

# Changes in Physico-Chemical Quality and Volatile Compounds of Orange-Carrot Juice Blends During storage

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## Abstract

The present study aimed to determine the changes in physico-chemical parameters (pH, total soluble solids “TSS”, total acidity, vitamin C, total carotene, total phenolic and antioxidant activity) as well as sensory evaluation and volatile constituents of the orange juice samples mixed with carrot juice at ratios (1:3, 3:1 and 1:1). While, the polyphenol content was determined using Folin–Ciocalteu, antioxidant activity was measured using two in vitro assays 2,2'-diphenyl-1-picrylhydrazyl (DPPH<sup>0</sup>) and metal chelating assays. While, the acidity increased, total carotene and pH of the juice decreased during the storage period. There were no significant changes in total phenolics throughout the storage period at the three blending ratios. A slight increase in antioxidant capacity during the storage period had been observed. The headspace volatiles of fresh orange and carrot juices as well as fresh and stored blend juice with ratio (1:3) for 14 days at refrigerator were collected and subjected to Gas chromatography (GC) and Gas chromatography-Mass spectrometry (GC-MS) analysis. A total of 26 volatile compounds were identified in samples of fresh orange and carrot juices as well as fresh and stored blend including 7 alcohols, 4 aldehydes, 9 monoterpene hydrocarbon and 6 esters. Limonene was the one most abundant monoterpene, representing in orange, carrot, fresh and stored blend 47.38, 42.37, 39.24 and 37.25%, respectively.

**Keywords:** Orange- carrot juice, Blend, Antioxidant, Volatile compounds

## 1. INTRODUCTION

The health benefits associated with drinking fruit juices on a daily basis are related to the ingestion of bioactive components such as essential vitamins and polyphenolic compounds. Accumulating evidence demonstrates that polyphenols in fruits are effective agents against degenerative diseases of humans such as cancer, inflammation, atherosclerosis and aging (Leja *et al.*, 2013). Furthermore, natural compounds in fruits and vegetables such as polyphenols, flavonoids and tannins have shown very promising results in combating bacteria, fungus and viral infections (Rosnah *et al.*, 2012).

Consumer demand for functional foods has led to the processing of derivatives of orange juice, refrigerated mixed fruit and vegetable juices. These products supply antioxidants, vitamins, nutritive and functional compounds. It is clear that, in addition to the particular excellent sensory and nutritive characteristics of orange juice, the incorporation of a proportion of carrot provides a considerable contribution to the health of the consumer since oranges have high vitamin C content, and carrots have the highest content of carotenoids of fresh foods (Campos *et al.*, 2010).

Citrus juices contain a high level of carotene but not all are precursors of vitamin A. The mixture of orange juice and carrot juice is rich in antioxidants and therefore is a rich dietetic source of them. Orange juice is a very important source of ascorbic acid, a nutrient that, besides its vitamin action, is valuable for its antioxidant effect, stimulation of the immune system and other health benefits which are being actively investigated and reported, such as inhibition of formation of cancer-causing N-nitroso compounds in the stomach (Bezman *et al.*,

2001).

Carrots (*Daucus carota*) are major vegetables in diets worldwide mainly due to their pleasant flavour (Vervoort *et al.*, 2013) and perceived health benefits (it is good for eye disorders, skin care, nervous disorders and indigestion), which have been associated with their vitamin, mineral and dietary fiber content. Carrot juice is rich in vitamin A,  $\beta$ -carotene, minerals such calcium, potassium, and it is easier to digest than raw or cooked vegetables only (Verbeyst *et al.*, 2012).

Juice blending is one of the best methods to improve the nutritional quality of the juice. It can improve the vitamin and mineral content depending on the kind and quality of fruits and vegetables used (De-Carvalho *et al.*, 2007). Apart from nutritional quality improvement, blended juice can be improved in its sensory and flavour characteristics according to their raw materials. Furthermore, there has been no detailed report on the volatile compounds in blended carrot-orange juice. Therefore, in this study, physico-chemical and volatile compounds assessment of blended juice with various proportions of orange and carrot juice was carried out to determine the most acceptable blend organoleptic properties and studied shelf life of such a blend.

## MATERIALS AND METHODS

### Plant materials

The freshly picked large mature orange and carrots were purchased from private farms at El-kalubia and El-sharkia governorates, respectively, Egypt during the seasons of 2013. The fruits had good maturity, colour and were free from any defects or spoiled parts by microorganisms or injury.

### Juice samples preparation and their blends

Unblemished orange (*Citrus Sinesis*) and carrots (*Daucus carota*) were selected, washed and processed for juices separately using a commercial juice extractor. The oranges were cleaned with tap water, peeled and then orange juice was extracted using juice blender (Citromatic Deluxe MPZ-22 Braun, Spain for orange juice).

The carrots were washed with tap water, and peeled using Sodium hydroxide (40 g/L) at 95°C for 1 min then washed again in tap water. This was followed by blanching in citric acid solution (60 g/L) at 95°C for 5 min then cooled in iced water to inactivate their endogenous enzymes and soften their tissues. At the end, they were sliced and grounded with addition of distilled water 1:1 (v/w) and filtered on cheese cloth under vacuum to get fresh juice.

Carrot and orange juices were blended at various ratios of 1:1, 1:3 and 3:1, respectively.

