academicJournals

Vol. 10(32), pp. 529-537, 25 August, 2016
DOI: 10.5897/JMPR2016.6146
Article Number: 6E7C4DA60300
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Impact of Colombian yellow fruits and tropical fruits drinks consumption on the antioxidant status of healthy women

Ana Cristina Gómez-García[1], Jeanine Peñaloza[2], Julio Cañas[2], Benjamín Rojano[3], Ana Rosa Ramos[1] and Maria Elena Maldonado[4]*

[1]School of Nutrition and Dietetics, Universidad de Antioquia, Medellín Colombia.
[2]Extracts, Antioxidants and Bioactives Department, Tecnas S.A., Medellin, Colombia.
[3]School of Chemistry, Universidad Nacional de Colombia, Medellín, Colombia.
[4]School of Nutrition and Dietetics, Universidad de Antioquia, Medellin, Colombia.

Received 13 May, 2016; Accepted 4 August, 2016

Reactive oxygen species are responsible for causing different types of damage in the body which are associated with the onset of chronic non-communicable diseases. A strategy to counteract these effects is by a diet rich in antioxidants compounds found in fruits. The aim of this study was to determine the antioxidant capacity in plasma and the content of total phenolic compounds in healthy women who consumed a drink prepared with Nativanox® Colombian tropical fruits or a drink prepared with Nativanox® Colombian yellow fruits during a short period of time. Nineteen healthy women received a daily 200 mL drink with Nativanox® Colombian tropical fruits or a drink with Nativanox® Colombian yellow fruits for 14 days. Before and after the intervention period blood and plasma were obtained to analyze C-reactive protein levels, lipid profile, total phenolic content and antioxidant status through FRAP, ABTS and EROS methods. Regular consumption of a drink with Nativanox® Colombian tropical fruits had a positive impact on the lipid profile and the antioxidant capacity on plasma of healthy women. The consumption of a drink with Nativanox® Colombian yellow fruits diminished diastolic blood pressure. These results showed that a Nativanox® Colombian yellow and tropical fruit contains bioactive compounds that can improve oxidative status in plasma and contribute to reduce the risk of cardiovascular disease.

Key words: Antioxidant, polyphenols, tropical fruits, yellow fruits, lipid profile.

INTRODUCTION

Epidemiological studies have found that eating foods rich in antioxidants, such as fruits and vegetables, has been associated with a low risk of oxidative stress associated with non communicable chronic diseases such as cancer and cardiovascular disease (Limón-Pacheco and Gonsebatt, 2009). The oxidative damage theory suggests

*Corresponding author. E-mail: maria.maldonado@udea.edu.co.

that mitochondrion produce reactive oxygen species (ROS) or free radicals from electron transporter chain (Harman, 1956). Free radicals are also produced by enzymatic reactions such as NADPH oxidase reactions of phagocytes to destroy invading microbes or xanthine oxidase. In addition, external sources such as pollution, cigarette smoke and sunlight produce ROS which are incorporated into organisms and cells. Excessive production of ROS leads to damage of lipids, proteins, carbohydrates and DNA (Dean et al., 1997). The membranes exposed to free radicals lose their ability to transport nutrients properly; lipoproteins become oxidized forms; and DNA damage has the potential to accumulate consecutive mutations, which can lead to carcinogenesis (Ames et al., 1993). Therefore, the oxidative damage theory strictly recalls the concept that antioxidant molecules are capable to slow oxidative process and very important for homeostasis normal body metabolism.

An antioxidant with biological function is defined like a substance present at low concentrations able to decreases or prevents oxidation of a substrate. Such substances may have direct action by the neutralization of free radicals and non-radical reactive species or indirect, through induction of enzyme systems such as glutathione reductase, catalase and superoxide dismutase (Halliwell and Whiteman, 2004; Hicks et al., 2006). Among the antioxidants of dietary origin, that is, present in fruits and vegetables are carotenoids and polyphenols, the phenolic acids and flavonoids comprise 60% of the phenolic compounds obtained from diet (Ramos, 2007). Polyphenols are secondary plan metabolites that contribute to the organoleptic qualities, color and defense against pathogens attacks. The chemical structure of phenols having one or more aromatic rings with one or more hydroxyl groups which confer activity radical scavenging inactivating directly ROS or by binding to pro-oxidant metal ions through their groups OH (Rice-Evans et al., 1997). In recent years, it has been shown that a diet rich in polyphenols can improve health and reduce the incidence of cardiovascular disease (Shroeter et al., 2006). These effects are primarily due to its antioxidant properties that can usually justify their vasodilatory and vasoprotective actions and their antithrombotic, antilipemics, antiarterosclerotics, anti-inflammatory and anti-apoptotic (Potenza et al., 2007) shares. In addition, some studies have shown that these compounds can also inhibit angiotensin converting enzyme (ACE) and inhibition of this enzyme would justify its vasodilating and cardio-protective effects (Ojeda et al., 2010; Andriambeloson et al., 1997). One of the most studied properties of polyphenols is the ability to improve the lipid profile (Aviram and Rosenblat,1994), thus, can prevent the development and occurrence of atherosclerosis, a disease characterized by progressive clogging of the arteries as a result of lipid accumulation in the arterial wall. These compounds are capable of attenuating the onset and progression of this disease due to their ability to mitigate LDL oxidation by increasing the concentration of HDL-cholesterol and inhibit the proliferation of vascular smooth muscle (Osakabe et al., 2001).

