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Bioactive Compounds and Physicochemical Properties of Fruit – Orange Juice Mixes with Rosemary Extract

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ABSTRACT

In the present study a variety of fruit juices (pomegranate, strawberry, apple, plums and blackberries) each was mixed with equal amounts of orange juice (1:1) and different levels of rosemary extract (5, 10, 15, ml/L) were developed. Sensory evaluation tests were performed to choose the most acceptable level of rosemary added to the fruit-orange juices. Physicochemical and bioactive determinations were also assessed.

Sensory evaluation tests showed that 10ml/L rosemary extract added to all tested fruit-orange juices was highly appreciated by panelists except blackberry-orange juice at 5ml/L. The physicochemical tests as measured by TTA%, pH and TSS revealed that the addition of rosemary extract did not significantly affect most of the tested parameters for all tested fruit-orange juices and their controls, and they were at the normal range of commercial juices. Vitamin C the total sugar content showed a variation ranging between 2.54 -28.11mg/100ml and 6.51-19.35%, respectively. Results also indicated that the addition of rosemary extract significantly enhanced the increase in total phenolics (TP) and flavonoids (TF), where blackberry (BO5), strawberry (SO10) and pomegranate (PO10) exhibited the highest TP values, respectively whereas, SO10, BO10 and RO10 exhibited the highest TF values, respectively. SO10, exhibited the highest anthocyanin content (800mg/L) as compared to all other fruit- orange juices. The antioxidant activity (% inhibition) measured by DPPH radical scavenging assay showed that SO10, PO10, and RO10 (plum) exhibited the highest antioxidant activities as compared to their controls and the other fruit-orange mixes. The study postulates that these beverages could provide health promoting benefits against oxidative stress related diseases.

Keywords: fruit – orange juice – rosemary extract-bioactive compounds – antioxidant activity.

INTRODUCTION

Recently, there is increased consumer need for high-antioxidant foods. Drinking high-antioxidant beverages may help to protect against aging, Alzheimer's disease, and other chronic diseases. (Alharbi *et al.,* 2016).

Natural antioxidants have been known to play a major role in stopping oxidative stress caused by free radicals. Natural foods and food-derived components, such as antioxidative vitamins and phenolic phytochemicals, have shown a great deal of importance because they are safe and not concerned as "medicine"; some of these are known to function as chemopreventive agents against oxidative damage. (Lee *et al.*, 2002).

Lately, there has been much attention in the natural sources of antioxidant phytochemicals from plant origin, especially fruits juices (Akazone., 2004). Fruit juices have a neutral effect and give the body a boost of energy so that it can overcome a number of health-related problems (Nandal and Meena, 2012).There is no great difference between fruit and fruit juice in the proximate composition and nutritional properties (Landon, 2007). The major substance groups are carbohydrates, watersoluble vitamins, minerals (potassium, calcium and magnesium), organic acids, amino acids, fibers and polyphenols like colorful anthocyanins, aroma compounds, carotenoids and other bioactive substances (Wardlaw, 2000).

Compounds such as flavonoids and ascorbic acid found in fruit juices have an important role in cognitive development of the brain by increasing its neurological activity several times. Many research reports demonstrated that grape juice, berries juice and citrus juice play an important role in maintaining cognition,

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controlling brain ageing, and lowering the progress of Alzheimer's disease (Dai *et al.,* 2006).

Flavonoids and polyphenols are the most important components of different fruit juices that have the power to increase their antioxidant potential, affect lipid metabolism (Akazone, 2004), and cholesterol absorption (Aprikian*et al.*,2001) . Polyphenols are derivatives, and/or isomers of flavones, isoflavones, flavonols, catechins, lignins, tannins and phenolic acids.

Dietary polyphenols may give indirect protection by activating endogenous defense systems and by controlling cellular signaling processes. They also contain phenolic constituents such as chlorogenic, arbutin, caffeic, p-coumaroyl quinic and p-coumaric acids, and a number of procyanidins and flavonol glycosides (Ma, 2005).

Much research revealed that polyphenols with other nutrients found in fruit juice help to retard the ageing process and decrease the risk of many diseases including cancer, heart disease, stroke, high blood pressure, cataracts, urinary tract infections and Alzheimer's disease (Granic*et al*., 2003).

Attention has concentrated to phenolic compounds derived from different fruits including citrus. Whereas, some studies reported that in addition to vitamin C and carotenoids, phenolic compounds play an integral role in total antioxidant capacity of citrus fruits (Gorinstein*et al.*,2004). The major phenolic compounds traced in different citrus fruits are categorized as flavonoids and phenolic acids (Balasundram*et al.,* 2006).

Rosemary extract contains several bioactive compounds which have known to produce antioxidant properties belonging mainly to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes. In comparison to other phenolic antioxidants, the presence of phenolic diterpenes such as carnosic acid and its derivative carnosol in rosemary extract are able to interact with lipid oxidation by donation of hydrogen atoms to lipid free radicals. (Liu *et al.,* 2013)

Honey is a natural sweetener widely used in various applications, contain approximately 200 different chemical compounds (Raman auskien *et al.,* 2012). Nearly all honey worldwide contains the same types of phenolic acids which has unique nutritional and medicinal properties which act synergistically to extend the utility of honey in a variety of applications (Patricia *et al.,* 2015)

Gonzales-Molina *et al*.(2009) designed beverages, based on pomegranate and lemon juices, offering a high antioxidant capacity the mix showed an attractive product with organoleptic (colour) properties.

El-Abasy *et al*.(2010) formulated functional juice mixes made from carrots (yellow and red varieties and orange juice), results indicated that mixing orange juice with red carrot juice and yellow carrot juice elevated the antioxidant content.