### Storage conditions

The treated juice samples were packaged in 100 ml sterilized glass bottles at about 100 °C for 10 minutes. They were stored in refrigeration; samples were subjected for analysis at different intervals (zero, 2, 4, 8 and 14) days of storage.

**Chemicals:** All chemicals used for experiments were of analytical grade and procured from Sigma Merck, Aldrich and Fluka. For the determination of kovat indices, a hydrocarbon mixture (Supelco, Bellefonte, PA, USA) ranging from C<sub>6</sub>-C<sub>22</sub> was used.

### Juice Characterization

#### **pH, °Brix, Total acidity (TA) and minerals content**

The pH was determined with a digital pH meter (Hanna pH-meter HI 9021 m Germany) at 20 °C. The °Brix was determined by measurement of the refractive index with a digital refract meter (JEN way) at 20 °C.

Total acidity was determined by means of a potentiometric titration of the acidity of the juice, with a solution of 0.1mol/l NaOH up to pH = 8.1. It was determined by means of a three replicates. The results were expressed as g/100ml with reference to citric acid (Kimball, 1999). Potassium, magnesium, sodium, calcium,

iron, copper and manganese were determined using perking Elmer 2380, atomic absorption spectrophotometer according to the method of **AOAC (2004)**.

#### **Ascorbic acid**

Ascorbic acid was determined by the direct colourimetric method using 2, 6- dichlorophenol-indophenols as decolourizing agent by ascorbic acid in juice sample (**AOAC, 2004**). The results are expressed as mg/100 ml.

#### **Determination of total carotene (TC)**

The measurement of carotenoids was carried out according to the method of (**Liao et al., 2007**) by measuring the  $A_{450}$  (absorbance at 450 nm) at ambient temperature by a UV-Vis Shimadzu Spectrophotometer (UV-1601 PC). The standard curve was drawn using the  $\beta$ -carotene solution at different concentrations and expressed as mg  $\beta$ -carotene/ml of juice.

#### **Total phenolic content (T.PH)**

The amount of total phenolic compounds in studied samples was determined according to the Folin-Ciocalteu procedure (**Singleton et al., 1999**). Total phenolic content data were obtained from the calibration curve prepared with gallic acid at concentrations of 8-80 mg/L and are expressed as gallic acid equivalents (mg GAE/L). Two trays were taken at each sampling time to perform replicate analyses throughout 14 d of storage.

#### **Antioxidant activity measurements**

##### **DPPH<sup>0</sup> radical scavenging activity**

Free radical scavenging activity of methanolic extract for treatment under investigation was determined using the 2,2'-diphenyl-1-picrylhydrazyl (DPPH<sup>0</sup>) method (**Lim et al., 2007**). A methanol solutions (150, 300 and 450  $\mu$ L) containing crude extracts was added to 3.9 ml of freshly prepared DPPH<sup>0</sup> methanol solution (0.1 mM). An equal amount of methanol was used as a control. After incubation for 30 min at room temperature in the dark, the optical density (OD) was measured at 517 nm using a UV-Vis Shimadzu (UV-1601 PC) Spectrophotometer. Scavenging activity (%) was calculated using the following formula:

$$\% \text{ DPPH}^0 \text{ Inhibition} = \frac{[(\text{OD}_{\text{control}} - \text{OD}_{\text{sample}})]}{\text{OD}_{\text{control}}} \times 100$$

BHA and TBHQ were used as a positive control.

#### **Metal chelating assay**

The metal chelating ability of the blended juice was estimated by method of **Dinis et al. (1994)**. Briefly, 50  $\mu$ l of 2 mM FeCl<sub>2</sub> was added to 1 ml of different concentrations of the juice samples. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine solution. The mixture was vigorously shaken and left to stand at room temperature for 10 min. The absorbance of the solution was thereafter measured at 562 nm. The ability of the extract to chelate ferrous ion was calculated using the following equation:

$$\% \text{ chelating effect} = [1 - \text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}}] \times 100$$

#### **Sensory evaluation**

A panel of 10 semi-trained members carried out the overall acceptance test for the juice 9-point Hedonic scale, where 9 is "like extremely" and 1 is "dislike extremely" as described by **Amerine et al. (1965)**.

#### **Volatile compounds analysis**

##### **Volatile isolation**

The volatiles in headspace of treatments under investigation were isolated by using a dynamic

headspace system. The samples were purged for ~3 h with nitrogen gas (grade of N<sub>2</sub> > 99.99 %). The headspace volatiles were swept into cold traps containing diethyl ether and pentane (1:1, v/v) and hold at 10 °C. The solvents containing the volatiles were dried over sodium sulfate anhydrous over night. The volatiles were obtained by evaporation of the solvents under reduced pressure.

### **Gas Chromatography/Mass Spectrometry (GC-MS)**

GC-MS analyses were performed with a Varian (Perkin-Elmer Autosystem gas chromatograph equipped with a DB-5 capillary column (60 m X 0.25 mm X 0.25 µm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperatures were 250 and 240 °C, respectively; oven temperature was programmed from 40 to 250 °C at 3 °C/ min; carrier gas was helium at 1 ml/min; splitless injection.

GC-MS analysis was performed on a Perkin-Elmer quadrupole MS system (Model 910) coupled with the above gas chromatograph, equipped with a DB-5 capillary column and operating under the same conditions described above. The MS operating parameters were: ionization 70 eV; ion source temperature, 230 °C; scan mass range, 40–400 Da.

