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# Improvement of serum antioxidant status in humans after the acute intake of apple juices

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#### Abstract

It is hypothesized that apples of 2 Brazilian cultivars with different content of sugars and antioxidant compounds promote similar effects on the antioxidant status and lipid peroxidation in human serum after acute intake. Nine healthy women ingested 300 mL of Golden Delicious or Catarina apple juice (AJ) or water, and blood samples were collected before and 1 hour after intake. After intake of both AJ, a similar and significant increase in serum antioxidant capacity and ascorbic and uric acid levels and a significant decrease in serum lipid peroxidation was observed. The increase in serum antioxidant capacity after consumption of both AJ was correlated directly with the uric acid levels and inversely with serum lipid peroxidation. In summary, the acute intake of AJ of 2 cultivars promoted a similar effect on the antioxidant status and lipid peroxidation in human blood serum. © 2012 Elsevier Inc. All rights reserved.

Keywords: Abbreviations: Apple juice; Acute intake; Antioxidant capacity; Human intervention; Lipid peroxidation AJ, apple juice; ABTS, 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric reducing antioxidant power; LH, lipid hydroperoxides; TBARS, thiobarbituric acid reactive substances.

#### 1. Introduction

Several studies have shown that apple cultivar may influence the content of sugars, phenols, ascorbic acid, and in vitro antioxidant capacity [1-3]. Based on this context, it has been suggested that some apple cultivars may be healthier than others.

Apple consumption has been associated with reduced risk for several diseases [4,5]. Such association may be attributed, at least in part, to the high content of polyphenol compounds, which can protect against the deleterious effect of oxidative stress [6]. However, few human studies have

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shown the effects of fruit intake of different cultivars on the biomarkers of oxidative stress. Furthermore, inconsistent results can be found when in vivo data are compared with in vitro antioxidant response or the fruit antioxidant content [7,8]. These inconsistencies indicate that the modulation of the physiologic process in humans cannot be predicted only by the chemical analysis of fruit. Moreover, it is unknown as to whether apples from different cultivars differentially affect the markers of the antioxidant status and of lipid peroxidation in humans.

Here, we hypothesized that the acute consumption of apple juice (AJ) of 2 Brazilian cultivars with different content of sugars, ascorbic acid, phenols, and antioxidant capacities promotes similar effects on the antioxidant status and lipid peroxidation in humans. Therefore, the objective of this study was to determine the antioxidant capacity and the levels of ascorbic and uric acids, total phenols, lipid

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hydroperoxides (LH), and thiobarbituric acid-reactive substances (TBARS) in the serum of 9 healthy individuals 1 hour after the intake of Golden Delicious or Catarina AJ.

#### 2. Methods and materials

#### 2.1. Apple cultivars

The Golden Delicious and Catarina apple cultivars produced and harvested under the same standard conditions were obtained from Rural Issues and Agricultural Research Institute of Santa Catarina, Brazil. To allow for a quick and easy intake of apples in large amount, 300 mL of AJ, equivalent to 5 apples, was used. For the preparation of the AJ, the fruits were washed with deionized water 3 times, and unpeeled apples without seeds were ground in a centrifugal juice extractor with no water addition. Each AJ was consumed immediately by the subjects after preparation. The total sugars, fructose, glucose, sucrose, total phenols, flavanols, anthocyanins, and ascorbic acid contents and antioxidant capacities of the AJ (Table 1) were assessed as described previously [2,3].

#### 2.2. Subjects, study design, and blood samples

The Federal University of Santa Catarina Ethics Committee approved the study, and all the participants gave their written informed consent. Nine healthy women, aged 23.6 years (range, 21-27 years) and body mass index of 20.6 kg/m² (range, 19.0-23.7 kg/m²) participated in this randomized crossover study. The exclusion criteria were the current use of any medication, alcohol or antioxidant supplements, smoking status, and history of major illness that affects the oxidative status.

