

Effect of Storage Time and Temperature on the Health-Promoting Substances and Antioxidant Activity of Two Commercial Fruit Based-Beverages

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Abstract

In the current study, changes in health-promoting substances (phenolics and flavonoids) and antioxidant activity of carrot and mango beverages during storage at 25 and 35°C for 30, 60 and 90 days were investigated. The total phenolic content was determined using Folin–Ciocalteu method. The antioxidant activity was based on the evaluation of free–radical scavenging activity (FRSA) using DPPH radical and ferric reducing power (FRP). At the end of storage, the analysed beverages exhibited a significant loss of phytochemicals and antiradical activity. Extremely significant correlation ($p < 0.001$) was observed between phenolic and flavonoid contents, and the antioxidant activity (FRSA and FRP) of carrot and mango beverages.

Keywords

Carrot and Mango Beverages, Storage, Phenolics, DPPH, Ferric Reducing Power

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1. Introduction

Currently, the food industry searches for market opportunities and tends to diversify its products by the preparation of new foods, enhancing sensorial, nutritional and functional attributes (Wootton-Beard and Ryan, 2011; Andrés *et al.*, 2015). Innovation, convenience and quality are considered as important marketing tools in the food industry (Rodríguez-Roque *et al.*, 2015). In this regard, the soft beverages sector has rapidly developed new ready-to-drink presentations, among which are beverages based on fruits and vegetables that are becoming more popular since they represent an easy and convenient way of consuming fruits, which are important sources of health-promoting compounds, such as phenolics (Gironés-Vilaplana *et al.*, 2015). Phenolic compounds are widely distributed in plants; they have been shown as the main contributors to the antioxidant activity of most of foods and beverages (Balasundram *et al.*, 2006). These compounds

possess numerous biological properties such as antioxidant and free radical scavenging activities which can prevent cancers, cardiovascular diseases, inflammation, Alzheimer's disease, diabetes and other oxidative stress-induced diseases (Klimczak *et al.*, 2007; Rodríguez-Roque *et al.*, 2015).

Literature data indicate that quality parameters of foods are affected by many factors including storage temperature and duration, packaging material, and oxygen concentration. To the best of our knowledge, there have been relatively few studies about the effect of storage temperature on the phytochemical content and antioxidant capacity of commercial fruits-based beverages when compared to the various foods and juices. Therefore, the purpose of the current study was to monitor the changes in the phytochemical (total phenolics and flavonoids) content and antioxidant activity of commercial carrot and mango fruit-based beverages marketed in Algeria, during storage at 25 and 35°C.

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2. Materials and Methods

2.1. Chemicals

Folin–Ciocalteu reagent, methanol and ethanol were from Biochem, Chemopharma (Montreal, Quebec); sodium carbonate was from Sigma–Aldrich (Switzerland); aluminium chloride and potassium ferricyanide were from Biochem, Chemopharma (Georgia, USA); gallic acid was from Prolabo (Montreuil, France) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) was from Sigma–Aldrich (Germany).

2.2. Samples and Preparation of Extracts

Three bottles from each of two batches of commercial fruit-based beverages largely consumed in Algeria (carrot and mango drinks) were used in the current study. Based on the details indicated on the label, carrot beverage is composed of orange juice concentrate, carrot juice, lemon juice, water, sugar (sucrose), etc. Mango beverage is composed of mango puree and mango puree concentrate, water, sugar (sucrose), etc.

The samples were divided into two groups. The first group was stored in the dark at 25°C and the second at 35°C. The investigated parameters were determined in the freshly manufactured samples of each batch, and after 30, 60 and 90 days.

Extracts were prepared by mixing equal volumes of sample and 70% ethanol. After agitation and centrifugation at 5000 rpm for 20 min, the extracts were recovered after filtration.

2.3. Quantification of Total Phenolic Contents (TPC)

The phenolic content of the extracts was determined according to Singleton and Rossi (1965). Briefly, ethanolic extract (200 µL) was mixed with 1/10 (v/v) diluted Folin–Ciocalteu reagent (700 µL) and 7.5% sodium carbonate (400 µL). The mixture was incubated at room temperature for 90 min, and the absorbance was measured at 765 nm (Uviline 9400 UV-visible spectrophotometer, Secomam, France). TPC were expressed as mg gallic acid equivalents per 100 mL of beverage (mg GAE/100 mL).

2.4. Quantification of Total Flavonoid Contents (TFC)

The flavonoid content of the extracts was determined according to Quettier-Deleu *et al.* (2000). One thousand microlitres of sample were mixed with 1 mL of aluminium chloride. The mixtures were allowed to stand at room temperature for 10 min. Absorbance was measured against the blank at 430 nm. TFC were expressed as mg quercetin equivalents per 100 mL of beverage (mgQE/100mL).

