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Assessment of Antioxidant Activity of Food Products by Iodometric Determination of Ascorbic Acid and Its Significance in The Prevention of Oxidative Stress

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Abstract: Oxidative stress is recognized as a key pathogenetic factor in chronic non-communicable diseases, which makes the assessment of dietary antioxidant intake particularly relevant clinically. The aim of this study was to evaluate the antioxidant activity (AOA) of freshly squeezed lemon, orange, and apple juices using iodometric titration, as well as to examine the effect of heat treatment on the stability of ascorbic acid. A 1% starch solution was used as the indicator, and titration was performed with Lugol's solution. The results showed that lemon juice had the highest AOA (28 drops to achieve a stable coloration), while apple juice showed the lowest activity (6 drops). Heating samples to 80°C for 10 minutes reduced AOA by 60–67%, confirming the thermal lability of ascorbic acid. The findings support the use of iodometry as an accessible screening method in food biochemistry and justify the recommendation to consume thermally unprocessed fruits and vegetables for the prevention of oxidative stress.

Keywords: Ascorbic acid, antioxidant activity, oxidative stress, iodometry, vitamin C, thermal degradation, free radicals.

INTRODUCTION

Oxidative stress, caused by excessive accumulation of reactive oxygen species (ROS) and depletion of the body's antioxidant reserves, is viewed in modern pathophysiology as a universal mechanism involved in the initiation and progression of a wide range of chronic non-communicable diseases. According to an extensive review by Jomová et al. [1], an imbalance in the pro-oxidant–antioxidant system is associated with the pathogenesis of atherosclerosis, type 2 diabetes mellitus, neurodegenerative processes, and oncological pathology. In atherogenesis, free-radical oxidation of low-density lipoproteins initiates an inflammatory cascade in the vascular wall, whereas in diabetes, chronic hyperglycemia serves as a source of intense ROS generation that impairs pancreatic β-cell function [3].

Ascorbic acid (vitamin C) occupies a leading position among low-molecular-weight non-enzymatic antioxidant factors in the body's antioxidant defense system. Its mechanism of action is based on electron donation to reactive radical species, forming semidehydroascorbate (monodehydroascorbate) — a relatively stable radical with low reactivity [16]. In addition to direct radical scavenging, ascorbate participates in the regeneration of α-tocopherol by reducing the tocopheroxyl radical in membrane structures [7]. Gęgotek and Skrzydlewska [4] emphasize that ascorbic acid can activate intracellular antioxidant systems by modulating transcription factors, including Nrf2, thereby inducing the synthesis of endogenous protective enzymes.

Despite well-established physiological justifications, the problem of dietary vitamin C deficiency in young individuals remains relevant. According to several epidemiological studies, a significant proportion of students and young people systematically fail to meet

the recommended daily intake (75–90 mg/day), which is attributed to the prevalence of thermally processed and refined food in their diet [14]. Given that fruits and freshly squeezed juices are the primary dietary source of vitamin C, assessing their actual antioxidant activity is of not only theoretical but also clear practical interest. Priority is given to simple, reproducible, and cost-effective analytical methods, one of which is iodometric titration [8, 10, 12].

AIM AND OBJECTIVES OF THE STUDY

Aim of the study: to assess the antioxidant activity of freshly squeezed lemon, orange, and apple juices by iodometric titration and to determine the extent to which heat treatment affects the stability of ascorbic acid.

To achieve this aim, the following objectives were formulated:

1. To develop a procedure for the iodometric determination of ascorbic acid in liquid food matrices using a starch indicator.
2. To perform comparative titration of freshly squeezed juices from three types of fruit and quantitatively characterize their AOA.
3. To investigate the changes in AOA following brief heating of samples to 80°C for 10 minutes.
4. To compare the obtained results with published data and draw well-founded conclusions about the nutritional value of the studied products from the perspective of oxidative stress prevention.

