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INFLUENCE OF ULTRASOUND PARAMETERS ON SOME QUALITIES OF FROZEN AFRICAN SPINACH

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Abstract: *Consumption of fresh and healthy vegetables in our diets aids protection from chronic diseases in humans. This study determined the effects of ultrasound as a pre-treatment technique on vitamin C, beta-carotene contents, and the weight loss of frozen African spinach (Amaranthus hybridus), and thereafter compared with the control. All the samples except the control were subjected to the ultrasound effects prior to freezing at frequency, time, and power ranges of 40-120 kHz, 10-30 min, and 50-150 W respectively. Result obtained indicated that the maximum vitamin C (99.5%) was obtained from the sample stored for 5 days under the treatment condition of 40 kHz, 50 W, and 20 min. However, the same sample retained more vitamin C within 5-20 days' storage, while the pre-treated sample at 80 kHz, 100 W, and 10 min retained the maximum (80.9%) at 25 days of storage. The sample under the treatment condition of 80 kHz, 100 W, and 20 min lost more vitamin C (67.6, 66.6, and 59.2%) between 15-25 storage days. While for the beta-carotene, the sample with the treatment condition of 40 kHz, 50 W, and 20 min before freezing had the highest retention (99.8%) when stored for 5 days, and the same sample retained more at 10, and 15 days than others (97.6, and 92.4%). All pre-treated samples retained more beta-carotene at 25 days of storage when compared to the control with the least retention between 15-25 days. Furthermore, the least obtained content from the pre-treated samples was 64.9%, that is, a sample treated at 80 kHz, 100 W, and 20 min. The result revealed that Amaranthus hybridus was best preserved in pre-treated Amaranthus hybridus leaves. The optimum quality response occurs at 80kHz, and 50 W for 30 min. This gives a vitamin C content of 31.1 mg/100g and a Beta-carotene content of 10.5 mg/g at 0.694 desirability.*

Key words: *Ultrasound pretreatment, Freezing, Food quality, African spinach.*

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1. Introduction

African spinach (*Amaranthus hybridus* L.) is one of the common leafy vegetables that lose their quality very rapidly after harvesting. It contains vitamin A, vitamin C, micronutrients, and minerals [6, 14, 16, 20]. Indigenous African vegetables (IAV) are excellent sources of minerals, fiber, bioactive compounds, beta-carotene, and iron. In addition, leafy greens are gaining significant attention as an inexpensive and readily available source of micronutrients for low-income rural communities [2, 19]. Green leafy vegetables are an important part of the diet in most tropical and temperate countries [7, 11, 13]. It has diversified the monotonous diet of many consumers with its natural flavors that provide alternative suitable tastes and aromas. Moreover, leafy greens have long been a source of income for the peasant farmers in Nigeria and other African countries. However, they have been used throughout history to this day as food and their extracts as medicine [15]. African spinach has been considered an important source of micro- and macronutrients in many households due to its nutritional quality, especially for those with increasing malnutrition [4, 8, 13]. Furthermore, African leafy vegetables have gained great attention as cheap, and readily available sources of micronutrients for low-income rural communities [19]. These plants are generally protected and can be eaten as soft vegetables. Meanwhile, the leaves are regularly harvested manually by hand. The inappropriate use of leafy greens as raw materials in an industry leads to the underutilization of the many embedded nutritional contents. Ndukwe et al. [13] and Kiremire et al. [8], recently stated that, due to the growing awareness of its nutritional and health benefits, IAV has gained commercial importance and is now manufactured through both formal and informal marketing channels. One of the challenges in IAV production, marketing, and consumption is its perishability and no adequate facility to keep it fresh [9]. Most storage techniques require low temperatures which is not readily available in all communities. Remotely manufactured African vegetables with minimal handling, can be stabilized and transported to urban markets. However, poor handling can affect the color, texture, taste, and nutritional value of the vegetable [3, 10, 17]. There is, therefore, the need for adequate post-harvest handling in order to ensure the extended shelf-life of the vegetable. However, proper measures must be put in place in preserving green leafy vegetables so that they can be made available all year round. African spinach leaves have a large surface area, require a large amount of water in their soft tissue, and have vigorous metabolism, so they tend to lose moisture during harvesting, transportation, and sale, and lose freshness. Improper storage can also lead to quality deterioration, which can result in a significant loss in nutritional quality. In addition, the leaves sold in the market are commonly stored in refrigerators and ordinary shelves, and the rate of spoilage varies depending on the temperature, so spinach leaves can be stored longer at lower temperatures [5, 7, 12]. *Amaranthus hybridus* (L.) is a seasonal leafy vegetable that is usually available in abundance during the rainy season.

