PRODUCTION OF INSTANT POWDER GREEN TEA ENRICHED WITH HERBAL MIXTURES

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ABSTRACT

PRODUCTION OF INSTANT POWDER GREEN TEA ENRICHED WITH HERBAL MIXTURES

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Black tea is the most consumed beverage in Türkiye following water. Due to the benefits of green tea for human health, the consumption of green tea is also gradually increasing. In addition to the diverse range of tea variations, it is also widely known that Türkiye has not yet generated a market for instant green powdered tea yet and that this product category has not yet been extensively developed. During tea production, a significant amount of tea waste is generated. This waste is also called as the *tea fiber* and is not utilized in the further blending and packaging processes. In this study, instant tea from tea waste fibers were produced. Instant tea was produced by "super grinding" to create a powder tea with high dispersibility.

Within the scope of this study, tea was dried and blended with rockrose herbs to give functional properties to instant teas.

To obtain the instant powder teas, tea fibers were brewed after super grinding using 2 different approaches. Hot brewing was performed at 70 °C for 20 minutes and cold brewing was performed at 25 °C and 2 hours. Following the brewing, drying was performed on the filtrates obtained with brewing. Instant tea powder was produced by using 2 drying methods: *freeze and spray drying*. Unlike the freeze-drying method, the drying process was carried out by adding inulin to the solution during spray drying, thus increasing the amount of dry matter content.

As physical characterization tests, moisture content, water activity, hygroscopicity, particle size, solubility, morphological and turbidity experiments were performed for the final products. Antioxidant activity, total phenolic substance, gallic acid, caffeine and L-theanine content were determined as the chemical characterization experiments. A detailed analysis of the epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC), and epigallocatechin gallate (EGCG) by HPLC was also carried out.

In general, the physical properties are almost similar to the teas obtained by freeze & spray drying methods. In terms of chemical analysis, the antioxidant capacities were found to be higher in freeze dried samples. Scanning electron images showed that spray dried powders were spherical in shape indicating that inulin encapsulated the extracts.

Keywords: Instant green tea, waste tea fiber, super grinding, rockrose

ÖZ

BİTKİLERLE ZENGİNLEŞTİRİLMİŞ HAZIR TOZ YEŞİL ÇAY ÜRETİMİ

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Türkiye'de sudan sonra en çok tüketilen içecek siyah çaydır. Yeşil çayın insan sağlığına olan faydaları nedeniyle yeşil çay tüketimi de giderek artmaktadır Çok çeşitli çay çeşitlerine sahip olmasının yanı sıra, Türkiye'de hazır kahvenin aksine hazır yeşil toz çay için henüz bir pazar oluşmadığı da bilinmektedir ve bu ürün kategorisi henüz kapsamlı bir şekilde geliştirilmemiştir. Çay üretimi sırasında önemli miktarda çay atığı oluşmaktadır. Bu atık aynı zamanda çay lifi olarak da adlandırılır ve sonraki harmanlama ve paketleme işlemlerinde kullanılmamaktadır. Bu çalışmada, çay atık liflerinden hazır toz çay üretilmiştir. Hazır çay, yüksek difüzyona sahip toz çaylar oluşturmak için "süper öğütme" ile üretildi.

Bu çalışma kapsamında çay kurutularak laden bitkisi ile harmanlanarak hazır çaylara fonksiyonel özellikler kazandırılmıştır.

Hazır toz çayları elde etmek için, çay lifleri süper öğütme işleminden sonra 2 farklı yöntem kullanılarak demlenmiştir. Sıcak demleme 70 °C'de 20 dakika ve soğuk demleme 25 °C'de ve 2 saatte gerçekleştirilmiştir. Demlemenin ardından demleme ile elde edilen sıvılar üzerinde kurutma işlemi yapılmıştır. Hazır çay tozu, dondurarak ve püskürterek kurutma olmak üzere 2 kurutma yöntemi kullanılarak üretilmiştir: Dondurarak kurutma yönteminden farklı olarak kurutma işlemi, püskürterek kurutma sırasında çözeltiye inülin ilave edilerek gerçekleştirilmiş ve böylece sıvının kuru madde miktarı artırılmıştır.

Çıkan son ürünlere fiziksel olarak nem analizi, su aktivitesi, higroskopisite, parçacık boyutu, çözünürlük, morfolojik analizler ve bulanıklık analizleri; kimyasal olarak da antioksidan aktivite, toplam fenolik madde, gallik asit, kafein ve L-theanin tayin analizleri yapılmıştır. Bunun yanı sıra, HPLC kullanılarak, epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC) ve epigallocatechin gallate (EGCG) analizleri yapılmıştır.

Genel olarak, dondurarak ve püskürterek kurutma yöntemleriyle elde edilen çaylar için fiziksel özellikler benzerdir. Kimyasal analizler açısından, dondurularak kurutulmuş örneklerde antioksidan, fenolik bileşen, kateşin miktarları kapasitelerinin daha yüksek olduğu görülmüştür. Morfolojik analizlerde taramalı elektron görüntüleri, püskürtmeli kurutulmuş tozların küre şeklinde olup, dondurularak kurutulmuş toz numunelerin daha şekilsiz ve pürüzlü olduğunu göstermiştir, püskürtmeli kurutmada kullanılan inülinin morfolojik analiz sonucuna etkisi görülmüştür.

Anahtar Kelimeler: hazır toz yeşil çay, atık çay lifi, süper öğütme, laden otu

I dedicate my thesis to my dear sister & twin, and my love for their constant support…

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Last but **not the** least, I express my deepest gratitude to my family. I am unable to sufficiently express their contribution on this research and my entire life. My deary sister Elif Çelik (cücük) & my lovely twin Emre Çelik.. I appreciate all that you have done for me. I appreciate you being the best siblings ever. Thank you for guiding me when I get lost and for your constant love and support. Without your help, I could not have completed my study.

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LIST OF ABBREVIATIONS

ABTS: 2.20-azino-bis-3-ethyl-benzothiazoline-6-sulfonic acid

D_{10} : The portion of particles with diameters smaller than this value is 10%

D_{50} : The portions of particles with diameters smaller and larger than this value are 50%.

D_{90} : The portion of particles with diameters below this value is 90%.

DPPH: 2.2-diphenyl-1-picryl hydrazyl

EC: Epicatechin

ECG: Epicatechin 3-gallate

EGC: Epigallocatechin

EGCG: Epigallocatechin-3-gallate

FNU: Formazine nephelometric unit

GAE: Gallic acid equivalent

GTWF: Green tea waste fiber

HPLC: High performance liquid chromatography

SEM: Scanning electron microscope

TPC: Total phenolic content

CHAPTER 1

INTRODUCTION

1.1 Camellia Sinensis

Camellia sinensis (see Figure 1.1.) is the species of plant that belongs to the *Theaceae* family (Üstün & Demirci, 2013). China, Sri Lanka, Indonesia, Japan, Taiwan, and central African countries are the main tea producing countries in the world (Elmas & Gezer, 2019). Türkiye was one of the world's top tea markets in 2019 with 1.45 million tons of tea produced (4% of global tea production) (Strateji Geliştirme Başkanlığı (TEPGE), 2021). There are basically 3 specific -species of *Camellia sinensis* plant which are *Camellia sinensis var. Sinensis* knowns as Chinese tea, *Camellia sinensis var. Assamica* known as Indian tea or Assam tea and, *Camellia sinensis var. Cambodiensis* (Tounekti et al., 2013).

The *sinensis* variety grown in Türkiye is among the first variety described above and mostly grown in Black Sea region. Tea plants in Türkiye can withstand temperatures below 0 °C in the north (Sinensis et al. 2007). *Sinensis* grows in small shrubs on the cold, high -altitude hills of Asia. Teas produced from this variant have a sweet, aromatic, and strong odor. Harvesting of the tea leaves usually begins early summer.

Figure 1.1 Camellia Sinensis

1.2 Tea Production Process and Tea Types

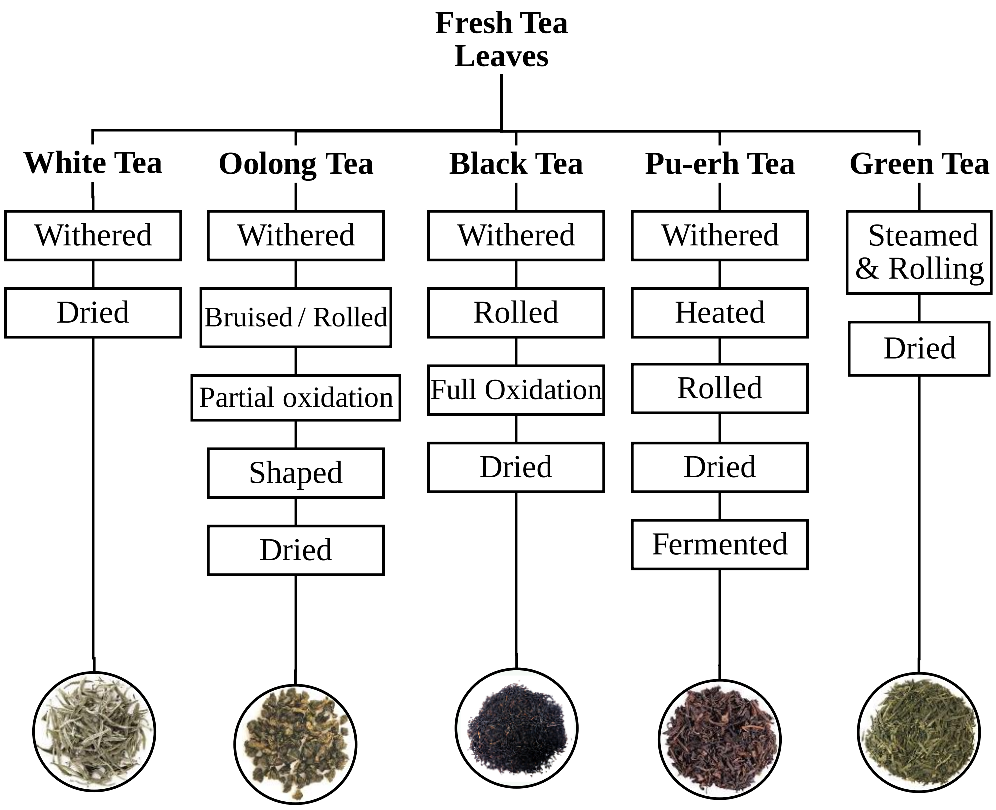

Figure 1.2 The production flow chart of all tea types

Camellia sinensis is the source of all tea varieties. However, they are distinguished from one another in accordance with the degree of fermentation which is basically the *enzymatic browning* (Namita et al., 2012). In tea processing terminology, '*fermentation'* refers to oxidation occurring due to enzymatic browning. Different tea types are given in Figure 1.2 The '*real fermentation*' (*fermentation by micro-organisms)* is performed only for Pu-erh tea.

Fresh tea leaves plucked as two leaves and a bud set, and then dried at 32-34 °C to remove excess water from the leaves. This process is known as withering. The first crucial stage in raising the quality of the finished tea is withering or partial desiccation. Typically, the selected leaves are spread out in a series of enclosed or open troughs equipped with perforated trays while being forced to circulate air (Deb & Jolvis Pou, 2016).

Following withering, steps differ for all tea types; pan firing or steaming is applied for green tea to stop oxidation and ensure the leaves stay as green as possible (Singh et al, 2014). Otherwise, to produce black or oolong tea, wilted tea leaves are bruised and rolled to increase the oxidation level; since rolling breaks down the enzymes in the tea leaves. Extent of the oxidation determines the types of tea; oolong tea is partially oxidized, black tea is fully oxidized (Elmas & Gezer, 2019).

Unlike other teas except black tea , Pu-erh tea leaves requires the leaves to be moistened causes to increase microbial activity , that is why the leaves are fermented (Z. J. Zhao et al., 2015).

1.2.1 White Tea

White tea is manufactured from young *Camellia sinensis* buds that have been saved from sunlight to prevent polyphenol breakdown, and has undergone the least processing of all the tea varieties (T. R. et al., 2013). The leaves are usually manually collected with minimal damage to the tea leaves. That makes this type of tea the most expensive one among the others.

Typically, tea leaves are just plucked and dried minimally. Drying usually takes place in open troughs equipped with perforated trays. The optimum leaf is either an additional leaf at the top of the first harvest of the year or the top bud alone (Figure 1.3.). Contrary to black and green tea, white tea is not rolled. Fresh tea leaves are just plucked and gently dried to produce high-quality white tea. Panning , rolling , shaking is not applied to these leaves (A Pawar, 2018).

As there is no oxidation, white tea contains high antioxidants, less caffeine, and has a pale color with a sweet taste. Catechins levels are higher than those of green tea derived from the same plant (A Pawar, 2018).

Minimal processing results in a large quantity of phytochemicals with a variety of health benefits. Two to four cups of white tea per day promotes health benefits. White tea consumption has been shown to prevent joint pains, softening of bones, also to promote elastic skin due to its higher anti-collagenase and antioxidative activity (Pastoriza et al., 2017). White tea helps to increase metabolism by lowering blood glucose levels and stress levels.

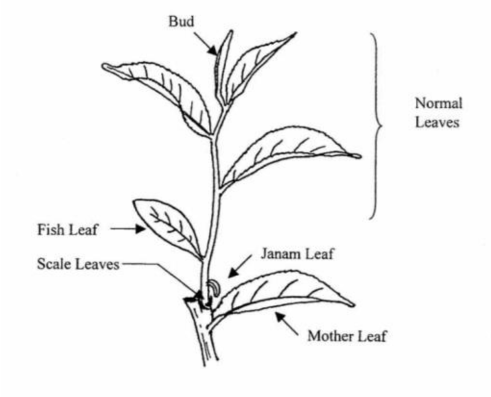

Figure 1. 3: The parts of *Camellia sinensis*

1.2.2 Oolong Tea

Oolong tea is a partially oxidized tea (Wang et al., 2016) . It is subjected to moderate level of oxidation during processing and drying. It has a taste and color between green and black tea. The main flavor and aroma compounds of oolong tea are catechins, amino acids and sugars.