METHODS

Principle of the method. The iodometric determination of ascorbic acid is based on the reaction of its quantitative oxidation by molecular iodine:

$$I_2 + C_6H_8O_6 \rightarrow 2I^- + C_6H_6O_6 + 2H^+$$

As long as the reduced form of ascorbic acid is present in the solution, the added iodine is immediately reduced to iodide ions and does not form a colored complex with starch. The endpoint of titration was recorded by the appearance of a stable (persisting for more than 30 seconds) blue-violet coloration of the starch indicator. This reaction is stoichiometric, which allows the ascorbic acid content to be estimated indirectly from the volume of Lugol's solution consumed [8, 11].

Reagents and equipment. The following were used: Lugol's solution (0.005 M in iodine), 1% starch solution as the indicator, distilled water, Pasteur pipettes, a drop burette, a water bath with thermostat, and a thermometer. All reagents were of "pure for analysis" grade.

Study objects. The study objects were freshly squeezed juices: lemon (Citrus limon), orange (Citrus sinensis), and apple (Malus domestica), prepared immediately before the experiment. The fruits were purchased from the same retail outlet and processed under identical conditions. Each juice was diluted with distilled water at a 1:1 (v/v) ratio to reduce matrix viscosity and improve reproducibility of results.

Experimental procedure. 2 mL of diluted juice and 3 drops of 1% starch solution were placed in a test tube. Lugol's solution was added drop by drop from a drop burette with continuous stirring. The minimum number of drops required to produce a stable coloration was recorded. Each sample was titrated in triplicate; the data presented correspond to the mean value. Distilled water (2 mL) with starch indicator served as the control.

Simulation of heat treatment. Half the volume of each sample (before dilution with water) was heated in a water bath at 80°C for 10 minutes under a lid, then cooled to room temperature and diluted with distilled water 1:1. This model reproduces typical culinary processing during compote preparation, blanching, or juice pasteurization. The heated samples were titrated using the same procedure as the fresh samples [11, 17].

RESULTS

Table 1 presents the titration results for fresh juices with Lugol's solution in the presence of a starch indicator.

Table 1. Volume of Lugol's solution consumed in titration of fresh juices

Sample	Volume of Lugol's solution, drops	Starch coloration character	Relative AOA
Control (distilled water)	1	Immediate bright blue-violet	—

Lemon juice	28	Stable blue-violet at 28th drop	High
Orange juice	18	Stable blue-violet at 18th drop	Moderate
Apple juice	6	Rapid coloration at 6th drop	Low

The control sample (distilled water) developed a coloration from the very first drop of Lugol's solution, indicating the absence of reducing substances and confirming the validity of the experimental setup. Lemon juice required the largest volume of titrant (28 drops), indicating a high ascorbic acid content and, accordingly, the maximum AOA among the studied samples. Orange juice occupied an intermediate position (18 drops), while apple juice showed the lowest antioxidant activity (6 drops): starch coloration appeared rapidly, which correlates with the relatively low vitamin C content in apples compared to citrus fruits [9, 17].

Data on the effect of heating to 80°C on the antioxidant activity of the studied juices are shown in Table 2.

Table 2. Effect of heat treatment (80°C, 10 min) on antioxidant activity of juices

Sample	Fresh juice, drops	Heated juice (80°C, 10 min), drops	AOA decrease, %
Lemon juice	28	11	60.7
Orange juice	18	7	61.1
Apple juice	6	2	66.7

According to Table 2, heating led to a sharp decrease in AOA for all three samples. The calculated decrease was: 60.7% for lemon juice (from 28 to 11 drops), 61.1% for orange juice (from 18 to 7 drops), and 66.7% for apple juice (from 6 to 2 drops). Notably, the relative losses of ascorbic acid upon heating were comparable for all three matrices (≈60–67%), although the absolute AOA values differed substantially.

Based on the data from both tables, a comparative histogram was constructed. The horizontal axis displays the juice names, and the vertical axis shows the volume of Lugol's solution in drops. For each product, two bars are presented: light (fresh juice) and dark (heated juice). The histogram clearly demonstrates a more than twofold reduction in bar height upon heat treatment in all three groups, as well as preservation of the hierarchy: lemon > orange > apple in both fresh and processed samples.