Nansombat *et al.* (2015) formulated ten beverages containing fresh grapes and dried medicinal plants. Results indicated that grape fruit juice with the medicinal plants of *A.polycephala* fruit, *ternatea* flowers, *biloba leaves*, *P. emblica* fruits and *chebula* fruits was abundant in bioactive phenolics, flavonoids and tannins, thus suggesting that these beverages could provide health-promoting benefits when consumed by preventing against Alzheimer's disease and other oxidative stress related diseases.

In a study conducted by Haskell-Ramsay *et al.* (2017) for evaluating the effect of purple grape juice, results of the study suggested that dense consumption of purple grape juice can improve aspects of cognitive performance and mood in healthy adults.

The present research aimed at developing beverages using different fruit juices (pomegranate, strawberry, apple, plums and blackberries) each mixed with equal amounts of orange juice (1:1) and different levels of rosemary extract. These juice mixes are going to be assessed sensorically, physicochemically and evaluated for their bioactive components (total phenolics, flavonoids, anthocyanin and vitamin C) as well as measuring their antioxidant activity.

MATERIALS AND METHODS

Plant Material:

About ten kg of strawberry (var. sweatcher) and ten kg of black berries (var. Rubusfructicosus) and Twenty kg of Pomegranate (var. Wonderful) were purchased from a private farm in Badr city- El Behira, Egypt. Twenty kg of Apples (var. Anna) and ten kg of Plumes, and Sixteen kg of Oranges (var. Citrus) were purchased fresh from the local market, Badr City – El Behira, Egypt. The fruits were selected on the basis of size uniformity, maturity stage (based on skin color and firmness and absence of physical damage).

Hundred grams of rosemary (*Rosemarinus officinalis*) oil extract were purchased from El-Hedaya group, El-Mehala Al Koubra, El Gharbeya governerate. Four Kg of Honey(Emtenane) were purchased from a health-shop in Karfour,Alexandria

Preparation of fruit-orange herb mixes:-

All fruits were washed, peels and/or seeds removed, cut into small pieces and blended using a Molinex blender. Each kind of fruit juice was mixed with orange juice at (1:1) v / v (after several sensory tests conducted to select the mot acceptable blend ratio). The juice mixes from each treatment were filtered through a sieve to remove any suspended matters. Then the rosemary extract and honey were added to each treatment (Table1) and mixed for 6 seconds in a high speed blender with 2g/L Lecithene to achieve homogenous distribution of all ingredients with rosemary extract.

All fruit-orange mixes under study were kept cool and refrigerated at -18°C, until further analysis. In the present study no dilutions were used.

Table 1. Treatments and ingredients used in the preparation of the different fruit juice mixes

Treatments	Fruit juice	Orange juice	Honey	Rosemary extract
Control	500ml	500ml	30ml	Zero
Treat 1	500ml	500ml	30ml	5ml
Treat 2	500ml	500ml	30ml	10ml
Treat 3	500ml	500ml	30ml	15ml

METHODS

Sensory evaluation:

After preparation of different fruit juice orange mixes with rosmary, sensory evalution tests were conducted to choose the most acceptable and highly scored (% level) of juice mix with rosemary for further chemical analysis.

Colour, flavor, consistency and overall acceptance of all tested juices were evaluated by 10 panelists of the members and postgraduate students of the Food Science department, Faculty of Agriculture, Saba Basha, Alexandria University. A 10 – point hedonic scale was used where (8-10) express extremely like and (4-1) extremely dislike as described by (Piggott 1998).

Determination of pH, total titratable acidity, total sugars, total soluble solides

The pH was measured at 20°C using 2ml juice with 25 ml distilled water by a digital pH meter (Metrohm 744, MetrohmLTd., CH-g 101 Herisan, Switzerland).

Total titrable acidity (TTA) was determined using 0.1 M NaoH to the titration end-point of pH 8.2 and values were expressed as percentage citric acid equivalents (AOAC, 2005).

Total sugars were determined according to AOAC (2005) by phenol – sulphonic acid method. The intense yellow-orange colour resulted was measured at 490nm with a Spectrophotometer (model# 2380, per king Elmer, England).

Total sugars were calculated as follows: T. sugar (%) = O.D X Dilution factor X 100) /K X 106
Where:
OD = spectrophotometer reading, K = the slope of the standard curve.

Total soluble solids (TSS) were determined by using digital refractometer (Boeco, Gemany) adjusted at 20°C.

Determination of total phenolic content

Total phenolic content of each juice sample was determined according to the method described by Singleton *et al.* (1999). Folin – ciocalteu's phenol reagent was added, shaken and allowed to stand for 1 min. Then, 1.5 ml of 20% (Na_2Co_3) and 1.9 ml of ultra-pure water were added. After incubation for 30 min. at 25 ° C, the absorbance was measured at 760 nm using UV – Visible spectrophotometer (Shimadzu Pty.Ltd. Australia).

Gallic acid was used as a standard and the results were expressed as mg gallic acid equivalents (GAE)/L .

Determination of flavonoids:

Total flavonoid content of the juice were analyzed spectrophotometrically using (UV- vis shmadzu, Pty, Ltd., Austalia) at a wave length 430 nm as described by Yang *et al*.(1999) Rutin was used as a standard and flavonoid level was expressed as mg of rutin equivalent/L of juice.

Determination of Anthocyanin

Total anthocyanin content was determined by the pH differential method of the AOAC official method as described by Lee *et al.* (2005). The anthocyanin pigment concentration was calculated and expressed as mg/L cyanidin- 3- glucoside equivelents.

