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# Antioxidant Vitamins and Minerals Composition of Fresh Carrot-Cabbage Juice

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# HISTORY

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# ABSTRACT

Antioxidants are substances that protect cells from the damages caused by free radicals. Fruit and vegetables are rich in antioxidants and provide important sources of antioxidants. While the quest for an antioxidant supplement is ongoing, this study investigated the antioxidant vitamins and minerals composition of fresh carrot-cabbage juice. Antioxidant vitamins and minerals compositions of the juice were determined by standard methods using spectrophotometry and atomic absorption spectrophotometry (AAS) respectively. The concentration of antioxidant vitamins was found to be in the order; vitamin C > vitamin A > vitamin E. However, manganese has the highest concentration (P < 0.05) among minerals. There is no significant difference between the concentrations of manganese and copper (P > 0.05). The concentration of chromium is significantly lower (P < 0.05) than that of manganese and copper but greater (P < 0.05) than that of zinc. The study showed that fresh carrot-cabbage juice contains a respective concentration of antioxidant vitamins (A, C and E) and antioxidant minerals (Cu, Cr, Mn and Zn). The juice is hence a good source of antioxidant vitamins and minerals and may help complement the antioxidant system of the body.

# INTRODUCTION

Antioxidants are substances that protect the cells against damages caused by a highly reactive and unstable molecule called free radical. They confer their protection upon cells by stabilizing free radicals through direct or indirect interaction with the free radicals [1]. Common examples of antioxidants are vitamins (A, C and E), beta carotene, some minerals (Mg, Cu, Zn and Cr) and other substances. Antioxidants act by reacting with the free radicals thereby interfering with the oxidation process. They may also act by halting the propagation process and providing an electron or hydrogen atom to the free radical and receiving the excess energy obtained by the activated molecule [2]. Antioxidant vitamins are obtained from natural food or dietary supplement and are needed for normal metabolism. They include vitamins A, C, E, carotenoids, and trace elements [3]. Antioxidant minerals - are cofactors of antioxidant enzymes. The deficiency of antioxidant minerals will affect the metabolism of macromolecules such as carbohydrates and proteins. Examples include manganese, zinc and chromium etc [3]. Free radicals are highly reactive, hazardous molecules that are implicated in the causation and progression of many diseases. They cause deleterious damage to cells and may play role in the pathogenesis

or progression of diseases such as cardiovascular diseases, neurological disorders, arthritis, muscle damage and cancer [1]. They can be generated by normal physiological processes and are hence inevitable to living systems. The normal metabolic and oxidative processes produce free radicals in addition to other external factors that raise them. Vegetables and fruits are good sources of antioxidants including vitamins and minerals. Different studies reveal ed that, natural antioxidant intake has a positive health benefit which lowers the risk of cancer and cardiovascular diseases [4].

Carrot botanically called *Daucus carota*, is a vegetable planted for its root (root vegetable). It is native to Europe and southwestern Asia [5]. It has bright orange color due to its  $\beta$ carotene content. It has little amounts of  $\alpha$ -carotene,  $\gamma$ -carotene, lutein and zeaxanthin [6]. The  $\alpha$ - and  $\beta$ -carotenes are partly metabolized into vitamin A [6]. Carrot contains vitamins such as ascorbic acid, riboflavin, thiamine, folic acid, niacin, and alphatocopherol [7]. Some health benefits of carrots include boosting the immune system, increasing cardiovascular health, detoxification and boosting oral health [8]. Cabbage is a biennial plant that is **a** leafy green, or purple and composed of superimposed layers of dense leaves. It contains significant amounts of vitamin C and vitamin A and can serve as a source of folate and vitamin A [9]. To counteract the deleterious effect of free radicals, the natural antioxidant system needs to be complemented with an antioxidant-rich supplement. While the quest for an antioxidant-rich supplement is ongoing, the present study is designed to investigate the antioxidant vitamins and minerals of juice formulated from fresh carrot-cabbage juice.