Oolong tea has higher total phenolic substances than black tea, but less than green tea (Koca et al., 2014).

Higher antimutagenic activity has been shown compared to green or black tea (Su et al., 2007).

Oolong tea is not recognized in Türkiye well, but in the world its popularity is gradually increasing (Koca et al., 2014).

1.2.3 Black Tea

The second most consumed beverage after water is the black tea (Üstün & Demirci, 2013).

Black tea is obtained by the full oxidation of the tea leaves. In black tea production, the leaves are curled and oxidized due to the action of the polyphenol oxidase. Oxidation decreases the catechin levels of the tea (Demir, 2018; Elmas & Gezer, 2019).

Rolling, oxidation and drying are the processing steps of black tea. Especially during drying the flavor of black tea develops significantly (Çelik, 2006)**.**

1.2.4 Pu-erh Tea

The majority of the world's supply of Pu-erh tea comes from the Yunnan province of China. It is made by roasting unprocessed green tea leaves, which is followed by a second fermentation with microbes to produce a special kind of tea (H. M. Zhang et al., 2012). It is a kind of post-fermented tea (Z. J. Zhao et al., 2015) The reaction between microorganisms like *Aspergillus* sp. enables a more smooth taste and it develops aroma and color (Pedan et al., 2018).

Pu-erh tea has changed throughout time from being predominantly used as a medicinal plant taken from woods to a beverage obtained from agricultural systems. (Ahmed & Stepp, 2012).

Pu-erh tea has other health advantages as well, such as promoting healthy digestion, managing weight, relieving stress, and providing critical nutrients. (H. M. Zhang et al., 2012)

1.2.5 Green Tea

Green tea is the first tea variety developed in history. It is produced quickly by drying the tea leaves following plucking. Unlike black tea it is not let to react with oxygen during the drying process (Yilmaz et al., 2016).

Alternatively, green tea is also referred to as a non-fermented tea (Sinija & Mishra, 2008). Thus, it protects its antioxidant capacity and green leaf appearance, and caffeine amount is usually less compared to black tea (Jigisha et al., 2012).

Caffeine is a natural component of all tea leaves. However, withering is an important process to determine whether tea leaves have more or less caffeine. While black tea undergoes withering, rolling, fermentation and drying processes, green tea undergoes only rolling and drying processes. Withering causes the amount of caffeine to rise, which enhances the flavor and astringency of tea. It is clarified that the rise in the quantity of amino acids is responsible for the rise in caffeine. Ribonucleic acid is a recognized component of caffeine. Some of the ribonucleic acid in the tea is changed into caffeine during the withering process (Akkuş, 2019).

Green tea accounts for 20%-22 % of world tea production and is primarily consumed in China, Japan, Korea and Morocco (Cabrera et al., 2006).

The composition of Green Tea

The composition of green tea is summarized in Table 1.1 below:

Table 1. 1: Composition of green tea leaves (% dry basis) (Cabrera et al., 2006)

Compound	**Green Tea**
Protein	15
Amino Acids	4
Fiber	26
Other Carbohydrates	7
Lipids	7
Pigments	2
Minerals	5
Phenolic Compounds	30
Oxidized Phenolic Compounds	0

The polyphenols in green tea include phenolic acids, flavandiols, flavonoids, and flavanols and they can account for up to 28-30% of the beverage's dry weight. Most of the green tea polyphenols are flavanols, commonly known as *catechins*. Epicatechin (EC), epicatechin 3-gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) are the four principal catechins in green tea. The chemical structure of these catechins are shown in Figure 1.4 (Balci & Özdemir, 2016; Cabrera et al., 2006).

In addition to catechins, green tea also contains minerals, amino acids, fiber, carbohydrates, proteins, lipids, and pigments.

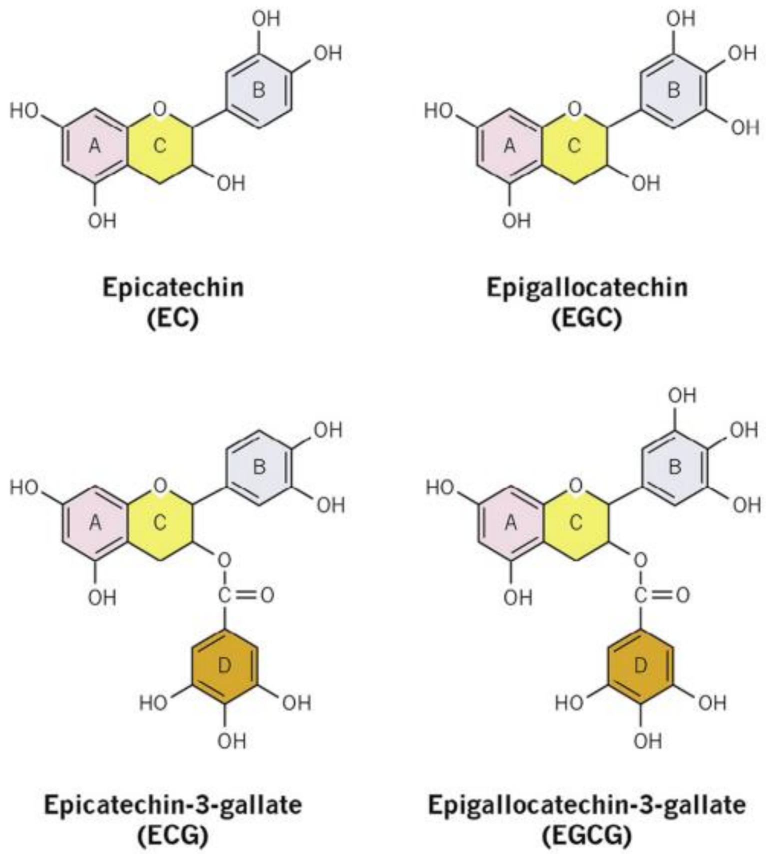

Figure 1. 4: Chemical structure of the four catechins (Roychoudhury et al., 2017)

1.2.6 Health Benefits of Green Tea

Tea has many beneficial effects in terms of health due to the chemical components it contains.

Although both black and green tea contains caffeine and antioxidants, the caffeine content in green tea is lower, because there is no withering process in green tea; but the number of antioxidants and polyphenolic components is higher.

Green tea shows antioxidant activity due to its high level of flavanols. Antioxidant substances prevent the formation of active oxygen or by keeping the formed active oxygen, prevent the damages promoted by oxidation on a cellular basis, and can slow down the formation of degenerative diseases. These antioxidants also decrease relative risks of cancer, heart disease, and accelerated aging (Cabrera et al., 2006).

It is well known that green tea helps with weight loss by boosting metabolism and energy expenditure (Basu & Lucas, 2007) . It is considered as a natural herb that can induce weight loss (Westerterp-Plantenga, 2010).

Due to its high vitamin C, E, and lutein content, green tea is also beneficial for skin care, oral health, and healthy vision, halting hair loss, and increasing insulin sensitivity. (Jigisha et al., 2012). Briefly, green tea is considered as the natural remedy to many health related problems (Namita et al., 2012).

1.2.7 Rockrose (*Cistus* spp.)

The *Cistaceae* family, which includes shrubs, semi-shrubs, and herbaceous plants, is typically found in the Mediterranean temperature zone. Rockrose is a significant species of this family (Şekeroğlu & Gezici, 2021).

Rockrose (see Figure 1.5.) has flavonoids, phenolics, tannins, hydrocarbons, lipids, vitamins, carbonic compounds and terpenoids (Şekeroğlu & Gezici, 2021).

It is used in the treatment of diarrhea, peptic ulcer, high fever, infertility, various skin disorders such as antispasmodic, hemostatic, antidiabetic and anti-flammatory (Sargın & Seyid Ahmet, 2016).

Figure 1. 5 Rockrose

1.3 Commercial Tea Types

Tea is the most consumed beverage in our country following water. The recommended brewing time and temperature for each tea type are different. In general, we leave dry tea in the upper teapot and boil water in the lower teapot. Then, when the water in the lower teapot boils, we add it to the tea and brew. This method is the *traditional hot brewing*. In general, tea steeps quicker in warm water. Cold water offers flexibility for longer brew durations while hot water requires less time for infusion. Usually, high temperatures and long brewing time results in a tea that is bitter. Using extremely hot water for some tea types can be advantageous since heat brings out more aromatics and complexity. Otherwise, you'll receive more flavor from your tea the longer you steep it.

Cold water and longer steeping times produce a rich tea with enticing texture. Along with hot brewing, cold brewing has recently gained popularity.

Over the past century, there has been many changes in how tea is processed, starting with blended loose tea, moving on to tea bags, packaged teas, and eventually instant teas, ready-to-drink teas, and flavored ones (Sinija & Mishra, 2008) .

Instant tea market (*excluding tea bags*) in Türkiye has not grown so much. In countries like India, Sri Lanka, Kenya and USA, instant is consumed in a great extent. Recently a powder instant black tea has been launched to the market and got good attention from the consumers.

To our knowledge, an instant green powdered tea product has not available in the Turkish market yet and in fact and there is no market in this product category yet like instant soluble coffee. Companies are looking for new approaches to produce instant green tea.

The brew from processed leaves, tea waste (see Figure 1.8.), or undried fermented leaves can be extracted to create instant tea. During tea production, significant amount of dust and tea fibers remain on the production line. According to the data obtained from Doğadan Tea Co. (Akyurt, Ankara), approximately 25 tons of tea waste fibers are generated annually. Tea "waste" is by definition worth *little to nothing* economically. Dust, twig fragments, leaf fragments that have been damaged, floor sweepings, stalks, and other debris that doesn't adhere to the criteria or go through the procedures necessary to be included in the packaged final product are all considered waste. Tea waste presents a chance to develop products that are useful in different sectors. Turning waste into revenue is a step in the right direction for business innovation, given the tremendous financial challenges that tea growers in all major regions face.

Figure 1. 6 The image of the green tea leaves (left side) and green tea waste fibers

There are three different traditional ways to make instant tea: spray-, freeze-, or vacuum-drying. A popular and well-known method for making powder from liquid and semi-liquid meals is spray-drying. The freeze-drying method involves sublimating a solution or wet solid product under vacuum to separate the liquid water from it (Bhatta et al., 2020). By removing moisture from food products under low pressure during vacuum drying, the drying temperature can be lowered (Pinedo & Murr , 2007). The most popular of these methods are spray- and freeze-drying. The nutritional and functional properties of instant tea may vary depending on the drying method.

It is also recognized that a range of factors, including the growth environment, production circumstances, and also particle size can alter the release of tea's active ingredients and functional properties . A crucial factor in the diffusion of active ingredients from tea leaves is the particle size. Food ingredients' particle sizes can be decreased through superfine grinding. To increase the extraction of a sample's bioactive components, super grinding can reduce particle size. It can reduce the particle size of food samples in the range between 1 nm to 100 μm (Xiao et al., 2017). Thus, super grinding process has potential in producing powder food products. This technique significantly alters the morphological features of the particles compared to the crude ones, giving them good surface qualities, water holding capacity, solubility, and oil holding capacity (X. Zhao et al., 2009). It is stated that super grinding process would not affect chemical composition polyphenols and flavonoids (H. Zhang et al., 2019).

1.4 Objective of the Study

The main objective of this thesis is to produce and characterize an instant powdered green tea blended with herbs by utilizing the green tea waste fiber (*GTWF*) generated during tea leaves production. To our knowledge, a study which investigated the production of a soluble instant tea with *rockrose* has not been found in the literature. In that scope, the specific objective of the study is listed as follows.

- To show that, GTWF can be used to produce instant tea powder.
- To test the effect of different brewing methods (*cold vs. hot brewing on the physicochemical properties of instant tea*.)
- To test the effect of drying methods (freeze vs. spray drying on the physicochemical properties of instant tea.)
- To test the effect of super grinding on the physicochemical properties of tea.
- To evaluate the effect of *rockrose* on the chemical properties of tea.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Waste and normal green tea leaves, *rockrose* as the herbal plant were provided from Doğadan Tea Co. Plant located in Akyurt, Ankara, Turkey. Gallic acid and Folin & Ciocalteu's phenol reagent were purchased from Merck (Darmstadt, Germany). Potassium per sulfate, sodium bicarbonate, methanol, and DPPH, ABTS were all provided by Sigma-Aldrich (St. Louis, MO, USA). Inulin was purchased from Tito (İzmir, Turkey).

2.2 Production of instant tea powder

The overall production flow chart is given in Figure 2.1. Different parameters were used in each step. That is why parameters of the processes are not listed in the diagram. They will be explained separately in the later sections.

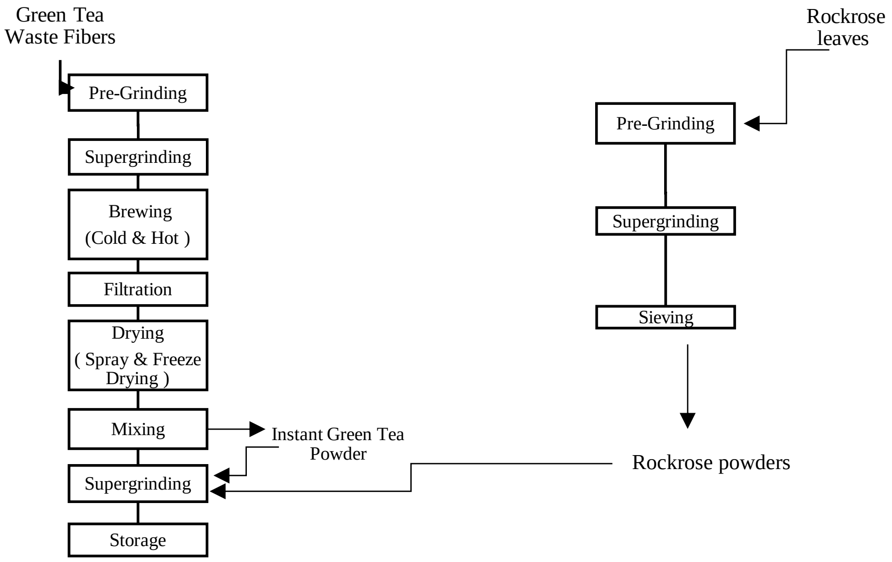

Figure 2. 1 The overall production chart of the instant green tea powder production

Pre-grinding of GTWF

The waste green tea leaves were ground at 10,700 rpm for 3 minutes using a high shear mixer (TM5, Vorwerk Thermomix, Turkey).

