

Vitamin Content and Storage Studies of Cookies Produced from Wheat Almond and Carrot Flour Blends

ABSTRACT

The purpose of this study was to produce cookies from wheat, almond and carrot flour blend, evaluate the vitamin content and storage parameters. Wheat, almond and carrot flour were blended in the ratio: 100:0:0, 90:10:0, 90:0:10, 80:15:5, 70:20:10 and were labeled A, B, C, D and E respectively to produce cookies. The control sample A was without treatment. The cookies produced were analysed for vitamin content and were stored for 7 weeks at relative humidity corresponding to wet and dry season (70% and 30% respectively) within which there were analysed for pH, moisture and fungi content in an interval of every 2 weeks using standard methods. At the end of the storage, the sensory attributes vitamin content of the cookies was analysed. The vitamin content range respectively: from 341.53 to 653.27 µg/100g for vitamin A, 1.523 to 2.450 mg/g for vitamin B1, 0.65 to 0.92 mg/g for vitamin B2, 3.12 to 3.52 mg/g for vitamin B3 and 2.093 to 3.007 mg/g for vitamin C. All cookies samples were generally accepted by sensory panelist before storage and at the end of storage time. At the end of storage for wet and dry season conditions respectively, pH value ranged from 5.5 to 7.8 and from 5.5 to 5.7, moisture content ranged from 4.5 to 6.17 % and 1.33 to 1.63%, vitamin A ranged from 341.53 to 653.23 IU/100g and 336.61 to 653.01 IU/100g, vitamin C ranged from 2.093 to 3.007 mg/g and 2.11 to 3.01 mg/g. 1CFU of fungi was identified for each sample and all samples generally accepted by panelist at the end of the storage. The study provides evidence that wheat, almond and carrot are suitable for cookies production and variation of storage conditions did not cause spoilage of cookies.

Keywords: vitamin, relative humidity, sensory attributes.

1. INTRODUCTION

Bakery cookies are very popular, ready to eat, convenient, inexpensive and also important product in human diet and are usually eaten with tea and used as weaning food for infants. It is also used as a snack in school for the school going children who are often underweight. It may be used as a nutrient supplement during emergency situation [1]. Cookie is also known as an excellent vehicle for incorporation of different nutritionally rich ingredients, thus making it a useful tool in meeting the nutritional requirements of the increasing population.

Wheat ranks first among the cereals used for baking in Nigeria and is associated with growth and survival of the people of the country. More than 60% of the total daily requirements of protein and calories are met through wheat; wheat provides 360 kilo calories [2]. It contributes 68-75% of the total food intake in the daily diet and provides 75% of the total protein requirements; the proximate composition of wheat can be seen on Table. 2. It is a staple food, consumed worldwide in the form of bread and biscuits etc. [3]. The edible portion of almonds (*Prunus amygdalus*) is its nuts, which are commonly known as almonds or badam and it is a popular, nutritious food. Almond seeds contain approximately 51% lipid, 21% protein, 20% carbohydrate and 12% fiber. The majority of lipids are monounsaturated (~67%) and

polyunsaturated (~25%) fatty acids (MUFA and PUFA, respectively). Previous studies indicate that the MUFAs from almond seeds may reduce total cholesterol and low-density lipoproteins (LDL, “bad cholesterol”) while maintaining healthy high-density lipoproteins (HDL, “good cholesterol”) levels (6-15). Carrots are a root vegetable also known as *Daucus carota* is one of the top ten foods that food consumers identify as having “health benefits beyond basic nutrition”. This reputation comes with good reason, as carrots are rich in carotenoids which are used to make vitamin A during digestion, and vitamins B, C, D, and E. They’re also high in folic acid, fiber, and minerals like Potassium (K) and Sodium (Na). Sweet and succulent carrot are notably rich in anti-oxidants, vitamins and dietary fiber; however, they provide only 41 calories per 100 g, negligible amount of fat and no cholesterol. They are exceptionally rich source of carotenes and vitamin-A. 100 g fresh carrot contains 8285 mcg of beta-carotene and 16706 IU of vitamin A [4].

