

## Evaluation of total polyphenols content and antioxidant capacity of different commercial cocoa (*theobroma cacao*) powders)

## Avaliação do teor de polifenóis totais e capacidade antioxidante de diferentes pós comerciais de cacau (*theobroma cacao*)

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## ABSTRACT

Polyphenols from cocoa have been demonstrated to reduce blood pressure and improve endothelial function in hypertensive individuals. However, polyphenols content in commercial cocoa products may be lost during fermentation, alkalization, and/or roasting process, impairing its bioactivity. Therefore, the current study sought to investigate whether total polyphenols content and antioxidant capacity are preserved among commercial cocoa powders. Four best-selling 100% cocoa powder products in Brazil (Garoto®, Nestle®, Mãe Terra®, and Hershey®) and one alkalized 100% cocoa powder product (Hershey®) were examined. The total polyphenols content was determined by using the Folin-Ciocalteu's phenol reagent and antioxidant capacity by using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) method. No significant difference in total polyphenols and antioxidant capacity among the brand of natural powder products (Garoto®, Nestle®, Mãe Terra®, and Hershey®) was observed. However, Alkalized cocoa powder (Hershey®) exhibited significantly lower total polyphenols content and antioxidant capacity compared to other brands of natural cocoa powder products. The current study showed that total polyphenols content and antioxidant capacity are consistent between natural cocoa powder products. However, total polyphenols content and antioxidant capacity of cocoa are reduced during the alkalization process.

**Key words:** functional food; food quality, oxidative stress, cardiovascular disease.

## RESUMO

Têm sido demonstrado que polifenóis do cacau podem reduzir a pressão arterial e melhorar a função endotelial em indivíduos hipertensos. No entanto, o teor de polifenóis em produtos comerciais de cacau pode ser perdido durante o processo de fermentação, alcalinização e/ou torra, prejudicando sua bioatividade. Portanto, o presente estudo buscou investigar se o teor de polifenóis totais e a capacidade antioxidante são preservados entre pós comerciais de cacau. Foram examinados quatro produtos 100% de cacau em pó mais vendidos no Brasil (Garoto®, Nestle®, Mãe Terra® e Hershey®) e um produto 100% de cacau em pó alcalinizado (Hershey®). O conteúdo total de polifenóis foi determinado usando o reagente fenol de Folin-Ciocalteu e a capacidade antioxidante usando o método 2,2'-azino-bis (ácido 3-etilbenzotiazolina-6-sulfônico) (ABTS). Não foi observada diferença significativa nos polifenóis totais e capacidade antioxidante entre as marcas de produtos em pó (Garoto®, Nestle®, Mãe Terra® e Hershey®). No entanto, o pó de cacau alcalinizado (Hershey®) exibiu um conteúdo de polifenóis totais e capacidade antioxidante significativamente inferior em comparação com outras marcas de produtos de cacau em pó. O estudo atual mostrou que os polifenóis totais e a capacidade antioxidante são consistentes entre os produtos de cacau em pó. No entanto, os polifenóis totais e a capacidade antioxidante do cacau são reduzidos durante o processo de alcalinização.

**Palavras-chave:** alimento funcional, qualidade alimentar, estresse oxidativo; doença cardiovascular.

## 1 INTRODUCTION

The consumption of polyphenols-rich foods has been linked to improvements in vascular health in clinical population (LUDOVICI et al., 2017; GOSZCZ, K. et al., 2017). Polyphenols are bioactive compounds that naturally exist in plants and plant products, including vegetables, fruits, herbs, and cocoa (TENA et al., 2020). Polyphenols content in cocoa powder have gained attention due to its positive effect on reducing inflammation, oxidative stress, and cardiovascular disease (CVD) (TENA et al., 2020). Regarding the effect of polyphenols on vascular system, previous studies have reported that individuals who ingest a high content of polyphenols are less susceptible to CVD (LÔBO et al., 2020; GRASSI et al., 2015). Therefore, the daily inclusion of foods rich in polyphenols are recommended in order to improve cardiovascular health.

