

Comparative Study of Total Polyphenol and Antioxidant Activity in Different Forms of Al-Kbous Black Tea

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Abstract

This study aimed to compare total polyphenol content and antioxidant activity of different forms of Al-Kbous black tea: teabag (TB), loose tea (LT), and opened teabag (OTB). Total polyphenol content was assessed by the Folin-Ciocalteu method, and DPPH and FRAP assays were used to determine antioxidant activity. The results revealed that TB and OTB have significantly more polyphenol than LT at the 5- and 15-minute brewing times. In addition, the data indicated that TB and OTB exhibit significantly greater DPPH radical scavenging activity than LT at all brewing times, while the results of the FRAP assay revealed that TB and LT had significantly greater reducing antioxidant power than OTB at all brewing times. In conclusion, the present results revealed that Al-Kbous black TB brewed for 5-20 minutes produces greater quantities of polyphenol and has either a greater or similar antioxidant capacity in comparison to LT. Additionally, the given results showed no effect on total polyphenol content or the radical scavenging activity of tea extract when prepared by opening the teabag.

Key words: Antioxidants, Black tea, DPPH, FRAP, Polyphenols

Introduction

Tea is the most consumed beverage in the world after water (Gardner et al., 2007; Khozaymeh et al., 2017). Moreover, data from the International Tea Committee survey of 2018 indicates there will be a global increase in annual tea consumption. Specifically, this statistic reveals that, in 2018, the global tea

consumption was approximately 273 billion liters, which is estimated to increase to 297 billion liters by 2021 (Annual tea consumption worldwide 2013-2021, n.d.). Tea has a complex structure, consisting of polyphenols, amino acids, proteins, alkaloids, minerals, carbohydrates, trace elements, and volatile compounds (Karori et al., 2007). In recent years, several scientific studies have proved that the polyphenol content of tea has beneficial health effects, such as anti-inflammatory, anti-bacterial, anti-mutagenic, anti-viral, anti-aging, and anti-carcinogenic properties (Chacko et al., 2010; Lakshmi et al., 2017). In addition, studies have shown that this high polyphenol content contributes to tea's high antioxidant activity (Karori et al., 2007; Cleverdon et al., 2018; Khan and Mukhtar, 2019; Pourhaji and Sahraiyen, 2018).

Different factors, such as the growth environment, manufacturing conditions, leaf age, and preparation methods are known to affect the antioxidant potential of commercial teas (Astill et al., 2001). Other studies have argued that particle size use of teabag versus loose tea can also modulate the antioxidant activity of the tea and its polyphenol content (Cleverdon et al., 2018; Astill et al., 2001; Nikniaz et al., 2016). However, few studies have been conducted in regard to comparing the polyphenol content and antioxidant property of different forms of tea such as teabag (TB) and loose tea (LT). Moreover, currently, there are no published studies that compare these factors for TB and LT with the opened teabag (OTB). Black tea has not been analyzed as frequently as green tea; therefore, this study aimed to compare the total polyphenol content and antioxidant properties of different forms of Al-Kbous black tea including the teabag (TB), loose tea (LT), and the opened teabag (OTB).

Materials and Methods

Chemicals

The chemicals used in this study included standard Galic Acid, Catechin, Ferrous Sulfate, and Trolox, which were purchased from Fisher Scientific (Loughborough, UK). Sodium carbonate (Na_2CO_3), Folin-Ciocalteu reagent, Sodium nitrate (NaNO_2), Aluminum chloride (AlCl_3), Sodium hydroxide (NaOH), Ethanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Acetate buffer, Tripyridyl triazine (TPTZ), Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), Copper sulfate (CuSO_4), and Neocuproine were purchased from Sigma-Aldrich Chemical Co. (Pool, UK).

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Tea samples

Al-Kbous, Yemeni black tea was purchased from the supermarket in Saudi Arabia and stored at room temperature in a dry place. Three different forms of tea were examined in the study: teabag (TB), opened teabag (OTB), and loose tea (LT).