However, the supply is always in short supply during the dry season and the cost is higher during this time. Therefore, to ensure its availability throughout the year, proper preservation and storage practices must be used to prevent off-season shortages.

Literature has revealed the retention capability of the previously applied methods of storage which include; sun-drying, solar drying, freezing, etc. Sun-drying happened to be the commonest method used, particularly in the local environment with poor reported retention record (10-20%), meanwhile, solar-drying also retained less than 50%. Kiremire et al. [8], in his research, compared the effects of sun drying, oven drying, and solar drying on vitamin C, and the β-Carotene content in amaranthus hybridus. The reported highest vitamin C was obtained from the oven-dried sample, while the maximum quality of β-Carotene was retained in the solar-dried sample. Nyambaka et al. [18] also made findings on the quality of β-Carotene using solar drying to obtain improved results as compared to those reported in previous studies, though there were no specified storage times by any one of the two authors. In a study by Wulansarie et al. [21], it was revealed that fast freezing retained more vitamin C than slow freezing though with about a 10% difference. According to Adepoju et al. [1], there was a magnificent loss in vitamin C with boiling but retained good content of β-Carotene. In the same study, there was poor retention of vitamin C through shade drying with a 50% retention of β-Carotene. However, in recent years, frozen vegetables are reported to retain more nutritional content than others. The limitation in the application of purely freezing operation is the lack of inactivation of microorganisms in foods. It is therefore of utmost importance to design a pre-treatment system that can get rid of enzymatic activities before freezing. In addition, freezing has good retention but varied with storage time. However, the limitation of freezing is that, it can only reduce the enzymatic activities but cannot inactivate them. The design of ultrasound of such attributes for the pre-treatment of vegetables has not been reported. Also, there has not been a reported study on the use of ultrasound-assisted freezing for the preservation of Amaranthus hybridus. This research aimed to proffer a solution to the post-harvest losses of leafy vegetables in order to aid their extended shelf life. The objectives of this research are to; design an ultrasound device, and then determine the effects of the pre-treatment-assisted technology on weight loss, and the nutritional qualities (vitamin C, and ß-carotene) of amaranthus hybridus at storage.

2. Materials and Methods

2.1. General Information

A 300 g amount of matured green African spinach was bought from the commercial market at Tanke, Ilorin Kwara state, Nigeria, and transported to the Central Research Lab and Diagnostic Center. The ultrasound system comprises a high-intensity ultrasound generator (HIUG) with a maximal input power of 150 W and operating frequency of 40kHz, the machine's frame (stand) is made of angle irons and a stainless-steel plate as well as three (3) transducers (40kHz, 50W each). The fresh spinach leaves were of the same maturity level and had the same physical, and chemical characteristics. The measuring apparatus used was a weighing balance and thermometer. There were also sample containers (rubber mesh), and a freezer.