### **Compounds Identification**

The linear retention index (RI) values for unknowns were determined based on retention time data obtained by analyzing a series of normal alkanes (C<sub>6</sub>-C<sub>22</sub>). Volatile components were positively identified by matching their RI values and mass spectra with those of standards, also run under identical chromatographic conditions in the laboratory (Adams, 2007).

### **Statistical analysis**

Analysis of variance (ANOVA) was carried out by using the software SPSS 16.0. Significant differences ( $P < 0.05$ ) among treatments were detected using Least Significant Difference (LSD) test (Steel and Torrie, 1980). Values expressed are means ± standard deviation of triplicate measurements.

## **RESULTS AND DISCUSSION**

### **Chemical composition of fresh juices**

The chemical attributes of fresh orange and carrot juices are shown in (Table 1); the results showed that carrot was a good source of carotene content and orange a good source of vitamin C content. This confirms the previous researches done on carrot and orange (Esteve *et al.*, 2005; Krishan *et al.*, 2012). The low acidity of carrot around 0.38 and high acidity of orange pH around 1.12 are also important parameters of the raw materials.

Mineral constituents of fruits and vegetables are one of the key quality parameters of the raw material. Mineral content in carrot and orange varied from 0.01 to 11.5 mg/100g and 0.08 to 64.2 mg/ 100g respectively. Potassium was the most abundant element followed by magnesium, calcium and sodium in carrot with 11.5; 10.6; 8.2 and 3.17 mg/100g, respectively. The same distribution in orange juice with the following values respectively; 64.2; 15.3; 14.5; and 8.18 mg/100g. The results showed that orange are rich in calcium which is necessary in bones and teeth build up, and blood clotting. (Dauthy, 1995). Iron helps to build red blood cells and aids the blood in carrying oxygen to the cells. While, Copper is of particular interest since it is a cofactor for PPO and can also serve as a catalyst for numerous oxidative reactions.

### **Main physico-chemical composition of blended orange-carrot juice**

#### **pH**

pH plays an important role in the flavour of the juice product, also acts as a factor of preservation and describes the stability of bioactive compounds in fruit juice. The results pertaining to the response of storage and

different blending ratio on pH are present in **Table 2**.

There was a significant ( $P \leq 0.05$ ) decrease in pH during storage (**Table 2**); this might be due to increase in titrable acidity, as acidity and pH are inversely proportional to each other **Bhardwaj and Mukherjee (2005)**.

It was observed that the maximum pH (3.72) was recorded in the juice blended from orange and carrot juice at ratio (1:3). The obtained results are in contrast with **Rivas et al. (2006)**, who reported no pH variations, in thermally treated juice (blended orange and carrot juice) during refrigerated storage at 2 and 12 °C. **Yeom et al. (2000)** also did not observe significant changes in heated orange juice during storage at 4 and 22 °C. A similar study that described the pH of thermally processed Valencia and Navel orange juice found no significant modifications during storage at 4 and 10 °C (**Bull et al., 2004**). On the other hand, our results are in agreement with **Nidhi et al. (2008)** who found slight decrease in pH was observed in ready-to-serve bael-guava blended beverage during 60 days of storage.

### **Titrrable Acidity (TA)**

Organic acids are found in foods as a result of biochemical processes, or in the case of fermentations, through the development of certain microorganisms. These acids contribute to the particular flavour and palatability of each juice, modifying the degree of sweetness of the sugars present and also acting to preserve the juice from spoilage. There was a significant increase in titratable acidity content during storage of all blends rations (**Table 2**). This might be due to conversion of acids into salts and sugars by enzymes particularly invertase. Maximum value was observed at the end of storage with blending ratio 3:1 of orange-carrot.

**Safdar et al. (1999)** observed gradual increase in acidity during storage of tomato concentrate at three different temperatures. The rise in acidity may be explained by the fact that the concentration of weakly ionized acid and their salts increased during storage. Another explanation for the acidity increase due to formation of acid by degradation of polysaccharides and oxidation of reducing sugars or by breakdown of pectic substances. Similar views were expressed by **Iqbal et al. (2001)**, who reported that gradual increase in acidity, which may be due to degradation of pectic substances and formation of uronic acid. Statistical analysis of the data revealed significant ( $P < 0.05$ ) effect of various blending ratio and storage period on titrateable acidity of orange-carrot blends. Similar results were reported by **Bajwa et al. (2002)** who found that acidity in citrus juices increased and pH decreased during processing and storage.

### **Total Soluble Solids (TSS)**

Retention or minimum increase in total soluble solids content of juice during storage is desirable for preservation of good juice quality. The total soluble solids increased with gradual passage of storage time (**Table 2**), which might be due to hydrolysis of polysaccharides into monosaccharide and oligosaccharides or consumption of sugars as a result of the onset of fermentation. The results revealed that the total soluble solids were significantly ( $P \leq 0.05$ ) affected by blending ratio and storage period.

Similar trend of increase in total soluble solids with advancement of storage period were observed in mandarin, sweet orange and lemon juice by **Bhardwaj and Mukherjee (2005)** who reported that the minimum increase in total soluble solids (12.50 to 13.38<sup>0</sup> Brix), when juice was blended with kinnow juice (87%) + pomegranate juice (10%) + ginger juice (3%) as compared to (12.00 to 13.67<sup>0</sup> Brix) kinnow juice (100%). Also, **Deka (2000)** found an increasing trend in total soluble solids during storage at ambient and low temperature in lime-aonla and mango-pineapple spiced ready-to-serve (RTS) beverages. However, the rate of increase was more at ambient temperature (12.5–36<sup>0</sup> C) as compared to low temperature (4<sup>0</sup> C).

