Research Article

Received: 13 November 2017 Revised: 1 March 2018 Accepted article published: 23 May 2018 Published online in Wiley Online Library: 12 July 2018

(wileyonlinelibrary.com) DOI 10.1002/jsfa.9146

Can we conserve *trans*-resveratrol content and antioxidant activity during industrial production of chocolate?

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Abstract

BACKGROUND: Cocoa exhibits high content of phenolic compounds, among which *trans*-resveratrol stands out, associated with several bioactive activities such as antioxidant properties. Chocolate contains reduced amounts of these bioactive compounds due to losses during the production process. Therefore, this study aimed to assess changes in total phenolic content, and specifically *trans*-resveratrol, as well as changes in the antioxidant activity of cocoa and its products during industrial production of chocolate.

RESULTS: A total of ten different cocoa products were analyzed. The processes of fermentation and roasting caused significant loss of total phenolic compounds and antioxidant activity. The high temperature of roasting had a major influence on this loss (71% for total phenolic compounds and 53–77% for antioxidant activity), except for *trans*-resveratrol. The *trans*-resveratrol content formed after fermentation (9.8 μg kg^{-1}) showed little variation during the processes, and it was detected in higher concentrations both in natural (11.4 μg kg^{-1}) and in alkalized cocoa powder (13.5 μg kg^{-1}). Alkalization of cocoa products led to loss of capacity of deactivating superoxide radical.

CONCLUSION: These findings contribute to the optimization of the production process of chocolate and other food products containing cocoa and its derivatives, aiming to better preserve their bioactive compounds.

Keywords: cocoa products; stilbenes; HPLC; reactive oxygen species

INTRODUCTION

Currently, natural antioxidants, including vitamins and phenolic compounds, are highly valued because they are protective agents against oxidative damage. Therefore, they may contribute to preventing the development of cancer and degenerative illnesses, including cardiovascular and neurological diseases, through multiple mechanisms.[1–3]

Since the 1980s, the search for natural antioxidants to replace synthetic ones has led to studies of these compounds in plant products. A broad range of plant foods, including cocoa (*Theobroma cacao* L.), have been considered sources of phenolic compounds. Worldwide dissemination and popularization of cocoa favored the discovery of new processes and different ways to consume it. To obtain products derived from cocoa, after harvest, the pods are split open and cocoa beans undergo several processing steps, such as fermentation, drying, and roasting. Cocoa roasted beans or nibs are ground to obtain a cocoa mass or finely ground to obtain cocoa liquor. The latter is pressed to obtain cocoa butter and cocoa draff, which is milled to produce cocoa powder. Both cocoa liquor and cocoa powder can be alkalized (Fig. 1).[4]

Cocoa not only is a type of energetic food, but also constitutes a source of antioxidants, displayed in amounts higher than those found in tea and red wine.[1,5] Moreover, it contains some compounds exclusive to certain plant species, such as *trans*-resveratrol (3,5,4′-trihydroxy-*trans*-stilbene),[6] a phenolic compound that belongs to the stilbene class, known principally for its cardioprotective activity. This phenolic compound is also frequently associated with prevention of cancer, ischemias, diabetes, inflammations, and viral infections, properties that have aroused great interest among scientists.[7]

The steps involved in cocoa processing during chocolate production, which include fermentation, roasting, and alkalinization, reduce the contents of phenolic compounds naturally present in cocoa beans, due to factors such as enzyme action and time/temperature. Therefore, it is desirable to minimize phenolic compound losses during chocolate production, more specifically the contents of flavanols and procyanidins, the major phenolic compounds in cocoa beans.[8–10] Special attention has been given to the polyphenols found in cocoa beans that belong to the stilbene class, mainly *trans*-resveratrol.[6,11] Nonetheless, so far, the effect of industrial processing of cocoa beans and derived products on the content of this stilbene is unknown.

