washed with several portions of boiling water using a twofold muslin cloth to trap the particles until the particles will be no longer acidic. The residues were transferred to a beaker and boiled for 30 minutes with 200 ml of a solution containing 1.25 g of carbonate free sodium hydroxide (NaOH) per 100ml and washed as before with boiling water. Then it was carefully transferred to a weighed porcelain and dried in the oven at 105 ^{0}C for 2 hours. Then, it was incinerated (until it turns ash), cooled in a desiccator and weighed. The crude fiber content of the sample was calculated as:

$$\text{\% crude fiber} = \frac{W_2 - W_1}{\text{weight of sample}} \times \frac{100}{1} \quad (2)$$

Where W_2 = weight of crucible + sample after dying

Where W_1 = weight of crucible + sample ash

Protein content determination.

The protein content of the samples was determined using the Kjeldahl method as described by Onwuka (2018). A 2 gram of each sample was weighed into a Kjeldahl flask and 4 tablets of Kjeldahl catalyst was added. It will be followed up by copper sulphate and a speck of selenium or 1 tablet of Kjeldahl catalyst (tablet containing 1g Na_2SO_4 + 0.05g selenium). In the mixture, 25 ml concentrated sulphuric acid and 5 glass beads (glass beads

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prevent bumping during heating) was introduced and heated in a fume cupboard, until a clear solution assumes a green colour. The heating continued until the colour disappeared, and it was allowed to cool. After cooling, the digest was washed severally and transferred into a 250 ml volumetric flask and then made up to the mark with distilled water. Distillation was carried out using Markham distillation setup. The apparatus was first steamed for about 15 min. A 10 ml portion of each digest was mixed with equal volume of 45% NaOH solution in Kjeldahl distilling unit. The mixture was distilled and the distillate collected into 100 ml conical flask containing 2-3 drops of 4 % boric acid solution indicator. Then, it was steamed through for about 5 – 7 min to collect enough ammonium sulphate, after which the receiving flask was removed. The condensed water in the condenser will be flushed out ready for another batch. The distillate was obtained and titrated against 0.02 M H_2SO_4 or 0.01 N HCl solution till a change in color of solution from initial green color to a deep red colour was observed at the end point. A blank will be run alongside the samples.

The nitrogen content of the samples was calculated as shown:

$$\% \text{ Nitrogen} = \frac{V_S - V_B \times 100 \times \text{N acid}}{\text{w}} \times \frac{14}{100} \quad (3)$$

Where: w = weight of sample analyzed in grams

V_S = total volume (ml) of acid required to titrate sample

V_B = total volume (ml) of acid required to titrate blank

N acid = normality of acid (0.1 N)

$$\% \, Crude\ protein = N\ X\ (Conversion\ factor)$$

Where: $$\text{Conversion factor} = \frac{100}{\% \, Nitrogen\ in\ protein} \quad (4)$$

Determination of ash content

The method described by Onwuka (2018) was adopted in the ash content determination of the samples. Exactly 2g of finely ground, dried sample was weighed into tarred silica or porcelain crucible, and made to char on a heater put inside a fume cupboard to drive off most of the smoke. The sample was transferred into a preheated muffle furnace at 550 ^{0}C. The sample was allowed to burn at this temperature for 2 hours until a white or light grey ash resulted. When the residue is black in color, it was wetted with a small amount of water to dissolve salts, dried in an oven and the ashing process repeated finally, the sample will be cooled in a desiccator and reweighed.