2.2.1 Super grinding

Super grinding was done using PB-100 Planet Ball Mill (LT-M0610) (Figure 2.2). Grinding process was carried out at 600 rpm for 5, 15 and 30 minutes respectively.

Figure 2. 2 PB-100 Planet Ball Mill (LT-M0610)

2.2.2 Pre-Grinding for the herbal ingredients

Rockrose was ground at 10,700 rpm for 3 minutes using the high shear mixer (Vorwerk Thermomix, Turkey).

Following the pre-grinding, the herbs were separated from their branches and roots using Fritsch Vibrating Sieve Shaker with 2000, 500-, 400, 335-, 250- and 200-micron sieves. Following sieving, the rockrose powders were ready to be mixed with the green tea powders.

2.2.3 Mean particle size measurement & cell wall disruption rate

Particle size of grinded tea and herbal powders were determined by using a Laser Scattering Instrument (Mastersizer 2000, Kinexus, Germany). Particle size measurement was carried out at METU Central Laboratory using the powder analyzer unit.

Refractive index value for green tea was used as 1.530 (Abraham et al., 2021) Refractive index value for herbal samples were accepted as 1.52.

D_{90}, D_{10} and median diameter D_{50} values were determined by particle size measurements, and the span factor was calculating using the formula (Xiao et al., 2017) :

$Span = \frac{D_{90} - D_{10}}{D_{50}}$ (Eqn.1)

Basically, breaking open cells to collect intracellular fluid is a process known as cell disruption. For intracellular components like proteins, metabolites, or nucleic acids to be released, it is important to know the cell disruption rate (Otto, 2012). Cell wall disruption increases the rate of chemical component diffusion while lowering the resistance to mass transfer. Cell wall disruption rate was calculated by the following equations (Xiao et al., 2017) :

$D_{50} > 10$ µm; % Cell wall disruption rate = 1-(1-10/ D_{50}) *3 (Eqn.2)

$D_{50} < 10$ µm; % Cell wall disruption rate = 100 %

2.2.4 Hot & cold brewing

The first parameter to be determined in the experiments was the tea/water ratio that will be used. The proper tea/water ratio was determined testing 1/10, 1/20, 1/30 and 1/40 tea/water ratios at 90 ^{o}C for 20 minutes which are the extreme conditions that will be used for hot brewing as will be seen in Table 2.1 The criteria to find the proper ratio was selected as the '*brewing efficiency*'.

Hot brewing was performed at the conditions determined in Table 2.1 for the tea/water ratio that gave the maximum efficiency.

Brewing was conducted both on pre-grinded & super grinded form of the GTWF. Rockrose was not used at this stage (Damiani et al., 2014;Castiglioni et al., 2015; Lin et al., 2014).

Brewing was performed in a hot water bath using beakers. Infusions were mixed for 15 seconds every 3 minutes to prevent sedimentation.

Table 2. 1: Hot Brewing Parameters

Process	**Temperature**	**Brewing Time**	**Tea/water Ratio**
Hot Brewing	70° C	7 & 20 mins.	1/40
Hot Brewing	90° C	7 & 20 mins.	1/40

Cold brewing was performed at the conditions listed in Table 2.2 using the tea/water ratio that yielded maximum brewing efficiency in hot brewing (De Carvalho Rodrigues et al., 2015; Hajiaghaalipour et al., 2016; Lantano et al., 2015)

Table 2. 2: Cold Brewing parameters

Process	**Temperature**	**Brewing Time**	**Tea/water Ratios**
Cold Brewing	4 °C	6 & 12 hours	1/40
Cold Brewing	25 °C	2 hours	1/40

2.3 Determination of the brewing efficiency

Filtration of the brewed teas was performed using a 100-micron filter cloth for pre-grinded teas and 50-micron filter cloth for super grinded teas. 15 ml of the filtrate of each sample was taken and kept in an oven (MST-120, Mikrotest Lab., Türkiye) at 65 °C for 48 hours and dried. The dry matter content of extract from the oven was considered as (W_1). Initial moisture content of GTWF was also considered in the efficiency calculations, and its moisture content was determined by drying the tea waste at 105° C for 48 hours. The dry matter content of the tea is considered as (W_2).

The following equation was used to determine the process efficiency (Balci & Özdemir, 2016) ;

$Efficiency\ (\%) = \frac{W_1*15}{W_2} * 100$ (Eqn.4)

2.4 Production of instant green tea powder

Instant green tea powder was produced from the filtrate obtained by the brewing of super grinded GTWF due to high brewing efficiencies.

Instant tea powder was produced by using two different drying methods.

2.4.1 Spray drying

A laboratory scale spray dryer was used for the experiment (Unopex B 15 Mini Spray Dryer, İzmir, Turkey). Spray drying was carried out with the following parameters:

- The inlet and outlet temperatures of 160 °C and 82 °C respectively (was determined by preliminary experiments);
- Pump flow rate 11 ml / min;
- Aspiration rate: 80%

Preliminary experiments of GTWF drying yielded a very low amount of tea powder that is why inulin was selected with the suggestions of Doğadan engineers as a coating material to be used for spray drying. Inulin was also selected as a carrier material to move in this direction and add an additional functional component to the end product. This will increase the spray drying process' efficiency. Inulin is a polysaccharide of the fructan type that is a prebiotic and has antioxidant and anticancer properties (Wan et al., 2020). We employed inulin in a variety of concentrations (2 %, 5 %, 7 % (w/v)).

The powder products were stored in glass jars with vacuum lids until further analysis.

2.4.2 Freeze drying

Freeze drying was carried out by using a laboratory scale freeze dryer (TF-10D Manifold Top Press Type Vacuum Freeze Dryer, Turkey). Following the filtration of the brewed tea, filtrate was frozen at -18 °C. The frozen samples were dried for 48 hours.

The powder products were stored in glass jars with vacuum lids until further analysis.

2.4.3 Mixing with the herbal ingredient

Herb was added to the dried tea powders. Ratios of the added herbs were determined by the sensory panelists of Doğadan Tea Co. Instant green tea powders and pre-grinded and sieved herb powders were mixed for 1 minute at 600 rpm by using PB-100 Planet Ball Mill (LT-M0610).

2.5 Physical characterization analysis of instant powder green tea

2.5.1 Determination of moisture content

Moisture contents of the powders were determined using an oven at 105 °C. The samples were kept in the oven for 48 hours to reach constant weight.

2.5.2 Measurement of water activity

Water activity values of instant tea powders were measured by a water activity meter (Novasina Lab Start -a_w, Switzerland).

2.5.3 Turbidity measurements

For turbidity measurements in tea, the turbidity values of the teas obtained as a result of brewing using the turbidimeter (Milwaukee Mi 415 , Hungary). The measuring range values of the device are 0.00 - 1000 FNU. Samples were placed in glass cuvette containers and results were obtained within seconds. Since the measurement could not be made for the teas with a turbidity ratio above 1000 FNU, the values were read by making 1/10 dilution (1 ml of sample + 9 ml of distilled water).

2.5.4 Solubility analysis

Solubility of the powders was determined following the procedure of Şahin Nadeem et al. (2011). 1 g of sample was added to 100 ml of distilled water and the mixture was stirred at 600 rpm for 5 minutes. The solution was centrifuged at 1792 g for 5 minutes. Then, 15 ml of supernatant were transferred to the petri dishes and waited to be stabilized at a 105 °C oven. Solubility was calculating as following (Şahin Nadeem et al., 2011):

$$\text{Solubility } (\%) = \frac{Dry\ matter\ of\ supernatant}{Dry\ matter\ of\ Sample} * 100 \text{ (Eqn.6)}$$

2.5.5 Hygroscopicity

Hygroscopicity analyzes were made in a test cabinet shown in Figure 2.4 (Nüve TK 120, Türkiye). One gram of sample was kept in the cabinet at 75% relative humidity, 20^{o}C for 48 hours to reach constant weight. Hygroscopicity was calculated as kg moisture/100 kg dry sample (Balkır, 2022).

Figure 2. 3 Nüve TK 120 Test Cabinet

2.5.6 Morphological analysis

Morphological analysis of the powders was performed using a Scanning Electron Microscope (QUANTA 400F Field Emission SEM). Before analysis, the samples were coated with gold and palladium at a thickness of 3 nm. Experiments were carried out at METU Central Laboratory. The microscope has a resolution of 1.2 nm.

2.6 Chemical analysis of instant powder green tea

2.6.1 Extraction of the polyphenols

Following the procedure outlined in ISO 14502-1the extract's polyphenols were extracted in accordance with its specifications. Basically, 200 mg of each sample were weighed and added to an extraction tube containing 5 mL of 70% methanol at 70 °C. A water bath was used to heat the instant tea for 10 minutes at 70 °C. The extract was vortexed for 5 min at 3500 rpm; while cooling at room temperature and then filtered into a 10 mL volumetric flask. After one additional round of extraction, the extracts were mixed. Using 70% methanol, volume was then adjusted to 10 mL. 100 mL of water was added to 1 mL of the extract for dilution (Odumosu, Ojerinde & Egbuchiem, 2015).

2.6.2 Antioxidant capacity by DPPH method

The antioxidant capacity of the extracts was assessed spectrophotometrically using a modified version of the DPPH method described by Odumosu, Ojerinde & Egbuchiem (2015). DPPH solution was prepared by dissolving 9.85 mg of DPPH in 500 ml of 70% methanol. In triplicate, 4 mL of DPPH solution in methanol were combined with 2 mL of each diluted sample. Test tubes were incubated for 30 minutes at room temperature in the dark after the mixture was vortexed for 10 seconds for homogenization. Spectrophotometer (Optizen Pop Nano Bio, Korea) was then used to detect the absorbance at 515 nm.

By using the absorbance values, the percentages of free radical quenching were calculated with the equation given later in the report.

$Percent\ inhibition\ (\%)\ =\ 100\ \ *(A_0 - A)\ /\ A_0$ (Eqn.7)

In the equation, A_0 represents the absorbance values of the control solution and A of the samples.

Using the data obtained, IC_{50} values (*the sample concentration that can quench half of the DPPH radical in the environment, were determined*).

2.6.3 Antioxidant capacity by the ABTS method

A technique based on the decolonization of the radical cation of 2.20-azino bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was used to assess the antioxidant capabilities of the samples (Kelebek, 2016). For this study, a modified version of this procedure used. After incubating at room temperature for 12–16 hours, the reaction between 38.4 mg of ABTS and 6.9 mg of potassium persulfate with 10 ml distilled water produced the ABTS radical cation. Dilution was made by using methanol. 0.8 mL of each sample was mixed with 3.2 mL of the diluted ABTS solution. After keeping at dark for 10 minutes at room temperature, absorbance at 734 nm was immediately measured by the spectrophotometer (Optizen Pop Nano Bio, Korea).

IC_{50} values were determined by using the free radical quenching percentages calculated with the obtained results.

2.6.4 Total phenolic content

With a few minor adjustments, the total phenolic content of the samples was determined spectrophotometrically using the Folin-Ciocalteu method described in (Özdemir et al., 2018). For this, 5 ml of the 0.2 N Folin Ciocalteu's reagent and 4 mL of Na_2CO_3 (75 g L^{-1}) were added to 1 mL of the sample phenolic extract. The mixture was vortexed well and then let to sit at room temperature for 1 hour in dark. Using a blank of 1 mL of 70% methanol as the reference, the absorbance of the final solution was measured with a spectrophotometer (UV Spectrophotometer (Optizen Pop Nano Bio, Korea) at 765 nm. Results were expressed in terms of gallic acid equivalent (mg of GAE/100 g dm)

2.6.5 Quantification of catechins and L-theanine, gallic acid and caffeine content

With some adjustments, the approach followed by the procedure of Serpen et al. (2012) (Serpen, 2012) was used to extract catechin, gallic acid, caffeine and L-theanine components. Five mL of distilled water (80 °C) was used to extract from 500 mg of dry tea samples. After thoroughly mixing the tea infusion for 5 minutes, it was centrifuged at 10.000 rpm for 5 minutes. The residue was further extracted with 5 mL of distilled water (80 °C) after the supernatant was collected. Through a 0.45 m nylon filter, combined supernatants were filtered before being transferred to vials. An HPLC system (Shimadzu Scientific Instruments, Japan) comprised of a degasser (DG-20A5), a pump (LC-20AD), an autosampler (SIL-20AHT), a column oven (CTO-20A), and a PDA detector (SPD-M20A) was used to measure the amount of catechins and L-theanine in tea samples.

The Hypersil GOLD Phenyl HPLC column (250 x 4.6, 5 m, Termo Fisher Scientific, USA) was used for the chromatographic analysis of catechins and L-theanine. The sample injection volume and column temperature were 10 L and 25°C, respectively. With a mobile phase of acetonitrile (Solvent A) and acetic acid (Solvent B) (%0.1 v/v) at a flow rate of 1 mL/min, catechins were separated using a linear gradient technique (Serpen, 2012). The program for gradient elution was decided upon as follows: 0–15 min, linear gradient from 10–20 % A; 15–25 min, linear gradient elution from 20–40 % A; and 25–30 min, linear gradient elution from 40–10 % A. By comparing the tea sample's retention period to that of the standard chemicals, the peaks at 275 nm were found, and calibration curves were made using standard solutions. L-theanine was analyzed chromatographically in accordance with the procedure of Murugesh et al. (2018) (Murugesh, 2018) published procedure. Analysis was performed by a linear gradient elution with mobile phase of acetonitrile (Solvent A) and water (Solvent B) at a flow rate of 1 mL/min. The gradient elution program was adopted as: 0-5 min, 2% A; 5-12 min, linear gradient elution from 2 to 50% A; 12-30 min, 50% A; 30-40 min, linear gradient elution from 50 to 2% A; 40-45 min, 2% A. The peaks were identified at 200 nm and calibration curve was prepared by using L-theanine standard.