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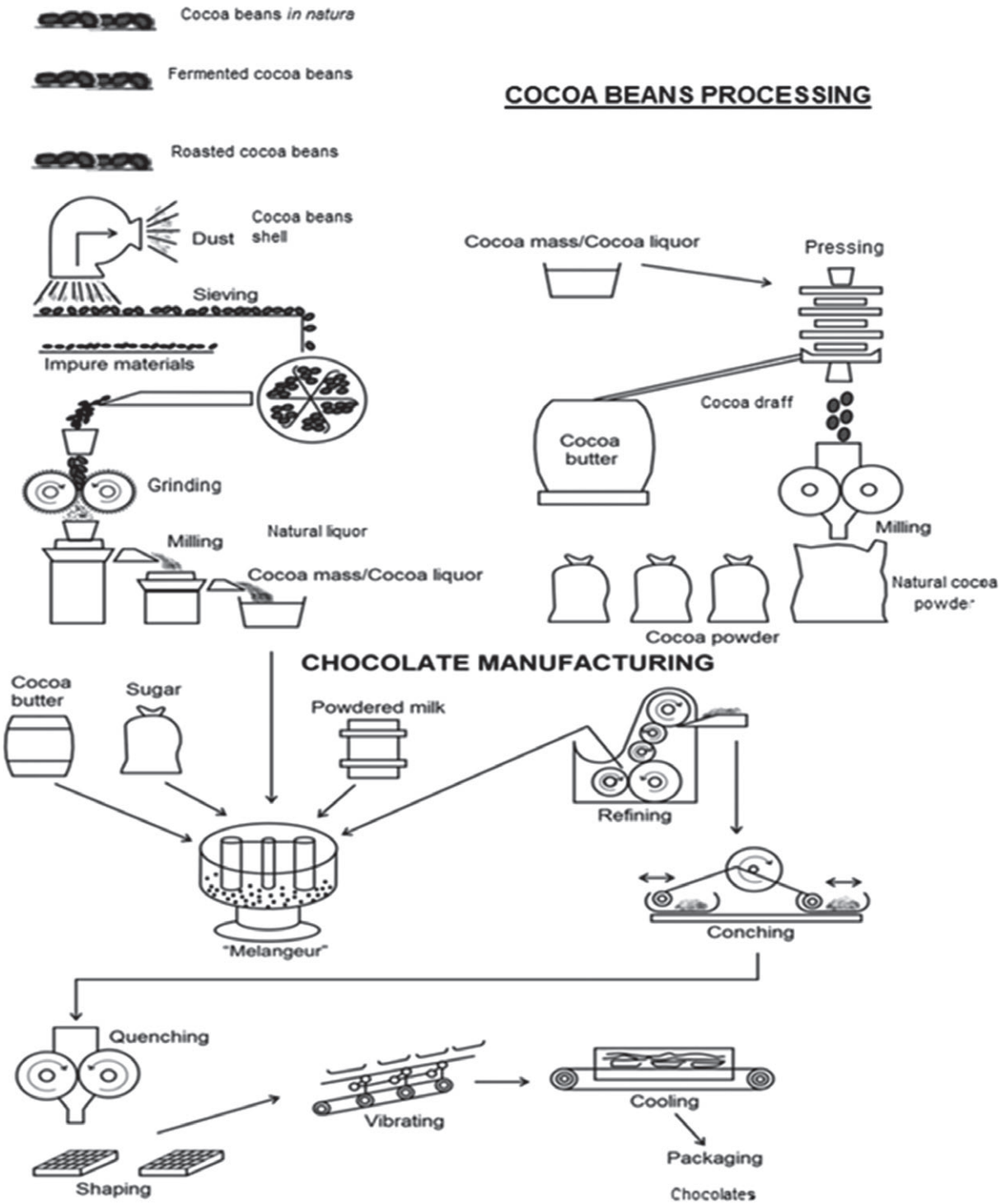

Figure 1. Process flow diagram for cocoa bean processing. Adapted from Oetterer *et al.*[4]

Considering that cocoa and its derivatives are important sources of phenolic compounds, this study aimed to assess the changes in the contents of total phenolic compounds, mainly *trans*-resveratrol, and antioxidant activity caused by chemical modifications that take place during chocolate manufacturing process. To the best of our knowledge, this is the first time that changes in the content of *trans*-resveratrol in cocoa during the chocolate manufacturing process have been studied.

MATERIAL AND METHODS

Samples

The cocoa products used in this study were raw cocoa beans, fermented cocoa beans, roasted cocoa beans, natural cocoa liquor, alkalized cocoa liquor, natural cocoa powder, alkalized cocoa powder, cocoa draff, cocoa bean shells, and dark chocolate 55% cocoa (Fig. 2) obtained from the same production line. These products were acquired from one batch of an industry located in Rio das Pedras, SP, Brazil.

