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# Phytochemical Contents and *In Vitro* Antioxidant Activity of Algerian Orange Juices

**Hayette Louaileche*, Yazid Khaled Khodja, Mostapha Bachir Bey**

Laboratory of Applied Biochemistry, Faculty of Life and Natural sciences, University of Bejaia, Bejaia, Algeria

## Abstract

The harmful effects of the free radicals on our organism can be inhibited by several antioxidant molecules (phenolic compounds, vitamins and carotenoids) found in fruits such as citrus. In the present study, the content of some antioxidants as well as the antioxidant power of juices of six orange varieties and one lemon variety were evaluated. The results show that the juices are richer in ascorbic acid, phenolic compounds and flavonoids. The juices which present the best antiradical capacity and reducing power are that of the Bitter orange and Sanguinelli varieties. The results of this study allow us to conclude that the juices of oranges constitute a good source of various antioxidants.

## Keywords

Antioxidants, Orange, Variety, Juice, Phenolics, Carotenoids, Ascorbic Acid

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## 1. Introduction

Consumption of fruits and vegetables, particularly vitamin C-rich fruits and vegetables, appears to have protective effect against coronary heart diseases (Joshipura *et al.,* 2001). The citrus fruits are one of the most important fruit harvests of the world; they are generally consumed in the form of product or of fresh juice because of their food value and their special flavour. The health beneficial effects of citrus fruits are mainly attributed to the presence of bioactive compounds such as the carotenoids, phenolic compounds, flavonoids and ascorbic acid (Miller and Rice-Evans, 1997; Ross *et al.,* 2000; Oboh *et al*., 2015).

The aim of the present study was to evaluate and to compare the antioxidant properties of juices of some orange varieties harvested from Bejaia department of Algeria. The contents of some bioactive phytochemicals (ascorbic acid, carotenoids, total polyphenol, and flavonoids) present in the orange juices were also measured.

## 2. Material and Methods

### 2.1. Plant Material

Six orange varieties (Washington Navel, Thomson Navel, Double fine, Sanguinelli, Valencia Late, Bitter orange) and one lemon variety (Eureka) were harvested from Bejaia region (Algeria). The fruits are washed, then peeled before being squeezed using a fruit squeezer. The juices were obtained after centrifugation for 20min at 4500rpm at 5°C (Sigma 2-16K, Osterode, Germany).

### 2.2. Determination of Antioxidants

#### 2.2.1. Ascorbic Acid

Ascorbic acid was determined according to the method of Mau *et al*. (2005). The ascorbic acid was extracted from 1ml of juice with 30ml of oxalic acid (0.4%). 300µl of juice extract were mixed with 2.7ml of 2,6-dichloroindophenol (0.006%). The absorbance was measured at 515 nm using an UV-visible spectrophotometer (UVmini 1240, Schimadzu, China). The content of ascorbic acid was calculated on the

* Corresponding author
E-mail address: haylouaileche@yahoo.fr (H. Louaileche)

basis of the calibration curve of ascorbic acid (y = -0.0298x + 0.8632; $R^2$ = 0.99) and the results were expressed as mg/100ml of juice.

### 2.2.2. Carotenoids

Juice samples (20ml) were homogenized with 20ml of extracting solvent (hexane/acetone/ ethanol, 2:1:1). After stirring during 30min, the upper phase was recovered. 10ml of hexane were added and a second extraction was carried out. The mixture of the two extractions was centrifuged for 5min at 6500 rpm at 5°C. The absorbance of hexanic extract was measured at 440nm (Sass-Kiss *et al.,* 2005); carotenoid content was calculated from the β-carotene calibration curve (y = 0.1264x; $R^2$ = 0.99). The results were expressed as µg of β-carotene per 100ml of juice.

### 2.2.3. Total Phenolics

Citrus juices (100µl) were mixed with 2.2ml of 2% sodium carbonate solution; after 3min, 100µl of 50% Folin-Ciocalteu reagent were added to the mixture. After 30min, the absorbance was measured at 720 nm (Naithani *et al.*, 2006). The total phenolic content calculated from the gallic acid calibration curve (y = 1.5481x; $R^2$ = 0.99) was expressed as mg of gallic acid equivalents per 100ml of juice.

