

## Screening of phytochemical content of commercial apricot- and orange-based beverages and its relationship with antioxidant capacity

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### Abstract

The aim of the present research study was to compare the antioxidant properties of beverages based on apricot and orange fruits as well as to establish if the combination of these resulted in a beverage with high antioxidant content. For this purpose, the quantification of some antioxidants (total phenolic compounds (TP), ascorbic acid (AA) and proanthocyanidins (PA)) using colorimetric assays and the determination of antioxidant activities of apricot- and orange-based beverages as well as their mixture were performed. The results revealed that the combination of apricot- and orange-based beverages had antagonistic effect on TP and AA content, unlike PA content. Moreover, these antioxidants correlated positively with total antioxidant capacity. The principal component analysis showed cumulative variance of approximately 85% which mean that the chosen parameters had a high discriminate power and they can be a useful criterion for the determination of quality of commercial beverages.

### Keywords

Bioactive compounds

Antioxidant capacity

Apricot- and orange-based beverages

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### Introduction

In the last two decades there has been an increasing interest in determining relevant dietary sources of antioxidant compounds. Thus, in some countries like Algeria, *Prunus* and *Citrus* fruits have received attention due to their high consumption as they can be a good source of bioactive compounds such as phenolics and ascorbic acid (Ouchemoukh *et al.*, 2013; Stinco *et al.*, 2013) which have been implicated in the reduction of degenerative human diseases, mainly because of their antioxidant potential (Del Caro *et al.*, 2004; Klimczak *et al.*, 2007).

The agricultural food industry is now one of the most dynamic in Algeria, mainly in the field of juices and beverages. In 2008, the production achieved 19 million hectolitres while it was 12 million hectolitres in 2002 (Ministère du commerce, Algérie, 2012), indicating that the consumption of beverages has rapidly increased, especially apricot- and orange-based beverages, which are widely consumed in Algeria. Fruit-based beverages, which are not juices, are known to constitute the primary source of naturally occurring antioxidants in the human diet; they possess many bioactive components that exert antioxidant and anticancer effects (Liu, 2003; Chira *et al.*, 2008). Therefore, for health promotion and disease prevention, it could be of importance to know the total antioxidant capacity (TAC) of fruit-based beverages that represents the cumulative capacity of the dietary components to scavenge free radicals

(Serrano *et al.*, 2007; Pérez-Jiménez *et al.*, 2008). This concept reflects the integrated and/or synergistic effects of all antioxidants (Wu *et al.*, 2004). The TAC may be viewed as a functional measure of a food or beverage, as it includes all the components with antioxidant capacity (e.g. vitamin C and phenolic compounds). A determination of the TAC of food beverages could provide a possibly useful measure of their functionality (Barba *et al.*, 2013).

Several published articles have been conducted to study bioactive compounds and antioxidant capacity in apricot and orange juices (Aider and De Halleux, 2008; Versari *et al.*, 2008; Stinco *et al.*, 2013). However, in the literature available at present, there is a lack of information about the activity of antioxidants in apricot- and orange-based beverages. The aim of our study was to evaluate bioactive compounds (ascorbic acid, total phenolic component and proanthocyanidins) of commercial apricot- and orange-based beverages and their relationship with antioxidant capacity measured using eight *in vitro* antioxidant assays ( $H_2O_2$ , TBARS, FRP, CP, DPPH, SOSC, NO and  $HO^\bullet$  tests), as there is an increasing number of this kind of food beverage commercialised in Algeria supermarkets, with new formulations, and the following nutritional interest by consumer.

### Material and Methods

#### Sample material

Three units from each of two batches of

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commercial fruit-based beverages largely consumed in Algeria (apricot nectar, orange drink and cocktail) were used for the determinations. Apricot- and orange-based beverage had 40 and 12% of fruit content, respectively. The combination apricot- and orange-based beverages (cocktail) had 12% of fruit content with proportion of 20 and 80%, respectively. Samples were centrifuged (20 min, 3000 rpm) to remove pulp.