Figure 2. Samples of cocoa products analyzed in this study: (A) raw cocoa beans; (B) fermented cocoa beans; (C) roasted cocoa beans; (D) natural cocoa liquor; (E) alkalized cocoa liquor; (F) natural cocoa powder; (G) alkalized cocoa powder; (H) cocoa draff; (I) cocoa bean shells; (J) dark chocolate 55% cocoa.

Chemicals

The following reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA): hexane, ethyl alcohol, ethyl acetate, methanol, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), (±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-tris(2-pyridyl)-*s*-triazine, iron(III) chloride, 2,4,6-tripyridyl-*s*-triazine (TPTZ), iron(II) sulfate heptahydrate, monobasic potassium sulfate, dibasic potassium sulfate, Folin–Ciocalteu reagent, gallic acid, fluorescein sodium salt, 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH), reduced nicotinamide adenine dinucleotide phosphate (NADH), phenazinemethosulfate (PMS), nitro blue tetrazolium (NBT), acetonitrile, formic acid, and *trans*-resveratrol authentic standard. All the chemicals used were of analytical grade of purity.

Extraction of phenolic compounds

Cocoa products samples were previously milled and defatted.[12] Aliquots of 25 mL hexane were added to 5 g of each sample and centrifuged at 5000 × *g* for 15 min, and the supernatant was discarded. This procedure was carried out twice; after the total evaporation of the solvent, the extracts were obtained in triplicate. Phenolic compounds were extracted from 1 g of the sample using 10 mL of aqueous ethanol solution (80% v/v), followed by sonication for 30 min and centrifugation at 5000 × *g* for 10 min. The supernatant was collected, filtered, and used for the assessment of antioxidant activity applying different methods. All extracts and analyses were performed in triplicate for each sample of cocoa product.

Extraction of *trans*-resveratrol

Previously milled and defatted samples underwent the extraction process following the method described by Ragab *et al.*,[13] with modifications. Aliquots of 10 mL ethyl acetate were added to 1 g of each sample, extracted in triplicate in a water bath at 70 °C for 30 min, and centrifuged at 9500 × *g* for 6 min. The supernatant was collected, filtered, and evaporated under nitrogen flow. After adding 1 mL methanol, the samples were filtered again and *trans*-resveratrol was quantified using high-performance liquid chromatography (HPLC). All extracts and analyses were carried out in triplicate for each sample of cocoa product.

Total phenolic content

Total phenolic content analysis was performed following the spectrophotometric Folin–Ciocalteu method with some changes.[14] Aliquots of 20 μL of the standard or sample and 100 μL Folin–Ciocalteu aqueous solution (10% v/v) were pipetted into each microplate well. After 5 min of reaction, 75 μL sodium carbonate aqueous solution (7.5% v/v) was pipetted into the wells and the mixture was stirred. After 40 min of reaction in the dark, absorbance was read at 734 nm in a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA). A calibration curve was plotted using gallic acid as standard at concentrations ranging from 5 to 80 μg mL^{-1}. The results are expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE g^{-1}).

Quantification of *trans*-resveratrol using reversed-phase HPLC

Reversed-phase (RP)-HPLC analyses were carried out in triplicate using the method described by Counet *et al.*,[6] with modifications. Each extract was injected at a volume ranging from 20 to 30 μL into an HPLC system (Shimadzu Co., Kyoto, Japan) coupled to a photodiode-array detector (SPD-M10AVp, Shimadzu) at 300 nm and a reversed-phase C18 column (250 mm × 4.6 mm; particle size of 5 μm). The mobile phase used was water : acetonitrile : formic acid (98.9 : 1.0 : 0.1, v/v/v) (solvent A) and acetonitrile (solvent B)

at a constant flow of 1 mL min^{-1}. The gradient profile used was as follows: 5% B to 45% B for 23 min, 100% B for 7 min, remaining in this isocratic condition for another 10 min and returning to 5% B for 5 min, with a total run time of 45 min. The column was kept at 30 °C. The chromatograms were analyzed using Class-VP® software. *trans*-Resveratrol was identified based on the characteristics of its electronic absorption spectra (200–400 nm), chromatographic retention time, and overlay of the corresponding standard. The detection limit and quantification limit were 0.001 μg mL^{-1} and 0.004 μg mL^{-1} respectively.

