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Effect of Storage on the Nutritional Quality, Carotenoid and Ascorbic Acid Contents of Two Commercial Beverages

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Abstract

The effect of storage time and temperature on the total sugar (TS), free amino acid (FAA), protein, hydroxymethylfurfural (HMF), carotenoid and ascorbic acid (AA) contents of commercial carrot and mango beverages was investigated. The decreasing levels for carrot and mango beverages after 90 days of storage at 25°C and 35°C, were 29.57%, 31.41% and 42.54%, 47.68% for total sugars, 23.81%, 17.26% and 30.24%, 21.94% for free amino acids, and 12.71%, 14.89% and 17.88%, 23.88% for proteins, respectively. Our results revealed that ascorbic acid was the most affected compound by both duration and temperature of storage. Ascorbic acid contents of carrot and mango beverages decreased by 42.10, 72.62% and 58.55, 79.31%, after storage at 25 °C and 35°C, respectively. Since HMF is one of the compounds of sugar and ascorbic acid degradation, its formation was also investigated. HMF accumulation in carrot and mango beverages was of 1.32, 1.46 mg/100 ml at 25°C, and 1.80, 1.82 mg/100ml at 35°C, respectively.

Keywords

Fruit Beverages, Sugars, Amino Acids, Ascorbic Acid, HMF, Carotenoids, Storage

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1. Introduction

Adapting to new trends and consumer demands is one of the main objectives of fruit Beverage producers. Current developments in new ready-to-drink beverages are based on drinks containing combinations of various fruit juices which are becoming common in markets and have been receiving considerable attention as their market potential grows [1-2]. 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, indicating that the consumption of beverages has rapidly increased, especially carrot and mango 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 [3].

During storage, liquid foods suffer an important number of deterioration reactions, with an important quality loss [2]. It is well known that ascorbic acid (AA) or vitamin C is present in most natural juices and is an important component of our nutrition because of its antioxidant capacity. On the other hand, ascorbic acid degradation in foods is widespread phenomenon that takes place during processing and storage. Ascorbic acid degradation reactions are often responsible for important quality changes that occur during the storage of foods, limiting their shelf-life [4], with formation of unstable intermediate compounds, such as furfural [5]. Hydroxy-methylfurfural (HMF) is one of the decomposition products of ascorbic acid and suggested as a precursor of brown pigments. It is used to evaluate severity of a heating

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treatment applied to fruit juices during processing and taken into account for quality control [6]. Carotenoids, one of the main classes of natural pigments, represent important biological compounds that are widely distributed in fruits and vegetables. They possess structural diversity and numerous important functions for human health. Apart from being responsible for the color of a wide variety of foods, they are important, from a nutritional point of view, because some of them have provitamin A activity.

To our knowledge, literature data regarding the evolution of nutritional quality of some commercial beverages during storage is scarce. Thus, the current study aimed to assess and monitor the chemical composition, carotenoid and ascorbic acid contents of carrot and mango beverages during storage, and to evaluate the effect of the interaction time-temperature.

2. Materials and Methods

2.1. Samples

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 investigation. 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 puree and puree concentrate mango, water, sugar (sucrose), etc.

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

2.2. Total Sugar Content

The total sugars were determined according to Dubois et al. [7]. 0.5 ml of phenol (5%) and 2.5 ml of sulfuric acid (95%) were added to 0.5 ml of the diluted beverage. After stirring, the reaction mixture was incubated at 105°C for 5min. The absorbance was measured at 490 nm (spectrophotometer Uvline 9400, Secomam, Alès, France). Total sugar content was calculated using a standard curve of sucrose.

2.3. Amino Acid and Soluble Protein Contents

Free amino acids content was determined using the method of Yemm & Cocking [8]. One milliliter of an ethanol extract [same volume of the beverage and 80% ethanol were mixed then centrifuged (Nüve NF 200, Ankara, Turkey) for 20 min, 5000 rpm) was added to 0.5 ml of citrate buffer (0.2M, pH = 5] was added to 1 ml of potassium cyanide (0.01 M) and 200µL of ninhydrin (1%). The mixture was incubated at 100°C for 15min. After cooling, 2.3 ml of ethanol 70% were added. The absorbance was measured at 570nm and results were expressed as mg glycine equivalent per 100ml of beverage with reference to a calibration curve.

The protein content was determined using the method of Bradford [9]. Bradford reagent (2.5ml) was added to 0.1ml of the analyzed beverage, and then stirred on vortex. After 5 min, the absorbance was measured at 595nm. The results are expressed as mg of bovine serum albumin equivalent per 100ml of beverage with reference to a calibration curve.

