*Research Article*

# PRODUCTION OF COLD GREEN TEA WITH NATURAL ADDITIVES

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## ABSTRACT

Tea is the product that goes through production stages such as withering, rolling, shredding, oxidation and drying after the harvest of 2.5 leaves of the *Camellia sinensis* species. Green tea, known for its health benefits, is consumed less than black tea due to sensory features. Cold tea is a product that is widely used in the world. The cold teas on the market are made with black tea extract and contain various additives to improve its sensory properties. In this study, it was aimed to increase the nutritional and sensory properties of cold green tea by adding natural substances: honey and lemon juice, and peel. Also, the effects of brewing methods on the quality of tea were investigated. Green tea samples containing lemon peel were infused by heat-assisted method and ultrasound method. Cold tea samples were produced with different concentrations of honey and lemon juice addition. The physiochemical parameters (total dry matter, pH, and color properties), total phenolic content, antioxidant activity, and sensory analysis of the cold tea samples were evaluated. The optimal tea formula determined according to the results is the sample of containing high amount of lemon juice. While the total phenolic content was 285.76 ± 2.80 mg GAE/ 1mL tea and the antioxidant activity was 62.42 ± 6.15 (DPPH radical scavenging activity, %) in the green tea sample, the total phenolic content was found to be 300.76 ± 3.34 mg GAE/ 1mL tea and the antioxidant activity 91.46 ± 1.94 (DPPH radical scavenging activity, %) in the sample containing high amount of lemon juice. It was observed that the honey and lemon juice addition increased the total phenolic and antioxidant content of the control sample. When the results of the study and the

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sustainability approach are evaluated, a product that will meet the market needs has been obtained.

**Keywords:** Green tea, Honey, Lemon, Ultrasound, Antioxidant activity.

## INTRODUCTION

Tea, which is stated in the sources of China to be found by chance falling into boiling water, has spread all over the world and reached many different cultures. About 30 countries, mainly India, Taiwan, and China, are involved in tea production. The presentation, consumption, and brewing of tea differ from country to country. Merkez Tea Factory started production in 1947 as Türkiye's first tea factory with a capacity of 60 tons/day. The private sector serves a daily processing capacity of approximately 9 thousand tons **[1].**

Tea polyphenols (catechins) create 30% to 42% of the dry weight of solids in brewed green tea. Flavones and flavonols have been detected in tea. Quercetin, kaempferol, myricetin, and glycosides are the main flavonols. The caffeine level is affected from the brewing time, temperature, and leaf size. Flavins are responsible for the bright red color of black tea. Thearubigins, the brown pigment in black tea, are heterogeneous polymers of tea catechins. They constitute 60% of the water-extractable material of black tea by dry weight. The dominant minerals contained in the water-soluble ash of tea are potassium, calcium, magnesium, and aluminum **[2].** Green tea is loaded with antioxidants and nutritional components; therefore, optimum brewing conditions are very important to increase the efficacy of green tea **[3].**

Epigallocatechin-3-gallate (EGCG) is a substance that has important properties such as antioxidant, anticancer, anti-inflammatory, and anti-proliferative effects and is widely used in traditional medicine. It prevents the formation of various types of cancer by inhibiting the effect of carcinogenic substances **[4].** Studies have proven that the antioxidant EGCG in tea has anti-carcinogenic effects. The effects of green tea on cardiovascular diseases are also known. It has proven antimicrobial properties against gram-positive and gram-negative bacteria. These include *Listeria*, *E. coli*, *Salmonella,* and *Pseudomonas* species. It is proven that it prevents oral wounds due to its anti-inflammatory properties **[5].**

Türkiye is the second biggest honey producer in the world after China. It has a 6.9% share in the world market **[6].** While honey consumption is common, its adulteration is widespread **[7].** Since ancient times, honey consumption was common, and its health effects were known **[8].** It is a natural bee product that has antioxidant, antimicrobial, and anti-inflammatory properties **[9].** The major components are complex of sugars and also have enzymes, minerals, vitamins, amino acids, and organic acids. Honey has low protein content (0.5%) and the proteins are mainly enzymes **[10].** The chemical composition may differ with climate, flowers, and geography **[11].** While the major components are carbohydrates, the glycemic index of honey ranges from 32 to 87 **[12].** It is known that heat treatment damages the enzymes and increases HMF (*hydroxymethylfurfural*) content in honey **[13].**

