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Stability of total phenolic and antioxidant capacity in ready-to-drink black and green tea formulations

Estabilidade de fenólicos totais e da capacidade antioxidante em formulações de chá preto e verde pronto para beber

Estabilidad de los fenólicos totales y la capacidad antioxidante en formulaciones de té negro y verde listas para beber

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Abstract

Teas are rich in antioxidant compounds and many ready-to-drink teas are being developed with great acceptance in the market. However, the bioactive potential for these products should be preserved during the storage. The aim of this work was to evaluate changes in total phenolic content (TPC) and antioxidant capacity (AC) from six formulations of green and black ready-to-drink teas, stored in the dark or exposed to light during eight weeks at room temperature. The stability was evaluated every two weeks by measuring TPC by Folin-Ciocalteu and AC by ABTS and FRAP assays. In the absence of light TPC of green tea remained stable throughout the storage, but it dropped after two weeks when exposed to light. TPC for black tea decreased after four and two weeks, in the absence and presence of light, respectively. The AC for green tea decreased after two weeks, in the presence of light, however the decay was greater for the FRAP assay. In the absence of light, changes in AC for black tea were observed only for samples assayed by FRAP after two weeks. A reduction in the AC (FRAP and ABTS) occurred after four weeks for both samples exposed to light. The results show that to retain the majority of their TPC and AC these tea drinks should be commercialized in opaque packages and stored for up to eight weeks.

Keywords: Polyphenols; Antioxidant capacity; Storage; Ready-to-drink tea.

Resumo

Os chás são ricos em compostos antioxidantes e muitos chás prontos para beber estão sendo desenvolvidos com grande aceitação no mercado. No entanto, o potencial bioativo desses produtos deve ser preservado durante o armazenamento. O objetivo deste trabalho foi avaliar mudanças no conteúdo fenólico total (CFT) e na capacidade antioxidante (CA) de seis formulações de chás verde e preto prontos para beber, armazenados no escuro ou expostos à luz durante oito semanas em temperatura ambiente. A estabilidade foi avaliada a cada duas semanas medindo o CFT por Folin-Ciocalteu e a CA pelos ensaios ABTS e FRAP. Na ausência de luz, o CFT do chá verde permaneceu estável durante todo o armazenamento, mas caiu após duas semanas quando as formulações foram expostas à luz. O CFT para o chá preto diminuiu após quatro e duas semanas, na ausência e na presença de luz, respectivamente. A CA para o chá verde diminuiu após duas semanas, na presença de luz, porém a queda foi maior para o ensaio FRAP. Na ausência de luz, as alterações na CA para o chá verde foram observadas apenas para as amostras analisadas por FRAP após 2 semanas. Uma redução na CA (FRAP e ABTS) ocorreu após quatro semanas para ambas as amostras expostas à luz. Os resultados mostram que, para reter a maioria de seus CFT e CA, essas bebidas de chá devem ser comercializadas em embalagens âmbar e armazenadas por até oito semanas.

Palavras-chave: Polifenóis; Capacidade antioxidante; Armazenamento; Chá pronto para beber.

Resumen

Los tés son ricos en compuestos antioxidantes y muchos tés listos para beber se están desarrollando con gran aceptación en el mercado. Sin embargo, el potencial bioactivo de estos productos debe preservarse durante el almacenamiento. El objetivo de este trabajo fue evaluar los cambios en el contenido fenólico total (CFT) y la capacidad antioxidante (CA) de seis formulaciones de tés verdes y negros listos para beber, almacenados en la oscuridad o expuestos a la luz durante ocho semanas a temperatura ambiente. La estabilidad se evaluó cada dos semanas midiendo CFT por Folin-Ciocalteu y AC por ensayos ABTS y FRAP. En ausencia de luz, el CFT de té verde se mantuvo estable durante todo el almacenamiento, pero disminuyó después de dos semanas cuando se expuso a la luz. La CFT para te negro disminuyó después de cuatro y dos semanas, en ausencia y presencia de luz, respectivamente. La CA para té verde disminuyó después de dos semanas, en presencia de luz, sin embargo, la descomposición fue mayor para el ensayo FRAP. En ausencia de luz, se observaron cambios en CA para te negro solo para muestras analizadas por FRAP después de dos semanas. Se produjo una reducción de la CA (FRAP y ABTS) después de cuatro semanas para ambas muestras expuestas a la luz. Los resultados muestran que, para retener la mayor parte de su CFT y CA, estas bebidas de té deben comercializarse en paquetes opacos y almacenarse hasta por ocho semanas.

Palabras clave: Polifenoles; Capacidad antioxidante; Almacenamiento; Té listo para beber.

