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Effects of Moisture Content and Storage Period on Proximate Composition, Microbial Counts and Total Carotenoids of Cassava Flour

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Abstract

The effects of moisture content and storage period on proximate composition, microbial counts and total carotenoids of cassava flour was investigated. Cassava cultivar TMS 3000 purchased from Kure Market in Minna, Nigeria was processed into high quality flour. The initial moisture content of the flour was 3.50%. The flour was divided into four samples; the first sample was used as control while the remaining three samples were labelled A, B and C and their moisture contents reconditioned to 5%, 7% and 9%, respectively. The samples were packaged into plastic containers and stored under the same condition of 25ºC and 60% RH. Proximate composition, microbial counts and total carotenoids of the flour samples were analysed weekly for three weeks. Results showed that moisture content and storage period had significant effects on the proximate composition, total plate counts for aerobic mesophilic bacteria and fungi, and total carotenoids of cassava flour ($p<0.05$).

Keywords: Moisture content, Proximate composition, Microbial count, Carotenoids, Cassava flour

1. Introduction

Cassava (*Manihot esculenta crantz*) belongs to the family *Euphorbiaceae* and is grown almost entirely within the tropics [1]. The cassava plant is a perennial shrub grown for its edible tubers. It accounts for over half of the root and tuber crops grown in African countries and is one of the major food crops produced in Nigeria, Brazil, Democratic Republic of Congo and Thailand. Despite its low protein content (1– 3%), cassava is however an excellent source of carbohydrate in the form of starch (80 – 90% of root parenchyma by dry weight).

Due to its high moisture content, cassava root is highly perishable with a postharvest life less than 72 hours. However, processing cassava root into food forms and raw materials such as flour, chips and pellets can extend the shelf life, facilitate trade and promote industrial use [2]. High quality cassava flour is white or creamy, unfermented and gluten-free flour obtained from cassava root and it is used in the food industry for the production of pasta and confectionery [3]. When wheat was substituted by up to 20% in bread, it was found that cassava flour added no foreign odour or taste to the product formed and no significant changes were observed in other bread characteristics [4].

During storage, fungal moulds may infest cassava flour and cause spoilage. The development of moulds leads to great modification in the chemical composition of infested stored produce. One of the most significant changes is an increase in free fatty acids (FFA) and reducing sugars as well as a loss in protein content. It was reported that all the food content of cassava flour diminished following infection [5]. Certain mould species have been isolated from cassava flour samples under different storage and marketing conditions [5]. The most important single factor encouraging mould contamination of cassava flour is an initial high moisture content or increase in moisture content during storage. It is on this note that this study was carried out to determine the effect of moisture content and storage period on storage stability of cassava flour.

The physicochemical properties of cassava flour offer the benefit of good functionality as raw material for the manufacturing of various food products. For instance, the high starch content of cassava flour contributes to crispy texture of processed products [6], while its low fat content is an excellent attribute for controlling rancidity and enhancing shelf-life stability of the product [7]. Flour and other materials used in manufacturing food products need to be packaged and stored properly prior to utilisation to ensure the quality, safety and storage stability. To realise the full potential of cassava flour in food processing, either alone or in combination with other raw materials such as wheat flour, knowledge of the effect of moisture content and storage period on proximate composition, microbial counts and total carotenoids of cassava flour is important.

2. Materials and Methods

2.1 Materials

Roots of cassava cultivar, TMS 3000 (white roots), were purchased from Kure Market, in Minna, Niger State, Nigeria.

2.2 Methods

2.2.1 Cassava flour processing, packaging and storage

Fresh cassava roots without breaks or cuts were sorted, and clean tap water was used to wash the sorted roots. The roots were peeled and re-washed with running tap water. The peeled roots were sliced into chips of about 2 – 3 cm in length using kitchen knife and oven-dried (85 ± 2 °C) for 24 hours [6]. Dried cassava chips obtained from the cultivar were packaged in sterile polyethylene bag of 0.4 mm thickness and transported under ambient conditions to the food processing laboratory of the Department of Agricultural and Bioresources Engineering, Federal University of Technology Minna, for further processing.

