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Chemical composition of cupuassu (*Theobroma grandiflorum*) and cocoa (*Theobroma cacao*) liquors and their effects on streptozotocin-induced diabetic rats

Thiago Belchior de Oliveira, Maria Inés Genovese *

Department of Food and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of São Paulo. Av. Prof. Lineu Prestes, 580, Bloco 14. 05508–900. São Paulo, SP, Brazil

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ABSTRACT

Cupuassu (*Theobroma grandiflorum* Willd. Ex Spreng) Shum. is a Brazilian Amazon rainforest fruit, phylogenetically close to cocoa (*Theobroma cacao* L.), with excellent flavor and high agro-economic potential, whose seeds can also be used to prepare a product similar to chocolate. Here, we compared the chemical composition and *in vitro* antioxidant capacity of cupuassu and cocoa liquors. In addition, we evaluated the effect of the daily intake of cupuassu and cocoa liquors (3.6 and 7.2 g/kg body weight) on oxidative stress and lipid profile in streptozotocin (STZ)-induced diabetic rats. Although cocoa liquor presented higher polyphenol, caffeine contents and *in vitro* antioxidant capacity, saturated fatty acid contents were also higher than in cupuassu liquor. Daily administration of both cocoa and cupuassu liquors resulted in ameliorated body weight gain and liver weight/body weight ratio in STZ-diabetic rats. Plasma triacylglycerol levels were strongly reduced by both doses of liquors, while HDL-c was raised in 27% when the highest doses of cupuassu and cocoa were administrated. Plasma antioxidant capacity was improved, and lipid peroxidation in plasma and tissues decreased in a dose-dependent manner. In spite of the lower polyphenol content of cupuassu, these results indicate advantages of cupuassu compared to cocoa in relation to potential health benefits.

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Cupuassu (*Theobroma grandiflorum* Willd. Ex Spreng) Shum. is a Brazilian Amazon Rainforest fruit phylogenetically close to cocoa (*Theobroma cacao* L.). Cupuassu seed-surrounding pulp is manufactured to produce juice, jam and ice-cream, being highly appreciated due to its characteristic flavor. Up to 45% of cupuassu fresh-fruit is comprised of seed pulp. The seeds can be used for chocolate-like production while the cupuassu butter has been used as cocoa butter substitute in foods and as a cosmetic ingredient (Alves, Sebbenn, Artero, Clement, & Figueira, 2007; Genovese & Lannes, 2009).

Cocoa-products are an important source of phenolic compounds such as proanthocyanidins (PAC) and monomeric flavan-3-ols. The consumption of cocoa with high levels of procyanidins and epicatechins, despite the high contents of saturated fatty acids, has been associated with benefits to human health including increased plasma antioxidant capacity and lower lipid peroxidation, a mechanism of protection in heart disease (Katz, Doughty, & Ali, 2011; Wang et al., 2000; Weisburger, 2001). Despite the phylogenetic proximity between cocoa and cupuassu, flavonoids not detected in cocoa seeds, such as theograndins, are present in cupuassu (Pugliese, Tomas-Barberan, Truchado, & Genovese, in press; Yang et al., 2003), which may have different antioxidant potential against oxidative stress associated with chronic diseases.

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laritim, Sanders, & Watkins, 2003). The oxidative stress is due to imbalance between free radicals generation and capture systems and is intrinsically linked with non-transmissible chronic diseases (Mullarkey, Edelstein, & Brownlee, 1990).

Although promising, studies about cupuassu are scarce and mainly focused on the fruit pulp (GonÇalves, Lajolo, & Genovese, 2010). The potential health benefits of the chocolate-like product obtained from cupuassu seeds were never addressed. Based on this, the objectives of our study were to evaluate and compare the chemical composition and the effect of chronic ingestion of cupuassu and cocoa liquors on antioxidant status of streptozotocin-diabetic rats (STZ-diabetic rats).

2. Material and methods

2.1. Chemicals

The hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Aldrich (Milwaukee, WI, USA). Folin-Ciocalteu reagent, malondialdehyde, fluorescein, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2-azobis(2-methylpropionamide) dihydrochloride (AAPH), xanthine oxidase (from bovine milk), cytochrome C (from horse heart) and xanthine were purchased from Sigma Chemical Co. (St Louis, MO, USA). Glutathione, glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH) were obtained from Merck Chemical Co. (Darmstadt, Germany). TPTZ (2, 4, 6-tripyridyl-S-triazine) was purchased

* Corresponding author. Tel.: +55 11 3091 1525; fax: +55 11 3815 4410.
E-mail address: genovese@usp.br (M.I. Genovese).