2.7 Experimental design

The parameters for brewing and production of instant green powder tea are summarized in Table 2.3, Table 2.4.

Table 2. 3: Experimental Design for hot and cold brewing (tea / water ratio 1/40)

Process	**Temperature**	**Brewing Time**
Hot Brewing	70° C	7 & 20 mins.
Hot Brewing	90° C	7 & 20 mins.
Cold Brewing	4 °C	6 & 12 hours
Cold Brewing	25 °C	2 hours

Table 2. 4: Experimental Design for production of tea powder with rockrose

Herb Type	**Herb Ratio**	**T(°C)**	**Time**	**Drying Method**	**Analyzes**
-	-	70 °C	20 mins	Freeze & Spray	**Particle Size Measurement** **Moisture Content** **Water Activity** **Turbidity Measurement** **Solubility** **Hygroscopicity** **Total Phenolic Content** **Antioxidant Activity** **Quantification of Catechins and L-theanine, Gallic Acid and Caffeine Content** **Morphological Analysis**
-	-	25 °C	2 h	Freeze & Spray	
Rockrose	25&35 %	70 °C	20 mins	Freeze & Spray	
Rockrose	25&35 %	25 °C	2 h	Freeze & Spray	

2.8 Statistical Analysis

Analysis of Variance (ANOVA) was carried out for data analysis (Minitab Inc., Coventry, UK). Tukey's comparison test with a 95% confidence interval was used for paired comparisons. For every experiment, at least three replicates were employed. Different letters ($p<0.05$) are used to denote significant differences between the samples.

CHAPTER 3

RESULTS AND DISCUSSION

This chapter was reported in three sections in accordance with the experimental design as:

- *The determination of brewing efficiency;*
- *The analysis of spray dried instant green tea powders mixed with rockrose;*
- *The analysis of freeze dried instant green tea powders mixed with rockrose;*

3.1 The determination of brewing efficiency

3.1.1 Determination of tea / water ratio

To determine the tea water ratio that will be used throughout the study, GTWF was used at the extreme temperature/time combinations (90 ^{o}C/20 min) of hot brewing using tea/water ratios of **1/10, 1/20, 1/30 and 1/40**.

It is expected that as the tea amount increases efficiency will increase. The ratios were determined such that the ratios were also feasible in large scale production.

At the same time, normal green tea leaves were used as the control samples and were brewed at the same conditions using the same ratios. Results are given in Table 3.1.

Table 3. 1 Brewing Efficiency

Brewing Efficiency (%)		
Ratio	**Green Tea Waste Fibers**	**Sample Green Tea Leaves**
1/10	26.04 ± 0.18^{c}	39.4 ±0.05^{b}
1/20	26.69 ± 0.28^{c}	47.9 ±0.02^{a}
1/30	29.43 ± 0.46^{b}	48.1 ±0.01^{a}
1/40	33.18 ± 0.89^{a}	48.8 ± 0.01^{a}

Lower case letters denote significance difference at 95 % within columns separately.

At the extreme brewing conditions of 90 °C & 20 mins, for GTWF and normal green tea, the ratio with the highest extraction efficiency overlapped and this ratio was determined as 1/40.

3.1.2 Pre-grinding of GTWF

The results showed that the first milling process applied to the waste tea fibers reduced the particle sizes of the GTWF to values lower than those of the normal tea leaves (Table 3.2). The span values of the control samples were narrower as expected.

Table 3.2 Particle sizes of the GTWF and normal tea leaves following pre-grinding

Sample	**D_{50} (μm)**	**D_{90}(μm)**	**D_{10}(μm)**	**Span**
GTWF	276.7	901.8	37.7	3.1
Normal green tea leaves	730.9	1394.2	247.7	1.6

As the goal of the study is to produce instant tea powder from the waste tea fibers, extraction yield after the brewing is crucial. As particle size decreases, extraction will be more and brewing efficiency will increase. That is why super grinding (*milling the samples in a ball-mill*) was also performed for the GTWF samples. Results are given in Table 3.3.

Table 3.3 Particle sizes and cellular disruption rates of the samples following super grinding

Sample	**Super grinding Time**	**D_{50} (µm)**	**D_{90} (µm)**	**D_{10} (µm)**	**Span**	**Cellular Disruption Ratio**
GTWF	5 mins.	112.1	758.2	8.3	6.7	24.4
	15 mins.	32.5	136.9	6.2	4.0	66.8
	30 mins.	**18.2**	**64.2**	**4.2**	**3.3**	**90.9**

Super grinding was performed at 3 different times (Table 3.3). It is expected that the extraction and dispersion properties of GWTF will increase, and thus the brewing efficiency will increase, due to the increased surface area and fragmented cell walls (Zhao et al., 2009). The results showed that as the super grinding time increased, the particle size decreased, and the cell wall fragmentation rates increased. Based on the results, it was decided to subject the GTWF to super-grinding process for 30 minutes before brewing and brewing efficiencies were compared with the pre-grinded samples.

3.1.3 Comparison of brewing efficiencies of pre and super grinded samples

At the tea/water ratio of 1/40, all *temperature-time* combinations were tested to compare the brewing efficiency of pre and super grinded samples (Table 3.4).

Table 3.4 Brewing efficiency after pre & super grinding*

Sample No	**Brewing Temperature**	**Brewing Time**	**Brewing Efficiency pre-grinded samples**	**Brewing Efficiency super grinded samples**
1	90 °C	7 mins	34.3 ±0.0.6^{a}	29.7 ±0.7^{ab}
		20 mins	30.2 ±1.1^{ab}	33.8 ±3.1^{a}
2	70 °C	7 mins	28.1 ±2.4^{b}	27.9 ±0.1^{b}
		20 mins	28.5 ±1.1^{b}	32.1 ±0.3^{ab}
3	25 °C	2 hours.	9.2 ±1.0^{a}	10.3 ±0.1^{a}
4	4 °C	6 hours	7.1 ±0.8^{b}	6.3 ±0.4^{b}
		12 hours	10.5 ±0.4^{a}	6.2 ±0.6^{b}

**Lower case letters denote significance difference at 95 % within columns separately*

As expected, the increased surface area and cell disruption rate were able to increase the brewing yields for most temperature-time combinations.

For cold brewing conditions, the brewing efficiency has the highest value @ 25 °C for 2 hours. As the temperature drops, a decrease in brewing yield has been observed. It was found that the brewing time @4°C did not have an effect on the brewing efficiency either ($p > 0.05$).

In a study of Lin et al. (2014), it is reported that soluble solids, caffeine, catechins, and antioxidant active ingredients were all reduced during cold brewing @4°C (Lin et al., 2014).

For hot brewing conditions, there was no significant difference of brewing temperature and times statistically ($p > 0.05$). In brewing step, low temperatures had the ability to prevent bioactive chemicals from degrading while *longer extraction times* helped transfer higher amounts of active molecules. Green tea's polyphenols can be damaged and its sensory properties can be diminished at temperatures above 90 °C, hence it is advised to extract antioxidant chemicals from the tea at *low temperatures* (Lantano et al., 2015).

By considering all suggestions mentioned above, the hot brewing parameters were determined for green tea at a ratio of 1/40 for 20 minutes at 70 °C, and at a ratio of 1/40 for 2 hours at 25 °C for cold brewing, and all analyzes for powder products were made according to these parameters.

3.1.4 Super grinding of herbs

Tea powders produced by spray drying and freeze drying processes were mixed with *rockrose* which had undergone pre-grinding and sieving processes, by super grinding at the rates given in the Table 3.5. The super grinding-mixing process was carried out at 600 rpm for 1 minute. It was expected that the super grinding process at this stage will improve the dispersion and dissolution properties of the powders (X. Zhao et al., 2017).

Rockrose were added at 2 different concentrations following the taste panels performed at Doğadan Tea company.

Table 3. 5 Herb ratio added to the dried green tea powders (w/w %)

Herb Type	**Ratio 1**	**Ratio 2**
Rockrose	25 %	35 %

3.1.5 Effect of inulin on spray drying

The main idea behind spray drying is that the liquid that enters the system is turned into atomized droplets and sprayed into the drying chamber, where the droplets come into contact with heated air and get dehydrated. Spray drying system was first tested with the green tea infusions obtained. However, the powders sticked to the spray dryer chamber, and very low powder yield was achieved. Adhesion of the dried powders to the drying chamber during spray drying is a frequently observed condition in foods containing sugar and acid (Adhikari et al., 2005). This is explained by the low glass transition temperatures of the obtained powders (Cano-Chauca et al., 2005); (Rezaul et al., 2017)). Due to the presence of certain sugars, green tea extracts are hygroscopic and become sticky after drying (Zokti et al., 2016).

The stickiness problem encountered during spray drying is usually solved using carrier materials with high molecular weight and, glass transition temperature, crystallinity. Use of such carrier materials increases the powder recovery in spray drying (Azhar et al., 2021). To put it another way, spray drying is used to encapsulate the active ingredient that is intended to be acquired with a carrier material.

Green tea has the highest concentration of healthy polyphenols and the least amount of caffeine. It contains a lot of catechins (polyphenols), which are powerful antioxidants. EPCG is the primary catechin present in green tea. Due to their strong astringency and bitter flavor, polyphenols are challenging to include into food products. Encapsulation could also solve these problems by masking the flavors (Tengse et al., 2017). In order to advance in this direction and give the finished product an extra functional component, it was decided to employ inulin as a carrier material. Inulin also has a 'prebiotic' feature *thus has the potential to promote the activity of healthy microorganisms in the c*olon, (Laurenço et al., 2020).

Having antioxidant and anticancer effects, inulin is a prebiotic polysaccharide of the fructan type (Wan et al., 2020). Inulin was used at different concentrations (2 %, 5 %, and 7 %). At 2 % (w/w) stickiness problem was not solved; and at 7 % (w/w) product became too sweet; that is why 5 % (w/w) was selected as the inulin concentration.

Table 3.6 Dry matter content of tea extract after hot & cold brewing before & after inulin addition

Sample	**Brewing Temp.**	**Brewing Time**	**Dry matter content without inulin (g dry content / g sample)**	**Dry matter content with inulin (g dry content / g sample)**
GTWF	70 °C	20 mins	0.59 ± 0.00^a	5.21 ± 0.02^a
GTWF	25 °C	2 hours	0.44 ± 0.07^b	5.02 ± 0.02^b

Lower case letters denote significance difference at 95 % within columns separately

As seen in the tables, the addition of inulin significantly increased the dry matter content of the extract at both brewing temperatures ($p < 0.05$).

3.2 Analysis of spray dried instant green tea powders mixed with *rockrose*

Physical characterization analysis of spray dried instant green tea with *rockrose*

3.2.1 Moisture content measurements

Moisture content is closely related to the product's quality and shelf life. The moisture content of the food samples is crucial as it influences both the cosmetic appearance and microbiological deterioration (Jung et al., 2018). According to the Turkish Food Codex (Turkish Food Codex, 2015), the moisture content of green tea should be maximum 7 % (g/g).

In Table 3.7 moisture content results are given for the green teas prepared with rockrose. Rockrose significantly increased the moisture content values as expected

($p < 0.05$). However, the herb ratio and brewing parameters did not have any effect on the moisture content of the samples ($p > 0.05$).

Table 3. 7 Moisture content of spray dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Moisture Content
GTWF	70 °C	20 mins	-	-	5.05 ± 0.13^{b}
GTWF	25 °C	2 hours	-	-	5.63 ± 0.085^{b}
GTWF	70 °C	20 mins	Rockrose	25 %	11.18 ± 0.99^{a}
				35 %	16.43 ± 10.98^{a}
GTWF	25 °C	2 hours	Rockrose	25%	11.04 ± 0.95^{a}
				35 %	12.97 ± 2.72^{a}

Lower case letters denote significance difference at 95 % within columns separately

3.2.2 Water activity measurements

In food products, water is the most crucial component. It has a direct impact on both the product's quality and shelf life. Moisture level has a significant impact on both the physical appearance and microbiological deterioration of food samples (Alp & Bulantekin, 2021)

Water activity results of the samples from spray dried powder green tea is shown in Table 3.8. Generally speaking, food with an a_w of less than 0.6 is thought to be microbiologically stable, and any deterioration that does occur is caused by chemical reactions rather than microorganisms. In a previous study where spray dried green tea powder which is encapsulated with maltodextrin is examined water activity is calculated in the range of 0.2-0.29 (Susantikarn & Donlao, 2016). Water activity of the tea samples with and without *rockrose* changed in the range of 0.2-0.365 which was still below the critical value of 0.6. *Rockrose* addition increased the water activity values as expected ($p < 0.05$). However, the herb ratio and brewing parameters had no effect on the water activity of the samples ($p > 0.05$).

Table 3. 8 Water activity of spray dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Water Activity
GTWF	70 °C	20 mins	-	-	0.205 ± 0.017^{c}
GTWF	25 °C	2 hours	-	-	0.200 ± 0.011^{c}
GTWF	70 °C	20 mins	Rockrose	25 %	0.335 ± 0.007^{ab}
				35 %	0.330 ± 0.00^{b}
GTWF	25 °C	2 hours	Rockrose	25%	0.340 ± 0.014^{ab}
				35 %	0.365 ± 0.007^{a}

Lower case letters denote significance difference at 95 % within columns separately

3.2.3 Turbidity measurements

Turbidity is a crucial aspect of tea infusions (Wang et al., 2016). Turbidity results of spray dried powders are given in the Table 3.9.

