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Supervisor

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by

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Rabia YILDIZ

ABSTRACT

INVESTIGATION OF CONSUMPTION OF HONEY AND POLLEN TOGETHER WITH GREEN TEA

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Green tea *(Camellia sinensis)* is a tea species originated in China and has common production in many countries in Asia. This plant species has polyphenols with natural antioxidant properties which are very beneficial for human health in its structure. In addition, it has been reported that antioxidant substances, which are available, are good for the health problems caused by smoking. The aim of the study was to develop a likable product made with green tea, honey, and pollen which are also rich in natural antioxidants. The ratio of honey to pollen and percentage of non-tea substances constitute 2 factors of design. The results were compared with untreated green tea after the sensory analysis, total phenolic content, antioxidant capacity determination, ascorbic acid determination, and sugar analysis were carried out. The optimization of the final product was carried out by considering the highest total phenolic content, total antioxidant, and overall sensory evaluation. The optimized conditions were 4:1 honey to pollen ratio and 6% total concentration of non-tea substances with TPC of 578.674 mg GAE/L sample and TAC of 94.463 DPPH•IC_{50}. Optimum score determined for sensorial evaluation was 3.8.

Keywords: Green Tea, Honey, Bee Pollen, Antioxidant

ÖZET

YEŞİL ÇAYIN BAL VE POLEN İLE BİRLİKTE TÜKETİMİ ÜZERİNE BİR ARAŞTIRMA

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Mayıs 2019

50 sayfa

Yeşil çay *(Camellia sinensis)*, anavatanı Çin olmakla birlikte üretimi Asya'daki çoğu ülkede yaygın olan bir çay türüdür. Bu bitki türünün özelliği, yapısında insan sağlığı açısından son derece faydalı olan doğal antioksidan özelliğe sahip polifenolleri barındırmasıdır. Bununla birlikte, sahip olduğu antioksidan maddeler sayesinde sigara kullanımının yol açtığı sağlık problemlerine karşı iyi geldiği belirtilmiştir. Çalışmanın amacı, doğal antioksidanlar açısından zengin yeşil çayı, bir başka doğal antioksidan olan bal ve polen ile karıştırarak yeni bir ürün geliştirmektir. Bal:polen oranı ve çay dışı maddelerin yüzdesi tasarımın 2 faktörünü oluşturmaktadır. Geliştirilen ürünün, toplam fenolik madde tayini, antioksidan kapasitesi tayini, askorbik asit tayini ve şeker analiziyle birlikte duyusal analizi yapıldıktan sonra, sonuçlar ekleme yapılmamış yeşil çay ile karşılaştırılmıştır. Son ürünün optimizasyonu, en yüksek toplam fenolik içerik, toplam antioksidan aktivitesi ve genel duyusal değerlendirme göz önüne alınarak gerçekleştirildi. Optimize edilmiş şartlar; bal:polen oranı 4:1 ve çay dışı maddelerin toplam konsantrasyonu %6 olmakla birlikte, proses değişkenlerinin bu oranlardaki toplam fenolik içeriği 578.67 mg GAE/L ve toplam antioksidan kapasitesi 94.46 DPPH•IC_{50} 'dir. Duyusal analiz için belirlenen optimum puan 3.8'dir.

Anahtar Kelimeler: Yeşil Çay, Bal, Polen, Antioksidan

To my family…

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LIST OF ABBREVIATIONS

ANOVA : Analysis of Variance

FAO : Food and Agricultural Organization

TPC : Total Phenolic Content

TAC : Total Antioxidant Capacity

QE : Quercetine Equivalent

DPPH : 1,1-Diphenyl -2-picrylhydrazyl radical

GAE : Gallic Acid Equivalent

EGCG : Epigallocatechin gallate

ECG : Epicatechin gallate

HPLC : High Performance Liquid Chromotography

SFE : Supercritical Fluid Extraction

NMR : Nuclear Magnetic Resonance

MS : Mass Chromatography

HMF : Hydroxymethylfurfural

ppm : Parts per million

ppb : Parts per billion

WHC : Water Holding Capacity

EC_{50} : Concentration of Half-maximal Response

IC_{50} : Concentration of an indicator where the response is reduced by half

RSM : Response Surface Methodology

CCD : Central Composite Design

CHAPTER 1

INTRODUCTION

1.1 General

Green tea *(Camellia sinensis)* is a tea species that is originated in China and has common production in many countries in Asia. This plant species, which is also very common in consumption, has polyphenols with natural antioxidant properties which are very beneficial for human health in its structure and has anti-carcinogenic properties against various types of cancer such as lung and liver. In addition, it has been reported that antioxidant substance is good for the health problems caused by smoking. However, bee products (honey, pollen, propolis, and royal jelly) used in many prescriptions for human health thanks to their therapeutic effect come out as miraculous nutrients we can consume not only at breakfast but also at other meals. These products supply the requirements of the body such as vitamin, mineral, enzyme and amino acid when they consumed regularly. Moreover, they provide antioxidant for the body.

When the effects of these products on health is taken into consideration, it is proposed that their consumptions should be expanded. Despite the availability of various consumption forms of these products, further diversification is possible.

As a result of preliminary experiments performed in the laboratory, it was observed that bee pollen is soluble in brewed green tea. However, the solubility of propolis is fairly low in water. For this reason, it was decided to investigate the consumption of green tea with honey and pollen. Analysis of the final product includes; total phenolic content, antioxidant capacity, ascorbic acid, sugar content, and sensory analysis.

1.2 Objectives of the Study

The aim of the work was to develop a likable product made with green tea, honey and bee pollen which is rich in natural antioxidants and shown in various studies that have anti-carcinogenic, antimicrobial and anti-inflammatory effects. The antioxidant and sensory properties of this product will be enhanced in comparison with those of the base green tea. This tea with added bee products has not been commercially available in the market, and as far as we know no study has been conducted on the addition of two bee products to the green tea. Also, it is targeted to determinate the optimum mixing ratios of acceptable products and verifying the experiment with these conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Green Tea

Tea *(Camellia sinensis)* is a very common culture in terms of production and consumption all over the world, especially in Asian countries. According to the Food and Agriculture Organization of the United Nations (FAO), China is ranked as the world's highest tea producing country with 1.9 million tons. Turkey is the 5th place with 227 thousand tons. In still another information obtained from FAO, green tea (Figure 2.1) production is 8.2% higher than black tea. The amount of green tea production for 2023 is expected to be 2.97 million tons (Figure 2.2).

Figure 2.1. A picture of green tea leaves

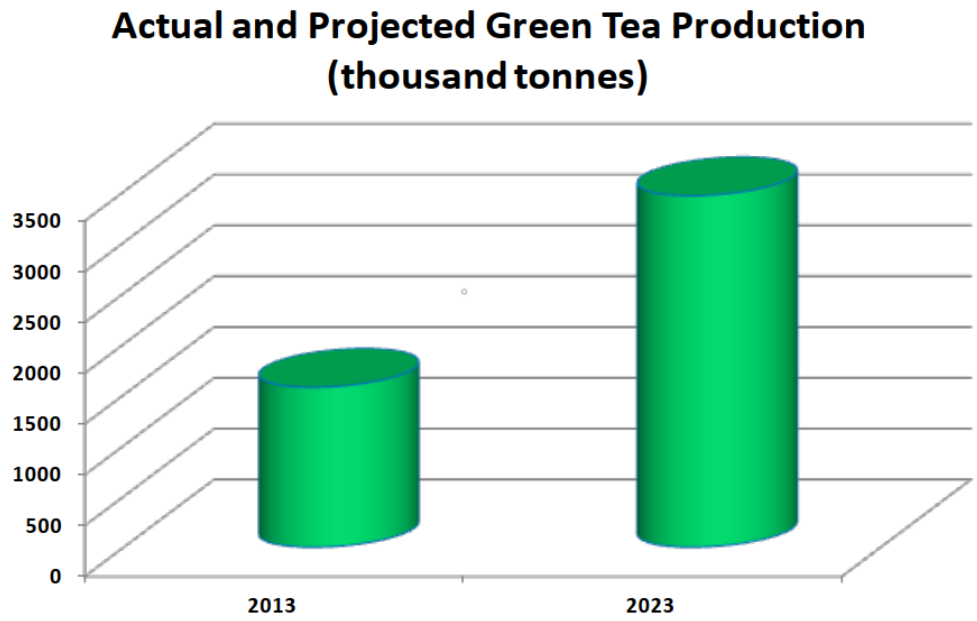

Figure 2.2. Actual and expected green tea production (FAO, 2015)

Turkish Food Codex expressed green tea as the non-oxidized product obtained as a result of production steps such as enzyme inactivation, curling, shredding, and drying. According to Wheeler's (2004) study, green tea production steps are ordered as steam treatment, rolling, drying and frying. During the preparation of green tea, the dried leaves are subjected to evaporation which causes the inactivation of intracellular enzymes. After the drying process, leaves are shredded. Thus, the activation of intracellular enzymes is ensured (Layher et al., 2013).

2.1.1 Phenolic Compounds in Green Tea

The reasons why green tea is one of the most consumed beverage are that polyphenols with natural antioxidant properties, anti-carcinogenic, anti-microbial and anti-inflammatory effects (Coppock and Dziwenka, 2016).

According to a study made by Atoui et al. (2005), it was tried to determine the phenolic profile and antioxidant activity of black tea, green tea, and other herbals. Infusion time for each herb was 3 minutes. Total phenolic content (TPC) was determined by using the Folin-Ciocalteu method and antioxidant activity by DPPH method. TPC value for green tea was 1216±32.0 mg GA/cup and 1 cup is equal to 240 ml. Antioxidant activity was 0.38 Quercetin Equivalents (QE). When these values are compared with those of other herbs, polyphenol content and antioxidant of green tea are the highest.

Mostly in literature, studies on catechin which is the most important polyphenol have been done. In a study, caffeine and catechin were extracted by Supercritical Fluid Extraction (SFE) method at different pressures (10, 20, 25, 30 Mpa), different temperatures (30, 40, 50, 60°C) and different extraction periods (1, 2, 3, 5 h) (Sokmen et al., 2017). It was determined that the optimum conditions for caffeine extraction were 60°C and 25 Mpa with 3 hours of extraction time. The conditions for the highest catechin was obtained under the same conditions using an ethanol modifier at a flow rate of 0.5 ml/min.

Green tea catechins can be exposed to oxidation or polymerization during processing. Ananingsih et al. (2011) investigated how these operations and storage conditions such as temperature and relative humidity affect the stability of green tea. Consequently, the aqueous solution of tea catechins was stable at pH below 4, but non-stable at pH above 6 and may undergo degradation. However, it has been also reported in the research that EGCG (Epigallocatechin gallate) was stabilized with a reduction of only 5% after 6 hours with a low oxygen concentration at 37°C and a pH of 7.4. Friedman et al. (2009) observed that EGCG decreased by 28% and ECG (Epicatechin gallate) decreased by 51% when tea leaves stored at 20°C for 6 months.

Polyphenols with high antioxidant capacity are quite important to prevent oxidative reactions in foods. Besides, antioxidants are helpful to increase shelf life. To determine the phenolic profile of green tea, there are some methods such as liquid chromatography (HPLC), spectrophotometry, mass spectrometry (MS) and nuclear magnetic resonance (NMR) (Lorenzo and Munekata, 2016). According to this study, most abundant flavonoids in green tea are gallocatechin, catechin gallate, gallocatechin gallate, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate. In addition, the glycoside structure of kaempferol and quercetin are also available in flavonol content of green tea as kaempferol-3-O-(glucose-(1,3-rhamnose-1,6-glucose)) and quercetin-3-O-(glucose-(1,3-rhamnose-1,6-galactose)).