Vitamin C content

Vitamin C was determined by a volumetric method using (2.6 dichlorophenol indophenol). Results were expressed as mg/100ml. according to (AOAC, 2007).

Antioxidant activity

DPPH radical scavenging activity assay was determined according to the method of Costa *et al*. (2012) with some modificahon. After incubation for 30 min in the dark at room temperature, the absorbance was measured at 517 nm against blank.The results were expressed as percentage of inhibition of the DPPH using the following equation:

Antioxidant activity % = 1- (Abs. sample at 517nm/Abs blank at 517 nm x 100

Statistical analysis:

All results were presented as means ± standard deviation (SD). Data was subjected to one-way analysis of variance (ANOVA) using SPSS 22 software package. Means were further differentiated using Duncansmulty range test (Steel *et.al.,* 1997).

RESULTS AND DISCUSSION

Sensory evaluation of fruit – orange juices with rosemary extract

Table (2) presents the sensory scores of panelists towards the different juice-orange mixes (controls) and with added rosemary extracts at different levels (5, 10, 15 ml/L).

Results clearly showed that panelist significantly accepted all fruit-orange juices at 10 ml/L addition of rosemary extract (RE) except blackberry- orange juice which was accepted at 5ml/L of RE.

As a matter of fact, the addition of RE did not affect significantly the colour of the juice mixes. However, the addition of orange juice to the fruit used in the present study may be responsible for the stability of anthocyanins containing fruits thus affecting the colour and overall acceptability of all fruit-orange juices, these results were similar to Gonzales- Moline (2009). Meanwhile, all juices showed homogenous consistency.

Table 2. Sensory evaluation of different juice-orange-mixes with rosemary extract at different concentration levels*

conc.level	Color	Flavor	Consistency	Overall acceptability
Pomegranate orange juice				
PO	8.6 ± 0.51^{a}	9.00 ± 0.62^{a}	8.80 ± 0.79^{a}	8.60 ± 0.51^{a}
PO-5	8.8 ± 0.74^{a}	8.60 ± 0.79^{a}	8.40 ± 0.67^{a}	8.80 ± 0.39^{a}
PO-10	8.80 ± 0.47^{a}	9.20 ± 0.51^{a}	9.40 ± 0.93^{a}	9.60 ± 0.49^{a}
PO-15	7.80 ± 0.69^{b}	5.00 ± 0.72^{b}	8.60 ± 0.51^{a}	6.40 ± 0.55^{b}
Strawberry orange juice				
SO	8.50 ± 0.47^{a}	8.50 ± 0.63^{a}	9.00 ± 00.64^{a}	8.83 ± 0.79^{a}
SO-5	8.30 ± 0.69^{a}	8.30 ± 0.79^{a}	8.60 ± 0.76^{a}	8.50 ± 0.83^{a}
SO-10	8.20 ± 0.63^{a}	8.80 ± 0.43^{a}	7.60 ± 1.15^{ab}	8.50 ± 0.83^{a}
SO-15	8.10 ± 0.74^{b}	7.30 ± 0.69^{b}	7.50 ± 1.19^{ab}	7.20 ± 0.85^{ab}
Apple orange juice				
AO	8.20 ± 0.63^{b}	8.80 ± 0.62^{a}	8.40 ± 1.12^{ab}	8.80 ± 0.85^{a}
AO-5	8.30 ± 0.79^{b}	9.00 ± 0.63^{a}	8.80 ± 1.07^{a}	8.60 ± 0.58^{a}
AO-10	8.60 ± 0.43^{ab}	8.60 ± 0.54^{a}	9.00 ± 0.65^{a}	9.00 ± 0.62^{a}
AO-15	7.80 ± 0.87^{bc}	8.00 ± 0.65^{b}	8.60 ± 0.63^{ab}	8.10 ± 067^{b}
Prune orange juice				
RO	7.80 ± 0.87^{b}	8.00 ± 0.72^{a}	8.80 ± 1.07^{a}	8.40 ± 0.67^{a}
RO-5	7.60 ± 0.58^{b}	7.50 ± 0.64^{b}	8.60 ± 1.02^{a}	8.60 ± 0.85^{a}
RO-10	8.20 ± 0.54^{ab}	8.00 ± 0.76^{a}	8.60 ± 1.02^{a}	7.00 ± 0.58^{b}
RO-15	8.50 ± 0.57^{a}	6.60 ± 1.15^{c}	8.20 ± 1.21^{ab}	6.80 ± 0.62^{c}
Black berry orange juice				
BO	6.80 ± 0.43^{b}	6.50 ± 0.87^{b}	8.50 ± 0.76^{a}	6.80 ± 0.81^{b}
BO-5	6.80 ± 0.43^{b}	7.00 ± 0.49^{a}	8.50 ± 0.76^{a}	7.80 ± 0.89^{a}
BO-10	7.00 ± 0.65^{a}	6.50 ± 0.71^{b}	8.70 ± 0.95^{a}	6.80 ± 0.55^{b}
BO-15	7.20 ± 0.79^{a}	4.80 ± 0.72^{c}	8.20 ± 0.94^{ab}	5.00 ± 0.49^{c}

Means with different superscript in a raw are significantly different $P<0.05$

PO, SO, AO, RO and BO= Pomegranate-orange, Strawberry-orange, Apple-orange, Plum-orange and Blackberry-orange juices (controls)

*All juice-orange mixes with 5, 10, and 15 ml/L rosemary extract.