Adequate nutrition during infancy and early childhood is fundamental to the development of each child’s full human potential. It is well recognized that the period from birth to two years of age is a “critical window” for the promotion of optimal growth, health and behavioral development. Longitudinal studies have consistently shown that this is the peak age for growth faltering, deficiencies of certain micronutrients, and common childhood illnesses such as diarrhea [5]. Food fortification refers to the addition of micronutrients to processed foods. In many situations, this strategy can lead to relatively rapid improvements in the micronutrient status of a population, and at a very reasonable cost, especially if advantage can be taken of existing technology and local distribution networks. Since the benefits are potentially large, food fortification can be cost-effective in public health intervention.

2. MATERIAL AND METHODS

2.1. Procurement of raw materials

Carrots, almond seeds, margarine, sugar, eggs, baking powder and refined wheat flour were purchased from the modern market Makurdi, Benue State of Nigeria. Procured materials were taken to the Chemistry department, Benue state University laboratory for processing.

2.2. Preparation of flour

Almond seeds was sorted/cleaned, washed and then oven dried at 60 °C for 2 hours and then ground into flour using laboratory grinders (M/S Sujata: New Delhi India). The almond flour was sieved through a 0.5 mm size mesh. Carrots will be sorted and cleaned, washed, cut into smaller sizes of 1 cm cubes and then blanched at 70 °C for 5 minutes. The carrot cubes was dried at 50 °C for 12 hours and then milled into flour. The carrot flour was then sieved through a 0.5 mm size mesh.

2.3. Preparation of composite flour blends

Composite flour was prepared from refined wheat flour fortified with almond seeds flour and carrot flour at different levels (Table 1). Cookies were prepared from supplemented flour with a little modification of the method described in [6] is indicated in Table 2.

Table: 1. Composition of flour blends

Samples	Wheat: almond: carrot flour ratio
A	100:0:0
B	90:10:0
C	90:0:10
D	85:15:5
E	70:20:10

Table 2: Recipe for the formulation of cookies from wheat flour, almond seed and carrot flour blends.

Component	Cookies composition
Flour (g)	100
Butter (g)	45
Sugar (g)	45
Egg (mL)	30 ml
Baking powder (g)	2

2.4. Method

The creaming method was used for the preparation of dough where vegetable fat and sugar were creamed together using a Kenwood mixer (United Kingdom) at medium speed for two (2) min. After creaming, such ingredients as flour, baking powder and egg were added and mixed to form dough and properly mixed (Table 2). The dough was manually kneaded to ensure uniformity and then transferred to a clean tray and gently rolled using a roller, the dough sheath was cut into round shapes using a cutter. Shaped dough pieces were placed into a greased pan and baked in an oven at 180 °C for 40min. The baked cookies were placed on a cooling rack for 30 min to cool before packaging [6].

2.4.1. Vitamin analysis

a. Vitamin A

Vitamin A was determined by the calorimetric method of Kirk and [11]. Approximately 1 g of the sample and standard was mixed with 30 ml of absolute alcohol and 3 ml of 5% KOH solution is added to it and is boiled for 30 min under reflux. After washing with distilled water, vitamin A is extracted with 150ml of diethyl ether. The extract is evaporated to dryness at low temperature and then dissolved in 10 ml of isopropyl alcohol. Exactly 1 ml of standard vitamin A solution is prepared and that of the dissolved extract is transferred to separate cuvettes and their respective absorbance are read in a spectrophotometer at 325 nm with a reagent blank at zero.

$$\text{Conc. of vit A in sample} = \frac{\text{Abs of sample}}{\text{Abs of std}} \times \text{conc. of STD} \quad (12)$$

b. Determination of thiamine (VitaminB1)