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from Fluka Chemie AG (Buchs, Switzerland). Ketamine chloride and xylidine chloride (Bayer, Leverkusen, Germany). Drabkin's reagent (Sigma Diagnostics, Poole, UK). All chemicals/solvents were analytical or HPLC grade, according to the requirement.

2.2. Samples

Cupuassu and cocoa liquors were obtained from CEPLAC (Comissão Executiva do Plano da Lavoura Cacaueira) in collaboration with Riachuelo Farm in Ilhéus – BA – Brazil, and produced simultaneously on a pilot scale for this work. Cupuassu and cocoa liquors consisted of the crushed seeds after fermentation and roasting.

2.3. Centesimal and mineral composition

The methods recommended by the Association of Official Analytical Chemists AOAC (1995) were used to determine ash, crude lipid, and protein contents. Fiber content was determined according to Garcia, Infante, and Rivera (1997). Mineral content and composition were determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) according to Duxbury (2003).

2.4. Fatty acid composition

Fatty acid composition was determined after saponification and esterification with boron trifluoride according to AOAC (1995). Gas chromatography analysis was carried out using Shimadzu GC17A equipped with injector AOC20i, software Class GC10, SP-2560 column (100 m × 0.25 mm i.d, 0.25 μm) (Supelco, PA, USA).

2.5. Sample extraction for total phenolic and antioxidant capacity assays

Samples were extracted in 70% aqueous methanol for 2 h at room temperature, at a 1:20 (w/v) ratio of sample-to-solvent ratio. After filtration (Whatman nº 1), extracts were kept at −80 °C until analyses.

2.6. Total phenolic (TP) content

Total phenolic content was determined by means of the Folin-Ciocalteu assay (Singleton, Orthofer, Lamuela-Raventos, & Lester, 1999) using (+)-catechin as the standard. The absorbance readings were performed at 750 nm in a spectrophotometer Ultrospec 2000 UV–visible spectrophotometer (Amersham Biosciences, Cambridge, UK) and results expressed as mg equivalents of catechin per 100 g of sample dry weight (DW).

2.7. Proanthocyanidin (PAC) content

Total PAC contents were determined by oxidative depolymerization to anthocyanidins in acid butanol (Porter, Hrstich, & Chan, 1985), and by means of the 4-dimethylaminocinnamaldehyde (DMAC) assay (Payne et al., 2010). Results were expressed as mg of quebracho tannin equivalents/100 g sample (DW).

2.8. Caffeine content

Caffeine quantification was performed in 70% aqueous methanolic extracts using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector. The column used was a Prodigy ODS3 reversed-phase silica (250 × 4.6 mm, 5 μm; Phenomenex Ltd., Torrance, CA) and elution solvents were: (A) water:tetrahydrofuran:trifluoroacetic acid (98:2:0.1) and (B) acetonitrile. Solvent gradient was in the proportion of 15% B for 2 min, increasing to 25% B after 5 min, to 35% B after 8 min, to 50% B after 5 min, to 90% B after 5 min, 90% for further 5 min and back to 15% B after 5 min. The temperature was set to 25 °C and flow rate at 1 mL/min. Samples were injected in duplicate. Calibration was performed by injecting an appropriate amount of standard solutions of caffeine three times at five different concentrations. Results were expressed as mg of caffeine/100 g of sample DW.

2.9. Antioxidant capacity

The *in vitro* antioxidant capacity was determined by the DPPH radical-scavenging method according to Brand-Williams, Cuvelier, and Berset (1995), with some modifications (Duarte-Almeida, Novoa, Linares, Lajolo, & Genovese, 2006), using a microplate spectrophotometer (Benchmark Plus, BioRad, Hercules, CA). The standard curve was prepared using a methanolic solution of Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) at different concentrations. The oxygen radical absorbance capacity (ORAC) was carried out according to Huang, Ou, Hampsch-Woodill, Flanagan, and Prior (2002) using fluorescein as a probe. Results were expressed as μmoles Trolox equivalents/100 g sample (DW).