Cocoa is one of the richest sources of the polyphenols and its protective effect in cardiovascular system is widely recognized by many cultures over the years (LUDOVICI et al., 2017). Given that polyphenols are likely the pivotal cocoa-derived component responsible for inducing improvement in blood pressure and endothelial function, the preservation of polyphenols content in cocoa-derived products during the conventional manufacturing process is important (GRASSI et al., 2015; LUDOVICI et al., 2017). For instance, the polyphenols content in cocoa may be lost during fermentation, alkalization, and roasting process (GONZÁLEZ-BARRIO, R. et al. 2020). Currently, people can easily find several brands of cocoa-based products (cocoa powder and dark chocolate) available on the market. Since the health benefit of cocoa ingestion on vascular system depends on their polyphenols content, the products should possess a sufficient quantity of polyphenols so that an antioxidant effect may be achieved. For example, studies have reported that ingesting a range of 500 to 1000 mg of polyphenols may improve vascular function parameters (GRASSI et al., 2015; LUDOVICI et al., 2017). As part of an ongoing multidisciplinary collaboration to promote food quality and nutrients bioactivity, the purpose of the current study was to evaluate the total polyphenols content, as well as the antioxidant capacity in different commercial cocoa powder products.

## 2 MATERIALS AND METHODS

### Sample acquisition

Cocoa powder products for this study comprised the top four best-selling brands in Brazil. Four natural cocoa powder products (Garoto®, Nestle®, Mãe Terra®, and

Hershey®) and one brand of alkaline cocoa powder (Hershey®) were examined. The inclusion criteria for cocoa powder products were that the product be within 6 months of manufacturing, stored in dry and cool conditions, and that it be 100% natural cocoa. Efforts to achieve a random sampling included purchasing three different batches for each selected product. Two sample were acquired from each batch of cocoa powder product and utilized in analysis in order to obtain a homogenous powder.

#### Determination of total polyphenols

Total polyphenols and flavonoids of cocoa products were determined by using the Folin-Ciocalteu (F-C) reagent, as previously described (MORGADO et al., 2016). Briefly, samples (4 g) were homogenized with 8 mL of methanol following centrifugation to 10 000 g, for 15 min. Afterwards, 500  $\mu$ L of the supernatant was mixed with 300  $\mu$ L of 1.5N hydrogen peroxide to oxidize the interfering compounds. The sample was vortexed and underwent the F-C assay by diluting 15  $\mu$ L of the sample mixture with 240  $\mu$ L of distilled water and 15  $\mu$ L of 0.25N F-C reagent. After 4 min, 30  $\mu$ L of 1N sodium carbonate was added. The mixture was incubated for 2 h in the dark and the absorbance values were determined at 765 nm. The outcome data were expressed as gallic acid equivalents in mg/100g of dry weight (mg GAE/100g dry weight).

#### Determination of total antioxidant capacity

Total antioxidant capacity was evaluated by using Trolox equivalent antioxidant capacity (TEAC) assay, as described by MORGADO et al., 2016. The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) for stock solution was prepared from 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate in a volume ratio of 1:1, and then incubated in the dark at room temperature for 16 h and used within 2 days. A 100 mL of the sample was mixed with 3.8 mL ABTS working solution and the absorbance was taken at 734 nm after 6 min of incubation at room temperature. The percent of inhibition of absorbance at 734 nm was calculated and the results were expressed as  $\mu$ mol of Trolox equivalents ( $\mu$ mol TE/100 g dry weight).

#### Statistical analysis

To detect statistical differences in total polyphenols content and total antioxidant capacity among the brands of cocoa powder products, as well as among batches, a one-way ANOVA test was performed. Statistical significance was set at a P value  $\leq 0.05$  and the results were expressed as mean  $\pm$  standard deviation (SD). A commercially available

statistical package (IBM SPSS Statistics version 22 for Mac, Armonk, N.Y., USA) was used for statistical analysis.

### 3 RESULTS AND DISCUSSION

No significant difference ( $P > 0.05$ ) in total polyphenols content and antioxidant capacity was found when comparing the batches of each brands of cocoa powder products (Table 1). These data suggest great stability of polyphenols present in cocoa powder products, which is important for those who expect such nutrients.