### Chemicals

Hydrogen peroxide, 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and ferrozine were from Sigma–Aldrich (Sternheim, Germany). Folin–Ciocalteu reagent was from Biochem, Chemopharma (Montreal, Quebec); sodium carbonate, potassium ferricyanide, trichloroacetic acid (TCA) and hydrochloric acid were from Biochem, Chemopharma (Georgia, USA); 2-Deoxy-D-ribose and sodium nitroprusside were from Fluka BioChemica (Germany); ferric chloride was from Panreac (Barcelona, Spain); tris and ethylene-diamine-tetraacetic acid (EDTA) were from BDH, Prolabo (CE). All chemicals and solvents used were of analytical grade.

### Bioactive compounds quantification

The total phenolic (TP) content was assessed according to Singleton and Rossi (1965). Aliquots (200  $\mu$ L) of sample were mixed with 1 mL of 10% Folin-Ciocalteu reagent and 800  $\mu$ L of 7.5% sodium carbonate solution, and the absorbance (Shimadzu Uvi-mini 1240 spectrophotometer) was recorded at 765nm. The results were expressed as mg gallic acid equivalent (GAE) per 100 mL of beverage. Proanthocyanidins (PA) content was determined by the method based on acid hydrolysis and colour formation (Porter *et al.*, 1986). Two millilitres of sample were mixed with 20 mL of Fe sulphate solution (77 mg of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 500 mL of HCl: n-butanol/2:3) and incubated for 15 min at 95°C. After cooling to room temperature, absorbance was measured at 530 nm. The results were expressed as mg cyanidin equivalent (CE) per 100 mL of beverage. Ascorbic acid (AA) content was assessed by the DCPIP method (Klein and Perry, 1982). One millilitre of sample was mixed with 9 mL of 2,6-dichloro-indophenol (15  $\mu$ g/mL); the absorbance was read at 515 nm and the results were expressed as mg AA/100 mL of beverage.

### Antioxidant activity

#### Hydrogen peroxide scavenging capacity

The scavenging capacity of fruit beverages

against hydrogen peroxide was determined according to Ruch *et al.* (1989). The test tubes were prepared with 1.5 mL of beverage and  $\text{H}_2\text{O}_2$  (1 mL, 40 mM) in phosphate buffer (0.1 M, pH 7.4). After 10 min incubation, the absorbance was measured at 230 nm. The percentage of  $\text{H}_2\text{O}_2$  inhibition effect was calculated using the following equation:

$$\text{Activity (\%)} = [(A_c - A_s)/A_c] \times 100 \quad (\text{Eq. A})$$

Where  $A_c$  and  $A_s$  are the absorbance of the control and the absorbance of the sample, respectively.

#### Thiobarbituric acid reactive species test (TBARS)

The determination of TBARS was based on the method reported by Daker *et al.* (2008). Briefly, 100  $\mu$ L of beverage was added to a mixture that contained 1 mL of egg yolk in 0.1 M phosphate buffer pH 7.4, and 100  $\mu$ L of 1 mM  $\text{Fe}^{2+}$ . After incubation at 37°C for 1 h, 500  $\mu$ L of 15% TCA and 1 mL of 1% thiobarbituric acid were added to the mixture. After incubation at 95°C for 10 min, the tubes were centrifuged at 3500 rpm for 10 min, and the absorbance recorded at 532 nm. The inhibition of reactive species was calculated according to Eq. A.

#### Reducing power (RP)

The ferric reducing power of beverages was assessed according to Oyaizu (1986). Samples (2.5 mL) were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. After incubation (20 min at 50°C), 2.5 mL of 10% TCA were added to the mixture. After centrifugation, 2.5 mL of the upper layer were diluted with distilled water (v/v) and 500  $\mu$ L of 0.1% ferric chloride were added. The absorbance was measured at 700 nm and the results were expressed as mg AAE/100 mL.

