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Studies Regarding Non-enzymatic Browning of Orange and Apple Juices during Storage

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Abstract

The aim of this paper was to evaluate the quality of two juices with or without antioxidants added. The quality of juices was assessed during storage by measuring the rate of non-enzymatic browning and by microbiological analysis. The non-enzymatic browning was monitored by spectrophotometric measurements at 420 nm (A_{420}) for apple juice and at 640 nm (A_{640}) for orange juice. The results demonstrated that the quality of juices during storage could efficiently be improved by adding antioxidants.

Keywords: non-enzymatic browning, orange and apple juice, antioxidant, storage

1. Introduction

The need to ensure food safety and a higher quality level for food requires the development of some strategies that allows the monitoring of possible microbiological, chemical and physical risks. Such a risk is browning, a phenomenon frequently encountered during food processing and storage, being due to oxidative or non-oxidative reactions [1]. Non-oxidative or non-enzymatic browning is a reaction involving the caramelization phenomenon, the degradation of ascorbic acid and / or the interaction of proteins or amines with carbohydrates [2,3]. The non-enzymatic browning reactions affect the organoleptic characteristics and are responsible for the most important qualitative changes in food during storage, leading to decrease of their shelf life [4]. The microbial spoilage of juices can lead to changes in flavor, odor, turbidity and carbon dioxide formation [5].

In general, browning causes destruction of nutrients and formation of undesirable intermediate compounds, like furfural and 5-hydroxymethylfurfural [6].

Apple and orange juices are the most consumed soft drinks in the world. The nutritional value of orange juice is primarily related to the content of vitamin C. Two of the biggest changes occurring during storage of orange juice are flavor degradation and browning. The ascorbic acid is an antioxidant with the role to slow down the browning reaction. However, the ascorbic acid is easily oxidized and decomposes at high temperatures contributing to the food's browning. The decomposition of ascorbic acid together with the non-enzymatic browning is the main degradation reaction occurring during storage of orange juice, in particular. Factors influencing the degradation of vitamin C are: oxygen, ascorbic acid concentration, temperature, light, metals, citric acid, etc [7]. The addition of synthetic or natural compounds provides rich resources of antioxidants in pharmaceuticals, cosmetics, including food products [8]. Most often, people add preservatives, antioxidants or conservation methods without knowing how they protect food against spoilage. Benzoic acid, the presence of antioxidants, carbon dioxide, and the lack of oxygen contributes to microbial inhibition [9]. The purpose of the present work was to examine the influence of several natural and synthetic antioxidants on the degree of nonenzimatic browning of two juices varieties (apple juice and orange juice) during storage.

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2. Materials and Method

Sample preparation. Two drinks have been prepared: *Apple juice 100% fruit – sample A*, made from apple concentrate (S.U.=70.4 ^{0}Brix.) - 164.37 g/l, deionized water – 835.63 ml, sodium benzoate, 9% solution - 0.8 g/l. *Orange juice 100% fruit – sample B*, made from orange concentrate (S.U.=63.7 ^{0}Brix.) – 185.79 g/l, deionized water – 814.21 ml, sodium benzoate, 9% solution – 0.8 g/l.Practically 1 l of orange juice, respectively apple juice, was obtained and divided into 10 samples of 100 ml each. In these samples synthetic antioxidants and natural extracts (powdered extracts used in soft drinks industry) were added as indicated in table 1.

Table 1. Sample identification

Sample	1	2	3	4	5	6	7	8	9	10
Antioxidant	-	Tocopherol acetate	Dihydro quercetin	Ascorbic acid	Lutein	Extract				
						Coenzyme Q_{10}	Green tea Teavigo	Black tea	Extra green tea	Rooibos

According to the recommendations from the technical specifications of substances with antioxidant role, the concentration used was 0.1 g/l, but for lutein it was of 0.01 g/l, because it changes the product's color to red-orange in higher concentration. The samples were pasteurized at 85^{0}C for 30 s. Thus there were obtained 20 samples that were kept at room temperature in PET bottles and were analyzed immediately after preparation (t_0), after 3 months (t_3), and respectively 6 months (t_6), after preparation.

