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Nutritional composition and antioxidant properties of three varieties of carrot (*Daucus carota*)

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ABSTRACT

Carrots are a potentially rich source of antioxidants in addition to their nutritional properties. In this study, the nutritional composition and antioxidant properties of three varieties of carrots, namely Kuroda, Pamela and Amazonia cultivated in Ghana were determined. The peroxide scavenging method was used to determine the antioxidant properties of the ethanolic extracts of the carrot varieties at different concentrations. The protein, crude fibre, fat and carbohydrate contents of the carrot varieties ranged 6.46 – 10.73 %, 7.18 – 8.87 %, 1.97 – 4.31 % and 6.25 – 8.39 % respectively. The three varieties had high moisture contents ranging from 69.06 to 75.30 %. The antioxidant properties were high even at low concentrations of extract, and their activity increased with time in the order Amazonia > kuroda > pamela. Amazonia had the highest fibre, protein and carbohydrate contents and the lowest moisture content, making it the most preferred variety.

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Introduction

Human nutrition's importance cannot be overemphasized since it is responsible for energy, growth, repair, immunity to diseases, and other metabolic processes in the human system [1]. The human body requires proteins, fats and oils, carbohydrates, fibre, vitamins, minerals and water for its metabolic processes. The body also needs some plant metabolites, which serve as antioxidants and protect it from oxidative stress.

In recent times, antioxidants have been used as food additives to increase the shelf life of some food products and improve the stability of fats and lipid-rich foods [2]. They have also been used to control the loss of taste and nutrients in food.

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The free radical acceptors are antioxidants that are often used in food products [3]. Some artificial antioxidants that include butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are common food additives for the protection of vegetables, milk powder, baked products, unsaturated fats and oils and nuts [4]. However, these products may pose some health threats such as hypersensitivity, allergy, asthma, hyperactivity, neurological damage and cancer [5], making it necessary to consider natural sources of antioxidants.

Phytoconstituents such as flavonoids, which are secondary plant metabolites with naturally occurring pigments, are free radical acceptors and, sometimes, chelating ligands that bind to metals [6]. The multiple phenolic groups present in these compounds are responsible for their antioxidant properties [7]. Flavonoids inhibit non-enzymatic and enzymatic oxidative processes [8].

Antioxidants in vegetables play a vital role in the prevention of oxidative stress-related diseases [9]. Oxidative stress releases free oxygen radicals in the body, which causes various disorders such as auto-immune diseases besides ageing, cataracts, cardiovascular malfunction, rheumatism and cancers [10]. These antioxidants remove free radicals in the body cells. Some studies indicate that eating vegetables reduces cancers, ageing and death from the heart and other degenerative diseases [11].

Carrots are known to have originated from Afghanistan and Persia about 1100 years ago [12]. The crop is among the ten leading vegetables grown globally [13]. About 1.2 million hectares of land are currently used for carrot cultivation globally, costing about $14 billion. Carrot is a cool-season vegetable crop grown globally but mainly in Europe and Asia in the last 50 years [14]. Carrots could be sensitive to high temperatures, limiting their growth in the tropics [15]. Carrots are cherished mainly for their vision enhancement attributes besides other benefits due to the possession of vital bioactive compounds such as carotenoids, a precursor to vitamin A formation [16]. Carotenoids, as well as anthocyanins, are non-enzymatic antioxidants [17].

Carrots are rich in antioxidants, such as alpha- and beta-carotene, lycopene, and lutein. These give the respective orange, red, and yellow colours to the carrots [18]. Anthocyanins confer purple colouration in fruits and vegetables [19]. These photosynthetic pigments accumulate in carrots' roots due to a photosensitive defect that allows the metabolic pathways of carotenoids and anthocyanins to be expressed in the dark [20].