Preparation of Tea Extract

Extracts of TB, OTB, and LT were prepared in a similar manner. Three different beakers containing 200 ml of boiled water were prepared. Then, one teabag was infused in one beaker, while a new teabag was opened, weighed (1.9126 g), and infused in another. For the loose tea preparation, an amount corresponding to the open teabag's weight (1.9126 g) was collected and infused in the third beaker. Samples were incubated for different brewing times (5, 10, 15, 20, and 25 minutes) at room temperature (24°C). These time intervals were chosen to resemble one of the traditional ways of preparing tea in Saudi Arabia, where TB or LT are left in teapot/cup until finishing the drink. The preparation for each tea type extract was conducted in triplicate.

Determination of Total Polyphenol Content

The total polyphenol content of the tea extracts was determined by using the Folin–Ciocalteu method (Nikniaz et al., 2016). Briefly, 0.5 ml of tea extract or a standard solution of gallic acid (0.2, 0.4, 0.6, 0.8, and 1 mg/ml) were decanted in a volumetric flask containing 2.4 ml of deionized water. Then, 0.1 ml of the Folin–Ciocalteu reagent was added and left at room temperature for 5 minutes. Finally, 2 ml Na₂CO₃ solution (2%) was added and the solution was put at room temperature for 60 minutes. This was followed by the absorbance being measured at 750 nm. The assay was conducted in triplicate.

DPPH Radical Scavenging Assay

This method was based on the ability of an antioxidant compound in a plant to donate a hydrogen ion and scavenge DPPH-free radicals. It is adopted with certain modifications by Omar et al., 2016. First, 500 µl of tea extract was diluted with 500 µl of ethanol (99.5%). Then, 125 µl of DPPH (0.02% in ethanol) was added to the mixture and put at room temperature for one hour in a dark place. The absorbance was measured at 517nm and the experiment was conducted in triplicate. Additionally, blank samples contained all reagents except the extract. Radical scavenging activity was expressed as an inhibition percentage and was calculated using the following formula: % inhibition = $100 \times [(A \text{ of blank} - A \text{ of the sample}) / A \text{ of blank}]$.

Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing/antioxidant power (FRAP) assay was carried out according to Benzie & Strain, 1996 with minor modifications (Benzie and Strain, 1996). The FRAP assay is based on the reduction of the Fe³⁺-2,4,6-tripyridyl-S-triazine complex into the ferrous form (Fe²⁺). The FRAP reagent was made by mixing an acetate buffer (0.3 mol/l), TPTZ, and FeCl₃.6H₂O (20 mmol/l

water solution) at a ratio of 10:1:1. Then, 1.5 ml of freshly prepared FRAP reagent was added to 50 µl tea extract. After incubation at 37 °C for 30 minutes, the intensity of the formed blue color was measured against a blank sample with a spectrophotometer at 595nm. All measurements were conducted in triplicate.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 6.0, and all data were expressed as the mean ± SD for each of the three independent experiments conducted in triplicate. The analysis of variance was performed using a one-way analysis of variance (ANOVA), and differences between means were evaluated via the Bonferroni test. Results with P < 0.05 were considered statistically significant.

Results

Total polyphenol content

The total polyphenol content of the three different tea extracts at different brewing times are presented in Figure 1. As demonstrated, TB and OTB significantly exhibited more polyphenol than LT at the 5-, 10-, and 15-minute brewing times. Furthermore, although there were no significant changes among the three forms of the tea at the 20-minute brewing time, the results showed that LT and OTB had significantly more polyphenol than TB (1.28±0.18 µg/ml) once brewing time increased to 25 minutes.

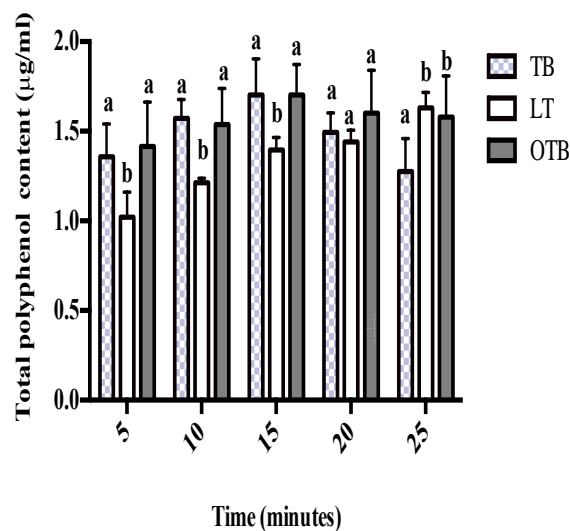


Figure 1. Total polyphenol content (µg/ml) of teabag (TB), loose tea (LT), and opened teabag (OTB) at different brewing times.