Antioxidant activity

Scavenging of synthetic free radicals: DPPH and ABTS

The measurement of the free radical scavenging activity using DPPH followed the method described by Melo *et al.*,[15] with some modifications. Aliquots of 66 μL of the standard, control, or samples and 134 μL of 150 μmol L^{-1} ethanol solution of DPPH were transferred to microplate wells. The plate remained for 45 min in the dark and, after that, the absorbance was measured at 517 nm in a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA). The blank was composed of ethanol, and the standard curve was generated using Trolox at concentrations ranging from 20 to 140 μmol L^{-1}. The results are expressed as micromoles of Trolox equivalents per gram of sample (mg TE g^{-1}).

The antioxidant capacity was assessed applying the free radical ABTS method following the procedure recommended by Oldoni *et al.*,[16] with some modifications. ABTS radical was prepared adding 5 mL of 7 mmol L^{-1} $ABTS^{\cdot+}$ solution to 88 μL of 140 mmol L^{-1} potassium persulfate solution, incubated at 25 °C in the dark for 12–16 h. Once formed, the radical was diluted with 75 mmol L^{-1} potassium phosphate buffer, pH 7.4, to an absorbance of 0.700 ± 0.01 at 734 nm. Aliquots of 20 μL of the samples and 220 μL ABTS radical solution were transferred to microplate wells and mixed at room temperature in the dark. The absorbance was read at 734 nm after 6 min of reaction, and potassium phosphate buffer was used as blank. Trolox was employed as reference at concentrations ranging from 12.5 to 200 μmol L^{-1}. The results are expressed as micromoles of Trolox equivalents per gram of sample (mg TE g^{-1}).

Ferric reducing antioxidant power

The antioxidant capacity was determined using ferric reducing antioxidant power (FRAP) as described by Benzie and Strain,[17] with some modifications. FRAP reagent was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution, and 2.5 mL $FeCl_3 \cdot 6H_2O$ solution. Aliquots of 20 μL of the samples were mixed with 30 μL distilled water and 200 μL FRAP reagent and kept at 37 °C for 8 min. The readings were carried out at 595 nm in a microplate reader SpectraMax® M3 (Molecular Devices LLC, Sunnyvale, CA, USA). A calibration curve was built with ferrous sulfate at concentrations ranging from 100 to 700 μmol L^{-1}. The results are expressed as micromoles Fe^{2+} per gram of sample.

Scavenging of reactive oxygen species: peroxyl and superoxide radicals

Peroxyl radical ($ROO^{\cdot}$) scavenging capacity was measured according to Melo *et al.*[15] with changes. This assay monitored the antioxidant action of cocoa products extracts on the fluorescence decay by $ROO^{\cdot}$-induced oxidation of fluorescein, expressed as the oxygen radical absorbance capacity. Aliquots of 30 μL of the samples, 60 μL of 508.25 nmol L^{-1} fluorescein, and 110 μL of 76 mmol L^{-1} AAPH solution were mixed and transferred to microplates. The solutions were diluted with 75 mmol L^{-1} potassium phosphate buffer, pH 7.4, also used as blank. The reaction was performed at 37 °C, and the readings were made every minute for 2 h, at excitation and emission wavelengths of 485 nm and 528 nm respectively, using a microplate reader SpectraMax® M3 (Molecular Devices LLC, Sunnyvale, CA, USA). Trolox was used as standard at concentrations ranging from 12.5 to 400 μmol L^{-1}. The results are expressed as micromoles of Trolox equivalents per gram of sample (mg TE g^{-1}).

The capacity of cocoa products extracts to scavenge the superoxide radical ($O_2^{\cdot-}$), generated by the NADH–PMS system was measured according to Melo *et al.*,[15] with modifications. Different concentrations of cocoa products extracts were mixed with 166 μmol L^{-1} NADH, 107.5 μmol L^{-1} NBT, and 2.7 μmol L^{-1} PMS dissolved in 19 mmol L^{-1} potassium phosphate buffer, pH 7.4, to a final volume of 300 μL. The assay was conducted at 25 °C. After 5 min, the absorbance was read at 560 nm using a microplate reader SpectraMax® M3 (Molecular Devices LLC, Sunnyvale, CA, USA). A control was made by replacing the sample with buffer, and a blank corresponding to each sample dilution was prepared by replacing PMS and NADH with buffer. The results are expressed as EC_{50}, the mean quantity of the sample required to quench 50% of the superoxide radical.

Statistical analysis

Analysis of variance and Tukey's test were employed to determine the differences of the means between cocoa products ($P < 0.05$) using the Statistical Analysis System 2002 (SAS) software (SAS Institute Inc., Cary, NC, USA). The results are expressed as means plus/minus standard deviation.

