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Effects of pre-storage treatments (using brine and vinegar) and refrigerated storage on vitamin c, β-carotene, microbial load and shelf-life of red pepper (*Capsicum frutescens*)

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ABSTRACT

This study was carried out to determine the effects of pre-storage treatments using antimicrobial agents such as vinegar, brine and refrigeration (4°C) on concentrations of vitamin C, β-carotene, microbial load and shelf-life of red pepper (Capsicum frutescens). The freshly harvested red pepper (C. frutescens) samples were immersed in different concentrations of brine (10% and 15%) and vinegar (0.8% and 1.5%) for about 20 minutes, air dried and kept at room temperature (28°C). Samples without treatments were also stored at room temperature (control) and refrigerated conditions for quality and shelf life studies. The results showed that the concentrations of vitamin C and β-carotene of the fresh untreated red pepper samples decreased considerably within the storage period of 39 days. Concentrations of vitamin C and β-carotene in samples treated with 0.8% vinegar (VI), 1.5% vinegar (V2), 10% brine (B1) and 15% brine (B2) also decreased considerably within the storage period of 42 days. However, refrigerated samples (R) showed a decreased in vitamin C concentration but an increase in β-carotene concentration within the storage period of within 48 days. The various pre-storage treatments caused significant decreases ($P<0.05$) in the microbial load (total coliforms and total plate counts) on day 0 (immediately after treatment) but increased gradually within the storage period except refrigeration which kept the microbial load stable within the storage period. It can be concluded from the results that essential nutrients (Vitamin C and β-carotene) were favourably maintained in the refrigerated conditions as compared to the other pre-storage treatments.

Keywords: *Capsicum frutescens*, vitamin C, β-carotene, coliforms, total plate counts

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INTRODUCTION

Vegetables are referred to as group of crops which are eaten as complements to staple foods [1]. These vegetables contain various kinds of dietary nutrients necessary for human health. For example, 90% of the world's total amount of vitamin C comes from vegetables and in the developing countries retinol provided by vegetables accounts for 80% of the retinol intake [2]. Also the colours and flavours obtained from vegetables meet the taste of different categories of people. Vegetables are also good sources of active substances and fibre, which play an active part in improving human health, increasing human immunity and promoting metabolism [3]. Some vegetables are used in

traditional medicine as curative and protective means of health [3]. Increase vegetable production may improve food security on one hand, and on the other hand offer income opportunities to small-scale farmers and especially to women who still lag behind with regards to development benefits.

It is required that vegetables are preserved fresh and made available throughout the year to fulfil the human dietary requirements. As living entities, their metabolic activities continue even after harvest with a sum total effect on their degrading quality and composition. During storage, the produce deteriorates in quality due to physiological activities such as respiration and loss of moisture. They are susceptible to microbial spoilage leading to changes in their structure, texture, colour and appearance. The type and intensity of the post harvest physiological activity and the kind of vegetable determine to a large extent, the storage life of the produce. The postharvest losses of vegetables in the developing countries lie between 20% and 50% and between 5% and 25% in the developed countries [4]. The high perishability of vegetables, lack of storage facilities, mechanical injuries due to improper handling, packaging, transportation, and microbial infections are the major causes of post harvest losses in vegetables [5]. In order to meet today's health conscious consumers' demand for fresh natural food; concerted efforts are being made to develop methods for preserving vegetables [6].

From the quality standpoint, it is desirable to preserve the characteristics of fresh vegetables at their peak. What the consumer perceives as the most appealing attributes of these products include their fresh-like appearance, taste and flavour, in addition to convenience [7]. Among the limitations to quality and shelf life of fresh vegetables are; microbial spoilage, desiccation, discolouration, mechanical injuries and development of off-flavour or off-odour [8]. Red peppers (*Capsicum frutescens*) belong to the family *Solanaceae*. They are native of tropical South America, where pre-historic peppers are known from burial sites in Peru. They spread through the New World tropics in pre-Columbian times [9]. They were introduced into West Africa by the Portuguese in the 13th century [10]. Red pepper is eaten as a raw and cooked vegetable and also used commonly in making paste, pickle and sauce. Red ground pepper made by drying and pulverizing is used as a spice and flavour ingredient in the food industry [11]. It is one of the most widely used food colorants for culinary and industrial purposes. Because of its high colouring capacity and in some cases its peculiar pungency, red pepper is used to modify the colour and flavour of soups, stews, sausage, cheese, snacks, salad dressing, sauces, pizza and confectionary products, among others. Peppers are also used as condiments, and as ingredients in medicine for both internal and external use [12].

It is an excellent source of vitamin C, vitamin B6, phytochemicals such as lycopene and β-carotene (the precursor for vitamin A), folate, potassium and plenty of fiber [13]. The antioxidant substances such as β-carotene (provitamin A) and vitamin C contained in pepper confer protection against carcinogenic components and delay the aging process [14].