The ash content of the samples was calculated as shown:

$$\% \text{ ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1} \quad (5)$$

Where W_1 = weight of empty crucible

W_2 = weight of crucible + sample

W_3 = weight of crucible + ash

Fat content determination

The fat content of the samples was determined using the method described by Onwuka (2018). A 250 ml clean boiling flask will be dried in an oven at 105 ^{0}C for about 30 minutes. It was then transferred into a desiccator and allowed to cool. A 2 g of samples will be weighed into labeled thimbles. The boiling flask was filled with

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about 300 ml of petroleum ether with boiling point of about 60 ^{0}C. The extraction thimble was plugged in lightly with cotton wool. The soxhlet apparatus were assembled and allowed to reflux for about 6 h. The thimble was carefully removed and the petroleum ether collected in the top container of the set – up and drained in a container to be re – used. When the flask was almost free of petroleum ether, it was removed and dried at 105 ^{0}C for 1 h. Then, the flask was transferred from the desiccator and allowed to cooled, then reweighed. The fat content of the samples was calculated as shown:

$$\% \text{ Fat} = \frac{\text{weight of fat}}{\text{weight of sample}} \times \frac{100}{1} \quad (6)$$

Carbohydrate determination

The carbohydrate content of the samples was calculated by difference, having estimated all other fractions, as described by Onwuka (2018).

$$\% \text{ Carbohydrate} = 100 - \% (a + b + c + d + e) \quad (7)$$

Where: a = % moisture

b = % ash

c = % protein

d = % fiber

e = % fat

Dry matter determination

The dry matter content of the samples was determined using the method described by Onwuka (2018)

% dry matter = 100 - % moisture

Experimental design and statistical analysis

The experimental design used in this study is completely randomized design (CRD). Microsoft Excel were used for the presentation of data and determination of sample means.

RESULTS AND DISCUSSIONS

Microbial count of high-fiber composite flours made from wheat, tiger nut fiber and cassava blends

The values for the microbial counts were presented in Table 2. The microbial loads for the total viable count (TVC), total coliform count (TCC) and total fungi count (TFC) were all determined from 0 (Day of production) to 192 storage days. High microbial loads can deteriorate flour quality through spoilage. The total viable count for day 0 ranged from 0.40 – 1.00 x 10^2 CFU/g, day 48: 1.20 – 2.50 x 10^3 CFU/g, day 96: 3.50 – 4.90 x 10^3 CFU/g, day 144: 3.00 – 9.50 x 10^3 CFU/g and day 192: 0.05 – 13.30 x 10^8 CFU/g.

Sample WTC2 (75 g Wheat flour; 20 g Tiger nut fiber; 5 g cassava flour) had the least microbial count for all the days of storage while WTC4 (100 g Wheat flour) had the highest microbial count. The microbial count from 0 – 144 days had safe limits when compared to Kenya Bureau of Standards (KEBS) of ≤ 1 x 10^5 CFU/g for composite flours (KEBS, 2021), until there was a spike at day 192 above this safe limit. Elevated total viable count at day 192 (0.05 – 13.30 x 10^8 CFU/g) indicates potential enzymatic activity that can degrade the flour's nutrients and sensory properties, such as taste, texture and aroma leading to a shorter shelf life (Okorie and Anosike, 2023). This is especially critical for composite flours that include cassava and tiger nut, as these ingredients may introduce more moisture or sugars that promote microbial growth (Adeyemi and Idowu, 2021).

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In contrast, TVC (Day 0 – 144) indicates better stability and longer storage potential. The presence of coliforms (TCC) having *Escherichia coli* as the indicator organism is an important food safety concern. Total coliform count for day 0 was not detected, day 48 varied from 0.30 – 1.00 x 10^1 CFU/g, day 96: 0.15 – 0.55 x 10^2 CFU/g, day 144: 0.28 – 0.95 x 10^2 CFU/g and elevated TCC levels at day 192 (0.10 – 12.22 x 10^8 CFU/g) from the trend of increasing microbial count with increasing storage periods with the safe limit pegged at 1.00 x 10^2 CFU/g (KEBS, 2021).

This spike of contamination at day 192 could arise from post – process contaminations like storage containers and utensils, environmental contamination due to prolonged storage and inadequate control of storage temperature (KEBS, 2021). Such contamination can lead to foodborne illnesses if consumed without proper cooking or processing.