Thus, it may be concluded that all fruit-orange juices in the present study were accepted with the addition of 10 ml/L RE except for blackberry –orange juice which is accepted with the addition of 5 ml/L rosemary extract.

Accordingly, all determinations in the present study will be performed on the accepted fruit-orange mixes with RE compared to their controls.

Physicohemical properties of fruit-orange juices with rosemary extract

Table (3) introduce the physicochemical parameters of all accepted fruit – orange juices with added rosemary extract against their counterparts without rosemary addition.

Total acidity is a measure of total acid present in the juice, accordingly, results indicate that the total titratable acidity (TTA%) in all tested fruit-orange juices under study ranged between 0.22- 1.10% citric acid equivalent. Both pomegranate-orange juice (PO) and (PO10) as well as strawberry-orange juice (SO) and (SO10) recorded the highest total titratable acidity with no significant difference between them ranging between 0.92 -1.10%. On the other hand, the TTA% of apple-orange juice (AO and AO10), plum-orange juice (RO and RO10) as well blackberry-orange juice and BO5 ranged between 0.22 -0.26%. These variability in TTA% may be due to the differences of the addition effect, also it was reported by Ghana standard Board (1995) that non-alcoholic beverages should have acidity between 0.50% and 1.90% calculated as anhydrous citric acid. It was also reported by FAO that juices containing more than 1.2% acid are sour independent of Brix / Acid value (Bates *et al.*, 2001). Accordingly, values obtained in the present study are within the reported ranges.

It is also observed that the addition of rosemary extract did not significantly affect the TTA of all juices except for RO and RO10 and BO and BO5.

Total soluble solids TSS measured as Brix value of all fruit-orange juices in the present study ranged between 14.80-18.50 Brix (Table 3), where BO and BO5 recorded the highest Brix values being 18.50 and 18.10, respectively and SO and SO10 recorded the least being 14.80 and 15.05, respectively. It was reported by Labbe (2016) that the TSS of moderate mature pomegranate juice (*var. Wonderful*) was 17.0 which was quite near to our results of PO and PO10 which ranged between 16.60 and 16.70, respectively.

It was reported by Ghana Standard Board (1995) that non alcoholic beverages should have a refractive value of not less than 8 Brix. Highr Brix value may be due to the high simple sugar content. It was also reported by Zaouay*et al*.(2012) that 12° Brix is the minimum value required for commercial juices

pH is a measure of the degree of acidity or alkalinity of a product. The pH of 3-4 may give juice a good potential of inhibiting the growth of pathogenic bacteria (Jay, 2000). In the present study the pH of all fruit-orange juices (Table 3) ranged between 3.32 and 4.79, where AO, AO10, RO and RO10 recorded the least pH values ranging between 3.32 and 3.88. On the other hand, PO, PO10, SO, SO10 and BO, BO5 recorded the highest pH values ranging between 4.32-4.79. As it can be seen, the addition of rosemary extract did not significantly affect the pH values except for AO and AO10.

Table 3. Physicochemical Properties of fruit orange juices with rosemary extract as compared to their controls

Treatment	TTA%	pH	Total sugar %	TSS
PO	1.10±0.02a	4.75±0.01a	6.82±0.18a	16.70
PO10	1.05±0.05a	4.72±0.00b	6.51±0.36a	16.60
SO	1.05±0.05a	4.81±0.00a	6.76±0.36a	14.80
SO10	0.92±0.08a	4.79±0.00a	6.16±0.25a	15.05
AO	0.45±0.02a	3.85±0.06a	9.54±0.42a	17.00
AO10	0.47±0.01a	3.88±0.04b	7.75±0.27a	17.90
RO	0.62±0.02b	3.32±0.15a	7.83±0.05a	16.60
RO10	0.69±0.01a	3.44±0.01a	7.83±0.14a	16.20
BO	0.22±0.00b	4.30±0.02a	19.35±0.37a	18.50
BO5	0.26±0.1a	4.32±0.02a	16.44±0.07b	18.10

Means with different superscript in a raw are significantly different P<0.05

PO, SO, AO, RO and BO= Pomegranate-orange, Strawberry-orange, Apple-orange, Plume-orange and Blackberry-orange Juices (controls).

PO10, SO10, AO10, RO10 and BO5: fruit orange juices with rosemary at 10mg/L except BO at 5 mg/L

The pH of the studied juices showed some differences this may be due to the fruit used. pH is the major factor affecting the stability of vitamin C, thus high values of pH enhance the oxidation process of vitamin C (Leahu*et al.,* 2013). It was reported by Bates *et al.* (2001) that pH, TSS and acidity assess the quality of the juices.

The sugar content of all fruit orange juices under study ranged between 6.51 - 19.35 % where BO and BO5 recorded the highest values.

Bioactive compounds in fruit-orange juices with added rosemary extract.

Total phenolic compounds

Figures (Fig 1, 2, 3, 4, 5) show the bioactive compounds as determined by total phenolics (TP) total flavonoides (TF) total anthocyanins (TA), vitamin C and antioxidant activity of all fruit-orange juices with added rosemary extract and their controls.

As can be seen, the addition of equal amount of orange juice to all fruits (1:1) and 10 ml/ L of rosemary extract greatly and significantly enhanced the increase in total phenolics (TP) of all tested fruit-orange juices (Fig 1) where BO5, SO10 and PO10 recorded the highest amounts being 2595, 2425,2335 mg GAE/ L, respectively . As a matter of fact Abdel Salam *et al*.(2016) reported that the total phenolics from arils of four varieties of pomegranate grown in Egypt ranged between 1900 – 3000 mg/L according to variety. Labbe *et al*.(2016) evaluated the relation between maturity stage in three cultivars of pomegranate and TPC. Results showed that the highest TPC was present in juice from fruits harvested in the medium ripening stage which ranged between 1300-2270 mg GAE/L.