1. Introduction

Tea is the most consumed flavored beverage in the world, and those produced from the *Camellia sinensis* plant extracts can be classified according to the manufacturing process into nonfermented green tea, semi fermented oolong tea and fermented black tea (Xing et al., 2019). Green and black tea account for about 20% and 78% of worldwide tea consumption, respectively (Li et al., 2013).

Regular consumption of this beverage is associated with reduction of risk of cardiovascular and inflammatory diseases, some sorts of cancer, diabetes and even lead to weight loss (Carloni et al., 2013; Cavalcante et al., 2020; Pinto, 2013). These properties are linked to the presence of antioxidant compounds such as polyphenols, especially flavonoids, and in the case of green teas, catechins in greater quantity (Bazinet et al., 2010; Coppock & Dziwenka, 2016; Li et al., 2013; Xing et al., 2019).

Consumers are increasingly looking for healthy foods like those rich in bioactive compounds that bring benefits, in addition to more convenient products. Ready-to-drink beverages are used both for hydration and nutrient intake, and those obtained from tea extracts fulfill these requirements to respond to the increasing market for functional foods. However, these beverages must preserve their bioactive compounds during the shelf-life period.

Although there are several studies of quantitative measure of bioactive compounds and antioxidant capacity in black and green teas (Nakamura et al., 2013; Paula et al., 2015; Yang & Liu, 2013) very few investigations have been listed in the literature about the stability of these compounds throughout the storage.

Friedman et al. (2009) studied catechins degradation in bags of green tea in a period of six months and Bazinet et al. (2010) did not observed significant degradation of catechins in green tea drink at 4 °C, for six months. Kim et al. (2011) reported that green tea infusions maintain their catechins levels stable up to 6 weeks, when stored in the absence of light at the temperature of 3 °C.

For black tea, Li et al. (2013) reported great stability for catechins in solid tea leaves and tea bags concluding that they could be stored for years and according to Din et al. (2014) retention of bioactive compounds was achieved at 5 °C storage conditions for tea drink, during 90 days. Chang et al. (2020) concluded that the antioxidant activity and total phenolic content of black tea infusions, presented negligible variation during 15 days at 4, 9 and 25 °C storage temperatures.

Commercial tea-based drinks are mostly sold in polyethylene terephthalate bottles (exposure to light) and aluminum cans (absence of light) at room temperature. However, compounds responsible for tea health benefit must be retained during the shelf-life. Thus, the main objective of this study was to simulate marketing conditions by monitoring changes in total phenolics content as well as in the antioxidant capacity associated to them, during the storage of ready for consumption tea beverages prepared with black and green tea extracts.

2. Methodology

Chemicals

The reagents ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid), TPTZ (2,4,6 -Tris (acid) were obtained from Sigma-Aldrich®. Folin-Ciocateu reagent was from Dinâmica®. All the other reagents were of analytical grade.

Raw materials

Tea bags of finely minced leaves of black and green tea, commercial sugar, fresh lemons and concentrated peach juice were purchased from several local markets in Minas

Gerais (MG, Brazil).

Production of the teas

Tea bags from the same batch were opened, homogenized, and a total weight of 21.6 g was mixed with 3000 mL of boiling water, followed by slight agitation for 10 min. Then the mixture was transferred through a paper filter number 02 and cooled to room temperature.

Different formulations were prepared from a same brew, altering the type of tea (green or black), with or without the addition of fruit juice (lemon or peach). All formulations were added with sucrose syrup to reach a final of total soluble solid of 7º Brix and pH was adjusted to 3.6, using citric acid 0.1 $mol.L^{-1}$. The final concentration of the tea extract in the beverages was 0.57% (w/v). At the end, six different formulations were obtained being two for control, two for lemon and two for peach flavor, each one contained green or black tea extract.

After formulation, sodium benzoate and potassium sorbate at 0.05% (w/v) were added to the tea drinks followed by pasteurization at 85 °C for 60 s. Finally, the beverages were hot filled into sterilized bottles.

Stability study

One half of each preparation, packed in amber glass bottles, was stored in the dark; while the other part bottled in transparent glass flasks was exposed to LED light, both at room temperature for 8 weeks. At day zero and every 2 weeks, three bottles of each formulation stored at different conditions were assayed in triplicate for total phenolic content and antioxidant capacity by FRAP and ABTS.