Analytical methods. ***A***. The study of fruit juice browning is based on absorbance measurement using the Perkin Elmer UV/VIS spectrophotometer, Lambda 20 and 10 mm thick quartz cuvettes. All tests were performed in duplicate.

1. For clear soft drinks (apple juice – *sample A*), the absorbance was measured and read directly at the 420 nm wavelength. The clear sample is diluted with distilled water to obtain a solution of 11.5^{0}Brix, and therefore, in a Berzelius beaker the required amount of juice is weighed, calculated by the Peasson square, for an amount of 100g of solution. Water is added until it reaches 100 g. The solution is homogenized by means of a rod. The concentration of the prepared solution is verified (the content in soluble solid substances) with Abbe RFM800 refractometer. If necessary, it can be adjusted by adding water or juice, which ever the case, so that concentration is exactly 11.5^{0}Brix [10].

2. For opalescent juices (orange juice – *sample B*), absorbance measurement is made after the samples were diluted to 3^{0}Brix prior the readings using Peasson's square. Color evaluation is measured at λ=640 nm wavelength [11].

B. The total bacteria count (TCB) [12] in the soft drinks with fruit content is calculated by introducing 1 ml of analysed sample in the Petri plate (9 cm in diameter), on which 12-15 ml of Orange Serum Agar (Merck) culture, melted and cold at 45^{0}C is added. The content is mixed up by stirring. The incubation is done at 30^{0}C for 72±2 hours. After incubation period all the colonies growth on the Petri plate are counted. Evaluation: maximum 30 CFU/ml.

3. Results and Discussion

In order to establish a correlation between quality and the browning degree of juices several key physical-chemical and microbiological parameters were monitored: absorbance at 420 nm for apple juice and 640 nm respectively for the orange juice (in order to determine the speed of browning in citrus juices) [13], acidity with a Mettler Toledo DL53 automatic titrator and the total bacteria count.The browning of the apple and orange juice samples over the storage time is shown in Table 2.

The increase of absorbance values over time indicates the products' browning, due in this case to the storage period. The results obtained show that the addition of synthetic and plant extract antioxidants protect the color of apple juice, respectively of orange juice, over time.

Overall, the experimental results show that the absorbance at 420 nm increases with increasing storage time of samples A (apple juice). For sample B, color assessment is more difficult, due to the opalescence of samples, namely of carotenoid pigments found in chromoplasts (give color to oranges) [14].

Table 2. Temporal variation in absorbance at 420 nm for apple juice samples (sample A) and at 640 nm for the orange juice (sample B)

Sample	Absorbance (A_{420}) – Sample A			Absorbance (A_{640}) – Sample B		
	t_0	t_3	t_6	t_0	t_3	t_6
1	0.454	0.492	0.502	1.693	1.696	1.697
2	0.425	0.447	0.469	1.750	1.730	1.712
3	0.428	0.451	0.492	1.660	1.664	1.674
4	0.395	0.416	0.456	1.753	1.731	1.704
5	0.542	0.550	0.557	1.749	1.702	1.652
6	0.485	0.503	0.516	1.695	1.649	1.598
7	0.418	0.468	0.556	2.034	1.959	1.748
8	0.494	0.525	0.537	1.734	1.750	1.771
9	0.435	0.442	0.458	1.671	1.713	1.764
10	0.487	0.496	0.515	1.729	1.722	1.719

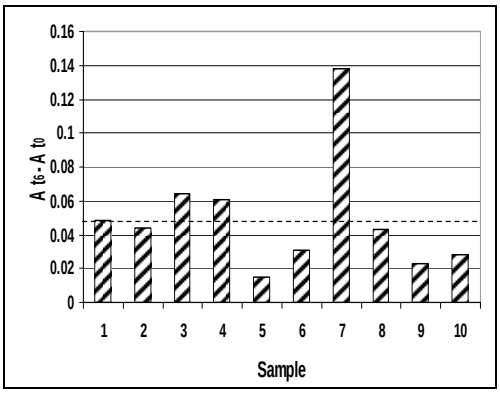

Figure 1. The variation in absorbance of samples A treated with various antioxidants during storage