2.5. Antioxidant Activities

2.5.1. DPPH Free–Radical Scavenging Activity (FRSA)

The 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical was used to measure the antioxidant activity of beverages (Tezcan *et al.*, 2009). Each extract (100 µL) was added to 1mL of methanolic DPPH solution. The decolorizing process was measured at 517 nm after 30 min of reaction and compared to a blank control. The antioxidant activity was expressed as mg gallic acid equivalents per 100 mL of beverage (mg GAE/100mL).

2.5.2. Ferric Reducing Power (FRP)

This method is based on the measurement of the ferric reducing activity of beverages. The reducing power of the extracts was measured according to Oyaizu (1986). One hundred microlitres of extract were mixed with 250 µL of phosphate buffer (0.2 M, pH 6.6) and 250 µL of 1% potassium ferricyanide. After incubation for 20 min at 50°C, 250 µL of 10% trichloroacetic acid and 850 µL of distilled water were added to the mixture, and finally 170 µL of 0.1% ferric chloride were added. The absorbance was measured at 700 nm. Gallic acid was used as a standard and ferric reducing power was expressed as mg GAE/100mL of beverage.

2.6. Statistical Analysis

The results were submitted to a bi-factorial (time and temperature) analysis of variance (ANOVA). The mean values were compared using the least significant difference test (LSD) at 5% level using infostat software. All the determinations were carried out in triplicate. Correlations were performed using the correlation matrix of the STATISTICA 5.5 software at three different significance levels (0.05, 0.01 and 0.001).

3. Results and Discussion

3.1. Effect of Storage on Phenolic Compound Content

Phenolic compounds, ubiquitous in fruits and vegetables are the most abundant antioxidants in the human diet, and are of considerable interest due to their antioxidant properties. Initial phenolic compound contents of carrot and mango beverages were 78.72 and 41.35 mgGAE/100mL, respectively. As it was shown in Table 1, time and temperature of storage affected the total phenolic content as determined by Folin-Ciocalteu assay. Content of these compounds decreased significantly during storage under the experimental conditions applied. After 90 days storage at 25

and 35°C, phenolic content decreased by 36.05%, 42.61% for carrot drinks and 28%, 41.64% for mango drinks, respectively. These results were consistent with the findings of Lee *et al.* (2007) and Laorko *et al.* (2013) who reported a decrease in phenolic content in clarified banana juice after 90 days at 25 and 35°C and 6 months at 27 and 38°C, respectively. In the same sense, Mgaya-Kilima *et al.* (2014) concluded a decrease in total phenolic compounds of roselle-fruit juice blends stored at 28°C for 6 months. The decrease in polyphenols could mainly be resulted from oxidation of these compounds and polymerization with proteins (Liu *et al.*, 2014). Also, Klimczak *et al.* (2007) found a decrease in total phenolics in orange juices after 4 months at 18, 28 and 38°C.

3.2. Effect of Storage on Flavonoid Content

In the literature, little information is available on the changes of flavonoid content of beverages during storage. In the current investigation, a significant ($p < 0.05$) decrease in total flavonoid contents of carrot and mango beverages was observed during the first month of storage (Table 1). Subsequently, after 90 days storage at 25 and 35°C, the content of these bioactive compounds decreased by 33.12%, 26.38% for carrot drinks and 75%, 70.83% for mango drinks, respectively. Statistical analysis revealed that time-temperature interaction factor had a significant effect ($p < 0.05$). Our results were higher than those reported by Hamedani *et al.* (2012) for the blood orange juice conserved at 22°C during 85 days. Oszmianski and Wojdylo (2009) found a loss from 37.28 to 50.50% of flavonols in apple juice after 6 months storage at 30°C.

3.3. Effect of Storage on Antioxidant Activity

Estimation of the antioxidant capacity of foods and beverages has been accomplished by various methods based on different reaction mechanisms. Among them, those involving stable radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the ferric reducing power (FRP) are some of the most used to assess the antioxidant capacity of a variety of foods and beverages.

3.3.1. Free Radical Scavenging Activity

In humans, fruit and vegetable consumption seems to play an important role as a health-protecting factor. This beneficial effect is mainly associated with the radical scavenging activity of phytochemicals, which are largely present in fresh fruits and their processed products. Initial antioxidant activity of carrot and mango beverages was 61.22 and 36.98 mg GAE/100mL, respectively (Table 1). After 90 days storage at 25 and 35°C, free-radical scavenging activity of carrot and mango beverages decreased by 64.71, 71.20% and 51.28, 59.43%, respectively. Antioxidant activity decreased significantly ($p < 0.05$) with increased storage temperature and time. The results of the current investigation are in line with the data obtained by Klimczak *et al.* (2007) who reported a decrease in antioxidant activity of commercial orange juices after 6 months of storage (45% at 28°C and 84% at 38°C). However, a slight decline (5.49%) was reported by Oszmianski and Wojdylo (2009) in apple juice stored at 30°C after 6 months.