2.2. Sample Preparation

The fresh *Amaranthus hybridus* (L.) leaves were obtained from a local market in Ilorin, Nigeria. The fresh leafy vegetable samples were de-stalked, and sorted while the damaged, withered, and yellowish leaves were discarded. The leaves were washed with clean tap water to remove external contaminants and allowed moisture evaporation from the leaves at room temperature before pre-treatment with ultrasound. 10 g per sample was taken for 25 samples grouped into five (5) as A1, A2, B1, B2, and C where each group contains five samples (replicates). However, the samples in their groups were contained in rubber mesh containers labeled with the group names. Four of the five groups had undergone pre-treatment with ultrasound under variable parameters of time (minutes), frequency (kHz), and power (W) at a constant temperature of 60°C as shown in Table 1, while group C was the control (non-pretreated samples). Haven determined the initial mass and temperature of the samples (pretreated and non-pretreated), they were frozen at a freezing temperature of -13. The samples were freeze-stored at intervals of 5 days for 25 days.

Samples to be pre-treated schedule with ultrasound variable parameters Table 1

Spinach leaves (samples)	Sub-samples	Frequency [kHz]	Power [W]	Treatment time [min]
A	A_1	40	50	10
	A_2	80	100	10
B	B_1	40	50	20
	B_2	80	100	20

2.3. Description of the Ultrasound Device

The ultrasound device (Figure 1) is of high-power intensity (HPI) type that consists mainly of the ultrasound generator (USG) of power, and a frequency of 150 W, and 40 kHz respectively, three (3) transducers (50 W, 40 kHz each), a treatment chamber (3269 cm^3), and the frame. The control buttons which are meant for temperature and time settings, and regulations. To operate the US system, it needs to be connected to an electrical source, then powered through a designated power button, as the machine begins to operate, the ultrasound generator transfers the electrical energy to the transducers which convert it to ultrasonic wave-inducing vibration in the treatment chamber which eventually generates heat energy, the intensity of the heat depends on frequency, power, and length (time) of operation.

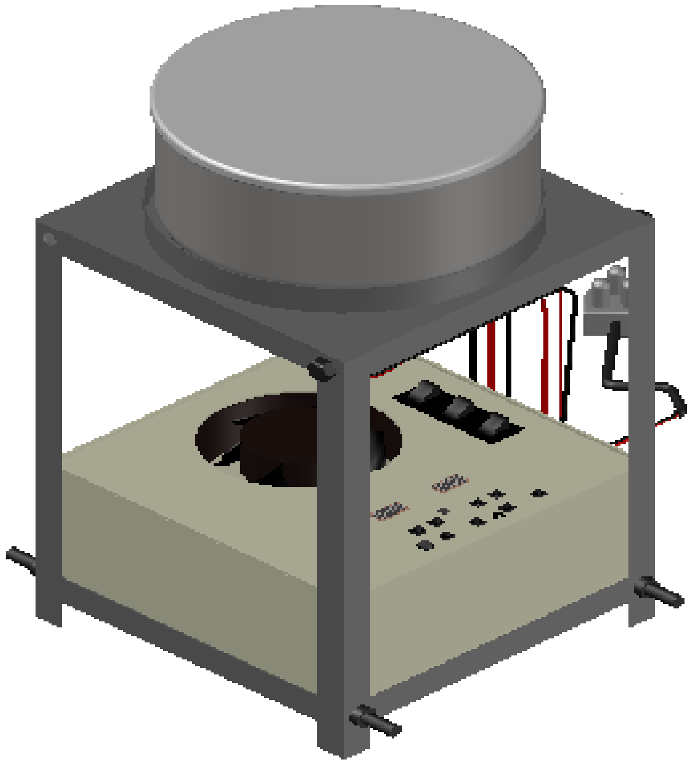

Fig. 1. *Ultrasound system*

2.4. Determination of the Quality Parameter

The quality and shelf life of the spinach was determined as a function of their physical, and biochemical properties. Weight loss, Vitamin C, and β-Carotene were determined from each frozen sample.

The content of vitamin C in each sample of the vegetable (African Spinach) was determined by the extraction method according to Owusu et al. [20]. A 0.1 g of the sample was taken into a 15ml test tube. This was extracted with 1 ml of 4% trichloroacetic acid (TCA). This was stirred with a vortex mixer and allowed to stay for 15 minutes. The component was centrifuged at 2000 rpm for 5 minutes. 500 microliters of Vitamin C color reagent (Dichlorophenolindophenol) was added to 250 microliter of the supernatant. The orange color that developed was measured at 700 nm. The blank was prepared the same way as the sample, but TCA was used in place of sample supernatant. The standard was prepared by using ascorbic acid at various concentrations. The vitamin C content in each sample was calculated from the standard curve prepared using the standard.