When rockrose was added to the control sample, the turbidity values increased ($p < 0.05$). However, it was interesting to see that with the increase in the concentration of rockrose, turbidity values decreased ($p<0.05$). The effect of inulin seemed to be compensated by the presence of *rockrose* which could have affected the turbidity values.

Table 3. 9 Turbidity of the spray dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Turbidity (FNU)
GTWF	70 °C	20 mins	-	-	85.70 ± 1,15[d]
	25 °C	2 hours	-	-	81.4 ± 0,53 [d]
GTWF	70 °C	20 mins	Rockrose	25 %	407.33 ± 9.07[b]
				35 %	262.50 ± 6.36[c]
GTWF	25 °C	2 hours	Rockrose	25%	540.66 ± 7.15[a]
				35 %	382.50 ± 31.81[b]

Lower case letters denote significance difference at 95 % within columns separately

3.2.4 Solubility Measurement

The ability of a powder to dissolve in water is referred to as solubility. In the study of Susantikarn & Donlao (2016) , solubility of the spray dried green tea powder was in the range of 94-98% (Susantikarn & Donlao, 2016). Control samples' solubility was also in a similar range as shown in Table 3.10.

Addition of *rockrose* powders decreased the solubility compared to the control samples ($p<0.05$). Rockrose powder was added directly without exposing the herb to the extraction. The presence of insoluble polysaccharides in the herb could have also decreased the solubility. Brewing type did not influence the solubility for the same concentrations ($p>0.05$).

Table 3. 10 Solubility of spray dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Solubility
GTWF	70 °C	20 mins	-	-	96.78 ± 0.92[a]
GTWF	25 °C	2 hours	-	-	93.55 ± 0.99[a]
GTWF	70 °C	20 mins	Rockrose	25 %	80.36 ± 1.08[b]
				35 %	72.99 ± 5.04[c]
GTWF	25 °C	2 hours	Rockrose	25 %	73.94 ± 0.28[bc]
				35 %	68.14 ± 2.47[c]

Lower case letters denote significance difference at 95 % within columns separately

A correlation analysis was also conducted between the solubility and the turbidity values of the powders. A negative correlation between the solubility and turbidity values of the powders was detected with a correlation coefficient of ~80% ($p \leq 0.05$). When herb ratio increased, turbidity value increased while the solubility decreased.

3.2.5 Hygroscopicity Analysis

Hygroscopicity is known as the ability to absorb water. Hygroscopicity is an important parameter of how powder products are affected by the relative humidity of the environment during storage or packaging material selection (Yüksel, 2018).

Hygroscopicity values of the samples shown in Table 3.11. The results showed that the hygroscopicity values of green powder teas dried by spray drying were in the range of 14%-25%.

Addition of herbal powders decreased the hygroscopicity compared to the control samples for *rockrose* ($p<0.05$). Furthermore, with *rockrose* addition; at the same quantities, there was not a significant difference between the hot and cold brew samples with the exception of hot brewed sample with *rockrose* ($p>0.05$).

The addition of *rockrose* to the control sample for cold & hot brewing decreased the hygroscopicity values. This was an expected case since the moisture content increased with herbal addition and it resulted in less water absorption. There is an inverse correlation between moisture content and water absorption ability (Mohammadi & Nokken, 2013). A correlation analysis was also conducted between the moisture content and the hygroscopicity values. The correlation coefficient between hygroscopicity and moisture content results was quite high ($r > 0.89$) and significant ($p < 0.05$) confirming the study of Mohammadi et al (2013).

Table 3. 11 Hygroscopicity of spray dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Hygroscopicity (%)
GTWF	70 °C	20 mins	-	-	25.19±1.27[a]
GTWF	25 °C	2 hours	-	-	25.51±1.23[a]
GTWF	70 °C	20 mins	Rockrose	25 %	17.63±0.21[b]
				35 %	14.51±0.22[c]
GTWF	25 °C	2 hours	Rockrose	25 %	14.10±0.44[c]
				35 %	16.25±0.17[bc]

Lower case letters denote significance difference at 95 % within columns separately

Chemical analysis of spray dried instant green tea with *Rockrose*

3.2.6 Determination of antioxidant activity values

DPPH & ABTS | Radical Extinguisher Capacity Method

ABTS (*2,20-azino-bis-3-ethyl-benzothiazoline-6-sulfonic acid*) and DPPH (*2,2-diphenyl-1-picryl hydrazyl*) tests were used to measure the antioxidant capacity of the tea infusions (Kelebek, 2016).

The amount of an antioxidant-containing material needed to scavenge 50% of the initial DPPH radicals is known as the IC_{50}. The more effective a substance is at scavenging DPPH, and hence, the lower the IC_{50} value, the greater the antioxidant activity of the material (Olugbami et al., 2014). The results of IC_{50} values from ABTS and DPPH assay are given in Table 3.12. Also, there is a correlation between the result of ABTS and DPPH methods ($r > 0.99$, $p<0.05$).

In both brewing parameters and both DPPH and ABTS method, adding *rockrose* to the control sample decreased the level of IC_{50} ($p < 0.05$); but there was no significant difference if the ratio increased to the 35 % concentration ($p > 0.05$).

Table 3. 12 IC_{50} values of spray dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	DPPH	ABTS
					IC_{50} (µg/mL)	IC_{50} (µg/mL)
GTWF	70 °C	20 mins	-	-	13.23 ± 1.02^{b}	11.92 ± 1.92^{c}
GTWF	25 °C	2 hours	-	-	31.71 ± 2.84^{a}	29.89 ± 1.17^{a}
GTWF	70 °C	20 mins	Rockrose	25%	5.95 ± 0.11^{c}	6.11 ± 0.09^{d}
				35 %	5.67 ± 0.68^{c}	5.41 ± 0.35^{d}
GTWF	25 °C	2 hours	Rockrose	25 %	14.92 ± 1.80^{b}	16.52 ± 3.61^{b}
				35 %	14.54 ± 1.33^{b}	15.20 ± 0.93^{bc}

Lower case letters denote significance difference at 95 % within columns separately

3.2.7 Determination of total phenolic content

Total phenolic content (TPC) is expressed as gallic acid equivalents (GAE) in g/100 g of material (Kopjar et al., 2015). Studies have shown that green tea leaves' phenolic content is found in the range of 11-19 g GAE/100 g (Anesini et al., 2008). In spray drying, the values were below the expected range for both brewing parameters (seen in Table 3.13). The presence of inulin is one of the main reasons of these low values.

Addition of *rockrose* significantly increased the total phenolic substances of powdered teas ($p<0.05$). For green tea , the TPC value of hot brewing was higher than cold brewing (Venditti et al., 2010). The result showed that there was a significant difference between hot & cold brewed control samples ($p<0.05$). Further addition of rockrose also increased the total phenolic content of the samples for both brewing type ($p<0.05$).

Table 3. 13 Total phenolic content of spray dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Total Phenolic Content (g GAE / 100 g)
GTWF	70 °C	20 mins	-	-	1.92±0.04^{e}
GTWF	25 °C	2 hours	-	-	1.00±0.06^{f}
GTWF	70 °C	20 mins	Rockrose	25 %	4.34±0.17^{a}
				35 %	3.40±0.04^{b}
GTWF	25 °C	2 hours	Rockrose	25 %	2.14±0.06^{d}
				35 %	2.58±0.05^{c}

Lower case letters denote significance difference at 95 % within columns separately

The relationship between total phenolic content (TPC) and antioxidant capacity (DPPH & ABTS essay) of instant tea powders with *rockrose* was evaluated by The correlation coefficient of both ABTS & DPPH and TPC were high ($r > 0.87$) and significant ($p < 0.05$) ; suggested that the DPPH & ABTS assay results captured similar compounds.

3.2.8 HPLC Method for determination of Catechins, Gallic Acid, Caffeine and L – Theanine

Flavonoids (and their component, catechins), which serve as antioxidants, are the main phenolic components in green tea. Most of the green tea polyphenols are catechins like epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) (Jigisha et al., 2012). The main catechin and thought to be the most potent catechin in green tea infusion is *epigallocatechin* *gallate* (EGCG). As an antioxidant, anti-inflammatory, antibacterial, and antiviral agent, EGCG is a promising chemical for both prevention and treatment (Can Agca et al., 2020).

Tea plants also contain L-theanine, a water-soluble, non-protein amino acid. It is a distinct flavor element with a caramel flavor that might lessen the caffeine's harshness (Li et al., 2022). L-theanine is the main source of tea flavor. Green tea also includes caffeine. Caffeine can alter the qualities of tea, such as the flavor, adding to the acidity and bringing out the astringency and bitterness (Tfouni et al., 2018). Gallic acid is a typical phenolic acid that is present in many plants and tea preparations.

The analytical method typically employed to determine green tea catechins is high performance liquid chromatography (HPLC). Results are given in Table 3.14.

Table 3. 14: Catechins, Gallic Acid, Caffeine & L-theanine content of spray dried instant green tea samples with rockrose

Sample	Temp.	Time	Herb Type	Herb Ratio	EC (mg / 100 g)	ECG (mg / 100 g)	EGC (mg / 100 g)	EGCG (mg / 100 g)	Gallic Acid (mg / 100 g)	Caffeine (mg / 100 g)	L-theanine (mg / 100 g)
GTWF	70 °C	20 mins	-	-	422.25 ±0.07^{a}	61.13 ±0.03^{a}	449.64 ±0.11^{a}	175.42 ±0.05^{a}	69.59±0.03^{a}	442.79±0.23^{a}	472.65±0.35^{a}
GTWF	25 °C	2 hours	-	-	276.50 ±0.15^{b}	27.94 ±0.02^{c}	170.82 ±0.41^{d}	49.75 ±0.01^{c}	56.95±0.02^{b}	319.29±0.29^{b}	367.71±0.01^{b}
GTWF	70 °C	20 mins	Rockrose	25 %	264.32±0.23^{b}	37.78±0.00^{b}	432.14±0.41^{ab}	77.69±0.06^{b}	34.92±0.00^{d}	226.99±0.05^{c}	365.97±0.04^{b}
				35 %	217.37±0.07^{c}	35.86±0.02^{b}	381.54±0.33^{b}	81.85±0.05^{b}	37.29±0.01^{d}	190.83±0.03^{cd}	332.92±0.02^{b}
GTWF	25 °C	2 hours	Rockrose	25 %	205.34±0.11^{c}	16.60±0.01^{d}	256.57±0.07^{c}	36.37±0.02^{d}	50.55±0.01^{c}	187.93±0.08^{cd}	348.55±0.11^{b}
				35 %	158.36±0.13^{d}	12.86±0.01^{d}	229.71±0.13^{cd}	34.10±0.03^{d}	52.42±0.03^{bc}	150.84±0.07^{d}	333.27±0.12^{b}

Lower case letters denote significance difference at 95 % within columns separately

For *rockrose*, except EC concentration, there is no significant difference on ECG, EGC and EPCG content when herbal ratio is increased from 25 % to 35 % for hot & cold brewing ($p > 0.05$). On the other hand, all catechins concentration for the powder with *rockrose* decreased when brewing temperature decreased in control samples ($p < 0.05$).

The highest value of L-theanine is found in the control sample in hot brewing for *rockrose*. In cold brewing, rockrose amount was insignificant on the L-theanine content ($p > 0.05$). There was no significant difference on the gallic acid and caffeine content when herbal ratio increased from 25 % to 35 % for hot & cold brewing ($p > 0.05$). On the other hand, gallic acid, caffeine and L-theanine concentration for the powder with *rockrose* decreased when temperature decreased in the control samples ($p< 0.05$).

3.3 Analysis of spray dried instant green tea powders mixed with *Rockrose*

Physical characterization analysis of freeze dried instant green tea with *rockrose*

3.3.1 Moisture Content Measurement

The moisture content of freeze dried instant green tea powders is shown in the Table 3.15.

With the addition of *rockrose*, the moisture content of the freeze-dried powder samples increased ($p < 0.05$). There was no significant interaction between herb ratio with the same brewing conditions in hot brewing ($p > 0.05$). However in cold brewing there was a significant difference between the moisture content with herb ratio ($p < 0.05$).

Table 3. 15 Moisture content of freeze dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Moisture Content
GTWF	70 °C	20 mins	-	-	9.22 ± 0.05^{c}
GTWF	25 °C	2 hours	-	-	9.87 ± 0.03^{c}
GTWF	70 °C	20 mins	Rockrose	25 %	13.84 ± 2.09^{b}
				35 %	13.60 ± 2.83^{b}
GTWF	25 °C	2 hours	Rockrose	25%	17.90 ± 1.48^{a}
				35 %	14.00 ± 1.81^{b}

Lower case letters denote significance difference at 95 % within columns separately

Due to the increase in feed solids and a decrease in free water, spray drying with more encapsulating material caused a noticeable decrease in the moisture content. It was discovered that spray dried powders had lower moisture content values than freeze dried powders. Heat transmission is more rapid at higher inlet temperatures, which created a strong pushing force for moisture evaporation. As a result, the powders' moisture content decreased (Caliskan & Dirim, 2016)..

3.3.2 Water Activity Measurement

The water activity values were in the range of 0.25-0.36 in the freeze-drying method. Microbiologically, the samples were found safe. Results shown in Table 3.16.

The addition of *rockrose* improved the water activity in comparison to the control samples as expected ($p < 0.05$).

Table 3. 16 Water activity of freeze dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Water Activity
GTWF	70 °C	20 mins	-	-	0.26 ± 0.02^{b}
GTWF	25 °C	2 hours	-	-	0.256 ± 0.005^{b}
GTWF	70 °C	20 mins	Rockrose	25 %	0.350 ± 0.007^{a}
				35 %	0.350 ± 0.00^{a}
GTWF	25 °C	2 hours	Rockrose	25%	0.325 ± 0.014^{a}
				35 %	0.360 ± 0.007^{a}

Lower case letters denote significance difference at 95 % within columns separately

In the study, the result showed that the water activity value of the freeze-dried powder samples was higher than spray dried powder samples.