### 2.2.4. Flavonoids

Total flavonoid contents were estimated using the method of Woisky and Salatino (1998). Orange juice (0.5 ml) was added to 2ml of 2% aluminium chloride methanol solution. After 20 min at room temperature, the absorbance was measured at 390nm. The total flavonoid content calculated from the calibration curve of quercetin (y= 0.0046x; $R^2$ = 0.99) was expressed as mg of quercetin equivalents per 100ml of juice.

## 2.3. Antioxidant Activity

### 2.3.1. Antiradical Activity

One milliliter of juice was added to 2ml of DPPH solution (0.002%). The mixture was left in dark during 30min and the discolouration was compared with the control, containing the DPPH and solvent, was measured at 517 nm (Peschel *et al.,* 2005). The antiradical activity calculated from the quercetin calibration curve (y = -0.0577x + 0.3659; $R^2$ = 0.99) was expressed as mg quercetin equivalent per 100ml of juice.

### 2.3.2. Reducing Power

The ferric reducing power of the samples was evaluated according to Oyaizu (1986). One milliliter of juice was mixed with 2.5ml of 0.2M phosphate buffer pH6.6 and 2.5ml of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20min. Then 2.5ml of 10% trichloroacetic acid were added, and the mixture was centrifuged at 300g for 10min. The upper layer (2.5ml) was mixed with 2.5ml of distilled water and 0.5ml of 0.1% ferric chloride, and the absorbance was measured at 700nm. The reducing power calculated from the quercetin calibration curve (y = 10.613x; $R^2$ = 0.99) was expressed as mg quercetin equivalent per 100ml of juice.

## 2.4. Statistical Analysis

For statistical analysis, Statistica v.5.5 software (STATSOFT, 1999) was used. Significant differences were evaluated by means of analysis of variance (ANOVA) for repeated measurements ($p<0.05$).

# 3. Results and Discussion

## 3.1. Phytochemical Content

**Table 1.** Phytochemical contents of the analyzed juices.

| Varieties | Ascorbic acid§ | Carotenoids* | Total phenolics§ | Flavonoids§ |
|---|---|---|---|---|
| Washington Navel | 48.99 ± 0.39$^{c}$ | 109.77 ± 1.90$^{f}$ | 41.02 ± 0.26$^{b}$ | 7.67 ± 0.07$^{c}$ |
| Thomson Navel | 68.08 ± 0.11$^{f}$ | 59.06 ± 1.27$^{d}$ | 47.53 ± 0.28$^{d}$ | 9.36 ± 0.07$^{e}$ |
| Double fine | 57.12 ± 0.24$^{d}$ | 47.60 ± 1.18$^{b}$ | 37.79 ± 0.23$^{a}$ | 6.17 ± 0.04$^{a}$ |
| Sanguinelli | 46.34 ± 0.20$^{b}$ | 26.35 ± 1.07$^{a}$ | 70.77 ± 0.86$^{f}$ | 8.82 ± 0.09$^{d}$ |
| Valencia late | 79.19 ± 0.25$^{g}$ | 59.94 ± 1.18$^{d}$ | 44.85 ± 0.16$^{c}$ | 7.19 ± 0.07$^{b}$ |
| Bitter orange | 36.03 ± 0.17$^{a}$ | 81.75 ± 1.40$^{e}$ | 117.93 ± 1.13$^{g}$ | 29.84 ± 0.29$^{g}$ |
| Eureka | 66.16 ± 0.29$^{e}$ | 55.60 ± 1.31$^{c}$ | 56.07 ± 0.19$^{e}$ | 10.58 ± 0.09$^{f}$ |
| Min | 36.03 | 26.35 | 37.79 | 6.17 |
| Max | 79.19 | 109.77 | 117.93 | 29.84 |

§: mg/100 ml of juice; *: µg/100 ml of juice.
a-g : Within a column, different letters indicate significant differences ($p < 0.05$) between varieties.
The results are ranged in ascending order (a<b<c<d<e<f<g).