2.4. Hydroxymethylfurfural Content

The HMF content was determined according to White [10]. Samples were placed in a flask of 10 ml; 100 µl each of Carrez I (potassium ferricyanide 15%) and II (zinc acetate 30%) reagents were added and the volume was adjusted with distilled water. After decantation, the supernatant was paper filtered. With a clarified solution containing 0.2% sodium bisulfite as reference and a similar solution without bisulfite as sample, a difference spectrum was obtained which represents only the HMF in the sample, without the interfering absorption of the fruit beverage. The HMF content was determined according to the following equation:

$$\text{HMF (mg/100ml)} = (\text{A284} - \text{A336})^{*}\ 74.87$$

A284, A336: absorbance values, 284 and 336 nm respectively. 74.87: Specific Factor.

2.5. Ascorbic Acid Content

Estimation of the ascorbic acid content was performed according to Mau et al. [11]. Five milliliters of each sample were extracted with 5ml of oxalic acid (1%); 100µl of the supernatant were mixed with 900µl of dichloroindophenol. The absorbance was measured at 515nm. The results were expressed in mg of ascorbic acid per 100ml of beverage, with reference to a calibration curve obtained with ascorbic acid.

2.6. Total Carotenoid Content

Carotenoid content was determined according to Choi et al. [12]. The beverage (1ml) was mixed with 5ml of extracting solvent (hexane: acetone: ethanol, 50:25:25, v/v/v). After centrifugation for 10 min at 5000 rpm, the top layer of hexane containing the carotenoids was recovered, and the absorbance was measured at 450nm. The carotenoid content was expressed in mg β-carotene equivalent per 100ml of beverage with reference to a calibration curve.

2.7. 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 tests were performed in triplicates.

3. Results and discussion

3.1. Effect of Storage on Sugar Contents

As shown in Table 1, time and temperature of storage affected the total sugar content as determined by Dubois assay. There was a significant decrease in total sugar contents during storage under the experimental conditions applied. After 90 days of storage at 25 °C and 35°C, content of these compounds decreased by 29.57% and 42.54% for carrot beverage, and 31.41% and 47.68% for mango beverage. The decrease was higher with longer time storage and higher temperature. The decrease of sugar content might be due to the contribution of the reducing sugars to non-enzymatic browning phenomenon.

Table 1. Effect of storage on total sugar, free amino acid, protein and HMF contents of carrot and mango beverages.

Storage duration (Days)	Total sugars (g/100ml)		Free amino acids (mg/100ml)		Proteins (mg/100ml)		HMF (mg/100ml)	
	25°C	35°C	25°C	35°C	25°C	35°C	25°C	35°C
Carrot beverage								
0	11.87Aa	11.87Aa	22.72Aa	22.72Aa	27.29Aa	27.29Aa	0.00Da	0.00Da
30	10.32Ba	10.09Ba	20.60Ba	20.38Ba	25.61Ba	24.39Bb	0.90Cb	1.12Ca
60	9.56Ca	7.99Cb	19.64Ca	19.50Ca	24.75Ca	23.40Cb	1.20Bb	1.47Ba
90	8.36Da	6.82Db	17.31Da	15.85Db	23.82Da	22.41Db	1.32Ab	1.80Aa
Mango beverage								
0	12.98Aa	12.98Aa	2.79Aa	2.79Aa	24.91Aa	24.91Aa	0.00Da	0.00Da
30	11.05Ba	9.49Bb	2.67Ba	2.50Bb	23.66Ba	22.37Bb	1.00Cb	1.12Ca
60	9.43Ca	7.73Cb	2.36Ca	2.27Cb	22.39Ca	20.49Cb	1.20Bb	1.42Ba
90	8.77Ba	6.79Db	2.30Da	2.18Db	21.20Da	19.11Db	1.46Ab	1.82Aa

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 total sugars, free amino acids, proteins and HMF of carrot beverage are 0.2356, 0.3800, 0.4323, 0.8064 and 0.0934, respectively.
LSD interaction time-temperature factor of total sugars, free amino acids, proteins and HMF of mango beverage are 0.2866, 0.0506, 0.4882, and 0.1584, respectively.

3.2. Effect of Storage on Free Amino Acid and Protein Contents

In the literature, little information is available on the changes in free amino acid and protein content of beverages during storage. Amino acids, a class of biologically active compounds present in food, are important for human nutrition and affect the quality of foods. Because amino acids take an active part in the Maillard reaction and browning processes, which determine the sensorial quality of foods, their determination is becoming more necessary as they can influence the quality of fruit-derived products [13-14]. Statistical analysis revealed that interaction time-temperature had significant effect for free amino acids of mango beverage ($p<0.05$). Nevertheless, temperature storage had not a significant effect for carrot beverage. After 90 days, total free amino acid content of carrot and mango beverages decreased by 23.81%, 17.26% and 30.24%, 21.94% at 25°C and 35°C, respectively. This decrease might be due to the contribution of amino acids in non-enzymatic browning reactions.

