IMPACT OF HEAT TREATMENT AND FLAVORINGS ON TEA ANTIOXIDANT CAPACITY
SHORT RUNNING TITLE: HEAT AND FLAVORINGS IMPACT ON TEA ANTIOXIDANTS

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Abstract

The high antioxidant capacity of tea is well-known, but the effect of flavoring materials like honey or lemon has been less studied. These add extra antioxidants to that can interact with each other, the global result being also affected by water temperature.

The research focuses on the combined effect of heat and flavorings (acacia and honeydew honeys, lemon juice) on the total polyphenol, total flavonoid content and antioxidant capacity of black and green teas.

In many cases higher antioxidant capacity was obtained at 80°C, which can be explained by Maillard reaction products. Tea prepared with honeydew honey has significantly higher antioxidant capacity than the tea with acacia honey. Addition of lemon decreased the antioxidant capacity of tea with honey. No synergies were confirmed in any of the compositions investigated. Vitamin C content of in lemon tea was reduced by half at higher temperature; its level was strongly affected by honey.

Keywords: tea, heat treatment, flavoring, antioxidant, Maillard

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1. Introduction

The use of natural antioxidants, especially those derived from plants, was found to successfully combat stress-related diseases. Tea, whether green or black, is widely known for its valuable health-promoting effects (Gardner et al., 2007, Annunziata et al., 2018). The bioactive compounds in green teas are mainly flavan-3-ols (catechins), proanthocyanidins (tannins) and flavonols, while black tea contains theaflavins and thearubigins (Malongane et al., 2017). Lemon and honey are also rich in antioxidants (mainly polyphenols, respectively ascorbic acid in lemon) and are popular tea flavorings, used as natural home remedies, especially for preventing infective respiratory diseases. Honey is a rich source of phytochemicals, especially phenolic compounds that vary depending on the type and origin of honey. It is known that honeydew honeys, darker in color, are higher in phenolics than floral honeys, especially the lightly colored ones like acacia honey (Jose et al., 2009, Kaszab et al., 2017).

However, the proper timing and temperature of addition of either lemon or honey has generated debates over generations due to the concerns related to heat damage of bioactive materials from both honey and lemon.

A research dealing with the effect of brewing temperature stated that various teas brewed at room temperature had significantly higher antioxidant capacities and flavonoid contents than those prepared with hot water (Damiani et al., 2014; Lantano et al., 2015).

Antioxidant capacity of commercial lemon-flavored black teas sweetened with different types of honey was also investigated (Pereira et al., 2013). Results showed that the use of honey, especially the darker type enhances the antioxidant activity of lemon-flavored black tea.

Effect of honey addition to green tea and herbal teas was also examined at different temperatures (Toydemir et al., 2015). The results obtained for green tea showed that polyphenol content increased continuously upon honey addition as temperature increased, especially when honeydew honey was used, while the increase was not consistent when antioxidant capacity and especially total flavonoid content were examined. The trends depended on the matrix, i.e. green or different herbal teas and different honey types. This effects were attributed to the Maillard processes taking place at higher temperatures, when antioxidant melanoidins are formed that also contribute to the measured polyphenol content.

Antioxidant properties of fruit teas were examined in combination with ascorbic acid and honey. The results are somehow intriguing, due to the fact that ascorbic acid contributed to the increase of antioxidant capacity and polyphenol content of teas, while supplementary addition of honey proved to have a detrimental effect on these parameters (approximately 20%

decrease). The supposed cause of the observed phenomenon is the formation of glycosides from honey sugars and phenolic compounds as aglycons (Belščak et al., 2011).

The present work focuses on the investigation of the impact of honey and lemon added to black and green teas at different temperatures, in order to define the conditions enabling optimal antioxidant capacity, polyphenol and flavonoid levels. Possible synergistic effects are also object of the research presented.

2. Materials and methods

2.1 Tea and honey samples

In this study unflavored black (FT) and green (ZT) teas were purchased from retail commerce, while acacia (A) and honeydew (E) honeys were obtained from domestic beekeepers. Lemon (C) was bought in regular supermarket, then its juice was squeezed and stored at -18°C.

2.2 Methods

2.2.1 Preparation of tea brews

Teas were for 3 minutes brewed using boiling distilled water (1 g tea for 100 ml water). The usually used amounts of honey (5 g) and/or lemon juice (5 ml) were added to the cooling teas at two different temperatures: 80°C or 55°C. The prepared tea samples were stored at -18°C until the analytical measurements.