2.1.2 Functional Properties of Green Tea

David Jockers who is a doctor of natural medicine made a search on green tea and its benefits which are shown in Figures 2.3 and 2.4 (DrJockers, 2016). He emphasized DNA regenerative effects, anti-inflammatory and anti-carcinogenic effects of green tea under the favor of a polyphenol which is known as EGCG.

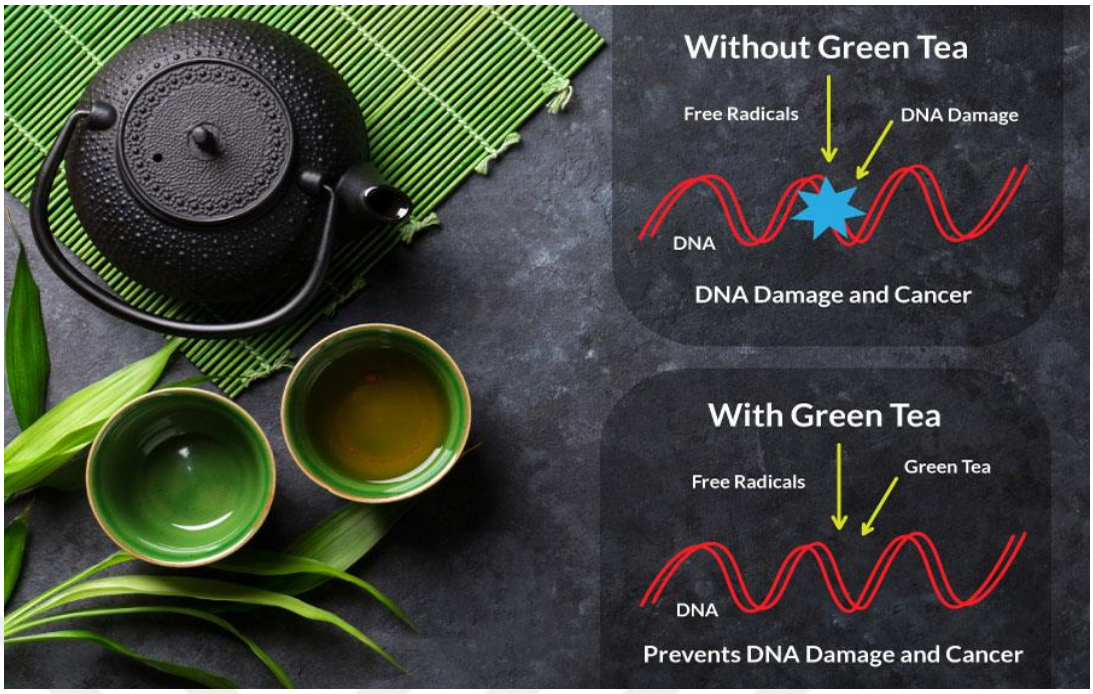

Figure 2.3. Schematic of DNA regenerative effects of green tea

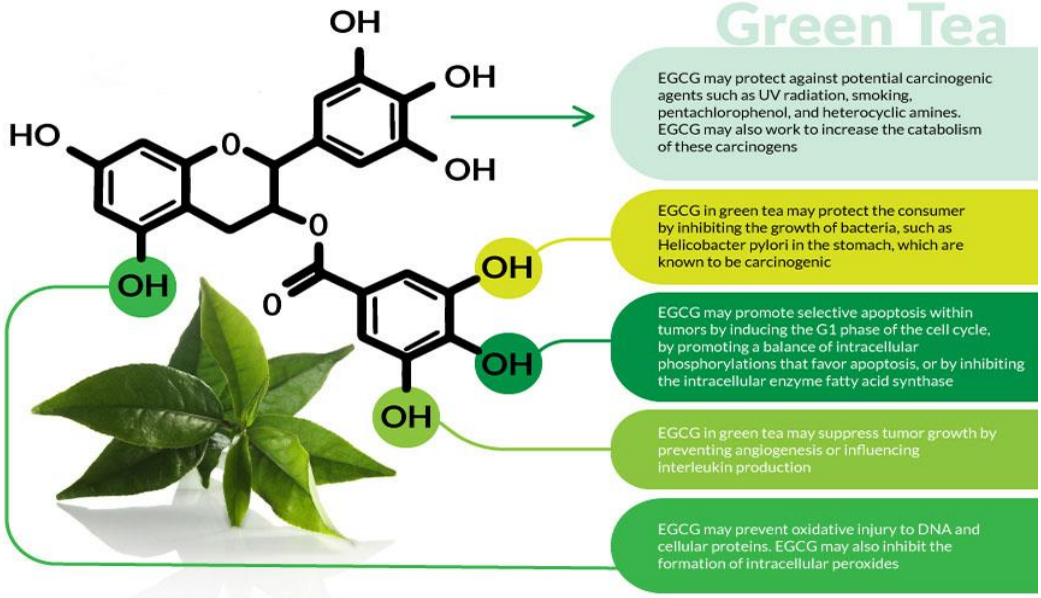

Figure 2.4. Chemical structure of EGCG *(Epigallocatechin gallate)* and its benefits

Another important benefit of green tea is the effect of reducing cigarette use. Considering the damages caused by smoking, cancer is the most harmful effect of it. For this reason, Azimi et al. (2017) conducted a study to compare salivary antioxidant

levels of smokers who consumed green tea with those of non-smokers. It was found that the change in TAC (Total Antioxidant Capacity) of non-smokers was 16.5 (3.47) mM/50µl and of smokers were 145.9 (14.07) mM/50µl.

2.2 Honey

Honey (Figure 2.5) is a product that has been used for years and believing that it is "curative nutrient". In the literature, anti-carcinogenic, anti-microbial and anti-inflammatory effects of honey have been frequently emphasized (Manyi-Loh et al., 2011; Mahmoudi et al., 2012; Can et al., 2015). The chemical composition of honey consists of sugar, water, proteins, vitamins, organic acid, mineral, phenolic compounds, and free amino acids (da Silva et al., 2015). The elements of honey are shown in Figure 2.6 (Jaganathan and Mandal, 2009).

Figure 2.5. A picture of honey

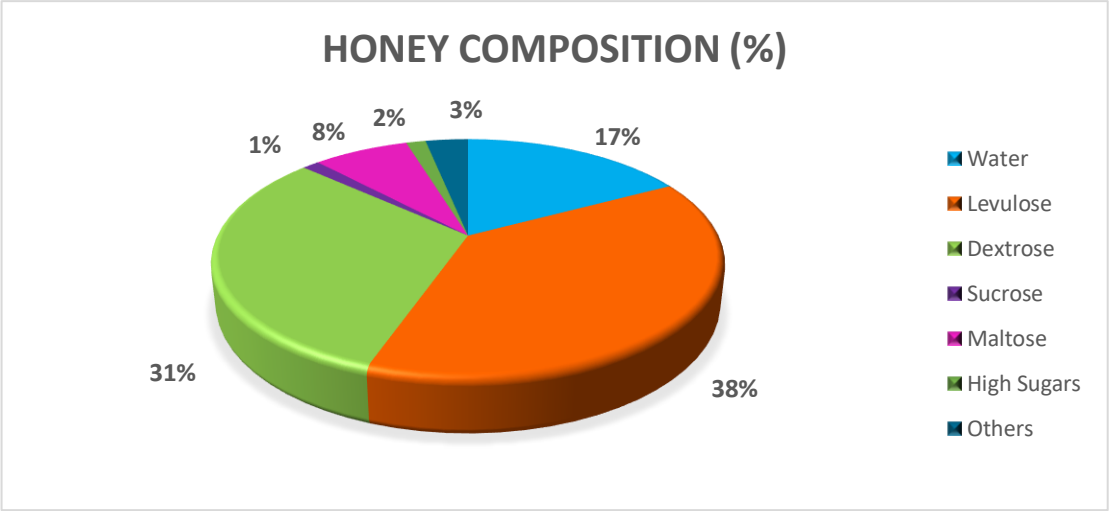

Figure 2.6. Elements of honey as percentages

Honey can be categorized in accordance with its source. Blossom honey is the nectar of flowers, but honeydew honey is formed of secretion of insects that live on the plant (Mutlu, 2016).

Turkish Food Codex Honey Communiqué (No: 2012/58) has edited characteristics of honey as shown in Table 2.1.

Table 2.1. Characteristics of honey according to Turkish Food Codex

Properties	Blossom	Honeydew	Blossom& Honeydew
Moisture (max. %)	20-23	20	20
Sucrose (max. %)	5-15	5-10	5
Fructose+ Glucose (min. %)	60	45	45
Water Insolubles (max. %)	0.1	0.1	0.1
Free Acidity (max. meq/kg)	50	50	50

Table 2.1. (Continue)

Properties	Blossom	Honeydew	Blossom& Honeydew
Electrical Conductivity (mS/cm)	Max. 0.8	Min. 0.8	Max. 0.8
Diastase Number (min.)	8	8	8
HMF (max. ppm)	40	40	40
Proline (min. ppm)	300	300	300
Naphthalene (max. ppb)	10	10	10

Quality parameters of honey are determined by examining the physicochemical properties which have been shown above-mentioned table. These properties given in the Table 2.1 can be interpreted as follow;

► Water content affects the quality, viscosity, and crystallization of honey (Nombre et al., 2010). Crystallization leads to an increment in water activity and thereby the fermentation of honey (Mahmoudi et al., 2012). Due to the fermentation, chemical structure and sensory properties of honey deteriorate. Therefore, the water content is very important for the quality of honey.

► The acidity increases with low pH value. The acidic pH value makes the honey more resistant to spoilage caused by microorganisms growing in neutral environments (Ahmed et al., 2016).

► The number of diastases was defined as the amount of starch that amylase enzymes in 100 grams of honey breakdown within 1 hour at 40°C (Chuttong et al., 2016). It is a quality parameter for honey due to the denaturation of diastase enzymes during heat treatment (Sramek et al., 2016).

► Crystallization mostly depends on the fructose/glucose ratio in honey. As long as the concentration of glucose increases, crystallization rate also increases (Escuredo et al., 2014). Since crystallization is undesirable by consumers, the heat treatment is

applied to honey by some producers (Costa et al., 2015). Thereby, HMF formation occurs.

► Another important quality parameter is *5-Hydroxymethylfurfural* (HMF) and it is a substance that has high toxication potential (Tornuk et al., 2013). HMF is the by-product of the *Maillard* reaction and is formed due to the dehydration of sugar during heat treatment (Barra et al., 2010). Also, it can be generated during extended storage (Wang et al., 2009). During the dehydration of glucose and fructose, *3-Deoxyosone* which is the causative component of 5-HMF is formed (da Silva et al., 2015). Turkish Food Codex established that HMF content must not exceed 40 mg/kg honey.

In a study in Tunisia (Boussaid et al., 2014), psychochemical properties of honey samples were determined. Mint, rosemary, eucalyptus, horehound, thyme and orange honey samples were collected from different regions of Tunisia. It has been reported that water contents ranged from 17.27 to 19.80%, protein contents were 0.13 to 0.16%, proline contents were 39.62 to 102.60 ppm, electrical conductivities were 0.39 to 0.89 mS. cm^{-1}, pH values were 3.67 to 4.11, and HMF contents were ranged from 12.07 to 27.43 ppm. In another similar study, characteristics of Spanish Acacia honey were examined. According to the results, HMF content was 3.3 ppm which is quite low (Juan-Borras et al., 2014).