In Ghana, fresh peppers are used in almost all Ghanaian dishes; however, the shelf life of these peppers is very short, ranging from a period of days to a week where firmness and flavour are lost. Some of the practices that are used to extend its shelf-life include blanching and drying which tend to result in the huge loss of the essential vitamins in the pepper. There is therefore the need to explore other means of preserving the pepper for the dry or lean season while maintaining its nutritive value. The objective of this study is to determine the effect of pre-storage treatments with vinegar, brine and refrigeration on the vitamin C, β-carotene concentrations, microbial quality and shelf-life of red pepper.

MATERIALS AND METHODS

Fresh peppers were obtained from the University farm, School of Agriculture, University of Cape Coast. The peppers were sorted out to remove the anthracnose affected, over matured and damaged ones. Care was taken to select only fresh produce with no indications of chilling stress for the study. The samples were sent immediately to the Chemistry laboratory of the Department of Chemistry, University of Cape Coast for treatment and analysis.

About 200 g of samples were weighed and immersed into 0.8% and 1.5% white vinegar solutions and 10% and 15% brine solutions for 20 minutes. The samples were removed and placed in well labelled perforated baskets and kept at room temperature. 200 g of the sample were also washed and placed in perforated baskets and kept in the refrigerator at 4^{o}C while another batch was kept as control (untreated) at room temperature. Vitamin C and β-

carotene concentrations, total coliforms and total plate counts were determined every three days interval for the stored samples.

Vitamin C and β-carotene concentrations were determined by spectrophotometric methods described by Agbemafle *et al.*, 2012 [15]. Microbial load was determined by the APHA (American Public Health Association) (1992) [16] standard analysis methods.

The data obtained were analyzed using Analysis of Variance (ANOVA) from SPSS 16.0 statistical tool. Fisher's least significant difference (LSD) test was used to identify significant differences among treatment means ($P<0.05$).

RESULTS

The results of the experiment showing the changes in Vitamin C, β-carotene, Total coliforms and Total plate counts of the treated and stored red pepper samples are shown in Figures 1 - 8.

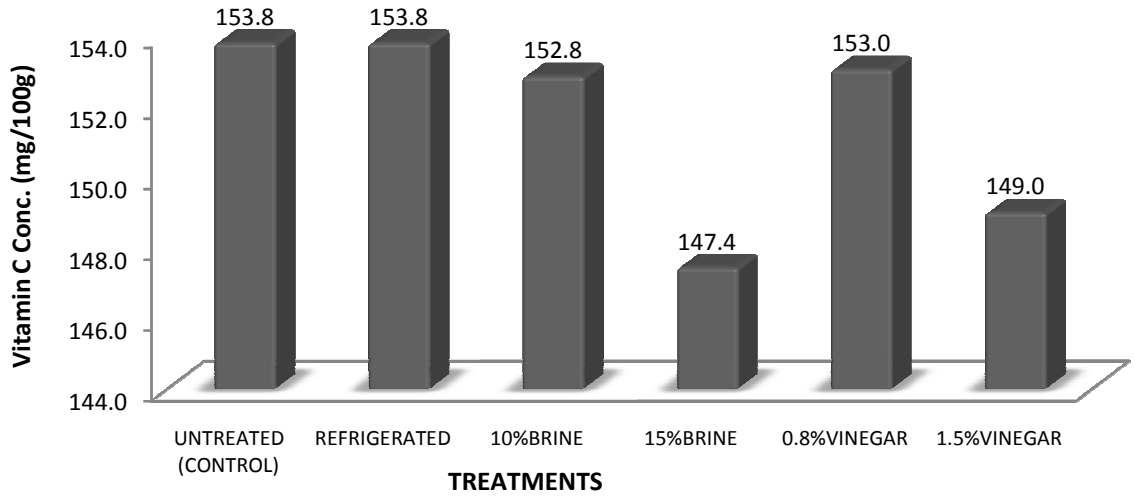

Figure 1: Variations in the amount of vitamin C at Day-0 as affected by the different pre-storage treatments and refrigeration.

Samples treated with 15% brine recorded the least amount of vitamin C, followed by those treated with 1.5% vinegar on day 0.