2.10. Animals and experimental design

The Faculty of Pharmaceutical Sciences/USP Ethical Committee for Animal Research approved all the adopted procedures (Protocol CEUA/FCF/USP no. 222). Sixty Wistar male rats weighing 200 ± 10 g were obtained from Animal House of Faculty of Pharmaceutical Sciences and Chemistry Institute of University of São Paulo. Animals were kept under standard laboratory conditions of temperature (23 ± 2 °C), relative humidity (50 ± 5%), 12 h light–dark cycle. Diet and water were provided *ad libitum*. For diabetes induction, overnight fasted rats received intraperitoneal injection (*i.p.*) of STZ (65 mg/kg) in citrate buffer (pH 4.5), followed by an aqueous solution of 10% glucose for eight hours. After three days the glycaemia was measured and all rats presented glucose levels upper than 200 mg/dL. The STZ-diabetic rats were divided into five groups of 12 animals, as follows:

- *Control*: animals receiving water by gavage during 40 days;
- *Cup1*: animals receiving 3.6 g/kg body weight of cupuassu liquor dispersed in water, by gavage, during 40 days;
- *Cup2*: animals receiving 7.2 g/kg body weight of cupuassu liquor dispersed in water, by gavage, during 40 days;
- *Coc1*: animals receiving 3.6 g/kg body weight of cocoa liquor dispersed in water, by gavage, during 40 days;
- *Coc2*: animals receiving 7.2 g/kg body weight of cocoa liquor dispersed in water, by gavage, during 40 days;

The food consumption was recorded daily. Animals were weighed and had the fast blood glucose measured once a week.

2.11. Blood and tissue samples

After 40 days the animals were anesthetized with ketamine chloride and xylidine chloride. Blood was collected by cardiac puncture into tubes with EDTA. The plasma was separated by centrifugation at 2000 *g* for 10 min at 4 °C. Erythrocytes were washed three times with ice-cold 9 g/L NaCl solution and hemolyzed with distilled water (1:4 v/v). The tissues were exhaustively perfused with sterilized ice-cold 9 g/L NaCl solution through heart puncture until the liver was uniformly pale. The kidney, brain and liver were removed, weighed and immediately frozen under liquid nitrogen and stored at −80 °C for further biochemical analysis. At the time of analysis the tissues homogenates were prepared with ice-cold 50 mM phosphate buffer (pH 7.4) (1:4 w/v) and centrifuged at 10000 *g* for 10 min at 4 °C.

2.12. Plasma and tissues antioxidant capacity

Total antioxidant capacity of plasma, erythrocytes and tissues homogenates was evaluated by the ORAC method described by Huang et al. (2002) and the ferric reducing ability of plasma (FRAP) assay was determined according to Benzie and Strain (1996). Both methods were performed on a microplate reader Synergy H1 Hybrid Multi-Mode (BioTek Instruments, Winooski, VT) and the results expressed in μmol Trolox equivalents/mL plasma.

2.13. Lipid peroxidation levels

Thiobarbituric acid reactive substances (TBARS) levels in plasma and tissues were measured according to Ohkawa, Ohishi, and Yagi (1979). An aliquot of plasma or tissue homogenate was mixed with 8.1% sodium dodecyl sulphate (SDS), 20% acetic acid, 0.67% thiobarbituric acid and water. The mixture was heated for 1 h at 95 °C and the pink chromogen formed was extracted into 1.4 ml of n-butanol. The absorbance of the organic phase was measured at 532 nm using a microplate reader Synergy H1 Hybrid Multi-Mode (BioTek Instruments Inc, Winooski, VT, USA). Malondialdehyde (MDA) was used as standard. Results were expressed as nmol MDA/mL plasma or nmol MDA/mg protein.

2.14. Antioxidant enzymes

Antioxidant enzymes activities were measured in plasma, erythrocytes and tissues (liver, brain and kidney). Briefly, CAT activity was assayed at 25 °C by a method based on the disappearance of 10 mM H_2O_2. The decomposition of H_2O_2 by CAT contained in the samples follows a first-order kinetic and changes in absorbance were measured 60 s after addition of H_2O_2 and then at 60 s intervals over 4 min (Hugo & Lester, 1984). Glutathione peroxidase (GPx) catalyses the oxidation of glutathione by tert-butyl hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$, which is reflected as a decrease in the absorbance at 340 nm (ε340 6.22 L/mmol/cm). Changes in absorbance were measured at 60 s intervals over 6 min (Albrecht & William, 1981). Superoxide dismutase (SOD) activity was measured by the decrease in the rate of cytochrome c reduction in a xanthine/xanthine oxidase superoxide-generating system consisting of 10 mM cytochrome c, 100 mM xanthine, 50 mM sodium phosphate buffer (pH 7.8) and the necessary quantity of xanthine oxidase to yield a variation of 0.025 absorbance/min at 550 nm (Gunzler et al., 1984). Enzymatic activities were expressed as units of activity (UA, corresponding to 0.1 absorbance changes for CAT and GPx and to 0.0125 absorbance change for SOD) min^{-1} g^{-1}.protein or mg^{-1} hemoglobin (Hb). Protein concentration in plasma samples was determined by the method described by Lowry, Rosebrough, Farr, & Randall (1951). Hemoglobin was measured using Drabkin's reagent.