3.3.3 Turbidity Measurement

In cold brewed *rockrose* samples, there was no significant difference between the ratios ($p>0.05$).

There was a significant difference between cold brewing and hot brewing samples in terms of turbidity ($p<0.05$).

Table 3. 17 Turbidity of freeze dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Turbidity (FNU)
GTWF	70 °C	20 mins	-	-	61.3 ± 2.08^{d}
	25 °C	2 hours	-	-	99.7 ± 1.70^{c}
GTWF	70 °C	20 mins	Rockrose	25 %	103.00 ± 3.46^{c}
				35 %	178.67 ± 13.28^{a}
GTWF	25 °C	2 hours	Rockrose	25%	156.50 ± 0.71^{b}
				35 %	167.00 ± 3.46^{ab}

Lower case letters denote significance difference at 95 % within columns separately

3.3.4 Solubility Measurement

The solubility of freeze dried sampled are shown in Table 3.18. The solubility ratio of instant powdered teas obtained by adding *rockrose* decreased as the herb ratio was added compared to the control sample ($p<0.05$). It was observed that the plant ratios did not have a significant effect in cold brewing ($p>0.05$). Significant interaction was observed between the brewing parameters at the same concentrations of *rockrose* (@ 25 % concentration). As mentioned before in spray drying, the addition of herb decreased the solubility of the control samples more, because the herbs added to the green tea instant powder as itself without any extraction process.

Table 3. 18 Solubility of freeze dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Solubility
GTWF	70 °C	20 mins	-	-	89.35 ± 0.88[a]
GTWF	25 °C	2 hours	-	-	86.55 ± 1.10[a]
GTWF	70 °C	20 mins	Rockrose	25 %	74.75 ± 0.63[b]
				35 %	70.83 ± 1.06[c]
GTWF	25 °C	2 hours	Rockrose	25 %	71.34 ± 0.65[c]
				35 %	69.17 ± 1.07[c]

Lower case letters denote significance difference at 95 % within columns separately

As seen in the spray drying results, there was a strong correlation between the solubility and the turbidity of the powders (r > 0.89 and p < 0.05). As the herbs are added in control sample, their solubility rate decreased.

3.3.5 Hygroscopicity Analysis

The hygroscopicity results of freeze-dried samples were found in the range of 12-26 % (Table 3.19).

Hygroscopicity values are important for the shelf life of the products. Hygroscopic powdered products tend to cake which is not desirable as it affects the flow behavior of the powdered products during processing. That is why samples were stored at a relatively high environment and moisture uptake values were calculated.

The hygroscopicity values of powdered teas mixed with *rockrose* decreased as the herb was added (p< 0.05). There was no significant interaction between brewing parameters and herb ratios (p>0.05).

While there is no significant difference between hot and cold brewing in spray-dried control sample teas (p > 0.05), the hygroscopicity of cold-brewed teas in freeze-dried teas was higher than hot brewed samples (p < 0.05).

Table 3. 19 Hygroscopicity of freeze dried instant green tea samples mixed with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Hygroscopicity (%)
GTWF	70 °C	20 mins	-	-	20.58±0.55^{b}
GTWF	25 °C	2 hours	-	-	26.04±0.06^{a}
GTWF	70 °C	20 mins	Rockrose	25 %	12.86±0.02^{cd}
				35 %	15.10±0.71^{c}
GTWF	25 °C	2 hours	Rockrose	25 %	12.58±0.11^{d}
				35 %	14.74±1.25^{cd}

Lower case letters denote significance difference at 95 % within columns separately

Chemical Analysis of Freeze-Dried Instant Green Tea with *rockrose*

3.3.6 Determination of Antioxidant Activity Values

DPPH & ABTS | Radical Extinguisher Capacity Method

For freeze dried samples, the results of IC_{50} values from DPPH and ABTS methods are given in Table 3.20.

According to the results, brewing parameters and changing the ratio of *rockrose* did not have a significant effect on the value of IC_{50} for both methods ($p > 0.05$). In hot brewing, teas mixed with *rockrose* had the lowest IC_{50} values.

There was a positive correlation between DPPH and ABTS assay for freeze dried samples. It is evaluated by Pearson correlation matrix with high correlation coefficient ($r > 0.95$).

Also, experimental results showed that the IC_{50} values of freeze-dried instant tea powders were higher than the spray dried powder results. This indicated that the antioxidant capacity of freeze-dried samples was higher.

Table 3. 20 IC_{50} values of freeze dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	DPPH	ABTS
					IC_{50} (µg/mL)	IC_{50} (µg/mL)
GTWF	70 °C	20 mins	-	-	1.02 ± 0.04^{bc}	0.70 ± 0.06^{bc}
GTWF	25 °C	2 hours	-	-	1.25 ± 0.22^{ab}	1.47 ± 0.53^{a}
GTWF	70 °C	20 mins	Rockrose	25%	0.29 ± 0.12^{d}	0.33 ± 0.00^{c}
				35 %	0.37 ± 0.04^{cd}	0.42 ± 0.02^{c}
GTWF	25 °C	2 hours	Rockrose	25 %	1.15 ± 0.45^{ab}	1.21 ± 0.27^{ab}
				35 %	1.70 ± 0.20^{a}	1.78 ± 0.42^{a}

Lower case letters denote significance difference at 95 % within columns separately.

3.3.7 Determination of Total Phenolic Content

Total phenolic contents results are given in Table 3.21 for freeze dried green tea powders mixed with *rockrose*. The further addition of rockrose to the control sample increased the total phenolic content of the sample in hot brewing ($p < 0.05$). In addition, the addition of rockrose increased the total phenolic content ($p < 0.05$). The total phenolic content of freeze drying was found to be higher than the powder that was spray dried. It is obvious that with spray drying , high temperatures resulted in the degradation of the phenolic compounds (Can et al., 2022). This may have caused lower values on the TPC.

Table 3. 21 Total phenolic content of freeze dried instant green tea samples with rockrose

Sample	Brewing Temp.	Brewing Time	Herb Type	Herb Ratio	Total Phenolic Content (g GAE / 100 g)
GTWF	70 °C	20 mins	-	-	16.68 ± 0.53^{b}
GTWF	25 °C	2 hours	-	-	12.90 ± 0.22^{c}
GTWF	70 °C	20 mins	Rockrose	25 %	16.46 ± 0.34^{b}
				35 %	20.45 ± 0.46^{a}
GTWF	25 °C	2 hours	Rockrose	25 %	13.20 ± 0.05^{d}
				35 %	14.23 ± 0.40^{c}

Lower case letters denote significance difference at 95 % within columns separately

Measurement of phenolic concentration is essential for antioxidants because this characteristic is frequently linked to phenolic structure. Therefore, the correlation as checked between TPC and both ABTS & DPPH method. A positive correlation between the antioxidant capacity and total phenolic content of the samples was detected (for ABTS $r > 0.78$ and $p > 0.05$ & for DPPH $r > 0.73$ and $p > 0.05$).

3.3.8 HPLC results for catechins, gallic acid, caffeine, and L – Theanine

All HPLC results are provided in Table 3.22. In hot brewed samples prepared with *rockrose*, EC, ECG and EGCG contents decreased when the herb ratio increased. For all types of catechin, there was no effect of the herbal ratio (from 25 % to 35 %) in the cold brewed samples ($p > 0.05$).

In cold brewed samples, the caffeine and L – theanine content decreased with *rockrose* addition ($p < 0.05$). Gallic acid content was not affected from the herbal ratio ($p > 0.05$). It was also observed that the gallic acid content was the highest in the 25 % her ratio for the hot brewed samples. Also, for gallic acid, caffeine and L-theanine content no significant difference was observed was observed for the samples prepared with cold brewing when the ratio increased from 25 % to 35 % ($p > 0.05$).

The change in the L-theanine contents for both brewing methods did not change significantly as the herb ratios increased ($p > 0.05$). Cold brewed samples had significantly higher L-theanine values ($p<0.05$).

While the amount of caffeine in the products obtained by hot brewing changed with respect to the herb ratios ($p<0.05$), the herb ratio did not have a significant effect in cold brewing ($p > 0.05$) . At high herb ratio, brewing type did not have a significant effect on the amount of caffeine ($p > 0.05$) , while the amount of caffeine at lower concentration decreases as the brewing temperature decreases ($p < 0.05$).

The drying method had a significant effect for the recovery of catechins. In the study of Vuong et al. (2012) where green tea was examined, it was stated that the yield of green tea powders produced by freeze drying was higher than that of spray drying, as was the recovery of catechins (Vuong et al., 2012). Due to their susceptibility to high temperatures, the catechins may have been partially deteriorated as a result of the 160 °C inlet and 82 °C outlet air temperatures they encountered during spray drying. In this study, the results showed that freeze drying was better than spray drying in terms of keeping the phenolic compounds.

Table 3. 22 : Catechins, Gallic Acid, Caffeine & L-theanine content of freeze dried instant green tea samples with rockrose

Sample	Temp.	Time	Herb Type	Herb Ratio	EC (mg / 100 g)	ECG (mg / 100 g)	EGC (mg / 100 g)	EGCG (mg / 100 g)	Gallic Acid (mg / 100 g)	Caffeine (mg / 100 g)	L-theanine (mg / 100 g)
GTWF	70 °C	20 mins	-	-	3756.06 ± 3.36^{a}	1164.64 ± 0.97^{a}	4270.14 ± 3.74^{a}	4867.56 ± 4.67^{a}	187.75 ± 0.09^{bc}	3331.59 ± 1.70^{a}	1904.53 ± 0.49^{bc}
GTWF	25 °C	2 hours	-	-	3317.06 ± 0.56^{ab}	473.61 ± 0.15^{c}	4031.65 ± 3.61^{a}	1249.90 ± 0.76^{d}	208.68 ± 0.15^{b}	2844.83 ± 2.10^{ab}	3044.40 ± 2.97^{a}
GTWF	70 °C	20 mins	Rockrose	25 %	2923.68 ± 0.98^{b}	768.21 ± 0.77^{b}	4180.59 ± 1.89^{a}	3973.69 ± 1.88^{b}	254.60 ± 0.097^{a}	2598.86 ± 2.50^{b}	1227.25 ± 0.39^{d}
				35 %	2368.17 ± 1.09^{c}	582.64 ± 0.40^{c}	3635.00 ± 2.58^{ab}	2839.57 ± 2.61^{c}	151.44 ± 0.13^{c}	1945.75 ± 0.36^{c}	1607.54 ± 0.91^{cd}
GTWF	25 °C	2 hours	Rockrose	25 %	2191.81 ± 1.62^{c}	228.43 ± 0.16^{d}	2790.08 ± 0.31^{c}	752.18 ± 0.63^{d}	194.07 ± 0.01^{bc}	1990.04 ± 1.80^{c}	2235.61 ± 1.85^{b}
				35 %	2103.49 ± 0.84^{c}	224.95 ± 0.06^{d}	2801.29 ± 1.78^{bc}	724.22 ± 0.55^{d}	194.07 ± 0.181^{bc}	1913.80 ± 0.29^{c}	2262.93 ± 0.94^{b}

Lower case letters denote significance difference at 95 % within columns separately.

3.4 Morphological Analysis of Spray & Freeze-Dried Instant Tea Powders

Morphological analysis of the powders was performed with Scanning Electron Microscopy at METU Central Laboratory. During the analysis, the samples were coated with gold and palladium at a thickness of 3 nm.

Powders that have been freeze-dried have an irregular morphology, an extremely porous surface, and a cake-like appearance. The pores in the structures are likely to be caused by the formation of ice crystals during the freezing stage, which sublimated during freeze drying (Dolly et al., 2011).

Unlike freeze-dried powders, the structure of spray-dried powders was similar regardless of the brewing parameters. It has been observed that the powders dried by spray drying are in the form of irregular spherical particles with a rough surface. It was also observed that inulin, which was used as a carrier material, results in larger particle sizes. Also, in the study of Jirayucharoensak et al. (n.d.) , the morphological structure of inulin was spherical in shape (Jirayucharoensak et al., n.d.).