Phenolic compounds are the largest non-energy plant substances group, so that products derived from fruit can be consumed to improve health. Furthermore, the combined food can have more benefits to consume the active components alone, because the set of nutrients can have a synergistic effect (Betoret et al., 2009). Andean berries and yellow Colombian native fruits with trade name Nativanox® prepared by the company Tecnas SA, rich in flavonoids, anthocyanins, tannins, polyphenols and carotenoids were determined ORAC value (Oxygen Radical Absorbance capacity) whose values were 55.000 µmol Trolox/100 g for Nativanox® tropical fruits, 40,000 µmol Trolox/100 g for Nativanox® Andean berries and 40,000 µmol Trolox/100 g for fruits Nativanox® yellow (Peñaloza and Rojano, 2014). These results suggested that the consumption of a drink rich in bioactive compounds such as polyphenols, with high antioxidant capacity determined by ORAC can improve antioxidant status after consumption regularly for a short-time in healthy women.

According to data reported by the National Survey of Nutritional Status of Colombia (ENSIN) in 2010, 33.2 and 71.9%, a low percentage of Colombians consume daily fruits and vegetables, respectively. Only 1 in 5 Colombians consume whole foods, and just 25% of the population eats fruits and vegetables daily. Therefore, as a strategy to encourage the consumption of fruits and vegetables, has been proposed based on drinks fruit as an option accepted by the population (Wootton-Beard and Ryan, 2011). Fruits or fruit juices are good sources of antioxidants such as polyphenols. These could fulfill the role of mediating biological processes, resulting in the prevention of non-communicable chronic diseases such as dyslipidemia, hypertension, atherosclerosis and cancer (Morton et al., 2000). The aim of this study was to determine the impact on the antioxidant status of healthy women who consumed a drink prepared with Nativanox® colombian tropical fruits or consumed a drink prepared with Nativanox® colombian yellow fruits, obtaining a significant antioxidant activity for 2 weeks.

MATERIALS AND METHODS

Characterization of Nativanox

The bioactives of Nativanox were characterized by carotenoids, tannins, flavonoids, polyphenols, scavenging capacity hydroxyls •OH radicals and reactive oxygen species (ROS).

Determination of carotenoids

A representative portion of the sample was taken in a test tube. 4 ml of acetone was added and stirred in a vortexer for 2 min. The

Table 1. Bioactive compounds contained in Nativanox®.

Nativanox®	Flavonoids (mg catechin/mg dry weight)	Anthocyanins (mg cyanidin-3-glycosid/kg dry weight)	Tanins (mg catechin (mg dry weight)	Carotenoids (mg β-carotene/100 g sample)	Total polyphenols (mg gallic acid/100 g dry weight)	DPPH (TEAC µmol Trolox/100 g sample)	Recommended dose/day by USDA-FDA/OMS
Colombian tropical fruits	1203-1500	0	8000-8492.40	0	4500-5012.8	18000[a] 19700	5-8 g
Colombian yellow fruits	530	0	4000-6000	6.12	2100-2500	8000[a] 9000	8-12 g

mixture was centrifuged at 4000 rpm/10 min and the supernatant was collected in another test tube. The absorbance of the solution was determined at 449 nm.

Determination of tannins

The analysis was performed according to the methods of the pharmacopoeia and the AOAC method after some modifications. 25 ml of the Nativanox extract were measured in a 1 L conical flask, and 25 ml of indigo solution and 750 ml of deionized water were added. Titration was performed with an aqueous solution of potassium permanganate ($KMnO_4$) 0.1 N until the blue solution turned green. The target of the test was made with 25 ml of indigo carmine solution in 750 ml of deionized water. The samples were analyzed in triplicate.

Determination of flavonoids

Determining flavonoids was performed following the method described by Debnath et al. (2011). A standard curve using catechin was constructed. Results were expressed as mg catechin/100 g Nativanox®. Readings were taken at 510 nm.

Evaluation of scavenging capacity hydroxyls •OH radicals

The hydroxyl radical scavenging activity was determined by fluorescence (Yang and Guo, 2001). The reaction was carried out in phosphate buffer (pH 7.4). 300 µl of sodium terephthalate, 420 µl of buffer, 100 µl of Nativanox®, 90 µl EDTA solution and 90 µl of iron solution (Fe^{+2}) were mixed. The mixture was allowed to stand for 6 min with constant aeration and at room temperature (26°C), and then the fluorescence intensity was measured. Results are expressed as mg of DMSO/100 g of Nativanox®, by constructing a standard curve using different concentrations of DMSO.