In brief, the characteristic of honey may vary depending on the region where it was produced. Many researchers studied on the characteristics of honey from various areas (Aazza et al., 2014; Ahmed et al., 2016; Akbulut et al., 2009; Alves et al., 2013). Table 2.2 demonstrates the physicochemical properties of honey samples collected from different countries.

Table 2.2: Physicochemical properties of honey samples from different countries

Countries	Moisture (%)	Electrical Conductivity (µS/cm)	Color	HMF (mg/kg)	Diastase Number	References
Turkey	17 – 20.8	0.2 – 3.1	42.9 – 88.5 (L*)	<40	6.3 – 13.2	**(Can et al., 2015)**
Spain	15.4 – 17.4	0.24 – 0.99	Light Amber – Dark Amber	5.4 – 15.0	11.5 – 45.8	**(Manzaranes et al., 2014)**
Greece	10.5 – 20.5	0.8 – 1.75	60.8 – 72.7 (L*)	-	-	**(Karabagias et al., 2014)**
Algeria	11.6 – 14.1	0.4 – 0.8	-	15.2 – 24.2	-	**(Khalil et al., 2012)**
Argentina	14.1 – 18.8	0.12 – 0.7	Extra White – Dark Amber	4.0 – 26.3	-	**(Isla et al., 2011)**
Morocco	14.6 – 18.6	0.3 – 1.12	White Amber – Dark Amber	7.2 – 30.4	6.1 – 19.1	**(Chakir et al., 2011)**

2.2.1 Phenolic Compounds in Honey

Honey is an extremely beneficial nutrient for human health due to its phenolic compounds with the natural antioxidant property. These are flavonoids, carotenoids, thiamin, riboflavin, ascorbic acid and also benzoic, ferulic and caffeic acid (Khalil et al., 2012). Many researchers have studied the phenolic compound profile of honey. They (Alvarez-Suarez et al., 2012; Trautvetter, Koelling-Speer & Speer, 2009) determined "Vanillic acid, Caffeic acid, β-coumaric acid, 3-Hydroxybenzoic acid" as the major phenolics. Özcan and Al Juhaimi (2015) reported that the region where honey produced affects the antioxidant activity as well as the other properties. Ferreira et al. (2009) expressed that phenolic compounds affect the color of honey, therefore dark-colored honey has a higher amount of phenolic compounds than light-colored honey.

Honey has anti-carcinogenic activity due to the effects of flavonoids, p-coumaric acid, caffeic acid, p-hydroxybenzoic acid and vanillic acid in its structure according to the literature reports (Spilioti et al., 2014; Eteraf-Oskouei and Najafi, 2013). The chemical structure of some organic acids, flavonoids and phenolic compounds which are present in honey have been shown Figures 2.7 and 2.8 (Erejuwa et al., 2012).

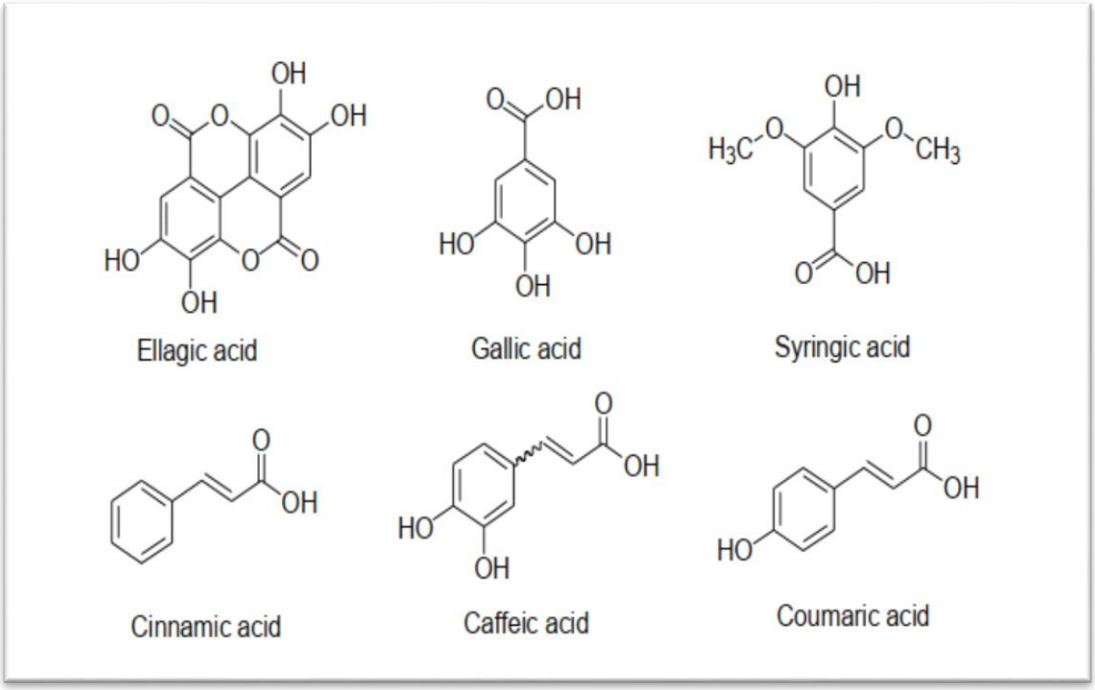

Figure 2.7. Chemical structure of organic acids present in honey (Erejuwa et al., 2012)

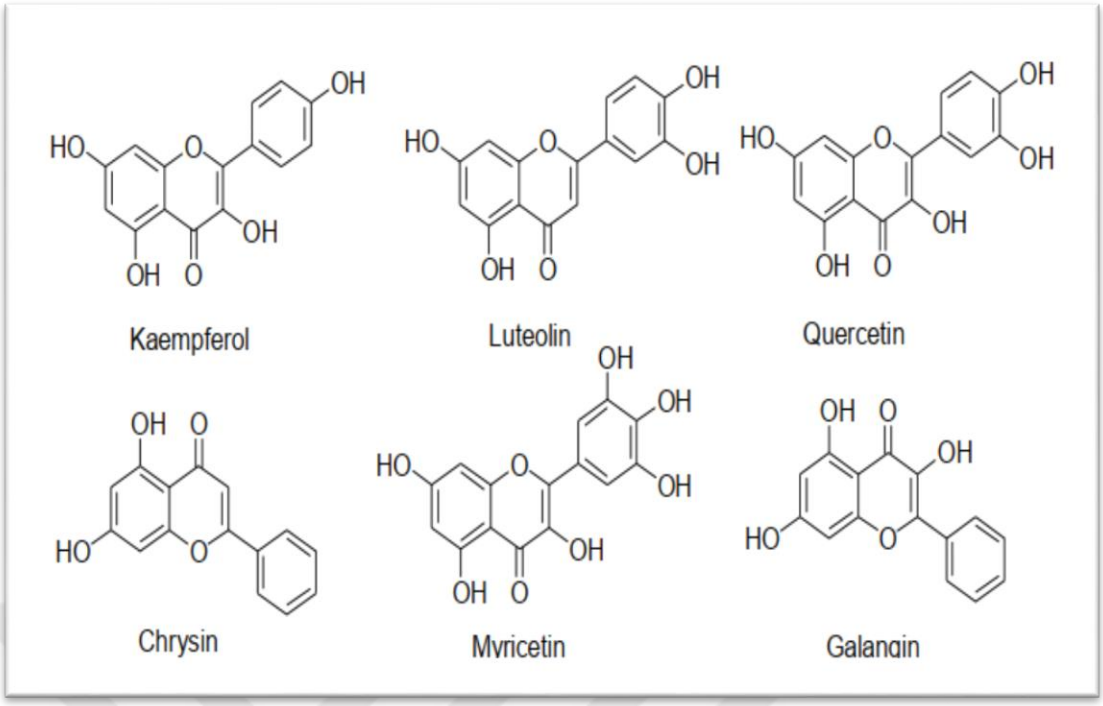

Figure 2.8. Chemical structure of flavonoids and phenolic compounds in honey (Erejuwa et al., 2012)

There are a few studies on honey added products such as foods and beverages in literature.

In a study conducted by Toydemir et al. (2015), it was discussed how honey changes the antioxidant activities when added to herbal teas. In this context, flower and pine honey were added into 9 types of herbal tea which were Green tea, Melissa, Rosehip, Sage, Echinacea, Fennel, Linden, Ginger, and Daisy) at 55°C, 65°C, 75°C and 85°C. Infusion time was determined as 3 minutes. HMF ranges were determined as 4.6-8.1 mg/kg for both types of honey. Besides, it was concluded that the addition of both honey species increased the TPC and antioxidant activity up to 57%. Total phenols were expressed by Gallic acid equivalent (GAE). It was observed that Melissa, Green tea and Rosehip had the highest phenolic content (465 mg GAE/L of green tea) (Toydemir et al., 2015).

Jaya et al. (2017) investigated that how it affects to add honey to the kefir whey beverage as sensorial and turbidity of the outcome. The results analyzed by ANOVA showed that 40% honey with color 3.25±0.78, aroma 3.50±1.14, taste 3.75±1.01, and turbidity 306.7±6.65 NTU was the most acceptable ratio.

In another study in Indonesia (Krisnaningsih and Yulianti, 2015), different amounts of honey (2, 4, 6, 8 and 10%) were added to yogurt to determine the quality effects which are protein content and pH value. It has been reported that the highest preference level belonged to 10% honey with the protein content of 4.3% and pH at 4.

In still another similar study (Stijepic et al., 2013), various levels of honey (2, 4, 6%) were added to probiotic yogurt to enrich its content. Water holding capacity (WHC), syneresis and viscosity of produced samples were analyzed during storage at 4±1°C for 21 days. The results revealed that the best level was 4% honey and it was observed that this ratio increased viscosity and WHC, however, decreased syneresis.

Celep and Yeşilada (2017) made a search on the effects of honey added to different coffee types in terms of bioaccessibility of phenolic contents and antioxidant capacity. According to the results, brewed coffee has a higher antioxidant capacity than the soluble one. However, total phenolic contents were on the decline. They expressed that such a decrease would be based on diversity in pH during digestion.

In a study examining the antibacterial effects of adding honey to lime juice, it was observed 50% of lime juice was an effective antibacterial, but the addition of honey did not show any alteration on results (Eveline and Yuliana, 2014).

Belscak et al. (2009) investigated that how it affects to add honey and ascorbic acid to ten different fruit teas (blueberry, peach and tangerine, pink grape, apple and cinnamon, orange, rose hip, forest fruit, apricot, cherry and strawberry) as antioxidant properties. 2 grams of all tea samples were brewed with 100 ml of water for 8 mins. Three types of the run were prepared: only tea, tea with ascorbic acid, and tea with ascorbic acid and honey. Optimum amounts of honey and Vitamin C were determined as 1.5 g and 22.5 mg. While the addition of Vitamin C increased total phenolic content, honey addition to ascorbic acid containing sample decreased 28%.

2.3 Bee Pollen

Bee pollen (Figure 2.9) is another important nutrient produced by bees, even though its consumption is not common as much as honey.

Figure 2.9. A picture of bee pollen

Bee pollen is an essential nutrient for the growth and reproduction of honey bees. The pollen is formed in the anther of the plant and grows in vesicles on anther. After formation, it is collected by traps called 'perforated honeycomb grid'. The collected pollen is dried at temperatures less than 45°C in order to preserve its nutritional value. Pollen grains can be varied by size, color and weight such as round, triangle cylindrical or spiny (Shubharani et al., 2013). This diversity depends on the plant species and also environmental conditions (Arruda et al., 2013; Melo et al., 2009).

