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Study on physico-chemical properties, antioxidant activity and shelf stability of carrot *(Daucus carota)* and pineapple *(Ananas comosus)* juice blend

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Abstract

The pineapple-carrot juice is a fruit juice blend developed to provide a new variety of shelf-stable drink for beverage market. Fruit and vegetables consumption constitute a major portion of diet in many parts of the world and they play a significant role in human nutrition. Juice blending is becoming a popular method of improving the nutritional quality of fruit based drink products. This study was carried out to produce a drink beverage from juice blend of carrot *(Daucus carota)* and pineapple *(Ananas comosus)* of different formulation of 100% pineapple juice (T_1), 100% carrot juice (T_2) and pineapple-carrot blend at ratio 2:1(T_3) and 1:1 (T_4) respectively. The Juice drinks produced were analyzed for its physico-chemical properties, antioxidant activity, sensory and microbial qualities. The result of analysis shows that mean PH value for T_1, T_2 T_3 and T_4 was 3.90, 4.30, 4.00 and 3.94 respectively. The mean total soluble solid for (T_1)was 13.8% and 3.60% for (T_2).The 100% pineapple (T_1) has the least beta-carotene content of 0.932mg/100ml which is significantly lower ($p<0.05$) than (T_2), (T_3) and (T_4) respectively. The microbial loads of the fruit juice were below 106cfu/ml, thus, within acceptable limit for human consumption.

Introduction

Fruits and vegetables are among the most important foods of mankind as they are not only nutritive but are also indispensable for the maintenance of health (Wong *et al.*, 2003). They play important roles in the diet of most people in the tropics, providing essential minerals and vitamins and adding colour, flavour and variety to diet (Ragaert *et al.*, 2004). Several reports have shown that adequate intake of fruits and vegetables form an important part of a healthy diet and low fruit and vegetable intake constitute a risk factor for chronic diseases such as cancer, coronary heart disease, stroke and cataract formation (VanDuyn and Pivonka, 2000).

According to the 2007 World Health Report unbalanced diets with low fruit intake and low consumption of dietary fiber are estimated to cause some 2.7 million deaths each year, and were among the top 10 risk factors contributing to mortality (Dias, 2011). Although, the exact mechanisms by which vegetable consumption reduces human diseases have not yet been fully understood, however the general consensus among physicians and nutritionists is that phytonutriceuticals in vegetables are responsible for mitigating some of these diseases (Dias, 2011).

Fruits and vegetables are abundant during their various seasons, with over 50% lost to wastage due to deterioration under tropical conditions due to the high ambient temperatures, humidity, pest and disease infestations, poor handling and storage facilities (Aworh and Olorunda, 1988). In 1998, World Resources Institute reported that not only are losses clearly a waste of food, but they also represent a similar waste of human effort, farm inputs, livelihood and investments. It implies that fruits, most often, do not attain their maximum market value thereby leading to less return to the grower as an individual and economic loss to the nation as a whole. Processing of fruits and vegetables to juices and other value-added products are the alternative ways in which excess fruits and vegetables can be utilized to reduced wastage and bring economic returns to farmers (FAO, 2011).

Carrot *(Daucus carota)* is a worldwide root vegetable that is highly nutritional, and an important source of b-carotene besides its appreciable amount of vitamins and minerals, it is often used for juice production (Walde *et al.*, 1992; Demir *et al.*, 2004). In recent years, a steady increase of carrot juice consumption has been reported in many countries (Schieber *et al.*, 2001). Among common fruits and vegetables, carrots are high in fibers, carotenoids, vitamins C and E, and phenolics compounds (Alasalvar *et al.*, 2001). Oral intake of carrot juice has some beneficial physiological effects which include

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reduced oxidative DNA damage (Pool-Zobel *et al*., 1998) and increased levels of plasma antioxidants (Törrönen *et al*., 1996). Carrots hold an important place in the nutrition of the Western industrial nations because of their high dietary value and generally good storage attributes (Berger *et al*., 2008). Carrots have been ranked tenth in terms of their nutritional value among 38 other fruits and vegetables, and seventh for their contribution to nutrition (Alasalvar *et al.*, 2005).