Composite flours containing cassava and tiger nut are more prone to microbial growth due to their higher moisture absorption capacity. Cassava flour, in particular, is known to be hygroscopic, creating an ideal environment for microbial growth if not stored properly (Adejumo *et al.*, 2020). Tiger nut, being high in natural sugars, can also support rapid microbial multiplication (Nwachukwu and Ezeigbo, 2022).

The total fungal count for day 0 ranged from 3.50 – 9.28 x 10^2 CFU/g, day 48: 1.20 – 2.85 x 10^3 CFU/g, day 96: 2.55 – 4.15 x 10^3 CFU/g, day 144: 6.33 – 8.20 x 10^3 CFU/g and day 192: 0.30 – 20.24 x 10^8 CFU/g. Sample WTC2 (75 g Wheat flour; 20 g Tiger nut fiber; 5 g cassava flour) had the least microbial count for all the days of storage while WTC4 (100 g Wheat flour) had the highest microbial count for total fungal count. The total fungal count of the composite mix followed the trend of increasing microbial count with increasing storage period.

Table 2: Microbial counts of the composite mix made from wheat, tiger nut fiber and cassava flours

		Days				
	Samples	0	48	96	144	192
TVC (CFU/g)	WTC1	0.70 x 10^2	1.50 x 10^3	4.00 x 10^3	6.00 x 10^3	1.00 x 10^8
	WTC2	0.40 x 10^2	1.20 x 10^3	3.50 x 10^3	3.00 x 10^3	0.05 x 10^8
	WTC3	0.85 x 10^2	2.00 x 10^3	4.30 x 10^3	8.00 x 10^3	1.56 x 10^8
	WTC4	1.00 x 10^2	2.50 x 10^3	4.90 x 10^3	9.50 x 10^3	13.30 x 10^8
	KEBS	≤ 1 x 10^5	≤ 1 x 10^5	≤ 1 x 10^5	≤ 1 x 10^5	≤1 x 10^5
TCC (CFU/g)	WTC1	ND	0.62 x 10^1	0.20 x 10^2	0.40 x 10^2	1.45 x 10^8
	WTC2	ND	0.30 x 10^1	0.15 x 10^2	0.28 x 10^2	0.10 x 10^8
	WTC3	ND	0.82 x 10^1	0.42 x 10^2	0.75 x 10^2	4.90 x 10^8
	WTC4	ND	1.00 x 10^1	0.55 x 10^2	0.95 x 10^2	12.22 x 10^8
	KEBS	< 1 x 10^2	< 1 x 10^2	< 1 x 10^2	< 1 x 10^2	< 1 x 10^2
TFC (CFU/g)	WTC1	5.50 x 10^2	1.50 x 10^3	3.10 x 10^3	7.15 x 10^3	1.25 x 10^8
	WTC2	3.50 x 10^2	1.20 x 10^3	2.55 x 10^3	6.33 x 10^3	0.30 x 10^8
	WTC3	8.75 x 10^2	1.65 x 10^3	3.80 x 10^3	7.85 x 10^3	5.61 x 10^8

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	WTC4	9.28×10^2	1.85×10^3	4.15×10^3	8.20×10^3	20.24×10^8
	KEBS	$\leq 1 \times 10^4$	$\leq 1 \times 10^4$	$\leq 1 \times 10^4$	$\leq 1 \times 10^4$	$\leq 1 \times 10^4$

Keys:

WTC1: Wheat (74.3 g), tiger nut fiber (20 g), cassava flour (5.7 g)

WTC2: Wheat (75 g), tiger nut fiber (20 g), cassava flour (5 g)

WTC3: Wheat (71.86 g), tiger nut (18.14 g), cassava flour (10 g)

WTC4: 100 % wheat flour (control)

KEBS: Kenya Bureau of Standards

ND: Not detectable

The total fungal count of the composite mix was within the safe limit for composite flours when compared with the KEBS of $\leq 1 \times 10^4$ CFU/g (KEBS, 2021) except for the spike observed at day 192.