2.15. Biochemical analysis

The concentrations of total cholesterol (TC), triacylglicerols (TAG), HDL cholesterol (HDL-c), glucose, urea and creatinine in plasma were determined using commercial kits LABTEST (Lagoa Santa, MG, Brazil). The fast blood glucose was determined with Accu-Check Performa®.

2.16. Statistical analysis

Data were expressed as the mean ± SEM. Biomarkers relative to the cocoa and cupuassu groups were compared by one-way ANOVA followed by Tukey's HSD test. Before all statistical procedures, data were checked for homogeneity of variances (Hartley test). The $p<0.05$ value was chosen as threshold of significance. The analyses

Table 1
Chemical composition (moisture, ashes, lipids, proteins and fibers), mineral content (g/kg DW) and fatty acid composition (%) of cupuassu and cocoa liquors.

	Moisture	Ashes	Lipids	Proteins	Fibers	Minerals												
						N	P	K	Ca	Mg	S	B	Zn	Fe	Mn	Cu	Na	Se
Cupuassu	2.64 ± 0.03	3.0 ± 0.2	54 ± 2	10.5 ± 0.3	27 ± 2	23.3	2.0	7.5	0.3	2.0	1.1	0.01	0.03	0.03	0.02	0.02	0.01	nd
Cocoa	2.26 ± 0.02***	0.73 ± 0.01***	52 ± 3	15.2 ± 0.8***	32 ± 2*	25.9**	3.6**	6.8*	0.5*	2.0	0.2***	0.01	0.03	0.07**	0.08**	0.02	0.02*	nd

Fatty acid composition

	Palmitic	Margaric	Estearic	Oleic	Vacenic	Linoleic	α-linoleic	Eicosanoic	Eicosaenoic	Docosanoic	Tetracosanoic
	16:0	17:0	18:0	18:1 (n-9)	18:1(n-7)	18:2 (n-6)	18:3 (n-3)	20:0	20:1 (n-9)	22:0	24:0
Cupuassu	6.9 ± 0.1	0.18 ± 0.01	31.4 ± 0.2	43.5 ± 0.1	0.46 ± 0.01	5.0 ± 0.1	0.13 ± 0.01	10.23 ± 0.09	0.37 ± 0.01	1.70 ± 0.05	0.17 ± 0.01
Cocoa	25.8 ± 0.7***	0.28 ± 0.01**	34.8 ± 0.5*	34.4 ± 0.2***	nd***	2.9 ± 0.1**	0.21 ± 0.02*	0.95 ± 0.05***	nd***	0.17 ± 0.01***	0.07 ± 0.01**

nd: not detected; n = 3. Student t test was performed to compare two samples at the same parameter.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$.

Table 2
Caffeine, total phenolic, and total proanthocyanidin contents, and *in vitro* antioxidant capacity of cupuassu and cocoa liquors.

	Cupuassu liquor	Cocoa liquor
Phenolic compounds (mg/100 g DW)		
Total phenolics	784 ± 54	2845 ± 245*
PAC – butanol	1950 ± 86	7059 ± 246*
PAC – DMAC	870 ± 75	2200 ± 185*
Caffeine (mg/100 g DW)	nd	270 ± 12*
Antioxidant capacity (μmol TE/100 g DW)		
DPPH	1913 ± 228	7957 ± 589*
ORAC	13628 ± 184	45193 ± 272*

nd: not detected; DPPH: radical scavenging capacity; ORAC: oxygen radical absorbance capacity; PAC – butanol: Proanthocyanidin content determined by oxidative depolymerization to anthocyanidins in acid butanol; PAC-DMAC: proanthocyanidin content determined with DMAC reagent.
* $p<0.05$.

were performed using Graphpad Prism for Windows (La Jolla, CA, USA).