Total phenolic content

Total phenol content (TPC) was determined according to Singleton and Rossi (1965). Samples of the beverages appropriately diluted were mixed with 2.5 mL of Folin-Ciocalteu reagent (10% v/v) and 2 mL of sodium carbonate solution (2% w/v). The mixture was stirred and kept at room temperature for 1 h in the dark. The absorbance was measured at 750 nm using a digital UV-vis spectrophotometer (Global Analyzer Model GTA 97, São Paulo, Brazil). Results were expressed as mg of gallic acid equivalent ($GAE.mL^{-1}$) of drink tea, using a six point calibration curve (43, 85, 128, 170, 213 and 255 $mg.mL^{-1}$).

Measurement of antioxidant capacity (AC)

Analysis of cation free radical scavenging capacity ($ABTS^{\bullet+}$) was determined according to the methodology described by Re et al. (1999). First, 7 µmol.L^{-1} of ABTS was reacted with 2.45 µmol.L^{-1} of potassium persulfate for 16 h, in the dark, at room temperature. The reduction of absorbance at 754 nm (initial absorbance = 0.700±0.020) in the presence of the samples was measured after 7 min. For construction of the calibration curve, Trolox (13, 25, 75, 125, 175 and 225 mg.mL^{-1}) was used as standard antioxidant and the Trolox Equivalent Antioxidant Capacity (TEAC) expressed as mg Trolox.mL^{-1} of beverage.

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out by the method of Benzie and Strain (1996) with minor modifications. The FRAP reagent was generated by the reaction of 2.1 mL of TPTZ 10 mmol.L^{-1} with 2.1 mL of 20 mmol.L^{-1} ferric chloride in the presence of 25 mL of 0.3 mol.L^{-1} acetate buffer. The FRAP reagent (3.6 mL) was added to the properly diluted beverage samples (0.4 mL) and incubated at 37 °C for 30 min. The absorbance of the reaction mixture was measured at 595 nm, using the FRAP reagent added of distilled water as a blank. Trolox (25, 50, 100, 150, 200 and 250 mg.mL^{-1}) was used for the calibration and the TEAC expressed as mg trolox.mL^{-1} of beverage.

Statistical analysis

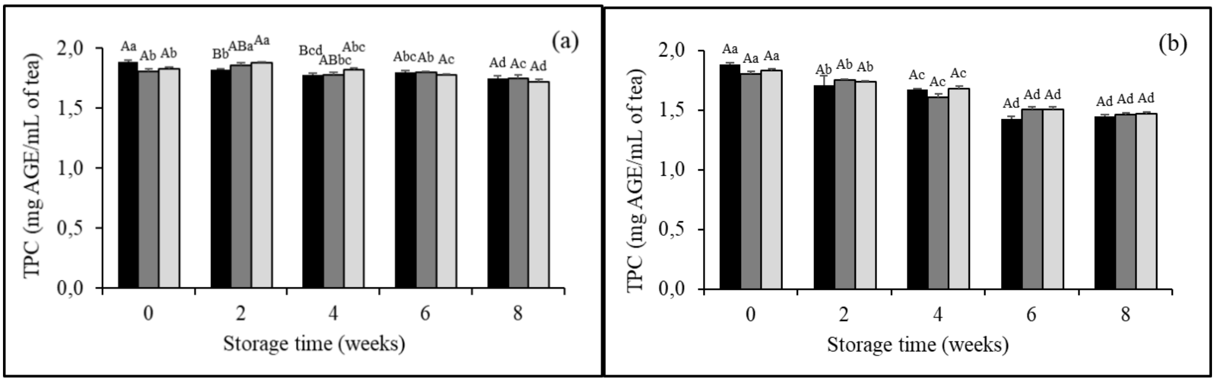
All the results were presented as the average ± standard deviation. The data obtained from the different formulations were submitted to analysis of variance (ANOVA) and the means compared by the Tukey test, with a probability of 5%. All the data analyses were performed using the R software.

3. Results and Discussion

Phenolics for green and black drinks

The results obtained for total phenolic content (TPC) of drinks made from green tea extract, in the absence (a) and exposure (b) to light, are shown in Figure 1. The total phenolic values at day zero were 1.89, 1.81 e 1.83 mg GAE.mL^{-1}, for natural, lemon and peach, respectively. No significant differences ($p>0.05$) were observed for TPC among the three formulations, showing that the presence of juice did not significantly affect the phenolic concentration. Yang and Liu (2013) and Oh et al. (2013) reported values for total phenolics in green tea infusion of 1.16 and 0.82 mg $GAE.mL^{-1}$ respectively, which are lower than our results for green tea formulations. These differences may be due to the lower infusion time and extraction temperature employed by these authors, besides the geographic region. Indeed, it is important to consider that another factors can affect the content of phenolic compounds found in infusions, such as the ratio of mass to solvent, stage of development and part of the plant used and the characteristics of soil, climate, stresses (Yang & Liu, 2013; Zhao et al., 2019).