The chips were milled into flour with an appropriate milling machine. Baseline or initial (week 0) analyses of cassava flour quality attributes were carried out on the flour prior to packaging and storage. The cassava flour was packaged in 200 g each in four separate plastic containers under aseptic conditions. The duration of storage was 3 weeks and the content of each container was taken for analysis weekly. Storage was stopped after 3 weeks due to some logistics and also due to the fact that most of the quality attributes such as carotenoid had declined rapidly. All the analyses for proximate composition, carotenoids and microbial count on the cassava flour were performed in triplicates and results reported as mean ± standard deviation.

2.2.2 Determination of proximate composition, microbial count and total carotenoids

Moisture content was determined using the standard oven method of [8]. Initial moisture content of the flour was determined after which the sample was divided into four parts; one part was left as it was (the control) while the remaining three parts were reconditioned to desired moisture content levels of 5%, 7% and 9% and labelled as samples A, B and C respectively as described by [9]. Distilled water was added to the cassava flour samples as calculated from equation 1 to increase the moisture content of the flour according [9].

$$Q = \frac{A(b-a)}{100-b} \qquad (1)$$

Where: A = initial mass of the sample (g), a = initial moisture content of the sample, b = desired moisture content of sample, Q = mass of water to be added (kg).

Ash, crude protein, fat, crude fibre and carbohydrate contents of the cassava flour were determined according to the nutritional guidelines of [8] while dry matter content was determined using the method of [10]. Microbial analysis was done to determine the microbial stability of the cassava flour over time according to the method of [11] while total carotenoid content was determined using the method of [12]. The data obtained were analysed using SPSS 20.0 statistical packages; a one-way ANOVA was carried out to determine significant differences; and Duncan's multiple range test was used to separate means.

4 Results and Discussion

4.1 Results

The effects of moisture content on the proximate composition of cassava flour are presented in Table 1 while the results for the microbial counts of cassava flour are shown in Figures 1 and 2.

Table 1: Changes in proximate composition of cassava flour at different moisture contents