In Ghana, carrot is one of the vegetables cultivated in most parts of the country and has become a regular vegetable in most dishes. Some of the commonly grown varieties are Kuroda, Amazonia and Pamela. Although these varieties are mostly consumed, there is no report on their nutritional properties. This study determined the nutrient composition and antioxidant properties of three Ghanaian carrot cultivars, namely Kuroda, Pamela and Amazonia.

Materials and methods

Chemicals

The reagents and chemicals were procured from Huge Ltd, Accra, Ghana and were used as received. The water used was double-distilled.

Sample collection and preparation

The carrot varieties were identified by a horticulturist, and the samples collected at the Department of Horticulture of the Kwame Nkrumah University of Science and Technology. The nature of soil from which the samples were harvested is sandy-loam [21]. Five carrots, each of three varieties, namely Kuroda, Pamela and Amazonia, were collected. The samples were harvested on an average of 95 days after cultivation. The samples were then thoroughly rinsed, air-dried, and the same varieties were pulverized together and stored in labelled sterile-clean transparent Ziploc plastic bags and preserved in a refrigerator before analysis.

Phytochemical screening

The Phytochemical screening was conducted on the pulverized carrot samples or ethanolic extracts to determine six phytoconstituents: carotenoids, coumarins, saponins, general glycosides, flavonoids and tannins [22]. The procedures for the various tests are as follows:

Test for flavonoids

A 5ml aliquot of the ethanolic extract was added 3-5 drops of concentrated hydrochloric acid and about 0.5 g magnesium ribbon. A magenta-red colour obtained after about 3 min indicated the presence of flavonoids.

Test for saponins

To about 0.5 g of the extract was added water in a test tube and vigorously shaken with the hand to froth. The test tube was kept on the rack for about 15 min to observe the froth. The presence of the froth after 15 min indicated the presence of saponins.

Test for tannins

About 1 g of the ethanolic extract was evaporated to dryness, and the residue extracted with a 10 ml aliquot of 0.9% w/v NaCl solution. The extract was filtered into a test tube. A few drops of $FeCl_3$ solution (1% w/v) was added to the extract. An intense green colouration of the extract indicated the presence of tannins.

Test for glycosides

A 5 ml aliquot of the ethanolic extract was hydrolyzed in concentrated hydrochloric acid (5 ml) at boiling temperature for 2 h. To 1 ml of the hydrolysate was added I ml of 10% NaOH. A yellow colouration was indicative of glycosides present.

Test for coumarins

0.5 g of the moistened extract was placed in a test tube. The tube was covered with a filter paper pretreated with 1 N NaOH solution and placed in a hot water bath for about 45 min. The covered test tube was allowed to cool on a rack, and the filter paper removed and observed under UV light for yellow fluorescence, which indicated the presence of coumarins.

Test for carotenoids

A 10 ml aliquot of chloroform was added to 1 g of the pulverized sample in a test tube and vigorously shaken for 30 min. The mixture was filtered into a fresh test tube, and to the filtrate was added few drops of concentrated sulphuric acid. The blue colouration of the solution indicated the presence of carotenoids.

Proximate analysis

The various carrot species' chemical composition was determined using the Official Methods of Analysis of the Association of Official Analytical Chemists [23] standard methods. The parameters under investigation were protein, total carbohydrate, fat, moisture, ash and fibre contents. All the parameters analyzed were expressed in percentages.

Determination of moisture content

In this method, the weight loss due to evaporation of moisture was measured. Labelled crucibles were washed and dried in an oven at 110 °C for 10 min and cooled in a desiccator. Each ground sample's 2.0 g weight was put in their respective crucibles, placed in an oven and dried to constant weight at 105 °C for 72 h. The difference in weight was estimated as the moisture content.

Determination of ash content

The organic matter in the samples was burnt away, leaving the inorganic residue. Well labelled ceramic crucibles were heated in a muffle furnace at 500 °C for 1 h, cooled in a desiccator and weighed. A 2.0 g weight of each of the ground samples was put into the respective crucibles. These were then ignited in the furnace at 500 °C for 3 h. The crucibles and their contents were then cooled in a desiccator to room temperature and weighed. The difference in weight was estimated as the ash content.