### **Ascorbic acid**

Ascorbic acid is excellent natural antioxidants that participate in the prevention of degenerative

illnesses. The main source of vitamin C for the consumers is citrus fruit, it is extremely labile, a characteristic that makes the concentration of this vitamin in foods a guarantee that other vitamins and nutritive elements are still present, and it is considered a control parameter for the nutritional quality of the finished product (**Bull et al., 2004**). The vitamin C concentrations in the studied blends (**Table 2**) were higher than the minimum values recommended for industrially processed orange juices (40 mg/100 mL) (**Lopez 1995**).

It decreased during storage with the advancement of storage period (**Table 2**), which was probably due to the fact that ascorbic acid being sensitive to oxygen, light and heat was easily oxidized in presence of oxygen by both enzymatic and non-enzymatic catalyst. These findings are in conformity with the studies of **Jain et al. (2005)** in Indian gooseberry juice blends. **Inyang and Abah (1997)** reported that the extracted juice of cashew apple when blended with various proportions of sweet orange juice, their ascorbic acid losses decreased after mixing of juice as compared to the juice of cashew apple alone.

#### **Total carotene (TC)**

The carotenoids are one of the major sources of colour and human nutrition as some of them convert into vitamin A. Linear decrease in carotene content was found under investigated samples (**Table 2**); these results are in agreement with **Gowda and Jalali (1995)** who observed that there was significant reduction in the carotenoid content in the RTS beverage prepared from the banganpalli variety of mango was 0.42 mg/100 g juice, while in the RTS beverage prepared from dashehari mango was 0.66 mg/100 g juice.

**Tandon et al. (2007)** reported that the addition of papaya RTS beverage with bael RTS beverage increased the carotenoids content. They also observed decrease in the carotenoids content of papaya-bael juice blend around 11–55% after six months of storage. **Deka et al. (2005)** reported that the total carotenoid content decrease minimally over a period of six months storage in mango-pineapple spiced beverage.

#### **Total Phenolics**

Phenolic compounds provide antioxidant potential and health-promoting properties and contribute to the flavour and colour attributes of fruits and vegetables. The levels of phenolic compounds also used to gauge the physical stages and potential loss in the quality of fruit products due to browning, formation of hazes and sediments (**Savikin et al., 2009**). As it was shown in **Table 2**, time of storage significantly affected the total polyphenol content as determined by Folin–Ciocalteu assay. There was a nonsignificant decrease in total polyphenols during 14 days under the experimental conditions applied.

At the end of storage, all blends of juices showed a stable or slight significant increase in total phenolic content (**Table 2**). It is possible that during blends storage, some compounds are formed that react with Folin–Ciocalteu reagent and significantly enhance total phenolic content. This observation is supported by the findings of **Klimczak et al. (2007)**, who reported that the total phenols of orange juice decreased after 4 months of storage and increased significantly at the end of 6 months' storage time. Another similar finding was reported by **Tavarini et al. (2008)**, who found that the phenols in kiwi fruits remained stable during the initial 2 months of storage at 0 °C and increased significantly after 6 months of storage.

It has been reported that there is a direct relationship between the phenolic content and antioxidant capacity of plants. They are known to constitute one of the most important groups of natural antioxidants due to their diversity and extensive distribution. They possess biological and chemical properties which include; reducing character, capacity of sequestering reactive oxygen species and several electrophiles, chelating metallic ions and capacity for modulating the activity of some cell enzymes (**Al-Mamary et al., 2002**).

#### **Antioxidant activity**

The evaluation of antioxidant activity in food sample is becoming increasingly important in the field of

nutritional research as it provides useful information with regard to health promoting functional quality of food material without the analysis of each antioxidant compound. In this experiment antioxidant activities of orange-carrots blends, measured by DPPH<sup>0</sup> and metal chelating assays (**Fig. 1**). Various natural antioxidants in the complex food matrix work synergistically and/or antagonistically through multiple reaction mechanism under different phase locations. Therefore a simple universal method by which total antioxidant activity can be measured accurately and quantitatively does not exist. At least two different antioxidant methods allows to compare samples identify variations in response under various reaction mechanisms (**Sun et al., 2009**).

In the test with DPPH<sup>0</sup> radical, there was a slight increase in antioxidant capacity during storage. The results presented are in line with the data obtained by **Arena et al. (2001)** and **Piga et al. (2002)**. They showed the increase in the antioxidant activity after 2 months of storage in orange juices reconstituted from concentrate. According to **Piga et al. (2002)**, storage of mandarin juices during 15 days at 4 °C also resulted in the increase in the DPPH<sup>0</sup> antioxidant activity. The obtained results are in contrast to **Del-Caro et al. (2004)** who described a slight decrease in the TEAC (trolox equivalent antioxidant capacity) value obtained by DPPH<sup>0</sup> method for orange juice stored in the same conditions. The decrease in the antioxidant activity may be linked to a lower content of phenolic compounds and vitamin C in stored juice as compared to fresh, the increase in the antioxidant activity is usually ascribed to Maillard's reaction products.