Evaluation of the total capacity to trap reactive oxygen species (ROS) of Nativanox®

Capacity of Nativanox® to trap reactive oxygen species (ROS) was evaluated. ROS are generated by the azo compound, 2,2'-diazobis (2- amidinopropane) dihydrochloride) (AAPH), which in aqueous medium free radicals produced at a constant speed (Martín-Romero, 2008). The reaction was carried out in phosphate buffer (pH 7.4). 50 ml of a solution of AAPH, 50 ml of an ethanolic solution of dichlorofluorescein diacetate, 2850 ml of buffer and 50 µl of Nativanox® were mixed. Immediately, fluorescence intensity was emitted during the first 10 min and compared to the intensity emitted in the absence of the sample which was read. Results are expressed as values TEAC (µmol Trolox/100 g Nativanox®), by constructing a standard curve using different concentrations of Trolox.

Preparation of drinks

A powder enriched in polyphenolic compounds was obtained, packaged in foil pouch protected from light, with trade name Nativanox® produced by the company TECNAS S.A. Passion fruit (*Passiflora edulis*), Anana (*Ananas comosus*) smooth cayenne variety, Granadilla (*Passiflora ligularis*), Common Guava (*Psidium guajava L*.) and Feijoa (*Acca sellowiana*) were used. The edible portion of each fruit was obtained and homogenized in a vegetable processor Black & Decker model FP1550S and homogenized in Ultraturrax T-50 Basic IKA-WERK containing the bioactive compounds in Table 1. The drink was prepared from the powdered extract. Following the recommendations of USDA and FDA for ORAC units/d indicated for preventing degenerative diseases, 5 g of Nativanox® colombian tropical fruits or 8.2 g of Nativanox® colombian yellow fruits representing 5,000 ORAC which were diluted in 200 mL of a Colombian blend mineral water and stored at 4°C until consumption were used. These preparations were made daily and consummed 1 h after dilution.

Study design

Double-blind trial with an intervention period of 14 days (2 weeks) was used. Twenty healthy women were recruited and given 200 ml/day a drink with tropical fruits (n = 9) or a drink with yellow fruits (n =11). During the study, the diet or lifestyle of the participants did not change though they were instructed to avoid the consumption of fruits or similar elements contained in the juices. Each subject's weight and height were measured for calculating the body mass index (BMI). The subjects' diets were assessed before and at the end of the intervention using 72 h recall. Women received the drink packed in plastic bottles sterilized for consumption weekend, they were asked refrigerated, protected from direct light and heat. Fasting venous blood samples were collected before and after 2 weeks of intervention from each subject. Blood was collected in

heparinized tubes and centrifuged to obtain plasma for antioxidants analysis and non-heparinized tubes to obtain serum for lipid profile, glucose and C reactive protein analysis.

Subjects

Twenty healthy women (aged 18 to 60 years) from the Universiy of Antioquia at Eastern Region were recruited in the study. The study protocol was approved by the Human Ethics Committee of Denstistry Faculty from the University of Antioquia University, and informed consent was obtained from each subject. All the subjects were in good health, with a regular medical history, and none of the subjects were on any medication, smoking, heavy physical exercise or took vitamin/mineral, antioxidant or herbal supplements.

Antropometrics and blood pressure

Before (initial time, day 1) and after the intervention (final time, day 14), weight, size, body mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using Tanita BC-1500 Ironman Radio Wireless, measuring tape Seca 201 and digital sphygmomanometer Welch Allyn, respectively.

Total phenols in blood plasma

The total phenolic content was determined according to the adapted Folin–Ciocalteu method (Singleton, 1999). Plasma deproteinized by perchloric acid (50 µl) were mixed with 125 µl of Folin–Ciocalteu reagent and 400 µl of sodium carbonate solution (7.1% p/v), and the resulting solution was brought to a final volume of 1000 µl. The mixture was stirred and stored at room temperature for 30 min in the dark. The absorbance was measured at 760 nm against a control sample. Aqueous solutions of gallic acid were used to build a calibration curve. The results were expressed as gallic acid equivalents (GAE)/ml.

FRAP assay (Ferric Reducing/Antioxidant Power) in blood plasma

The antioxidant capacity of wine was estimated according to the procedure described by Benzie and Strain (1996), with some modifications. This method is based on the increase of absorbance due to the formation of 2, 4, 6-tripyridil-s-triazine (TPTZ)-Fe (II) in the presence of reducing agents. A volume of 50 µl of deproteinized plasma was mixed with 950 µl FRAP reagent previously dissolved in acetate buffer (pH 3.6). The absorbance increase was measured at 590 nm. The FRAP values were expressed as ascorbic acid equivalent antioxidant capacity (AEAC: mg ascorbic acid per ml) using an ascorbic acid standard curve.

ABTS assay in blood plasma

ABTS radical was produced through an oxidative reaction of ABTS using potassium persulphate. The capacity of plasma samples to scavenger ABTS radical was evaluated by reducing the absorbance after 30 min at 732 nm. Results were presented as TEAC values/L solution using a Trolox standard curve (Van der Berg et al., 1999).