#### Chelating power (CP)

The chelating power of beverages on ferrous ions was examined as described by Dinis *et al.* (1994). Volume of sample was mixed with same volume of 1 mM  $\text{FeCl}_2$  and 0.25 mM ferrozine. After incubation for 10 min, the absorbance was read at 562 nm. The ferrous ion chelating capacity was calculated according to Eq. A.

#### DPPH antiradical activity

The antiradical activity of beverages on DPPH free radical was performed as reported by Brand-Williams *et al.* (1995). Aliquot (200  $\mu$ L) of sample was added to 1 mL DPPH methanolic solution (60  $\mu$ M). After incubation at room temperature for 30 min, the absorbance was recorded at 517 nm. The results were expressed as mg AAE/100 mL.

### *Superoxide scavenging capacity (SOSC)*

The superoxide scavenging capacity of beverages was measured according to the method of Jiao *et al.* (2005). Aliquot (200  $\mu$ L) of sample was added to 1.8 mL of Tris-HCl (50 mM) and EDTA (2 mM) buffer solution (pH 8.2). After incubation (25°C for 10 min), 80  $\mu$ L of pyrogallol (5 mM) were added. The mixture was again incubated at 25°C for 3 min and the reaction was stopped by addition of drops of ascorbic acid. The absorbance was recorded at 420 nm and the SOSC was calculated according to Eq. A.

### *Nitric oxide scavenging capacity (NO)*

The nitric oxide radical scavenging capacity of beverages was assayed using method reported by Ebrahimzadeh *et al.* (2010). The reaction mixture (800  $\mu$ L) containing sodium nitroprusside (10 mM) and sample was incubated at 25°C for 150 min. A volume of the mixture was removed and added with to an equal volume of the Griess reagent. The absorbance was measured at 540 nm and the inhibition of nitric oxide was calculated according to Eq. A.

### *Hydroxyl radical scavenging activity (HO•)*

Hydroxyl radical scavenging capacity of beverages was determined according to the method described by Halliwell *et al.* (1987). The reaction mixture (100  $\mu$ L of sample diluted with phosphate buffer 50 mM, pH 7.4, 100  $\mu$ L of 60 mM 2-deoxyribose, 200  $\mu$ L of a premixed 4 mM FeCl<sub>3</sub> and 4 mM EDTA 1:1, v/v, 100  $\mu$ L of 20 mM H<sub>2</sub>O<sub>2</sub> and 100  $\mu$ L of 4 mM ascorbic acid) was incubated at 37°C for 1 h. Then, 1 mL of 20% TCA and 1 mL of 0.8% TBA were added. The reaction mixture was heated in a boiling water bath for 15 min. The absorbance was recorded at 532nm. The hydroxyl radical scavenging capacity was calculated according to Eq. A.

### *Statistical analysis*

The results were submitted to a factorial analysis of variance. The mean values were compared using the least significance difference test (LSD) at 5% level. All the tests were performed in triplicates and the results averaged. Multivariate statistical treatments (Hierarchical Cluster Analysis HCA and Principal Component Analysis PCA) were carried out using Statistica®. This data analysis provides graphic representations which constitute the best possible summary of lot of information. Also, they enable us to represent measured variables on a graph in order to study their proximity and classify them.

## **Results and Discussion**

### *Antioxidants*

#### *Total phenolic content (TP)*

The total phenolic content can be considered as an important indicator of antioxidant capacity which can be used as a preliminary screen for any product when intended as a natural source of antioxidants in functional foods (Barba *et al.*, 2013). The total phenolic content of beverages is presented in Table 1. Apricot nectar showed a higher TP content (70.55 mg GAE/100 mL) than orange drink and cocktail (20.36 mg GAE/100 mL and 17.97 mg GAE/100 mL, respectively). Our result for apricot nectar was in the interval reported by Wootton-Beard *et al.* (2011) for total polyphenol content of 23 commercially vegetable juices; however the TP content for orange drink and cocktail were markedly lower. Likewise, the extrapolation of the results reported by Zabidah *et al.* (2011) on the tropical juices, the assessed beverages in this study might contribute to the protection effect against LDL oxidation since they contain high TP content than tropical juice which ranked from 10.01 to 24.64 mg/100 mL.