The ß-carotene was determined according to the methods outlined by Owusu et al. [20] with slight modification. 0.1 g of the samples were weighed into test tubes. 10ml of a mixture of hexane:

acetone [6:4 (v/v)] was used for the extraction. The mixture was mixed and allowed to extract for 10 minutes after which it was centrifuged for 3minutes at 2000 rpm. Thereafter, the absorbance was measured spectrophotometrically at 505, 453 and 663 nm respectively. The solvent of extraction was used as the blank. The ß-carotene content in the sample was calculated empirically.

2.5. Statistical Analysis

The statistical analysis of the obtained data (physical and biochemical) was performed with Microsoft Excel 2016 to obtain the inferential and quantitative values of the samples before and after treatment. All data were statistically analyzed at a significance of $P < 0.05$. However, a post-ANOVA comparison of the mean test was performed with the Tukey test to determine the pairs of freezing time which were statistically significant.

3. Results and Discussions

3.1. General Results

The effects of pretreatment characteristics and storage on the weight differences in the samples were shown in Figure 2. There were significant differences in the weight of samples with respect to storage time. However, the sample with the least pretreatment characteristic has more weight loss than others. While the minimum weight loss was observed from the control, this may be attributed to the limited mass, and energy transfer in the cells of the sample unlike the treated sample with the improved mass transfer which allowed the incorporation of water.

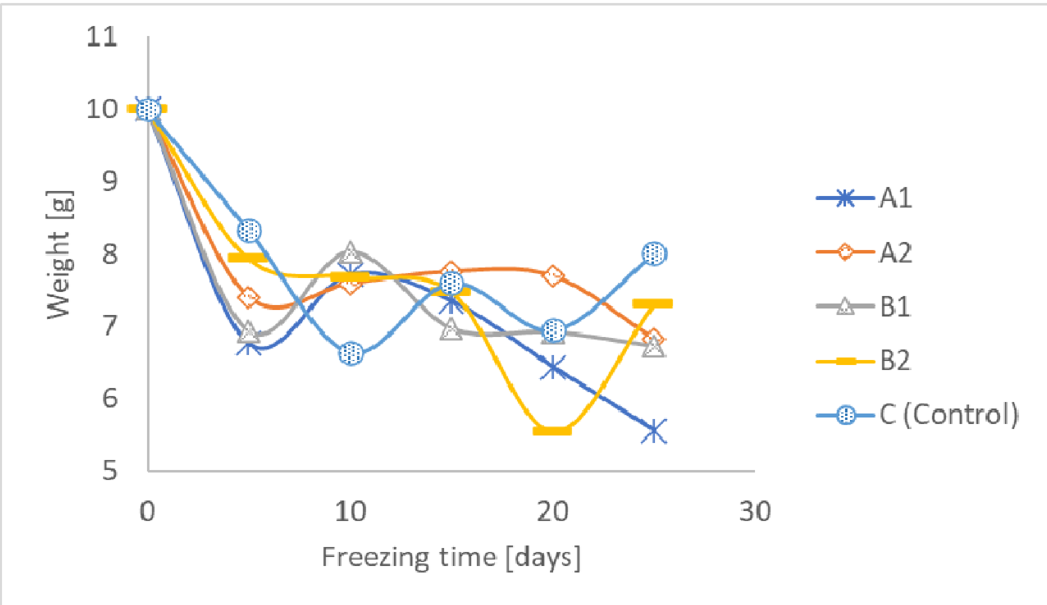

Fig. 2. *Weight differences in frozen spinach*

Temperature changes in the frozen *Amaranthus hybridus* (L.) as shown in Figure 3, the pre-treatment has an impact on the freezing characteristics of the vegetable. The cooling rate of the ultrasound-assisted frozen samples increased when compared with the control, however, the samples with more freezing quality were those that have undergone the ultrasound effect.