Sample	Duration (weeks)	Moisture (%)	Ash (%)	Fat (%)	Crude fibre (%)	Crude protein (%)	CHO (%)	Dry matter (%)
Control	0	3.50 ± 0.12^{d}	2.42 ± 0.09^{b}	3.81 ± 0.70^{a}	1.92 ± 0.06^{a}	11.16 ± 0.05^{c}	70.62 ± 0.89^{a}	89.92 ± 0.12^{a}
	1	4.72 ± 0.61^{a}	1.00 ± 0.00^{a}	3.24 ± 0.26^{c}	1.99 ± 0.01^{a}	10.76 ± 0.44^{a}	79.40 ± 1.83^{c}	95.28 ± 0.61^{c}
	2	6.31 ± 0.51^{a}	3.17 ± 0.33^{b}	2.22 ± 0.28^{b}	2.00 ± 0.00^{a}	5.64 ± 0.64^{a}	80.66 ± 0.54^{b}	93.69 ± 0.51^{c}
	3	5.69 ± 0.31^{d}	2.99 ± 0.01^{a}	2.28 ± 0.00^{b}	1.99 ± 0.01^{a}	3.42 ± 0.08^{a}	83.63 ± 0.41^{b}	94.31 ± 0.31^{a}
A	0	4.91 ± 0.10^{a}	2.36 ± 0.15^{b}	3.86 ± 0.65^{a}	2.31 ± 0.33^{ab}	10.05 ± 0.64^{a}	76.53 ± 1.85^{c}	95.09 ± 0.10^{d}
	1	5.84 ± 0.44^{b}	2.44 ± 0.06^{b}	1.10 ± 0.10^{a}	2.34 ± 0.34^{ab}	10.35 ± 0.51^{a}	77.93 ± 0.37^{c}	94.16 ± 0.44^{b}
	2	9.86 ± 0.15^{c}	2.67 ± 0.17^{a}	1.41 ± 0.08^{a}	1.85 ± 0.16^{a}	6.62 ± 0.50^{b}	77.60 ± 0.44^{a}	90.14 ± 0.15^{b}
	3	3.66 ± 0.46^{b}	4.11 ± 0.90^{b}	1.61 ± 0.33^{a}	4.83 ± 0.50^{b}	4.16 ± 0.66^{b}	81.64 ± 0.05^{a}	96.34 ± 0.46^{c}
B	0	7.61 ± 0.61^{b}	2.82 ± 0.19^{d}	3.16 ± 0.35^{a}	2.46 ± 0.22^{b}	10.27 ± 0.06^{ab}	73.69 ± 0.20^{b}	92.39 ± 0.61^{c}
	1	6.72 ± 0.48^{b}	2.42 ± 0.08^{b}	2.50 ± 0.50^{b}	2.53 ± 0.22^{b}	10.50 ± 0.00^{a}	75.33 ± 1.28^{b}	93.28 ± 0.48^{b}
	2	7.04 ± 0.24^{b}	2.72 ± 0.22^{ab}	2.00 ± 0.01^{b}	1.78 ± 0.77^{a}	5.50 ± 0.50^{a}	80.01 ± 2.39^{ab}	92.96 ± 0.24^{b}
	3	2.90 ± 0.10^{a}	4.86 ± 0.14^{b}	1.66 ± 0.33^{a}	1.99 ± 0.01^{a}	3.39 ± 0.11^{a}	85.20 ± 0.49^{c}	97.10 ± 0.10^{d}
C	0	9.03 ± 0.03^{c}	1.97 ± 0.03^{a}	3.99 ± 0.51^{a}	1.97 ± 0.03^{a}	10.92 ± 0.29^{bc}	72.14 ± 0.77^{ab}	90.97 ± 0.03^{b}
	1	8.67 ± 0.34^{c}	2.31 ± 0.20^{b}	3.22 ± 0.28^{c}	3.00 ± 0.00^{c}	10.36 ± 0.14^{a}	72.45 ± 0.95^{a}	91.33 ± 0.34^{a}
	2	7.66 ± 0.46^{b}	3.75 ± 0.25^{c}	2.00 ± 0.00^{b}	1.44 ± 0.11^{a}	7.38 ± 0.03^{b}	77.78 ± 0.34^{a}	92.34 ± 0.46^{a}
	3	5.09 ± 0.11^{c}	4.89 ± 0.73^{b}	1.66 ± 0.34^{a}	1.62 ± 0.38^{a}	3.17 ± 0.02^{a}	83.24 ± 0.29^{b}	94.91 ± 0.11^{b}

The values are given as means of triplicate determinations ± standard deviation. Means with the same letters in columns are not significantly different ($p < 0.05$).