The spectrophotometric method, described by [8] was used for determination of the B Vitamins. Exactly 5 g of each sample was homogenized with 50 ml of 1N ethanolic sodium hydroxide and the homogenate was filtered to obtain the filtrate to be used for the analysis. An aliquot (10 ml) of the filtrate was treated with equal volume of 0.1N K₂Cr₂O₇ solution in a flask. Standard thiamine solution was prepared and diluted to a chosen concentration (0.5). An aliquot of the standard thiamine solution was also treated with 10 ml of the dichromate solution (K₂Cr₂O₇) in a separate flask while a reagent blank was set up by treating 10 ml of the ethanolic sodium hydroxide with the potassium dichromate solution. The absorbance of the sample and the standard solutions was measured in a spectrophotometer at a wavelength of 360 nm with the reagent blank to be used to calibrate the instrument at zero. The thiamine content was calculated using the formula:

$$\text{Thiamine} \frac{\text{mg}}{100} = \frac{100}{W} \times \frac{Au}{As} \times \frac{C}{1} \times \frac{Vf}{Va} \times D \quad (13)$$

Where: W - Weight of sample analyzed, Au = Absorbance of sample, As = Absorbance of standard solution, C = Concentration (mg/ml) of standard solution, Vf= Total volume of filtrate, Va = Volume of filtrate analyzed, D = Dilution factor where applicable

c. Determination of riboflavin (Vitamin B2)

Approximately 1 g of sample was weighed into a conical flask and is dissolved with 100 ml of deionized water. This is shaken thoroughly and heated for 5 min and allowed to cool and then filtered. The filtrate is poured into cuvettes and their respective wavelengths for the vitamins set to read the absorbance using spectrophotometer.

Vitamin B1 = 261nm, Vitamin B2 = 242nm

$$\text{Vitamin conc. (mg/\%)} = A \times Df \times \text{Vol. of cuvette} \quad (14)$$

Where: A = Absorbance, E = Extinction co-efficient = 25 for B1 and B2, Df = Dilution factor

d. Determination of niacin (Vitamin B3)

A measured weight (5 g) of each sample was treated with 50 ml of 1N sulphuric acid (H₂SO₄ solution) and is shaken for 30 min. The mixture is treated further with 3 drops of aqueous ammonia and filtered. The filtrate (extract) is used for the analysis. Standard niacin (nicotinic acid) solution is prepared and diluted as desired. 10 ml portion of the standard solution, sample extract and 10 ml of the acid solution (treated with a drop of ammonia) is dispensed into separate flasks to serve as standard, the sample and reagent blank respectively. Each of them is treated with 5 ml of normal potassium cyanide solution and acidified with 5 ml of 0.02N H₂SO₄ solution; its absorbance is read in a spectrophotometer at a wavelength of 470 nm. The reagent blank is used to calibrate the instrument at zero. Niacin content was calculated using the formula;

$$\text{Niacine} \frac{\text{mg}}{100} = \frac{100}{W} \times \frac{A_u}{A_s} \times \frac{C}{1} \times \frac{V_f}{V_a} \times D \quad (15)$$

Where; W - Weight of sample analyzed, Au = Absorbance of sample, As= Absorbance of standard solution, C = Concentration (mg/ml) of standard solution, Vf = Total volume of filtrate, Va = Volume of filtrate analyzed, D = Dilution factor where applicable, C =Concentration of standard solution

e. Determination of ascorbic acid (Vitamin C)

The method described by [8] was used. Exactly 10g of the sample was extracted with 50ml EDTA/TCA (50g in 50ml of water) extracting Solution for 1 hour and filtered through a Whatman filter paper into a 50ml volumetric flask and made up to the mark with the extracting solution. Twenty (20ml) of the-extract is pipetted into a 250ml conical flask and 10ml of 30% KI is added and also 50ml of distilled water added. This was followed by 2 mL of 1% starch indicator. This is titrated against 0.01 mL CuSO₄ solution to a dark end point.

$$\text{Vit. C} \frac{\text{mg}}{100\text{g}} = 0.88 \times \frac{100}{5} \times \frac{V_f}{20} \times \frac{T}{1} \quad (16)$$

Where: Vf = Volume of extract, T = Sample titre – blank titre.