Figure 1. Effect of storage time in total phenolics content of green tea drinks, in the absence (a) and exposure (b) to light. The flavors are represented by the following colors: ■ Natural; ▪ Lemon and □ Peach. Different capital and lower-case letters represent significant difference by Tukey test (p<0.05), among tea flavors and throughout storage period, respectively.



Source: Research Data (2020).

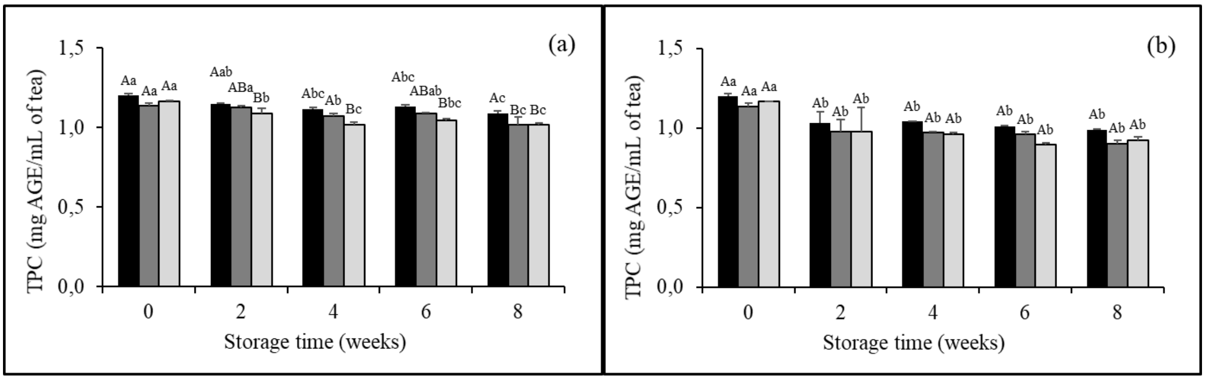
Regarding stability, in the absence of light there was observed a significant difference ($p < 0.05$) for phenolic content of drinks only at 8 weeks of storage that corresponded to less than 6% of the initial values, while the formulations exposed to light registered a drop of 21% for phenolic contents at 6 and 8 weeks of storage (Figure 1b).

Black tea beverages exhibited TPC of 1.30, 1.14 and 1.17 mg $GAE.mL^{-1}$ for natural, lemon and peach, respectively (Figure 2). In accordance with these values, Rechner et al. (2002) reported an average of 1.14 mg $GAE.mL^{-1}$ for seven brands of black tea infusions. Otherwise, smaller values of 0.94 and 0.85 mg $GAE.mL^{-1}$ were found in the literature for black tea infusion (Oh et al., 2013; Yang & Liu, 2013). This can be explained by the variation in the raw materials (conditions of cultivation and harvest) and by the processing conditions, thus influencing the characteristics of the final product offered to the consumer.

All the reported phenolic content values were lower for black teas infusions compared to the green tea. During the commercial production, leaves of *Camellia sinensis* undergo different degrees of processing, giving rise to various types of tea with different phenolic composition. For green tea predominate catechins specially epigallocatechin gallate (Bazinet et al., 2010) and polymerized catechins such as theaflavins (3-6%) and thearubigins (10-30%) dictate in black tea (Li et al., 2013; Yang & Liu, 2013). Yao et al. (2006) also reported higher values for TPC in green tea bags than those encountered in black tea bags and suggest the possibility that some of the oxidation products, although still phenolic compounds, might not be detected by the methods commonly used. That way, over-fermentation during the manufacturing process of black tea may reduce the phenolic concentration.

Figure 2 also presents the results of stability study for black teas formulations storage in the absence (a) and presence of light (b). A significant decrease ($p < 0.05$) in total phenolic contents for all formulations was observed after 4 and 2 weeks for the drinks maintained in the absence and presence of light, respectively, leading to a drop of 11 and 20% after 8 weeks of storage. Although the percentages of the decreases were the same compared to the TPC for green tea formulations, they occurred in a later period of the time, especially for the beverages exposed to light (Figure 2a). These observations allow us to conclude that even in the absence of light, it was not possible to maintain the whole initial phenolic content for black tea formulations, at room temperature.

Figure 2. Effect of storage time in total phenolics content of black tea drinks, in the absence (a) and exposure (b) to light. The flavors are represented by the following colors: ■ Natural; ▪ Lemon and □ Peach. Different capital and lower-case letters represent significant difference by Tukey test (p<0.05), among tea flavors and throughout storage period, respectively.



Source: Research Data (2020).