Microbial activity can affect the functional properties of composite flours, such as their ability to form dough or absorb water. Bacterial spoilage may lead to changes in pH and enzymatic breakdown of starch, reducing the flour's elasticity and swelling capacity.

Proximate composition, dry matter and energy content of the composite mix

The result of the proximate composition, dry matter and energy of the optimum composite mix is presented in Table 3. Moisture, protein, fats, crude fiber, ash and carbohydrate contents of the optimum composite mix were reported.

The result of the moisture content of the optimum blends varied from 4.65 to 5.89 %. Sample WTC4 (100 g wheat flour) had the lowest mean value for moisture content while sample WTC1 (70.3 g wheat flour; 20 g tiger nut fiber; 5.7 g cassava flour) had the highest moisture content. The moisture content of the optimum blends was low as several researchers had recommended the range of 12 to 14 % moisture content for flours as levels higher than this limit will encourage microbial growth that deteriorate the shelf keeping the flour (Finnie and Atwell, 2016). Ojo *et al*. (2017) reported the maximum allowable limit of 10 % for long – term storage which is in line with the moisture content of the optimum composite blends. There was significant ($p<0.05$) difference in the moisture content of the blends. The control sample WTC4 (100 g wheat flour) at the lower spectrum which indicates the best choice based on the moisture content attributes. followed by sample WTC3 (71.86 g wheat flour; 18.14 g tiger nut fiber; 10.00 g cassava flour), and sample WTC2 (75 g wheat flour; 20 g tiger nut fiber; 5 g cassava flour) while WTC1 (70.3 g wheat flour; 20 g tiger nut fiber; 5.7 g cassava flour) had the least moisture content.

The result of the protein content of the optimum blends is presented in Table 3. The protein contents ranged from 8.50 to 13.84 %. Sample WTC2 (75 g wheat flour; 20 g tiger nut fiber; 5 g cassava flour) had the lowest mean value for protein content while the control sample WTC4 (100 g wheat flour) had the highest protein content. The protein content of the optimum blends compared favourably with the control sample WTC4 (100 g wheat flour).

The protein content of WTC3 (71.86 g wheat flour; 18.14 g tiger nut fiber; 10.00 g cassava flour) was higher than the values obtained for WTC1 (70.3 g wheat flour; 20 g tiger nut fiber; 5.7 g cassava flour) followed by WTC2 (5 g wheat flour; 20 g tiger nut fiber; 5 g cassava flour) that had the lowest mean score. There was significant ($p<0.05$) difference in the moisture content of the blends. The high protein content of the optimum composite mix is an indication that, it is balanced in its nutrient compositions.

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The result of the fat content of the optimum blends is presented in Table 3. The fat contents oscillated from 1.37 to 1.77 %.

Table 3: Proximate composition, dry matter and energy content of the optimum composite mix made from wheat, tiger nut fiber and cassava flours

Parameters	WTC1	WTC2	WTC3	WTC4	LSD
Moisture (%)	5.89^{a}±0.06	5.71^{b}±0.06	5.46^{c}±0.03	4.65^{d}±0.04	0.08
Protein (%)	8.71^{c}±0.08	8.50^{d}±0.09	9.73^{b}±0.03	13.84^{a}±0.04	0.12
Fat (%)	1.41^{c}±0.02	1.37^{d}±0.03	1.77^{a}±0.03	1.65^{b}±0.02	0.06
Crude fiber (%)	3.44^{b}±0.04	3.42^{b}±0.04	3.50^{a}±0.02	2.10^{c}±0.02	0.06
Ash (%)	0.42^{b}±0.03	0.35^{c}±0.03	0.57^{a}±0.02	0.26^{d}±0.03	0.06
Carbohydrates (%)	80.13^{b}±0.04	80.65^{a}±0.12	78.95^{c}±0.12	77.50^{d}±0.06	0.17
Dry matter (%)	94.11^{d}±0.06	94.29^{c}±0.06	94.54^{b}±0.03	95.35^{a}±0.04	0.08
Energy (Kcal)	368.08^{d}±0.18	368.90^{c}±0.36	370.73^{b}±0.12	380.24^{a}±0.07	0.40