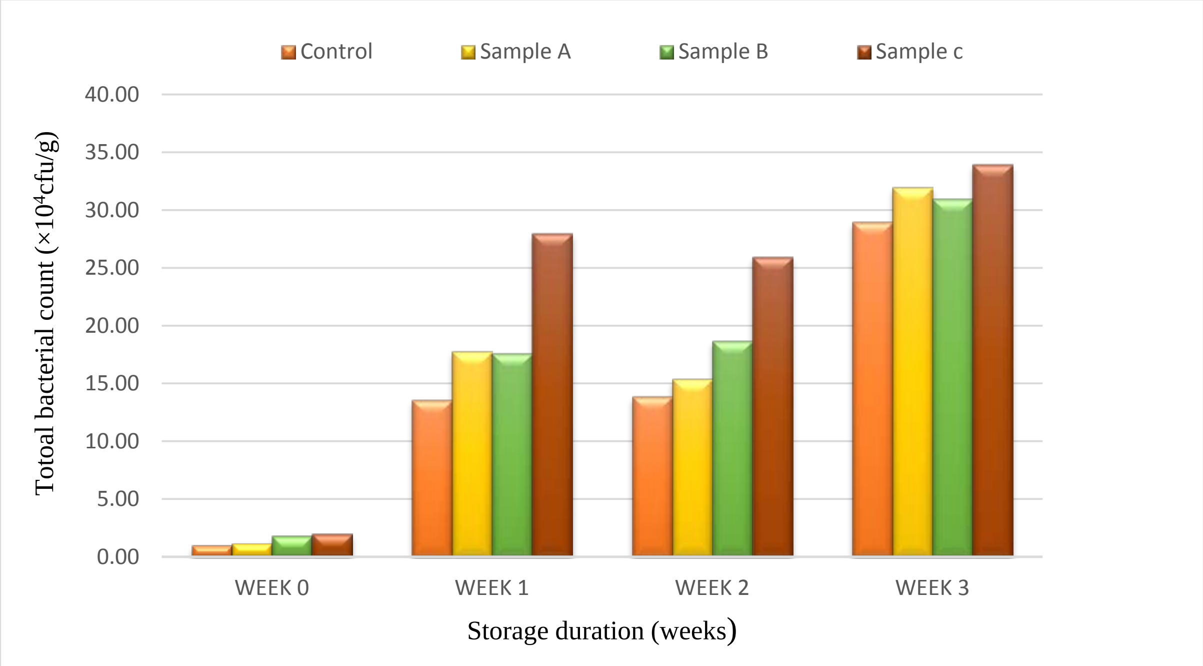

Fig. 1: Effect of storage period on the total viable bacterial count of cassava flour

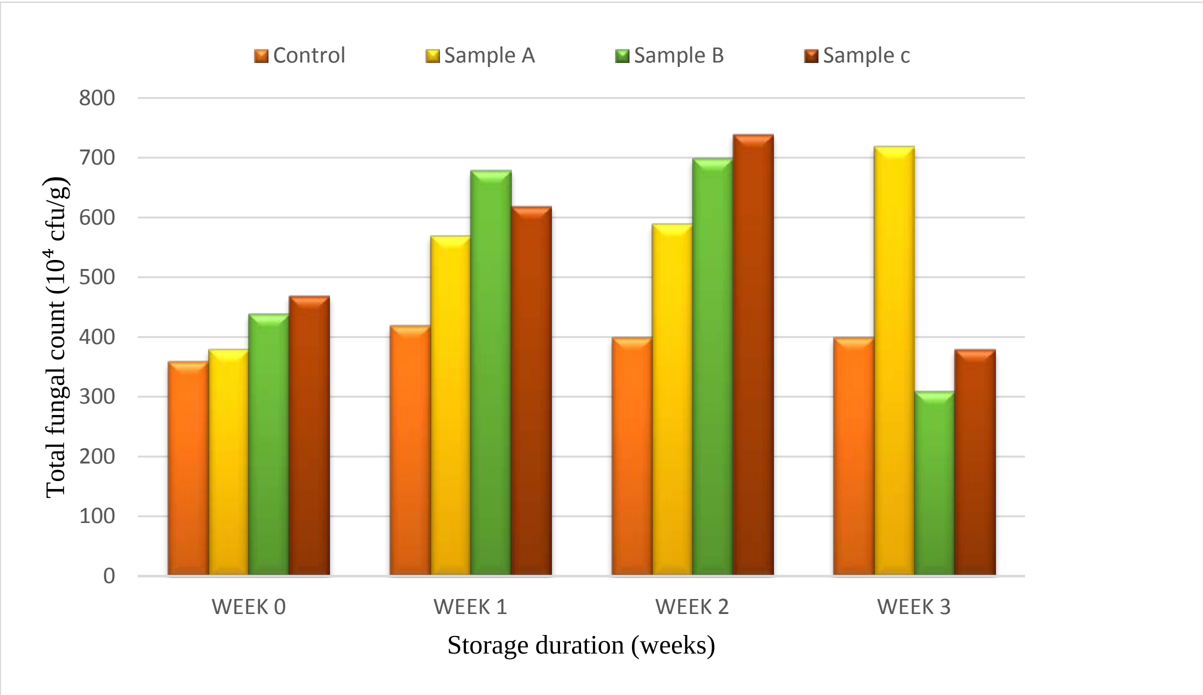

Fig. 2: Effect of storage period on the total viable fungal counts of cassava flour

The results for the total carotenoids content of cassava flour samples during storage are presented in Figure 3.