The initial protein content of carrot and mango beverages decreased by 12.71%, 14.89% and 17.88%, 23.28% after 90 days of storage at 25°C and 35°C, respectively (Table 1). Statistical analysis revealed that the interaction time-temperature had a significant effect ($p<0.05$) on the protein content of the analyzed samples. Hamedani et al. [15] indicated that protein content of the blood orange juice decreased by 23.57% after storage for 85 days at 22°C.

3.3. Effect of Storage on HMF Content

HMF is recognized as an indicator of quality deterioration resulting from excessive heating or storage, in a wide range of foods containing carbohydrates [2]. In the present study, the investigated beverages do not contain hydroxylmethyl-furfural. However, this compound appears during storage with increasing temperature. After 90 days of storage, a significant increase ($p<0.05$) of HMF content is observed over time; HMF values recorded at the end of storage were 1.32 and 1.46 mg/100 ml at 25°C, and 1.80 and 1.82 mg/100 ml at 35°C for carrot and mango beverages, respectively. The HMF content increased with time with a concurrent depletion of sugar content.

3.4. Effect of Storage on Ascorbic Acid Content

Vitamin C or ascorbic acid is an essential nutrient for health maintenance. Nearly all species of animals synthesize ascorbic acid and do not require it in their diet; however, humans cannot synthesize this vitamin [16].

Since ascorbic acid is highly unstable, its content has not only been used as a nutritional index, but also for evaluating

processing effects [1]. Initial ascorbic acid contents of carrot and mango beverages were 45.09 and 17.74 mg/100ml, respectively (Table 2). After 90 days storage, ascorbic acid contents of these beverages decreased by 42.10 and 72.62% and 58.55 and 79.31%, at 25 and 35°C, respectively. Among all compounds investigated, ascorbic acid was the most affected by temperature and time of storage. Increase of temperature by 10°C caused a distinct decrease in the concentration of this compound. The results obtained are in line with the data found by Burdurlu et al. [6] and Klimczak et al. [17] who reported a decrease in ascorbic acid content in fruit juice concentrates after eight weeks of storage at 28°C and 37°C and in commercial orange juices stored at 28°C and 38°C during 6 months, respectively.

Table 2. Effect of storage on ascorbic acid and carotenoid contents of carrot and mango beverages.

Storage duration (Days)	Ascorbic acid (mg/100ml)		Carotenoids (mg/100ml)	
	25°C	35°C	25°C	35°C
Carrot beverage				
0	45.09Aa	45.09Aa	2.06Aa	2.06Aa
30	38.72Ba	33.99Bb	1.98Ba	1.94Bb
60	32.13Ca	26.50Cb	1.98Ba	1.94Bb
90	26.10Da	18.69Db	1.97Ba	1.94Bb
Mango beverage				
0	17.74Aa	17.74Aa	0.29Aa	0.29Aa
30	10.50Ba	7.68Bb	0.24Ba	0.23Ba
60	6.96Ca	4.32Cb	0.24Ba	0.22Bb
90	4.85Da	3.67Db	0.24Ba	0.20Cb

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 ascorbic acid and carotenoids of carrot beverage are 0.8064 and 0.0223, respectively.
LSD interaction time-temperature factor of ascorbic acid and carotenoids of mango beverage are 0.6523 and 0.0146, respectively.

3.5. Effect of Storage on Carotenoid Content

Carotenoids are natural pigments that contribute to both the appearance and attractiveness of beverages as well as additional nutritional value in the form of powerful dietary antioxidants. These bioactive compounds play key roles in many important nutritional functions such as being converted into vitamin A.

Initial carotenoid contents of carrot and mango beverages were of 2.06 and 0.29mg/100ml, respectively (Table 2). Total carotenoid content decreased significantly ($p<0.05$) after storage. After 90 days, carotenoid contents of the studied samples decreased by 3.90, 21.40% and 5.85, 28.57%, at 25°C and 35°C, respectively. According to Sanchez-Moreno [18], oxidation is the major cause of carotenoid loss. The relative stability of carrot beverage carotenoids showed during storage could be probably related to the protection that ascorbic acid offers to them from oxidation.

4. Conclusion

The results of the current study supplied detailed information regarding the physicochemical stability, carotenoid and ascorbic acid contents of commercial carrot and mango beverages. The decrease of free amino acid content was concomitant with sugar level indicating their implication in the non-enzymatic browning process. The interaction time-temperature had a significant effect on the carrot and mango beverages stability. Ascorbic acid was the most affected by time and temperature of storage. On the other hand, HMF accumulation in fruit beverages increased depending on storage temperature. Small changes in carotenoid content were observed indicating a relative stability of these compounds upon storage. The results revealed that the nutritional quality depletion of the investigated beverages was more pronounced at 35°C than at 25°C. Thus, it can be concluded that fruit beverages should be stored at temperatures lower than 25°C, in order to assure a good stability of the nutritional quality.

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