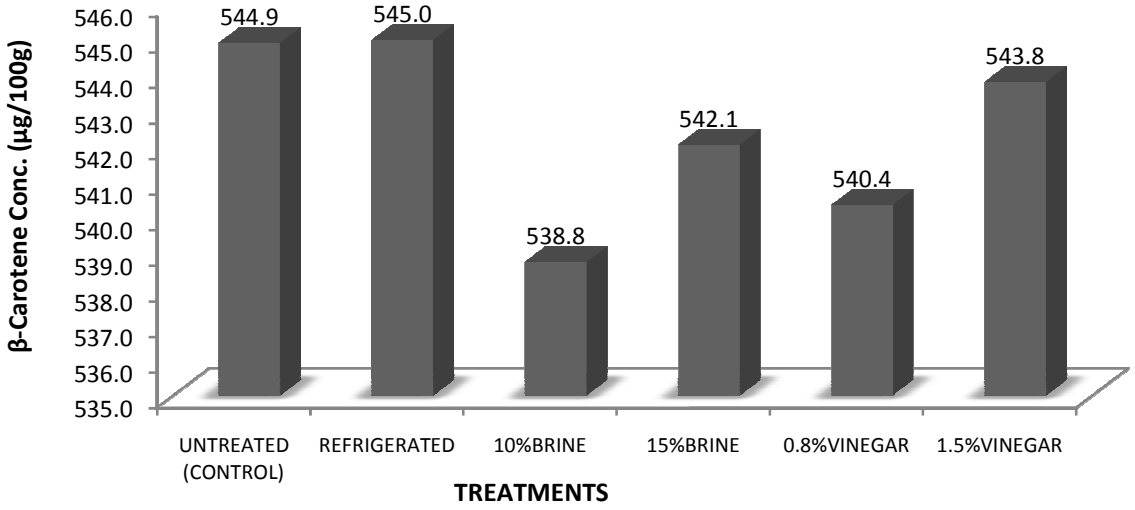

Figure 2: Variations in the amount of β-Carotene at Day-0 as affected by the different pre-storage treatments and refrigeration.

Samples treated with 10% brine recorded the least amount of β-carotene, followed by those treated with 0.8% vinegar on day 0.

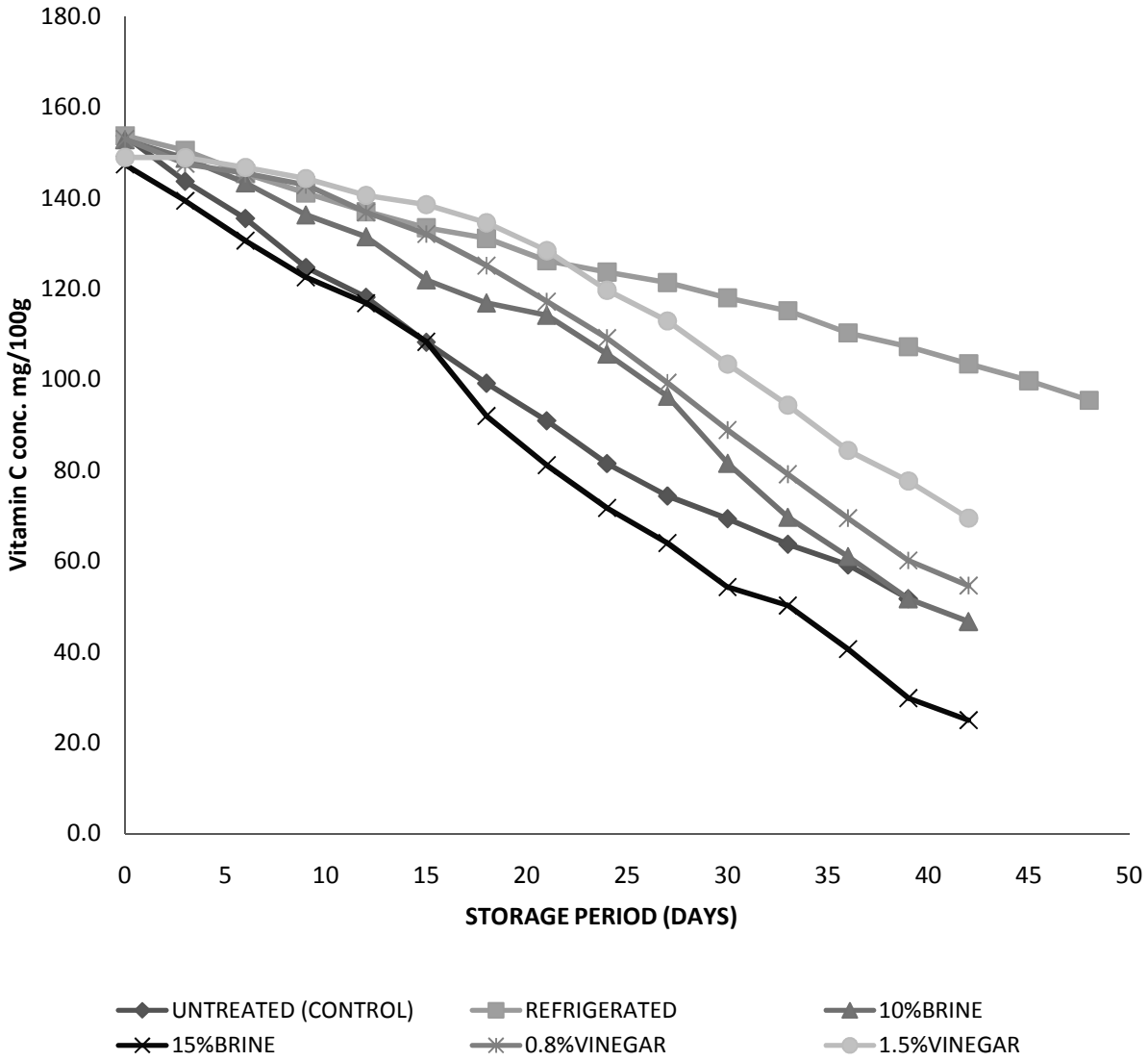

Figure 3: Variations in Vitamin C content of C. frutescens with time of storage as affected by different pre-storage treatments and refrigeration.