Bee pollen (Figure 2.10) contains carbohydrates, protein and amino acids, lipids, enzymes, coenzymes, vitamins and phenolic compounds (Campos et al., 2008; Campos et al., 2010). The composition of bee pollen is 37% of protein, 51% of carbohydrates, 1% of vitamins, 8% of lipids and 3% of phenolic compounds.

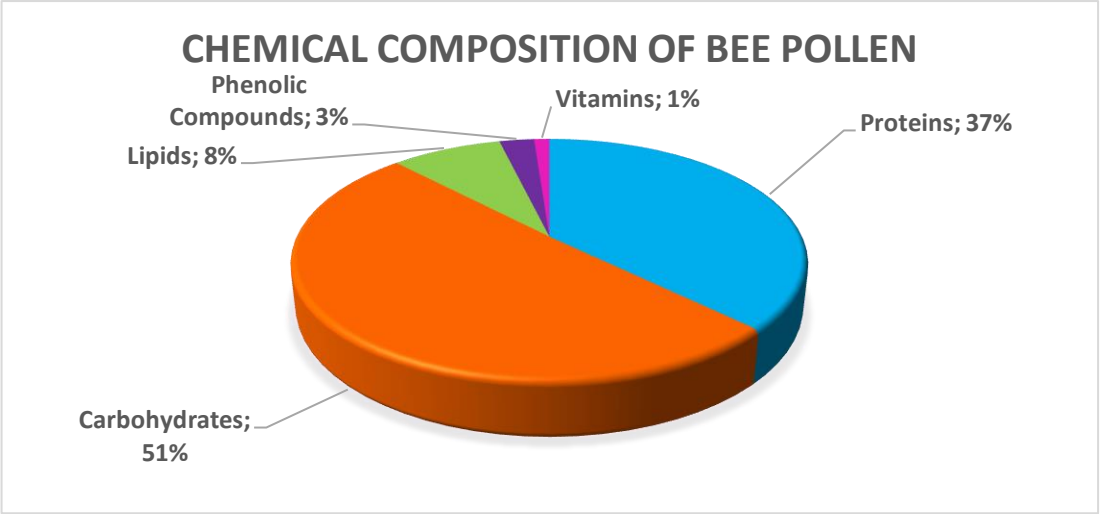

Figure 2.10. Chemical composition of bee pollen

2.3.1 Phenolic Compounds in Bee Pollen

The flavonoids found in bee pollen are "quercetin, naringenin, kaempferol, isorhamnetin, galangin, pinocembrin, luteolin, apigenin, rutin, catechin, delphinidin" (Mohdaly et al., 2015; Šarić et al., 2009; Zhang et al., 2016; Sun et al., 2017; Canale et al., 2016; Kedzia and Holderna-Kedzia, 2005; Roulston and Cane, 2000; Szczesna, 2006; Komosinska-Vassev and Olczyk, 2015). In addition, "syringic acid, gallic acid, *p*-coumaric acid, caffeic acid, and ferulic acid were found in bee pollen as phenolic acid (Silva et al., 2009; Negri et al., 2011; Mohdaly et al., 2015). Organic structures of some of these phenolic compounds were shown in Figure 2.11 and 2.12.

The flavonoids and phenolic acids which are present in the structure of pollen are responsible for its anti-inflammatory effects (Komosinska-Vassev and Olczyk, 2015). In addition to anti-inflammatory effects of pollen, it is reported that it has anti-microbial, anti-carcinogenic and antioxidant effects (Pascoal et al., 2014; Xua et al., 2012; Fatrcova-Sramkova et al., 2013).

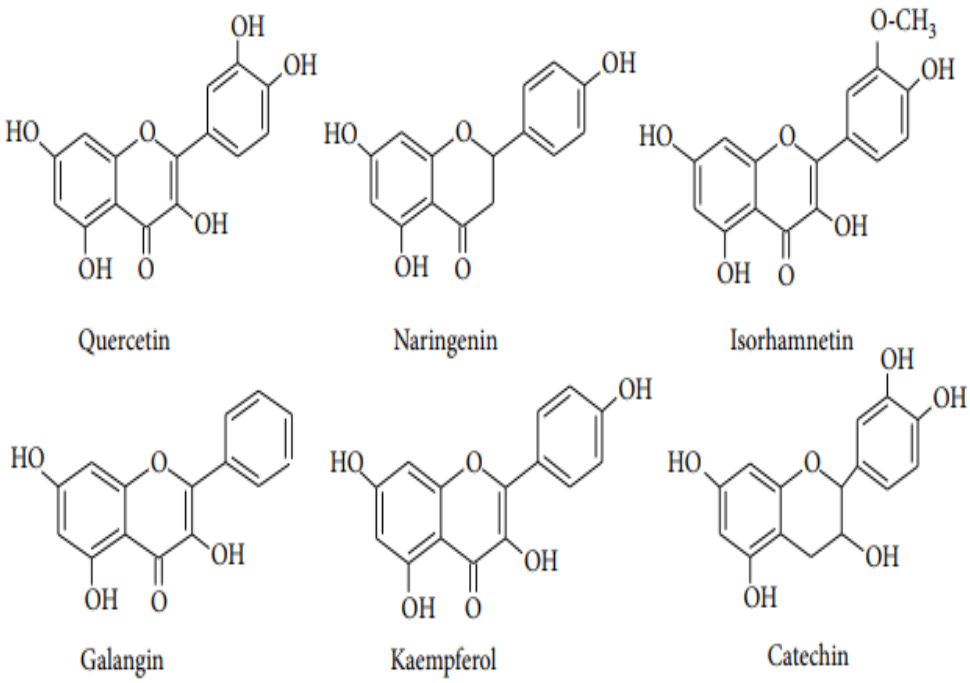

Figure 2.11. Organic structures of some flavonoids in bee pollen (Kocot et al.,2018)

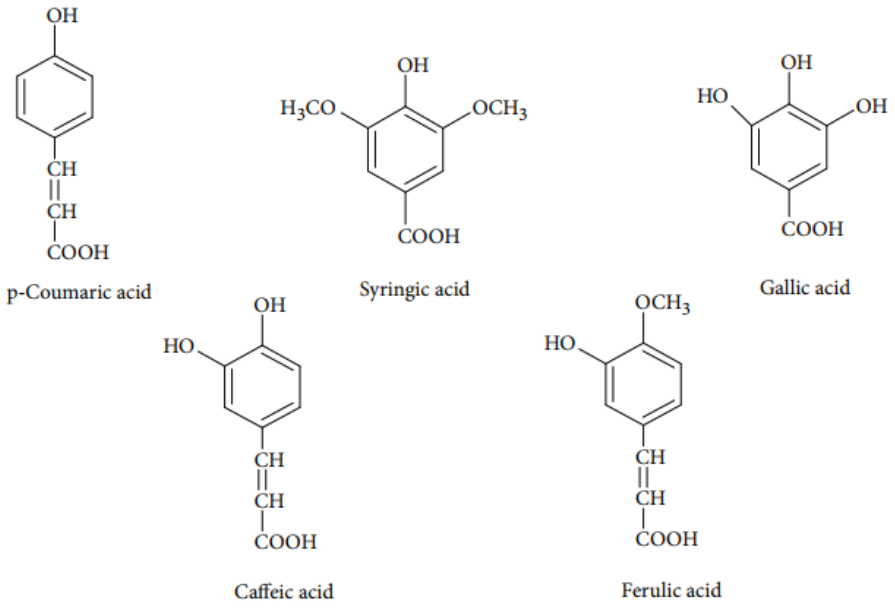

Figure 2.12. Organic structures of some phenolic acids in bee pollen (Kocot et al., 2018)

In a study, bioactive compounds and biological effects of 8 different pollen samples were investigated by Pascoal et al. (2013). The content of total phenolic compounds was expressed as Gallic Acid Equivalents (GAEs) and antioxidant activity as EC_{50} values (Pascoal et al., 2013). According to the results obtained from experiments, polyphenols ranged from 18.55 to 32.15 mg GAE/kg pollen. EC_{50} values were between 2.16 and 5.87 mg/mg extract.

Salgojova et al. (2014) made a search on the effects of rape bee pollen addition to a cookie on its quality. By this means, the nutritional properties of cookies provided to increase. Cookies were baked in the amount of 16% (1 g bee pollen/cookie) and 32% (2 g bee pollen/cookie) using bee pollen obtained from 2 different regions. Results showed that antioxidant activity increased by adding bee pollen. It means a higher amount of bee pollen addition (32%) has higher inhibition (55 and 67%). Also, the cookies baked with bee pollen obtained from the region Lenartovce have higher inhibition than the region Nove Zamky which means results vary across localities.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

In this study, zahter honey, multiflorous bee pollen, and green tea were used as raw material. Honey and Bee Pollen were procured by the firm called "Arı Hüneri" which operates beekeeping and sales of bee products in Gaziantep, Turkey. Green tea was purchased from a wholesale market in Gaziantep, Turkey. Honey and green tea were stored at room temperature and bee pollen was stored at +4°C until processing.

All other reagents and solvents which were used in analysis are; Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), gallic acid, methanol, 1,1 – Diphenyl – 2 – Picrylhydrazyl (DPPH), dinitrosalicylic acid reagent (DNS), hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide (KOH), fructose, acetic acid, 2,6 – Dichloroindophenol, sodium salt hydrate ($C_{12}H_{10}Cl_2NNaO_4$), meta phosphoric acid (HPO_3), L-Ascorbic acid, sodium bicarbonate ($NaHCO_3$). All these were analytically reagent grade and purchased from Merck (Merck, Darmstadt, Germany) and Sigma (Sigma-Aldrich, Steinheim, Germany).

3.2 Tea Sample Preparation

Green tea was infused by adding 200 ml of freshly boiled water on 4 grams of green tea (T_i=100°C). Brewing time was 3 minutes (T_f=70°C) (Toydemir et al., 2015). The residuum of the tea was filtered, then, honey and pollen were added to this filtrate. Brewing without any honey and pollen addition was used as control.

3.3 Experimental Design, Statistical Analysis, and Optimization

Response Surface Methodology (RSM) was applied for experimental data by using a commercial statistical package, Design-Expert (Version 10.0, Statease Inc., Minneapolis, MN, USA), for the generation of response surface plots. The experimental design was done by Central Composite Design (CCD) using two factors

and five levels. The process variables and the outline of the design with coded and actual levels were presented in Table 3.1 and 3.2. The independent variables were determined as honey to pollen ratio (1:4 – 4:1) and non-tea substances in the mixture (2% – 6%). Dependent variables were total phenolic content, total antioxidant capacity, ascorbic acid content, sugar content, and sensory analysis. The obtained design consists of 13 runs, including five replications of the center point in order to investigate the effect of honey and bee pollen on the properties of green tea samples. For each dependent variable, a mathematical model was obtained with multiple regression analysis method and significant terms in the model were found by analysis of variance (ANOVA). The goodness of fitness of the model was detected by Fisher test (F-test) value. The level of significance was set at 95% ($P<0.05$).

Moreover, numerical optimization was carried out for independent variables of green tea mixture. In parallel this purpose, desirability function of RSM was used. The desirability approach consists of;

a) Performing experiments and fitting response models (y_i) for all m (number of response),
b) Defining individual desirability functions for each response (d_i),
c) Maximizing the overall desirability.