Despite of this decrease, at the end of the storage, TPC values for black tea drinks in both presence and absence of light were around 1.0 mg $GAE.mL^{-1}$, and the remaining phenolic concentrations in green tea beverages were 1.73 and 1.46 mg $GAE.mL^{-1}$ in the presence and absence of light, respectively.

Those values suggest that green and black tea drinks may contribute for a healthy diet since the concentration of total phenolics obtained here are in the same range of results from the literature for commercial Brazilian grape fruits juices (0.3-3.4 $mg.mL^{-1}$, with mean values among 1.4 and 1.9 $mg.mL^{-1}$) (Burin et al., 2010; Sautter et al., 2005), whole grape juices, 1.34 mg $GAE.mL^{-1}$ (Lima et al., 2014) and also pomegranate juices, with average values of 1.8 mg $GAE.mL^{-1}$ (Akhavan et al., 2015), all beverages recognized as a good source of phenolics.

Therefore, both green and black tea beverages even at the end of 8 weeks storage still preserve a substantial phenolic content, especially if consumed in packs in the absence of light and in a portion of 300 mL per day, which provides half of recommended daily consumption of green (Dekant et al., 2017) and black (Rechner et al., 2002) tea phenolics.

There are very few studies about bioactive compounds stability of tea drinks. Bazinet et al. (2010) studied the stability of long-term storage on catechin contents in green tea drinks in HDPE plastic bottle. It was observed a decrease in some catechins (ECG and GCG) after 15 to 30 days of storage at 25 °C but no significant degradation occurred at 4 °C during 6 months of storage. In another study, Labbé et al. (2008) reported that catechins content in two green tea drinks exhibited great stability within 8 weeks of storage at 4 °C. Storage stability of green tea infusions was evaluated by Kim et al. (2011), depending on packaging material used (glass, PET, and retortable pouch). Tea drinks were stored in the absence of light at 3 °C and catechins were found to be stable up to 6 weeks. Chang et al. (2020) studied a short-term storage for black tea infusions and concluded that total phenolic content did not significantly change within 15 days for storage and temperatures of 4, 9 and 25 °C. Their findings corroborate with the results obtained in this study for the same period of storage (Figure 2a).

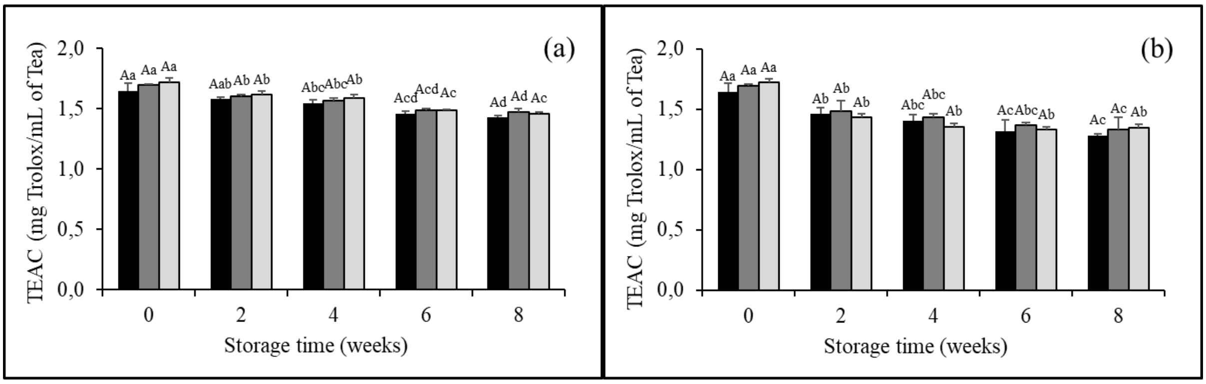
Din et al. (2014) investigated the effect of storage on phenolic content in carbonated black tea drinks with different concentrations of tea extract bottled in a plastic PET and stored at 5 °C. It was observed a TPC decrease over 90 days of storage, for all treatments. Those authors reported values of 1.30 mg $GAE.mL^{-1}$ at the first day of storage and 1.06 mg $GAE.mL^{-1}$ after 60 days, for beverages containing 0.6% of extract. These TPC values can be strictly compared to the black tea formulations developed in this study with 0.57% of tea extract and lead us to believe that if refrigeration conditions were adopted, the TPC values would remain stable at the end of the storage.

Antioxidant capacity ABTS for green and black drinks

Figure 3 presents the results of antiradical capacity for the green tea drinks maintained in the absence (a) and exposure (b) to light.