#### *Proanthocyanidins (PA)*

Proanthocyanidins are known to inhibit lipid peroxidation and information has been accumulated over the past few years demonstrating their ability to scavenge radicals such as hydroxyl, superoxide and peroxy, which are known to be important in cellular prooxidant states (Gyamfi and Aniya, 2002). The results of proanthocyanidins content in fruit-based beverages are presented in Table 1. Apricot nectar contains higher level of proanthocyanidins (17.95 mg CE/100 mL) than orange drink and cocktail (11.73 mg CE/100 mL and 10.48 mg CE/100 mL, respectively). The statistical analysis revealed significant differences at  $p < 0.05$  between the assessed beverages. In addition, our results revealed that the proanthocyanidins represent the major constituents of orange drink and cocktail phenolics with a PA/TP ratio higher than 50% (Table 1). In our previous investigation (Benmeddour *et al.*, 2013), we have also found that PA/TP ratio ranged from 44 to 82%, for Algerian date cultivars.

#### *Ascorbic acid content*

Ascorbic acid is one of the most important organic acids in fruits and vegetables. Since ascorbic

Table 1. Total phenolic (TP), proanthocyanidin (PA) and ascorbic acid (AA) contents of fruit-based beverages expressed in mg per 100 mL

	TP	PA	AA	(PA/TP)x100
Apricot	70.55 ± 3.61 <sup>a</sup>	17.95 ± 0.23 <sup>a</sup>	38.99 ± 0.93 <sup>a</sup>	25.44
Orange	20.36 ± 3.13 <sup>b</sup>	11.73 ± 1.05 <sup>b</sup>	14.56 ± 2.52 <sup>b</sup>	57.61
Cocktail	17.97 ± 0.42 <sup>b</sup>	10.48 ± 0.06 <sup>c</sup>	12.30 ± 1.05 <sup>b</sup>	58.32
Cocktail(Apricot+Orange)	0.876	1.00	0.879	-

Results are means of nine values (3 independent repetitions × triplicate analyses) ± standard deviation (S.D.). Means within a row designated by different letters are significantly different by the LSD test at  $p < 0.05$ .

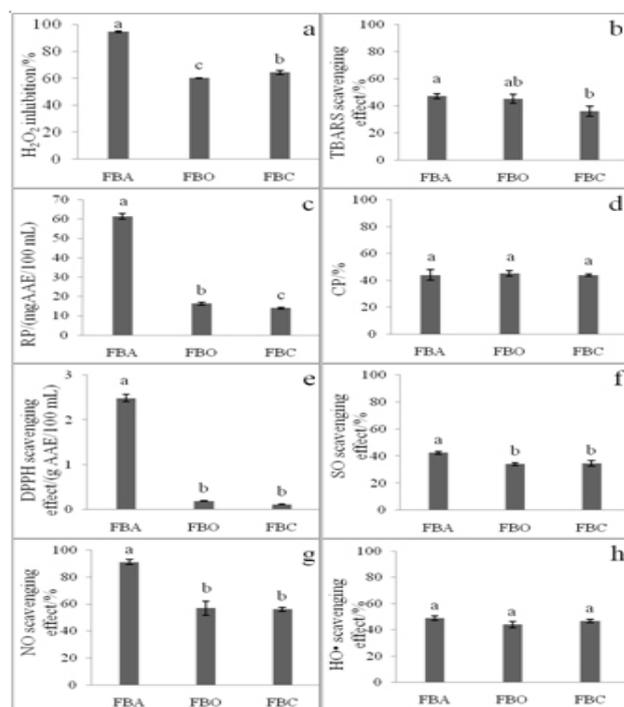


Figure 1. Antioxidant activities of fruit-based beverages: (a) hydrogen peroxide inhibition, (b) thiobarbituric acid species test, (c) reducing power, (d) chelating power, (e) DPPH, (f) superoxide anion radical, (g) nitric oxide and (h) hydroxyl radical scavenging effects