Evaluation of ROS scavenging capacity in blood plasma

The method described by Rojano (2008) was used. 2.7 dichloro-dihydrofluorescein (DCFH) reacts with ROS produced by 2,2'-diazobis (2- amidinopropane dihydrocloruro) (AAPH) in an aqueous medium and forms the compound 2,7 dichlorofluorescein (DCF) fluorescence. 50 µl of 0.3 M AAPH solution were mixed with 50 µl of 2,7-dichlorofluorescein diacetate ethanolic solution 2.4 mM, 2850 µl of 75 mM phosphate buffer, pH 7.4 and 50 µl of the sample were be evaluated. The intensity of fluorescence emitted during the first 10 min was analyzed and compared to the intensity emitted in the absence of the sample (λ excitation: 326 nm, one λ emission: 432 nm and 10 nm slit). Results were expressed as % relative value.

Statistical analysis

Data are presented as mean ± standard deviation (SD) for normally distributed data or median and p25- p75 for data with non-normal distribution. For statistical differences, Student's t-test for repeated samples and the Wilcoxon test were used. To determine the correlation between antioxidant activity values, blood biomarkers and the total content of polyphenolic compounds, the Pearson correlation coefficient was calculated. ANOVA with repeated measures was used to evaluate interaction between time and type of drink, this type of ANOVA was choosen beacause participants of the study were measured at two times to see the changes to the intervention with Nativanox® Colombian yellow fruits or Nativanox® Colombian tropical fruits. A post-test analysis was performed for significant differences. The level of significance was $p<0.05$. All analyses were performed using SPSS version 22.

RESULTS

After two weeks (14 days) of consumption of a drink prepared with Nativanox® Colombian tropical fruits and other prepared with Nativanox® Colombian yellow fruits, no significant changes were observed in the anthropometric variables weight and BMI, but significant decrease in diastolic blood pressure in the group of yellow fruits was observed (Table 2).

An improvement in lipid profile parameters was evidenced in both groups, but only a statistical significance was reached ($p <0.05$) in the group of Nativanox® colombian tropical fruits for total cholesterol and LDL-cholesterol (Table 3).

The antioxidant capacity measured in plasma using ABTS and ROS methods, and the total concentration of phenols in both groups showed no significant difference between day 0 and day 14 of intervention. However, in the group of participants who received the drink prepared with Nativanox ® Colombian tropical fruits, a significant difference in reducing activity of plasma determined by FRAP assay was observed (Table 4).

There was an inverse Pearson correlation between the total phenolic compounds content and triglyceride, VLDL-cholesterol, total cholesterol, FRAP and ROS values in the group Nativanox® Colombian tropical fruits. On the contrary, the group that received drink prepared with Nativanox® Colombian yellow fruits did not show significant Pearson correlation (Table 5).

A significant interaction time vs. intervention was found for cholesterol-LDL and FRAP values ($p <0.05$) (Table 6), indicating a different in time variation in every group, being significant for the group that received the drink

Table 2. Effect of treatment on antropometrics and blood pressure values.

Parameter	Yellow fruits			Tropical fruits		
	Initial time (day 0)	Final time (day 14)	P	Initial time (day 0)	Final time (day 14)	P
Weight (kg)	57.5 (57-60)*	57.6 (56.6-60.2)*	0.2	58.07±10.3	58.02±9.91	0.84
BMI (kg/m^2)	23.9 (23.1-24.4)*	23.4±2.8	0.3	22.38±2.43	22.35±2.32	0.62
SBP (mmHg)	110 (100-110)*	100 (100-112)*	0.14	110 (110-110)*	109.37±4.42	0.098
DBP (mmHg)	73.63±8.96	70.09±7.77	0.022**	70.5±7.61	68.5±9.22	0.45

Mean ± SD, T-student for repeated samples, *Me (Rq), Wilcoxon,**Statistical significance p<0.05. SAP: Systolic arterial pressure; DAP: diastolic arterial pressure.

Table 3. Lipid profile, reactive C protein and blood glucose.

Parameter	Yellow fruits			Tropical fruits		
	Initial time (day 0)	Final time (day 14)	p	Initial time (day 0)	Final time (day 14)	p
C reactive protein	0.09 (0.02-0.24)*	0.11 (0.04-0.17)*	0.75	0.13 (0.37-0.65)	0.26±0.27	0.61
Total cholesterol (mg/dl)	167 (142-183)*	159 (145-180)*	0.16	180.62±29.03	165±30.61	0.023**
Tricilglycerides (mg/dl)	99 (52-212)*	92 (49-162)*	0.05	96.25±51.71	66 (66-167.7)	0.88
Cholesterol-HDL (mg/dl)	50.54±10.21	52.83±9.04	0.171	48.18±9.23	50.23±8.48	0.07
Cholesterol-LDL (mg/dl)	91.3 (82.4-11.2)*	85.1 (76-105.2)*	0.79	113.18±23.95	96.15±24.34	0.012**
Cholesterol-VLDL (mg/dl)	19.8 (10.4-42.4)*	18.4 (9.8-32.4)*	0.05	19.25±10.34	13.2 (9.4-33.5)	0.89
Glucose (mg/dl)	74.81±7.93	74.63±7.71	0.91	72.37±4.53	74±8.08	0.54

Values are mean ± SD, T-test for repeated samples. *Me (Rq), Wilcoxon. **Statistically significative p<0.05.

prepared with Nativanox® Colombian tropical fruits. Additionally, the values of DBP, total cholesterol, HDL-cholesterol and LDL-cholesterol were significantly different between the two groups, likewise significant changes of FRAP values after the intervention period were evidenced (Table 6).