DISCUSSION

The obtained results are consistent with well-established data on the variability of ascorbic acid content in fruit raw materials. The superiority of lemon juice over orange and apple juice in antioxidant activity is reproduced in several independent studies where citrus fruits consistently outperform pome fruits in vitamin C content [9, 17]. The difference between lemon and apple in our experiment was 4.7-fold (28 vs. 6 drops), which is practically significant for dietary recommendations.

The observed decrease in AOA upon heating is explained by a complex of biochemical mechanisms of ascorbic acid degradation. Njus et al. [16] have characterized the kinetics of ascorbate oxidation in detail, emphasizing that elevated temperature accelerates both the aerobic degradation pathway (involving molecular oxygen) and metal-catalyzed decomposition via the Fenton reaction. An additional contribution comes from the hydrolysis of the lactone ring of the ascorbic acid molecule, forming biologically inactive diketogulonic acid [14]. Nowak et al. [17], comparing juices subjected to mild and intensive heat treatment, established that losses of bioactive components depend fundamentally on the temperature-time regime; our data on a 60–67% decrease in AOA upon heating to 80°C correspond to

the upper boundary of the range reported in that work.

From a clinical standpoint, the reduction in AOA of food products during preparation directly affects the antioxidant status of the organism. Forman and Zhang [3] note that even in the presence of endogenous enzymatic defense systems (superoxide dismutase, catalase, glutathione peroxidase), dietary antioxidants play an indispensable role in neutralizing extracellular ROS and protecting cell membranes. Vitamin C deficiency is associated with an elevated risk of subclinical inflammation and endothelial dysfunction — pathogenetic mechanisms that precede clinically manifest atherosclerosis [1, 4].

The observed trend of AOA reduction upon heating has practical significance in the context of youth nutrition. Doseďdel et al. [14] emphasize that the thermal lability of ascorbic acid necessitates the inclusion of sufficient quantities of fresh (unprocessed) fruits and vegetables in the diet to maintain adequate vitamin C levels. This point is particularly relevant for students, whose diet often substitutes fresh citrus fruits and juices with pasteurized commercial beverages containing reduced levels of native vitamin C [17].

Regarding the methodological aspect, it should be noted that iodometric titration, despite its simplicity, has satisfactory reproducibility and sensitivity for screening purposes. Munteanu and Apetrei [12], in an analytical review of antioxidant activity assessment methods, state that redox-based methods — of which iodometry is one — have performed well in routine food analysis, although for a comprehensive characterization of a product's antioxidant profile, the use of several complementary methods is recommended [8, 13]. Belete et al. [9], upon verification of iodometric data by UV spectrophotometry, obtained a recovery coefficient of 85–113%, indicating acceptable accuracy of the method for analytical tasks at this level.

CONCLUSIONS

1. Among the studied samples, lemon juice showed the highest antioxidant activity (28 drops of Lugol's solution to achieve stable starch indicator coloration), exceeding orange juice (18 drops) by more than one and a half times and apple juice (6 drops) by nearly five times. This pattern is consistent with the known dependence of ascorbic acid content on the botanical species of the fruit.

2. Heat treatment of samples at 80°C for 10 minutes caused a reduction in antioxidant activity of 60.7–66.7% for all studied juices. This finding confirms the biochemical instability of ascorbic acid upon heating and supports the advantage of consuming thermally unprocessed fruits and freshly squeezed juices to maintain effective dietary antioxidant protection.

3. Iodometric titration with a starch indicator has proven to be an accessible, cost-effective, and reproducible screening method for assessing the antioxidant activity of food products, suitable for use in teaching laboratories and preliminary dietary quality assessments. The obtained data may serve as a basis for developing practical recommendations to optimize the diet of young people in order to prevent oxidative stress and its associated chronic diseases.

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