2.4.2. Sensory evaluation

Sensory evaluation of cookies was carried out using 30 panelist comprising MSc/PhD students and staff of the Food Science and Technology course, CEFTER BSU Makurdi. Cookies were made with same quantity as commercially sold cookies. Panelist were required to evaluate the aroma, appearance, taste, mouth feel and overall acceptability of the cookies using a 9-point Hedonic scale with 1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, 9 =like extremely [9].

2.4.3. Storage stability study

The cookies were packaged in a LDPE (Low Density Polyethylene) packaging material at different relative humidity (relative humidity corresponding to the dry and wet season. That is 75% and 30%) and stored at ambient temperature for 7 weeks. After an interval of 14 days, samples were analyzed for pH, moisture content, microbial growth (mold and yeast) and sensory evaluation. The samples were later analyzed for vitamin A, C. content at the end of the storage period. Moisture content of cookies samples was determined by standard method described by [10].

3.4.5 Microbial Analysis

Potato dextrose agar (PDA) was used for yeast and mold. Serial dilutions were made for each sample and 1 ml of the appropriate dilution poured, 10^{-5} and 10^{-6} serial dilution were used for pour plate in triplicate on selective media. Culture media will be incubated at 37°C temperature for 7 days. The molds growth from the samples will be purely cultured and isolated/identified. Developed colonies were expressed as colony forming units per gram (cfu/g) of sample [10]. Vitamin A was analysis by the method described by [11] while vitamin C was determined [10].

2.5. Statistical analysis

Statistical package for social science (SPSS) V21 computer software was used to analyze the data. Mean and standard deviation will be calculated where appropriate. Analysis of variance (one way ANOVA) was used to determine the treatment that were different from the others in the various parameters tested; differences was considered at 95% ($p < 0.05$) significant level and 99% ($p < 0.01$) significant level where mentioned.

3. RESULTS AND DISCUSSION

3.1. Vitamin content of cookies

Vitamins play vital functions in the body: they are needed for formation of hormones, cell growth, needed for proper functioning of the immune system, enhances clear vision etc. There was a constant increase in the vitamin content of all the cookie samples as presented in Table 3. This increase is as a result of increase fortification of almond and carrot flour for wheat flour which is higher in micronutrients than wheat flour. The significant increase in this provitamin A and thiamine (vitamin B_1) profile with increase in almond and carrot flour suggests that vitamin A precursors and vitamin B_1 are concentrated in almond and carrot flour than wheat flour. This is expectedly augmented by the yellow maize purposely used for its carotenoid content). The vitamin B_1 values obtained are much higher than the 0.130 to 0.423 mg/100 g reported by [12] for ready to eat snacks made from blends of breadfruit, cashew nut and coconut. They are also in excess of the recommended minimum daily intake of 0.2 to 1.0 mg [13]. The vitamin B_2 (0.65 to 0.92mg/100g) values where in range with the recommended daily intake of 0.3 to 1.6mg [93]. This equally took similar pattern as in Vitamins B_1 and B_2 , and as before will be attributed to the rich deposit of the B vitamins in almond and carrot. The values are much higher than the 0.157 to 0.477 mg/100 g reported by [12] for ready to eat snacks produced with African breadfruit, cashew nut and coconut flour blends. The B vitamins are needed for carbohydrate and protein metabolism, and are essential for growth, well structuring and functioning of the cells. There was significant ($p < 0.05$) difference in Vitamin C content of the samples with Sample E excelling with 3.01 mg/100 g while sample A was the least with 2.09mg/100g. The values compared lower with the value 4.77 mg/100 g reported by [15]. Vitamin C is a strong water soluble antioxidant that helps the body develop resistance against infectious agents and scavenges harmful free radicals. The vitamin A and C content of the cookie samples were not affected by storage conditions or storage time of 7 weeks; the values did not show any level of increase from the initial vitamin values Table 4. Vitamins are mostly aromatic shaped chemical compounds and degrade with time due to exposure to various environmental conditions e.g. vitamin A is susceptible to degradation

by sun rays, other harsh environmental conditions can lead to the degradation of these vitamins such as temperature, radiation exposure and spoilage.