### 3.1.1. Ascorbic Acid

Ascorbic acid is a water-soluble vitamin. Health benefits of this micro-nutrient are antioxidant, anti-atherogenic, and immune-modulator effects. Another positive impact of this vitamin resides in reducing the incidence of stomach cancer,

and in preventing lung and colorectal cancer (Pham-Huy *et al*., 2008). According to the ascorbic acid content in juices, the orange varieties are ranged as follows: Valencia late (79.2mg/100ml)>Thomson Navel>Eureka>Double fine> Washington Navel > Sanguinelli> Bitter orange (36.0mg/100ml). These contents were higher than those obtained by Oboh *et al*. (2015) for lemon (35.0 mg AAE/l) and lime (29.5 mg AAE/l). The variability of the ascorbic acid contents of oranges and their derivatives is influenced by many parameters such as the climatic and processing factors, cultural practice, variety and maturity stage of the fruit (Nagy, 1980).

### 3.1.2. Carotenoids

Carotenoids are fat-soluble pigments. They are very efficient quenchers of singlet oxygen and scavengers of other reactive oxygen species. The carotenoid contents of the juices vary from 26.3 (Sanguinelli) to 109.8µg/100ml of juice (Washington Navel). The study of Lee (2001) on the juices of Sanguinelli and Navel varieties from Florida shows contents ranging between 600 to 990µg/100 ml and 380 to 570µg/100 ml, respectively. These concentrations are higher than those of our samples; this is probably due to the method of juice extraction. The carotenoid content of the orange juices is influenced by several factors such as the growing conditions, the geographical origin, the maturity of the fruits and especially the varietal factor (Fanciullino *et al.,* 2006).

### 3.1.3. Total Phenolics

The phenolic compound contents of the analyzed juices are significantly different ($p<0.05$); they vary from 37.8 (Double fine) to 117.9 mg/100ml (Bitter orange). These results are in agreement with those reported by Antolovich *et al.* (2000). However, our results were higher than those obtained by Oboh *et al*. (2015) for lemon (64.5 mg GAE/l) and lime (52.0 mg GAE/l). The differences of the phenolic contents in various varieties could be attributed to genetic factors.

### 3.1.4. Flavonoids

Flavonoids are phenolic compounds present in most plants. These phytochemicals have been reported to prevent or delay many chronic and degenerative ailments including cancer, cardiovascular diseases, arthritis, cataract, memory loss, Alzheimer disease, inflammation, stroke (Pham-Huy *et al*., 2008). The concentrations in flavonoids of the juices vary from 6.2 (Double fine) to 29.8mg/100ml (Bitter orange). The juices of the seven investigated varieties revealed significant differences ($p<0.05$). Our results were higher than those found by Oboh *et al*. (2015) for lemon (24.0 mg QE/l) and lime (29.5 mg QE/l). Lu *et al.* (2006) reported that the flavonoid composition of orange varieties varies according to their genetic origin, the period of harvest and the various analyzed parts of fruit.