The previous studies have shown that the antioxidant efficiency of orange juice may be attributed, in a significant part, to their total phenolic content. However, according to **Kahkonen et al. (2001)**, ascorbic acid could exert a synergistic effect with phenolic components. In work of (**Gonzalez-Molina et al., 2008**), the addition of 5% black chokeberry concentrate to lemon juice did not increase the antioxidant activity with respect to the control. According to data showed in the **Fig. 1**, it could be observed that stable or slight increase in antioxidant activity with increasing carrot juice ratio or prolonging the storage time.

### Sensory evaluation

From the point of view for consumers, the flavour, colour, and organoleptic taste of fruit juice is very important because it determines the marketability of juice. Organoleptic quality like colour, flavour, and nutritive value of fruit products generally reduces with the increase in storage period. In the present study, results indicated that odour, colour and organoleptic score of juice blends, decreased with increase the portion of orange juice (**Table 3**). The colour, odour, taste, texture, appearance and overall acceptability of the blends were found to be superior as the portion of carrot increased.

**Tannous and Lawn (1981)** reported that blends of American and Maharaji in the ratio of 1:2 with 2% added sugar had the highest organoleptic score. The black raspberry-apple blend stored at 25°C for up to 48 hrs resulted in increased polymeric colour and percent colour due to increased tannin.

**Tandon et al. (2007)** reported that the addition of papaya pulp with bael pulp was found to be very effective in checking the browning and improving the appearance of the beverage. They also observed that the beverage prepared from 2:3 blend of bael:papaya pulp scored maximum (7.4 out of 10.0) after six months of storage.

### Volatile compounds

The volatile compounds of fresh orange and carrot juices as well as fresh and stored selected blended were extracted using headspace and identified by GC and GC-MS and the data are given in **Table (4)**. The results showed a total of twenty six volatile compounds in samples of fresh orange and carrot juices as well as fresh and stored blend including 7 alcohols, 4 aldehydes, 9 monoterpene hydrocarbon and 6 esters. Results are in accordance with those of (**Kjeldsen et al., 2001; Arena et al., 2006**), who found several classes of volatiles in

carrot and orange cultivars.

As shown in **Table 4**, the volatile compounds were dominated by monoterpenes and sesquiterpenes with around 47.5-61.8% of the total volatiles of stored blend and fresh orange juice respectively. Among these components, Limonene was the one most abundant monoterpene, representing in orange, carrot, fresh and stored blend 47.38, 42.37, 39.24 and 37.25% respectively. These findings agree well with previous studies on carrot and orange volatiles (**Yu et al., 2010**).

The fresh orange juice flavour due to the complex combination of several odours components that include alcohols, aldehydes, esters, ketones and hydrocarbons has been extensively investigated. On the other hand, the characteristic flavour of carrots is mainly due to the volatile constituents which are mostly made up of terpenes and sesquiterpenes (**Kebede et al., 2014**).

It has been proposed that sabinene and particularly myrcene are responsible for notes on "green", "earthy" and "carrot top" flavours (**Duan et al., 2012**).

After two weeks of storage at refrigerator, only small changes in volatile compounds were detected (**Table 4**), suggesting that the blend quality was perfectly suitable for market. Similar studies report that fruit quality can be properly preserved in cold conditions for long periods of time, resulting in only a small reduction in flavour quality (**Abad et al., 2003**), and a small increase in the volatile compound content.

In the literature, little information is available on the changes in volatile constituents of orange-carrot juice blends during storage. To the best of our knowledge, this is the first study showing the changes in volatile compounds in orange-carrot juice blend during storage.

**Aldehydes:** Aldehydes are secondary metabolites formed during normal ripening and maturation of orange fruits. They are important in terms of orange odour quality and their concentrations increase with fruit maturity. Hexanal is not believed important to fresh orange juice flavour, except for some possible contribution to a green flavour note. Octanal and decanal are generally considered important contributors to orange flavour (**Boelens and van Gemert, 1987**), and one of the standards of identity for orange peel oil is its aldehyde content (mostly octanal and decanal). In the current study, fresh orange (**Table 4**) juice had relatively higher levels of both octanal and decanal, compared with fresh carrot juice or fresh and stored blend. **Moshonas and Shaw (2000)** concluded that decanal and octanal were important contributors to orange juice flavour. However, **Ahmed et al. (1978b)** found decanal to make a negative contribution to orange juice flavour at the level tested (0.72 ppm).

**Esters:** The six esters quantified in this study (**Table 4**), ethyl acetate, methyl butyrate, and ethyl butyrate, are known to contribute to the "top-note" of fruit flavours, including citrus. Ethyl acetate is generally the major volatile ester in orange juices and orange flavour fractions and is an important contributor to desirable flavour in orange products (**Ahmed et al., 1978a**). The results showed small reduction in some esters especially in ethyl acetate, ethyl hexanoate and ethyl butyrate. Similar reductions in concentrations have been reported by **Moshonas and Shaw (2000)** for ethyl butanoate, in orange juices stored in laminated cartons for 7 weeks at 2 °C. The concentrations of water-soluble compounds were found to be reduced to 30% of their original value and those of oil-soluble compounds to 70%, after storage for 5 weeks. These authors also found that for some compounds reduction in concentrations of up to 50% occurred when the juice was stored at -18 °C.