acid is highly unstable, its content has not only been used as a nutritional index, but also for evaluating processing effects (Barba *et al.*, 2012). Ascorbic acid was assayed by DCPIP which is a rapid, easy and cheap method. As indicated in Table 1, the highest AA content in beverages was exhibited by apricot nectar (38.99 mg/100 mL) which was significantly different ( $p < 0.05$ ) from both orange drink and cocktail (14.56 mg/100 mL and 12.31 mg/100 mL, respectively); however, non-significant differences were observed between orange drink and cocktail. Our results were higher than those reported in the published articles for several juices and beverages (Igual *et al.*, 2010; Barba *et al.*, 2013). The differences in ascorbic acid content may be due to the formulation (juice, nectar or drink), fabrication process (pasteurization), fruit type and storage conditions.

### Antioxidant activity

Evaluation of the total antioxidant capacity cannot be performed accurately by any single test due to the complex nature of phytochemicals (Frankel and Finley, 2008; Du *et al.*, 2009); so many methods have been proposed to evaluate the antioxidant potential of natural antioxidant source. Usually, these methods measure the ability of antioxidants to chelate metal ions, scavenge ROS or inhibit lipid peroxidation. In the present work, eight antioxidant activity assays (H<sub>2</sub>O<sub>2</sub>, TBARS, RP, CP, DPPH, SOSC, NO, HO<sup>•</sup> test) were used to evaluate the antioxidant capacity of the fruit beverage.

### Hydrogen peroxide scavenging capacity (H<sub>2</sub>O<sub>2</sub>)

Hydrogen peroxide can be formed *in vivo* by many oxidizing enzymes such as superoxide dismutase. Also, it is a relatively unstable metabolic product being responsible for the generation of hydroxyl radical and singlet oxygen. H<sub>2</sub>O<sub>2</sub> is formed by the Fenton reaction and initiates lipid peroxidation (toxic to cells). With reactive oxygen species (ROS), hydrogen peroxide can damage several cellular constituents. Thus, the removing of H<sub>2</sub>O<sub>2</sub> is very important for antioxidant defence in cells. Apricot nectar exhibited the best activity against H<sub>2</sub>O<sub>2</sub> with 94.67% removal, followed by cocktail and orange drink with 64.33% and 60.26%, respectively (Figure 1a). These differences were statistically significant at  $p < 0.05$ .

### Thiobarbituric acid reactive species test (TBARS)

The beverages used in this study were examined for their ability to act as radical scavenging agents. The highest antioxidant effect against reactive species was exhibited by apricot nectar with 47.10%, followed by orange drink and cocktail (45.21% and 35.92%, respectively). Statistically significant differences ( $p < 0.05$ ) were observed between apricot nectar and cocktail. However, orange drink did not exhibit any significant difference with both apricot nectar and cocktail (Figure 1b).

### Reducing power (RP)

The reducing power of compound may serve as a significant indicator of its potential antioxidant activity. The reducing activities are generally associated with the presence of reductones which have been shown to exhibit antioxidant action by breaking the chain reactions by donating hydrogen atom (Duh *et al.*, 1999). For the measurement of the reductive ability, we investigated the ferric-ferrous transformation in the presence of beverages. In this

method, antioxidant compounds form a coloured complex with potassium ferricyanide, trichloroacetic acid (TCA) and ferric chloride. The highest reducing power of beverages was achieved by apricot nectar with 61.36 mg AAE/100 mL, followed by orange drink and cocktail (16.34 mg AAE/100 mL and 13.92 mg AAE/100 mL, respectively). Statistically significant differences ( $p < 0.05$ ) were noted between the three beverages (Figure 1c).