Values are means ± standard deviation of the triplicate determination a-d. Means bearing the same superscript on the same row is not significantly ($p \geq 0.05$) different

Key:

WTC1: 70.3 g Wheat flour; 20 g Tiger nut fiber; 5.7 g cassava flour

WTC2: 75 g Wheat flour; 20 g Tiger nut fiber; 5 g cassava flour

WTC3 : 71.86 g wheat flour; 18.14 g tiger nut fiber; 10.00 g cassava flour

WTC4: 100 g Wheat flour (Control)

Sample WTC2 (75 g wheat flour; 20 g tiger nut fiber; 5 g cassava flour) had the lowest mean value for fat content while sample WTC3 (71.86 g wheat flour; 18.14 g tiger nut fiber; 10.00 g cassava flour) had the highest fat content. All the optimum blends had lower fat content than the control sample WTC4 (100 g Wheat flour) except the sample WTC3 (71.86 g wheat flour; 18.14 g tiger nut fiber; 10.00 g cassava flour). There was significant ($p<0.05$) difference in the fat contents of all the optimum composite blends. The fat content of the optimum composite mix was low and in agreement with the range of 0.47 to 5.75 % reported by Ahmed *et al.* (2018) for wheat – sweet potato composite flours. Also, the fat content of the optimum composite mix was within the allowable limits of 0.98 to 2.5% reported by USDA (2018) for whole and white wheat flour. Apart from the nutritional composition and impact on the function properties of the flour, high – fat flours are more prone to lipid oxidation and hydrolysis that can negatively impact the organoleptic properties of the flours (Delcour and Hoseney, 2010; Pareyt *et al.,* 2011). High – fat flours are also more susceptible to moisture absorption providing an environment conducive for the proliferation of deteriorative organisms that will reduce the shelf keeping of the flours (Cauvain and Young, 2008).

The result of the crude fiber content of the optimum blends is presented in Table 3. The crude fiber content varied from 2.10 to 3.50 %. Sample WTC4 (100 g Wheat flour) had the lowest mean score for crude fiber content while sample WTC3 (71.86 g wheat flour; 18.14 g tiger nut fiber; 10.00 g cassava flour) had the highest crude fiber content. All the optimum blends had higher crude fiber content than the control sample WTC4 (100 g

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Wheat flour). There was significant ($p<0.05$) difference in the crude fiber contents of the optimum composite blends except WTC1 (70.3 g wheat flour; 20 g tiger nut fiber; 5.7 g cassava flour) and WTC2 (5 g wheat flour; 20 g tiger nut fiber; 5 g cassava flour) which are not significant ($p>0.05$) with each other. The high crude fiber content of the optimum composite mix was high due to the high tiger nut fiber partially replacements of up to 20 % in the mix. The optimum composite flour combinations reflect a balance of crude fiber with the other nutritional and functional requirements as reported by Jones (2014). The high – fiber content can influence the texture and structure of baked goods, impacting their overall quality and consumer acceptance (Delcour and Hoseney, 2010). Nevertheless, the presence of high fiber can enhance the functional properties of flours such as water absorption and binding capacity (Slavin, 2013).