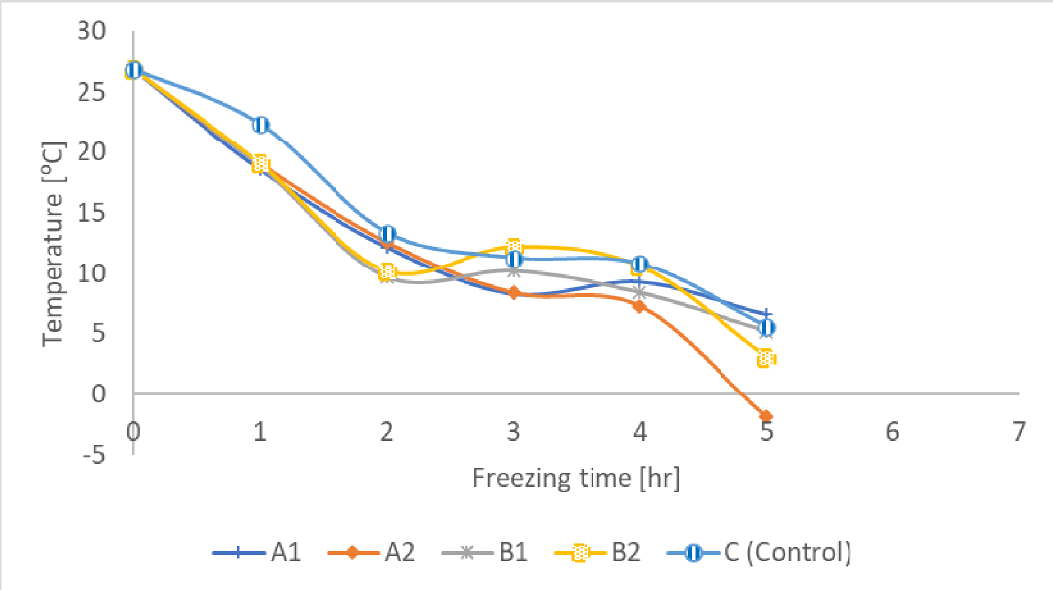

Fig. 3. *Freezing curve of frozen spinach*

3.2. Effects of Ultrasound-Assisted Freezing on the Vitamin C of *Amaranthus hybridus*

From the result obtained, all samples showed significant losses in vitamin C throughout the period of storage (Figure 4). However, samples with the treatment condition 40 kHz, 50 W, and 20 min retained more vitamin C than others at 5-, 10-, 15-, and 20-days' storage (99.5, 96.7, 84, and 83.5%), while the sample with the treatment characteristic of 80 kHz, 100 W, and 10 min retained the maximum after 25 days (80.9%). More so, samples under the pretreatment condition of 80 kHz, 100 W, and 20 min experienced more losses at 15, 20, and 25 days respectively, that is, 32.4, 33.4, and 40.9%. This can be because of induced heat in the sample during the pretreatment. Furthermore, samples under the pre-freezing treatment conditions of 80 kHz, 100 W, and 10 min, 40 kHz, 50 W, and 20 min, and 40 kHz, 50 W, and 10 min have more vitamin C quality than the control after 25 days of storage, and the percentage retentions were 80.9, 68.9, and 70.1% respectively. This implies that the pretreatment enhanced the samples with better retention of vitamin C compared to the control. Meanwhile, the sample with pretreatment characteristics is 40 kHz. 50 W and 10 min have less vitamin C losses between 15-25 days than others as shown in Figure 3.

The least vitamin C (59.2%) was obtained from samples under the treatment condition of 80 kHz, 100 W, and 20 min stored for 25 days Furthermore, the combined statistical mean ± standard error values of vitamin C for fresh *Amaranthus hybridus* (L.) was (51.58195 ± 0.12295) while the obtained values from the frozen groups were; A1 (37.31148 ± 0.49916), A2 (43.10657 ± 0.44451), B1 (44.67213 ± 2.81774), B2 (39.36884 ± 3.81696), and C (42.27048 ± 0.69689).