#### *Chelating power (CP)*

Elemental species such as ferrous iron ( $\text{Fe}^{2+}$ ) can facilitate the production of ROS and lipid peroxidation within animal and human systems; hence, the ability of substances to chelate iron can be a valuable antioxidant capacity. The chelating effect of ferrous ions by the investigated beverages was determined according to the method of Dinis *et al.* (1994); in the presence of chelating agents the complex formation (Ferrozine-  $\text{Fe}^{2+}$ ) is disrupted, resulting in a decrease of the complex colour. The data obtained from (Figure 1d) reveal that beverages demonstrate a marked capacity for iron binding (44.27, 45.23, and 43.92% for apricot nectar, orange drink, and cocktail, respectively). This fact indicates that fruit beverages interfered with the formation of ferrous and ferrozine complex. In addition, the statistical analysis revealed no significant difference between the beverages.

#### *DPPH antiradical activity*

The free radical scavenging activity of the fruit beverages was assessed by DPPH assay which is one of the most widely used method for screening antioxidant activity. The role of antioxidant is its interaction with oxidative free radicals. In the present study, apricot nectar exhibits a much higher antioxidant effect (2482.32 mg AAE/100 mL) than orange drink and cocktail (190.02 mg AAE/100 mL and 115.49 mg AAE/100 mL, respectively). No significant difference at  $p < 0.05$  was noted between orange drink and cocktail (Figure 1e). Wootton-Beard *et al.* (2011) reported an antiradical activity of 23 commercial juices between  $57.8 \pm 1.9$  and  $100 \pm 0.0\%$ .

#### *Superoxide scavenging capacity (SOSC)*

Superoxide anion is an initial free radical and plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems (Stief, 2003). It can also react with nitric oxide and form peroxyxynitrite, which can generate toxic compounds such as hydroxyl radical and nitric dioxide (Halliwell *et al.*, 1997). Scavenging

capacity of beverages on superoxide anion radicals was evaluated using a pyrogallol autoxidation system. Results obtained in this assay revealed that inhibition effects on pyrogallol autoxidation did not differ significantly at  $p < 0.05$  between orange drink (34.11%) and cocktail (34.64%). The apricot nectar exhibited the strongest scavenging effect (41.02%) which differs significantly from orange drink and cocktail (Figure 1f).

#### *Nitric oxide scavenging capacity (NO)*

Oxidative stress caused by the production of nitric oxide excess during infection or inflammation has been implicated in the pathogenesis of several diseases. Accordingly, the scavenging of NO radical may be a promising indicator in screening healthy foods (Tsai *et al.*, 2007). Our study proved that beverages are a potent scavenger of NO. The maximum nitrite radical scavenging effect was recorded for apricot nectar (91.25%) followed by orange drink and cocktail (57.04% and 56.17%, respectively) (Figure 1g). Statistically, orange drink and cocktail did not differ significantly at  $p < 0.05$ , while apricot nectar differs significantly from the other beverages.

#### *Hydroxyl radical scavenging activity (HO $\cdot$ )*

The hydroxyl radical was generated by using Fenton reaction which otherwise would degrade deoxyribose substrate if not inhibited by the sample. Formation of  $\text{HO}\cdot$  by the Fenton reaction is representative of the events that occur *in vivo* in iron rich tissues like liver where it contributes to the initiation of lipid peroxidation (Porter, 1984). Besides, its high reactivity can lead to DNA damage and inactivation of proteins (Stojs and Bagchi, 1995). Therefore, the scavenging activity of  $\text{HO}\cdot$  can be considered as one of the best indicators of the antioxidant potential. The highest antioxidant effect against  $\text{HO}\cdot$  was exhibited by apricot nectar with 49.06%, followed by cocktail and orange drink (44.17% and 46.60%, respectively). Although fruit beverages have a potent effect, no significant difference was revealed between apricot nectar, orange drink and cocktail at  $p < 0.05$  (Figure 1h). Lichtenthaler and Marx (2005) also found a high antioxidant capacity against hydroxyl radicals in fruit juices and nectars.