Table 3: vitamin content of cookies

Sample	Vitamin A (µg /100g)	Vitamin B ₁ (mg/g)	Vitamin B ₂ (mg/g)	Vitamin B ₃ (mg/g)	Vitamin C (mg/g)
A	341.53±0.02 ^e	1.523±0.006 ^e	0.65±0.01 ^d	3.12±0.00 ^e	2.093±0.01 ^e
B	416.25±0.01 ^d	2.153±0.006 ^d	0.67±0.01 ^c	3.25±0.00 ^d	2.437±0.01 ^d
C	512.35±0.01 ^c	2.227±0.006 ^c	0.75±0.00 ^b	3.27±0.01 ^c	2.460±0.00 ^c
D	611.05±0.01 ^b	2.417±0.006 ^b	0.75±0.01 ^b	3.41±0.00 ^b	2.773±0.01 ^b
E	653.27±0.02 ^a	2.450±0.000 ^a	0.92±0.01 ^a	3.52±0.00 ^a	3.007±0.01 ^a

All data are means of 3 triplicates expressed on dry weight basis. Different superscripts between columns depict significant difference ($p \leq 0.05$).

Table 4: Increase level of Vitamin A and Vitamin C

Samples	Vitamin A (µg/100g)			Vitamin C (mg/100g)		
	Initial	(R.H 75%)	(R.H 30%)	Initial	(R.H 75%)	(R.H 30%)
A	341.53±0.02 ^e	337.00±0.03 ^e	336.61±0.02 ^e	2.093±0.01 ^e	2.10±0.02 ^e	2.11±0.01 ^e
B	416.25±0.01 ^d	418.03±0.12 ^d	418.23±0.12 ^d	2.437±0.01 ^d	2.39±0.05 ^d	2.40±0.05 ^d
C	512.35±0.01 ^c	511.23±0.09 ^c	510.96±0.01 ^c	2.460±0.00 ^c	2.50±0.23 ^c	2.51±0.06 ^c
D	611.05±0.01 ^b	610.02±0.07 ^b	611.87±0.03 ^b	2.773±0.01 ^b	2.77±0.01 ^b	2.66±0.12 ^b
E	653.27±0.02 ^a	652.01±0.13 ^a	653.01±0.01 ^a	3.007±0.01 ^a	3.00±0.21 ^a	3.01±0.17 ^a

All data are means of 3 triplicates expressed on dry weight basis. Different superscripts between columns depict significant difference ($p \leq 0.05$).

3.2. Sensory attributes of cookies

Results of the sensory attributes of cookies are presented on Table 5 below. The values showed significant difference for its appearance and general acceptability while they were no significant difference in the texture, taste and aroma of the samples. All cookie samples were generally accepted by panelist, Sample A had the highest value of 7.74 closely followed by sample B (7.37). Sample A again had the highest reading for appearance 7.89 and least in sample C (5.94). The statistical results reveal a positive score for all samples in the various parameters. These results shows that cookies can be produced from wheat, almond and carrot flour blends at all substitution levels of 90:10:0, 90:0:10, 80:15:5, 70:20:10 (wheat, almond and carrot flour respectively). The results also show that at continuous flour substitution levels, the sensory attributes start declining. [16] and [17] reported the same trend of results for the sensory attributes of mushroom enriched biscuits and cookies produced from Sweet Potato- Maize Flour Blends respectively. The results for the sensory attributes of cookies stored at 75% and 30% relative humidity are shown in Table 5 and Table 6. below