Lemon is a medicinal plant belonging to the *Rutaceae* family that grows on small trees 10 to 20 feet high. The main producers of lemon, which are preferred frequently all over the world, are Mexico, Argentina, Brazil, Spain, China, USA, Türkiye, Iran, and Italy. Extracts from other parts of lemon, which have alkaloids with anticancer activity, have been reported in various studies to have antibacterial potential. The peel of citrus fruits is rich in glycosides, coumarins, and flavonoids **[14].** Lemon peel is rich in substances such as flavonoids, pectin, fiber, and essential oil. For this reason, it is important to evaluate lemon peels in the food industry. It shows important effects such as antioxidant, antimicrobial and anti-diabetic properties. Due to the positive effects of beneficial components in lemon peels on health, it can be used in the development of functional foods **[15].**

In the traditional brewing method, the solubility of tea components is increased by the heating or boiling method **[16].** Brewing temperature and time are considered the most effective factors for the composition and sensory properties of tea infusions. Increasing temperature and time applications make the infusions dark and bitter. In addition, the antioxidant activity, the total phenolic content, and the total flavonoid contents vary depending on the brewing temperature and duration. At this point, the composition of the tea before brewing is also important. Determining the optimum brewing conditions is very important in terms of the composition and sensory properties of the prepared tea **[17].**

Ultrasonic extraction is an effective process with wide application in food processing. It is characterized by high frequency and good penetrating power **[3].** Ultrasonic waves have a frequency of 20 kHz and higher. The ultrasonic method has become increasingly popular in recent years both to prevent loss due to the negative impact of the active ingredients on the temperature and to increase the extraction yield **[16].** In ultrasonic extraction techniques, the material transfer is supported by increasing the breaking effect of the cell walls **[3].** The advantages of ultrasonic extraction compared to other methods are that the equipment cost is cheap, the process is easy, and the time saving is the case. It is stated that it will be more effective in the extraction of antioxidants, phenolics, and anthocyanins compared to other methods that require high temperatures and a long time. In addition, studies have shown that alkaloids and flavonoids can be extracted in extracts obtained from ultrasonic extraction compared to the traditional method **[18].**

The study aims to analyze the effects of different infusion methods on green tea's physicochemical properties and improve sensory acceptance by adding lemon derivatives and honey. In the study, the traditional method of high-temperature infusion and the low-temperature method with ultrasound, which is a good extraction method, were used.

## MATERIALS AND METHOD

### Materials

The green tea used in the preparation of cold green tea was obtained from the Çaykur (Rize, Türkiye). The added honey is the Şemdinli flower honey of the Baltek company (Istanbul, Türkiye). The lemon and water used in the study were bought from a local market in Istanbul. Lemon peels were dried at 40 $^{o}$C for 8 hours in an oven (Suzen, Türkiye). Methanol (Merck, Germany), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrıchand, USA), Folin Ciocalteu reagents (Merck, Germany), Sodium carbonate (Merck, Germany), Gallic acid anhydrous (ISOLAB chemicals, Germany) were used in the study.

**Preparation of cold tea with traditional method**

Since there were 3 different tea formulations and 2 different brewing methods in the study, 6 different teas were obtained: Traditionally brewed green tea (control sample) (GT), Traditionally brewed green tea with high honey content (HGT), Traditionally brewed green tea with high lemon juice content (LJGT), Green tea brewed by ultrasonic method (control sample) (UGT), Green tea with high honey content brewed by ultrasonic method (U-HGT), Green tea with high lemon juice brewed by ultrasonic method (U-LJGT).