A Pearson's test was conducted to explore the relationships between the different antioxidant variables measured for beverages (Table 2A). The antioxidant capacity of beverages revealed to be largely influenced by the ascorbic acid and total phenolic levels. In this line, several authors reported significant correlation between antioxidant and total

Table 2. Lineal correlation coefficients between antioxidant composition and antioxidant capacity (A), and lineal correlation between the different methods for quantifying antioxidant capacity (B)

A	H <sub>2</sub> O <sub>2</sub>	TBARS	RP	CP	DPPH	SOSC	NO	HO•
TP	0.98***	0.60**	1.00***	-0.13 <sup>ns</sup>	0.99***	0.93***	0.99***	0.69**
PA	0.86***	0.59**	0.88***	-0.03 <sup>ns</sup>	0.86***	0.74**	0.86***	0.75**
AA	0.98***	0.64**	0.99***	-0.04 <sup>ns</sup>	0.99***	0.93***	0.99***	0.68**
B	H <sub>2</sub> O <sub>2</sub>	TBARS	RP	CP	DPPH	SOSC	NO	HO•
TBARS	0.47*	1.00						
RP	0.99***	0.60**	1.00					
CP	-0.12 <sup>ns</sup>	-0.01 <sup>ns</sup>	-0.13 <sup>ns</sup>	1.00				
DPPH	0.90***	0.57*	1.00***	-0.08 <sup>ns</sup>	1.00			
SOSC	0.93***	0.54*	0.94***	-0.18 <sup>ns</sup>	0.95***	1.00		
NO	0.97***	0.59**	0.98***	-0.00 <sup>ns</sup>	0.99***	0.93***	1.00	
HO•	0.73**	0.21 <sup>ns</sup>	0.67**	0.09 <sup>ns</sup>	0.67**	0.61**	0.74**	1.00

\*, \*\*, \*\*\* = significant correlation at  $p < 0.05$  or  $0.01$  or  $0.001$ , respectively.

<sup>ns</sup> = non significant

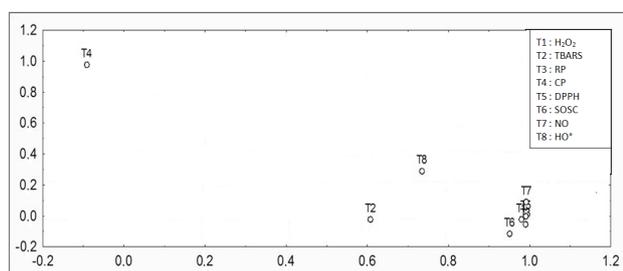


Figure 2. Plot of factorial weights in first factor versus factorial weights in second factor from the antioxidant activities of fruit-based beverages

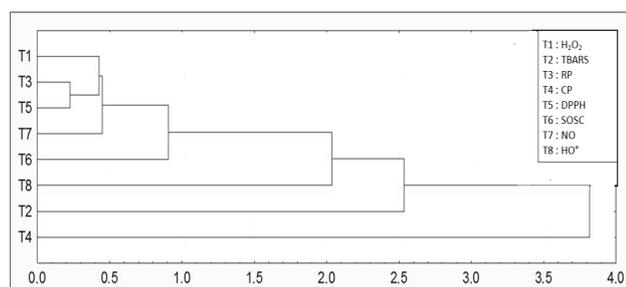


Figure 3. Hierarchical cluster analysis of antioxidant activity obtained by Euclid's distances

antioxidant capacity of commercial beverages (Barba *et al.*, 2013). RP, DPPH, H<sub>2</sub>O<sub>2</sub>, NO, SOSC, HO• and TBARS tests were highly correlated ( $p < 0.01$ ) with TP, PA and AA contents. However, no significant correlation was found with CP. These data are in agreement with Lee *et al.* (2003) and Serrano *et al.* (2005). Moreover, the combination of apricot- and orange-based beverages had antagonistic effect on the TP compound and ascorbic acid content, unlike proanthocyanidins content.