The result of the ash content of the optimum blends is presented in Table 3. The ash content varied from 0.26 to 0.57 %. Sample WTC4 (100 g Wheat flour) had the lowest mean score for ash content while sample WTC3 (71.86 g wheat flour; 18.14 g tiger nut fiber; 10.00 g cassava flour) had the highest ash content. All the optimum blends had higher crude fiber content than the control sample WTC4 (100 g Wheat flour). There was significant ($p<0.05$) difference in the ash contents of all the optimum composite blends According to Bilge et al. (2016), ash contents in flour reflects its mineral composition, including both macro – elements (sodium, potassium, calcium, magnesium etc.) and trace elements (iron, zinc, copper etc.). However, the range of ash content obtained in this study was higher than the stricter requirement of ≤ 0.35 % acid – insoluble ash for low – mineral wheat – cassava composite reported by East African Standards (EAS, 2010), except samples WTC2 (5 g wheat flour; 20 g tiger nut fiber; 5 g cassava flour) and WTC4 (100 g Wheat flour).

The result of the carbohydrate content of the optimum blends is presented in Table 3. The carbohydrate content varied from 77.50 to 80.65 %. Sample WTC4 (100 g wheat flour) had the lowest mean score for carbohydrate content while sample WTC2 (5 g wheat flour; 20 g tiger nut fiber; 5 g cassava flour) had the highest carbohydrate content. There was significant ($p<0.05$) difference in the carbohydrate contents of all the optimum composite blends. Carbohydrates are vital energy sources in flours, as they serve as "the primary sources of energy to the cells" (Irondi *et al.,* 2024). The carbohydrate content of the optimum blends was lower that the limits reported by FAO (2019) for cassava flours. Lower carbohydrate content of the optimum mix helps in blood sugar control aiding better insulin resistance (Feinman *et al.,* 2015) and nutrient density (lower carbs – higher protein contents) (Irondi *et al.,* 2024).

The result of the dry matter and energy content of the optimum blends is presented in Table 3. The dry matter content varied from 94.11 to 95.35 %. The dry matter followed an increasing trend with decreasing moisture content with sample WTC1 (70.3 g wheat flour; 20 g tiger nut fiber; 5.7 g cassava flour) having the lowest mean score for dry matter content while sample WTC4 (100 g wheat flour) had the highest dry matter content. Dry matter refers to the portion of flour that remains after removing its water content. A higher dry matter percentage indicates lower moisture content which increases the shelf stability of the flour (Liu *et al.,* 2018). In the other hand, the optimum composite blends had the energy content ranging from 368.08 to 380.24 Kcal. The energy of the blends is consistent with dry matter content with sample WTC1 (70.3 g wheat flour; 20 g tiger nut fiber; 5.7 g cassava flour) having the lowest mean score for energy content while sample WTC4 (100 g Wheat flour) had the highest energy content. The energy content of the optimum flours are combined estimations of the protein, fat and carbohydrate contents. The energy content influences the formulation of energy – dense products like biscuits and energy bars (Liu *et al.,* 2018). Also, the digestibility of dry matter in flour affects how efficiently the body can extract and utilize its energy. Flours with higher digestibility ensure better absorption and utilization of nutrients (Liu *et al.,* 2018). There was significant ($p<0.05$) difference in the dry matter and energy contents of all the optimum composite blends.

CONCLUSION

The study evaluated the microbial load of high-fiber composite flours made from wheat, tiger nut fiber and cassava blends. The microbial load for the total fungi counts (TFC), total bacterial count (TBC) and total coliform count (TCC) were all within acceptable limits for day 0 to 144 days of storage. The microbial counts generated for 192 days showed that it was not within the acceptable limits when compared with the Kenya Bureau of Standards (KEBS). WTC3 (71.86 g wheat flour; 18.14 g tiger nut fiber; 10 g cassava flour) had better proximate

compositions than the rest of the samples comparing favourably with WTC4 (Control) for moisture, protein, fat, carbohydrates, dry matter and energy compositions but, performed better in crude fiber and ash contents. The tiger nut fiber supplementation was achieved with partial replacement up to 20% presenting an excellent option for formulating healthier food alternatives.

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