There was a significant decrease ($P<0.05$) in the amount of vitamin C in all the samples. Brine-treated samples experienced the highest loss of vitamin C at the end of the storage period, followed by untreated (control) samples. The highest retention of vitamin C (95.5mg/100g) was observed in the refrigerated sample at the end of the storage period, followed by vinegar-treated samples.

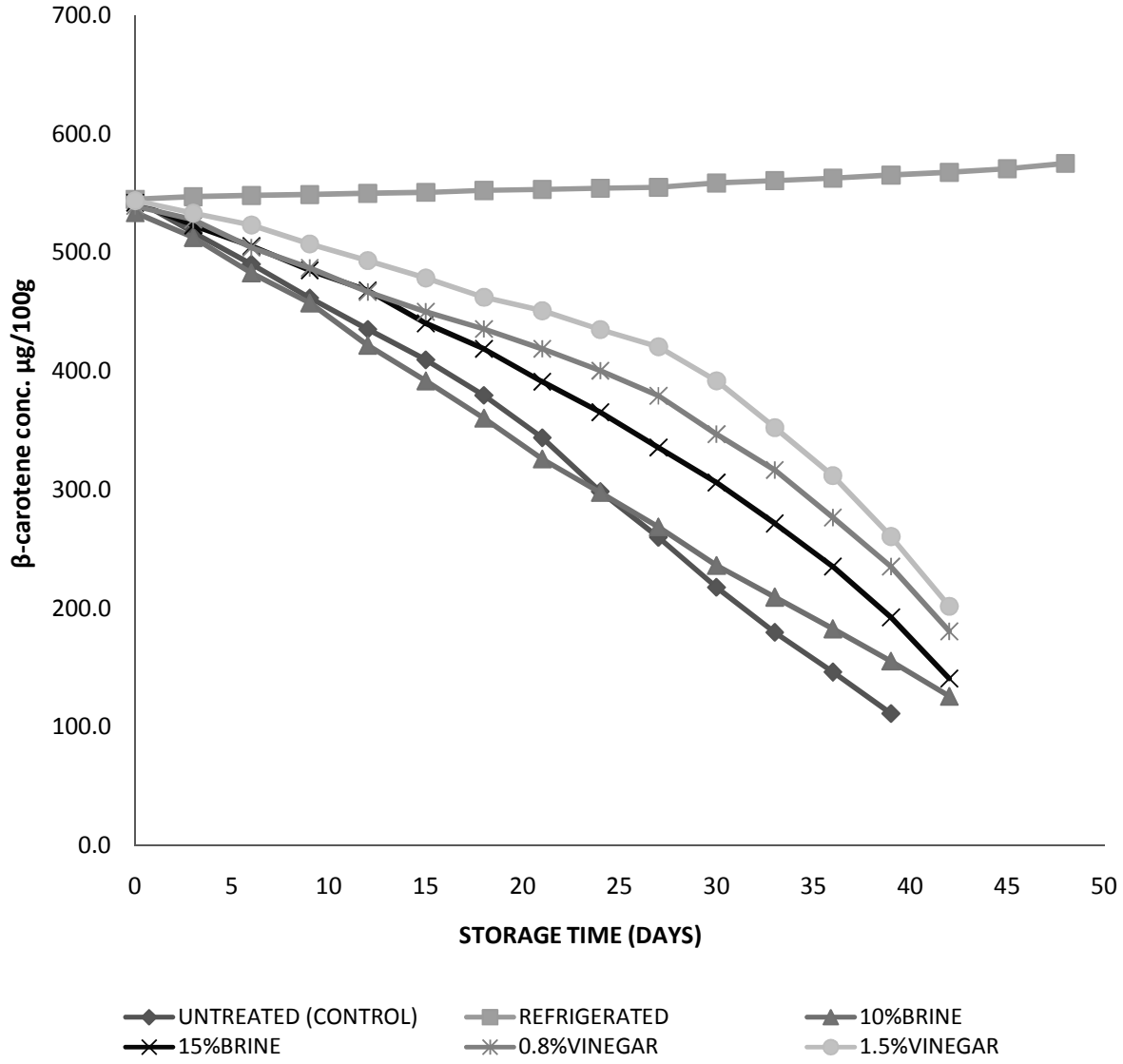

Figure 4: Variations in β-carotene content of *C. frutescens* with time of storage as affected by different pre-storage treatments and refrigeration.

Results showed that β-carotene content in pepper significantly decreased ($P<0.05$) with storage time with the exception of refrigerated sample which experienced an increment from 545.0 µg/100g to 575.4 µg/100g. Vinegar treated samples also maintained relatively higher amount of β-carotene as compared to brine treated samples at the end of the storage duration. The least amount of β-carotene was recorded in the untreated (control) sample.