Pineapple *(Ananas comosus)* is one of the most popular tropical non-citrus fruit, mainly because of its attractive aroma, refreshing flavour, and its balance between acidity and sweetness (Bartolomé *et al*., 1995). Its juice has been used in based fruit beverages individually, in the form of mixture, or combined with other fruit juices. As an ingredient, the concentrated pineapple juice blends well with other aromas of fruits resulting in a pleasant product with a competitive market price available to consumers of all ages. It stands out because of its energetic value on account of its high composition of sugars and nutritional value due to the presence of mineral salts - calcium, phosphorous, magnesium, potassium, sodium, copper, and vitamins (Cabral *et al.*, 2005). The pineapple –carrot fruit drink blend is not available on Nigeria supermarket shelf. The study is aim to develop a fruit juice drink from the juice blend of carrot and pineapple fruit; evaluate its nutritional composition and shelf –stability.

Materials and Methods

The fully matured, freshly harvested pineapple and carrot were procured from the Oje market which is a popular local fruit market in Ibadan and brought to National Horticultural Research Institute (NIHORT) for processing.

Juice preparation

The carrots were washed with tap water, peeled and sliced into smaller pieces (3-5 mm), then washed again in tap water. This was followed by blanching in hot water at 85^0Cfor 5min then cooled in cold water to inactivate enzymes that can cause spoilage and soften their tissues. They were extracted using juice extractor (Breville, model JE15 Breveill, USA) with addition of distilled water 1:1 (v/w). Sodium benzoate (300 ppm) was added as preservative. The pineapples were washed with tap water, peeled and blanched in hot water at 85^OC. The pineapple was extracted using manual juice extractor. After that the juice of pineapple and carrot was blended in different ratios of 100% pineapple juice, 100% carrot juice, pineapple-carrot at ratio 2:1 and 1:1 respectively. The products were stored at room temperature ($28+0.02^O$C) for subsequent analysis for period of 60 days at 15days intervals.

Physico-chemical analysis

Total soluble solids (TSS) were assayed using the refractometric method, Total soluble solid (TSS) was determined using hand refractometer (ATAGO - ATC1, Atago Co. Ltd., Tokyo, Japan).Total acidity (as % citric acid) was determined by titrimetric method (Ranganna 1986). Acidity was measured by titrating samples with 0.1 mol L^{-1} of NaOH solution up to pH 8.2, and was expressed as citric acid per 100 ml of juices. The values for pH were measured by pH meter (AOAC, 1990).

Vitamin C determination

The ascorbic acid was determined by iodine titration method (AOAC. 2000). The 10 ml of juice sample were taken in 250 mL conical flask, and then 75 mL of distilled water and 0.5 mL of starch indicator were added. The sample was titrated with 0.1 mol L^{-1} iodine solution. The endpoint of the titration was identified as the first permanent trace of a dark blue-black color due to the starch-iodine complex. The amount of ascorbic acid was expressed in mg/100 mL of juice.

Beta carotene content determination

The beta-carotene content was estimated following the procedure of (Sharma *et al*., 2009). The 25mg of beta-carotene was weighed and dissolved in 2.5 mL of chloroform and diluted to 250 mL with petroleum ether. Further, this solution was diluted with petroleum ether. The final concentrations of standards were 2, 10, 20, 30, 40 and 50 mg$\cdot L^{-1}$. The absorbance was measured at 452 nm, using 3% of acetone in petroleum ether as blank. The beta-carotene content in the juice sample was calculated using the standard curve.

Antioxidant activity

The antioxidant activities of juices were measured using DPPH ((2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging method. The DPPH assay was carried out following the method reported by (Burits *et al*., 2000). Various amounts of the samples dissolved in methanol were added to 5 mL of a 0.004% methanol solution of DPPH. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm (Hatano *et al.*, 1988). Vitamin C was used as positive controls. Percent inhibition of free radical DPPH (I %) was calculated as follows