Table 1. Effect of storage on the phytochemical and antioxidant activity of commercial carrot and mango beverages.

Storage (Days)	Total phenolics (mgGAE/100mL)		Flavonoids (mgQE/100mL)		FRSA (mgGAE/100mL)		FRP (mgGAE/100mL)	
	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C
Carrot beverage								
0	78.72Aa	78.72Aa	1.63Aa	1.63Aa	61.22Aa	61.22Aa	38.82Aa	38.82Aa
30	64.94Ba	60.76Bb	1.26Ba	1.16Bb	46.49Ba	42.21Bb	30.59Ba	27.63Bb
60	58.86Ca	53.96Cb	1.11Cb	1.21Ba	29.92Ca	25.42Cb	27.89Ca	25.92Cb
90	50.34Da	45.12Db	1.10Cb	1.20Ba	21.60Da	17.64Db	23.05Da	20.86Db
Mango beverage								
0	41.35Aa	41.35Aa	0.96Aa	0.96Aa	36.98Aa	36.98Aa	23.93Aa	23.93Aa
30	35.89Ba	31.73Bb	0.40Ba	0.24Bb	23.89Ba	22.37Bb	18.84Ba	15.60Bb
60	30.42Ca	27.04Cb	0.26Ca	0.28Ba	22.03Ca	20.23Cb	16.43Ca	14.05Cb
90	29.77Da	24.13Db	0.24Ca	0.28Ba	18.02Da	15.00Db	14.50Da	13.82Db

FRSA: free radical scavenging activity; FRP: ferric reducing power

A-D: Within a column, different letters indicate significant differences ($p < 0.05$).

a-b: Within a row, different letters indicate significant differences ($p < 0.05$).

LSD interaction time-temperature factor of phenolic compounds, flavonoids, radical scavenging activity and reducing power of carrot beverage are 1.1275, 0.0815, 1.5902 and 0.3158, respectively.

LSD interaction time-temperature factor total phenolic compounds, flavonoids, radical scavenging activity and reducing power of mango beverage are 0.8058, 0.0759, 0.9198, and 0.3352, respectively.

3.3.2. Ferric-Reducing Power

Initial values of ferric reducing potential of carrot and mango

beverages are of 38.82 and 23.92 mg GAE/100mL, respectively. At the end of storage at 25°C, this antioxidant activity decreased to 23.05 and 14.50 mg GAE/100 mL,

while it reached values of 20.86 and 13.82 mg GAE/100mL, respectively, at 35°C (Table 1). A significant ($p < 0.05$) decrease in FRP of the beverages investigated was observed during storage. Thus, after 90 days at 25 and 35°C the FRP decreased by 40.60%, 46.26% for carrot beverage and by 38.96%, 42.18% for mango drink, respectively. Statistical analysis revealed that time, temperature and interaction time-temperature had significant effects on FRP of the commercial beverages ($p < 0.05$). These results are in line with those of Klimczak *et al.* (2007) and Oszmianski and Wojdylo (2009).

3.4. Correlations

The correlation matrix of assessed parameters is presented in Table 2. Both antioxidant assays (FRSA and FRP) showed an extremely significant positive correlation ($p < 0.001$) with TPC ($R^2 = 0.85$ and $R^2 = 0.93$, respectively), which is in agreement with findings of Tezcan *et al.* (2009). A highly positive correlation between total phenolic compounds and antioxidant capacity indicated that these compounds are the major responsible for free radicals scavenging ability and ferric reducing power of the investigated beverages. However, Kim *et al.* (2014) and Piljac-Zegarac *et al.* (2009) have reported a weak correlation ($R^2 = 0.25$; $R^2 = 0.21$) between total phenolic content and the DPPH radical scavenging activity of commercial beverages and industrial dark fruit juices, respectively, implying that other compounds besides phenolics contribute to their antioxidant capacities. A positive significant correlation was also observed between flavonoid content and FRSA ($R^2 = 0.76$) and FRP ($R^2 = 0.90$) assays.

Table 2. Correlation matrix between phytochemical content and antioxidant activity of carrot and mango beverages.

Parameter	Total phenolics	Total flavonoids	FRSA	FRP
Total phenolics	1.00			
Total flavonoids	0.94***	1.00		
FRSA	0.85***	0.76***	1.00	
FRP	0.93***	0.90***	0.91***	1.00

FRSA : free radical scavenging activity; FRP : ferric reducing power
*** $p < 0.001$: extremely significant correlation.

4. Conclusion

The result of this study revealed that storage time and temperature had significant effects on the bioactive phytochemical (total phenolic compounds and total flavonoids) content and antioxidant activity (FRSA and FRP assays) of commercial carrot and mango fruit based beverages. The analysed parameters decreased with the increase of the storage time and temperature. Decrease in the level of health-promoting compounds like phenolics and flavonoids upon storage reflected the decrease of the health benefits of the investigated beverages.

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