DISCUSSION

The results of several studies show the beneficial effect of polyphenols on the cardiovascular system, through mechanisms that improve endothelial function, platelet aggregation and anti-inflammatory function (Vita, 2005). In this study, a positive effect of consumption drink prepared with Nativanox® Colombian yellow fruits on the diastolic blood pressure after ingesting daily for 14 days was observed, suggesting a health benefit of this product on the vascular homeostasis. Similar findings after consumption of a diet rich in berries showed a high antioxidant content (Erlund et al., 2008) or after consumption during 4 weeks of rich tomato lycopene (Engelhard et al., 2006).

Taking account of the bioactive compounds present in Nativanox® Colombian yellow fruits (Table 1), this preparation has an important content of tannins and carotenoids. Tannins are present in pommegranate juice (Ignarro et al., 2006; Stowe, 2011) and Sumac berries (*Rhus coriaria*) which are able to reduce diastolic pressure and the vascular smooth muscle cell (VSMC) migration by 62% (Zargham and Zargham, 2008). It has been shown that hydrolisable tannins are able to increase nitric oxide (NO) a vasoactive molecule endothelial-derived whose alteration is involved in the ocurrence of atheroesclerosis (Moncada and Higgs, 2006). In relation to the carotenoids present, only in Nativanox® Colombian yellow fruits, β-carotene is widely distributed in fruits in vegetables and is considered beneficial to endothelial functions and vascular health because of the ability to increase NO that leads to a downregulation of the expression of NF-kB-dependent adhesion molecules in endothelial cells involved in the proinflammatory response, by which (Aizawa et al., 2003; Gammone et al., 2015). Thus, it will be interesting to investigate the type of tannins (hydrolysable or condensed) and carotenoids present in Nativanox® Colombian yellow fruits and to determine its effects on nitric oxide metabolites after consumption.

In other hand, the findings in this study regarding lipid profile show that consumption of Nativanox® Colombian tropical fruits rich in polyphenols such as flavonoids and tannins, decreased the concentration of total cholesterol

Table 4. Antioxidants in plasma.

Parameter	Yellow fruits			Tropical fruits		
	Initial time (day 0)	Final time (day 14)	p	Initial time (day 0)	Final time (day 14)	p
Total phenols (GAE/ml)	1334.7 ± 157.33	1454.13 ± 123.3	0.05	1345.17 (1345.1 - 1411.1)	118716.75 ± 33342.0	0.67
FRAP (mg ascorbic acid/L solution)	76.71 ± 12.76	77.77 (61.86 - 80.2)*	0.85	75.28 ± 7.8	85.76 (79.6 - 91.7)	0.017**
ABTS TEAC (µmol Trolox/L solution)	4518.98 ± 211.9	4733 ± 377.2	0.07	4428.14 (4272.1 - 4606.7)	4305.43 (2354 - 4305.4)	0.78
ROS (% relative value)	0.0 (0.0-0.0)*	0.0 (0.0-0.0)*	0.59	0.0 (0.0 - 0.0)	0.0 (0.0-52.72)	0.18

Values are mean ± SD, T-test for repeated samples. *Me (Rq), Wilcoxon, **Statistically significant at p<0.05.

Table 5. Pearson's correlation coefficient.

Parameter	Yellow fruits		Tropical fruits	
	(r)	p	(r)	p
DBP (mmHg)	-0.37	0.26	0	0.9
SBP(mmHg)	-0.21	0.62	0.62	0.78
C reactive protein	-0.015	0.96	-0.35	0.38
Total cholesterol (mg/dl)	0.438	0.17	-0.59	0.12
Triglycerides (mg/dl)	0.348	0.29	-0.71	0.04*
Cholesterol-HDL (mg/dl)	0.208	0.62	0.208	0.62
Cholesterol-LDL (mg/dl)	-0.519	0.10	-0.44	0.27
Colesterol VLDL (mg/dl)	0.348	0.29	-0.71	0.04*
Glucose (mg/dl)	0.565	0.7	-0.92	0.48
FRAP (mg ascorbic acid/L solution)	-0.132	0.69	-0.84	0.008*
ABTS TEAC (µmol Trolox/L solution)	0.532	0.92	0.43	0.28
ROS (% relative value)	-0.191	0.57	-0.86	0.005*

*Statistically significant at p<0.05.

and LDL-cholesterol. A study shows similar findings of the relationship between cocoa catechins and epicatechins with the LDL-cholesterol concentration (Baba et al., 2007). Beneficial effects have been observed in humans after intake of chocolate (Baba et al., 2007; Mursu et al., 2004), grape juice (Freedman et al., 2001) and black tea (Duffy et al., 2001) with high content of phenolic compounds. Epidemiological studies suggest that the rate of cardiovascular events is reduced by about 1% for every 1% decrease in LDL (Brown et al., 2006), which is important in the prevention and management of these diseases. A possible mechanism involved in the results observed here on LDL-cholesterol levels is the evidenced obtained with phenolics of cocoa that decrease levels of LDL-cholesterol by reducing protein and mRNA expression of ApoB protein and mRNA which is contained in LDL-cholesterol lipoprotein, whereas cocoa polyphenols increase apolipoprotein (Apo) A1 and mRNA expression

Table 6. ANOVA repeated measures anthropometric, blood and plasma markers.

