INVESTIGATION OF APPLICABILITY OF UV-LIGHT EMITTING DIODES (UV-LEDs) AS AN ALTERNATIVE TECHNOLOGY IN PASTEURIZATION OF COLD-PRESSED AND NEWLY FORMULATED MIXED BEVERAGE

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ABSTRACT

INVESTIGATION OF APPLICABILITY OF UV-LIGHT EMITTING DIODES (UV-LEDs) AS AN ALTERNATIVE TECHNOLOGY IN PASTEURIZATION OF COLD-PRESSED AND NEWLY FORMULATED MIXED BEVERAGE

The aims of this thesis were to evaluate the cold pressing technique for juice extraction and to evaluate whether the cold pressed mixed beverage can be pasteurized with ultraviolet light emitting diodes (UV-LEDs) using *E. coli* K12 as the target microorganism. Initially, the physical, chemical and optical properties of cold pressed juice (CPJ) were compared with juice extracted by centrifugal juicer (CDJ). The CPJ was then exposed to UV-LEDs simultaneously and sequentially and the microbial inactivation was evaluated. Besides, barrier technologies have been tried to increase the UV-LED inactivation effect.

It has been found that the juice yield performance of the cold pressing technique is better than the centrifuge method. The total phenolic content of the CDJ and CPJ was 922 ± 0.01 mg GAE/L, and 867.25 ± 0.01 mg GAE/L, respectively. However, DPPH radical scavenging activity of both beverages were not significantly different.

The use of UV-LEDs in simultaneous or sequential mode could not achieve a 5-log reduction in pertinent microorganism required as part of HACCP mandated by US Federal regulations for juice issued by the Food and Drug Administration (FDA). However, filtration and addition of ginger juice revealed that the inactivation efficiency of UV-LEDs increased in highly turbid juice reaching to 3.65 ± 0.218 $\log_{10}$ CFU/ml. In conclusion, this study showed that UV-LEDs used simultaneously at different wavelengths may have the potential to be used in turbid cold press juice pasteurization when combined with different barrier technologies.

ÖZET

UV IŞIN YAYAN DİYOTLARIN (UV-LEDS) YENİ FORMÜLİZE SOĞUK SIKIM İÇECEK PASTÖRİZASYONUNDA ALTERNATİF BİR TEKNOLOJİ OLARAK KULLANILABİLİRLİĞİNİN ARAŞTIRILMASI

Bu tezin amacı, meyve suyu eldesinde soğuk sıkım yöntemini değerlendirmek, bu yöntem ile hazırlanmış içeceğin hedef mikroorganizma olarak *E. coli* K12 kullanılarak ultraviyole ışın yayan diyotlar (UV-LED's) ile pastörizasyonunu değerlendirmektir. Soğuk sıkım yöntemi (CPJ) ile hazırlanmış meyve suyunun fiziksel, kimyasal ve optik özellikleri, santrifüjlü meyve sıkacağı (CDJ) ile elde edilen meyve suyu ile karşılaştırılmıştır. CPJ ile hazırlanmış içecek daha sonra 280/365 nm, 280 nm ve 365 nm'de eş zamanlı ve sıralı UV-LED uygulamasına maruz bırakılmış, *E.coli* K12 üzerindeki mikrobiyal inaktivasyon değerlendirilmiştir. Ayrıca UV-LED'lerin inaktivasyon etkisini artırmak için filtrasyon ve zencefil suyu eklemesi gibi farklı bariyer (hurdle) teknolojileri de denenmiştir.

CPJ yönetminin meyve suyu veriminin CDJ yöntemine göre daha iyi olduğu tespit edilmiştir. CDJ ve CPJ ile elde edilen meyve suları ile hazırlanan içeceğin toplam fenolik bileşen miktarı sırasıyla 922 ± 0.01 mg GAE/L ve 867.25 ± 0.01 mg GAE/L'dir. Ayrıca, her iki yöntem ile hazırlanmış içeceğin DPPH radikal inhibisyon aktivitesi de ölçülmüş ve tespit edilen değerlerde önemli farklılık gözlemlenmemiştir.

UV-LED uygulamaları ile HACCP'in bir parçası olarak gerekli olan ilgili mikroorganizmada 5 log'luk inaktivasyon sağlanamamıştır. Bu nedenle, UV-LED'ler ile bariyer teknolojisi kullanılmış, bulanıklık değeri yüksek olan meyve suyunda 3.65 ± 0.218 $\log_{10}$CFU/ml inaktivasyon gözlemlenmiştir. Sonuç olarak, bu çalışma farklı dalga boylarında eş zamanlı uygulanan UV-LED'lerin farklı bariyer teknolojileri ile kullanılarak bulanık soğuk sıkım meyve sularının pastörizasyonunda kullanılma potansiyeline sahip olabileceğini ortaya koymuştur.