Both SO10 and BO5 recorded the highest TP content of all tested fruit –orange juices with RE. It was reported by (Fredes*et al*., 2014) that the TP of strawberries is approximate to values in raspberries and blackberries. Surprisingly, in the present study BO with added 5 mg/L of RE contained more TP than strawberry with 10 mg/L thus it was suggested by Shipp and Abdel-Aal (2010) that blackberries could be used for the production of functional foods to increase their biological value as they contribute positively to human health and prevention of illness.

The variability and the exact content of phenolic compounds of strawberry depends on many factors such as genetic qualities, cultivation conditions, ripeness stage, storage time and extraction conditions (Kim and Shin, 2015).

Results in the present study also showed considerable amounts of total phenolic compound in plum-orange (RO) and RO10 where RO10 recorded 895 mg GAE/L as compared to its counter part RO (660.5 mg GAE/L).

It was reported by Hernandez – Ruis*et al.* (2018) that the TP content obtained in two plum varities (red and yellow) ranged from 1 to 18 mg GAE/g where red plums contained the highest TP content. Other studies reported lower results where TP in plums were 1.88 and 1.08 mg GAE/g FW for red and yellow plums, respectively (Marille*et al.*, 2012) . Thus according to many environmental and analytical methods the content of phenolic acid in plums fall within a wide range of values (Paz *et al*., 2015).

Similarly, the total phenolic content in apple-orange juice with added 10mg/L RE showed that the corporation of RE to apple-orange juice AO significantly increased the total phenolics content (561.5 mg GAE/L) as compared to its counter part (504.5 mg GAE /L).

In a study, forty-one apple samples representing the most widely cultivated in Western Europe were collected for a various of bioactive determinations. Total polyphenol content measured by an optimized Folin-Ciocalteu assay ranged between 66.2 and 22.9 mg/ 100g of FW depending on variety (Vrhovsek*et al*., 2004). Results suggests that genotype is the main factor that determines the compositions of bioactive compounds in apples. Also, maturation degree of the fruit has an impact upon the content of bioactive compound and antioxidant capacity (Iordanescu*et al.*, 2012).

Total Flavonoids

Figure (2) show the total flavonoids (TF) measured as mg Rutin / L in all fruit-orange juices.

All fruit-orange juices in the present study exhibited significant higher flavonoid content with the addition of RE as compared to their counterparts.

As can be seen, SO10, BO10, RO10 recorded the highest flavonoid contents. Similarly, Mahmood (2012) studied the TF of different species of Strawberry variety mulberry from Pakistan. Results revealed that the TF ranged between 110 – 1021 mg CE/100g DW. It was reported by Wang*et al.* (2008) that the berries generally contain a great array of flavonoids that contribute to improved blood flow in the brain, there is also preliminary evidence that suggest the possibility of better brain functioning involving cognitive function. Fisetin, a naturally- occurring flavonoid most abundantly found in strawberry is involved in lowering the complication of many disease such as diabetes and cancer (Khan *et al.,* 2013). El-Beltagi (2019) in a study evaluated the TF content in two prune varieties Santa Rosa plum and African rose. Results indicated that TF in the first was 30.02 mg QE/g Dw, where it was 45.99 mg QE/g Dw in the second variety.

Meanwhile , it was stated by Hamid *et al*.(2013) that the range of TF in pomegranate juice ranged between 150-580 mg rutin / L, whereas, Guo *et al.* (2008) stated that TF in commercial pomegranate juice was 177 mg/ ml.

Concerning the apple juice, it was reported that the TF content as determined by a spectrophotometric method was in the range of 42.33-298.7 mg CE/L with mean value and standard deviation of 134± 69 (Wlodarska, 2017).. As a matter of fact, it may be suggested that the addition of orange juice to all tested fruit juices (1:1) may contribute to the enhanced level of flavonoid in the fruit juices, as the major phenolic compounds detected in different citrus fruits are categorized as flavonoids and phenolic acids (Pellegrini *et al.*, 2003 and Peterson *et al.*, 2006).