Water (100 mL) was taken into a clean jar and placed in a water bath (Stuart SWB2D, UK) previously set at 85 $^{o}$C. Green tea (2 g) was weighed for the sample named GT. Green tea (2 g) and lemon peel (0.2 g) were weighed for the sample named HGT and LJGT. All tea samples were transferred to jars in a constant temperature (85 $^{o}$C) water bath and infused for 6 minutes. The samples were filtered (Sartorius Stedim Biotech, France) with the help of a strainer and left at room temperature. 9 g of honey and 1 g of lemon juice were weighed in the beaker for the HGT sample and 6 g of honey and 4 g of lemon juice for the LJGT sample. These products were added to the tea and mixed homogeneously. The prepared samples were kept in the refrigerator until the analysis time. Production steps of the traditional brewing method were demonstrated in Figure 1.

**Preparation of cold tea by ultrasonic method**

The same amounts of the samples by the traditional method were prepared for the ultrasound method. The brewing was carried out in an ultrasonic water bath (SWB2D, UK) at 25 $^{o}$C for 30 min and outside the ultrasonic bath at 25 $^{o}$C for 30 min. The stages after brewing were the same as with the traditional method. The production steps of the ultrasound proses are similar to the traditional one. The only difference is the temperature and time.

**Physicochemical analyzes**

Total dry matter analysis of cold tea samples was made using a drying oven (BINDER, Germany) at 105 $^{o}$C. The color analysis of cold tea samples was made using Lovibond Tintometer (PFX-880, UK) according to the CIE Lab system (*L** Lightness, *a**: redness or greenness, *b**: yellowness or blueness). The pH analysis of the samples was performed with a pH meter (Mettler Toledo, S210, Switzerland).

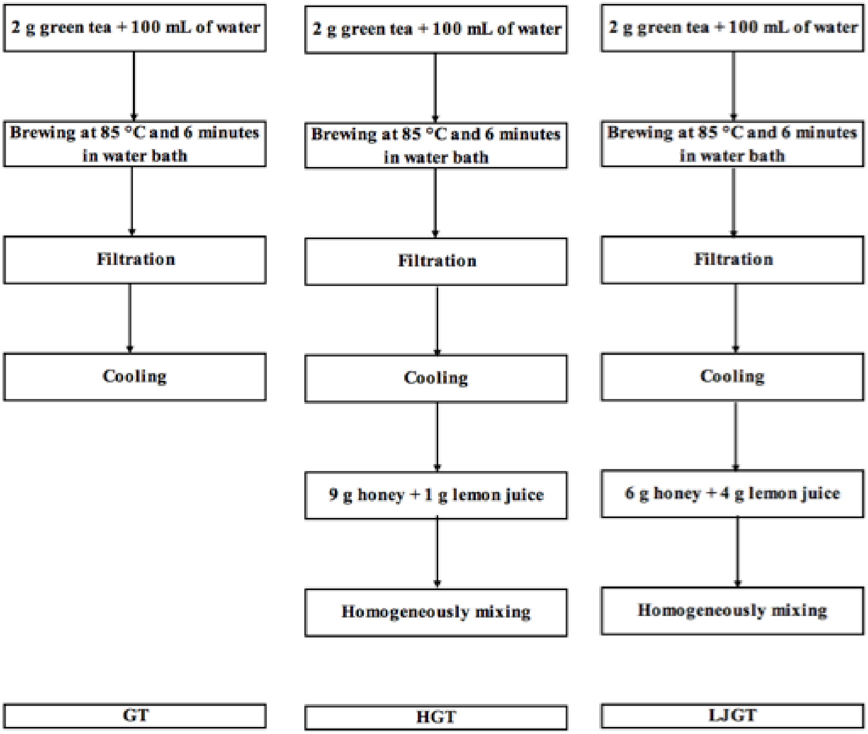


**Figure 1.** Production flow chart of traditionally brewed cold green teas (GT: control green tea; HGT: green tea with 9:1 ratio of honey: lemon juice; LJGT: green tea with 6:4 ratio of honey: lemon juice).