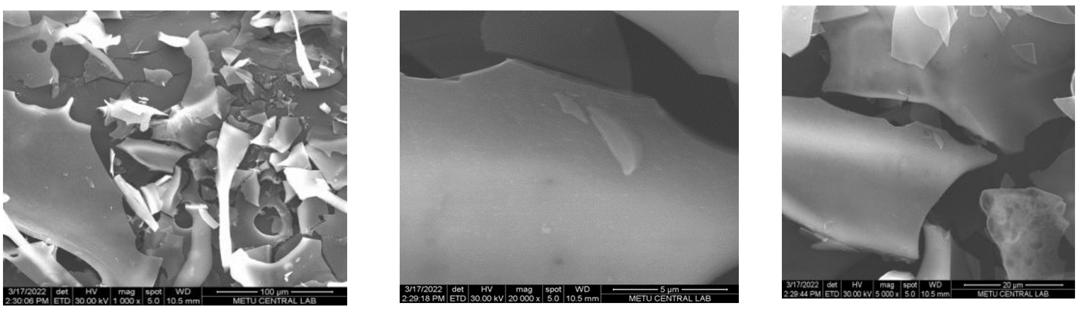

Figure 3. 1 Scanning Electron Microscope (SEM) images of freeze-dried powders obtained from waste green tea fiber (70°C & 20 minutes)

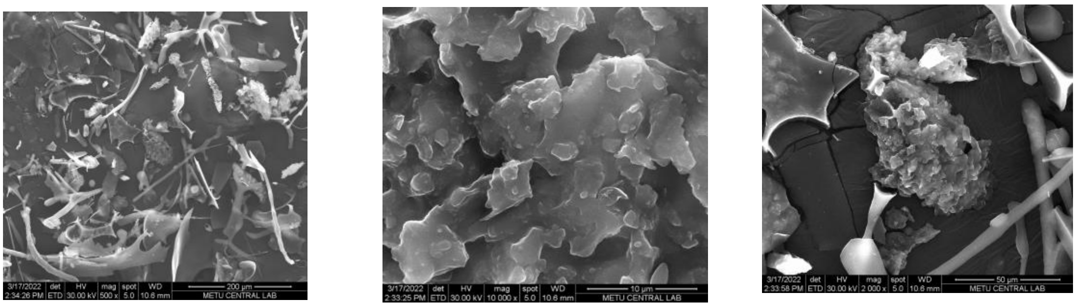

Figure 3. 2 Scanning Electron Microscope (SEM) images of freeze-dried powders obtained from waste green tea fiber (25℃ & 2 hours)

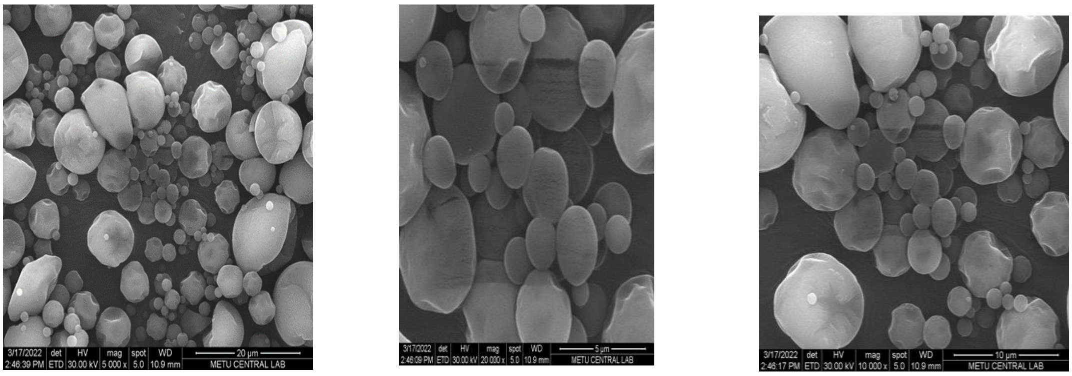

Figure 3. 3 Scanning Electron Microscope images of spray-dried powders obtained from waste green tea fiber (70℃ & 20 minutes)

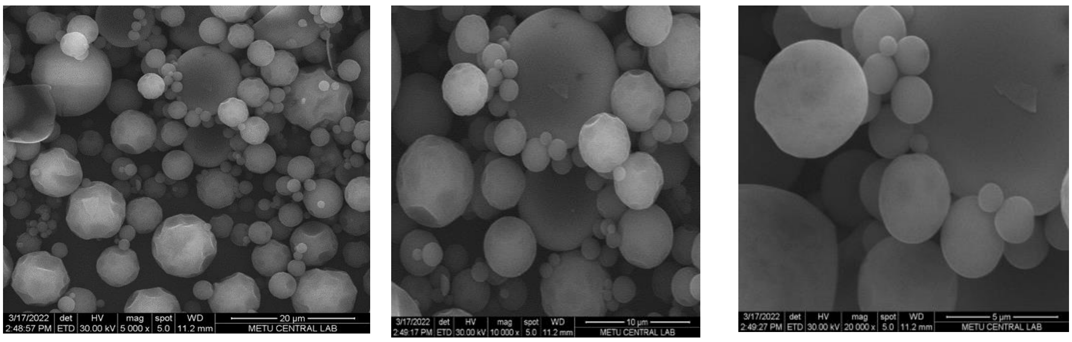

Figure 3. 4 Figure 3. 3: Scanning Electron Microscope images of spray-dried powders obtained from waste green tea fiber (25℃ & 2 hours)

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

The primary goal of this study was to utilise green tea waste fibers to produce an instant green tea powder with mixed herbs. Two separate kinds of brewing types which are cold brewing and hot brewing were used with two kinds of brewing times to achieve this goal. Also, for drying step ; spray and freeze drying method were used to obtain green tea powders. Rockrose was also added to the tea powders Efficiency, quality parameters, antioxidant activity, physical and chemical analyzes of tea were conducted.

In first part of the study, brewing efficiency was calculated to determine the hot brewing parameters and this was also used for also cold brewing.

In the second part of the study, the physical analysis was performed separately for two different drying methods as spray and freeze drying. Although there was no noticeable difference between drying types in terms of physical analysis, the solubility and moisture content of the samples from spray drying were more proper than the powders obtained by freeze drying.

The last part of the study included the chemical analysis of the powders. Antioxidant activity, total phenolics, catechins and caffeine content of the powders obtained by freeze drying were found higher than the spray dried ones.

To sum up, this study showed that ready-made powdered teas can be obtained by both drying methods. The goal of this study was to determine the optimum procedures for making a soluble green tea with *rockrose*. The thesis is intended to provide information for the industry and future academic research.

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APPENDICES

A. Brewing Efficiency

Table A. 1 ANOVA & Comparison test for the brewing efficiency at different ratios performed at 90°C at 20 minutes

Analysis of Variance for Brewing Efficiency

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	3	284.300	94.7668	34116041.05	0.003
Error	32	0.000	0.0000		
Total	35	284.300			

Tukey Pairwise Comparisons: Brewing Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping		
40	9	33.176	A		
30	9	29.427		B	
20	9	26,689			C
10	9	26.044			C

Table A. 2 One Way ANOVA test for brewing efficiency of control green tea leaves

Analysis of Variance for Brewing Efficiency

Source	DF	Adj SS	Adj MS	F-Value	P-Value
N	3	128.50	42.834	7.31	0.015
Error	7	40.99	5.856		
Total	10	169.50			

Tukey Pairwise Comparisons : Brewing Ratio

Grouping Information Using the Tukey Method and 95% Confidence

N	N	Mean	Grouping	
40	3	48.780	A	
30	3	48.060	A	
20	3	47.89	A	
10	2	39.43		B

Table A. 3 ANOVA and Comparison test for brewing efficiency after pre-grinding for hot brewing

Analysis of Variance for Brweing Efficiency

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	42.062	42.062	17.47	0.004
Time	1	9.490	9.490	3.94	0.087
Temperature*Time	1	14.022	14.022	5.82	0.047
Error	7	16.854	2408		
Total	10	72.505			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping	
90	5	32.2543	A	
70	6	28.2827		B

Tukey Pairwise Comparisons: Time

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping
7	5	31.2117	A
20	6	29.3253	A

Tukey Pairwise Comparisons: Temperature*Time

Temperature*Time	N	Mean	Grouping	
90 7	2	34.3441	A	
90 20	3	30.1645	A	B
70 20	3	28.4860		B
70 7	3	28.0794		B

Table A. 4 ANOVA and Comparison test for Brewing efficiency after pre-grinding for cold brewing

Analysis of Variance for Brewing Efficiency

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	18.139	9.0694	13.79	0.006
Error	6	3.945	0.6575		
Total	8	22.084			

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping	
4°C 12h	3	10.530	A	
25 °C	3	9.218	A	
4°C 6h	3	7.085		B

Table A. 5 ANOVA and Comparison test for the brewing efficiency after super grinding for hot brewing

Analysis of Variance for Brewing Efficiency

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	7.2602	7.2602	1.93	0.207
Time	1	42.3595	42.3595	11.28	0.012
Temperature* Time	1	0.0715	0.0715	0.02	0.894
Error	7	26.2794	3.7542		
Total	10	80.8955			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
90	5	31.6605	A
70	6	30.0104	A

Tukey Pairwise Comparisons: Time

Grouping Information Using the Tukey Method and 95% Confidence

Time	N	Mean	Grouping	
20	6	32.8282	A	
7	5	28.8427		B

Tukey Pairwise Comparisons: Temperature*Time

Grouping Information Using the Tukey Method and 95% Confidence

Temperature * Time	N	Mean	Grouping	
90 20	3	33.5714	A	
70 20	3	32.0851	A	B
90 7	2	29.7495	A	B
70 7	3	27.9358		B

Table A. 6 ANOVA and Comparison test for brewing efficiency after super grinding for cold temperatures

Analysis of Variance for Brewing Efficiency

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Sample	2	31.1611	15.5806	433.54	0.000
Error	5	0.1797	0.0359		
Total	7	31.3408			

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

Sample	N	Mean	Grouping	
4°C 12h	3	10.3185	A	
25 °C	2	6.333		B
4°C 6h	3	6.1839		B

B. Physical Characterization Analysis of Spray Dried Instant Tea

Table B. 1 ANOVA for moisture content analysis of spray dried instant powder tea with rockrose

Analysis of Variance for moisture content					
Source	**DF**	**Adj SS**	**Adj MS**	**F-Value**	**P-Value**
Temperature	1	4.606	4.6061	5.09	0.048
Herb Ratio	2	133.178	66.5888	73.65	0.000
Temperature*Herb Ratio	2	5.577	2.7886	3.8	0.091
Error	10	9.041	0.9041		
Total	15	150.850			

Table B. 2 Comparison test for moisture content of spray dried tea with rockrose

Tukey Pairwise Comparisons: Temperature

Temperature	N	Mean	Grouping
25	8	9.88167	A
70	8	8.78889	B

Tukey Pairwise Comparisons: Herb Ratio

Herb Ratio	N	Mean	Grouping
35	4	11.5525	A
25	6	11.1133	A
0	6	5.3400	B

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Temperature*Herb Ratio	N	Mean	Grouping
25 35	2	12.9750	A
70 25	3	11.1867	A
25 25	3	11.0400	A
70 35	2	10.1300	A
25 0	3	5.6300	B
70 0	3	5.0500	B

Table B. 3 ANOVA & Comparison test for the water activity of spray dried powder with rockrose

Analysis of Variance for water activity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	0.000408	0.000408	7.00	0.038
Herb Ratio	2	0.052467	0.026233	449.71	0.000
Temperature*Herb Ratio	2	0.000867	0.000433	7.43	0.024
Error	6	0,000350	0,000058		
Total	11	0.054092			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping	
25	6	0.301667	A	
70	6	0.290000		B

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping	
35	4	0.3475	A	
25	4	0.3375	A	
0	4	0.2025		B

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping		
25 35	2	0.365	A		
25 25	2	0.340	A	B	
70 25	2	0.335	A	B	
70 35	2	0.330		B	
70 0	2	0.205			C
25 0	2	0.200			C

Table B. 4 ANOVA & Comparison test for the turbidity of spray dried powder with rockrose

Analysis of Variance for turbidity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	38507	38506.7	32.27	0.001
Herb Ratio	1	55085	55085.4	46.16	0.000
Temperature*Herb Ratio	1	107	106.7	0.09	0.775
Error	6	7160	1193.4		
Total	9	103312			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping	
25	5	461.583	A	
70	5	334.917		B

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping	
25	6	474.0	A	
35	4	322.5		B

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping		
25 25	3	540.667	A		
70 25	3	407.333		B	
25 35	2	382.500		B	
70 35	2	262.500			C

Table B. 5 ANOVA & Comparison test for the solubility of spray dried powder with rockrose

Analysis of Variance for turbidity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	105.24	105.238	18.24	0.001
Herb Ratio	2	1946.27	973.134	168.65	0.000
Temperature*Herb Ratio	2	7.66	3.831	0.66	0.533
Error	12	69.24	5.770		
Total	17	2128.41			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping	
70	9	83.3784	A	
25	9	78.5424		B

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping		
0	6	95.1656	A		
25	6	77.1514		B	
35	6	70.5641			C

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping	
70 0	3	96.7797	A	
25 0	3	93.5515	A	
70 25	3	80.3637	B	
25 25	3	73.9392	B	C
70 35	3	72.9917		C
25 35	3	68.1366		C

Table B. 6 ANOVA & Comparison test for the hygroscopicity of spray dried powder with rockrose

Analysis of Variance for hygroscopicity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	0.725	0.725	1.27	0.303
Herb Ratio	2	252.698	126.349	221.05	0.000
Temperature*Herb Ratio	2	14.788	7.394	12.94	0.007
Error	6	3.430	0.572		
Total	11	271.640			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	6	19.1101	A
25	6	18.6186	A

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	4	25.3480	A
25	4	15.8652	B
35	4	15.3799	B

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping	
25 0	2	22.5057	A	
70 0	2	25.1904	A	
70 25	2	17.6266	B	
25 35	2	16.2463	B	C
70 35	2	14.5134		C
25 25	2	14.1038		C

C. Chemical Characterization Analysis of Spray Dried Instant Powder Tea

Table C. 1 ANOVA & Comparison test for the total phenolic of spray dried powder with rockrose

Analysis of Variance for total phenolic compund

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	7.9231	7.92315	1478.81	0.000
Herb Ratio	2	12.6362	6.31809	1179.24	0.000
Temperature*Herb Ratio	2	0.6098	0.30490	56.91	0.000
Error	12	0.0643	0.00536		
Total	17	21.2334			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	9	3.23523	A
25	9	1.90832	B

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb %	N	Mean	Grouping
35	6	3.48083	A
25	6	2.77546	B
0	6	1.45904	C

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb %	N	Mean	Grouping
70 35	3	4.38426	A
70 25	3	3.40625	B
25 35	3	2.57739	C
25 25	3	2.14468	D
70 0	3	1.91519	E
25 0	3	1.00289	F

Table C. 2 ANOVA & Comparison test for the HPLC methos for catechins of spray dried powder with rockrose

Analysis of Variance of HPLC for egicatechin gallate

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	1144.90	572.45	192.59	0.000
Temperature	1	2394.66	2394.66	805.62	0.000
Herb Ratio*Temperature	2	100.61	50.30	16.92	0.001
Error	9	26.75	2.97		
Total	14	4510.42			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	5	44.5323	A
25	5	27.1895	B
35	5	24.3629	B

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	7	44.9238	A
25	8	19.1327	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping
0 70	3	61.1287	A
25 70	2	37.7792	B
35 70	2	35.8635	B
0 25	2	27.9358	C
25 25	3	16.5999	D
35 25	3	12.8623	D