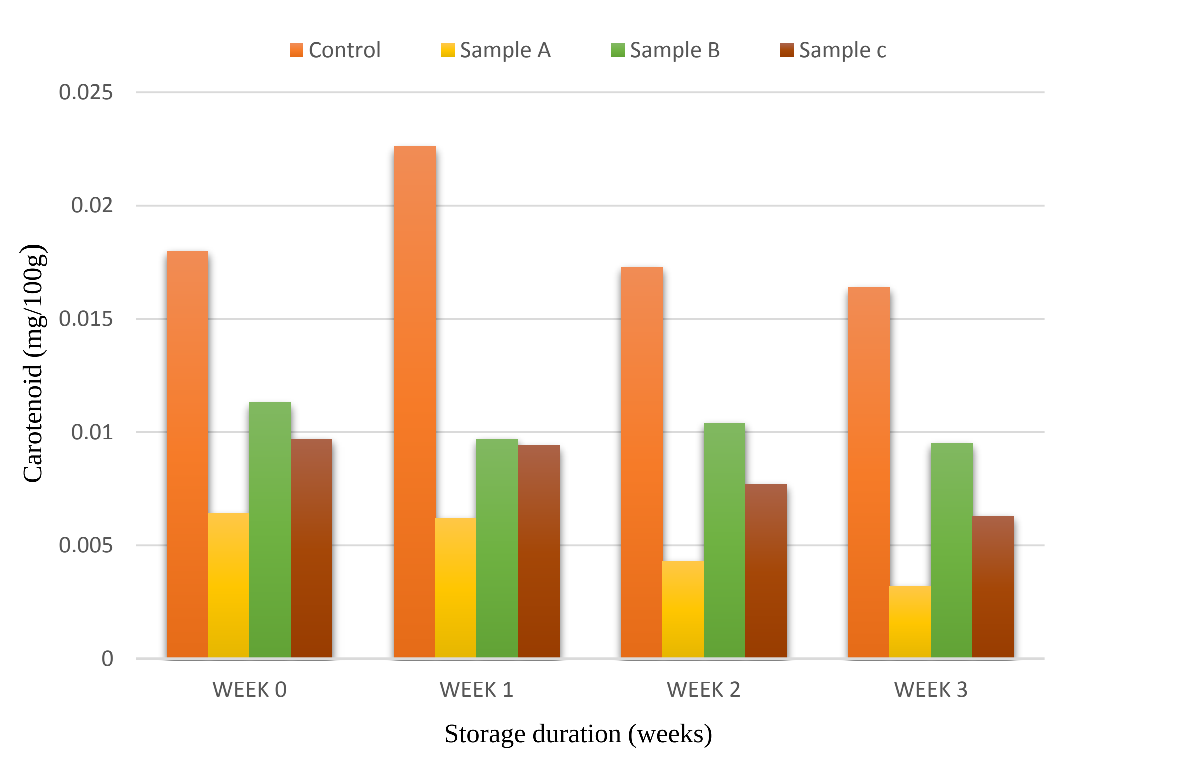

Fig. 3: Changes in the total carotenoids of cassava flour during storage

4.2 Discussion of results

4.2.1Proximate composition of cassava flour

Moisture content and storage duration had significant effect on the proximate composition of cassava flour ($p < 0.05$). Progressive increase in moisture content was observed in all the four samples during the first 2 weeks of storage and sudden decrease was observed in the third week. This could be as a result of variation of the storage temperature and relative humidity. It was observed that the moisture content of cassava flour (control) increased from 3.50 ± 0.12% to 5.69 ± 0.31% while that of samples A, B and C decreased from 4.91 ± 0.10% to 3.66 ± 0.46%, 7.61 ± 0.61% to 2.90 ± 0.10% and 9.03 ± 0.03% to 5.09 ± 0.11%, respectively after three weeks of storage. The increase in moisture content observed in the control sample could be as a result of increase in the relative humidity of the environment during the storage period. It has been reported that dry produce will exchange moisture with its storage environment until equilibrium is reached [5]. Results from the current study are in the range of the values reported for 31 different cassava flour cultivars (3.21 to 11.75%) by [13].