The general approach is to first convert each response y_i into an individual desirability function d_i that varies over the range $0 \leq d_i \leq 1$ where if response y_i is at its target value, then $d_i = 1$, and if it is outside an acceptable region, $d_i = 0$. The overall desirability defines in Equation 3.1 as follows;

$$D = (d_1 d_2 \ldots d_m)^{1/m} \tag{3.1}$$

(Myers and Montgomery, 2002).

In the optimization process it was aimed to obtain total phenolic content, total antioxidant capacity and overall acceptability to be as high as possible.

Table 3.1. Process variables used in central composite design

		Variable Level Codes				
	Codes	-1.68	-1	0	1	1.68
Honey: Pollen	X_1	0:1	1:4	1:1	4:1	1:0
Total Concentration (%)	X_2	0	2	4	6	7.36

Table 3.2. The experimental design with coded and actual levels

	Coded Levels		**Actual Levels**	
Number Of Runs	X_1	X_2	Honey: Pollen	Total Concentration of Non-Tea Substances (%)
1	0	-1.68	1:1	0
2	0	1.68	1:1	7.36
3	-1.68	0	0:1	4
4	1	1	4:1	6
5	0	0	1:1	4
6	0	0	1:1	4
7	0	0	1:1	4
8	0	0	1:1	4
9	0	0	1:1	4
10	1.68	0	1:0	4
11	1	-1	4:1	2
12	-1	1	1:4	6
13	-1	-1	1:4	2

3.4 Methods of Analysis

3.4.1 Determination of Total Phenolic Content (TPC)

Total phenolic content of the sample was determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Briefly, 0.5 ml of diluted sample (1:10) was mixed with 2.5 ml of diluted Folin-Ciocalteu reagent (1:10, v/v). After 4 minutes, 2 ml of saturated sodium carbonate solution was added to test tubes and allowed to stand for 2h in the dark room. Then, the absorbance of the sample was measured by a spectrophotometer (UV/Vis – SP3000nano, Optima, Tokio, Japan) at 760 nm. Results were expressed as gallic acid equivalents (mg GAE/L). Gallic acid curve was shown in Figure 3.1. All the measurements were done in triplicate. Phenolic content of sample was estimated from the calibration curve.

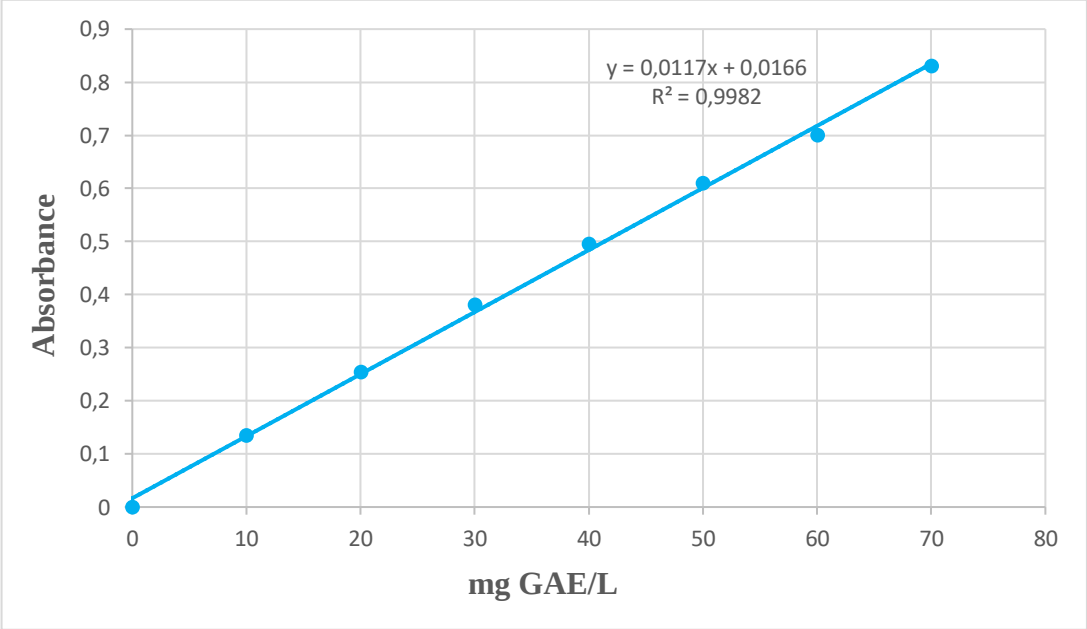

Figure 3.1. Gallic acid calibration curve

3.4.2 Total Antioxidant Capacity (TAC)

Total antioxidant capacities of the samples were determined by DPPH radical scavenging activity assay (Kumaran and Karunakaran, 2006). A 0.1 ml of each sample was completed with 3.9 ml of DPPH• methanolic solution. Also, a blank solution was prepared. This solution contains a DPPH solution without a sample. All tubes were left in a dark room for 30 minutes. After incubation, absorbances were read at 517 nm against pure methanol. Antioxidant activity was calculated according to Equation 3.2:

$$\% \text{ Inhibition of DPPH} = \left[1 - \left(\frac{Asample}{Ablank}\right)\right] x100 \quad (3.2)$$

Where

A_{sample} : Absorbance of the sample

A_{blank} : Absorbance of DPPH solution

The result was expressed as IC_{50} value which obtained from a curve drawn by measuring the absorbance of the final product at different concentrations. Different concentrations of final product were prepared and absorbances were read at 517 nm. These absorbances were used for calculation of inhibition percentages. The graph of concentration versus inhibition (%) gives the IC_{50} curve. IC_{50} refers to 50% inhibition of DPPH (Figure 3.2).

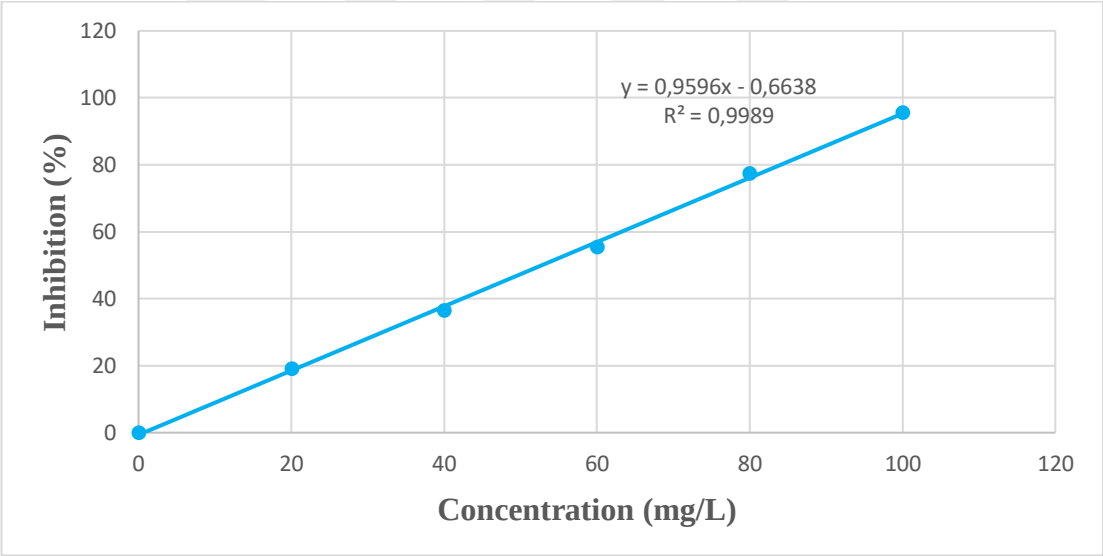

Figure 3.2. IC_{50} curve

3.4.3 Determination of Ascorbic Acid (Vitamin C)

Vitamin C contents of the samples were determined by the method described in AOAC (AOAC, 1968). Before making the experiment, the required solutions which are Metaphosphoric acid solution, Dye solution and Ascorbic acid solution were prepared.

- **Metaphosphoric Acid Solution:**

15 grams of metaphosphoric acid and 40 ml of acetic acid were mixed with distilled water in 500 ml of volumetric flask. After metaphosphoric acid dissolved completely, the solution was filtered to another volumetric flask. This solution was re-prepared every 7 days and stored in the refrigerator at +4°C.

- **Indophenol (Dye) Standard Solution:**

50 mg of 2,6 dichloroindophenol sodium salt hydrate (dye) and 42 mg of $NaHCO_3$ were dissolved, well and completed with distilled water in 200 ml. The solution was filled to amber glass and stored at +4°C until processing.

- **Ascorbic Acid Standard Solution:**

25 mg of L-ascorbic acid was dissolved with metaphosphoric acid in 25 ml of volumetric flask. This solution was prepared for standardization.

2 ml of ascorbic acid solution and 5 ml of metaphosphoric acid solution were taken into Erlenmeyer flask and titrated with dye solution. The spent volume of dye solution was recorded. Water as much as the spent volume of dye solution was mixed with 7 ml of metaphosphoric acid solution and titrated to obtain the volume of the blank. The difference between of spent volumes gives the final volume for the Equation 3.3:

$$\frac{mg\ Ascorbic\ acid}{ml\ sample} = \frac{X.F.V}{E.Y} \tag{3.3}$$

Where

X: spent volume of dye solution for sample analysis
F: mg ascorbic acid to 1 ml of dye solution
V: ml of diluted sample
E: the amount of sample (ml)
Y: volume of sample titrated

3.4.4 Determination of Sugar Content by DNS Method

Reducing sugars in the samples were determined by the DNS method (Saqib and Whitney, 2011). For the preparation of DNS (3,5 Dinitrosalicylic acid) reagent, 10 grams of DNS and 300 grams of sodium potassium tartrate were dissolved with 800 ml of 0.5N NaOH. Distilled water was added for the rest of 1000 ml of volumetric flask. The solution was stored at +4°C.

1 ml of diluted sample (1:10) was taken into the test tubes and 1 drop of 37.5% HCl acid was added. Samples were put in the water bath for 5 minutes at 90°C. After that, 3 drops of 5N KOH solution and 3 ml of DNS reagent were added to all tubes. The tubes were placed in boiling water for 5 minutes. Then, they were cooled at room temperature and added 6 ml of distilled water. Absorbance readings (UV/Vis – SP3000nano, Optima, Tokio, Japan) were taken at 540 nm. Results were expressed as mg fructose/L of tea mixture. The calibration curve for fructose given in Figure 3.3. All the measurements were done in triplicate.

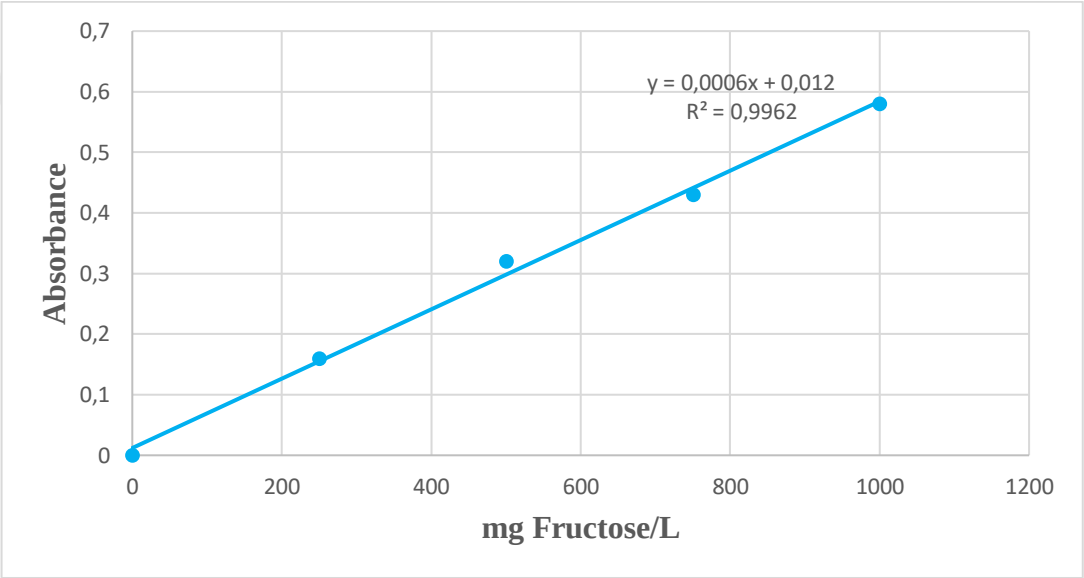

Figure 3.3. Fructose Calibration Curve

3.4.5 Sensory Analysis

8 samples (R1 and R8) were evaluated in terms of odor, taste, aroma, color and overall evaluation by 10 trained panelists, and scored over 5-point hedonic scale (1: dislike extremely and 5: like extremely). Samples were presented in white cups and they were labeled with random letters. The hedonic scale was given in Table 3.3.