Determination of protein content

The micro-Kjeldahl technique was used to determine the nitrogen and protein contents of each sample. The protein content was estimated using the relation, $\%\ protein\ content = N \times 6.25$, where N is the nitrogen content, and 6.25 is the protein conversion factor [23]. Approximately 1.0 g carrot powder of each variety was poured into a Kjeldahl digestion flask and followed by the addition of a catalyst (2.0 g of potassium sulfate, 1.0 g of copper sulfate and 0.1 g selenium powder) and 10 mL concentrated H_2SO_4. The flask was heated continuously in the fumehood until a green solution was obtained. The heating continued for about 30 min before cooling. Distilled water (10 ml) was added to the cooled digest and vigorously shaken. The digest was transferred into 100 mL volumetric flask and topped up to the mark with water. A mixture of 10 mL aliquot of the digest and 10 mL of 40% NaOH was distilled for 5 min into a receiver containing boric acid (10 mL, 2% w/v) using a Markham distillation unit. The distillate was titrated with 0.01 M HCl to determine the nitrogen content.

Determination of fibre content

Approximately 2.0 g of the sample was poured into a volumetric flask (1 L preheated) followed by the addition of 150 ml, 0.128 M H_2SO_4. The solution was refluxed for 30 min and cooled to room temperature. It was filtered through an ashless filter paper, and the residue washed with hot water (10 ml x 3). Exactly 150 mL of preheated 0.22 M KOH was added to the residue and refluxed for 30 min. It was cooled, filtered, and the residue was washed with acetone. The residue was oven-dried at 130 °C for an hour and weighed, and the dried residue was ignited in a muffled furnace at 500 °C for 3 h, cooled and weighed. The loss in weight was estimated as the fibre content [23].

Table 1
Phytoconstituents of carrot varieties cultivated in Ghana.

Phytoconstituents	Kuroda	Pamela	Amazonia
Carotenoids	+	+	+
Saponins	+	+	+
Glycosides	-	-	-
Flavonoids	+	+	+
Coumarins	-	-	-
Tannins	+	+	+

Note: + showing present and – showing absent

Determination of fat content

The fat content was determined by the Soxhlet extraction method [23]. A clean and pre-weighed 250 mL round bottom flask was fitted with a suitable column packed with a thimble containing 1.0 g portion of the sample. About 150 mL petroleum ether (boiling range 40-60 °C) was used for the extraction and heated at 60 °C for 2 h under reflux on a heating mantle. The solvent was recovered using a rotary evaporator. The extract was then dried in an oven at 100 °C for 2 h, cooled and weighed. The weight of empty flask was subtracted from the final weight. The estimated weight was expressed as the fat content.

Determination of carbohydrate content

The percentage carbohydrate content was expressed as the sum of the percent crude protein, moisture, ash, crude fibre and fat contents less 100 % [23].

Sample extraction

The carrot samples were extracted by soxhlet extraction method. Approximately 50 g of each pulverized sample was extracted with 400 mL aliquot of ethanol for 10 hours. The solvent removal was done with an R-114, Buchi (Switzerland) rotary evaporator at 60 °C temperature and reduced vacuum pressure and allowed to dry in the fume hood at room temperature.

Antioxidant assay by hydrogen peroxide scavenging

The antioxidant properties of the carrot samples were determined by the hydrogen peroxide scavenging method by iodometric titration [24]. 2 ml (20 mg/ml), 4 ml (40 mg/ml) and 8 ml (80 mg/ml) aliquots of 1% w/v ethanol extracts were added separately to 8 ml H_2O_2 (17mM) solution in different Erlenmeyer flasks with continuous stirring on a stir plate at room temperature. At 60 s intervals for 4 min, 1 ml aliquot of the reaction mixture was drawn with a pipette into a separate conical flask and quenched with 25 mL of water. Potassium iodide (2.2 g) and sulphuric acid (10 ml, 2M) were added to the mixture and titrated with 0.0519 M sodium thiosulphate solution, using a starch indicator. A blank titre was subtracted from the sample's titre, and the resultant was used to estimate the hydrogen peroxide scavenging. The H_2O_2 scavenging of the samples was then plotted against time.