At the beginning of the storage, the TEAC values were 1.65, 1.70 and 1.72 mg Trolox.mL^{-1} for natural, lemon and peach formulations, respectively. These values significantly decreased at 2 weeks for both conditions. The average reductions were 14 and 22% over 8 weeks of storage for the beverages, and the correspondent final values for antioxidant capacity were 1.45 and 1.34 mg Trolox.mL^{-1} under absence and exposition to the light, respectively. The intake of one serving of plant infusion could release the equivalent of up to 375.4 mg of Trolox, being this amount a good source of antioxidants for the human diet (Jiménez-Zamora et al., 2016). Therefore, those final values for the green tea beverages still retain their potential biological capacity.

Figure 3. Effect of storage time on the antioxidant capacity of green tea drinks in the absence (a) and exposure (b) to light measured by the ABTS method. The flavors are represented by the following colors: ■ Natural; ■ Lemon and □ Peach. Different capital and lower-case letters represent significant difference by Tukey test (p<0.05), among tea flavors and throughout storage period, respectively.



Source: Research Data (2020).

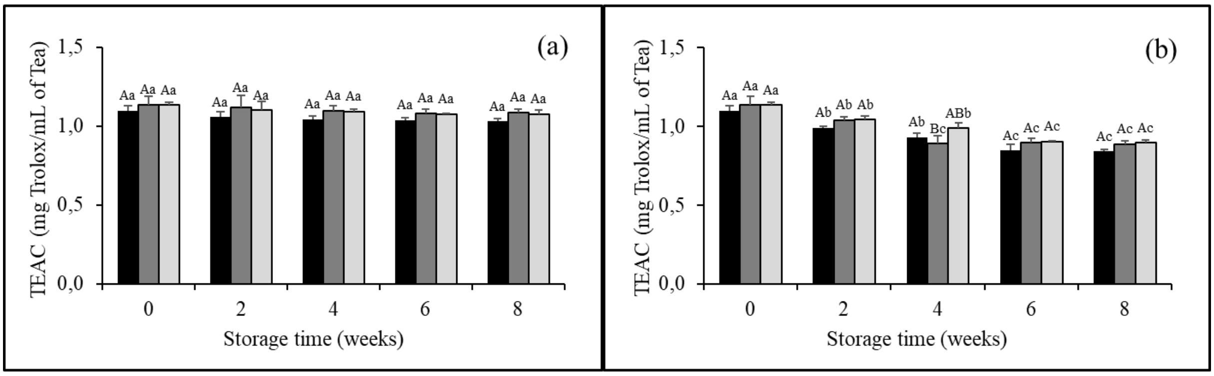
Interesting to note that, at the end of the storage, the decreases in the percentages for phenolic contents were around 6% in the absence of light and for the formulations exposed to light a drop of 21% were observed (Figure 1). The percentage reductions are close to those observed for the antioxidant capacity (14 and 22%) measured by the ABTS method, which suggests that the reductions in TPC were accompanied by the same proportions of loss in the capture of the free radical ABTS displayed by ready-to-drink teas.

No different behavior was observed on AC decay among formulations of teas added of fruit juices and the natural tea, contrary to the study of Nekvapil et al. (2012) where the addition of fruit juices as lemon and peach implied in a positive impact on the antioxidant capacity of beverages containing white, black and green tea extracts.

Data with changes in antioxidative capacity of teas during storage at room temperature are sparse. The antioxidant capacity of green tea aqueous infusions was investigated by Kim et al. (2011), during 12 weeks of storage in the dark at 3 °C. The AC decreased by 18, 14, and 30% in glass, PET and retortable pouch, respectively. The authors suggest that differences were caused by different oxygen and light permeability of packaging materials. Nekvapil et al. (2012) reported that cold storage at 4 °C of tea beverages in PET bottles ensures a slower decrease in antioxidant capacity in white, black, and green teas, compared to mild temperatures (22 °C).

For black teas of different flavors, TEAC values were 1.10 1.14 and 1.13 mg Trolox.mL^{-1} of drink (Figure 4) and these values are lower than green teas formulations. Both phenolics and AC for black tea were smaller compared to green tea. These observations agree with the studies of Lantano et al. (2015) and Hazra et al. (2017).

Figure 4. Effect of storage time on the antioxidant capacity of black tea drinks in the absence (a) and exposure (b) to light measured by the ABTS method. The flavors are represented by the following colors: ■ Natural; ■ Lemon and □ Peach. Different capital and lower-case letters represent significant difference by Tukey test (p<0.05), among tea flavors and throughout storage period, respectively.



Source: Research Data (2020).

The general trends of AC changes for black teas during the storage was somewhat the same for TPC (Figure 1). After 8 weeks in the dark the AC decreased only 5% meanwhile in the light it was observed respective reductions of 16 and 22% after 4 and 8 weeks of storage.