Table 3.3. Hedonic scale used for sensory analysis of tea samples

Name of Panelist								
	Samples							
	R1	R2	R3	R4	R5	R6	R7	R8
Color								
Odor								
Taste								
Overall evaluation								

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Preliminary Studies

Determination of factors and their upper and lower levels for optimization were based on preliminary studies. Factors in this study were determined according to honey to bee pollen ratio and the total concentration of non-tea substances. The levels for the fraction of honey to pollen ranged between 1:4 and 4:1. The ratio above the upper level of honey and bee pollen has masked the taste and changed the color of green tea. In conjunction with this, the range of total concentration of non-tea substances was chosen between 2% and 6%. Initial content of tea sample (sample with non-added honey and bee pollen) was 464.44 mg GAE/L sample of TPC, 93.79 mg IC_{50}/L sample of TAC and 0.0145 mg ascorbic acid/L sample of Vitamin C content. Sugar content was zero.

4.2 Experimental Design and Model Fitting

Response surface methodology was used for experimental design. ANOVA was applied to evaluate the significant effects of independent variables on the responses. The significance of coefficients of fitted models was assessed by using *F*-test, *p*-value, lack of fit and coefficient of variation.

Table 4.1 shows the effect of honey to pollen ratio (1:4 – 4:1) and total concentration of non-tea substances (2 – 6%) on the total phenolic content (TPC), total antioxidant capacity (TAC), ascorbic acid content and sugar content of final product (green tea with honey and bee pollen). Regression analysis showed that the fitted models had a coefficient of determination (R^2) that varied from 0.62 to 0.96 (Table 4.2).

Table 4.1. Results of responses obtained from two-factor, five-level central composite design

Run	Honey: Pollen	Tot. Conc. (%)	TPC (mg GAE/ L)	TAC	Ascorbic Acid Content (mg/ml)	Sugar Content (mg Fructose /L)	Sensory (Overall Evalua.)
1	1:1	4	549.92	92.26	0.062319	2216.67	2.78
2	1:4	6	556.75	91.45	0.068116	1300	1.78
3	1:1	4	544.79	90.56	0.063768	2266.67	2.78
4	1:1	4	549.92	90.53	0.062319	2233.33	2.78
5	4:1	2	498.63	92.5	0.018841	1800	4.44
6	4:1	6	614.87	95.53	0.072464	5633.33	3.89
7	1:1	7.36	559.32	93.66	0.069565	4300	2.33
8	1:1	0	464.44	89.34	0.014493	0	0
9	1:1	4	541.39	90.69	0.062319	2216.67	2.78
10	1:4	2	481.54	89.34	0.024638	4666.67	3.11
11	1:1	4	538.80	90.68	0.063768	2250	2.78
12	1:0	4	524.27	94.20	0.017391	4633.33	4.11
13	0:1	4	524.27	88.95	0.03913	633.33	2.11

Table 4.2. The regression models by using independent variables of honey to pollen ratio (X_1) and total concentration of non-tea substances (X_2)

Response	Regression Model	R^2
TPC	$534.53+36.35X_2$	0.766
TAC	$91.52+1.66X_1+1.28X_2$	0.875
Vit C	$0.063+0.020X_2-0.012X_1^2–7.088E–003X_2^2$	0.958
Sugar Cont.	$2626.92+848.56X_1+797.23X_2+1800X_1X_2$	0.764
Sensory	$2.92+0.71X_1$	0.620

4.3 Evaluation of the Effects of Process Variables on Responses

Five responses (TPC, TAC, Ascorbic Acid (Vitamin C), Sugar Content and Sensory Perception) were decided for determining the most beneficial and acceptable product. Green tea, honey and bee pollen are very rich in total phenolic content and have high antioxidant capacity. Due to this fact, total phenolic content, total antioxidant capacity and ascorbic acid content (as an important antioxidant) of the product were investigated. In addition, sugar content was examined since experimental samples include honey.

Acceptability of final product by consumers is as important as other variables. Therefore, sensory perception were chosen as the fifth response.

The effects of independent variables (honey to pollen ratio and total concentration of non-tea substances) on responses were evaluated to determine optimum conditions. According to the preliminary works, it determined that change in temperature does not affect the responses. Therefore, it was not accepted as independent variable. The influences of process variables were indicated by 3D response surface graph. The regression model graphs described the effects of process variables by linear and quadratic equations.

4.4 The Effect of Process Variables on Total Phenolic Content (TPC)

Honey to bee pollen range was selected between 1:4 and 4:1 while total concentration of non-tea substances was ranged from 2% to 6%. The levels of these factors were evaluated in order to determine their effect on responses. One of the responses is total phenolic content. The results were expressed as Gallic acid equivalent (mg GAE/L). In Table 4.3, it was seen that the process variables have linear effect on TPC, significantly ($p<0.05$). It means that if honey to pollen ratio and total concentration increase, TPC increases, as well. However, honey to pollen ratio was not as effective as other variable, total concentration (Figure 4.1). In another word, total concentration of non-tea substances is the significant term of model ($p<0.05$). In literature, there is no study in which both honey and pollen are added to herbal tea. However, in a work studied by Toydemir et al. (2015), honey was added to various types of herbal teas, and green tea was one of the three herbal tea to have highest TPC when compared with control sample. Similar results were found in this manner.

Table 4.3. ANOVA table and estimated coefficients for total phenolic content

Source	Coefficients	Sum of Squares	df	Mean Squares	F value	p-value Prob>F
Model		13345.23	2	6672.61	16.35	0.0007*
Intercept	534.53					
Linear						
Honey:Pollen	7.79	585.94	1	585.94	1.44	0.2584
Total Conc.	36.35	12759.29	1	12759.29	31.27	0.0002
Residual		4080.59	10	408.6		
Lack of Fit		3980.66	6	663.44	26.56	0.0035*

*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.7658

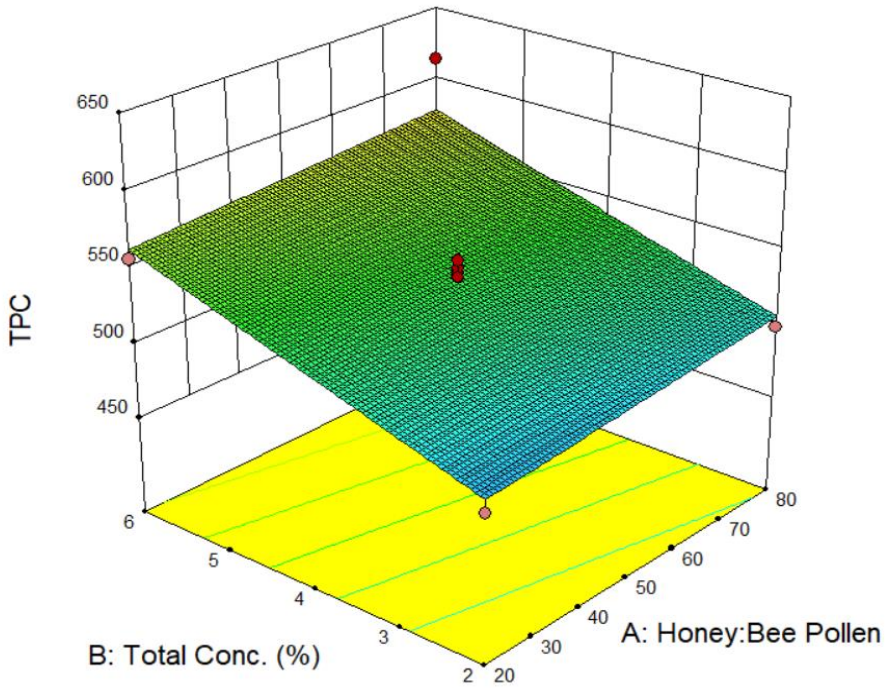

Figure 4.1. 3D response surface of the effects of total concentration and honey:pollen ratio on total phenolic content

4.5 The Effect of Process Variables on Total Antioxidant Capacity (TAC)

Second response was the total antioxidant capacity. The influences of total concentration of non-tea substances and honey to bee pollen ratio on TAC was investigated. ANOVA table and estimated coefficients for TAC was shown in Table 4.4. According to the results, independent variables (honey:bee pollen and total concentration) indicated a positive linear effect on TAC ($p<0.05$) (Figure 4.2). The lack of fit is not significant ($P>0.05$). That means the model fits well to actual values, i.e., model predicted data fit to actual experimental response data. Both honey to pollen ratio and total concentration of non-tea substances are significant model terms ($p<0.05$). It means when honey to pollen ratio and total concentration increased, increase in TAC can be noted. This was an expected result considering the relationship between TPC and TAC. There is not so much studies about this topic as mentioned above, however, a study made by Celep and Yesilada (2017), honey addition to coffee showed an increase in total antioxidant capacity. Our results are in agreement with this study.