2.2.2 Total polyphenol content and antioxidant capacity

Sample preparation

1 ml of tea sample was diluted 10 times with distilled water and this stock solution was used for the measurements. Spectrophotometric measurements were performed on a Thermo Helios Alpha UV-VIS spectrophotometer (±0.001 au), using cells of 1 cm path.

CUPRAC- Cupric Reducing Antioxidant Capacity assay

The reduction power of teas against copper(II) ion was estimated according to the procedure described by Apak et al. (Apak et al., 2004). To 1 ml 10^{-2} M $CuCl_2$ solution 1 ml of 1 M ammonium acetate buffer (pH= 7), 1 ml of $7.5*10^{-3}$ M neocuproine solution and 0.2 ml of tea stock solution were added, completed by 0.9 ml water. Measurements were performed after 30 minutes at 450 nm against a blank. Results were reported as mmol trolox equivalents (TE) /1 l tea.

Total polyphenol content

Total polyphenol content (TPC) was determined by the Folin–Ciocalteu method, following a procedure adapted from (Singleton & Rossi, 1965). 1 ml of the stock solution solution was put in a test tube, and 7.5 ml distilled water was added, followed by 0.5 ml of

Folin–Ciocalteu reagent. After 3 minutes 1 ml saturated Na_2CO_3 solution was added. Absorbances were read at 750 nm after a 30-minutes incubation period in the dark. Results were expressed as mg equivalents of gallic acid (GAE) per 1 l tea.

***Total flavonoid content*:**

Total flavonoid content was measured by the $AlCl_3$ method (HIV): to 1 ml of tea sample diluted at 1:4 1 ml water and 0.3 ml 5% $NaNO_2$ were added. After 5 minutes 0.3 ml 10% $AlCl_3$ was added. After 5 minutes of waiting 2 ml 1M NaOH was added, then the volume was brought to 10 ml with water. Measurements were performed at 415 nm against a blank, using catechin as calibrating standard (mg CE/ 1 l tea) (Zhisen et al., 1999).

Ascorbic acid content:

Ascorbic acid was quantified in some of the samples using an enzymatic kit procured from Megazyme.

Statistical analysis

Excel and SPSS were used for statistical evaluation. Normality of the variables was tested with Shapiro-Wilk test. Levene-test was used for checking the homogeneity of the variances. Due to the fact the homogeneity of variances was not assumed, Welch test was applied for pairwise comparison for temperature values, while Games-Howell test was applied for group comparison.

3. Results and discussion

3.1 In vitro antioxidant capacity (CUPRAC assay)

In the case of green teas (Figure 1) no significant differences were found for teas flavored with acacia honey and lemon prepared at 55°C (ZTAC_55) or 80°C (ZTAC_80). Moreover, antioxidant capacities were significantly higher at 80°C in the case of teas prepared with only lemon (ZTC) or honeydew honey and lemon (ZTEC), respectively acacia honey and lemon (ZTAC). Teas containing honeydew honey (ZTE) have higher overall antioxidant capacities compared to those flavored with acacia honey (ZTA). Teas containing both honey and lemon reached generally lower values than those containing only one of these ingredients, when the same temperatures are compared. Thus, no synergistic effects in relation to green tea, honey or lemon were observed. One flavored tea (ZTAC_55) showed significantly lower performance than pure green tea.

Similarly to green teas, black teas (FT) didn't show the expected clear decrease of antioxidant capacity with temperature. The highest values were obtained for teas prepared with

only lemon (FTC_80) or only honeydew honey (FTE_55). Some of the flavored teas (FTA_55, FTE_80, FTEC_80) had significantly lower antioxidant capacities than the original black tea.