**Alcohols:** Linalool and 1-octanol are responsible for fruity/floral and herbal notes, respectively. They are known as important contributors to the fresh orange juice odour (**Tonder et al., 1998**) even if their amount in orange juice is strictly dependent on orange variety (**Maccarone et al., 1998**). Where, alcohols accounted 25.01% stored blend, 24.89% fresh blend, 16.14% fresh orange and 15.97% in fresh carrot juice (**Table 4**) of the total volatile compounds. Ethanol was the most abundant alcohol in all samples with 15.16%, 11.24% 10.12% and 8.27% in aforementioned samples respectively.



**Terpene hydrocarbons;** Monoterpene and sesquiterpenes were also detected in high amounts with 47.5% in stored blend (**Table 4**) compared with the fresh blend one 49.48%. Limonene was identified as the main monoterpene found in common for all samples. Limonene is the most abundant terpene hydrocarbon in orange juice, and its concentration in processed orange juice is much higher than in fresh hand squeezed juices. This variability arises because most of the limonene comes from peel oil and is introduced into the juice during mechanical extraction. In spite of its high concentration, limonene is not a key flavor impact compound in orange juice. Nevertheless, limonene is a necessary component of any orange juice odor model, although its exact function is still uncertain.

## CONCLUSION

On the basis of the results of this study it may be concluded that formulation of mixed (blend) fruit juice from orange and carrot is possible to satisfy consumer taste and preferences. These juice blends can be stored effectively for a period of 14 days. It was concluded that the orange: carrot juice blend ratio (1:3) was most effective juice blend for minimum change in TSS (10.97 to 12.45 brix), acidity (1.26-1.32) and vitamin C (52.41-46.04 mg/100g). Sensory evaluation was also higher and better than the other ratios. However, further investigations are required for assessing microbiologically safety of this blend under different storage temperature. Also, more informations are in need on the changes in individual phenolic using HPLC constituents of blend during storage to clarify the changes in taste evaluation. The stability in polyphenol and vitamin C content upon storage is reflected by the stability or increase in DPPH<sup>0</sup> and B-carotene antioxidant capacities of orange-carrot selected blend. Several research were carried out on the volatile constituents of either orange or carrot juice, however this is the first trial to follow up the change in these compounds in orange-carrot blend.

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**Table 1. Chemical composition of fresh orange and carrot juices**

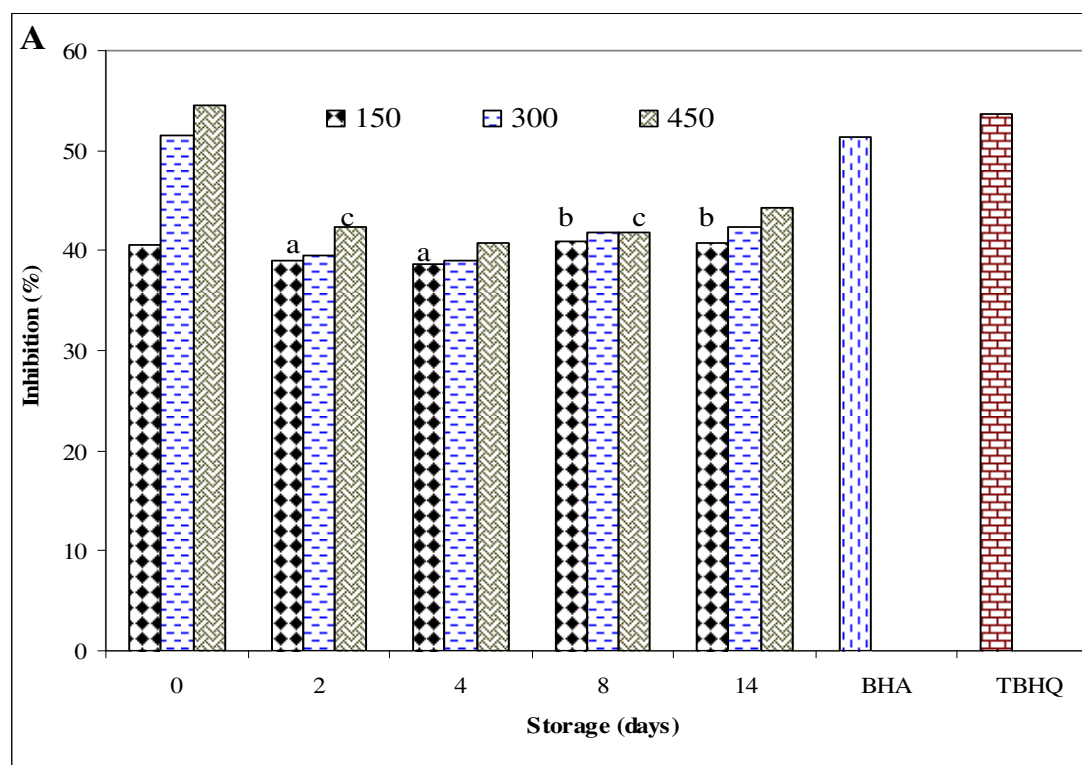
Parameters	Orange				Carrot		
pH	3.62 ±0.016				6.35±0.16		
TSS ( <sup>o</sup> Brix)	13.41±0.02				4.17±0.01		
Acidity %	1.12±0.01				0.38±0.17		
Ascorbic acid (mg/100ml)	78.4±0.15				16.8±0.11		
TC (mg/100 ml)	14.2±0.21				37.6±0.15		
T.PH (mg/g)	87.4±0.17				29.8±0.22		
Minerals (mg/100g)	Na	K	Fe	Mg	Ca	Cu	Mn
Orange	8.18	64.2	0.76	14.5	15.3	0.08	0.52
Carrot	3.17	11.5	0.36	10.6	8.2	0.01	0.61