3. Results and discussion

T. cacao is the most known specie within *Theobroma* genus. Phylogenetically close, cupuassu (*T. grandiflorum*), a Brazilian native fruit, has a greatly appreciated savory pulp and its seeds can also be used for the production of a chocolate-similar (Alves et al., 2007; Genovese & Lannes, 2009; Yang et al., 2003). Comparison among chemical composition of liquors prepared from the seeds of both species showed that moisture (2.3–2.6%), lipid (52–54%), and fiber (27–32%) contents of cocoa and cupuassu liquors were similar (Table 1). However, cocoa liquor presented approximately 4-fold more ashes and 45% more protein than cupuassu liquor. Additionally, cocoa liquor had 2.2, 4.7 and 1.8 more iron, manganese and sodium, respectively, compared to cupuassu liquor. The amount of saturated fatty acids was higher in cocoa (61%) compared to cupuassu liquor (51%). Cocoa liquor presented 26% palmitic acid, approximately 4-fold more than cupuassu liquor, which presented 10-fold more eicosanoic acid. Polyunsaturated fatty acid PUFA/MUFA ratio was similar for both liquors (~0.1) (Table 1).

The higher palmitic acid concentration seems a disadvantage of cocoa liquor compared to cupuassu liquor. This saturated fatty acid is known to induce atherogenesis, weight gain, insulin resistance, inflammation, and is also capable of increasing production of reactive oxygen species (Coll et al., 2006; Duval, Cámara, Hondares, Sibille, & Villarroya, 2007). On the other hand, the major fatty acid found

Table 3
Food intake (g/g body weight) and tissue weight (% body weight) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving cupuassu or cocoa liquors for 40 days by gavage.

Groups	Control	cup1	cup2	coc1	coc2
Food intake	0.20 ± 0.02^{a}	0.18 ± 0.01^{b*}	0.18 ± 0.03^{b*}	0.18 ± 0.02^{b*}	0.17 ± 0.01^{b*}
Tissues					
Liver	6.90 ± 1.14^{a}	5.05 ± 0.62^{b***}	5.03 ± 0.6^{b***}	5.57 ± 1.1^{b*}	5.57 ± 0.6^{b*}
Brain	0.71 ± 0.07^{a}	0.70 ± 0.06^{a}	0.68 ± 0.06^{a}	0.66 ± 0.09^{a}	0.71 ± 0.1^{a}
Kidneys	1.70 ± 0.15^{a}	1.50 ± 0.22^{a}	1.52 ± 0.2^{a}	1.55 ± 0.3^{a}	1.51 ± 0.2^{a}

Values are expressed as mean ± SD (n = 12/group). Different letters at the same line indicates statistic difference. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ always compared with control group.

in cupuassu liquor was oleic acid (~43%), a MUFA also detected in cocoa (~35%) and inversely correlated with insulin resistance in dyslipidemic individuals (Sala-Vila et al., 2011).

Cocoa beans and derivatives are widely known by the high content of polyphenols and are one of the major contributors of antioxidants to the American diet after fruits and vegetables (Rusconi & Conti, 2010). In spite of the phylogenetic proximity between cocoa and cupuassu, a significant difference was found in relation to phenolic contents and antioxidant capacity, which were higher in cocoa compared to cupuassu liquor. Total phenolic and proanthocyanidins contents were up to 4-fold higher in cocoa liquor and, as expected, the same trend was observed for both antioxidant capacity assays, DPPH scavenging and ORAC (Table 2).

Many studies have suggested that cocoa derivatives play a beneficial role in prevention of the oxidative stress damage associated with chronic diseases in animal models (Corti, Flammer, Hollenberg, & Lüscher, 2009; Ding, Hutfless, Ding, & Girotra, 2006; Jalil, Ismail, Pei, Hamid, & Kamaruddin, 2008). STZ-induced diabetes is an animal model used to promote metabolic dysfunctions related to oxidative stress (Raza & John, 2012). Daily intake of cupuassu and cocoa liquors showed a tendency to improve body weight gain of STZ-diabetic rats, despite of the lower food intake (Fig. 1, Table 3). Besides the effect on body weight gain, there were significant differences in liver weight for the groups supplemented with cupuassu and cocoa liquors compared to the control diabetic animals. It is known that streptozotocin, by producing diabetes (hyperglycaemia) and hypoinsulinemia, causes reduction in the body weight of diabetic animals and an increase (hypertrophy) in the weight of the liver in proportion to the body weight, attributed to increased triglyceride accumulation (Zafar & Naqvi, 2010). In this way, the liver to body weight ratio was also improved in STZ-treated rats supplemented with cupuassu and cocoa