The data represents the mean of the three independent experiments ± SD. Statistical analysis was performed with a one-way variance analysis (ANOVA) followed by Bonferroni's test. A significant difference ($p < 0.05$) between the different tea forms at each time point is indicated by differing letters.

DPPH Radical Scavenging Activity

Statistical analysis indicated that there was a significant difference in DPPH radical scavenging activity among the tea extracts at different brewing times (Figure 2). TB and OTB results showed significantly higher DPPH radical scavenging activity at all brewing times than the LT results. These results demonstrate there are no significant differences between TB and OTB, and both have a greater ability to scavenge free radicals than LT at all brewing times.

Ferric Reducing Antioxidant Power (FRAP)

As depicted in Figure 3, the results showed that the only significant difference in reducing power between TB and LT occurred at the 5-minute brewing time, while no difference was found between these same two tea forms as brewing time increased. However, the data revealed that TB and LT have significantly higher reducing antioxidant power than OTB at brewing times of 10, 15, 20, and 25 minutes.

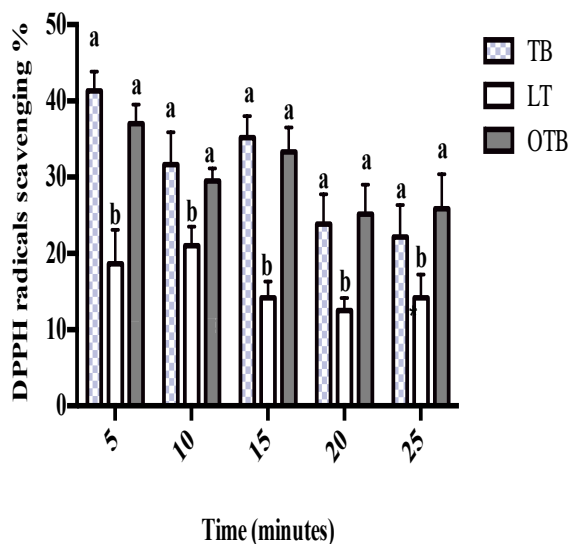


Figure 2. DPPH radical scavenging activity by teabag (TB), loose tea (LT), and opened teabag (OTB) at different brewing times. The data show the mean of the three independent experiments \pm SD. The statistical analysis was performed by a one-way variance analysis (ANOVA) followed by Bonferroni's test. A significant difference ($p < 0.05$) between tea forms at the same time point is indicated by differing letters.

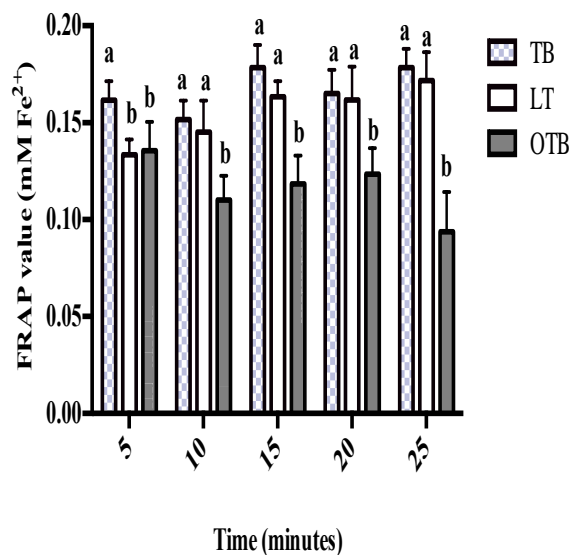


Figure 3. Ferric Reducing Antioxidant Power (FRAP) of teabag (TB), loose tea (LT), and opened teabag (OTB) at different brewing times. The data represents the mean of the three independent experiments \pm SD. Statistical analysis was performed by a one-way variance analysis (ANOVA) followed by Bonferroni's test. A significant difference ($p < 0.05$) between tea forms at each point is indicated by differing letters.