Statistical analysis

The variation between the carrot samples' antioxidant properties was determined by One-way analysis of variance (ANOVA) using MS Excel 2016 software. Values of $P<0.05$ were considered significant variations, and $P > 0.05$ were considered insignificant. A linear regression model was used to test the relationship of the antioxidant activity of the extracts with time.

Results and discussion

Phytoconstituents of the carrot samples

The Phytochemical screening indicated the presence of carotenoids, saponins, flavonoids and tannins in the carrot samples. There were, however, no glycosides and coumarins found (Table 1). Phytochemicals are essential medicinal natural products [25]. Tannins have been used effectively to treat diarrhoea. Tannins also enhance the antioxidant properties in vegetables [26]. Saponins act as food supplements and nutraceuticals [27]. They are also known to have anti-cancer properties and lower blood cholesterol level. Saponins are also amphipathic and promote protein penetration into the cells. Carotenoids and Flavonoids are known antioxidants [28].

The proximate composition of the carrot varieties has been summarised in Table 2. The moisture content spanned over 69.06 to 75.30 %, with Amazonia having the least moisture and Pamela having the highest moisture content. The differences

Table 2
Percentage composition of Proximate Compounds in Carrot Samples.

Samples	Kuroda	Pamela	Amazonia
Moisture (%)	74.04	75.3	69.06
Ash (%)	0.50	0.50	0.98
Fat (%)	2.44	4.31	1.97
Fibre (%)	7.80	7.18	8.87
Protein (%)	8.60	6.46	10.73
Carbohydrate(%)	6.62	6.25	8.39

in the moisture content of the samples may be due to varietal differences. The high moisture content in carrots may increase fungal growth and reduce storage time. The moisture content obtained in all samples compared favourably with the literature [29].

Amazonia had the highest ash content of 0.98%. Kuroda and Pamela had the same ash content of 0.50%. These values were generally lower than the reported values for carrot powder by Gazalli et al. [30]. The ash content indicates the mineral content in the sample. Low ash content, therefore, indicates low metals content. The difference in the ash content of the three varieties may result from varietal differences. The protein content ranged from 6.46 to 10.73%. Amazonia showed the highest percentage of crude protein. This implies that Amazonia can be used as a highly nutritious vegetable in malnourished human beings and livestock. These high protein levels may be due to the genetic improvement of the carrot varieties. Pamela, on the other hand, had the least protein content. The protein content was generally low compared with the value reported by Ramamoorthy et al. [31] of 22%. However, they compared favourably with the value obtained by Gazalli and Singh et al. [30,32].

The fibre content ranged from 7.18% to 8.87%, with Amazonia having the highest fibre content while the least was found in Pamela. The high crude fibre content aids in the digestion process of humans and livestock and prevents constipation [33]. High fibre content foods have been reported to prevent colon cancer. The values obtained for crude fibre in the carrot varieties were significantly lower than the reported value in the literature of 24.66% [30].

The fat content of the carrot samples ranged from 1.97% to 4.31%. Pamela had the highest fat content, with Amazonia having the least. The carrot's fat content indicates the amount of oil it contains and whether it is suitable for oil extraction. Fat content in the carrot varieties was generally low as compared with literature values of other carrot varieties studied [31]. However, it compared favourably with the value obtained for carrot powder [30].