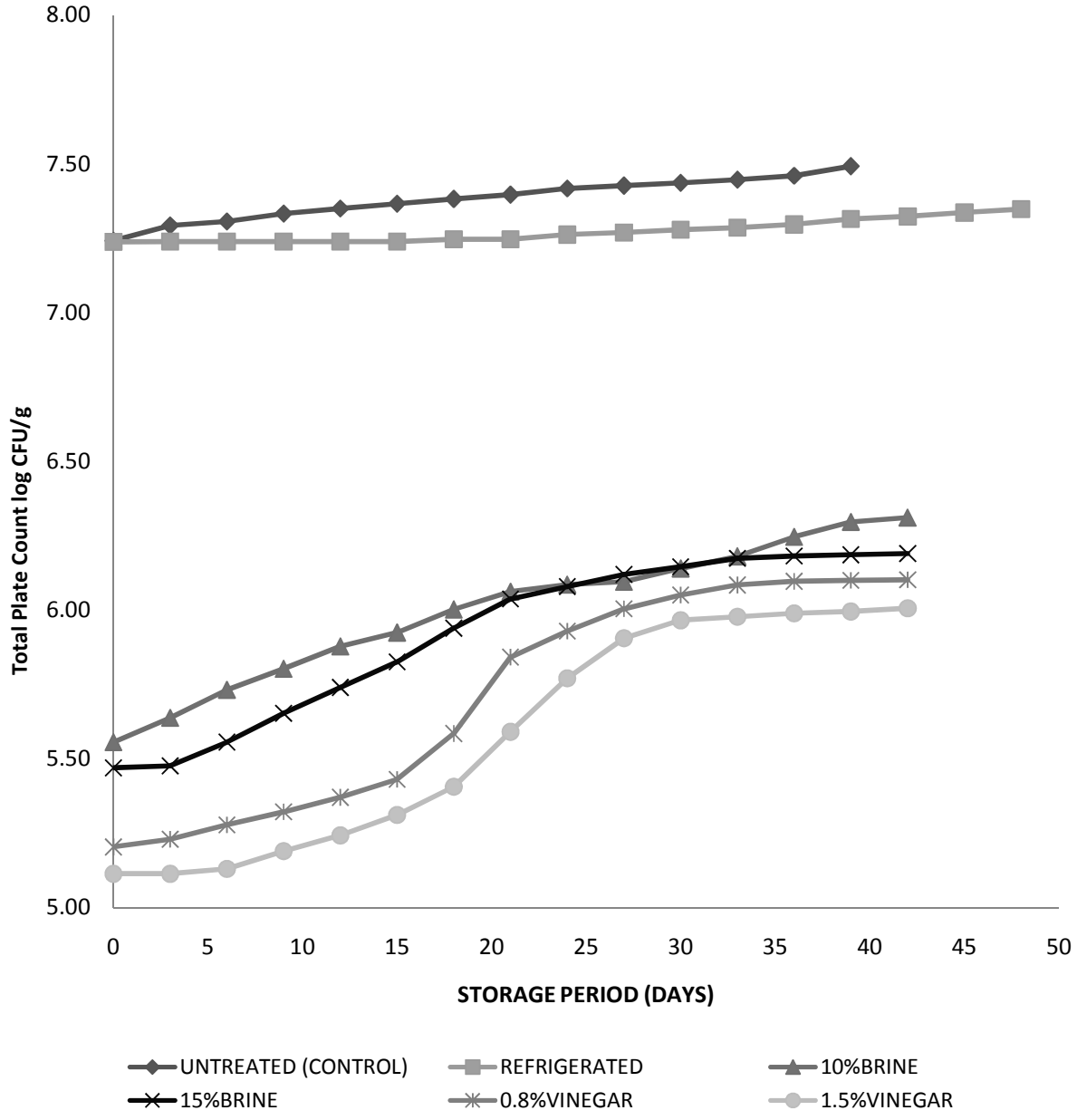

Figure 5: Variations in Total Plate Count of *C. frutescens* with time of storage as affected by different pre-storage treatments and refrigeration.

Treating the pepper samples (on Day 0) with the brine and vinegar solutions resulted in an appreciable drop of the total microbial load in the samples. The highest drop was recorded in samples treated with vinegar. Refrigerated samples had the minimum growth observed during the storage time.