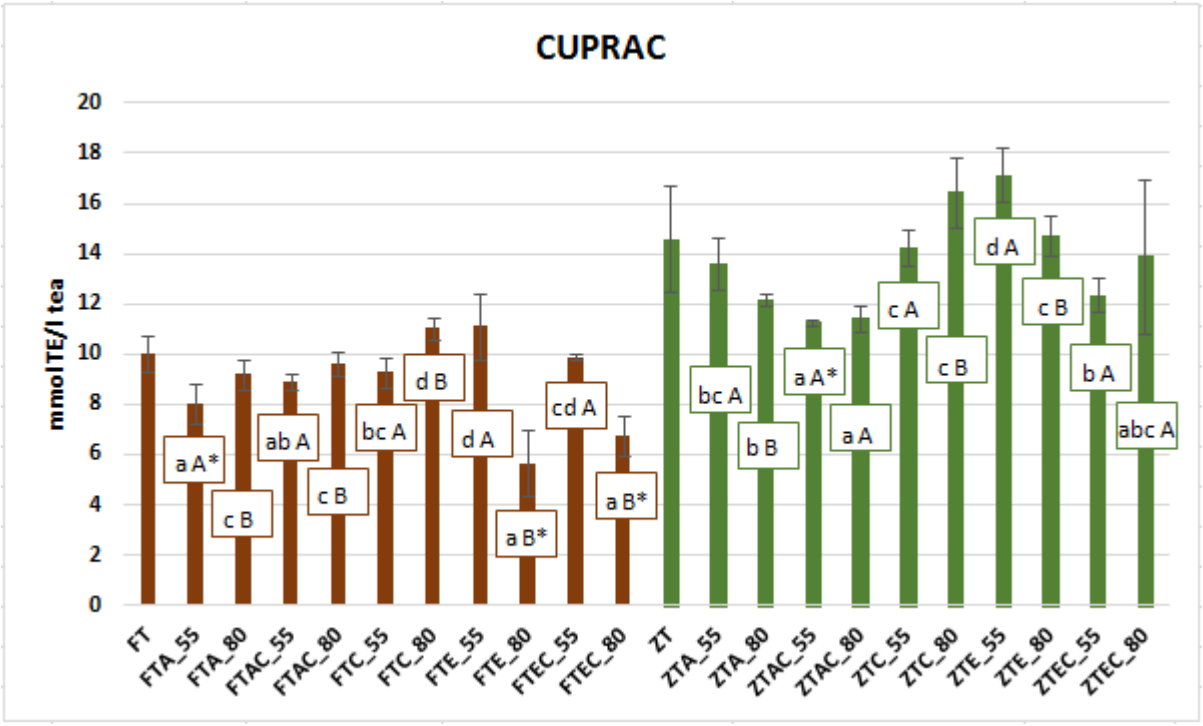

Figure 1. Mean±standard deviation of CUPRAC antioxidant capacity of tea samples
*: significantly different from control tea; low case letters: comparison of groups at the same temperature (Games-Howell test $p<0.05$); capital letters: comparison of temperature levels at the same tea types within groups(Welch-test ($p<0.05$)

3.2 Total polyphenol content (TPC, Folin-Ciocalteu assay)

Similarly to CUPRAC results, teas prepared with honeydew honey showed better performance than those containing acacia honey, this is due to the higher phenolic compound content of honeydew honey (Figure 2). Temperature difference affects polyphenol content in most of the cases, but in many cases higher temperatures led to higher polyphenol contents. This could be attributed to the the reaction between Maillard reaction products and the Folin-Ciocalteau reagent (Toydemir, 2015). Phenolic compound content in teas containing only lemon or only honey is either higher or does not differ significantly from those containing both ingredients, thus no synergistic effects were evidenced. The lower values measured for teas prepared with honey and lemon are attibuted to the formation of glycosides between the –OH moieties of phenols and sugars in honey, as bescribed in the literature (Belščak et al., 2011).

Use of honeydew honey resulted in higher polyphenol content in black teas compared to those containing acacia honey at 55°C. The addition of lemon led to polyphenol contents

significantly lower than in the case of original tea, this is due to the lower polyphenol content of lemon.