The carrot varieties' carbohydrate content ranged from 6.25% to 8.39%, with Amazonia having the highest carbohydrate content and Pamela having the lowest. The carbohydrate content compared favourably with the results obtained by Fanlégué et al. [34], which ranged 5.62-6.72%. Carrots contain carbohydrates that are mainly starch, sucrose and glucose. Sucrose being a disaccharide, may have a mixture of aldose and ketose, whereas glucose is an aldose [35]. Carbohydrates get absorbed by the body and provide energy. They also affect starch digestion and satiety, which control blood glucose and insulin [36]. Therefore, the proper intake of carbohydrates may reduce the risk of diabetes [37].

Antioxidant properties

Oxidative stress in humans results from decreasing antioxidative potential or increasing oxygen or nitrogen radicals production [38]. Most radicals that cause oxidative stress are reactive oxygen species (ROS) or reactive nitrogen species. ROS include oxygen-based free radicals such as superoxide ($0^{2-}\bullet$), hydroxyl (OH•), alkoxyl (RO•), peroxyl (ROO•) and hydroperoxyl (ROOH•). Hydrogen peroxide and lipid peroxides can be converted to free radicals by transition metals, either free in the cell or bound to protein. Reactive nitrogen species include the free radicals nitric oxide (NO•) and nitrogen dioxide ($NO_2\bullet$) and the potent oxidant peroxynitrite ($ONOO^-$) [39]. The Carrot extracts showed high antioxidant activities and recorded mean peroxide scavenging values of 80.10±8.35%, 83.27±9.04%, 86.28±7.64%, respectively, for Pamela, Kuroda, and Amazonia. The peroxide scavenging is shown in Figure 1. Amazonia recorded the highest antioxidant activity while Pamela recorded the lowest.

In general, the antioxidant properties increased as the concentration of extract increased for all the samples. A one-way ANOVA performed on the % H_2O_2 scavenging assay showed a significant difference ($P<0.05$) for Kuroda and Amazonia at different concentrations but no significant difference ($P>0.05$) for Pamela (Table 3). The results indicate that for Kuroda and Amazonia, the antioxidant activities are significantly higher at specific concentrations than others, whereas for Pamela, there is no significant difference in their antioxidant activities between different concentrations.

The significance between the three extracts of Kuroda and Amazonia was further tested with a two-sample t-Test. The test indicated a significant difference between the 20 mg/ml and 80 mg/ml ethanolic extracts and no significant differences between the 20 mg/ml and 40 mg/ml extracts and that of the 40 mg/ml and 80 mg/ml extracts of Kuroda. There were significant differences between the 20 mg/ml and 40 mg/ml extracts and the 20 mg/ml and 80 mg/ml extracts of Amazonia but, no significant difference between the 40 mg/ml and 80 mg/ml extracts. A Bonferroni correction conducted on the significant differences between Kuroda and Amazonia's extracts that showed significant differences indicated no significant difference