# MATERIALS AND METHODS

### Reagent and apparatus used

Xylene, Chloroform, phosphotungstate reagent, H3PO4 (All sourced from AnalarR, England), Ethanol, Bathophenanthroline, FeCl3, KOH solution (All sourced from BDH Chemical, England), Distilled water (Sourced from Biochemistry laboratory, UDUS), Dilute HC l (BDH Chemicals, Lobachemie PVT ltd), Vitamin C standard, Vitamin E standard (BDH Chemicals, Tokyo, Japan), Spectrophotometer (AE-350 ERMA Inc./Tokyo, Japan), Centrifuge Machine (800D/Metal), Muffle furnace (Lento Furnace Gallenkamp, USA), AAS Machine (AA-6300, Japan).

#### Sample preparation

Carrot and cabbage samples were obtained from the general market and Kasuwar-Daji Market Sokoto in fresh form. The samples were identified and authenticated by a botanist from the department of biological sciences at Usmanu Danfodiyo University Sokoto. The samples were weight in the ratio of one to six (1:6) of cabbage and carrot respectively (50 g of cabbage for 300 g of carrot), washed, sliced into small pieces and blended with the addition of 120 mL of distilled water into the blender. The blended sample was filtered, and the juice was collected. Honey, 20 mL was added to the juice to serve as a temporal preservative. The sample was preserved in a freezer at a temperature of 10-15°C.

# Determination of antioxidant vitamins

#### **Determination of Vitamin A**

A test tube was labelled as 'I' and 1 mL of the prepared juice sample was measured into the test tube. Potassium hydroxide (KOH) solution (1 mL) was added into the test tube and was plugged and shaken vigorously for 1 minute. The content of the test tube was heated in a water bath (60 °C for 20 minutes) and then cooled. To the cooled mixture, 1 mL of xylene was added and the tube was plugged and shaken vigorously for 1 minute. The tube was then centrifuged at 15000×g for 10 minutes. The supernatant was collected and put in another test tube labelled II and absorbance 'A1' was measured and recorded. The content in test tube II was then irradiated using ultraviolet (UV) light for 30 minutes and then the absorbance 'A2' was measured [10]. The concentration of vitamin A was calculated using the formula;

 $Ax = (A1 - A2) \times 22.23$ 

Where Ax is the absorbance of the test, A1 is absorbance before irradiation A2 is the absorbance after irradiation and 22.23 is a constant value

# **Determination of Vitamin C**

Three centrifugal test tubes labelled as a test, blank and standard were set. Analyzed sample (1 mL), 1 mL of standard vitamin C and 1 mL of distilled water were measured into the test, standard and blank test tubes respectively. To all the three test tubes, a phosphotungstate (1 mL) reagent was added. The content in each test tube was mixed and then incubated at room temperature for 30 minutes. The sample test tube was centrifuged at 7000×g for

10 minutes. The supernatant was collected which is then used for spectrophotometric measurement. Finally, the absorbance of the test sample 'Ax' and the standard sample 'As' were measured at 700nm against the reagent blank [11]. The concentration of vitamin C (Cx) was calculated in  $\mu$ M using the formula:

 $Cx = Absorbance of sample (Ax) \times Concentration of standard (56.8 \mu M)$ The absorbance of standard (As)

### **Determination of Vitamin E**

The juice sample (0.5 mL) was measured into a test tube labeled as I and anhydrous ethanol (0.5 mL) was then added. The contents were shaken vigorously, and the test tube was then plugged for sixty seconds. Xylene (3 mL) was then added, and the test tube was plugged for another sixty seconds. The tube was centrifuged at 1500 ×g for 10 minutes. Betaphenanthroline (0.25 mL) solution was put into a different test tube labeled as II. The extract (1.5 mL, upper layer/supernatant in test tube I) was added and the content was mixed into test tube II. Ion III chloride (FeCl<sub>3</sub>: 0.25 mL) was then added to test tube II. This provides the test sample for spectrophotometric measurement. Standard solution (0.5mL) was added into another test tube labeled as III. Distilled water (0.5 mL) was added to a separate test tube labeled as IV which served as a blank. Vitamin E concentration was found by measuring the absorbance of the test sample (Ax) and that of the standard sample (As) at 539 nm against the reagent blank [12]. Vitamin E concentration (Cx) was calculated in mg/dl using the formula.