Table 4.4. ANOVA table and estimated coefficients for total antioxidant capacity

Source	Coefficients	Sum of Squares	df	Mean Squares	F value	p-value Prob>F
Model		42.63	2	21.32	34.93	<0.0001*
Intercept	91.52					
Linear						
Honey:Pollen	1.66	26.69	1	26.69	43.74	<0.0001
Total Conc.	1.28	15.94	1	15.94	26.11	0.0005
Residual		6.10	10	0.61		
Lack of Fit		3.91	6	0.65	1.19	0.4545**

*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.8748

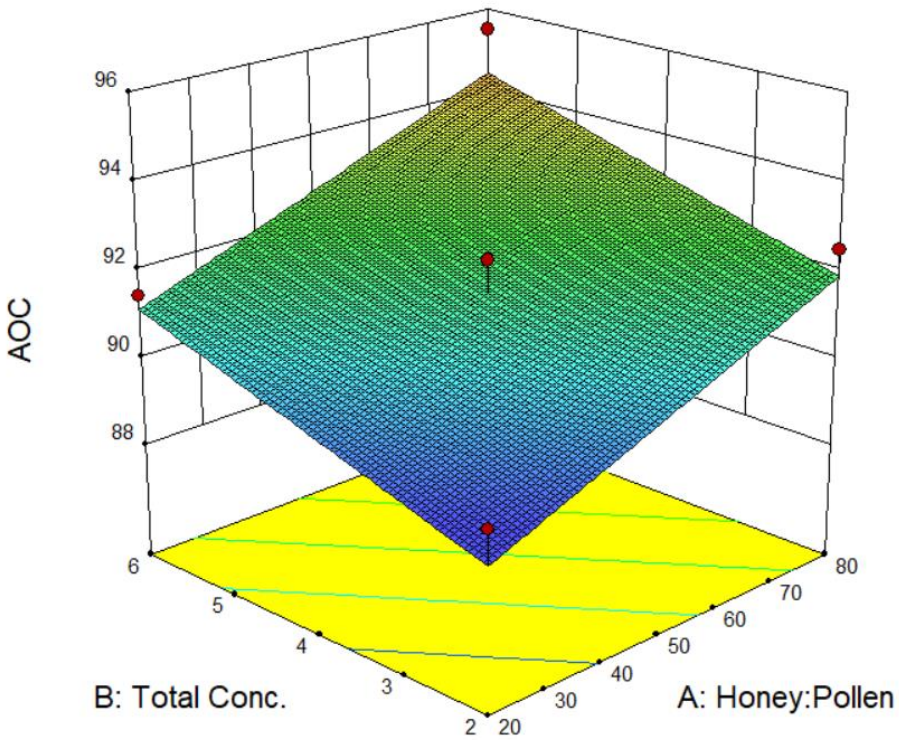

Figure 4.2. 3D response surface of the effects of total concentration and honey:pollen ratio on total antioxidant capacity

4.6 The Effect of Process Variables on Ascorbic Acid Content (Vitamin C)

Ascorbic acid is a well know antioxidant. Table 4.5 shows quadratic effect of process variables on ascorbic acid content. The model is significant ($p<0.05$). The effect of honey:pollen and total concentration on ascorbic acid are shown in Figure 4.3. According to the ANOVA results, honey to pollen ratio is not significant ($p>0.05$). However, total concentration affected ascorbic acid content of the tea significantly ($P<0.05$). It means that increase in total concentration of non-tea substances results in increased ascorbic acid content. There is also interaction between honey to pollen ratio and total concentration. The interactive effect between independent variables was not significant ($p>0.05$). In an article by Bogdanov et al. (2013), it was stated that recommended daily ascorbic acid intake for an adult is 100 mg. According to the results, the final product prepared in this study supplies 7% of daily ascorbic acid intake.

Table 4.5. ANOVA table and estimated coefficients for ascorbic acid content

Source	Coefficients	Sum of Squares	df	Mean Squares	F value	p-value Prob>F
Model		5.842E-003	5	1.168E-003	32.05	0.0001*
Intercept	0.063					
Quadratic						
A: Honey:Pollen	-3.936E-003	1.496E-004	1	1.496E-004	4.10	0.0824
B: Total Conc.	0.021	3.727E-003	1	3.727E-003	102.25	<0.0001
AB	2.536E-003	2.573E-005	1	2.573E-005	0.71	0.4286
A^2	-0.012	1.721E-003	1	1.721E-003	47.20	0.0002
B^2	-7.088E-003	6.046E-004	1	6.046E-004	16.58	0.0047
Residual		2.552E-004	7	3.646E-005		
Lack of Fit		2.527E-004	3	8.422E-005	133.71	0.0002*

*Significant at p-value<0.05; R^2=0.9581

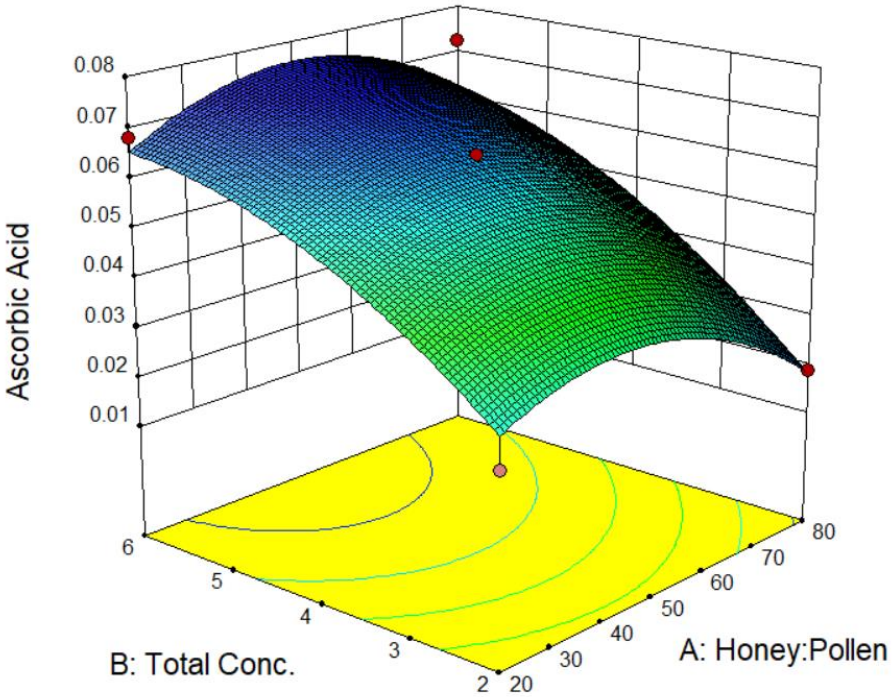

Figure 4.3. 3D response surface of the effects of total concentration and honey:pollen ratio on ascorbic acid content

4.7 The Effect of Process Variables on Sugar (Fructose) Content

Honey to pollen ratio and total concentration of non-tea substances with positive linear regression coefficient indicated that all the process variables are effective on sugar content (Table 4.6). The regression model (2FI) was significant ($p<0.05$). It can be interpreted that as honey to pollen ratio and total concentration increase, amount of sugar also increases. ANOVA results and Figure 4.4 reveal that the interaction effect between independent variables are positive and it has significant ($p<0.05$) effect. It was expected due to the fructose and glucose content of honey. Increase in concentration resulted in increase in the sugar content.

Table 4.6. ANOVA table and estimated coefficients for sugar content

Source	Coefficients	Sum of Squares	df	Mean Squares	F value	p-value Prob>F
Model		2.605E+07	3	8.683E+06	9.70	0.0035*
Intercept	2626.92					

2FI						
A: Honey:Pollen	848.56	6.952E+006	1	6.952E+006	7.76	0.0212
B: Total Conc.	797.23	6.136E+006	1	6.136E+006	6.85	0.0279
AB	1800	1.296E+007	1	1.296E+007	14.47	0.0042
Residual		8.059E+006	9	8.955E+005		
Lack of Fit		8.057E+006	5	1.611E+006	3412.55	<0.0001*

*Significant at p-value<0.05; R^2=0.7637

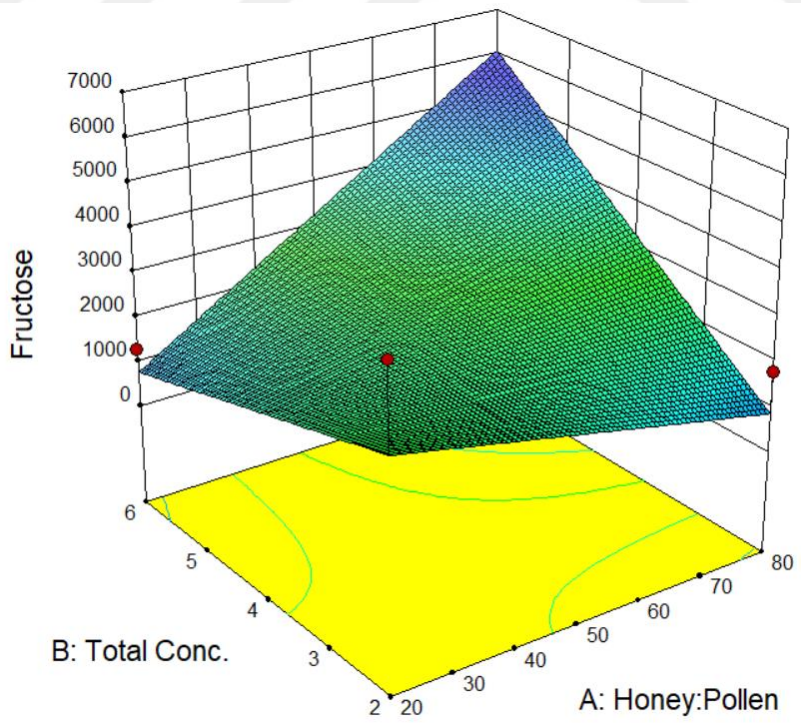

Figure 4.4. 3D response surface of the effects of total concentration and honey:pollen ratio on fructose content

4.8 The Effect of Process Variables on Sensory Perception

Another response is sensory perception. As it can be seen in Table 4.7, influences of honey to bee pollen and total concentration of non-tea substances on this response is not significant ($p>0.05$). When 3D model was examined, it is seen that variables have a quadratic effect on the response (Figure 4.5). In addition to this, honey to bee pollen ratio has significant effect on the sensory properties of tea ($p<0.05$). The panelists were able to distinguish tea from honey to pollen content.

Table 4.7. ANOVA table and estimated coefficients for sensory perception

Source	Coefficients	Sum of Squares	df	Mean Squares	F value	p-value Prob>F
Model		9.44	5	1.89	2.28	0.1563**
Intercept	2.92					
Quadratic						
A: Honey:Pollen	0.71	4.80	1	4.80	5.79	0.0470
B: Total Conc.	0.21	0.43	1	0.43	0.52	0.4952
AB	0.19	0.15	1	0.15	0.18	0.6820
A^2	0.22	0.60	1	0.60	0.72	0.4241
B^2	-0.47	2.60	1	2.60	3.14	0.1197
Residual		5.80	7	0.83		
Lack of Fit		5.80	3	1.93	1.699E+006	<0.0001*

*Significant at p-value<0.05; **Not significant at p-value>0.05 R^2=0.6195

Table 4.8. Results of sensory analysis

	Samples (honey:bee pollen)							
	4:1 (2%)	1:4 (6%)	1:4 (2%)	1:0 (4%)	1:1 (4%)	0:1 (4%)	4:1 (6%)	1:1 (7.36%)
Color	4.4	1.7	3.1	4.1	2.8	2.1	3.8	2.3
Odor	4.4	1.7	3.1	3.8	2.7	2.1	3.9	2.3
Taste	4.3	1.4	3.2	4.2	2.5	2	3.9	2.2
Overall	4.44*	1.77	3.11	4.11	2.77	2.11	3.89*	2.33

*The most acceptable samples by consumers

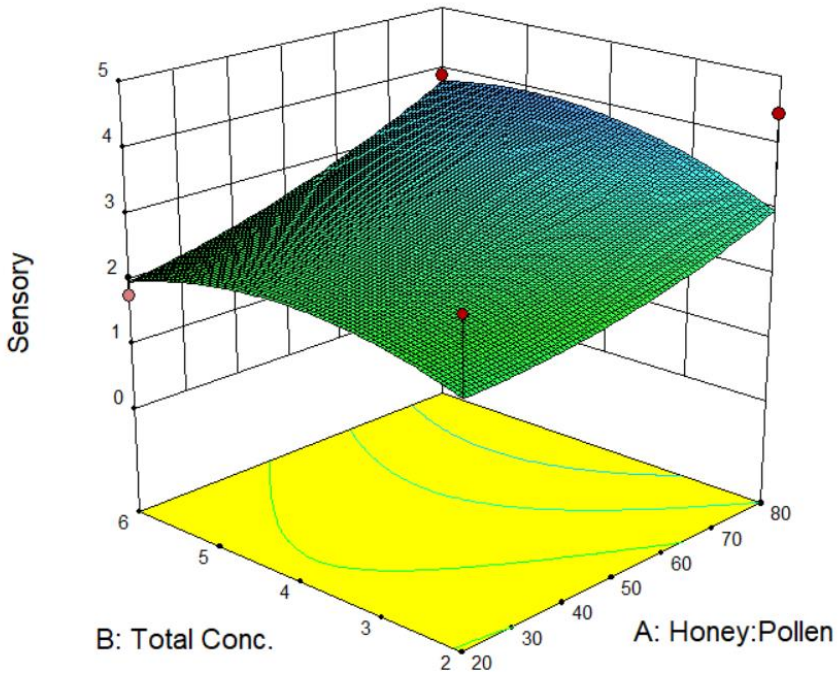

Figure 4.5. 3D response surface of the effects of total concentration and honey:pollen ratio on sensory perception

4.9 Model Optimization

Model optimization was carried out by central composite design. The optimization of final product (green tea with honey and bee pollen) was carried out by considering the highest TPC, TAC and sensory perception. Table 4.9 shows the optimized condition for the final product. The optimized condition for green tea with honey and bee pollen were 4:1 honey to pollen ratio and 6% total concentration of non-tea substances with TPC of 578.674 mg GAE/L sample and TAC of 94.463 DPPH•IC_{50}. Optimum score

determined for sensorial evaluation was 3.8 which is fairly close with sensory analysis done by the panelists. In these conditions, the desirability was 0.816.