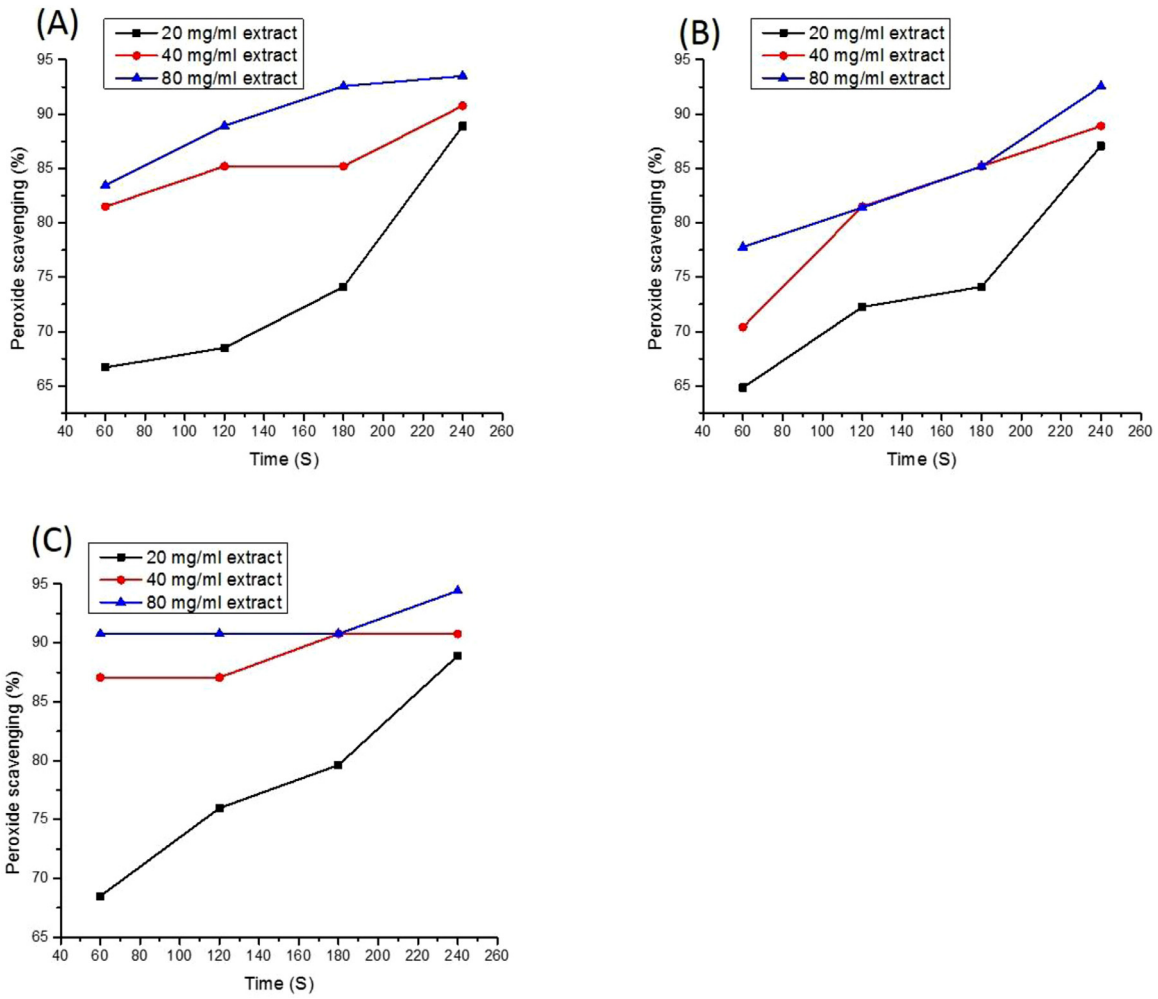

Figure 1. Peroxide scavenging for (A) Pamela, (B) Kuroda and (C) Amazonia carrots varieties cultivated in Ghana.

Table 3
One-way ANOVA of antioxidant properties between individual carrot varieties at different extract concentrations.

Carrot variety	*Source of Variation*	*SS*	*df*	*MS*	*F-value*	*P-value*	*F crit*
	Between Groups	487.325	2	243.66	5.33	0.030	4.26
Kuroda	Within Groups	411.285	9	45.70			
	Total	898.611	11				
	Between Groups	198.778	2	99.39	1.57	0.26	4.26
Pamela	Within Groups	568.46	9	63.16			
	Total	767.238	11				
	Between Groups	402.392	2	201.20	7.56	0.012	4.26
Amazonia	Within Groups	239.612	9	26.62			
	Total	642.003	11				

SS- Sum of squares
df-degrees of freedom
MS- Mean square

between them. There was also no significant difference between the different varieties of similar extract concentrations (Table 4). This statistical analysis implies that the preference of a particular variety of carrot among the three varieties with respect to their antioxidant properties is of little significance since any of the varieties is suitable. Studies carried out by Sun et al. [40] on the antioxidant activities of seven varieties of carrots with different colours showed high antioxidant activities in all the varieties, although purple carrots had the highest antioxidant capacity and antioxidant content.