 $C_x = Absorbance of sample \times Concentration of standard (100mg/dl)$ Absorbance of standard

# Determination of antioxidant minerals (AAS)

The empty crucible was weighed as W1. A specific amount of the sample was placed in the crucible. The crucible containing the sample was weighed again as W2 before being placed in a muffle furnace and heated at 600 °C for 5 hours. The crucible containing the ashed sample was finally weighed as W3 and the percentage of the ash was determined using the formula below;

%Ash =  $\underline{W_3 - W_1} \times 100$  $W_2 - W_1$ 

#### Digestion

Digestion of the ash was done by adding 5mL of 10% hydrochloric acid (HCl). The volume of the resulting mixture was made up to 50 mL with distilled H2O. The resulting sample was then subjected to atomic absorption spectroscopy to determine the concentration of the minerals [13] by multiplying the concentration of individual minerals by the dilution factor. The dilution factor was found by dividing the volume of digested sample (50 mL) by the weight of the ash found (0.42 g).

#### Atomic absorption spectroscopy (AAS)

Standard solutions were prepared by appropriate dilution of stock solutions using AAS standard solutions (1000 ppm) of Mn, Cu, Cr, Se, and Zn supplied by the manufacturers of AAS machines. The AAS machine (AA-6300 Model) was set up following the manufacturer's instructions for each element to be analyzed. The standard, blank and sample will be aspirated into the flame. Elemental ions were then atomized, and the atom absorbs radiation of a characteristic wavelength from a hollow cathode. The absorbance measured is proportional to the amount of the analyte in the sample solution [13].

# RESULTS

#### Antioxidant vitamins

**Table 1** shows the result of antioxidant vitamins. Vitamin C has the highest concentration followed by vitamin A. Vitamin E has the least concentration.

Table 1. Concentration of antioxidant vitamins.

Vitamins	Concentrations
Vitamin A equivalent (mg/100g)	0.83 <u>+</u> 0.000
Vitamin C (mg/100g)	1.67 <u>+</u> 0.00
Vitamin E (mg/100g)	0.128 <u>+</u> 0.00

Key: Values were presented as mean  $\pm$ standard error of the mean obtained from three replicate findings.

#### Antioxidant minerals

Table 2 shows the result of ash contents and antioxidant minerals. The Ash content of the juice was found to be approximately 1%. The juice contains more water since it was obtained by sieving the blended mixture. The concentrations of copper and manganese are statistically similar (P>0.05) but differ significantly (P<0.05) from the concentration of chromium and zinc.

Table 2. Ash content and concentration of antioxidant minerals.

Ash content (%)	1%
Antioxidant Minerals	Concentration
Copper (mg/100g)	4.75 <u>+</u> 0.04 <sup>c</sup>
Chromium (mg/100g)	3.05 <u>+</u> 0.52 <sup>b</sup>
Manganese (mg/100g)	4.95 <u>+</u> 0.07 <sup>c</sup>
Zinc (mg/100g)	$0.55 \pm 0.05^{a}$

Note: Values were presented as the mean  $\pm$ standard error of the mean obtained from three replicate findings. ANOVA was used to compare means. Turkey-Kramer multiple comparisons test was used as a post hoc test. Different superscripts indicate significantly different at P < 0.05

## DISCUSSION

Vegetables such as cabbage and carrot are good sources of phytochemicals, antioxidant vitamins and minerals [9]. Blending samples to form a juice can improve the vitamins and mineral content [14]. Vitamin A concentration was found to be  $0.83\pm0.00$  mg/100g. This concentration is possibly due to the higher proportion of carrots in the juice. This is in line with the report of Compos *et al.* [15] and Olalude *et al.* [16] that, carrot juice has a high content of carotenoids which are mostly vitamin A precursors. The result found for vitamin A is however, less than 2.8 mg/100 g found by Olalude *et al.* [16]. Consumption of food with high content of beta carotene (provitamin A) is associated with decreased risk of heart attack, cancer and some chronic diseases [17].