Parameter	p[2]		
	Treatment	Time	Interaction
Weight (kg)	0.283	0.823	0.426
BMI (kg/m^2)	0.307	0.457	0.427
SBP (mmHg)	0.61	0.837	0.504
PBD (mmHg)	0.049*	0.530	0.562
C reactive protein	0.721	0.689	0.782
Total cholesterol (mg/dl)	0.005*	0.817	0.071
Triglycerides (mg/dl)	0.220	0.334	0.355
Cholesterol-HDL (mg/dl)	0.045*	0.565	0.906
Cholesterol-LDL (mg/dl)	0.004*	0.482	0.016*
Cholesterol-VLDL (mg/dl)	0.220	0.334	0.355
Glucose (mg/dL)	0.623	0.624	0.54
Total phenols (GAE/ml)	0.408	0.687	0.074
FRAP (mg ascorbic acid/L solution)	0.286	0.041*	0.044*
ABTS TEAC (µmol Trolox/L solution)	0.731	0.052	0.145
ROS (% relative value)	0.233	0.241	0.205

*Statistically significant at p<0.05.

present in HDL-cholesterol lipoprotein (Andújar et al., 2012).

With regard to total phenol content in the drink prepared with Nativanox® Colombian tropical fruits and triglycerides and VLDL-cholesterol, a negative correlation was found, indicating that the higher concentration of total phenols lower levels of these parameters. These effects on triglycerides and VLDL has been observed in people following diets naturally rich in polyphenols (2776 to 2903 mg) for 8 weeks (Annuzzi et al., 2014) that significantly reduced fasting triglyceride concentrations (2-factor ANOVA) in plasma (P = 0.023) and large very-low-density lipoproteins (VLDLs) (P = 0.016) and postprandial triglyceride total area under the curve in plasma (P = 0.041) and large VLDLs (P = 0.004) (Anuzzi et al., 2014).

In the case of hydroxyl radicals, they are generated by the Fe^{2+}- EDTA/H_2O_2 system. The mechanism is carried out in three stages: first, the oxidation of the pair Fe^{2+} EDTA occurs with molecular oxygen to form Fe^{3+}- EDTA and superoxide radical. In the second, the superoxide radical in the presence of hydrogen is dismutated to H_2O_2; and in the last stage, the Fe (II)-EDTA catalyzes the decomposition of H_2O_2 to •OH (Yang and Guo, 2001). After being generated, hydroxyl radicals react with terephthalic acid to form a highly fluorescent product monohydric, 2-hidroxitereftalato acid. The capacity of Nativanox® to catch hydroxyl radicals decreases the amount of product 2-hidroxitereftalato, which can be evidenced on the decreased fluorescence intensity. Values for Nativanox® of 36147 mg DMSO/100 g dry weight have a considerable value as a bioactive expression.

Similarly, some bioactive of Nativanox have a similar behavior like anthocyanins. This actives compounds might provide a lot of effects such as the reactive oxygen species scavenger capacity, chelate metals, stimulating the expression of enzymes, reducing the formation of oxidative DNA adduct, reducing lipid peroxidation inhibiting toxins and environmental mutagenesis carcinogens, and reducing cell proliferation by modulating the signal transduction pathways (Wang and Jiao, 2000; Wang and Stoner, 2008). Nativanox contains bioactive with a high capacity to trap peroxyl free radicals (ROO•), hydroxyl (OH •), and generally reactive oxygen and nitrogen species (ROS and RNS). An intake of 200 ml Nativanox drinks provides about 4,000 IU ORAC, enough to maintain a good oxidative balance.

The study of antioxidant status in plasma of participants after 14 days of intake 200 ml of a drink prepared with Nativanox® colombian tropical fruits led to a significant increase in the reducing capacity of plasma determined by FRAP method, a trend also reported by Pedersen et al. (2000) in a group of 9 healthy women who consumed a cranberry juice for 1 week. This effect was attributed to the phenolic content and vitamin C of juice.

The correlation between the total phenol content in the beverage prepared with Nativanox® Colombian tropical fruits and FRAP value was inversely significant, suggesting that this antioxidant activity in plasma could be attributed to other polyphenolic compounds presents in drink such as tannins, carotenoids and vitamin C.