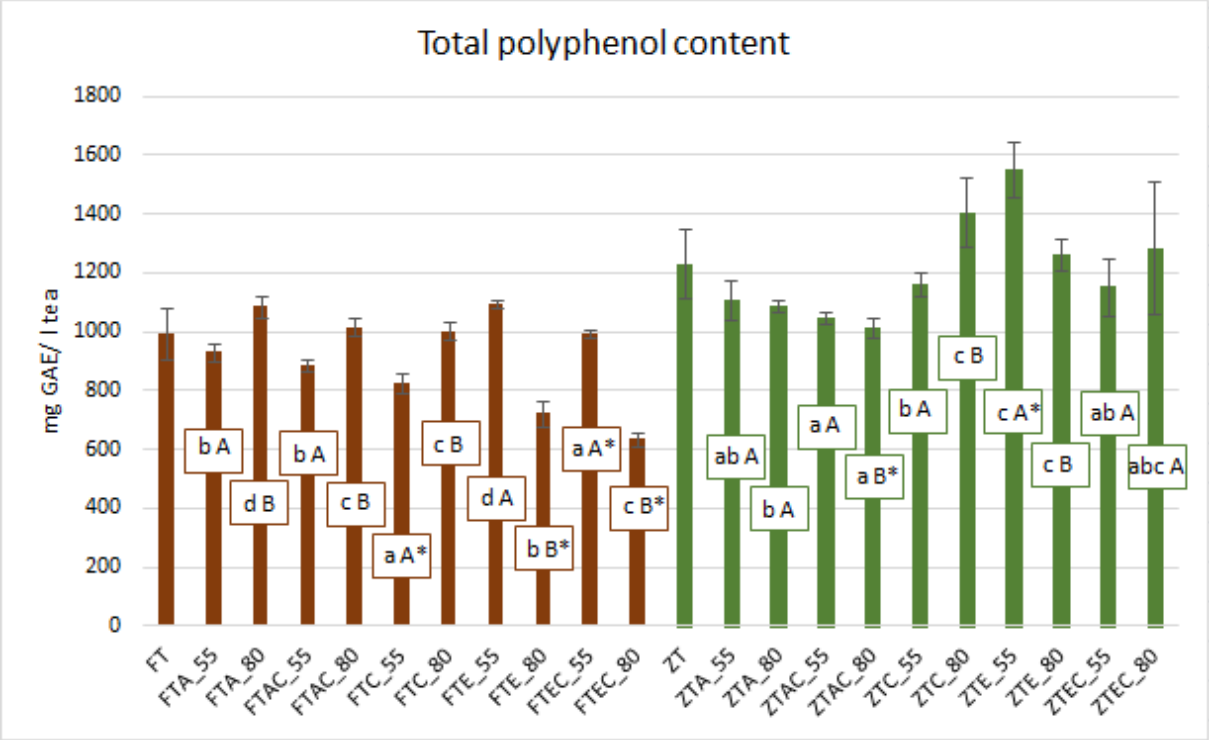

Figure 2. Mean±standard deviation of total polyphenol content of tea samples
*: significantly different from control tea; low case letters: comparison of groups at the same temperature (Games-Howell test $p<0.05$); capital letters: comparison of temperature levels at the same tea types within gorups(Welch-test ($p<0.05$)

3.3 Total flavonoid content (TF, $AlCl_3$ assay)

Excepting green tea with honeydew honey, no significant flavonoid losses were confirmed for green teas flavored with the same ingredients at lower or higher temperature (Figure 3), so no significant flavonoid losses are confirmed at these temperatures. Unlike for CUPRAC antioxidant capacity and Folin assay, here Maillard reaction products do not contribute to the global flavonoid content, thus values obtained at 80°C were not superior to those measured for teas flavored at 55°C. Instead of synergies between honey and lemon, a constant decrease was observed for teas with honey and lemon compared to those containing only honey or lemon. The well-known high flavonoid content of green tea was confirmed in comparison to any flavored tea containing either lemon or honey, however, the higher flavonoid content of honeydew honey (Lachman et al., 2010) was confirmed in our flavored tea samples.

The flavonoid content pattern for black teas was different from the one obtained for green teas. Excepting the teas flavored with honeydew honey, all the teas show higher flavonoid content at 80°C compared to their pairs prepared at 55°C, this suggesting that some compounds

are formed that contribute to the overall flavonoid content result. Teas containing both lemon and honey show lower values than those containing only one of these ingredients. The high flavonoid content of pure black tea is equaled only by the teas containing acacia or honeydew honey.