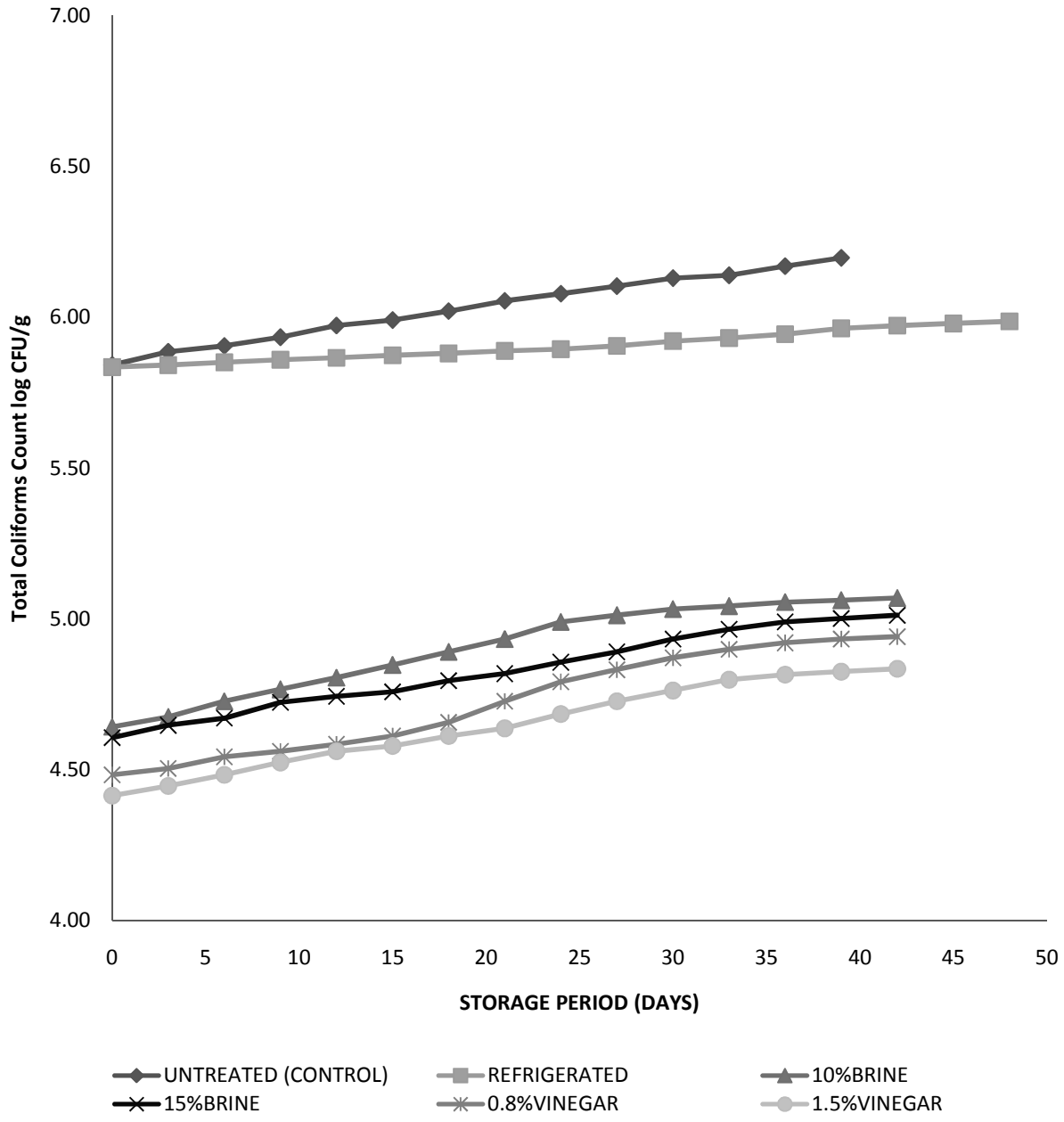

Figure 6: Variations in Total Coliform Count of *C. frutescens* with time of storage as affected by different pre-storage treatments and refrigeration.

Brine and vinegar treated samples recorded a drop in the Total Coliform Count on Day 0, with the highest drop occurring in samples treated with vinegar. Refrigerated samples had the minimum growth observed during the storage time.

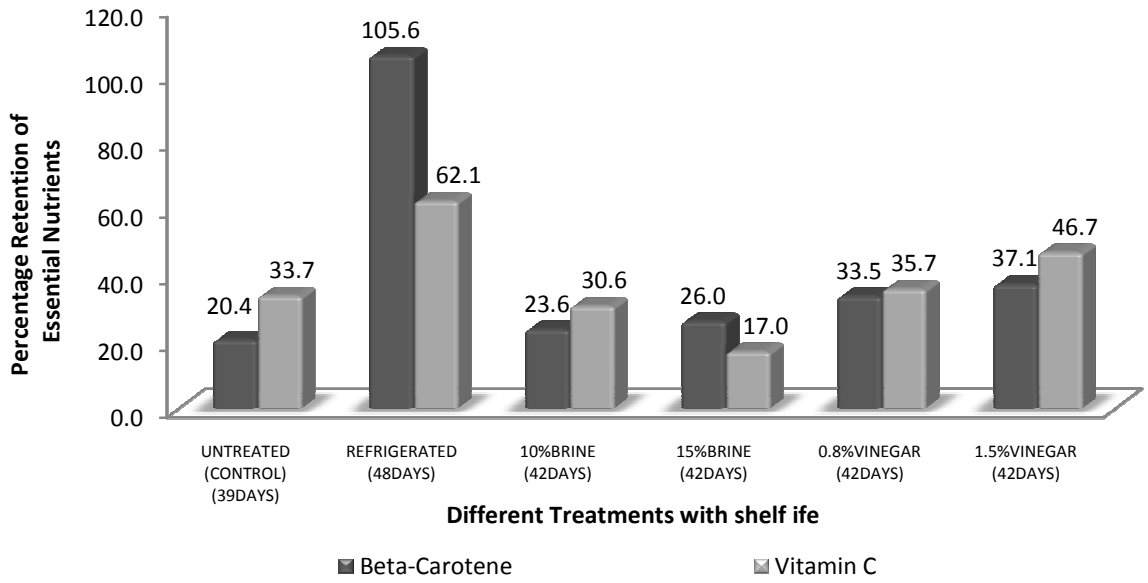

Figure 7: Percentage retention of Vitamin C and Beta-Carotene in *C. frutescens* as affected by different pre-storage treatments at the end of storage time.