Table 1
Sugars, phenols, and ascorbic acid content and antioxidant capacity of the Golden Delicious and Catarina AI

Parameter	Apple cultivar	
	Golden Delicious	Catarina
Total sugars (g·100 mL <sup>-1</sup> )	$10.32 \pm 0.06^{b}$	$12.35 \pm 0.07^{a}$
Fructose (g·100 mL <sup>-1</sup> )	$5.16 \pm 0.03^{b}$	$6.17 \pm 0.03^{a}$
Glucose (g·100 mL <sup>-1</sup> )	$1.55 \pm 0.02^{a}$	$1.03 \pm 0.03^{b}$
Sucrose (g·100 mL <sup>-1</sup> )	$3.61 \pm 0.05^{b}$	$5.15 \pm 0.06^{a}$
Total phenolic (mg GAE·100 mL <sup>-1</sup> )	$108.27 \pm 1.60^{b}$	$163.83 \pm 1.48^{a}$
Total flavanol (mg catechin·100 mL <sup>-1</sup> )	$16.47 \pm 0.45^{b}$	$30.86 \pm 4.05^{a}$
Total anthocyanin	ND	$1.02 \pm 0.03$
$(mg cy-3-gal\cdot 100 mL^{-1})$		
Ascorbic acid	$1.96 \pm 0.03^{a}$	$1.30 \pm 0.07^{b}$
(mg ascorbic acid·100 mL <sup>-1</sup> )		
Total antioxidant capacity		
ABTS ( $\mu$ mol TEAC·100 mL <sup>-1</sup> )	$359.63 \pm 3.98^{b}$	$709.70 \pm 11.89^{a}$
FRAP ( $\mu$ mol TEAC·100 mL <sup>-1</sup> )	$182.94 \pm 6.09^{b}$	$306.25 \pm 4.23^a$

Values are means  $\pm$  SEM of 5 determinations in triplicate. Different superscript letters a,b denote significant differences (Student t test, P < .05). ND indicates not detected; GAE, gallic acid equivalent; TEAC, Trolox equivalent antioxidant capacity.

After overnight fasting, all the subjects consumed 300 mL of Golden Delicious AJ, tap water, or Catarina AJ, and a blood sample was collected just before (baseline) and 1 hour after drinking. This protocol was chosen based on the maximal antioxidant capacity and phenolic concentration in serum 1 hour after intake of fruit or juice [6-13]. The participants served as their own control because we compared all the data obtained after either AJ or water consumption with the respective baseline values. A washout period of 2 weeks was maintained between the study stages. To verify the acute effects of the intake of 2 apple cultivars on the serum antioxidant status and the lipid peroxidation levels, the subjects were strongly instructed to maintain their usual diet during the study periods but to abstain from fruits, vegetables, tea, alcoholic beverages, and caffeine- or theobromine-containing food intake for 2 days before each study day. A 3-day dietary record showed satisfactory adherence to dietary counseling in the 3 study stages. Serum samples were isolated by centrifugation at 1000×g for 10 minutes and were used immediately for the measurements.

#### 2.3. Serum assays

Total phenolic content was analyzed by the Folin-Ciocalteau method [9]. Ascorbic acid was measured by the 2,4-dinitrophenylhydrazine method [14]. The antioxidant capacity was assessed by the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay [3] and the ferric reducing antioxidant power (FRAP) assay [15]. Uric acid and glucose were determined by commercially available enzymatic kits (Gold Analisa, Lagoa Santa-MG, Brazil). The lipid peroxidation was determined by the measurement of LH [16] and TBARS [17].

#### 2.4. Statistical analyses

All the analyses were performed in triplicate. Results are expressed as means  $\pm$  SEM. The parameters of the juices were compared by the Student t test. The effects of AJ and water intake on serum parameters were evaluated by the paired t test (intragroup comparison) and by analysis of variance and Tukey test for intergroup comparison. Pearson correlation was also used. Significance was set at P < .05. The STATISTICA software package (StatSoft Inc., Tulsa, Okla, USA) was used.

#### 3. Results and discussion

In the present study, we confirmed our hypothesis that the acute consumption of juices from 2 apple cultivars with different sugars, ascorbic acid, and phenols content and antioxidant capacities promoted a similar effect on the serum antioxidant status and lipid peroxidation of healthy subjects. Our results are in accordance with findings previously reported in human studies after acute intake of apple or AJ [10,12,13].

A significant and comparable increase in the antioxidant capacity of serum, measured by ABTS and FRAP assays,

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