CONCLUSION

Results of this study show favorable changes in diastolic

blood pressure, total cholesterol and LDL-cholesterol as well as improvement in antioxidant status in plasma measured by the FRAP method, and an inverse relationship between total phenolic content and triglycerides and VLDL-cholesterol levels after consumption of a drink containing Nativanox® Colombian tropical fruits and other drink with Nativanox® Colombian yellow fruits for a short period of time in healthy women. These findings are important because they can explain the protective role against cardiovascular risk of a diet that includes fruits, and propose the development of food matrices containing active ingredients mixtures of yellow and tropical fruits for the prevention of non-communicable chronic diseases associated with oxidative stress. It is also demonstrated that the combination of fruits has a synergistic effect on the antioxidant capacity, which opens the door to the development of nutraceuticals or functional foods from the Nativanox® Colombian yellow or tropical fruits. Additional studies are needed to identify the bioactive compounds and the mechanisms responsible for these observed effects and their effect on cardiovascular risk population.

Conflict of interests

The authors state that they have no conflict of interest.

AKNOWLEDGEMENTS

The author acknowledged the Estrategia de Sostenibilidad 2014–2015 de la Universidad de Antioquia, Universidad Nacional de Colombia (Medellín) and Tecnas S.A.

REFERENCES

Aizawa T, Wei H, Miano JM, Abe J (2003). Role of PDE-3 in NO/cGMP-mediated anti-inflammatory effects in vascular smooth muscle cells. Circ. Res. 93(5):406-413.

Ames BM, Shigena MK, Hagen TM (1993). Oxidants, antioxidants and the degenerative disease of aging. Proc. Natl. Acad. Sci. USA. 90(17):7915-7922.

Andriambeloson E, Kleschyov AL, Muller B, Beretz A, Stoclet JC, Andriantsitohaina R (1997). Nitric oxide production and endothelium-dependent vasorelaxation induced by wine polyphenols in rat aorta. Br. J. Pharmacol. 120(6):1053-1058.

Andújar I, Recio MC, Giner RM, Ríos JL (2012). Cocoa polyphenols and their potential benefits for human health. Oxid. Med. Cell Longev. 2012.

Annuzzi G, Bozzetto L, Costabile G, Giacco R, Mangione A, Anniballi G, Vitale M, Vetrani C, Cipriano P, Della Corte G, Pasanisi F, Riccardi G, Rivellese AA (2014). Diets naturally rich in polyphenols improve fasting and postprandial dyslipidemia and reduce oxidative stress: a randomized controlled trial. Am. J. Clin. Nutr. 99(3):463-471.

Aviram M, Rosenblat M (1994). Macrophage-mediated oxidation of extracellular low density lipoprotein requires an initial binding of the lipoprotein to its receptor. J. Lipid Res. 35(3):385-398.

Baba S, Osakabe N, Kato Y, Natsume M, Yasuda A, Kido T, Fukuda K, Muto Y, Kondo K (2007). Continuous intake of polyphenolic compounds containing cocoa powder reduces LDL oxidative susceptibility and has beneficial effects on plasma HDL-cholesterol, concentrations in humans. Am. J. Clin. Nutr. 85(3):709-717.

Benzie IF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal. Biochem. 239(1):70-76.

Betoret E, Betoret N, Carbonell JV, Fito P (2009). Effects of pressure homogenization on particle size and the functional properties of citrus juices. J. Food Eng. 92(1):18-23.

Brown BG, Stukovsky KH, Zhao XQ (2006). Simultaneous low-density lipoprotein-C lowering and high-density lipoprotein-C elevation for optimum cardiovascular disease prevention with various drug classes, and their combinations: a meta-analysis of 23 randomized lipid trials. Curr. Opin. Lipidol. 17(6):631-636.

Dean RT, Fu S, Stocker R, Davies MJ (1997). Biochemistry and pathology of radical-mediated protein oxidation. Biochem. J.15 (1):1-18.

Debnath T, Park P-J, DebNath NC, Samad NB, Park HW, Lim B (2011). Antioxidant activity of Gardenia jasminoides Ellis fruit extracts. Food Chem. 128(3):697-703.

Duffy SJ, Keaney JFJr, Holbrook M, Gokce N, Swerdloff PL, Frei B, Vita JA (2001). Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. Circulation 104(2):151-156.

Encuesta Nacional de la Situación Nutricional de Colombia (ENSIN) (2010). Instituto Colombiano de Bienestar Familiar (ICBF). ICBF: Bogotá DC.

Engelhard YN, Gazer B, Paran E (2006). Natural antioxidants from tomato extract reduce blood pressure in patients with grade-1 hypertension: A double-blind, placebo-controlled pilot study. Am. Heart J. 151(1):100.e66-100.e1.

Erlund I, Koli R, Alfthan G, Marniemi J, Puukka P, Mustonen P, Mattila P, Jula A (2008). Favorable effects of berry consumption on platelet function, blood pressure, and HDL cholesterol. Am. J. Clin. Nutr. 87(2):323-331.

Freedman JE, Parker C, Li L, Perlman JA, Frei B, Ivanov V, Deak LR, Lafrati MD, Folts JD (2001). Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation 103(23):2792-2798.

Gammone MA, Riccioni G, D'Orazio N (2015). Carotenoids: potential allies of cardiovascular health? Food Nutr. Res. 59 p.

Halliwell B, Whiteman M (2004). Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br. J. Pharmacol. 142(2):231-255.