RESULTS AND DISCUSSION

Phenolic content

Cocoa beans contain 6–8% phenolic compounds (dry weight), and the main ones (60%) are (+)-catechin, (−)-epicatechin, and procyanidins.[18] The steps in the industrial transformation of cocoa into chocolate may also influence the content of phenolic compounds in the final products. During cocoa processing for chocolate production, flavanols undergo a series of chemical reactions such as oxidation, complexation, and leaching. These, in association with other ongoing reactions such as protein hydrolysis into amino acids and sugar hydrolysis, lead to the formation of aromatic compounds, substances that significantly contribute to the formation of desirable flavor and reduction of bitterness and astringency.[19] In this study, changes in phenolic compound content were investigated during the industrial chain of chocolate production (Fig. 3).

Raw cocoa beans exhibited the highest content of phenolic compounds (64.1 ± 5.2 mg GAE g^{-1}), whereas cocoa bean shells displayed the lowest content (5.9 ± 0.1 mg GAE g^{-1}). However, this result was higher than that found in cocoa bean shells from Ecuador (1.54 mg GAE g^{-1}).[20]

A great influence of processing on cocoa content of total phenolic compounds was observed, mainly as a result of fermentation and roasting. The content of phenolic compounds in fermented cocoa beans was 35.7 ± 0.3 mg GAE g^{-1}, statistically different from that found for roasted cocoa beans (10.4 ± 0.9 mg GAE g^{-1}). These results show significant losses of phenolic compounds, of approximately 44% due to fermentation and 71% due to roasting

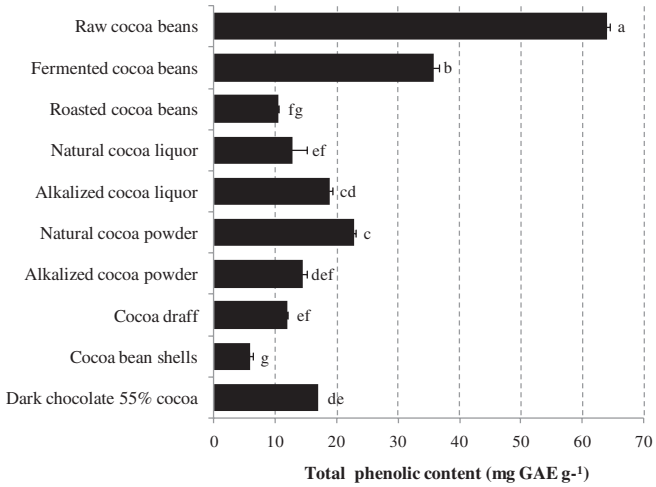

Figure 3. Content of total phenolic compounds in the samples of cocoa products analyzed in this study.

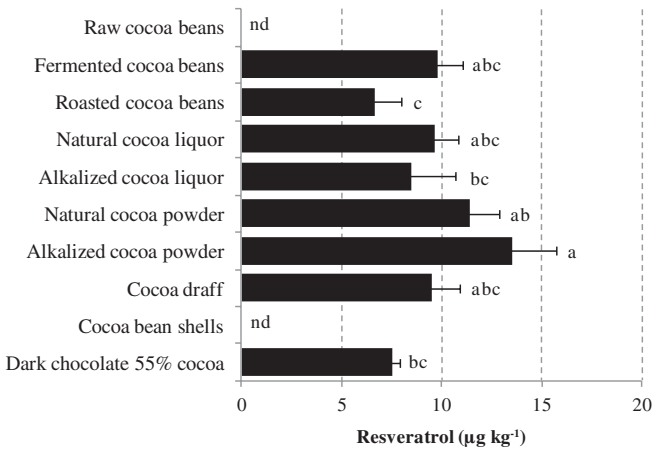

Figure 4. Content of *trans*-resveratrol determined by HPLC in the samples of cocoa products analyzed in this study. nd, not detected.

(140–150 °C for 20 min). Brito *et al.*[21] reported that fermentation of cocoa beans reduced the content of phenolic compounds by 31% after 7 days under this process, at temperatures ranging from 22 °C (initial) to 34 °C (final), reaching a maximum of 41 °C on the fifth day.