Table 4
One-way ANOVA of antioxidant properties between carrot varieties at different extract concentrations.

Concentration of extract	*Source of Variation*	*SS*	*df*	*MS*	*F-value*	*P-value*	*F crit*
20 mg/ml extract	Between Groups	36.38	2.00	18.19	0.21	0.81	4.26
	Within Groups	777.00	9.00	86.33			
	Total	813.39	11.00				
40 mg/ml extract	Between Groups	110.52	2.00	55.26	1.99	0.19	4.26
	Within Groups	249.50	9.00	27.72			
	Total	360.02	11.00				
80 mg/ml extract	Between Groups	118.20	2.00	59.10	2.76	0.12	4.26
	Within Groups	192.86	9.00	21.43			
	Total	311.06	11.00				

SS- Sum of squares
df-degrees of freedom
MS- Mean square

Table 5
Linear regression of the antioxidant activities of the ethanolic extracts with time.

Carrot variety	*EtOH Extracts*	*SS*	*MS*	*F-value*	*P-value*	*R Square*
Kuroda	20mg/ml	261.15	261.15	11.97	0.07	0.86
	40 mg/ml	38.75	38.75	14.99	0.06	0.88
	80 mg/ml	57.36	57.36	21.99	0.04	0.92
Pamela	20mg/ml	234.89	234.89	21.82	0.04	0.92
	40 mg/ml	175.47	175.47	21.48	0.04	0.91
	80 mg/ml	116.16	116.16	57.01	0.02	0.97
Amazonia	20mg/ml	210.54	210.54	79.97	0.01	0.98
	40 mg/ml	11.01	11.01	8.00	0.11	0.80
	80 mg/ml	6.03	6.03	3.00	0.23	0.60

SS- Sum of squares
df-degrees of freedom
MS- Mean square

A linear regression analysis of the antioxidant activities of the various EtOH extracts of the carrot varieties with time (Table 5) showed that for Kuroda, there was only a significant linear relationship ($P<0.05$) between the antioxidant activity of the 80 mg/ml EtOH extract and time. For Pamela, all three extracts showed significant linear relationships with time, and only the 20 mg/ml EtOH extract of Amazonia showed a significant linear relationship with time. This result implies that, while increasing time increased scavenging activity in the extracts, there were strong correlations between 80 mg/ml EtOH extract of Kuroda, all EtOH extracts of Pamela and 20 mg/ml EtOH extract of Amazonia and time.

The carotenoids, flavonoids and anthocyanins in the carrot contribute immensely to their antioxidant properties [9]. Therefore, carrot intake is vital and should be encouraged since it acts as a potent antioxidant and can reduce oxidative stress and prevent cardiovascular diseases and cancer.

Conclusion

In this study, the nutritional and antioxidant properties of three varieties of Daucus *carota* species, namely, Kuroda, Pamela and Americana, were determined. The three varieties had high moisture content and were generally rich in proteins and low in carbohydrates. The nutritional composition increased among the carrot varieties in the order Amazonia > Kuroda > Pamela.

The antioxidant properties were high even at low concentrations, and their activity increased with time. The trend of antioxidant activity in the carrot varieties was similar to their nutritional composition. This study will inform the general public, dieticians, nutritionists and other health practitioners in Ghana, Africa and beyond about the nutritional and antioxidant benefits of carrot. Dietitians and Nutritionists in both clinical and community settings can better utilize carrot in formulating therapeutic diets and nutraceuticals based on the outcome of this research.

Data availability statement

Data is available upon request

Public interest statement

Carrot is a common vegetable grown and eaten across the world. This study explores the nutritional composition and antioxidant properties of three common varieties (Kuroda, Pamela and Amazonia) of carrot cultivated in Ghana, a West

African country. The outcome of this study will inform stakeholders such as farmers, nutritionists and policymakers on the carrot varieties to cultivate for different nutritional purposes. It will also provide baseline information for further research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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