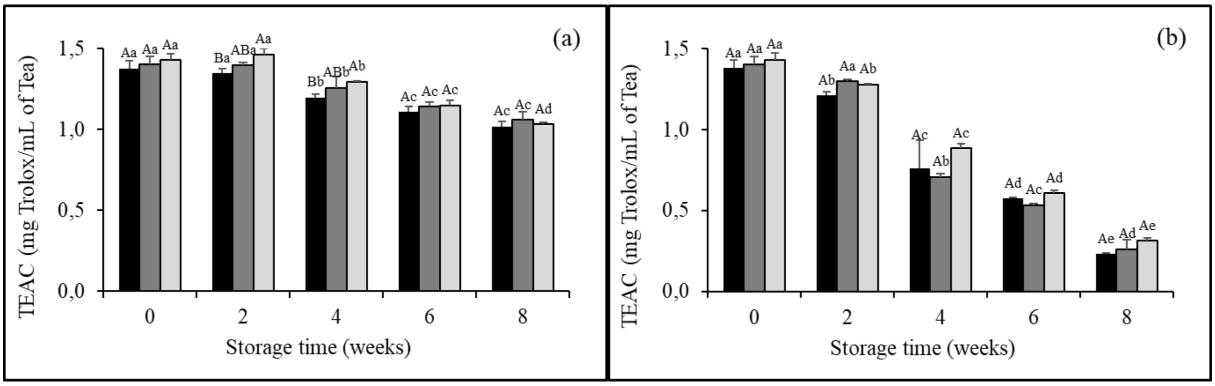
The same behavior was observed for the decreasing of TPC of black tea drinks when exposed to light. At the end of the storage, TEAC values were 0.87 mg Trolox.mL^{-1}, in the presence of light and 1.06 mg Trolox.mL^{-1} when protected from light.

Din et al. (2014) observed that DPPH free radical scavenger activity of black tea beverages decreased during the storage period of 90 days and was accompanied for a reduction in the percentages of theaflavins and thearubins. For black teas infusions, Chang et al. (2020) did not found perceptible difference in the scavenging activity (DPPH) among storage temperatures of 4, 9 and 25 °C. The authors attributed those results to the relatively short 15-day storage period.

Antioxidant capacity FRAP for green and black drinks

Figure 5 exhibits the results obtained for AC using the FRAP methodology. Initially the TEAC values for green tea formulations were 1.38, 1.40 and 1.43 mg Trolox.mL^{-1} of drink. As storage proceeded, in the absence of light the AC reduced 26% over 8 weeks. In the presence of light, a sharp decreased of 44% was observed in 4 weeks for AC and an 81% after 8 weeks of the storage period. The TEAC values dropped to 1.04 mg Trolox.mL^{-1} in the absence of light and to 0.27 mg Trolox.mL^{-1} under light exposition. Interesting to point out that the levels of phenolics for green teas exposed to the light did not decrease at the same proportion (reductions of only 21%) compared with AC decline at the end of storage period (Figure 1b).

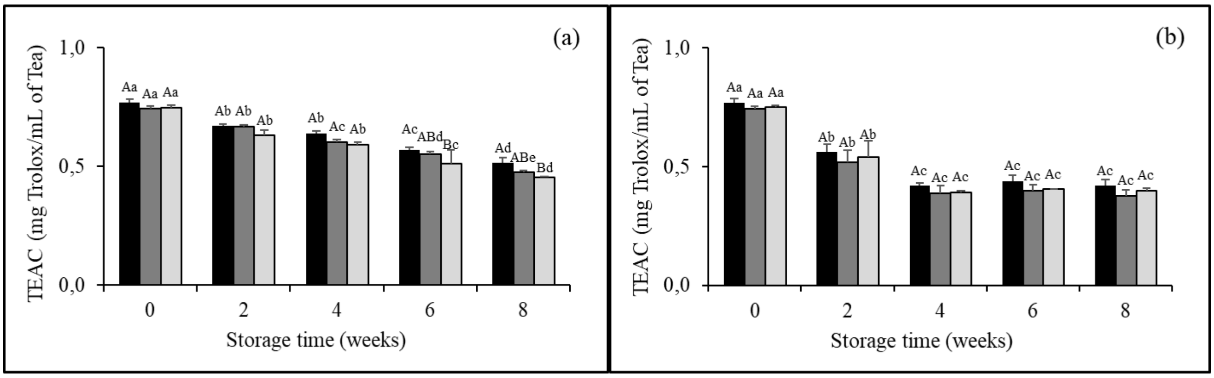
Figure 5. Effect of storage time on the antioxidant capacity of green teas in the absence (a) and exposure (b) to light measured by the FRAP method. The flavors are represented by the following colors: ■ Natural; ■ Lemon and □ Peach. Different capital and lower-case letters represent significant difference by Tukey test ($p<0.05$), among tea flavors and throughout storage period, respectively.