## 3.2. Antioxidant Activity

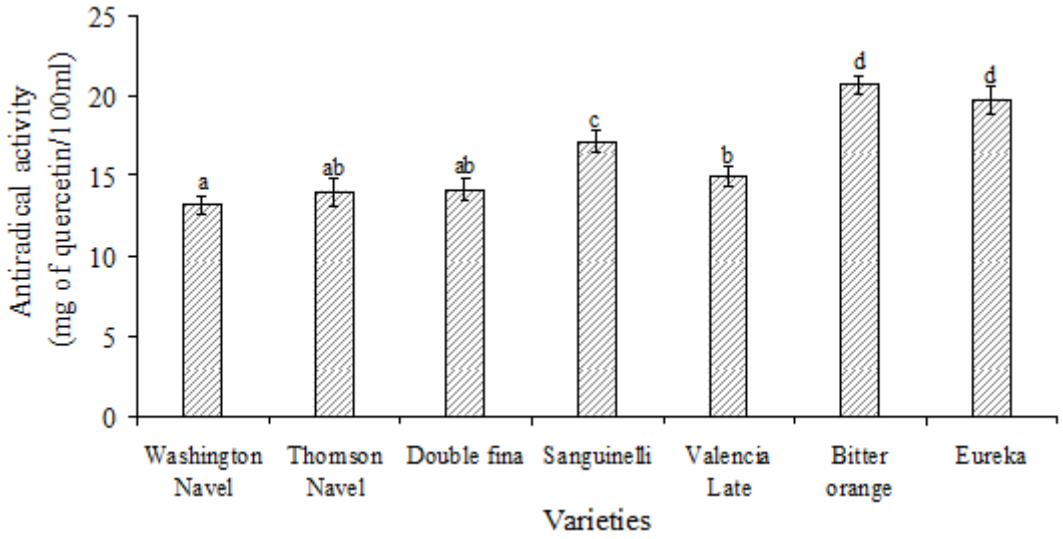


**Figure 1.** Antiradical activity of orange juices.

(a–d) Different letters indicate statistically significant differences ($p < 0.05$) between varieties.

### 3.2.1. Antiradical Activity

The best antiradical activity was recorded for Bitter orange variety followed by Sanguinelli then Valencia late, Double fine, Thomson Navel and Washington Navel varieties. Rapisarda *et al.* (1999) reported similar results for the juices of Washington Navel and Valencia late varieties. The antiradical activity of orange juice could be attributed primarily to its phenolic contents, while the ascorbic acid seems to play a minor role with a contribution usually lower than 15% (Wang *et al.,* 1996; Miller and Rice-Evans, 1997).

### 3.2.2. Reducing Power

The reducing power of the investigated citrus juices presents significant differences ($p<0.05$) except those of Thomson Navel and Valencia late varieties. The strongest ferric reducing power was recorded for Bitter orange juice, whereas the juice of Valencia late variety exhibited the lowest reducing power.

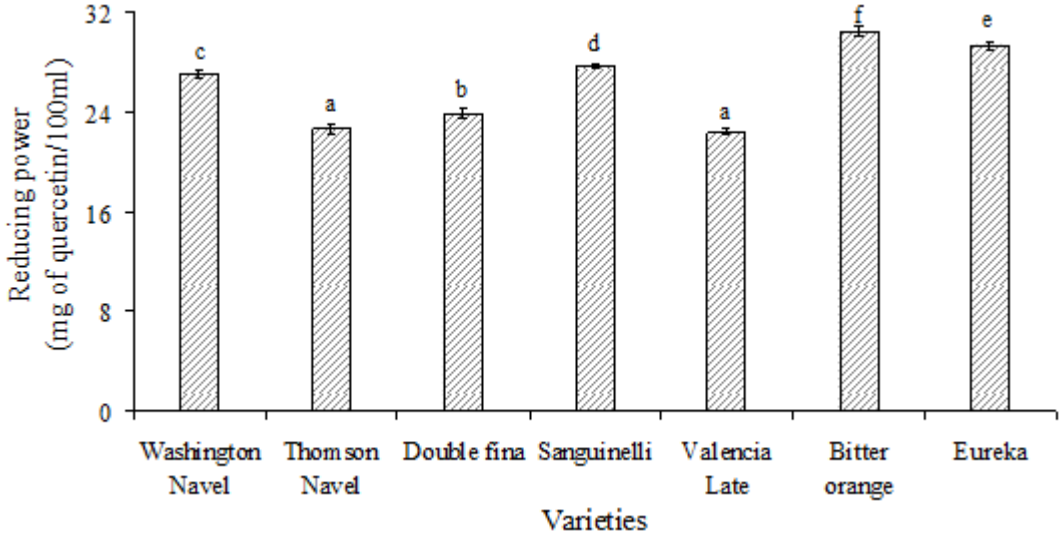


**Figure 2.** Reducing power of orange juices.

(a–f) Different letters indicate statistically significant differences ($p < 0.05$) between varieties.

## 4. Conclusion

The present study allowed the analysis of various bioactive phytochemicals including ascorbic acid, carotenoid, total phenolic, and flavonoids as well as the determination of the antiradical capacity and ferric reducing power of juices of six orange varieties (Washington Navel, Thomson Navel, Double fine, Sanguinelli, Valencia late and Bitter orange) and of one lemon variety (Eureka) harvested from Bejaia, department of Algeria. The juices of Bitter orange and Sanguinelli cultivars displayed the best reducing power and antiradical potential. Our results revealed that juices of the investigated orange varieties constitute a good source of health promoting substances and that contents the analysed compounds depend on the variety.

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