The results from the current study showed no significant difference in total polyphenols content among the brands of natural cocoa powder products (Garoto®:  $4,006 \pm 162.3$ ; Nestle®:  $3,919 \pm 125.7$ ; Mãe Terra®:  $3,889 \pm 65.3$ ; and Hershey®:  $3,844 \pm 14.3$  mg GAE/100g dry weight,  $P > 0.05$ ). However, total polyphenols content from the alkalized cocoa powder (Hershey Alkalinized®) was significant lower ( $1096 \pm 51.1$  mg GAE/100g dry weight,  $P < 0.05$ ) compared to the natural cocoa powder brands (approximately 30% less total polyphenols) (Table and Figure 1). These findings are in line with previous study demonstrating that alkalization, a procedure applied to change the color of the product and give it a milder taste, leads to considerable losses of total polyphenols present in cocoa powder (ANDRES-LACUEVA et al., 2008). Additionally, it is important to note that cocoa powder from the Mãe Terra® brand was marketed as an organic product, as per its label. It has been suggested that organic foods could exhibit higher polyphenols content, which may occur due to the absence of synthetic pesticides, resulting in higher exposure of the plant to stressful situations that lead to an improvement of natural defense substances such as phenolic compounds (WOESE, K. et al., 1997). In contrast with this idea, no significant difference in polyphenols among the non-organic brands of natural cocoa powder was found.

Table 1. Total polyphenols content and antioxidant capacity of batches of different brands of cocoa powder products

Cocoa Power brands	Batches	Total polyphenols (mg GAE/100g dw)	Total antioxidant capacity ( $\mu\text{mol TE}/100 \text{ g dw}$ )
Garoto®	#1	$4126 \pm 187.9$	$492.8 \pm 3.75$
	#2	$3946 \pm 179.4$	$476.9 \pm 6.04$
	#3	$3947 \pm 24.04$	$491.9 \pm 3.57$
	<i>Mean</i>	$4006 \pm 162.31$	$487.2 \pm 8.67$
Nestle®	#1	$3949 \pm 150.8$	$490.9 \pm 1.49$
	#2	$3911 \pm 96.18$	$491.1 \pm 0.95$
	#3	$3898 \pm 155.2$	$484.1 \pm 7.70$
	<i>Mean</i>	$3919 \pm 125.70$	$488.6 \pm 5.29$
Mãe Terra®	#1	$3898 \pm 27.02$	$484.2 \pm 9.01$

	#2	3889 ± 94.58	479.6 ± 8.31
	#3	3881 ± 106.70	488.9 ± 5.77
	<i>Mean</i>	3889 ± 65.38	483.7 ± 7.94
Hershey®	#1	3850 ± 4.49	483.5 ± 1.25
	#2	3830 ± 6.36	484.4 ± 1.53
	#3	3844 ± 16.04	485.7 ± 3.44
	<i>Mean</i>	3844 ± 14.34	484.6 ± 2.20
Hershey Alkalinized®	#1	1068 ± 51.07	388.4 ± 0.72
	#2	1093 ± 67.36	387.5 ± 0.70
	#3	1117 ± 43.15	387.0 ± 1.41
	<i>Mean</i>	1096 ± 51.18*	387.6 ± 0.99*

GAE, gallic acid equivalent; TE, Trolox equivalent; dw, dry weight. Data are shown as mean ± standard deviation. \* significant different from other brands of natural coco powder products (P < 0.05).