## Determination of total phenolic content

The total phenolic content of the cold tea samples was determined according to the Singleton and Rossi method with some minor changes. 0.1 mL of tea sample and 0.9 mL of distilled water were added to the test tubes **[19].** The tubes were vortexed by adding 4 mL of 0.2 N Folin-Ciocalteu reagent. The samples were covered with aluminum foil and kept for 2 hours at room temperature and in a dark place. At the end of the period, the absorbances were determined by a spectrophotometer (PG Instruments, T60UV-Visible, UK) at 760 nm. The total phenolic content of the samples was calculated using the standard curve of gallic acid. Results were expressed in mg gallic acid equivalent (GAE) per 1mL of tea sample. Analysis of total phenolic content was done in 4 parallels for each sample.

**Determination of DPPH Radical-Scavenging Activity**

Antioxidant activities of cold tea samples were determined according to the Brand – William et al. method with some modifications. 0.1 mL of tea samples were taken into test tubes and 3.9 mL of 6 x $10^5$ DPPH was added to each tube and then vortexed **[20].** The samples were wrapped with aluminum foil and kept in a dark place for 30 minutes at room temperature. The absorbances of mixtures were measured by the spectrophotometer in the 515 nm wavelength against blank. DPPH radical scavenging activity (%) was calculated according to the following formula:

DPPH radical scavenging activity (%) = ($Absorbance_{Blank}$ – $Absorbance_{sample}$) / $Absorbance_{blank}$) x 100

**Sensory analysis**

In the food industry, sensory tests are applied for the acceptability of the product **[21].** The hedonic scale is the most widely used method for evaluating product acceptance among sensory tests **[22].** The use of hedonic scales allows one sample to be preferred over other samples **[21].** In this way, panelists' comments and likes about the product are evaluated **[22].** A 5-point hedonic scale (1 = Very bad, 2 = Bad, 3 = Fair, 4 = Good, 5 = Very good) was used for sensory analysis of cold tea samples. The samples were evaluated in terms of odor, color, taste, and general acceptability. The samples were coded with randomly selected 3-digit numbers and presented for evaluation by panelists. The sensory analysis was carried out in a noiseless environment with a ventilation system and illuminated with white fluorescent light. It is important to provide appropriate environmental conditions in sensory analysis. The panelists were 17 people from Istanbul Aydın University faculty and students.

**Statistical analysis**

Analyzes were performed in 3 repetitions in parallel. Results are given as mean ± standard deviation. The results were evaluated statistically by applying ANOVA (analysis of variance) and Duncan Multiple Comparison test and SPSS (IBM, SPSS Statistics, Version 19) (p=0.05).

## RESULTS AND DISCUSSION

### Color and pH value of tea samples

The color and pH values of the tea samples are shown in Table 1. For the consumer, color is an important criterion. As the ratio of lemon juice increased, the *L** value of samples also increased, and it was statistically significant ($p<0.05$). After adding honey and lemon juice to green tea, a decrease in *b** value and an increase in *a** value was observed, and it was statistically significant ($p<0.05$).

**Table 1.** Color and pH value results of tea samples.

| Sample* | pH | *L** | *a** | *b** |
|---|---|---|---|---|
| GT | $5.68^{a} \pm 0.01$ | $89.66^{c} \pm 0.67$ | $-7.69^{c} \pm 0.50$ | $30.52^{a} \pm 0.68$ |
| HGT | $3.90^{c} \pm 0.01$ | $96.54^{a} \pm 0.64$ | $-3.38^{a} \pm 0.55$ | $15.09^{cd} \pm 0.71$ |
| LJGT | $3.15^{f} \pm 0.01$ | $96.58^{a} \pm 0.58$ | $-3.05^{a} \pm 0.48$ | $13.97^{d} \pm 0.55$ |
| U-GT | $5.51^{b} \pm 0.00$ | $81.02^{e} \pm 0.05$ | $-5.51^{b} \pm 0.01$ | $19.01^{b} \pm 0.01$ |
| U-HGT | $3.54^{d} \pm 0.01$ | $87.97^{d} \pm 0.59$ | $-2.90^{a} \pm 0.49$ | $15.50^{c} \pm 0.63$ |
| U-LJGT | $3.18^{e} \pm 0.00$ | $91.00^{b} \pm 0.66$ | $-2.81^{a} \pm 0.45$ | $14.54^{cd} \pm 0.69$ |

* Data with the same superscript in the same column does not show a significant difference according to Duncan's test ($p<0.05$).