Although alkalization caused little change in content of total phenolic compounds compared with fermentation and roasting (Fig. 3), in cocoa powder it caused a decrease by 24% compared with alkalized cocoa liquor. Jolić *et al.*[22] and Gültekin-Özgüven *et al.*[9] also observed losses of phenolic compounds (over 60%) after alkalization of nibs and cocoa power.

In fact, not only can alkalization decrease the content of total phenolic compounds, but it also may change their composition. Under alkaline conditions, polyphenols can undergo oxidative degradation, as well as monomerization and epimerization reactions. This leads to a decrease in the content and changes in the structure of phenolic compounds in the food product, modifying its phenolic composition. In cocoa, these changes may decrease the bioactive properties and bioavailability of phenolic compounds. This is particularly important in the epimerization of epicatechin to catechin during alkalization of cocoa. Epicatechin (*cis*-isomer) is more unstable in a heated alkaline medium and undergoes epimerization to the *trans*-isomer catechin, which is more stable, although less bioactive.[23]

Although cocoa industrial processing steps (mainly fermentation and roasting) promote loss of phenolic compounds, they are essential to obtaining good quality cocoa beans, due to the formation of flavor precursors in the final product through several chemical reactions.[24] In contrast, phenolic compounds have also been studied for decades because of their potentially negative influence on flavor, conferring bitterness and astringency to products with high contents of these substances, sensory attributes that are often unwanted.[18,25] Alkalization contributes to lessen the bitterness and astringency of cocoa products, and to improve their solubility and color.[23] Nevertheless, similar to fermentation and roasting, this process causes losses of phenolic compounds.

However, studies of the beneficial effects of phenolics on human health have aroused great interest in preserving them during the manufacturing process of cocoa products in order not to cause losses of flavor[26,27] or of any technological attributes of interest to the food industry.

trans-Resveratrol content

Despite the fact that raw cocoa beans are an important source of phenolic compounds (Fig. 3), *trans*-resveratrol was not detected in this product (Fig. 4). This is explained by the fact that *trans*-resveratrol is a stilbene aglycone, whereas in raw cocoa beans resveratrol is present in its glycosylated form (*trans*-piceid). In fact, after cocoa bean fermentation, certain enzymes, such as β-glucosidase, catalyze *trans*-piceid hydrolysis. Therefore, in all the other products obtained after this step, except cocoa bean shells, the stilbene aglycone form (*trans*-resveratrol) was detected (Fig. 4), which also has the highest degree of intestinal absorption.[28] Thus, to the best of our knowledge, this is the first time that *trans*-resveratrol behavior during cocoa industrial processing has been reported.

Different from our findings regarding the content of total phenolic compounds, high temperature did not significantly affect the content of *trans*-resveratrol during cocoa beans processing. The content of *trans*-resveratrol in fermented beans ($9.8 \pm 1.2\ \mu g\ kg^{-1}$) did not statistically differ from that in roasted beans ($6.63 \pm 1.36\ \mu g\ kg^{-1}$). In contrast, the production processes of alkalized cocoa powder and natural cocoa powder increased the content of *trans*-resveratrol compared with roasted beans ($13.53 \pm 2.14\ \mu g\ kg^{-1}$ and $11.4 \pm 1.49\ \mu g\ kg^{-1}$ respectively). This was due to the fact that, in this step, most of the cocoa butter was removed, resulting in a product with high content of nonfat cocoa solids. In summary, during the industrial processing of cocoa, the products with the highest contents of *trans*-resveratrol were alkalized cocoa powder and natural cocoa powder.

The content of *trans*-resveratrol in dark chocolate 55% cocoa was $7.53 \pm 0.35\ \mu g\ kg^{-1}$, which is not statistically different from that found in fermented cocoa beans ($9.8 \pm 1.2\ \mu g\ kg^{-1}$). Thus, the content of *trans*-resveratrol present in the chocolate studied did not vary according to the intensity of the initial heat treatment applied to the raw material. Despite the different percentages of ingredients such as alkalized cocoa powder, cocoa butter, sugar, and milk used in chocolate production,[29] the content of *trans*-resveratrol in the final product was preserved. It is known that, when in solution, the degradation rate of *trans*-resveratrol increases with the combination of high pH and high temperature.[30] However, the chemical stability of this stilbene observed in this study was maintained by the action of other phenolic compounds present in cocoa, possibly due to a mechanism of hydrogen transference and/or reducing power.