Source: Research Data (2020).

Samples containing black teas formulation exhibited values for AC of 0.77; 0.74 and 0.75 mg Trolox.mL^{-1} of drink (Figure 6), and these values are also lower than the encountered for green teas beverages, as noticed by Lantano et al. (2015). The storage of these samples revealed a respective decrease of 13 and 37% after 2 and 8 weeks in their antioxidant capacity for dark conditions. When exposed to light a decay of 29% in 2 weeks and 47% after 8 weeks was observed.

Figure 6. Effect of storage time on the antioxidant capacity of black teas in the absence (a) and exposure (b) to light measured by the FRAP method. The flavors are represented by the following colors: ■ Natural; ■ Lemon and □ Peach. Different capital and lower-case letters represent significant difference by Tukey test ($p<0.05$), among tea flavors and throughout storage period, respectively.



Source: Research Data (2020).

Nekvapil et al. (2012) employed a photochemiluminescence (PCL) method to capture superoxide radicals and found a most significant decrease in the AC for beverages containing green and black tea extract during the first 4 days of storage at 4 °C, in the absence of light.

To the best of our knowledge, this is the first research comparing formulations of green and black teas drinks in the absence and exposition to LED light. The highest decreases observed in antioxidant capacity for the green tea formulations exposed to light by using ABTS and FRAP methods, especially the last one, are relevant information for marketing of these beverages.

For green and black tea formulations, reductions on phenolic contents at the end of the storage time were proportionally accompanied by decreases on AC evaluated by ABTS both for dark and light conditions. When protected from light there was a tendency of maintenance of phenolics compounds and antioxidant capacity by ABTS, since the decays were smaller than 15%, at the end of 8 weeks of storage (Figure 3a). These results suggest that the phenolic compounds present in these tea formulations might be associated with the maintenance of the antiradical capacity to capture ABTS free radical during the storage period. However, in the presence of light, the remained 79% of total phenolic compounds, could not assure the ability of green tea drinks to maintain ferric reduction antioxidant power at the end of the storage period, since the phenolic content reduced only 21%, while the AC declined 81% relative to that of the initial time after 8 weeks of storage, using FRAP method (Figure 5b).

Other interesting finding concerns to the differences presented by the green and black teas in reducing their AC by the two methods through the storage. While a noticeable decline in AC (approximately 80%) was observed by the ferric reduction power for green tea, only 47% of reduction was noticed for black tea, in the presence of light.

Indeed, despite of the residual percentages of phenolic content were the same for black and green tea formulations at the end of the storage (around 20%), samples of the green tea lost 80% of their ability to donate an electron to reduce complexes of TPTZ-Fe^{+3} while only 22% of their capacity to neutralize free radicals generated from ABTS had decreased. Thus, the antioxidant compounds present in the green tea formulations exhibited different reducing capacity in the presence of light.

The analysis of antioxidant capacity applied to food matrices may exhibits different results depending on the method used. The FRAP assay is different from the others as there are no free radicals involved but the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) is monitored. Thus, results from this study evidenced the importance of evaluating AC using more than one method since the antioxidant compounds present in the tea formulations, mainly under incidence of light, showed a distinct ability to reduce the target molecules existing in the reaction medium for each assay.

4. Final Considerations

In general, the storage had no impact on phenolic concentrations for both green and black ready-to-drink teas formulations, maintained in the absence of light, and a retention of 94% and 89% of total phenolic was observed respectively for green and black teas during the storage of eight weeks at room temperature.

Decreases in phenolic compounds and antioxidant capacity occurred in the beverages exposed to the LED light. However, for green tea beverages the antioxidant capacity measured by FRAP were most impacted by light. While samples of green tea had a loss of 26% in the ability of capture ABTS radicals, their capacity to reduce TPTZ Fe^{+3} decrease

81% at the end of storage. These results evidenced the difference in the ability of the antioxidant compounds in tea beverages to respond at different storage conditions.

As shown in this study, in order to assure that ready to drink black and green teas reach a long retail shelf life with the protection of the compounds responsible for the potential health benefits, it is suggested to keep these beverages in the dark conditions when stored at room temperature.

Those results support future proposals for the development of ready-to-drink teas, complemented with microbiological and sensory analyzes. They can also contribute to the choice of packaging to be used in marketing and alert professionals in the area of nutrition and consumers that expect to obtaining health benefits from bioactive compounds present in those teas.

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