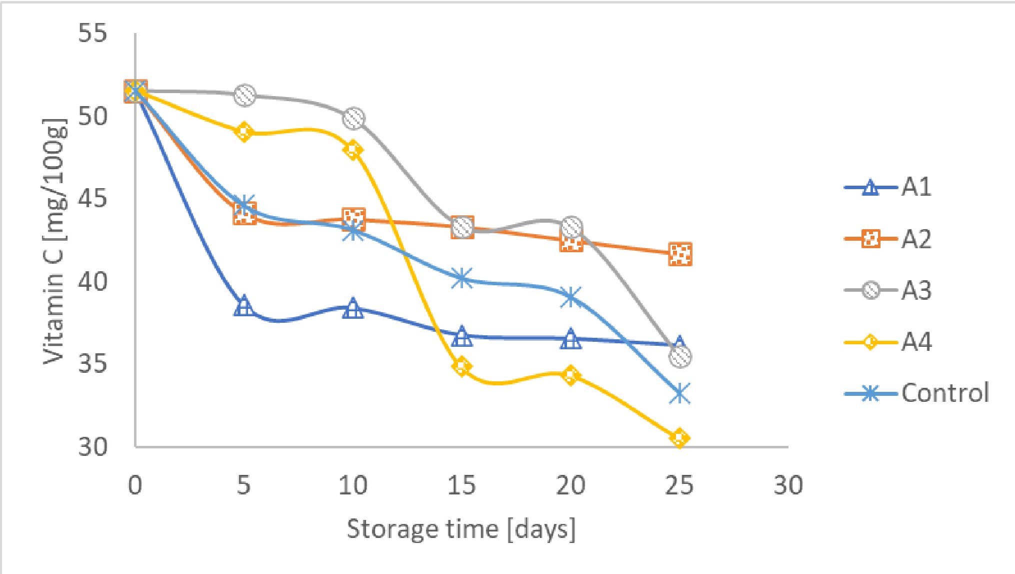

Fig. 4. *Content of vitamin C in frozen spinach*

This result corroborates the findings of Wulansarie et al. [21], but with improved quality, when tomatoes retained 87.84% content of vitamin C for quick freezing, and 77.77% under slow freezing. In another study by Adepoju et al. [1] the vitamin C content of fresh *Amaranthus hybridus* (L.) was 54.34 ± 0.95 which is in the same range as obtained in this research (51.58195 ± 0.12295). He however further stated that 8.25 ± 0.78, 12.40 ± 1.40, and 20.20 ± 0.69 were the mean and standard error of vitamin C contents obtained from different processing methods such as boiling, blanching, and shade drying. However, the percentage loss of vitamin C for the three reported methods of processing was huge when compared with the preservation and storage measures put in place in the present study. The percentage losses of vitamin C in samples processed by boiling, blanching, and shade drying were 84.8, 77.2, and 62.8% [1]. But in this study, the percentage losses in vitamin C content of the samples depend on the pre-treatment parameters and the storage time respectively. Generally, the reduction in vitamin C level of *Amaranthus hybridus* (L.) was less in most ultrasound-assisted frozen samples as compared with the previous methods of preservations. It is obvious that ultrasound-assisted freezing is a process with improved vitamin C retention ability.

3.3. Effects of Ultrasound-Assisted Freezing on of ß-Carotene *Amaranthus hybridus*

Ultrasound assisted-technology has a huge influence on the ß-carotene of frozen African spinach (*Amaranthus hybridus* L.). In this research, the result obtained indicated that the Beta-carotene of samples decreases significantly with increasing length of storage. Furthermore, the pre-treated sample under the ultrasonic condition of 40 kHz, 50 W, and 20 min has the maximum retention of Beta-carotene 99.8% when stored for 5 days, also, the treated sample at 40 kHz, 50 W, and 10 min retained more at 10 and 15 days' storage as 97.6, and 92.4%. However, the sample under the treatment condition of 40 kHz, 50 W, and 20 min has the maximum retention at 20, and 25 days' storage time. Meanwhile, the least content for 15, 20, and 25 days was obtained from the control as 67.6, 67.6, and 63.3% respectively (Figure 5).