Analysis of Variance of HPLC for epicatechin

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	63773	31886.5	152.70	0.000
Temperature	1	29812	29811.8	142.77	0.000
Herb Ratio*Temperature	2	5645	2822.3	13.52	0.001
Error	10	2088	208.8		
Total	15	97546			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	4	349.377	A
25	6	234.832	B
35	6	187.866	C

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	8	301.316	A
25	8	213.401	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping
0 70	2	422.252	A
0 25	2	276.502	B
25 70	3	264.324	B
35 70	3	217.370	C
25 25	3	205.340	C
35 25	3	158.361	D

Analysis of Variance of HPLC for epigallocatechin gallate

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	248402	124201	70.47	0.000
Temperature	1	780339	780339	442.77	0.000
Herb Ratio*Temperature	2	200977	100488	57.02	0.000
Error	8	14099	1762		
Total	13	1446488			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
25	5	414.939	A
35	5	403.036	A
0	4	112.583	B

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	8	550.609	A
25	6	69.764	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping	
25 70	3	752.185	A	
35 70	3	724.224	A	
0 70	2	175.417		B
35 25	2	81.848		B
25 25	2	77.694		B
0 25	2	49.750		B

Analysis of Variance of HPLC for epigallocatechin

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	4298	2149	4.28	0,049
Temperature	1	147005	147005	292.99	0,000
Herb Ratio*Temperature	2	10941	5470	10.90	0,004
Error	9	4516	502		
Total	14	163234			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
25	5	344.359	A
0	5	310.231	A
35	5	305.625	A

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping	
70	8	421.110	A	
25	7	219.034		B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping			
0 70	3	449.643	A			
25 70	2	432.144	A	B		
35 70	3	381.542		B		
25 25	3	256.574			C	
35 25	2	229.708			C	D
0 25	2	170.818				D

Table C. 3 : ANOVA & Comparison test for the HPLC methos for gallic acid, caffeine and L-theanine of spray dried powder with rockrose

Analysis of Variance of HPLC for gallic acid

	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	1224.64	612.321	138.24	0.000
Temperature	1	131.37	131.365	29.66	0.000
Herb Ratio*Temperature	2	628.31	314.153	70.93	0.000
Error	9	39.86	4.429		
Total	14	1737.00			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	5	63.2700	A
35	5	44.8528	B
25	5	42.7341	B

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
25	9	53.3060	A
70	6	47.2653	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping			
0 70	2	69.5900	A			
0 25	3	56.9499		B		
35 25	3	52.4155		B	C	
25 25	3	50.5526			C	
35 70	2	37.2901				D
25 70	2	34.9157				D

Analysis of Variance of HPLC for caffeine

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	151313	75656.3	299.02	0.000
Temperature	1	20514	20514.0	81.08	0.000
Herb Ratio*Temperature	2	7052	3526.0	13.94	0.001
Error	12	3036	253.0		
Total	17	181915			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	6	381.038	A
25	6	207.461	B
35	6	170.834	C

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	9	286.870	A
25	9	219.352	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping
0 70	3	442.788	A
0 25	3	319.288	B
25 70	3	226.989	C
35 70	3	190.833	C D
25 25	3	187.932	C D
35 25	3	150.835	D

Analysis of Variance of HPLF for L-theanine

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	16169	8084.4	31.78	0.001
Temperature	1	4962	4962.3	19.51	0.004
Herb Ratio*Temperature	2	6353	3176.4	12.49	0.007
Error	6	1526	254.4		
Total	11	29010			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	4	420.179	A
25	4	357.258	B
35	4	333.095	B

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	6	390.513	A
25	6	349.42	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping
0 70	2	472.648	A
0 25	2	367.711	B
25 70	2	365.966	B
25 25	2	348.550	B
35 25	2	333.265	B
35 70	2	332.925	B

D. Physical Characterization Analysis of Freeze Dried Instant Tea

Table D. 1 :ANOVA & Comparison test for the moisture content of freeze dried powder with rockrose

Analysis of Variance of moisture content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	10.390	10.390	9.57	0.013
Herb Ratio	2	105.572	52.786	48.62	0.000
Temperature*Herb Ratio	2	9.085	4.542	4.18	0.052
Error	9	9.770	1.086		
Total	14	133.326			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
25	8	13.9244	A
70	7	12.2256	B

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
25	4	15.8725	A
35	5	13.8025	B
0	6	9.5500	C

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping
25 25	2	17.9000	A
25 35	3	14.0000	B
70 25	2	13.8450	B
70 35	2	13.6050	B
25 0	3	9.8733	C
70 0	3	9.2267	C

Table D. 2: ANOVA & Comparison test for the water activity of freeze-dried powder with rockrose

Analysis of Variance of water activity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	0.000033	0.000033	0.40	0.550
Herb Ratio	2	0.021617	0.010808	129.70	0.000
Temperature*Herb Ratio	2	0.000717	0.000358	4.30	0.069
Error	6	0.000500	0.000083		
Total	11	0.022867			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	6	0.318333	A
25	6	0.315000	A

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
35	4	0.3550	A
25	4	0.3375	A
0	4	0.2575	B

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping
25 35	2	0.60	A
70 35	2	0.50	A
70 25	2	0.350	A
25 25	2	0.325	A
25 0	2	0.260	B
70 0	2	0.255	B

Table D. 3: ANOVA & Comparison test for the turbidity of freeze dried powder with rockrose

Analysis of Variance of turbidity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	1166.7	1166.69	20.36	0.003
Herb Ratio	1	4949.8	4949.80	86.37	0.000
Temperature*Herb Ratio	1	2831.1	2831.13	49.40	0.000
Error	7	401.2	57.31		
Total	10	10437.6			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
25	5	161.750	A
70	6	140.833	B

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
35	6	172.833	A
25	5	129.750	B

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping
70 35	3	178.667	A
25 35	3	167.000	A
25 25	2	156.500	A
70 25	3	103.000	B

Table D. 4: ANOVA & Comparison test for the solubility of freeze dried powder with rockrose

Analysis of Variance of solubility

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	31.02	31.015	26.10	0.000
Herb Ratio	2	1107.31	553.655	465.85	0.000
Temperature*Herb Ratio	2	2.34	1.171	0.99	0.401
Error	12	14.26	1.188		
Total	17	1154.93			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping	
70	9	78.3112	A	
25	9	75.6859		B

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping		
0	6	87.9503	A		
25	6	73.0459		B	
35	6	69.9994			C

Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping		
70 0	3	89.3498	A		
25 0	3	86.5508	A		
70 25	3	74.7505		B	
25 25	3	71.3413			C
70 35	3	70.8333			C
25 35	3	69.1656			C

Table D. 5 : ANOVA & Comparison test for the hygroscopicity of freeze dried powder with rockrose

Analysis of Variance of hygroscopicity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	7.770	7.770	19.65	0.004
Herb Ratio	2	249.957	124.979	316.07	0.000
Temperature*Herb Ratio	2	22.284	11.142	28.18	0.001
Error	6	2.372	0.395		
Total	11	282.384			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping	
25	6	17.7886	A	
70	6	16.1792		B

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping		
0	4	23.3117	A		
35	4	14.9216		B	
25	4	12.7184			C

. Tukey Pairwise Comparisons: Temperature*Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb Ratio	N	Mean	Grouping	
25 0	2	26.0435	A	
70 0	2	20.5800	B	
70 35	2	15.1000	C	
25 35	2	14.7432	C	D
70 25	2	12.8577	C	D
25 25	2	12.5791		D

E. Chemical Characterization Analysis of Freeze Dried Instant Powder Tea

Table E. 1:ANOVA & Comparison test for the total phenolic content of freeze dried powder with rockrose

Analysis of Variance of total phenolic content

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Temperature	1	87.784	87.7836	778.59	0.000
Herb %	2	25.552	12.7758	113.31	0.000
Temperature*Herb %	2	7.477	3.7385	33.16	0.000
Error	12	1.353	0.1127		
Total	17	122.165			

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	9	17.8643	A
25	9	13.4476	B

Tukey Pairwise Comparisons: Herb %

Grouping Information Using the Tukey Method and 95% Confidence

Herb %	N	Mean	Grouping	
35	6	17.3407	A	35
25	6	14.8355	B	25
0	6	14.7916	B	0

Tukey Pairwise Comparisons: Temperature*Herb %

Grouping Information Using the Tukey Method and 95% Confidence

Temperature*Herb %	N	Mean	Grouping
70 35	3	20.4481	A
70 0	3	16.6805	B
70 25	3	16.4643	B
25 35	3	14.2333	C
25 25	3	13.2067	D
25 0	3	12.9028	D

Table E. 2: ANOVA & Comparison test for the HPLC methos for catechins of freeze-dried powder with rockrose

Analysis of Variance of HPLC for epigallocatechin

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	2529407	1264704	17.85	0.001
Temperature	1	2599250	2599250	36.69	0.000
Herb Ratio*Temperature	2	890530	445265	6.29	0.017
Error	10	708356	70836		
Total	15	7167674			

Comparisons for Epigallocatechin

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	6	4150.89	A
25	5	3485.34	B
35	5	3218.15	B

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	7	4028.58	A
25	9	3207.68	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping		
0 70	3	4270.14	A		
25 70	2	4180.59	A		
0 25	3	4031.65	A		
35 70	2	3635.00	A	B	
35 25	3	2801.29		B	C
25 25	3	2790.08			C

Analysis of Variance of HPLC for epicatechin

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	3950198	1975099	83.30	0.000
Temperature	1	824307	824307	34.76	0.000
Herb Ratio*Temperature	2	150811	75405	3.18	0.090
Error	9	213402	23711		
Total	14	5417896			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping		
0	4	3536.56	A		
25	6	2557.74		B	
35	5	2235.83			C

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping	
70	7	3015.97	A	
25	8	2537.46		B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping		
0 70	2	3756.06	A		
0 25	2	3317.06	A	B	
25 70	3	2923.68		B	
35 70	2	2368.17			C
25 25	3	2191.81			C
35 25	3	2103.49			C

Analysis of Variance of HPLC for epicatechin gallate

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	403957	201979	91.08	0,000
Temperature	1	946234	946234	426.71	0,000
Herb Ratio*Temperature	2	61334	30667	13.83	0,003
Error	8	17740	2217		
Total	13	1500173			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping		
0	4	819.124	A		
25	5	498.317		B	
35	5	403.796			C

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	6	838.494	A
25	8	308.998	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping
0 70	2	1164.64	A
25 70	2	768.21	B
35 70	2	582.64	C
0 25	2	473.61	C
25 25	3	228.43	D
35 25	3	224.95	D

Analysis of Variance of HPLC for epigallocatechin gallate

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	4402185	2201092	38.93	0,000
Temperature	1	34364250	34364250	607.77	0,000
Herb Ratio*Temperature	2	1565903	782952	13.85	0,001
Error	10	565414	56541		
Total	15	44462211			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	6	3058.73	A
25	5	2362.94	B
35	5	1781.90	C

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	7	3893.60	A
25	9	908.77	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping
0 70	3	4867.56	A
25 70	2	3973.69	B
35 70	2	2839.57	C
0 25	3	1249.90	D
25 25	3	752.18	D
35 25	3	724.22	D

Table E. 3 : ANOVA & Comparison test for the HPLC methos for gallic acid, caffeine and L-theanine of freeze dried powder with rockrose

Analysis of Variance of HPLC for gallic acid

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	646.7	3231.87	17.53	0.001
Temperature	1	2.4	2.40	0.01	0.912
Herb Ratio*Temperature	2	7033.6	3516.82	19.08	0.001
Error	9	1659.2	184.35		
Total	14	13057.6			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping	
25	5	224.344	A		
0	5	198.214		B	
35	5	172.449			C

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
25	9	198.744	A
70	6	197.928	A

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping		
25 70	2	254.593	A		
0 25	3	208.677		B	
25 25	3	194.096		B	C
35 25	3	193.458		B	C
0 70	2	187.752		B	C
35 70	2	151.439			C

Analysis of Variance of HPLC for caffeine

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	3878635	1939318	62.93	0.000
Temperature	1	586761	586761	19.04	0.001
Herb Ratio*Temperature	2	239252	119626	3.88	0,053
Error	11	338990	30817		
Total	16	5205938			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping	
0	6	3088.21	A	
25	6	2294.45		B

35	5	1929.77	C

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
70	8	2625.40	A
25	9	2249.56	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping		
0 70	3	3331.59	A		
0 25	3	2844.83	A	B	
25 70	3	2598.86		B	
25 25	3	1990.04			C
35 70	2	1945.75			C
35 25	3	1913.80			C

Analysis of Variance of HPLC for L-theanine

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Herb Ratio	2	1179203	589602	24.64	0.001
Temperature	1	2620101	2620101	109.49	0.000
Herb Ratio*Temperature	2	125539	62769	2.62	0.152
Error	6	143580	23930		
Total	11	4068423			

Tukey Pairwise Comparisons: Herb Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio	N	Mean	Grouping
0	4	2474.46	A
35	4	1935.24	B
25	4	1731.43	B

Tukey Pairwise Comparisons: Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Temperature	N	Mean	Grouping
25	6	2514.31	A
70	6	1579.77	B

Tukey Pairwise Comparisons: Herb Ratio*Temperature

Grouping Information Using the Tukey Method and 95% Confidence

Herb Ratio*Temperature	N	Mean	Grouping			
0 25	2	3044.40	A			
35 25	2	2262.93		B		
25 25	2	2235.61		B		
0 70	2	1904.53		B	C	
35 70	2	1607.54			C	D
25 70	2	1227.25				D

Table F. 1: Particle size of rockrose after super grinding

Herb Type	D_{50} (µm)	D_{90}(µm)	D_{10}(µm)	Span	Cellular Disruption Ratio
Rockrose	70.68	207.65	7.41	2.83	36.72