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Investigation of the ascorbic acid content of cold-pressed orange and pomegranate juiceusing electroanalytical methods

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----------ABSTRACT----------
Cold pressing is a widely used extraction technique for producing high-quality fruit juices. In this study, cold-pressed orange and pomegranate juices were stored at 25°C for 10 days. When ascorbic acid reacts with oxygen gas under a Cu^{2+} catalyst, hydrogen peroxide is released. The rate of this reaction is temperature and pH dependent. Therefore, the reaction was carried out at 25°C using 0.1 M citrate buffer (pH=4.5) and a Cu^{2+} catalyst. Differential pulse polarography, which has high sensitivity and reproducibility, was used in the analysis of ascorbic acid. When citrate buffer was used as a supporting electrolyte, the hydrogen peroxide formed showed a characteristic peak at -1.0 V. The peak increases responded linearly to the addition of standard peroxide solutions. The amount of ascorbic acid in cold-pressed orange and pomegranate juices was calculated based on peroxide. The same samples were also analyzed using HPLC chromatography, and the results were found to be consistent.

KEYWORDS; Ascorbic acid, Hydrogen peroxide, Differential pulse polarography, Orange, Pomegranate.

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I. INTRODUCTION

Ascorbic acid, commonly known as vitamin C, is one of the basic and best-known compounds necessary for the proper functioning of the human body. Currently, it is known that ascorbic acid is necessary for the proper functioning of the human body. It contributes to many processes, including strengthening and sealing blood vessels, regulation of microbial absorption by leukocytes, lowering the level of cholesterol, as well as acceleration of wound healing [1-2].The intake and analysis of ascorbic acid from food, which is important for human health, is becoming increasingly important.

Analysis of ascorbic acid in food products was carried out using several methods.The ascorbic acid content of commercial fruit juices and their loss rate depending on storage time and temperature were determined. The ascorbic acid content of commercial fruit juices was determined using the titration method, showing that it ranged from 2.4 to 43 mg per 100 ml of fruit juice [3].Ascorbic acid in Emblica fruit was determined using the colorimetric method [4], iodometric titrationwith UV spectrophotometry in tropical fruits [5-6], HPLC in cabbage [7], voltammetry in fruits and soybean [8-9], titrimetric in cold pressed orange juice [10].

Fruit juice is an aqueous liquid extracted from fruit tissue, either manually or mechanically. Extraction of juice from fruits can enhance the usage of fruits because the extracted juice can be easily stored and transported [11]. The demand for high-quality, nutritious fruit juice is steadily increasing. Although various methods have been developed for juice extraction, nonthermal methods are particularly effective at preserving nutrients [12].Among these, cold pressing is the most widely used nonthermal technique in the commercial production of fruit juices [13].

In this study, differential pulse polarography was used for the determination of ascorbic acid. Conditions with high sensitivity of the method were determined. Its analytical performance was examined. The amounts of ascorbic acid in cold-pressed orange and pomegranate juices were measured.

II. MATERIAL AND METHODS

2.1 ***Reagents***

All chemicals used were of analytical-reagent grade (proanalysis). Triply distilled water was used in the preparation of all solutions. Solutions of 10^{-3} M and more dilute ones were prepared before every use in order to avoid aging of the solution.The mercury used in the dropping mercury electrode was obtained from Merck (Darmstadt, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO_3 (3.0 M) and water columns in the form of fine droplets by using a platinum sieve. The collected mercury was dried between sheets of filter paper. Before use, a DPP polarogram of this mercury was recorded in order to confirm the absence of impurities.

2.2 Apparatus

A PAR (Model 174A) polarographic analyzer system equipped with a PAR mercury drop timer was used. The natural drop time of the mercury electrode was in the range of 2 - 3 s (2.37 mg/s). A Kalousek electrolytic cell with a reference saturated calomel electrode (SCE), separated by a liquid junction, was used in the 3-electrode configuration, so that the IR drop can be eliminated. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY1600) X-Y recorder. DP polarograms were recorded under the conditions of a drop life of 1 s, a scan rate of 5 mV/s, and a pulse amplitude of 50 mV.

2.3 Preparing Membrane Electrode

Finike variety oranges of similar weight (150 ± 10 g) and ripeness were purchased from a hypermarket in Antalya. The fruits were thoroughly washed under tap water, the top and bottom were cut off with a knife, and the peels were removed by hand. The pulp was then processed into juice using a cold-press juicer (Ninja, Leeds, England). The fresh, cold-pressed orange juice was then divided into six equal portions (500 mL each) and stored in amber-colored glass bottles. The bottles were stored either at room temperature (25°C) or in the refrigerator (4°C). These storage conditions were chosen because they are commonly used for fruit juices at retail outlets [14]. The juices were stored for 12 days, as the shelf life of unprocessed orange juice is approximately 10 days [15].
The same procedures were applied to Hicaznar variety pomegranates.

III. RESULT VIEW

3.1 Indirect determination of ascorbic acid

When ascorbic acid reacts with oxygen gas under the catalyst of Cu^{2+}, hydrogen peroxide is released. The rate of this reaction depends on temperature and pH. Therefore, the reaction was carried out using 25°C and 0.1 M citrate buffer (pH=4.5) [16].The hydrogen peroxide formed showed a characteristic peak at -1.0 V when citrate buffer was used as a supporting electrolyte.The polarogram is given in Figure 1.