Table 1. Results of the physico-chemical analysis

	pH				TSS				TA			
days	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T3
0	3.5±0.05^{c}	4.4±0.05^{e}	4.1±0.05^{a}	4.0±0.04^{a}	14.0±0.04^{a}	4.0±0.05^{a}	9.0±0.04^{c}	10.0±0.05^{e}	4.4±0.10^{a}	2.2±0.00^{a}	2.50±0.0^{c}	3.2±0.00^{a}
15	3.5±0.05^{c}	4.4±0.05a	4.1±0.05^{b}	3.9±0.05^{b}	13.5±0.05^{a}	3.5±0.04^{d}	9.2±0.05^{c}	10.0±0.05^{b}	4.2±0.01^{a}	2.1±0.10^{d}	2.50±0.0^{c}	3.2±0.00^{b}
30	3.5±0.05^{c}	4.4±0.04^{a}	4.0±0.05^{b}	3.9±0.04^{b}	13.5±0.04^{a}	3.5±0.05^{d}	9.2±0.04^{c}	10.0±0.05^{b}	4.4±0.00^{a}	2.1±0.00^{d}	3.30±0.1^{c}	3.5±0.00^{b}
45	3.4±0.04^{d}	4.3±0.05^{a}	4.0±0.05^{b}	3.9±0.05^{b}	13.5±0.05^{a}	3.5±0.04^{d}	9.2±0.05^{c}	10.0±0.05^{b}	4.4±0.00^{a}	2.3±0.10^{d}	3.30±0.0^{c}	3.5±0.10^{b}
60	3.5±0.05^{c}	4.4±0.05^{a}	4.0±0.05^{b}	3.9±0.04^{b}	13.5±0.04^{a}	3.5±0.05^{d}	9.2±0.05^{c}	10.0±0.04^{b}	4.4±0.10^{a}	2.3±0.00^{d}	3.30±0.0^{c}	3.5±0.00^{b}

Data are a mean ± standard deviation of triplicate determination. Values in the same row with different superscripts are statistically significant ($p<0.05$)

$$I\ \% = (A_{control} - A_{sample} / A_{contro}l) \times 100.$$

Sensory evaluation

Sensory evaluation test on the samples was done with a panel of 20 semi-trained members using 7-point Hedonic scale, where 7 is "like very much" and 1 is "dislike very much". The parameters evaluated includes- colour, taste, odour, and over-acceptability respectively.

Microbial load evaluation

The microbial load was evaluated by method of (Speck, 1979).The samples were shaken vigorously to mix up well. From each of the samples, aliquots of 1.0ml was collected and inoculated into 9 ml of peptone water in screw caped test tubes for pre-enrichment of organisms Incubation of tubes was for 24 hours at 37^{0}C. Samples (0.2ml) was inoculated by spread plate methods onto Nutrient agar (Difco), MacConkey agar (Oxoid) and Sabauroud Dextrose agar (Fluka,Germany) for total viable count, coliform count and fungal count respectively. Plates were incubated for 24 - 48hours at 37^{0}C for colony formation, except however, Sabouraud Dextrose agar (SDA) that was left for 24-72 hours at 28 ± 2^{0}C. At the expiration of the incubation time, the colonies were counted using colony counter (StuartScientific, UK).

Statistical analysis

Analysis of variance (ANOVA) was carried out by using the software SPSS 16 (Chicago IL). Significant differences ($p<0.05$) were detected using Duncan"s multiple range tests. Values expressed are means ±standard deviation of triplicate measurements

Results and Discussion

Physico-chemical analysis

The results of the pH, TSS and TA value of different formulations of pineapple-carrot juice blend is presented in (Table 1). The pH value of a preserved product is becoming increasingly recognized for its important contribution to product quality. This is because it plays a key role in prevention of microbial spoilage. The mean pH value of the products as presented in (Table 1) ranged between 3.50 and 4.40 which are within the range of 3.0 to 5.0 for fruit and vegetable juices as reported by (Harris *et al*., 1991). There is no significance difference at ($p<0.05$) in pH value of T_3 and T_4 for the 60 days period of shelf -life study. Thus, the low pH observed during period could be one of the contributing factors responsible for shelf–stability of the products. The Total soluble solid (^{0}brix %) which is also a measure of sweetness varies among the different formulations of the juice blend. The 100% pineapple juice (T_1) has the highest means brix% value of 13.80% while T_2, T_3 and T_4 were 3.60, 9.20 and 10.00% respectively. The results shows there is a significance difference between the total soluble solid (^{0}brix %) value of unblended pineapple (T_1) and carrot (T_2) at ($p<0.05$).