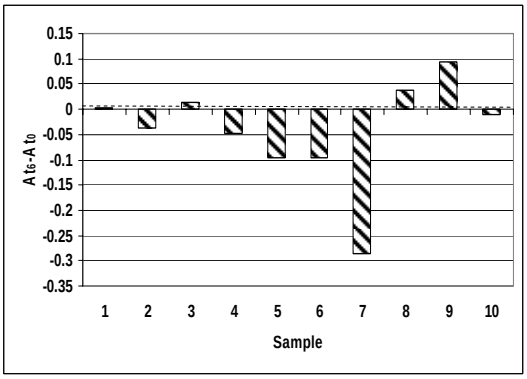

Figure 2. The variation in absorbance of samples B treated with various antioxidants during storage

Higher values of absorbance indicate products' browning due to oxidative degradation over time. Absorbance values of A studied samples (figure 1) shown an increase for samples 3, 4 and 7, compared with the untreated sample with antioxidants. In samples 2, 5, 6, 8, 9 and 10, synthetic and plant extract antioxidants have protected the product color, which is shown in the lower values of absorbance versus the blank sample. Comparing the sample absorbance values with those of the blank sample, substances with a protective role for the orange juice color against non-enzymatic browning reactions can be identified (figure 2). Thus, in samples 2, 4, 5, 6, 7 and 10, the addition of substances with antioxidant properties (tocopherol acetate, ascorbic acid, lutein, coenzyme Q10, Teavigo green tea and rooibos powder extract) has proven effective in protecting the color of orange juice, compared with samples 3, 8 and 9 with addition of dihydroquercetin, black tea powder extract, respectively Extra green tea.

The acidity of apple juice samples varied between 2.97 to 4.23 g/L citric acid, while that of orange juice was between 6.66 to 8.18 g/L citric acid. In order to determine the microbiological qualities of apple and orange juice samples, after heat treatment it was experimentally determined the total bacteria count [15] after preparation, at 3 months, and 6 months respectively (figure 3 and 4).

From the data on total bacteria count during the storage period (figure 3) it was found that for samples 1 and 5 the 30 CFU/ml limit was exceeded after 6 months from the preparation.

In samples B (figure 4), the increase of the total bacteria count above the allowed limit was found in samples 1, 5 and 9 after 6 months, and in sample 1 even after 3 months of production. Experimental data show a much higher microbial load for samples B compared to samples A. This leads to the assumption that the microorganisms' development depends on the nature of fruit concentrate added.

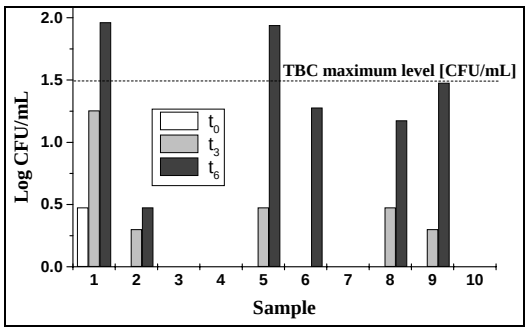

Figure 3. Total bacteria count over time for samples A

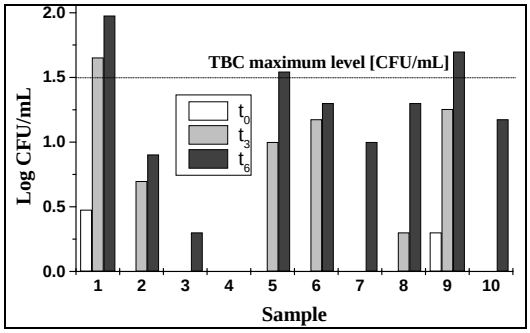

Figure 4. Total bacteria count over time for samples B

4. Conclusion

The quality and wholesomeness of soft drinks containing fruit is provided by the addition of substances with antioxidant role. The study allows correlation between the rate of non-enzymatic browning reactions and microbiological growth during storage fruit juice.

The addition of antioxidants protects, in some cases, the color of the samples. Overall, the addition of tocopherol acetate, lutein, coenzyme Q10 and rooibos powder extract has proven to be effective in protecting the clear juices (sample A) and in the opalescent juices (sample B).

Assessing the quality of samples based on total bacteria count, values of these indicators were recorded and have been falling in most cases within the limits imposed by law.

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