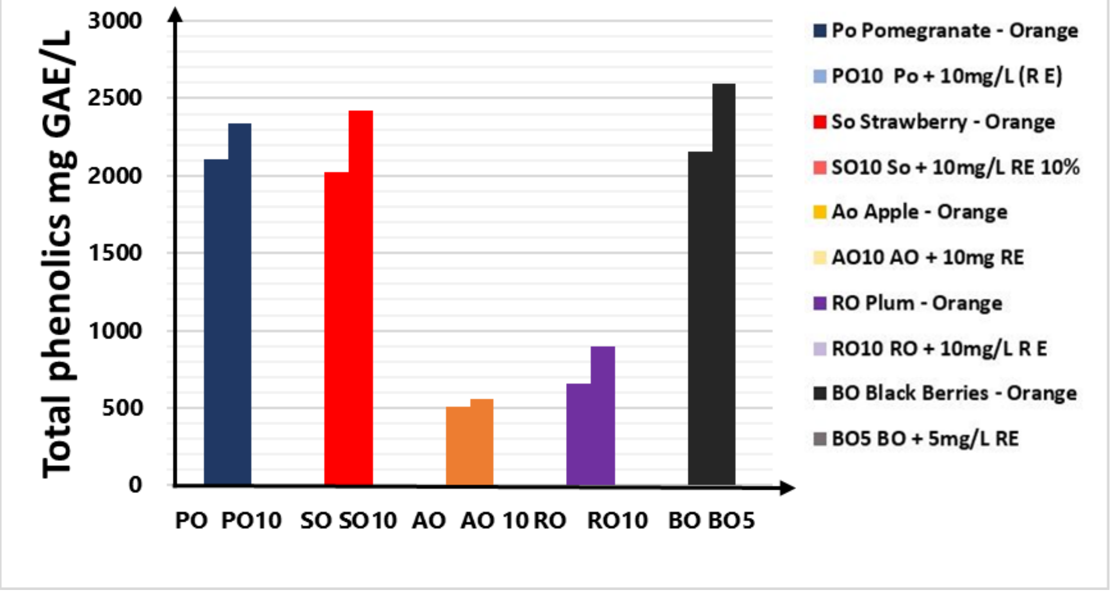

Fig. 1. Total phenolic compounds in fresh fruits - orange juices with added rosemary extract and their controls

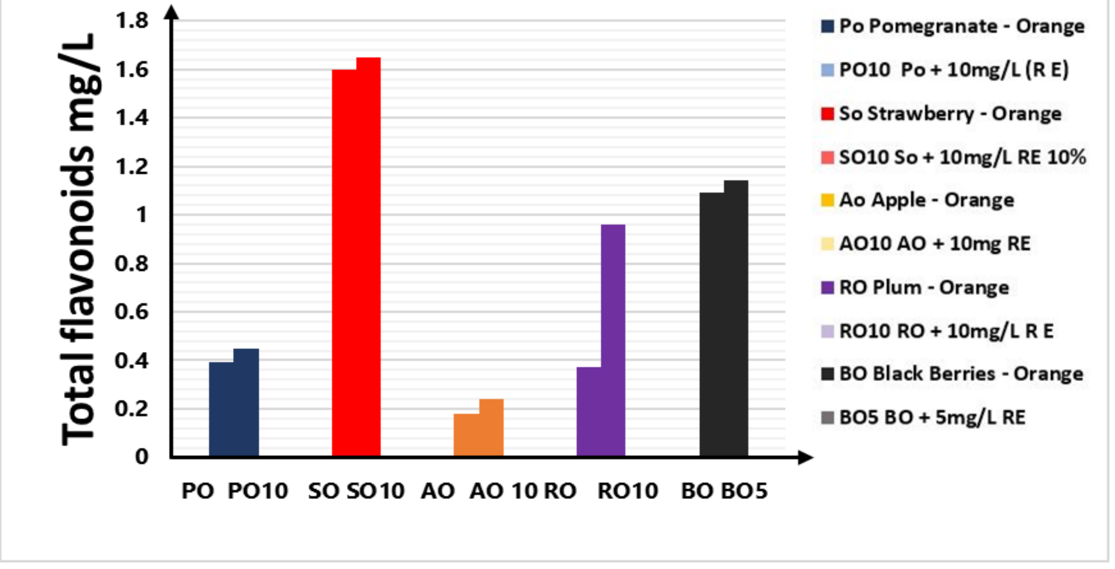

Fig. 2. Total flavonoids in fresh fruits - orange juices with added rosemary extract and their controls.

Anthocyanin content

Figure (3) also represents the anthocyanin content (mg/L) of all fruit – orange juices with added RE and their controls. Results showed that from all tested fruits, strawberry-orange juice SO10 exhibited the highest anthocyanin content being 805 mg/L, where all other fruit orange juice ranged between 51.16-99.55 mg/L.

Both AO10 and BO5 comes in the second place for the anthocyanin content. It is well documented that anthocyanin in strawberries are the major known polyphenolic compound responsible for fruit color, as well as blackberries and blueberries (Fredes*et al.*, 2014). Notwithstanding, their occurrence is influenced by cultivar selection and environmental factors (Kim and Shin, 2015).

Concerning apple juice, it was reported by Nunes *et al*.(2016) that the anthocyanins in Malay apple exhibited a high content of Monomeric anthocyanins reaching 278.34 mg/100g.

Plum juice is also known to contain anthocyanin together with other important nutrients (Hernandez-Ruiz *et al*., 2018) where Jaiswal *et al*. (2013) identified 5 anthocyanines in European varieties.

Vitamin C content

Figure (4) also reveals vitamin C content of all fruit-orange juices under study. The vitamin C content ranged between 2.54 and 28.11 mg/ 100ml. As a matter of fact, AO, AO10, RO, RO10 recorded the least vitamin C content ranging between 2.54 and 6.88 where as BO, BO5, PO, PO10 and SO, SO10 recorded higher vitamin C values ranging between 16.36 and 28.11 mg/100 ml. Losses of vitamin C in orange juice are the most obvious results as confirmed in many studies where Vitamin C becomes unstable in the presence of oxygen also oxidation depends on pH (Leahu*et al.*, 2013). Vitamin C also is an important antioxidant. It was also reported by Adrian *et al*.(2003) that vitamin C and flavonoid levels vary widely not only by species and variety, place of growth, harvesting period and storage, but they also vary depending on processing methods.

Antioxidant activity (AC)

The DPPH radical scavenging assay was applied to measure the ability of antioxidant to scavenge free radicals. The DPPH of fruit-orange juices are illustrated in Figure (4). Results revealed that SO10, PO10 and RO10 possessed the highest antioxidant activity where the addition of 10 mg/L RE significantly enhanced the rise of their antioxidant activity. It was reported by Van De velde*et al*.(2013) that the higher antioxidant capacity observed in berries was in accordance with the higher anthocyanin content, whereas, Ricci *et al*.(2006) reported that commercial pomegranate juices showed an antioxidant activity three times higher than red wine and green tea infusions when measured by four different methods such as ABTS, DPPH, DMPD and FRAP.