Beta-carotene and vitamin C content

The result of vitamin C and b-carotene and vitamin C is presented in (Table 2). Beta- carotene, a carotenoid is an important phytonutrients useful for human health (Castermiller and West, 1998). The main physiological function of carotenoids is as precursor of vitamin A (Nocolle *et al.*, 2003). The beta-carotene content of the products ranged from 0.932 to 5.55mg/100ml. The 100% pineapple juice (T_1) has the lowest beta-carotene value of 0.932mg/100ml which is significantly lower at

Table 2. Results of beta-carotene and vitamin C

	T_1		T_2		T_3		T_4	
days	b-carotene (mg/100ml)	Vitamin C(mg/100ml)	b-carotene (mg/100ml	Vitamin C(mg/100ml)	b-carotene (mg/100ml)	Vitamin C(mg/100m)	b-Carotene (mg/100ml)	Vitamin C(mg/1001)
0	0.950±0.47^{e}	45.84±0.00^{a}	9.34±0.05^{a}	23.56±0.47^{e}	4.94±0.05^{e}	30.81±0.05^{c}	3.86±0.00^{c}	36.91±0.47^{e}
15	0.950±0.05^{d}	45.82±0.05^{a}	9.22±0.47^{a}	23.25±0.05^{d}	4.83±0.47^{b}	28.82±0.05^{c}	3.72±0.05^{c}	34.71±0.05^{b}
30	0.934±0.05^{c}	38.89±0.47^{a}	4.17±0.05^{a}	19.71±0.47^{d}	3.24±0.47^{b}	24.54±0.47^{c}	3.05±0.47^{b}	28.61±0.05^{b}
45	0.920±0.00^{c}	34.91±0.05^{a}	3.01±0.47^{a}	17.43±0.05^{d}	2.97±0.05^{b}	22.440±.47^{c}	2.89±0.05^{b}	25.84±0.05^{b}
60	0.910±0.05^{d}	27.92±0.05^{a}	2.01±0.05^{c}	17.23±0.05^{d}	2.30±0.05^{b}	20.30±0.05^{c}	2.50±0.05^{a}	23.24±0.00^{b}

Data are a mean ± standard deviation of triplicate determination. Values in the same row with different superscripts are statistically significant ($p<0.05$)

Table 3. The result of antioxidant activity (DPPH-Assay)

	Antioxidant Activity $_{DPPH}$(%)			
Days	T1	T2	T3	T4
0	81.07+0.01^{a}	29.61+0.01^{d}	33.30+0.05^{c}	54.16+0.13^{b}
15	60.30 ±0.12^{a}	26.31±0.05^{d}	30.88±0.01^{c}	52.20±0.01^{b}
30	42.69 +0.05^{a}	26.31+0.05^{d}	25.88+0.13^{c}	44.13+0.13^{b}
45	36.67± 0.13^{a}	26.41±0.05^{c}	18.55±0.05^{d}	32.02±0.01^{b}
60	34.60 ±0.00^{a}	26.41±0.01^{c}	18.45±0.01^{d}	32.02±0.13^{b}

Data are a mean ± standard deviation of triplicate determination. Values in the same row with different superscripts are statistically significant ($p<0.05$).

($p<0.05$) than T_2, T_3 and T_4 respectively. However the addition of carrot juice to pineapple increase significantly its beta-carotene content at ($p<0.05$). This suggests therefore that blending of two or more fruit juices together to form a blend could improve the nutrient of a product. Sistrunk and Morris (1985) reported that the blend of apple and grape juices were highly acceptable in quality and retained acceptable flavour and colour during storage at 24^{o}C for 12 months. The gradual decrease in beta-carotene as observed in result presented in (Table 2) may be due to changes in surrounding storage temperature. This gradual decrease in beta-carotene during storage was also observed by (Awsi, 2012) in the study of quality evaluation of pineapple juice blended with orange and carrot juice.

Vitamins have some special roles which are essential for life and most of them are not produced by the body. Vitamin C, which is naturally present or added to most juices, is necessary for the body to form collagen, cartilage, muscle and blood vessels, and aids in the absorption of iron (Levine *et al*., 1993). The means ascorbic acid content as evaluated was 38.68, 20.23, 25.38 and 29.86mg/100ml for T_1, T_2, T_3 and T_4 respectively. The 100% pineapple juice (T_1) has the vitamin C content of 38.68mg/100ml which is significantly higher at ($p<0.05$) than unblended carrot (T_2).The consumption of pineapple –carrot blend could provide a measure of Vitamin C needed by the body for Its physiological functions.

Antioxidant activity

Antioxidants are secondary constituents or metabolites found naturally in the body and in plants such as fruits and vegetables (Hollman, 2001). Plant-based dietary antioxidants are believed to have an important role in the maintenance of human health because our endogenous antioxidants provide insufficient protection against the constant and unavoidable challenge of reactive oxygen species (Fridovich, 1998). The means antioxidant activity of T_1, T_2, T_3, and T_4 was 51.07, 27.03, 25.41 and 42.96 (Table 3). The antioxidant activity value of 100% pineapple (T_1) was significantly higher than Carrot (T_2) at ($p<0.05$). This provides the basis for the blending of the two or fruits and vegetables together for complimentary of nutrient of and functions.