High percentages of retention were observed in samples that were refrigerated. The least percentage retention of vitamin C and β-carotene were recorded in 15% brine treated and untreated (control) samples respectively.

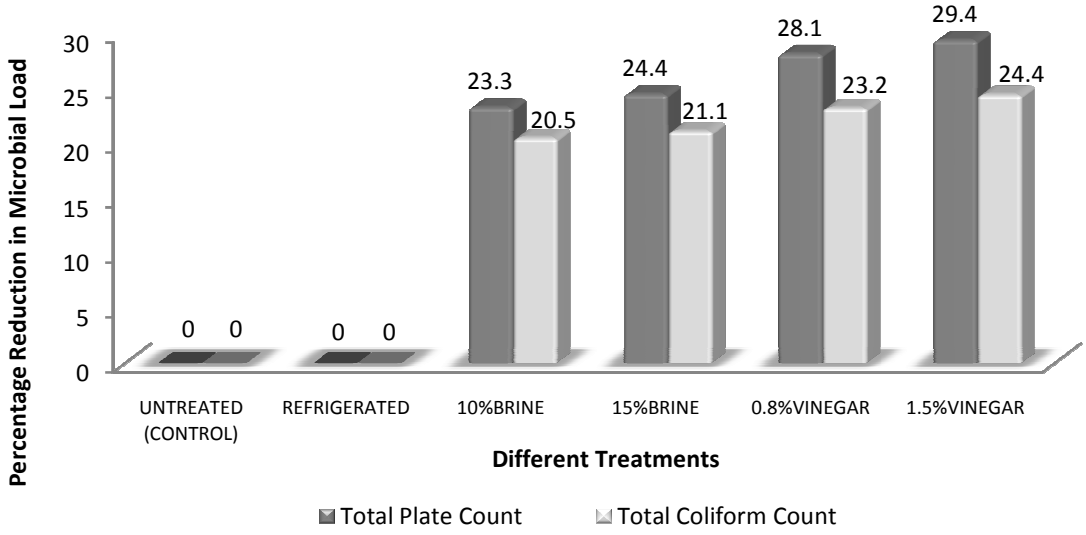

Figure 8: Percentage reduction in Microbial load (Total Plate and Coliform Counts) as affected by different pre-storage treatments on day 0 of storage time.

Samples treated with 1.5% vinegar recorded the highest percentage reduction in the total microbial load.

DISCUSSION

EFFECTS OF THE TREATMENTS ON VITAMIN C

The vitamin C content of the fresh red pepper was found to be 153.8 mg/100g and this value favourably compares with the US Department of Agriculture's National Nutrient Database which listed red *Capsicum* as having 143.7

mg/100g. The difference however could be as a result of different soil conditions and climate. At Day 0, samples treated with 15% brine recorded the least amount of vitamin C, followed by those treated with 1.5% vinegar (Figure 1). This could be due to the high salt of the brine concentration and low pH of the vinegar. Generally, vitamin C content in the peppers decreased gradually with storage time (Figure 3). This decrease may be due to the oxidation of ascorbic acid into dehydroascorbic acid by the enzyme ascorbic acid oxidase. This result corroborated with results of Eris *et al.,* (1994) [17]; Nazar *et al.,* (1996) [18]; and Pal *et al.,* (2002) [19] who reported a decrease in the Vitamin C content of vegetables on storage.

At the end of the storage period, brine treated samples had the least retention of vitamin C (46.8 mg/100g representing 17.0% and 25.0 mg/100g representing 30.6% for 10% brine and 15% brine respectively) as compared to the untreated sample (51.8 mg/100g representing 33.7%) (Figure7). The high loss of vitamin C in the samples treated with brine could be as a result of the fermentation processes caused by the salt-tolerated micro organisms, with the combined effect of temperature and water loss (shrinkage) during storage. The results favourably compares with studies conducted by Nunes *et al.* and (1998) [20]. The variation of vitamin C content in the brine treated and the untreated samples were however not significantly different ($P>0.05$).

On the other hand, peppers treated with vinegar maintained relatively high vitamin C (54.7 mg/100g representing 35.7% and 69.5 mg/100g representing 46.7% for 0.8% and 1.5% vinegar respectively) (Figure 7) at the end of the storage period as compared to brine treated, but not as high as recorded in refrigerated sample. This phenomenon could be due to the fact that, water loss is moderately lower in vinegar treatment than in brine treatment. Vinegar treated samples recorded high values of vitamin C than untreated samples (Control) at the end of the (42days) storage time. This result however is contrary to the findings of Gramlich *et al.,* (2002) [21], which reported that vitamin C degrades (or oxidizes) more quickly at low pH (acidic) medium.