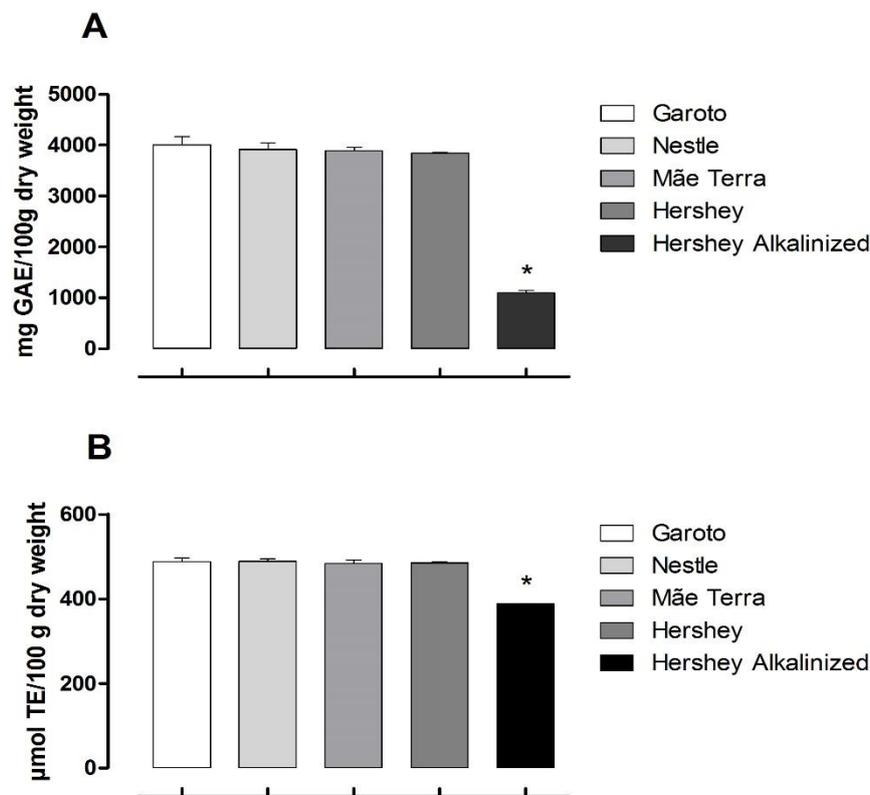


Figure 1. Total polyphenols content (A) and antioxidant capacity (B) of brands of natural cocoa powder products. GAE = gallic acid equivalent; TE = Trolox equivalent. Data are shown as mean ± standard deviation. \* significant different from other brands of natural coco powder products (P < 0.05).

Cocoa and cocoa products, including cocoa liquor, cocoa powder, and chocolate (dark chocolate and milk), are highly consumed due to its variety of polyphenols content and different levels of antioxidant proprieties (LUDOVICI et al., 2017). Many randomized and placebo-controlled studies have shown positive effect of cocoa beverage and/or dark chocolate consumption on blood pressure and endothelial function measurement, which are attributed to its total polyphenols content (DECROIX et al.,

2018; REES et al., 2018; SHAW et al., 2020). Typically, the total polyphenols content in the cocoa powder products are not informed on the label, which make it difficult to establish recommendations about how much cocoa powder is necessary to provide an optimal amount of polyphenols. A previous study reported that daily ingestion of approximately 500 mg of polyphenols present in cocoa powder can reduce inflammatory biomarkers involved in the process of atherosclerosis in individuals at high risk for CVD (MONAGAS et al., 2009). Therefore, considering that 100 g of cocoa powder possesses approximately 4,000 mg of total polyphenols (as observed in the present study in the Garoto® brand), it would be necessary to ingest at least 12.5 g of cocoa powder to achieve 500 mg of total polyphenols (MONAGAS et al., 2009; GRASSI et al., 2015).

Regarding the total antioxidant capacity of natural cocoa powder products, no significant difference was observed among the brands evaluated (Garoto®:  $487.2 \pm 8.6$ ; Nestle®:  $488.6 \pm 5.2$ ; Mãe Terra®:  $483.7 \pm 7.9$ ; and Hershey®:  $484.6 \pm 2.2$   $\mu\text{mol TE}/100$  g dry weight,  $P > 0.05$ ). On the other hand, the total antioxidant capacity of alkalized cocoa powder (i.e., Hershey Alkalinized®) was significant lower ( $387.6 \pm 0.9 \pm 5.7$   $\mu\text{mol TE}/100$  g dry weight,  $P < 0.05$ ) compared to natural cocoa powder brands (Table and Figure 1). These findings do not come as a surprise, since total polyphenols content (which were also lower in Hershey Alkalinized® brand) are linked to the antioxidant capacity of the cocoa powder (MILLER et al., 2009). Previous clinical studies interrogating the effect of cocoa powder ingestion on vascular health have determined the dose of cocoa-derived products (i.e. dark chocolate) based on its polyphenols content (LUDOVICI et al., 2017). GRASSI et al. (2014) demonstrated that a 2-week ingestion of dark chocolate containing 1,008 mg of polyphenols reduced systolic and diastolic blood pressure, as well as increased endothelial function in hypertensive individuals with impaired glucose tolerance. Moreover, MONAGAS et al. (2009) observed a reduction in inflammatory biomarkers related to atherosclerosis in patients at high risk for CVD after ingesting 495 mg of cocoa powder-derived total polyphenols throughout 4 weeks of intervention. The authors attributed this positive effect of cocoa powder ingestion to its antioxidant properties, which may counteract the higher production of reactive oxygen and nitrogen species that are often observed in individuals at risk for CVD (MILLER et al., 2009; GRASSI et al., 2015).

#### 4 CONCLUSION

In conclusion, the current study demonstrated that total polyphenols content and antioxidant capacity of natural cocoa powder product were not different among four best-selling brands, despite the alkalization of cocoa may reduce such characteristics. These findings provide valuable information on the total polyphenols content present in cocoa powder products, which may be useful in delivering an optimal amount of polyphenols to consumers in order to enhance vascular health.

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## **6 DECLARATION OF CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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