Table 1. Antioxidant activity of cocoa products measured using the synthetic 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free-radical scavenging, ferric-reducing ability of plasma (FRAP), and reactive oxygen species scavenging [peroxyl radical (ROO•)]

Sample	DPPH• (μmol TE g^{-1})	ABTS•+ (μmol TE g^{-1})	ROO• (μmol TE g^{-1})	FRAP (μmol Fe^{2+} g^{-1})
Raw cocoa beans	218.86 ± 27.53^{a}	1034.61 ± 13.12^{a}	1160.00 ± 2.43^{a}	581.55 ± 14.59^{a}
Fermented cocoa beans	134.58 ± 8.17^{b}	547.06 ± 8.59^{b}	849.66 ± 22.89^{b}	337.80 ± 4.72^{b}
Roasted cocoa beans	43.17 ± 5.70^{ef}	127.47 ± 12.44^{f}	399.98 ± 25.85^{e}	78.54 ± 5.79^{g}
Natural cocoa liquor	54.17 ± 7.66^{def}	155.57 ± 3.41^{f}	383.88 ± 4.24^{ef}	105.39 ± 0.77^{fg}
Alkalized cocoa liquor	84.57 ± 1.62^{cd}	374.32 ± 26.51^{c}	515.78 ± 8.13^{d}	156.40 ± 14.73^{e}
Natural cocoa powder	112.20 ± 10.97^{bc}	498.29 ± 27.65^{b}	708.95 ± 11.53^{c}	279.57 ± 17.69^{c}
Alkalized cocoa powder	76.07 ± 4.15^{d}	210.71 ± 33.28^{e}	494.83 ± 7.44^{d}	140.81 ± 11.71^{ef}
Cocoa draff	57.59 ± 0.16^{de}	135.52 ± 2.84^{f}	353.57 ± 6.02^{f}	103.71 ± 3.00^{g}
Cocoa bean shells	12.41 ± 0.20^{f}	52.89 ± 3.77^{g}	175.81 ± 1.39^{g}	26.49 ± 1.33^{h}
Dark chocolate 55% cocoa	71.40 ± 10.39^{de}	319.02 ± 34.36^{d}	399.58 ± 21.05^{e}	240.65 ± 26.05^{d}

Means followed by the same letter in the columns are not significantly different from each other by the Tukey's test ($P < 0.05$).
Values are the means of triplicates ± standard deviation.

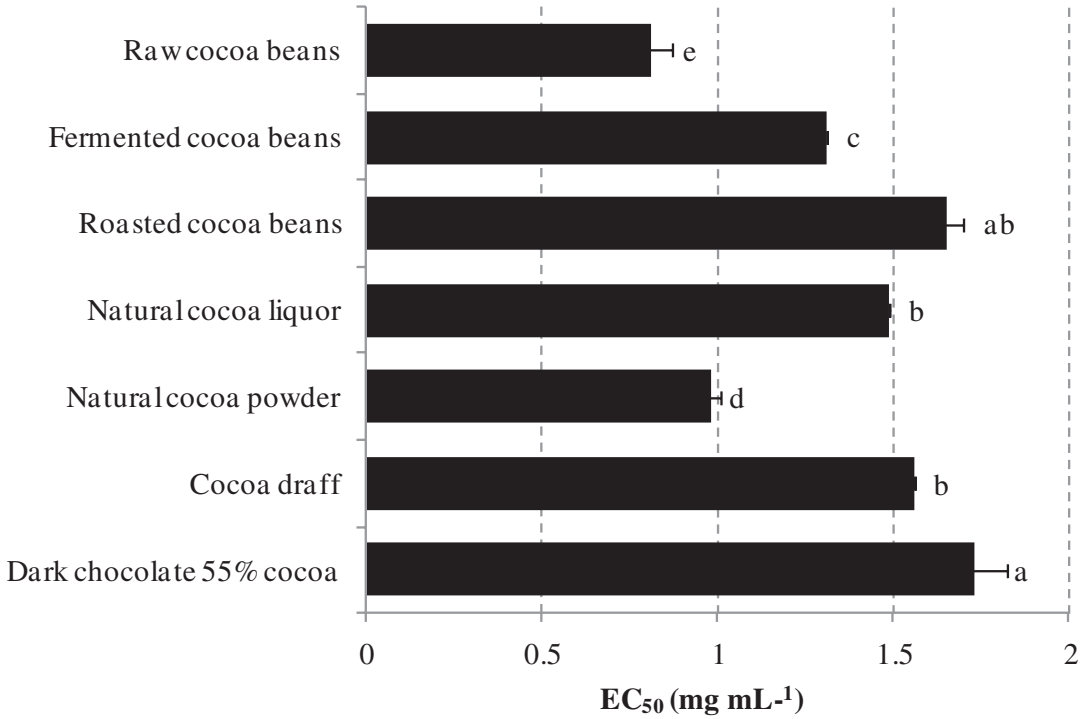

Figure 5. Antioxidant activity in the samples of cocoa products analyzed in this study, measured using reactive oxygen species scavenging [superoxide radical ($O_2^{\bullet-}$)].