Discussion

Tea is known to be a potent antioxidant, as it contains a high amount of polyphenol compounds. Recent studies have shown that different factors might affect the antioxidant property of tea products. Considering this, the present work aimed to study the effects of different forms of Al-Kbous black tea including TB, LT, and OTB in terms of total polyphenol content and antioxidant activity. The present results showed that 5-, 10-, and 15-minute brewing times were enough for both TB and OTB to release significantly higher amounts of total polyphenol than LT; however, the results shifted at the 25-minute brewing time, at which LT and OTB released more total phenol than TB. According to these results, it can be concluded that TB and OTB release more polyphenol at shorter brew times in comparison to LT, which requires more brewing time for the same effect. The tea particles of TB generally are finely ground leaves, which means that a greater surface area of these tea particles is in direct contact with the hot water during infusion, which, subsequently, could cause large amounts of polyphenol to be released at the shorter brewing times (Sharpe et al., 2016; Yang et al., 2013). On the other hand, LT, which has relatively larger particles, would require a longer time to allow for these particles to gradually open, hydrate, and release their polyphenol content. These findings are in line with other studies conducted on different brands of loosely packed and bagged teas, concluding that bagged teas are a richer source of phenolic compounds than loose tea (Nikniaz et al., 2016; Sharpe et al., 2016; Komes et al., 2010; Kyle et al., 2007; Rusak et al., 2008).

The antioxidant property of different forms of tea was determined using DPPH and FRAP tests. The DPPH assay showed that TB and

OTB possess higher scavenging activity than LT at all brewing times. Previous investigations showed that the antioxidant ability of tea was significantly affected by its form; bagged, powdered or loose; however, these results have been controversial. For example, Komes et al. (2010) studied different forms of green tea including powdered (Matcha), loose-leaf and bagged, stating that bagged tea exhibits the highest scavenging activity followed by powdered and then loose-leaf tea. On the other hand, Farooq and Sehgal (2018) found no significant difference between the radical scavenging ability of the different forms of tea, except bagged tea, which exhibited slightly higher scavenging potential than powdered (Matcha) and loose-leaf tea. In general, it can be concluded that bagged tea has a greater ability to scavenge free radicals than loose tea, and according to the present results regarding Al-Kbous black tea, opening the teabag (OTB) has no major effect on radical scavenging activity. This conclusion is supported by several studies that have indicated that antioxidant activity is dependent on particle size. Specifically, Zaiter et al. (2016) revealed that the best radical scavenging activity of green tea powders (GTPs) occurred with a fraction of 100–180 µm in comparison to larger and non-ground particles. Another study has shown that aqueous extraction of black tea with smaller particle sizes tend to have higher antioxidant properties (Makanjuola et al., 2017).

The FRAP results of this investigation showed that TB and LT have higher reducing antioxidant power than OTB. A study by Nikniaz et al. (2016) investigated loosely packed black teas as well as the teabags of five different tea brands and found that the ferric reducing antioxidant power of teabags was significantly higher than that of the loosely packed forms of the same brands after 5 minutes of brewing (Nikniaz et al., 2016). The difference in the results between Nikniaz et al. and the present study might be due to differences in the tea preparation methods. While they infused the tea in 240 ml of 80°C water and solutions were allowed to reach room temperature prior to the analysis, the present samples were prepared in 200 ml of boiled water and the analysis was run immediately after the designated brewing times. Accordingly, it can be concluded that preparation variables can greatly influence the ferric reducing antioxidant power of tea extracts.

Conclusions

This study is one of the first comparative studies on the total polyphenol content and antioxidant properties of three different forms of black tea (Al-Kbous brand): teabag (TB), loose tea (LT), and opened teabag (OTB). The data revealed that TB and OTB released much more polyphenol at shorter brewing times (5-15 minutes) than LT. Furthermore, both forms exhibited higher scavenging activity than LT at all brewing times. Moreover, TB and LT exhibited more reducing antioxidant power than OTB. Teabags have globally become more widely available in recent years, becoming a common choice for consumers. The present results allow one to conclude that Al-Kbous black TB brewed for 5-20 minutes would possess a greater quantity of polyphenols and either more or similar potent antioxidant capacity compared to LT. Moreover, there appeared to be no effect on total polyphenol

content or the radical scavenging activity of the tea extract when prepared by opening the teabag (OTB).

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Disclosure statement

No potential conflict of interest was reported by the authors.

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