Peppers stored in refrigerated condition maintained the highest of vitamin C content of 95.5 mg/100g after the storage period of 48days. This corresponds to 62.1% retentions of vitamin C. This phenomenon is due to the oxidation process correlating negatively with supply of oxygen. Similar result was reported by Lee and Kader (2000) [22].

EFFECTS OF THE TREATMENTS ON β- CAROTENE

β-carotene content of fresh red capsicum was found to be 544.9 µg/100g and this value is comparable with the reference value of 534 µg/100g by US Department of Agriculture's National Nutrient Database. The difference however could be as a result of different soil conditions and climate. At Day-0, samples treated with 10% brine recorded the least amount of β-carotene, followed by those treated with 0.8% vinegar (Figure 2). β-carotene generally decreased gradually in all the samples with storage time, with the exception of the refrigerated sample which experienced a gradual increase to about 5.6% (Figure 4). This gradual increase in β-carotene in the refrigerated sample could be attributed to the fact that the pepper turned redder when kept in the refrigerator. Hence its (β-carotene) percentage to other compound seemed to have increased. The variations in the beta-carotene content of the refrigerated sample and the treated samples were highly significant ($P<0.05$). Fabian and Blum (1943) [23] reported that vegetables differ in their ability to retain β-carotene and also the lower the brine concentration, the greater the loss in beta-carotene. Results of this study indicated that samples treated with 10% brine concentration recorded a lower percentage of retention (23.6% beta-carotene) at the end of the storage time than those treated with 15% brine concentration (26.0% β-carotene) (Figure 7). This result also favourably compares with Ivan *et al.,* (1944) [24] who found that during brine preservation, loss of β-carotene may occur during fermentation as a result of microbiological activity or through chemical destruction (such as oxidation).

Samples treated with vinegar maintained a relatively higher β-carotene content (33.5% and 37.1% retention for 0.8% and 1.5% vinegar concentration respectively) after the storage period as compared to those samples with brine treatment. This could be because vinegar is more effective in reducing the oxidation process through which β-carotene is lost.

EFFECTS OF THE TREATMENTS ON MICROBIAL LOAD

The microbial load of the fresh pepper samples was found to be 7.24 log CFU/g, with total coliform count recorded as 5.84 log CFU/g. These values are however within the range of specifications for fresh vegetables by the International Commission for Microbiological Specifications for Foods (ICMSF). The high microbial contamination

observed in the pepper may be a reflection of the sanitary quality of the cultivation water, the type of manure used, harvesting, transportation, and storage, of the produce. The results favourably compares with other studies (Beuchat, 1996 [25]; Ray and Bhunia, 2007 [26]; Uzeh *et al.*, 2009 [27] and Bukar *et al.*, 2010 [28]).

After treatment (on Day 0) with the brine solutions, the total plate count dropped to 4.56 log CFU/g, and 4.47 log CFU/g, in samples treated with 10% brine, 15% brine, respectively (Figure 5). With respect to total coliform count, there was also a drop to 3.64 log CFU/g and 3.61 log CFU/g, for 10% brine and 15% brine respectively (Figure 6). The results favorably compares with the results of Ivan *et al*., (1944). On the other hand, samples treated with the vinegar solutions, had their total plate count dropped to 4.20 log CFU/g and 4.11 log CFU/g in samples treated with 0.8% vinegar, and 1.5% vinegar respectively (Figure 6), while the total coliform count dropped to 3.48 log CFU/g, and 3.41 log CFU/g for 0.8% vinegar and 1.5% vinegar respectively (Figure 6). Refrigerated samples had relatively equal amount of total plate count (7.24 log CFU/g) and total coliform count (7.24 log CFU/g) as that observed in the untreated sample (Control). Vinegar-treated samples experienced a greater reduction in both total plate count and coliform count (Figure 8). Results of this study favourably compares with previous reports of microbial load reduction observed in vegetables washed and rinsed in vinegar [29, 30, 31]. The observed proportionate reduction in microbial loads was more in samples treated with high vinegar (1.5%) concentration. This can be attributed to the further reduction in pH and most bacteria cannot survive in acidic conditions. Similar findings were obtained by a study conducted by Eni *et al.*, (2010) [32]. The differences in the variations of microbial load in the vinegar-treated and untreated samples were highly significant ($P<0.05$). The total plate and coliform counts for the refrigerated sample did not experience much increase during the period of storage. This could be due to the fact that low temperature inhibited the activities of microorganisms. There was a significant difference between the variations of the microbial load of the refrigerated and untreated samples ($P<0.05$).

CONCLUSION

The study provides foundation for minimizing post harvest losses by using appropriate storage methods. It was found that quality of vegetables which were stored in refrigerated condition maintained better quality attributes and gave the highest shelf life (48 days). Results also indicated that shelf life of brine and vinegar-treated samples had their shelf life extended for at least 3 days (42 days) more than as compared to the untreated (39 days). The study also found that, essential nutrients (vitamin C and β-carotene) were favourably maintained in the refrigerated conditions.

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