In the present study RO10 recorded a high antioxidant activity similar to PO10 this result was also confirmed by Hernandez-Ruiz *et al*.(2018) who mentioned that red plum possessed higher DPPH capacities than yellow plums.

In comparison to the aforementioned results, the following juices showed relative lower DPPH as AO10 and BO5 but were greatly and significantly improved by the addition of 10mg and 5 mg/L RE, respectively. It is well documented that although apples are one of the most consumed fruits in the world, it is important to note that, they are not those with the greatest phenolic content and antioxidant capacity(Fu *et al*., 2011).

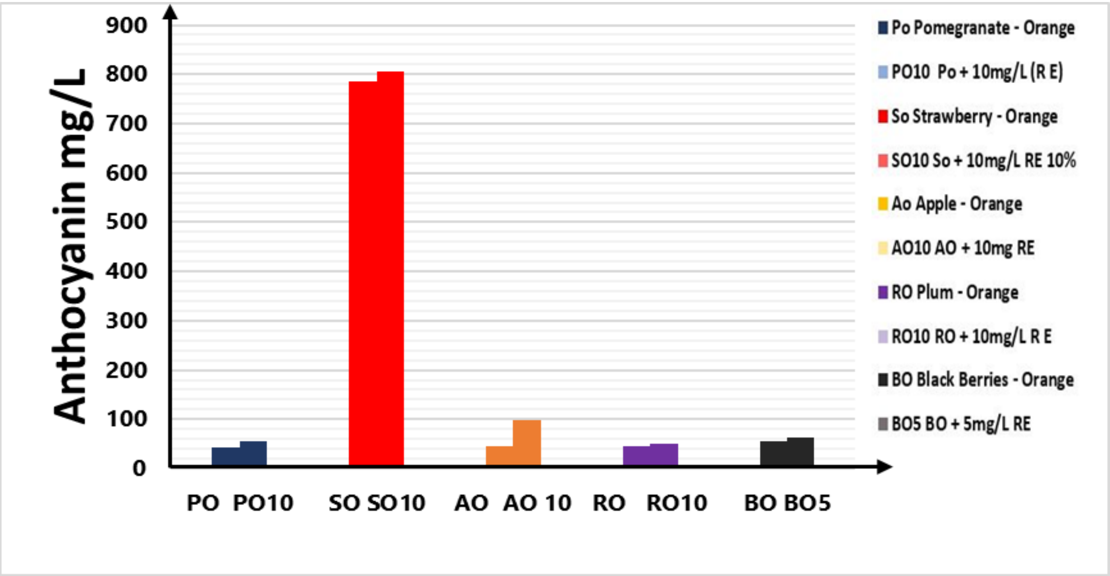

Fig. 3. Anthocyanin content in fresh fruit- orange juices with added rosemary extract and their controls

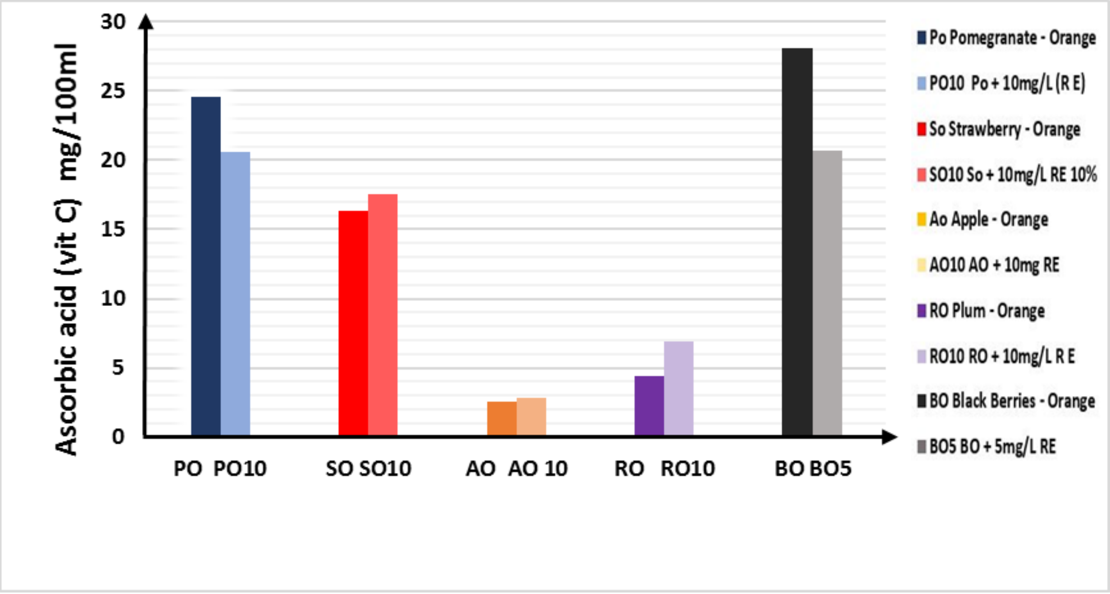

Fig. 5. Vitamin C content of fresh fruit - orange juice with added rosemary extract and their controls