Table 5: Initial sensory attributes of cookies

Sample	Texture	Taste	Aroma	Appearance	General Acceptability
A	7.50±1.29 ^a	7.56±1.20 ^a	7.11±1.49 ^a	7.89±0.96 ^a	7.74±1.10 ^a
B	7.50±1.50 ^a	7.44±1.50 ^a	6.94±1.59 ^a	7.50±1.10 ^{ab}	7.37±1.34 ^a
C	6.61±1.69 ^a	6.72±1.74 ^a	6.33±1.28 ^a	5.94±1.73 ^d	6.95±1.58 ^{ab}
D	7.00±1.53 ^a	7.33±1.37 ^a	6.94±1.30 ^a	6.72±1.27 ^{bcd}	7.05±1.27 ^a

E	7.06±1.59 ^a	7.17±1.58 ^a	6.67±1.78 ^a	6.17±1.72 ^{cd}	7.11±1.59 ^{ab}
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Table 6: Sensory attributes of cookies stored at 75% relative humidity after 7weeks

Samples	Texture	Taste	Aroma	Appearance	General acceptability
A	7.6±1.42 ^a	7.3±1.34 ^a	7.3±1.68 ^a	7.8±1.16 ^a	7.6±1.11 ^a
B	7.7±1.18 ^a	7.2±1.66 ^{ab}	7.0±1.34 ^{ab}	7.4±1.28 ^b	7.3±1.00 ^b
C	7.1±1.70 ^b	6.5±1.91 ^c	6.5±1.28 ^b	5.4±1.74 ^{bc}	6.8±1.25 ^{ab}
D	7.4±1.42 ^{ab}	7.2±1.47 ^{ab}	7.3±1.10 ^a	6.8±1.60 ^{ab}	6.9±1.30 ^{ab}
E	7.3±1.18 ^b	7.0±1.67 ^b	7.0±1.5 ^{ab}	6.1±1.70 ^c	7.3±1.90 ^b

Table 7: Sensory attributes of cookies stored at 30% relative humidity after 7weeks

Samples	Texture	Taste	Aroma	Appearance	General acceptability
A	7.5±1.14 ^b	7.3±1.32 ^a	7.2±1.61 ^a	7.8±1.23 ^a	7.65±1.43 ^a
B	7.8±1.23 ^a	7.15±1.45 ^a	7.0±1.27 ^a	7.3±1.45 ^b	7.2±1.12 ^b
C	6.8±1.26 ^{ab}	6.5±1.28 ^b	6.3±1.19 ^b	5.9±1.11 ^{ab}	6.8±1.56 ^{ab}
D	7.2±1.70 ^b	7.0±1.90 ^a	7.0±1.34 ^a	6.9±1.23 ^b	7.0±1.23 ^{ab}
E	7.4±1.54 ^b	6.9±1.34 ^a	7.0±1.56 ^a	6.3±1.45 ^{ab}	7.4±1.54 ^a

All data are means of 3 triplicates. Different superscripts between columns depict significant difference ($p \leq 0.05$).

3.3 Variation of pH for cookies stored at 75% and 30% relative humidity respectively

Cookies were stored for a period of 7 weeks at two different conditions that is; wet season condition (75% relative humidity) and dry season relative humidity (30% relative humidity). The pH values of cookies stored at 75% and 30% relative humidity were almost at the pH value of distilled water to the basic direction during the first 5 weeks of storage (Table 8). The cookies were within the acceptable range. Usually, pH changes toward a high acidity or basicity depict spoilage by microorganisms (yeast and molds). The pH values of cookies decreased during the 7th week of storage towards the acid scale for cookies stored at wet season condition (7.8 to 5.5) and an acid scale value of 5.5 to 5.7 was recorded for cookies stored at dry season conditions. This difference in pH values can be attributed to the high percentage of moisture lost for cookies stored at 30% relative humidity (Table 8). Decreases in moisture content (water lost by vaporization) of food product increases the concentration of substances present in it the food product. [18]