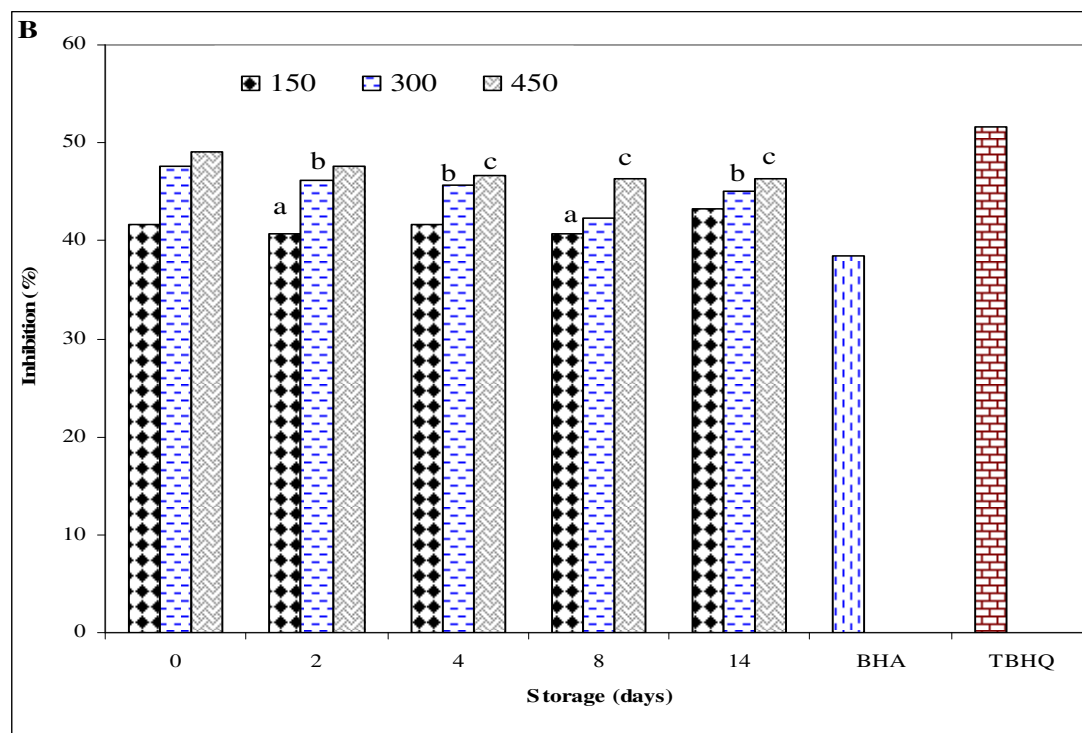
**Table 2. Chemical characteristics of orange-carrot juice blends recorded during storage for two weeks at 4°C.**

Storage (Days)	pH	TA	TSS	V.C	TC	T.PH
<b>Orange: carrot ratio (1:3)</b>						
0	3.72±0.18*	1.26±0.02 <sup>a</sup>	10.97±0.21 <sup>a</sup>	52.41±0.09	35.54±0.15	73.2±0.074 <sup>a</sup>
2	3.70± 0.25	1.22±0.07 <sup>b</sup>	11.16±0.15 <sup>b</sup>	51.51±0.13 <sup>a</sup>	34.22±0.06 <sup>a</sup>	71.53±0.32 <sup>b</sup>
4	3.68±0.36 <sup>a</sup>	1.25±0.03	11.4±0.26	50.60±0.12	33.28±0.03 <sup>b</sup>	73.32±0.11 <sup>a</sup>
8	3.67±0.11 <sup>a</sup>	1.33±0.02 <sup>c</sup>	11.73±73 <sup>c</sup>	49.07±0.55 <sup>b</sup>	32.16±0.04 <sup>c</sup>	73.72±0.39 <sup>a</sup>
14	3.65±0.01	1.32±0.02 <sup>c</sup>	12.45±0.21	46.04±0.42	31.32±0.09 <sup>d</sup>	74.78±0.41
<b>1:1</b>						
0	3.65±0.04	1.14±0.02	10.43±0.15	49.09±0.38 <sup>b</sup>	34.21±0.07 <sup>a</sup>	68.46±0.06 <sup>c</sup>
2	3.61±0.03	1.16±0.03	10.7±0.12 <sup>a</sup>	48.62±0.03 <sup>c</sup>	33.00±0.22 <sup>b</sup>	68.49±0.05 <sup>c</sup>
4	3.51±0.94	1.17±0.01	11.13±0.21 <sup>b</sup>	47.61±0.09	32.21±0.06 <sup>c</sup>	68.83±0.59 <sup>c</sup>
8	3.47±0.57	1.23±0.02 <sup>b</sup>	11.33±0.16	46.78±0.45	30.21±0.05 <sup>d</sup>	69.73±0.39
14	3.41±0.64 <sup>b</sup>	1.26±0.01 <sup>a</sup>	11.8±0.1	44.68±0.20	28.89±0.55	71.35±0.08 <sup>b</sup>
<b>3:1</b>						
0	3.41±0.11 <sup>b</sup>	1.27±0.01	11.07±0.15 <sup>c</sup>	53.55±0.33	31.24±0.02	72.3±0.36
2	3.37±0.27	1.29±0.02	11.33±0.12	51.48±0.18 <sup>a</sup>	29.59±0.13 <sup>d</sup>	71.53±0.25 <sup>b</sup>
4	3.22±0.56	1.33±0.03 <sup>c</sup>	11.7±0.26 <sup>c</sup>	49.07±0.54 <sup>b</sup>	28.43±0.15	72.39±0.07
8	3.18±0.13	1.35±0.04 <sup>d</sup>	12.13±0.21	48.96±0.31 <sup>c</sup>	27.29±0.16	72.87±0.59
14	3.14±0.19	1.36±0.02 <sup>d</sup>	12.6±0.26	47.94±0.07	26.13±0.06	72.79±0.36