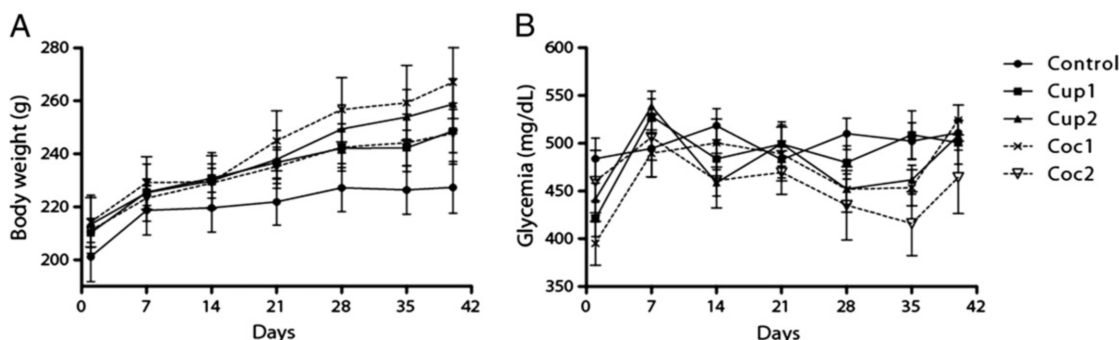

Fig. 1. Body weight evolution (A) and glucose levels (B) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving cupuassu (Cup) or cocoa (Coc) liquors for 40 days by gavage. Values were expressed as mean ± SEM (n = 12 rats/group).

Table 4
Lipid profile, glucose, urea and creatinine levels of STZ-diabetic rats fed *ad libitum* with chow diet and receiving cupuassu or cocoa liquors for 40 days by gavage.

Biochemical parameters (mg/dL)	Groups				
	control	cup1	cup2	coc1	coc2
Lipid profile					
TAG	513 ± 139[a]	286 ± 105[b*]	267 ± 112[b**]	328 ± 144[b*]	263 ± 122[b**]
TC	74 ± 16[a]	60 ± 11[a]	57 ± 10[a]	59 ± 14[a]	59 ± 26[a]
HDL-c	44 ± 8[a]	49 ± 6[a]	56 ± 5[b*]	49 ± 10[a]	56 ± 8[b*]
Blood glucose					
First day	484 ± 69[a]	421 ± 65[a]	442 ± 55[a]	395 ± 73[a]	485 ± 70[a]
Final day	511 ± 43[a]	501 ± 79[a]	508 ± 51[a]	524 ± 50[a]	497 ± 63[a]
Renal markers					
Urea	62 ± 6[a]	65 ± 5[a]	71 ± 7[a]	72 ± 15[a]	64 ± 20[a]
Creatinine	0.86 ± 0.3[a]	0.76 ± 0.1[a]	0.80 ± 0.07[a]	0.83 ± 0.1[a]	0.78 ± 0.1[a]

Values are expressed as mean ± SD (n = 12/group). Different letters at the same line indicates statistical difference. *$p<0.05$; **$p<0.01$, always compared with control group.

liquors. This ratio was previously reported to be of 3.4% in healthy rats and 6.6% in diabetic rats (Maritim, Dene, Sanders, & Watkins, 2003), and here a reduction to 5–5.6% was observed for groups receiving liquors (Table 3).

Diabetes is characterized by decreased protein synthesis and high muscle proteolysis levels, increasing nitrogen compounds such as urea and creatinine in blood and urine. In the present study, urea ranged from 62 to 72 mg/dL and creatinine from 0.76 to 0.86 mg/dL, with no differences between groups. It was previously reported that STZ-treated rats presented urea levels from 44 to 87 mg/dL, and creatinine from 0.55 to 0.8 mg/dL (Meyerovitch, Farfel, Sack, & Shechter, 1987). The increased levels of urea and creatinine in the control diabetic animals were not reverted by cupuassu and cocoa liquors (Table 4).