It was found that green tea samples (GT, U-GT) had the highest, honey-dense samples had the medium (HGT, U-HGT) and lemon juice-dense samples had the lowest (LJGT, U-LJGT) pH level and it was found statistically significant ($p<0.05$). It is believed that the reason for this change is the added products and the proportions of these products. It is known that the pH of honey is low (3.2-4.5) **[23].** For this reason, the sample containing a high amount of honey is more acidic than the green tea sample. It is known that the pH of lemon juice is 2.9 on average **[24].** Therefore, it is explained that the sample where the lemon juice is dense has a lower pH than the sample where the honey is dense.

## Total dry matter results of tea samples

The total dry matter results of tea products are shown in Table 2. The results of each product revealed statistically significant differences ($p<0.05$). For all samples, it was observed that the traditional method had a higher dry matter ratio than the ultrasound method. Ultrasound extraction time can be extended to achieve the level of solids transition in the conventional method. Among the product types, it has been proven that HGT has the highest dry matter and GT has the lowest dry matter. Because honey is a low-moisture food with an average moisture content of 17% **[23].** The reason why LJGT contains higher dry matter than GT can be because lemon contains 8.4% dry matter **[15].**

**Table 2.** Total dry matter results of tea samples.

| Sample | Total Dry Matter* (%) |
|---|---|
| GT | $0.53^{e} \pm 0.11$ |
| HGT | $4.82^{a} \pm 0.08$ |
| LJGT | $3.62^{c} \pm 0.08$ |
| U-GT | $0.32^{f} \pm 0.004$ |
| U-HGT | $4.60^{b} \pm 0.13$ |
| U-LJGT | $2.94^{d} \pm 0.06$ |

* Data with the same superscript in the same column does not show a significant difference according to Duncan's test ($p<0.05$).

## Phenolic material and antioxidant activity

The total phenolic substance and antioxidant activity results of tea samples are shown in Table 3. Although there is no statistically significant difference between the U-HGT and U-LJGT samples, the other samples showed a statistically significant difference ($p<0.05$). The most effective result was found in the LJGT sample. The ultrasonic extraction method did not show a positive effect in terms of extraction of phenolic compounds compared to the traditional brewing method.

Honey contains lots of bioactive constituents, such as enzymes (glucose oxidase, diastase, invertase, catalase, and peroxidase), ascorbic acid, vitamins, proteins, and organic acids **[25].** It is known that especially vitamin C and glucose oxidase have antioxidant activity in honey **[26].** In our study, there was no increase in the total phenolic content of the honey-added samples, while it was observed that the antioxidant activities increased. It can be concluded that not all antioxidant activities in honey come from the phenolic property. This information explains the relation between the antioxidant level and total phenolic compound.

**Table 3.** Phenolic substance and antioxidant activity results of tea samples.

| Sample | Total Phenolic Substance* (mg GAE/ 1mL tea) | Antioxidant Activity* (DPPH radical scavenging activity, %) |
|---|---|---|
| GT | $285.76^{b} \pm 2.80$ | $62.42^{b} \pm 6.15$ |
| HGT | $277.65^{c} \pm 1.64$ | $89.03^{a} \pm 2.38$ |
| LJGT | $300.76^{a} \pm 3.34$ | $91.46^{a} \pm 1.94$ |
| U-GT | $247.48^{d} \pm 2.03$ | $53.80^{c} \pm 3.58$ |
| U-HGT | $237.37^{e} \pm 4.10$ | $90.61^{a} \pm 0.56$ |
| U-LJGT | $237.32^{e} \pm 2.69$ | $92.72^{a} \pm 0.57$ |

*Data with the same superscript in the same column does not show a significant difference according to Duncan's test ($p<0.05$).