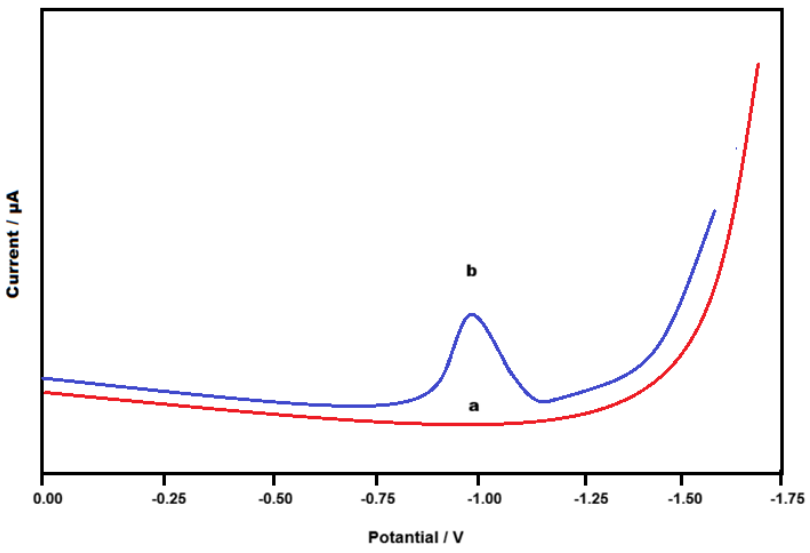

Figure 1: Polarographic peak of hydrogen peroxide:
a) 10 ml 0.1 M Citrate buffer (pH=4.5), b) + 0.1 ml $1x10^{-4}$ M hydrogen peroxide.

3.2 Analytical performance of the method

First, the polarogram of our supporting electrolyte, citrate buffer, was obtained. Then, additions of $1x10^{-4}$ M hydrogen peroxide solution were made to it. The increase in peak height was measured to be directly proportional to the concentration. The resulting polarograms are given in Figure 2.

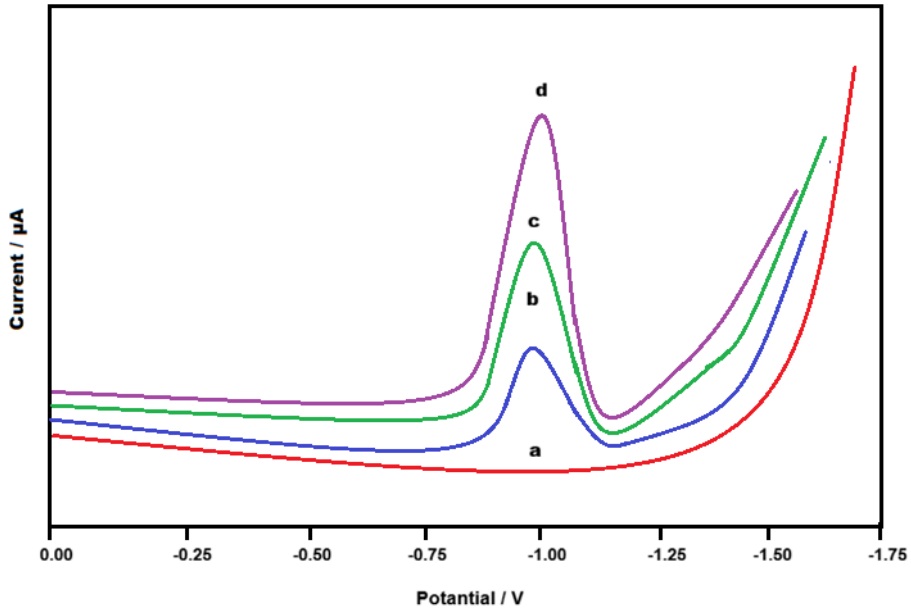

Figure 2: Sensitivity to increasing hydrogen peroxide concentrations:
a) 10 ml 0.1 M Citrate buffer (pH=4.5), b) + 0.1 ml $1x10^{-4}$ M hydrogen peroxide,
c)+ 0.1 ml $1x10^{-4}$ M hydrogen peroxide, d) + 0.1 ml $1x10^{-4}$ M hydrogen peroxide.

The analytical performance values of the method, based on measurements from polarograms, are given in Table 1.

Table 1.Performance values of the applied method.

Analyte	LOD, $molL^{-1}$	LQD, mgL^{-1}	R^2
Hydrogen peroxide	$1.2x10^{-5}$	$4.0x10^{-5}$	0.9991

3.3 Ascorbic acid levels in orange and pomegranate juice

A polarogram was taken of 10 ml of a 0.1 M citrate buffer (pH=4.5) solution. 0.2 ml of cold-pressed orange juice was added, and the resulting hydrogen peroxide peak height was measured. 0.1 ml of a $1x10^{-4}$ M hydrogen peroxide standard solution was added twice. The calculated peroxide concentration in the orange juice was equal to the ascorbic acid concentration. Similarly, the amount of ascorbic acid in the pomegranate juice was also measured.The same samples were analyzed using HPLC chromatography. The results obtained by both methods were found to be consistent.The results are given in Table 2.

Table 2.The amounts of ascorbic acid found in orange and pomegranate juices.

Sample	Ascorbic Acid: (mg/100 ml)	HPLC: (mg/100 ml)	t-test ($t_{critical}$ =3.18)	F-test ($F_{critical}$ = 9.28)
Orange Juice	46.5 ± 2. 41	44.8 ± 2. 32	1.81	5.73
Pomegranate Juice	0.56 ± 0.14	0.55 ± 0.17	2.15	6.22

IV. CONCLUSION

Cold pressing is a widely used extraction technique for producing high-quality fruit juices. In this study, cold-pressed orange and pomegranate juices were stored at 25°C for 10 days. Ascorbic acid in these samples was converted to hydrogen peroxide. Differential pulse polarography, which has high sensitivity and reproducibility, was used. The same samples were also analyzed using HPLC chromatography, and the results were found to be consistent.

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