Cupuassu and cocoa liquors intake did not affect fasting blood glucose levels. Similar results were observed in STZ-diabetic rats treated with cocoa proanthocyanidins (Osakabe, Yamagishi, Natsume, Yasuda, & Osawa, 2004). Diabetic individuals usually have elevated levels of free fatty acids and TAG in plasma. Cupuassu and cocoa liquors had positive effects on plasma TAG and HDL-c of diabetic rats. Plasma TAG was reduced (between 40 and 50%) for both cupuassu and cocoa liquors. HDL-c, on the contrary, had an increase of 27% for the highest doses of cupuassu and cocoa (Table 4). The high content of lipids in cupuassu and cocoa liquors suggests that they could contribute to the worsening of hyperlipidemia, especially because these fruits have high contents of saturated fatty acids (van Dijk et al., 2009). However, some studies reported that stearic acid (~32% of fatty acids of cupuassu and cocoa samples) did not change levels of total and LDL cholesterol (Hunter, Zhang, & Kris-Etherton, 2010) and also increased HDL-c (Wan et al., 2001). In addition, polyphenols such as proanthocyanidins and quercetin glycosides are able to *in vitro* and *in vivo* inhibit pancreatic lipase and to diminish plasma TAG and TC levels (Fernandes et al., 2010; Quesada et al., 2009; Yokozawa, Cho, Park, & Kim, 2012).

A significant increase of the plasma antioxidant capacity was observed for cupuassu and cocoa liquors supplemented animals, compared to control. Concomitantly, levels of lipid peroxidation were also significantly reduced in plasma (Fig. 2). The higher plasma antioxidant capacity and the lower levels of lipid peroxidation in animals receiving cupuassu and cocoa liquors, compared to control, suggest that bioactive compounds in both liquors can protect the STZ-diabetic rats against oxidative damage. This effect is intrinsically related to phenolic compounds absorption and specific fatty acids actions. According to Baba et al. (2000), (−)-epicatechin in cocoa powder is absorbed from the digestive tract, conjugated and distributed to the plasma, enhancing the antioxidant activity and reducing the accumulation of lipid peroxides of plasma. Long-term ingestion of chocolate by healthy humans decreased serum lipid peroxidation, increased serum HDL cholesterol, and affected the fatty acid content of serum and LDL, which was associated to the fatty acid composition of cocoa (Mursu et al., 2004). According to our results, antioxidant capacity was also improved in liver and kidneys, and lipid peroxidation was reduced in liver, kidneys and brain. However, a clear dose-effect relationship was not observed, except for the liver of diabetic rats receiving cupuassu liquor (Fig. 3).

Hyperglycemia can lead to glycation and inactivation of the antioxidant enzymes SOD, CAT and GPx causing oxidative stress, which is closely associated with lipid peroxidation (Bagri, Ali, Aeri, Bhowmik, & Sultana, 2009). Chronic ingestion of cupuassu and cocoa liquors did not show clear effects on CAT, SOD and GPx activities which varied according to cupuassu and cocoa doses and tissue analyzed. Fig. 4 shows significant increase of CAT activity in liver and brain for the Cup1 group, and in kidneys for both cupuassu and cocoa groups. SOD activity was also increased in brain for the cupuassu groups and plasma for both cupuassu groups and for the Coc1 group. The GPx activity was elevated in liver for the Coc1 group, kidneys for the Cup1, erythrocytes for both cupuassu groups and plasma for the Coc2 and Cup1 groups.

Similar to these results, Juskiewicz et al. (2008) found no significant changes in plasma SOD activity in diabetic rats treated with green tea extract. Some studies proposed that these conflicting results, related to increased or decreased antioxidant enzymes activities, are dependent on the severity, duration and treatment of diabetes (Armstrong, Chestnutt, Gormley, & Young, 1996; Ugochukwu, Bagayoko, & Antwi, 2004). According to Maritim, Dene, Sanders, and Watkins (2003), Maritim, Sanders, and Watkins (2003), the effect of STZ-induced diabetes on the activity of SOD is erratic, with no discernible pattern based on gender or species of animal, or duration of diabetes, or tissue studied.