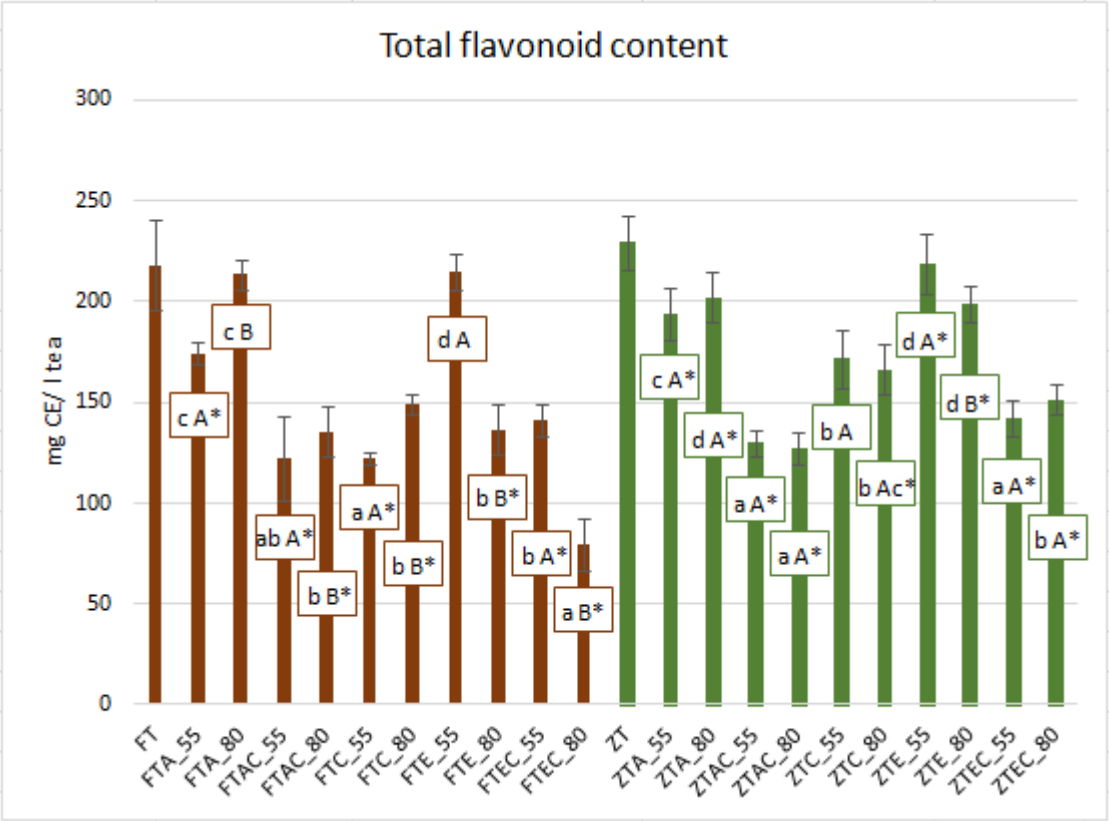

Figure 3. Mean±standard deviation of total flavonoid content of tea samples
*: significantly different from control tea; low case letters: comparison of groups at the same temperature (Games-Howell test $p<0.05$); capital letters: comparison between temperatures within groups (Welch-test ($p<0.05$)

3.4 Ascorbic acid content

Ascorbic acid was determined for the black tea samples containing lemon. A decrease of the original ascorbic acid content of lemons by 50% was observed for all the sample pairs investigated when teas prepared at 55 and 80 °C were compared. For teas containing only lemon, but not honey much lower values were obtained (FTC_55: 8.10±0.67 mg/l and FTC_80: 3.81±0.01 mg/l) than for either acacia honey-lemon (FTAC_55: 27.27±15.95 mg/l and FTAC_80: 12.73±12.21 mg/l) or honeydew honey-lemon (FTEC_55: 23.18±8.36 mg/l vs. FTEC_80: 10.91±1.29 mg/l) teas. For both tea types (only lemon and lemon-honey) the same degree of decrease was observed, however, honey-containing teas showed much higher ascorbic acid contents which can be explained by the ascorbic acid content of honey, which is around 0.9 mg/g for both honeys used, this corresponding to a theoretic content of 45 mg

ascorbic acid per liter tea (Dobrinas et al., 2006). On the other hand, the higher amounts of preserved ascorbic acid are due to the protective effects of phenolic antioxidants against oxidative decomposition of ascorbic acid (Miller et al., 1997). Thus, the use of honey potentiates significantly the ascorbic acid content of tea, which is, among other phytochemicals – i.e. mainly polyphenols – an additional advantage of the use of honey as a sweetener.

4. Conclusion

The comparison on the effect of honey and lemon addition to green and black tea samples at different temperatures revealed that the total polyphenol, flavonoid content and CUPRAC antioxidant capacity of the honey-added-tea samples were generally lower than those of the control tea samples. Combination of lemon and honey led to lower antioxidant, polyphenol and flavonoid values in many cases compared to either pure tea or teas containing only lemon or only honey. Temperature increase did not result in a consistent trend in the change of the parameters investigated. This is mainly attributed to the contribution of Maillard reaction products showing antioxidant properties. Unlike for antioxidant properties, the temperature increase had a negative impact on the ascorbic acid content, which dropped to half of the initial value in the samples investigated. Ascorbic acid content was much higher in teas containing honey and lemon than in those containing only lemon, due to the protective effects of phenolic antioxidants present in honey.

Our findings do not support any synergistic effects between antioxidant properties of tea, lemon and honey in terms of global in vitro antioxidant capacity and phenolic antioxidants, however, a protective effect these latter on the ascorbic acid content of lemon and honey was observed.

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