Vitamin C concentration was found to be  $1.67\pm0.00$  mg/100 g this is a relatively high concentration as compared to results of other antioxidant vitamins. This result agreed with the report of Keinath *et al.* [9] that both carrot and cabbage are important sources of vitamin C. The result is in line with  $1.67\pm1.33$ , reported by Olalude *et al* [16]. Carrot juice alone is relatively not an excellent source of vitamin C. Hence this concentration is possibly more contributed by cabbage. Vitamin C inhibits haemoglobin polymerization [18], boosts the immune system and also scavenges free radicals. It is also important for the absorption of non-heme iron, hence can play important role in the management of anaemia [19].

The concentration of vitamin E was found to be  $0.13\pm0.00$  mg/100 g. Carrot and cabbage are a good source of vitamin E [9] Vitamin E is important in the management of oxidative stress

initiated by free radicals, [19] prevent hemolytic crisis being it membrane antioxidant [20] and inhibit deoxyhemoglobin polymerization [18]. Among antioxidant vitamins, vitamin E has the least concentrated in the juice. Vitamin E is essential for normal body metabolism. Ash content of approximately 1% was found after the ashing of the juice sample. This is approximately similar to 1.33 found by Olalude *et al.* [16] on the ashing of carrot juice. The reason for the low value of ash might be a result of discarding the chaff when extracting the juice [16]. The ash provides the sample for mineral analysis by atomic absorption spectroscopy

The concentration of Cu was found to be  $(4.75\pm0.04)$  mg/100 g which is significantly higher than 0.56 mg/100g found by Domagała-Świątkiewicz *et al.* [21] in the analysis of carrot juice alone. Copper is a cofactor for enzymes that are involved in metabolism. It recommended that the dietary recommendation for Cu should not exceed 10 ppm per day [22]. Chromium was found to have a concentration of  $0.26\pm0.04$  mg /100 g. It is required for glucose metabolism [23]. It is recommended that daily intake of Cr should be within the range of 0.025 and 0.2 mg/day [24] The concentration found of Cr found in this study is therefore above the recommended limit. WHO (1998), [25] prescribed a maximum limit of 0.05 mg/L of Cr in drinking water.

The concentration of manganese was found to be  $(4.95\pm0.07 \text{ mg}/100 \text{ g})$ . Manganese is a cofactor of some enzymes of energy metabolism and antioxidant enzymes such as superoxide dismutase of mitochondria. It is involved in the synthesis of glycoproteins and bone formation [26]. It is recommended that the daily intake of Mn should be within the range of 2-3 mg/day. WHO (1998) [25] prescribed a maximum limit of 0.05 mg/L of Mn in drinking water. Zn has the least concentration  $(0.55\pm0.05 \text{ mg}/100 \text{ g})$  which is higher than that of Domagała-Świątkiewicz *et al.* [21] who found 0.205 mg/100 g of zinc in carrot juice alone. The slight variation could be due to the presence of cabbage in the juice. Zinc is a cofactor of various antioxidant enzymes such as SOD and enzymes of energy metabolism [23]. For an adult, the recommended daily intake of Zn is about 15 mg/day [27].

## CONCLUSION

The body's natural antioxidant system can be strengthened by taking an antioxidant-rich supplement to combat the damage caused by free radicals. We hope to learn more about the antioxidant vitamins and minerals found in a juice blend made from fresh carrots and cabbages as the search for an antioxidant-rich supplement continues. The study showed that fresh carrot-cabbage juice contains antioxidant vitamins in the order C > A > E and antioxidant minerals (Cu, Cr, Mn and Zn). The juice is therefore a good supplement of antioxidant vitamins and minerals.

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