Because multiple reaction characteristics and mechanisms are likely involved, no single assay will accurately reflect all antioxidants in a mixed or complex system. Thus, to elucidate a full profile of antioxidant capacity of beverages, different antioxidant capacity assays were chosen. Amongst the eight assays used, significant correlations ( $p < 0.05$ ) were found between H<sub>2</sub>O<sub>2</sub>, RP, DPPH, SOSC

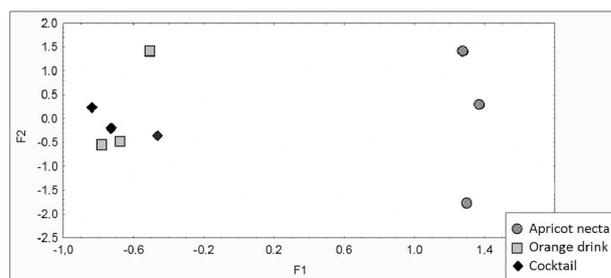


Figure 4. Plot of first factor versus second factor for the classification of fruit-based beverages to their antioxidant activities

and NO tests, while TBARS, HO• tests and CP exhibited weak or no correlation with other activities (Table 2B). As one would expect, H<sub>2</sub>O<sub>2</sub>, TBARS, RP, DPPH, SOSC, NO and HO• activities were well correlated since these tested methods for measuring antioxidant capacity are based on electron transfer.

Concerning the comparison between beverages, Principal Component Analysis (PCA) is applied to the antioxidant activity results in order to determine the differences between beverages. The first principal component accounts for 71.66% of the variance and the second for 13.22%. The cumulative variance is approximately 85%, which shows that the chosen beverages are well distinguished by the antioxidant activity assays.

A scatter plot was obtained correlating the factorial weights of features in the first factor against the factorial weights in the second factor. It can be seen from Figure 2 that H<sub>2</sub>O<sub>2</sub>, RP, DPPH, SOSC and NO tests present high correlation with the first factor which is superior to 90% while HO•, TBARS and CP tests revealed weak or no correlation.

The application of Hierarchical Cluster Analysis (Figure 3) confirmed that effect of beverages on H<sub>2</sub>O<sub>2</sub>, RP, DPPH, SOSC and NO tests is almost similar, with an averaged Euclidis distance of 0.6; their comparison by ANOVA revealed no significant difference at  $p < 0.05$ . However the CP test was distant from the first group by 2.9 Euclid's distance.

The graphic distribution of the fruit beverages according to their factor scores (Figure 4) showed that apricot nectar is differentiated from orange drink and cocktail, tending to higher values of antioxidant component as mentioned above. This means that the chosen parameters have a high discriminate power and they can be a useful criterion for the determination quality as well as avoid mistakes or fraudulency by the industry.

## Conclusion

In conclusion, this research study revealed that apricot- and orange-based beverages as well as their

mixture are good source of natural compounds with significant total antioxidant activity assessed by eight *in vitro* assays. The combination of apricot- and orange-based beverages had antagonistic effect on TP and AA content, unlike PA content. Moreover, these antioxidants correlated positively with total antioxidant capacity. The multivariate statistical treatments revealed that *in vitro* assays of total antioxidant activity had a high discriminate power. These assays could be a useful criterion when screening the total antioxidant activity of liquid foods.

As perspectives, we project to submit these fruit-based beverages to different storage conditions in order to determine their stability.

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