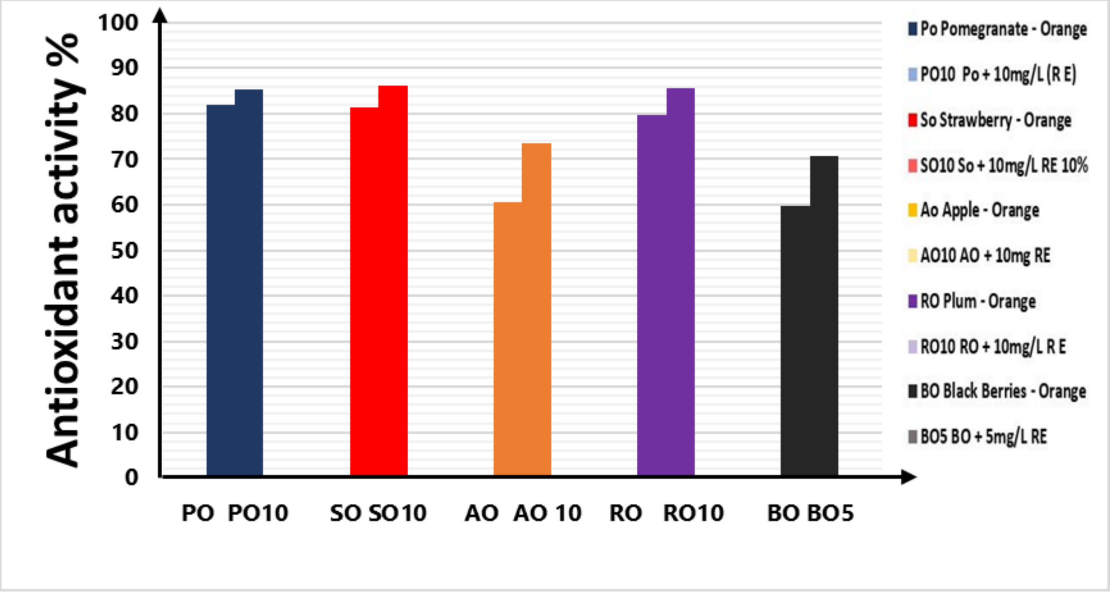

Fig. 4. Antioxidant activity of fresh fruit - orange juice with added rosemary extract and their controls

CONCLUSION

In the present study the addition of rosemary extract at 10mg/L significantly enhanced the content of total phenolics, flavonoids and anthocyanin of all fruit-orange mixes tested without affecting their physicochemical characters. Strawberry (SO10) exhibited the highest content of bioactive compounds and antioxidant activity followed by PO10 and RO10. Therefore, there is a possibility that these beverages could provide health-promoting benefits when consumed by protecting against oxidative stress related diseases.

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الملخص العربي

المركبات النشطة حيويًا والخصائص الفيزوكيماوية لخلطات عصائر الفواكه مع البرتقال ومستخلص الروزماري

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تم في هذه الدراسة تجهيز خلطات من مجموعة من الفواكه (الرومان والفراولة والتفاح والبرقوق والتوت الأسود) كلاً على حدي مع كمية متساوية من عصير البرتقال (1: 1) ومستويات مختلفة من مستخلص الروزماري (5، 10، 15 مل / للتر).

تم إجراء الاختبارات الحسية على جميع العصائر ومستخلصات الروزماري لاختيار أفضل الخلطات التي لاقت استحسان المحكمين والتي سوف يتم إجراء التحاليل الفيزوكيماوية والكيماوحيوية عليها.

أظهرت الاختبارات الحسية ان جميع خلطات العصائر تحت الدراسة قد لاقت استحسان وقبول المحكمين عند اضافة 10 مل/ لتر من مستخلص الروزماري لها ما عدا خلطة التوت البري عند 5 مل/ لتر.

كما بينت الاختبارات الفيزوكيماوية التي تم قياسها باستخدام نسبة الحموضة الكلية ورقم الاس الهيدروجيني والمواد الصلبة الكلية أن اضافة مستخلص الروزمارى لخلطات العصائر لم يظهر فروقا معنوية فى هذه القياسات مقارنة بالعينات الظابطة وكانت القياسات تقع فى المدى الطبيعى للعصائر التجارية.

كما اظهرت نتائج محتوى العصائر من السكريات الكلية وفيتامين C تباينا كبيرا فيما بينهم حيث يقع الاول فى مدي 6.51 و19.35% والثاني بين 2.54 و28.11 مجم/ 100مل.

واوضحت النتائج ايضا ان اضافة مستخلص الروزماري لخلطات العصائر قد ادي الي وجود زيادة معنوية في قيم كل من الفينولات الكلية والفلافونويدات الكلية والانثوسيانين مقارنة بالعينة الضابطة حيث تصدر كل من خلطات التوت الاسود والفراولة والرومان أعلي قيم بالنسبة للفينولات الكلية في حين تصدر كل من خلطات الفراولة والتوت والبرقوق أعلي قيم بالنسبة للفلافونويدات الكلية.احتلت خلطة عصير الفراولة أعلى قيمة معنوية بالنسبة للأنثوسيانين الكلى(800 مجم/لتر) مقارنتا بجميع خلطات العصائر تحت الدراسة والعينة الضابطة.

أوضحت نتائج النشاط المضاد للأكسدة (مقاسة على أساس % تثبيط) أن كل من خلطات عصائر الفراولة والرومان والبرقوق كان لهم أعلي نشاط مضاد للأكسدة مقارنة بباقي الخلطات. تتنبأ الدراسة أن اضافة مستخلص الروزمارى لخلطات عصائر الفواكه يمكن أن يحقق قيمة مضافة فى تعزيز الصحة والحماية من الأمراض.

تتنبأ هذه الدراسة أن خلطات عصائر الفواكه والروزماري من الممكن ان تعزز من الحالة الصحية ضد الأمراض التي لها علاقة بالضغط التأكسدي (Oxidative stress).