The moisture content range of the current study does not correspond to the recommended moisture range (12.0 – 13.0) for flour stability. Higher moisture content in the range (9.2 – 11.4%) has been reported by [10] in South Africa and [14] also reported higher moisture content (10.38 –

11.58) in Lagos. The moisture content of any produce will depend on factors such as location, season and processing method. A Lower moisture content in flour is a good indication of microbial stability and may also contribute to reducing the tendency of staling in baked food products [15]. Furthermore, the high moisture noticed in flour packed in plastic bucket after 12 weeks storage period would be related to its low water vapour transmission rate as well as the higher microbial activities [10].

A significant increase in ash content of the flour was observed in all the four samples across the storage durations. The increase in ash content was in the order: control (2.99 ± 0.01) < sample A (4.11 ± 0.90) < sample B (4.86 ± 0.14) < sample C (4.89 ± 0.73) at the end of three weeks storage. However the effect of moisture content was not significant in ash content for samples A, B and C. The observed results are in line with a similar study where an increase in cassava flour supplementation in bread making resulted in a corresponding increase in ash content [16].

Protein and fat contents consistently decreased during the storage period. The percentage decrease in protein (dry basis) was between 3.42 ± 0.08 – 11.16 ± 0.05 for the control sample, 10.05 ± 0.64 – 4.16 ± 0.66 for sample A, 10.27 ± 0.06 – 3.39 ± 0.11 for sample B and 10.92 ± 0.29 – 3.17 ± 0.02 for sample C. The results observed in this study could be as a result of effect of moisture and microbes in the package [10]. Similarly fat content decreased as the storage time increased. The decrease observed in the fat content of cassava flour was as a result of possible high proteolytic and lipolytic activities of the corresponding enzymes which in turn led to the loss in the nutrients [17]. Similar findings have been reported on garri [5]. Previous research on the storage of other flour products also reported decrease in protein and fat contents [14].

Cassava flour showed significantly high carbohydrate and dry matter contents in all the samples during storage. The increase in carbohydrate content could be as a result of the variation in the other parameters (protein, fat and ash) during the storage period. The highest carbohydrate (CHO) content was observed in flour sample B (85.20 ± 0.49%) at 25 °C, 60% RH storage condition. The lowest CHO content of cassava flour was observed in cassava flour sample A (81.64 ± 0.05). This was in the range reported for percentage CHO of cassava flour in Nigeria (84.1 ± 0.06-85.4 ± 0.07%) [18]. The dry matter content of cassava flour ranged from 89.92 ± 0.12 – 94.31 ± 0.31 for the control sample, 95.09 ± 0.10 – 96.34 ± 0.46 for sample A, 92.39 ± 0.61 – 97.10 ± 0.10 for sample B and 90.97 ± 0.03 – 94.91 ± 0.11 for sample C.

A significant increase was observed in the dry matter content of cassava flour for all the four samples at the end of three weeks storage period. This could be attributed to the decrease in moisture content of cassava flour during storage. However, cassava flour sample B stored at an initial moisture content of 7.61 ± 0.61 maintained the highest dry matter content of flour (92.39 ± 0.61 - 97.10 ± 0.10) throughout the three weeks storage period. This corresponds with the literature on the effect of packaging material and storage condition on functional properties and quality attributes of cassava flour [10]. The author suggested that cassava flour stored at high moisture content gave a corresponding decrease in percentage CHO and vice-versa. Therefore, cassava flour stored at a moisture content of 7.61 ± 0.61 in plastic container at 25 °C and 60% RH storage condition best maintained CHO, dry matter content and moisture content stability. This is an indication of longer shelf life and higher storage stability of cassava flour.

4.2.2 Microbial count of cassava flour

It was observed that microbial count of cassava flour samples stored at different moisture contents at ambient conditions (25 °C and 60% RH) was influenced by moisture content. Results from this study show a progressive increase in the total plate count for aerobic mesophilic bacterial and fungi in all the four samples of cassava flour. This could be due to the increase in moisture content during the period of storage. The total viable bacterial count increased from 1 to 29.0 × 10^4, 32.0 × 10^4, 31.0 × 10^4cfu/g and 34.0 × 10^4cfu/g while total viable fungal count increased from about 1 to 400 × 10^4, 720 × 10^4, 310 × 10^4cfu/g and 380 × 10^4cfu/g in cassava flour control sample, sample A, sample B, and sample C, respectively. The steady and gradual increase recorded in the total viable bacterial and fungal counts in all the samples stored at different initial moisture contents suggest a favourable micro environmental conditions and nutrient availability [11]. This is attributed to the permeability of the packaging materials to atmospheric gases such as oxygen, carbon dioxide and water vapour.