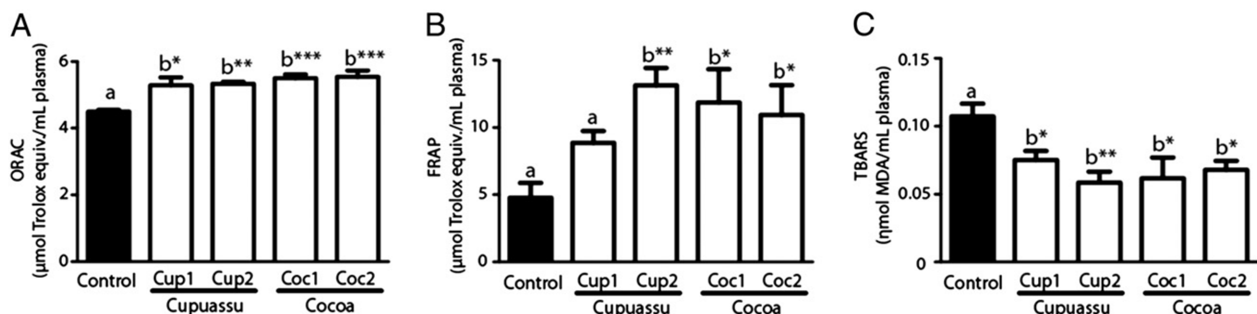

Fig. 2. Plasma antioxidant activity performed by ORAC (A) and FRAP (B) methodologies, and plasma lipid peroxidation (C) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving cupuassu (Cup) or cocoa (Coc) liquors for 40 days by gavage. Values were expressed as mean ± SEM (n = 12 rats/group). Different letters above the columns indicates statistical difference. The *p value* between supplemented and control groups was expressed as * ($p<0.05$) and ** ($p<0.01$).

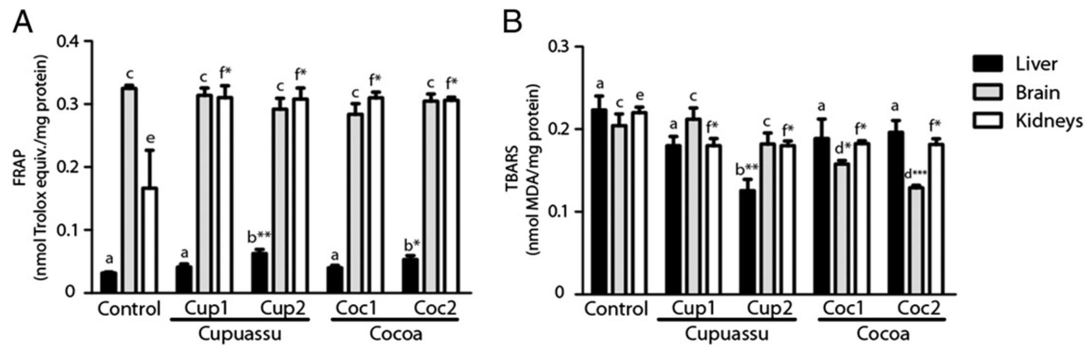

Fig. 3. Ferric reducing antioxidant power (FRAP) (A) and lipid peroxidation (B) in liver, brain, and kidneys of STZ-diabetic rats fed *ad libitum* with chow diet and receiving cupuassu (Cup) or cocoa (Coc) liquors for 40 days by gavage. Values were expressed as mean ± SEM (n = 12 rats/group). Different letters above the columns indicates statistical difference at the same tissue. The *p value* between supplemented and control groups was expressed as * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$).

Glutathione peroxidase, glutathione reductase and catalase can be elevated or decreased depending on the tissue considered. The effects of antioxidants would change depending on the administration prior to STZ, or concomitant with STZ, or after well-established diabetes (Maritim, Dene, Sanders, & Watkins, 2003; Maritim, Sanders, & Watkins, 2003).

4. Conclusion

In conclusion, both cupuassu and cocoa liquors demonstrated positive effects on lipid profile and antioxidant status of STZ-diabetic rats. Our results indicate that the synergism between fatty acids and phenolic compounds in cupuassu and cocoa liquors may contribute to

Fig. 4. Activity of antioxidant enzymes CAT (A and B), SOD (C and D) and GPx (E and F) of STZ-diabetic rats fed *ad libitum* with chow diet and receiving cupuassu (Cup) or cocoa (Coc) liquors for 40 days by gavage. Values were expressed as mean ± SEM (n = 12 rats/group). Different letters above the columns indicates statistical difference. The *p value* between supplemented and control groups was expressed as * ($p<0.05$), ** ($p<0.01$) and *** ($p<0.001$).

reduction of hypertriglyceridemia caused by lack of insulin. Although cupuassu liquor has approximately 3.5 fold less phenolic compounds than cocoa liquor, it also presents a much lower amount of palmitic acid, and chronic intake of cupuassu was equally effective on ameliorating lipid profile and improving antioxidant status in STZ-diabetic rats. This suggests that the specific fatty acid and phenolic profile in cupuassu liquor may be superior to cocoa.

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