Table 8: Variation of cookies pH during storage at 75% and 30% relative humidity

Samples	Dry season (PH at 75% and 30% R.H)							
	Week 1	Week 3	Week 5	Week 7	Week 1	Week 3	Week 5	Week 7
A	7.21±0.01 ^d	7.21±0.01 ^d	7.21±0.01 ^d	5.5±0.0 ^c	7.19±0.00 ^a	7.19±0.0 ^a	7.19±0.00 ^a	5.5±0.0 ^a
B	7.16±0.01 ^e	7.17±0.01 ^e	7.17±0.00 ^e	6.6±0.0 ^b	7.18±0.0 ^b	7.17±0.0 ^b	7.18±0.01 ^b	5.6±0.0 ^a
C	7.27±0.01 ^b	7.28±0.01 ^b	7.28±0.00 ^b	5.5±0.0 ^c	7.18±0.0 ^b	7.17±0.0 ^b	7.18±0.00 ^b	5.5±0.0 ^a
D	7.25±0.01 ^c	7.25±0.01 ^c	7.26±0.01 ^c	7.8±0.0 ^a	7.15±0.01 ^c	7.15±0.0 ^c	7.15±0.01 ^c	5.7±0.0 ^a

E	7.30±0.01 ^a	7.30±0.01 ^a	7.30±0.01 ^a	6.67±0.0 ^b	7.19±0.00 ^a	7.19±0.0 ^a	7.19±0.00 ^a	5.6±0.0 ^a
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All data are means of 3 replicates expressed on dry weight. Different superscripts between columns depict significant difference ($p \leq 0.05$).

3.4 Variation of moisture content for cookies stored at 75% and 30% relative humidity respectively

The results for cookies moisture content during storage are shown on table 9: within the first five weeks of storage, they was a slight increase in the moisture content of cookies stored at wet season condition with a minimum value of 9.11% and a maximum value of 11.07%, this values later dropped at the end of the 7th week of storage 4.45% to 6.4% in sample A and D respectively. At the end of the storage time, a moisture content of 1.33 to 1.63% was recorded in sample A and E respectively. This decrease in moisture content for samples stored at different conditions signifies that, the moisture content of cookies during storage can be affected by the environmental conditions of storage as well as the kind of packaging material used, this results shows that the cookies produced from wheat, almond and carrot flour blends where highly affected by humidity during the wet seasons (averagely 75% relative humidity) than dry seasons (averagely 30% relative humidity). Moisture (water) is very important for metabolic activities in living organisms. High moisture content foodstuff provides the relative environment for microbial activities. Nonetheless, the moisture content at the end of the 3th week slightly differ from the 9.05 to 10.25% initial moisture content of cookies produced from cassava, soybeans and mango flour blends reported by [19]. The values at the 5th week of storage where in range with a 12.59 moisture reported by [20]. On the other hand, a decrease in moisture content depict less activity of spoilage organisms in food samples and the greater chance of the food product lasting for a longer period of time. The results of 4.5% to 6.4% moisture content of cookies stored at 75% relative humidity were similar to 5.0 to 6.1% moisture content of cookies produced from sweet potato- maize flour blends [21]. 2.73 to 3.01% moisture content was reported by [22] for soya flour carrot pomace biscuits which were similar to the moisture content of cookies stored at 30% relative humidity at the end of the studies.

Table 9: Variation of moisture content for cookies stored at 75% and 30% relative humidity respectively