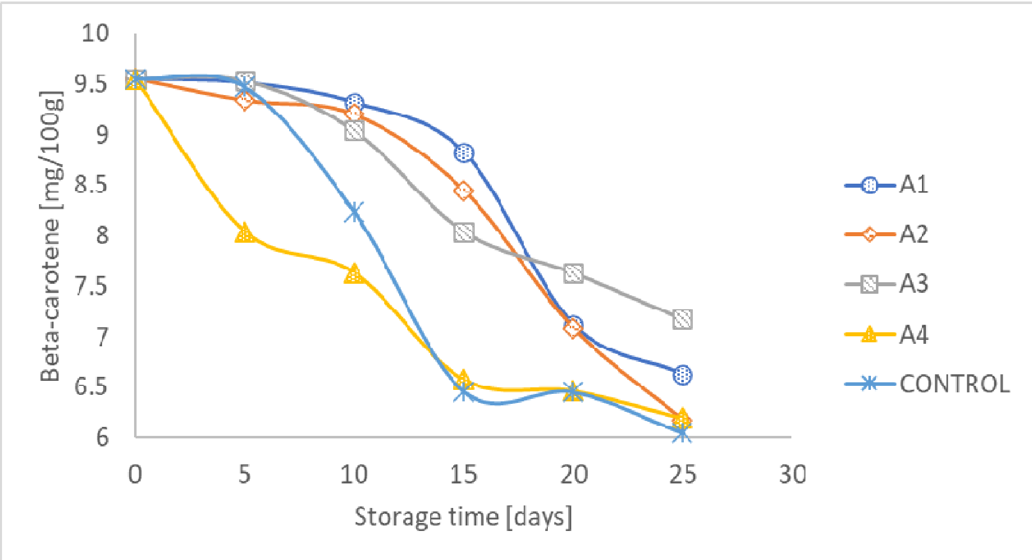

Fig. 5. *Content of ß-carotene in stored spinach*

Generally, beta-carotene losses in the pre-treated samples were minimized as compared to the losses in the control. The beta-carotene retention of this study is better than the reported study by Adepoju et al. [1], where 75 and 50% were the retention content for boiling and shade-drying. He stated that 0.04 ± 0.00 was the mean and standard error content of beta-carotene for fresh *Amaranthus hybridus* (L.) while 0.03 ± 0.00, 0.04 ± 0.00, and 0.02 ± 0.00 were obtained from boiling, blanching, and shade drying respectively. This implies that there were 25, and 50% losses of beta-carotene in *Amaranthus hybridus* (L.) leaves boiled, and shade dried while no loss in beta-carotene content for blanching. Also, in the solar-drying method applied by Nyambaka et al. [18], 68.07% was the reported beta-carotene retained from *Amaranthus hybridus* (L.), which also ranges with the result from the current study, though depending on the storage period.

Traditional sun-drying and oven-drying techniques caused a huge loss of beta-carotene with just 8.3, and 21.6% retention during storage.

4. Conclusions

Prolonged ultrasonic treatment of amaranth will change the color of the vegetables to pale green but with a complete reduction in moisture content in a short time. Reducing moisture levels leads to weight loss depending on the duration of treatment. There was, however, a high retention of vitamin C, and beta-carotene in the ultrasound-assisted frozen samples compared with the controls. The optimum quality response was obtained at 80 kHz, 50 W, and 30 min, which gives a mass of 6.5g, 31.1 mg/100g content of vitamin C, and a Beta-carotene content of 10.5 mg/g respectively, at 0.694 desirability.

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