\*: Values are expressed mean ± SD

Means followed by the same letter within each column are not significantly different (n = 3, P < 0.05).





**Figure 1. Antioxidant activity of orange-carrot blend (3:1) during storage for two weeks at 4 °C as determined by DPPH<sup>0</sup> (A) and metal chelating (B) assays. Values with the same letter are not significant ( $P \leq 0.05$ )**

**Table 3. Sensory quality of orange and carrot juice as a function of blending ratio**

Orange: carrot ratio	Colour		Odour		Taste		Texture		Appearance		OA	
	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S	$\bar{x}$	S
1:3	9.2	0.34	8.5	0.19	8.7	0.51	9.1	0.43	9.6	0.82	9.7	0.72
1:1	7.4	0.41	8.4	0.56	7.9	0.47	8.2	0.52	8.6	0.49	8.4	0.64
3:1	8.6	0.27	7.5	0.91	7.9	0.61	8.3	0.17	8.7	0.68	8.9	0.28

$\bar{x}$  =mean value; S =standard deviation; OA=overall acceptability;

**Table 4. Effect of storage for two weeks at 4 °C on the volatile compounds of orange-carrot blend (1:3) ratio.**

Compound	LRI <sup>a</sup>	Fresh orange	Fresh carrot	Fresh blend	Stored blend	Description <sup>c</sup>	FTV <sup>d</sup>
<b>Alcohols</b>							
Ethanol	614 <sup>b</sup>	10.12	8.27	11.24	15.16		53
1-Butanol	694	0.93	0.12	0.48	0.61		
1-Penten-3-ol	733	2.48	2.47	3.48	2.73		
α-terpinol	1229	0.29	1.84	2.82	0.29		0.3
Linalool	1091	0.75	1.19	3.64	1.19	<b>Floral, fruity, lemon</b>	0.0038
Octanol	1074	1.26	1.32	1.49	2.71		
Terpinene-4-ol	1217	0.31	0.76	1.74	2.32		
<b>Aldehydes</b>							
Hexenal	768	0.84	0.71	1.37	0.34	<b>fruity, orange, floral</b>	
Octanal	1023	1.29	1.19	0.95	0.31		0.0005
Decanal	1220	2.23	0.37	1.14	0.45		0.0032
Nonanal	1159	1.17	0.92	0.16	1.15		0.0043
<b>Terpene hydrocarbons</b>							
α-pinene	932	2.19	1.57	3.21	2.97	<b>citrus, spicy,woody</b>	1.0
α-thujene	936	0.28	1.18	0.71	0.82		
β-Pinene	948	3.37	1.37	1.16	0.95	<b>citrus, terpene-like</b>	
Sabinene	651	1.39	1.36	1.42	1.25		
β-Myrcene	993	2.25	2.41	0.62	1.64	<b>peel, medicine</b>	0.042
α-phelladrene	1005	2.91	n.d	1.34	0.29		
p-Cymene	1025	1.42	1.86	1.14	0.57		
Limonene	1032	47.38	42.37	39.24	37.25		0.21
α-Terpinene	1071	0.61	2.19	0.64	1.76		
<b>Esters</b>							
Ethyl acetate	641	4.21	3.29	3.59	2.49		
Methyl butyrate	752	3.68	1.67	0.26	2.18		
Ethyl Hexanoate	1014	2.18	0.57	1.92	1.85		
Ethyl butyrate	842	1.13	13.24	8.43	7.42		
Hexyl acetate	1016	0.35	2.83	1.13	2.37		
Ethyl octanoate	1084	3.46	3.23	2.87	4.69		
<b>Alcohols</b>		<b>16.16</b>	<b>15.97</b>	<b>24.89</b>	<b>25.01</b>		
<b>Aldehydes</b>		<b>5.43</b>	<b>3.29</b>	<b>3.62</b>	<b>2.25</b>		
<b>Terpene hydrocarbons</b>		<b>61.8</b>	<b>54.31</b>	<b>49.48</b>	<b>47.5</b>		
<b>Esters</b>		<b>15.01</b>	<b>24.83</b>	<b>18.2</b>	<b>21.0</b>		

<sup>a</sup>: Linear retention index

<sup>b</sup>: Values are expressed as relative area percentage; n.d : Not detected

<sup>c</sup>: Rega et al., 2003; Plotto et al., 2004

<sup>d</sup> FTV=flavour threshold value, as reported by Shaw (1991) (ug/ml; (water)

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