Samples	(moisture at 75% and 30% R.H)							
	Week 1	Week 3	Week 5	Week 7	Week 1	Week 3	Week 5	Week 7
A	6.42±0.43 ^c	7.29±0.63 ^b	11.07±0.11 ^a	4.50±1.50 ^c	6.42±0.43 ^c	6.51±0.50 ^c	9.28±0.83 _{bc}	1.33±0.58 ^b
B	7.43±0.45 ^{ab}	8.11±0.75 ^{ab}	10.98±0.06 ^a	6.33±0.58 _a	7.43±0.45 ^{ab}	7.67±0.29 ^{ab}	9.04±0.80 ^c	1.63±0.32 ^a
C	7.32±0.56 ^{ab}	7.71±0.63 ^{ab}	10.34±0.59 ^a	5.87±0.23 _b	7.32±0.56 ^{ab}	7.33±0.58 ^{ab} _c	11.94±0.8 _{2^a}	1.60±0.10 ^a
D	7.14±0.22 ^b	7.28±0.37 ^b	10.10±0.96 ^{ab}	6.40±1.00 _a	7.14±0.22 ^b	6.91±0.46 ^{bc}	10.45±0.5 _{0^b}	1.37±0.37 ^b
E	8.04±0.09 ^a	8.43±0.44 ^a	9.11±0.86 ^b	6.17±0.76	8.04±0.09 ^a	7.82±0.42 ^a	8.56±0.61 ^c	1.43±0.51 ^b

All data are means of 3 triplicates expressed on dry weight basis. Different superscripts between columns depict significant difference ($p \leq 0.05$).

3.5. Fungal coliforms identified cookies stored at 75% and 30% relative humidity

At the end of the storage period, 1cfu of fungi grew in each sample for both cookies stored at wet season condition and dry season condition (75 and 30 % R.H respectively). For sample A *Saccharomyces spp* was identified for cookies stored at both seasonal conditions. These are flat, smooth, moist, glistening

and creamy in colour, fungi of this group are good carbohydrate fermenters. They cause food spoilage of sugar rich foods such as cookies, syrup, juices etc. In sample B, C and D, *Asperigillus flavus* was identified. *A. flavus* are saprophytes that can be found in soil samples where they obtain their nutrition from dead and decaying matter. In particular, *A. flavus* are nuisance to farmers given that they tend to infect and contaminate crops (especially seeds) For sample E, *Asperigillus niger* was identified for cookies stored at wet and dry season conditions. *A. niger* largely exist as saprophytes, which means they obtain their nutrition from dead and decaying materials such as leaves, fruits, and other vegetables. As such they also contribute to the delay of various food products, given that their source of vegetation is virtually everywhere. The fungi species isolated and identified from the cookies can be attributed to infection of the raw plant products in trace amounts. These results were lower than the 9 to 10 cfu fungi species reported by [65] for biscuit incorporated with defatted soya flour and carrot pomace powder, these values were lower than the permissible level of 50 cfu reported by[23].

Table 10 Fungal coliforms identified cookies stored at 75% and 30% relative humidity

Samples	Fungi isolated at 70% humidity(CFU/g)				Fungi isolated at 30% humidity(CFU/g)			
	Week1	Week 3	Week 5	Week 7	Week 1	Week 3	Week 5	Week 7
A	–	–	1	1	–	–	1	1
B	–	–	1	1	–	–	1	1
C	–	–	1	1	–	–	1	1
D	–	–	1	1	–	–	1	1
E	–	–	1	1	–	–	1	1

All data are means of 3 triplicates expressed on dry weight basis. Different superscripts between columns depict significant difference ($p \leq 0.05$).

A = *Saccharomyces spp* ,B, C and D = *Asperigillus flavus* identified, E = *Asperigillus niger*
50 CFU = Permissible level by (Sindhu *et al.*, 2013).

4. CONCLUSION

The present results showed that, cookies can be produced from wheat, almond and carrot flour blends. Fortification of cookies with almond and carrot flour increased the vitamin content of cookies increasingly. All cookie samples were generally accepted by the sensory panelist before and after storage at all substitution levels. Cookies stored at 30% relative humidity had higher pH and lower moisture values than cookies stored at 75% relative humidity, the fungal coliforms identified in cookies were within permissible limits. Storage of cookies did not have any significant effect on the original vitamin A and C content of cookies. Variation of seasonal conditions (relative humidity) did not cause spoilage of cookies.

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UNDER PEER REVIEW