Table 4.9. Optimum process conditions for green tea with honey and bee pollen

	Honey:Bee Pollen	**Total Concentration**
Green tea with honey and pollen	4:1	6 %

The validation of the model was controlled by testing optimum response values. Experimental results and predicted values are shown in Table 4.10. It can been seen that the experimental results were quite similar with the predicted results. Therefore, the model is accurate. On the other hand, initial and final contents of the last product is presented in Table 4.11.

Table 4.10. Comparison of predicted and experimental values of determined responses at optimum conditions

	TPC[1]		**TAC[2]**		**Sensory**	
	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.
Green tea with honey and pollen	578.67	614.87	99.13	100.24	3.79	3.88

[1]mg GAE/L sample; [2]mg IC_{50}/L sample

Table 4.11. Comparison of initial and final contents of the last product

Variables	**Initial Content**	**Final Content**
TPC[1]	464.44	614.87
TAC[2]	93.79	100.24
Ascorbic Acid[3]	0.0145	0.0725
Fructose Content[4]	0	5633.33

[1]mg GAE/L sample; [2]mg IC_{50}/L sample; [3]mg ascorbic acid/L sample; [4]mg fructose/L sample

The following figures show the three dimensional graphs of responses at optimum conditions and desirability (Figure 4.6, 4.7, 4.8, 4.9). According to optimized responses, response surface methodology offers solutions. These solutions have

desirability values. The desirability is the closeness of responses to the ideal value and ranges from 0 to 1. As desirability approaches to 1, response gets close to ideality. Our desirability is 0.816 which is quite good and ideal.

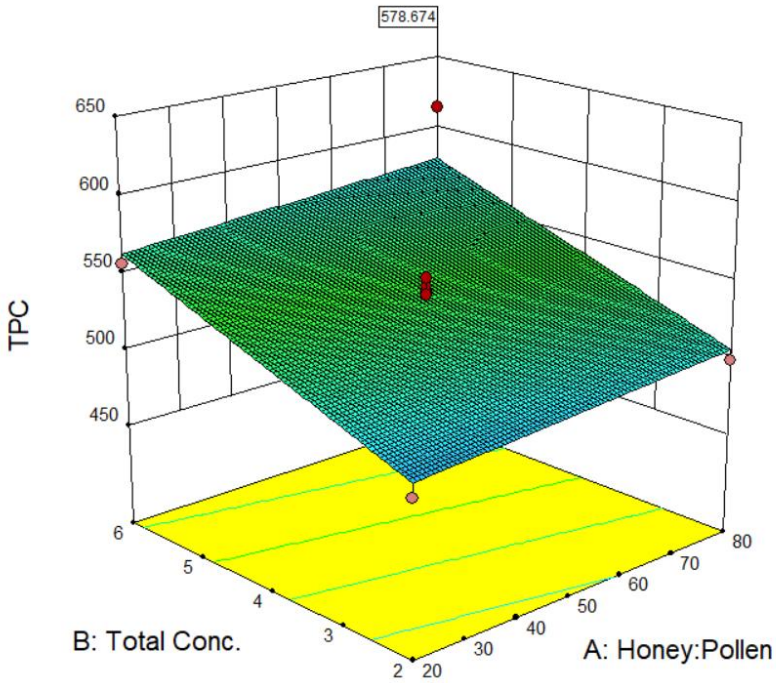

Figure 4.6. 3D response surface of the total phenolic content at optimum conditions

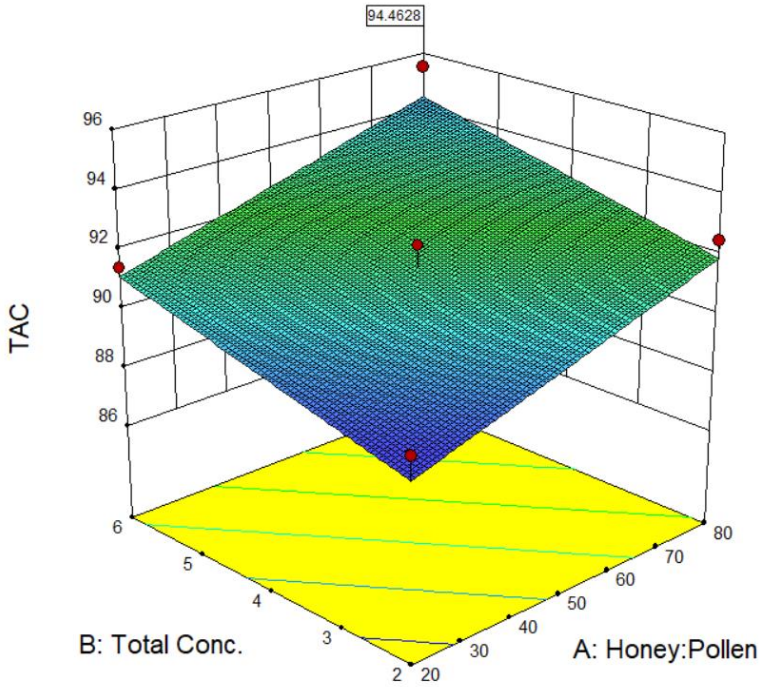

Figure 4.7. 3D response surface of the total antioxidant capacity at optimum conditions

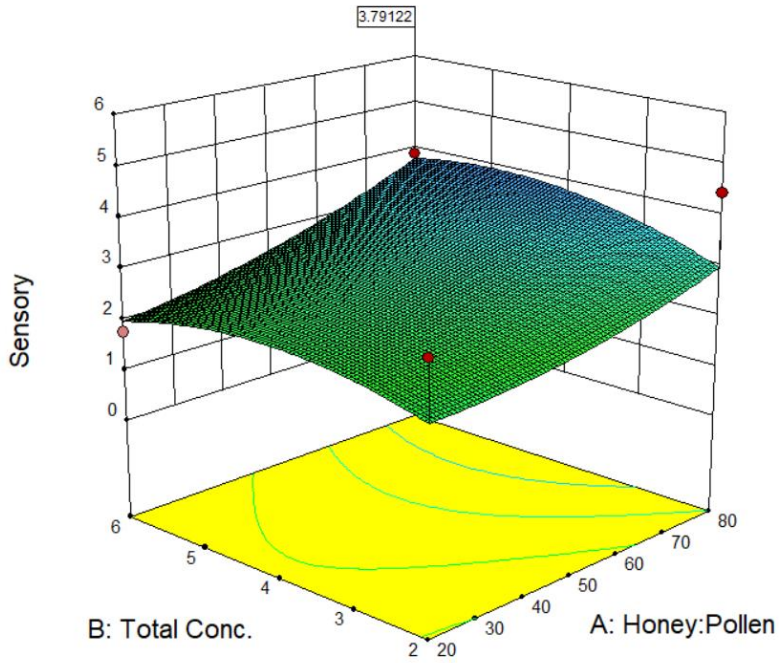

Figure 4.8. 3D response surface of the sensory perception at optimum conditions

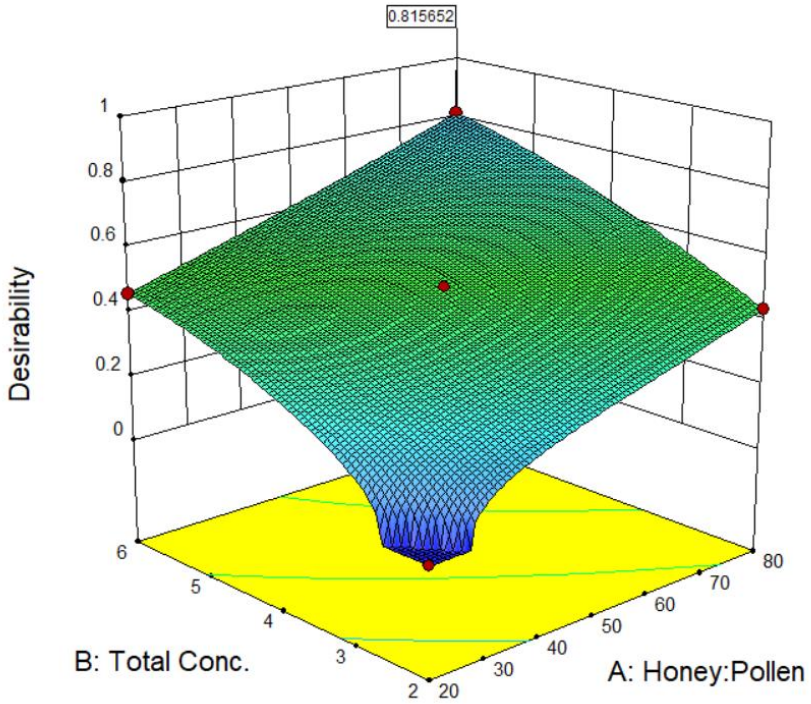

Figure 4.9. 3D response surface of desirability of optimum conditions

CHAPTER 5

CONCLUSION

5.1 Conclusion

This research attempted to develop a likable product made with green tea, honey and bee pollen which is rich in natural antioxidants. It has been shown in various studies that have anti-carcinogenic, antimicrobial and anti-inflammatory effects. The antioxidant and sensory properties of this product were enhanced in comparison with those of the base green tea. This tea with added bee products has not been commercially available in the market, and as far as we know no study has been conducted on the addition of two bee products to the green tea.

The different models were developed to describe effect of process variables on responses and determine the optimum mixing ratios of acceptable products and verifying the experiment with these conditions. Response surface methodology was used to determine the optimum conditions that maximize total phenolic content, antioxidant capacity and acceptability by the panelists from sensory evaluation.

The optimum conditions for the independent variables were honey to pollen ratio of 4:1 and total concentration of non-tea substances of 6%. At these optimal conditions, the values of responses were; total phenolic content of 614.872 mg GAE/L tea sample and total antioxidant capacity of 100.24 mg IC_{50}/L tea sample. One of the most acceptable products determined by panelists was the same with this ratio. Also, desirability of the optimum conditions was 0.816. The experimental values were compared with the predicted values and the results demonstrated that the RSM model was satisfactorily determined the optimum conditions.

5.2 Future Work

The results showed that it is possible to produce green tea with honey and bee pollen which is very rich in natural antioxidants. For the future work, it was targeted to provide this high quality and healthy beverage for the consumers by supplying in the vending machines. This has the potential to provide a significant economic gain for bee producers.

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