Table 4. Result of sensory evaluation

	T_1	T_2	T_3	T_4
Colour	6.00± 1.25^a	4.20 ±1.42^d	5.60± 1.07^c	5.90± 1.19^b
Taste	6.10± 0.99^a	3.64± 0.69^d	3.90 ±1.19^c	5.80± 0.91^b
Aroma	6.20 ± 0.63a	4.00± 1.05^d	5.04± 0.94^c	5.50± 1.17^b
OA	6.30± 0.94^a	3.70 ±0.82^d	5.0 ± 0.94^c	5.40± 1.17^b

OA= Overall acceptability.
Results are means + Standard deviation (SD) of evaluation score of 20 semi-trained panelists. Values in the same row with different superscripts are statistically significant ($p<0.05$)

Table 5. Result of microbiological evaluation

	Day 0			Day 15			Day 30			Day 45			Day 60		
Days	TVC	TFC	TCC	TVC	TFC	TCC	TVC	TFC	TCC	TVC	TFC	TCC	TVC	TFC	TCC
T_1	0.03 x10^3	0.02 x10^3	ND	0.08 x10^3	0.06 x10^3	ND	0.18 x10^3	0.15 x10^3	0.01 x10^3	0.29 x10^3	0.27 x10^3	0.03 x10^3	0.51 x10^3	0.40 x10^3	0.08 x10^3
T_2	0.03 x10^3	0.01 x10^3	ND	0.09 x10^3	0.04 x10^3	ND	0.13 x10^3	0.09 x10^3	ND	0.25 x10^3	0.20 x10^3	0.01 x10^3	0.49 x10^3	0.30 x10^3	0.01 x10^3
T_3	0.05 x10^3	0.03 x10^3	ND	0.09 x10^3	0.04 x10^3	ND	0.15 x10^3	0.11 x10^3	ND	0.22 x10^3	0.19 x10^3	0.01 x10^3	0.45 x10^3	0.30 x10^3	0.01 x10^3
T_4	0.04 x10^3	0.03 x10^3	ND	0.07 x10^3	0.03 x10^3	ND	0.16 x10^3	0.11 x10^3	ND	0.24 x10^3	0.17 x10^3	0.01 x10^3	0.44 x10^3	0.27 x10^3	0.01 x10^3

All counts as colony forming units (cfu) per ml of juice.
TVC – Total Viable Count
TFC – Total Fungal Count
TCC – Total Coliform Count
ND – None detected.

Sensory evaluation

The mean scores sensory result for the products with respect to color, aroma, taste, and overall acceptability is represented in (Table-4). The taste values ranged between 6.10 to 3.64. The 100% pineapple (T_1) has the highest taste value of 6.10 out of 7 point which is significantly higher ($p<0.05$) than unblended carrot juice (T_2).The unblended (T_1) was preferred to unblended carrot (T_2) due to its pleasant and refreshing taste.

Microbial load

The microbial load evaluation was done to ascertain the safety of the product for human consumption. The result of microbial loads observed for period of 60 days is presented in (Table 5). Microbial contaminants of the fruit juice were below 10^6cfu/ml thus within acceptable limit for human consumption (ICMSF, 1974). The addition of Benzoic acid as preservative coupled with the low pH of the juice could be some of the factors responsible for keeping the microbial load in check within acceptable level. The fungal count in all the products is within the range of $10^3 – 10^5$(cfu) per ml. This finding is similar in range to the result observed in assessment of microbiological quality of some commercially packed and fresh fruit juices available in Dhaka City (Rahman *et al.* 2011).The results of microbial load suggest that the products were fit for consumption.

Conclusions

The study shows the blending of carrot and pineapple juice to have a product with an improved nutritional and increased shelf-life quality. The analysis of the products revealed possible nutrients available on consumption. The pineapple- carrot blend could be stored successfully for period of 60 days at room temperature without any appreciable decrease in quality. Hence, this product could be a good addition to varieties of commercially produced

non-alcoholic beverages found in our stores and supermarkets. However, the principles of hygiene should be followed in the production to prevent microbial contamination that can affect its safety for consumption.

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