The agents that contaminate and spoil stored cassava flour are fungi, bacteria, insects and mite [11]. The storage quality of cassava flour therefore depends on the rate of reproduction and growth of these organisms which in turn depends on some biological and non-biological variables. The most important of these variables are temperature and moisture content. The survival of any of these organisms in any stored product depends on whether the intensity or level of these two variables is conducive. The microbial count of cassava flour at the end of three weeks storage period were still within the limits and in agreement with the reports of [11]. In addition, it was observed that the fungal count in cassava flour after three weeks storage period was higher than the bacterial count. This could be as a result of the ability of fungi to withstand harsh environmental condition such as low moisture content and eventually low water activity. These results are in agreement with previous reports of [10] which showed that the microbial load of cassava flour stored in plastic container for a period of 12 weeks was influenced by moisture content, water activity and pH. The results of the current study show that cassava flour sample B stored at an initial moisture content of 7.61 ± 0.61 exhibited higher microbial stability with a lower bacterial count of 31 × 10^4cfu/g compared to samples A and C and the lowest fungal count of 310 × 10^4cfu/g compared to the other samples at the end of three weeks storage period. The results therefore indicate that cassava flour stored in plastic container at 7.61 ± 0.61% moisture and 25 °C would best maintain microbial stability while maintaining other quality attributes of such as dry matter content and carbohydrate.

4.2.3 Total carotenoids content

Carotenoids content of cassava flour decreased with the storage duration in all the four samples. Moisture content and storage duration had significant effect on total carotenoids content of cassava flour ($p<0.05$). Degradation of carotenoid content during processing and storage is one major challenge associated with maintaining the health benefits of high carotenoid products [19]. Reduction in total carotenoid content observed from this study was experienced in two ways. First was during processing of flour which includes grating/chipping, oven-drying, and milling. These processing methods have been reported to be major contributing factors exacerbating carotenoid degradation in cassava flour [10]. The effect of exposure to heat during flour processing has been described as a catalyst for oxidation which leads to carotenoid degradation [20]. The authors also observed a continuous decrease (6.07 ± 0.01 – 0.16 ± 0.09) in carotenoid concentration during storage of cassava flour and recording a percentage loss of about 86% on the 19th day of storage. These investigations agree with the carotenoid reduction trend observed from this study and therefore justify that carotenoid retention is a function of the length of storage such that the longer the storage period the higher the percentage loss.

5 Conclusions

It can therefore be concluded that moisture content is an important factor to be considered in establishing the shelf life stability of cassava flour. Moisture content had significant effect on the carbohydrate and dry matter contents of the cassava flour studied. The moisture content of cassava flour also had significant effect on the microbial quality and carotenoid content of cassava flour during storage. Lower moisture content in flour corresponds to lower microbial growth and hence longer shelf-life stability and better quality attributes. Low moisture content also resulted in higher dry matter content, which is a good attribute for the food industries and cassava flour processing.

Cassava flour sample B stored in plastic container at an initial moisture content of 7.61 ± 0.61% provided the lowest moisture content of 2.90 ± 0.10% and the maximum stability of microbial quality of cassava flour during the three weeks storage period. Cassava flour sample B showed the highest CHO content of 85.20 ± 0.49% and the highest dry matter contents of 97.10 ± 0.10% at the end of three weeks storage period. Cassava flour control sample stored at an initial moisture content of 3.50 ± 0.12% had the lowest percentage loss in carotenoid content with about 9% followed by flour sample B with about 16%, flour sample C with about 35% and flour sample A had the highest percentage loss in carotenoid of about 50%.

However, based on the overall quality and microbial safety, a moisture content of 7.61 ± 0.61% would be recommended for long term storage of cassava flour in terms of quality attributes, shelf life and microbial stability all of which are essential to food industries.

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