Harman D (1956). Aging: A theory based on free radical and radiation chemistry. J. Gerontol. 11:298-300.

Hicks JJ, Torres-Ramos YD, Sierra-Vargas MP (2006). Estrés oxidante, concepto y clasificación. Rev. Endocrinol. Nutr. 14(4):223-226.

Ignarro LJ, Byrns RE, Sumi D, de Nigris F, Napoli C (2006). Pomegranate juice protects nitric oxide against oxidative destruction and enhances the biological actions of nitric oxide. Nitric Oxide 15(2):93-102.

Limón-Pacheco J, Gonsebatt ME (2009). The role of antioxidants and antioxidant-related enzymes in protective response to environmentally induced oxidative stress. Mutat. Res. 674:137-147.

Martín Romero FJ (2008). Contribution of culture media to oxidative stress and its effect on human oocytes. Reproductive Biomed. Online 17(5):652-661.

Moncada S, Higgs EA (2006). The discovery of nitric oxide and its role in vascular biology. Br. J. Pharmacol. 147(1):S193-S201.

Morton L, Abu-Amsha C, Puddey I, Croft K (2000). Chemistry and biological effects of dietary phenolic compounds: relevance to cardiovascular disease. Clin. Exp. Pharmacol. 27(3):152-159.

Mursu J, Voutilainen S, Nurmi T, Rissanen TH, Virtanen JK, Kaikkonen J, Nyyssönen K, Salonen JT (2004). Dark chocolate consumption HDL cholesterol concentration and chocolate fatty acids may inhibit lipid peroxidation in healthy humans. Free Radic. Biol. Med. 37(9):1351-1359.

Ojeda D, Jimenez-Ferrer E, Zamilpa A, Herrera-Arellano A, Tortoriello J, Alvarez L (2010). Inhibition of angiotensin convertin enzyme (ACE) activity by the anthocyanins delphinidin- and cyanidin-3- O-sambubiosides from *Hibiscus sabdariffa*. J. Ethnopharmacol. 127(1):7-10.

Osakabe N, Baba S, Yasuda A, Iwamoto T, Kamiyama M, Takizawa T, Itakura H, Kondo K (2001). Daily cocoa intake reduces the susceptibility of low density lipoprotein to oxidation as demonstrated in healthy human volunteers. Free Radic. Res. 34(1):93-99.
Pedersen CB, Kyle J, Jenkinson AMcE (2000). Effects of blueberry and cranberry juice consumption on the plasma antioxidant capacity of healthy female volunteers. Eur. J. Clin. Nutr. 54(5):405-408.
Peñaloza J, Rojano B (2014). Potenciación de la capacidad antioxidante mediante interacción sinergista entre bioactivos de frutas nativas colombianas. Alimentos Hoy 22(33):3-25.
Potenza MA, Marasciulo FL, Tarquinio M, Tiravanti E, Colantuono G, Federici A, Kim JA, Quon MJ, Montagnani M (2007). EGCG, a green tea polyphenol, improves endothelial function and insulin sensitivity, reduces blood pressure, and protects against myocardial I/R injury in SHR. Am. J. Physiol. Endocrinol. Metab. 292(5):E1378-E1387.
Ramos S (2007). Effects of dietary flavonoids on apoptotic pathways related to cancer chemoprevention. J. Nutr. Biochem. 18(7):427-442.
Rice-Evans CA, Miller NJ, Paganga G (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci. 2(4):152-159.
Rojano B (2008). Actividad antioxidante del isoespintanol en diferentes medios. Vitae 15(1):173-181.
Shroeter H, Heiss C, Balzer J, Kleinbongard P, Keen CL, Hollenberg NK, Sies H, Kwik-Uribe C, Schmitz HH, Kelm M (2006). (-)-Epicatechin mediates beneficial effects of flavanol-rich cocoa on vascular function in humans. Proc. Natl. Acad. Sci. USA. 103(4):1024-1029.
Singleton V (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Meth. Enzymol. 299:152-178.
Stowe CB (2011). The effects of pomegranate juice consumption on blood pressure and cardiovascular health. Complement. Ther. Clin. Pract. 17: 113-115.
Van den Berg R, Haenen GRMM, van den Berg H, Bast A (1999). Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chem. 66(4):511-517.
Vita JA. (2005). Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am. J. Clin. Nutr. 81(1):292S-297S.
Wang LS, Stoner GD (2008). Anthocyanins and their role in cancer prevention. Cancer Lett. 269(2):281-290.
Wang SY, Jiao H (2000). Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen, J. Agric. Food Chem. 48(11):5677-5684.
Wootton-Beard PC, Ryan L (2011). Improving public health?: The role of antioxidant-rich fruit and vegetable beverages. Food Res. Int. 44(10):3135-3148.
Yang XF, Guo XQ (2001). Fe (II) EDTA chelate-induced aromatic hydroxylation of terephthalate as a new method for the evaluation of hydroxyl radical-scavenging ability. Analyst 126(6):928-932.
Zargham H, Zargham R (2008). Tannin extracted from Sumac inhibits vascular smooth muscle cell migration. Mcgill. J. Med. 11(2):119-123.