When the antioxidant activity results of the samples were examined, it was determined that the U-LJGT had the highest value. However, it is seen that there is no statistically significant difference between LJGT and U-HGT ($p>0.05$). The lowest value belongs to the U-GT and then the GT sample. The lowest value reached in green tea samples shows the effect of added ingredients.

**Sensory analysis results of tea samples**

Sensory evaluation results of tea samples are given in Table 4 and Figure 2. There is no statistically significant difference ($p>0.05$) in terms of odor and color for all tea samples. A significant difference in taste was found in the samples with honey and lemon juice compared to the green tea sample. When evaluated in terms of general aspect, there is a statistically significant difference in the example of the high amount of lemon juice compared to the control green tea sample. The addition of honey and lemon to green tea has been shown to improve sensory properties.

**Table 4.** Sensory analysis results of tea samples.

| Sample | Odor | Color | Taste | General Aspect |
|---|---|---|---|---|
| GT | 4.12[a] ± 0.86 | 4.35[a] ± 0.79 | 3.12[b] ± 0.93 | 3.59[b] ± 0.71 |
| HGT | 4.06[a] ± 0.97 | 4.12[a] ± 0.93 | 3.88[a] ± 0.86 | 4.12[ab] ± 0.70 |
| LJGT | 4.29[a] ± 0,99 | 3.94[a] ± 0.83 | 3.88[a] ± 0.93 | 4.18[a] ± 0.88 |

[*] Data with the same superscript in the same column does not show a significant difference according to Duncan's test ($p<0.05$).

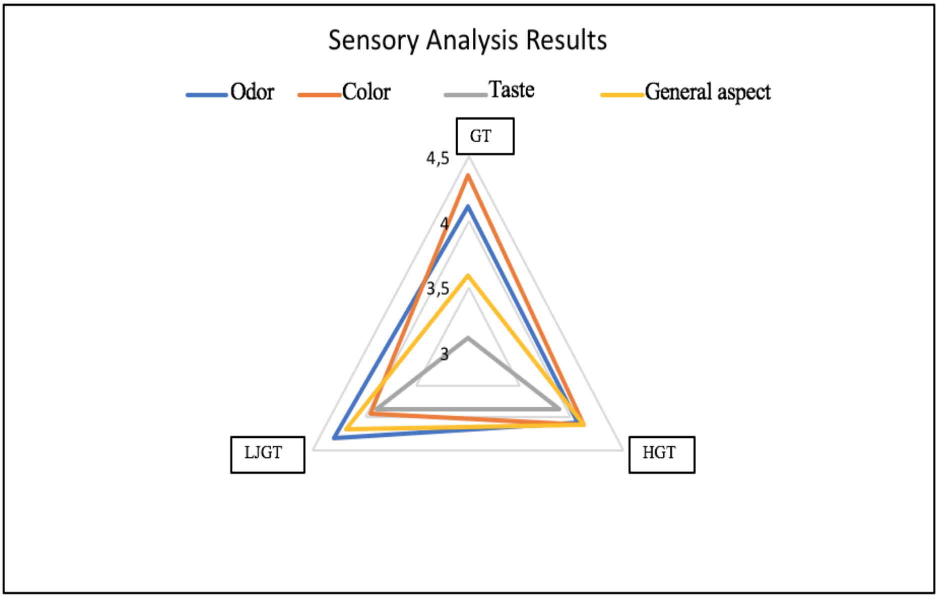


**Figure 2.** Sensory analysis results diagram.

## CONCLUSION

Cold tea is one of the drinks that is widely consumed in Türkiye and around the world. Various additives such as colorants, sweeteners, synthetic antioxidants, and preservatives are used in the products on the market. The interest in the consumption of healthy food products is increasing daily.

It is proven that the tea formulations prepared in this study are promising for the industry. When the total phenolic substance, antioxidant activity, and sensory analysis results were evaluated, it was concluded that the best formulation was green tea containing a high amount of lemon juice. Therefore, adding honey, lemon peel, and lemon juice to green tea, can meet the market needs because of the high nutritional and good sensory properties.

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