Scavenging of synthetic free radicals, FRAP, and reactive oxygen species

The phenolic compounds found in cocoa have been considered major chemopreventive agents, principally because of their strong antioxidant activity.[22] In this study, the antioxidant activity of the different products obtained during the industrial chain of chocolate production was assessed using five different methods: scavenging of free radicals DPPH and ABTS, scavenging of reactive oxygen species (ROO•) and FRAP (Table 1), and scavenging of reactive oxygen species ($O_2^{\bullet-}$) (Fig. 5).

For the same sample, the results differed in each of the different methods employed. The highest antioxidant activities using DPPH, ABTS, ROO•, and FRAP were registered for raw cocoa beans followed by fermented cocoa beans (Table 1). During fermentation, the antioxidant activity decreased by 38.5%, 47.1%, 26.7%, and 42% measured by DPPH, ABTS, ROO•, and FRAP respectively. The roasting phase caused a significant decrease in antioxidant activity, from 134.58 μmol TE g^{-1}, 547.06 μmol TE g^{-1}, and 849.66 μmol TE g^{-1} to 43.17, 127.47, and 399.98 μmol TE g^{-1} respectively, as measured by DPPH, ABTS, and ROO• respectively.

Cocoa bean shells displayed the lowest antioxidant activity in all methods used (Table 1), and it was also the sample with the lowest content of phenolic compounds (Fig. 3). Nonetheless, the study of the content of bioactive compounds in this by-product is highly relevant, as a way to find options to minimize the environmental impact caused by its inappropriate disposal, as well as to favor a strategy for its industrial reuse.

In the final product, dark chocolate 55% cocoa, the antioxidant activity decreased by 67.38%, 69.17%, and 65.56% as measured

by DPPH, ABTS, and ROO$^{\cdot}$ respectively compared with raw cocoa beans.

Raw cocoa beans displayed the highest antioxidant activity regarding deactivation of $O_2^{\cdot -}$ (0.81 ± 0.04 mg mL^{-1}) (Fig. 5). Using this method, no antioxidant activity was detected in the products that underwent alkalization (alkalized cocoa liquor and alkalized cocoa powder) or in cocoa bean shells. Since the process of alkalization decreases the content of phenolic compounds, the ones responsible for superoxide radical scavenging may have been chemically modified, as already observed by Jolić *et al.*[22]

A strong positive correlation was found between phenolic compounds and the results of antioxidant activity, as follows: phenolic compounds × DPPH – 0.98; phenolic compounds × ABTS – 0.97; phenolic compounds × FRAP – 0.98; phenolic compounds × ROO$^{\cdot}$ – 0.94. These results demonstrate that phenolic compounds are directly related to the antioxidant activity in cocoa and its derivative products. The high correlations suggest that the antioxidant compounds that contribute the most to the antioxidant capacity in cocoa and its products have a phenolic structure.

Knowledge about the factors that influence the loss of phenolic compounds in the industrial processing chain of cocoa may contribute to the optimization of the production process of other types of food containing cocoa and its derivatives aiming to preserve these bioactive compounds.

CONCLUSION

The loss of phenolic compounds and antioxidant activity during the processing chain of cocoa varied depending on the process, and was mainly influenced by fermentation and roasting. The content of the stilbene *trans*-resveratrol, produced by hydrolysis during cocoa fermentation, did not significantly decrease with the increase in temperature or the alkalinization during chocolate manufacturing process. Therefore, *trans*-resveratrol content is maintained during the industrial production of chocolate, but this does not happen to the other phenolic compounds and the antioxidant activity. The strict control of each step of cocoa processing is highly relevant to obtain a final product with the desirable sensory attributes and reduced losses of antioxidant compounds with important biological properties, thus adding value to it.

ACKNOWLEDGEMENT

This work was supported by the São Paulo Research Foundation (FAPESP, grant number 2011/50498-0).

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