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CHAPTER 1

INTRODUCTION

Fruit juices are non-alcoholic beverages that consumed frequently due to their nutritional values, taste, and easy consuming property (Koutchma, et al., 2016). On the other hand, potential health benefits of fruit juices are a trend topic subject that is considered by consumers. It is reported that fruit juices without any external substances protect the health-beneficial components of the fruit and thus become health-beneficial drinks (Ruxton, et al., 2016). Because of the increasing awareness about health effects of consuming fruit juices, demand of fruit juice industry increases day by day. However, squeezing technique of fruit juices becomes a considered point from the consumer side to be sure that health relevant substances are included in the final product. This demand pushes the fruit juice industry to find innovative techniques that could be used both household and industrial scales. Conventional centrifugal juicers are widely used at homes to yield juice from fruits and vegetables. They are easy to use and a quick method to have freshly squeezed fruit and vegetable juices. The technique works basically with the following steps: Fruit is crushed by the blades rotating at high speed and the fruit juice extraction is provided by centrifugal force (Khaksar, et al., 2019). However, when the metal blades rotate rapidly at high speed, heat is released after a while due to friction force. Generated heat may negatively affect the bioactive components of the fruit juices. In that point, customer demands different techniques to be sure that the health beneficial components are retained better.

In recent years, the cold press juice industry has become a rapidly growing sector in the world (Biancaniello, et al., 2018). People uses this method both at their home by using a household cold press juicer, or at small business by using commercial machines. Cold pressing takes place in two stages; fruit and vegetables are cut into small pieces, then small pieces of vegetables and fruits are extracted physically by applying pressure. The process is followed by filtration (Biancaniello, et al., 2018). It is claimed that no heat generation is observed during the squeezing of fruit and vegetables. The crushing and extraction are slow compared to centrifugal juicer. Almost no heat

generation is observed due to slow extraction. On the other hand, cold-pressing technique yields more juice than the conventional centrifugal juicing technique (Donaldson, 2020). This technique also let us to squeeze leafy greens that give a chance to create different juice formulations. In addition, the retention of vitamin C and antioxidant activity is higher than the centrifugal juicer (Gouws et al., 2019; Kim et al., 2017).

Table 1.1 Comparison of squeezing techniques

Cold Press Juicer	**Centrifugal Juicer**
Extraction is slow	Blading is at high speed
Almost no heat generation due to slow extraction	Heat is released with the effect of friction force
More juice yield than centrifugal juicer without segmentation	Segmentation is observed

Cold pressing technique is a rising trend among the people who adopt a healthy diet in their life. Demanding of that kind of juices increase day by day. It is projected that the global cold pressed juices market is estimated to reach USD 3.99 billion by the end of 2023 (Digital Journal, 2022). In Turkey, investments about cold press juicing started in 2015 and it goes on increase. Today, the production exceeds 3 tones/day. Fruit juices obtained by cold pressing are consumed without pasteurization to benefit more effectively from their bioactive components. However, this situation causes that the shelf life of unprocessed cold pressed juice is up to 3-days. Their shelf life is short since they do not contain preservatives.

In order to make the shelf life of freshly squeezed juice drinks a little longer and to make them microbially safe, it is necessary to apply a treatment. Heat treatment is the most common technique used to make products such as juice safe and extend their shelf life. However, since the heat treatment damages the organoleptic properties of the product, it cannot provide the cold pressed fruit juice properties demanded by the consumer, where fresh and similar properties are preserved. This situation has led researchers to develop alternative non-thermal processing techniques to be applied for fruit juices. Some commercially applied non-thermal preservation methods to eliminate or minimize the damage caused by heat treatment to the product include UV irradiation,

pulsed electric field (PEF), high hydrostatic pressure (HHP) and ionizing radiation technique (Bates et al., 2001). However, these techniques also have some disadvantages. For example, it has been shown that after the application of the PEF to orange juice, its color darkens throughout its shelf life, and its effect on the inactivation of yeasts is limited (Min et al., 2003). In addition, it has been reported that the industrial use of the PEF system requires high capital costs (Tahiri et al., 2006). In HHP application, it has been reported that very high pressures must be used to ensure sufficient microbial inactivation in fruit juice. However, it has been reported that its use in industrial scale is limited due to the high investment cost (L. Parekh et al., 2017; Tahiri et al., 2006). It has been reported that the application of ionizing radiation damages the sensory quality of the product and leaves a plastic taste that makes it difficult to drink, especially after the application of ionizing radiation to orange juice (Foley et al., 2002).

UV lights are widely used for surface disinfection, sterilization of water and various liquid foods. It has been revealed that the change in organoleptic properties such as odor, taste and flavour of the UV-treated product is minimal or no change is observed, and it also requires very little energy compared to heat treatment (Koutchma et al., 2016). The US Food and Drug Administration (US FDA) has approved the use of this system as a lethal agent in fruit juice pasteurization to achieve 5 log inactivation of the target pathogenic microorganism (US FDA 2000). There are many studies in the literature on the application of UV-C application to fruit juices. In these studies, it was revealed that UV-C treatment increased the number of phenolic components of fruit juice and increased the antioxidant level (Alothman et al., 2009; Bhat et al., 2011; Falguera et al., 2014; Santhirasegaram et al., 2015). However, the low penetration depth of UV-C rays in turbid and particulate liquids reduces the performance of the process. On the other hand, it is known that UV-C lamps contain mercury which is known as to have toxic effect for both environment and human body (Mori et al., 2007). In addition, UV-C lamps have large sizes that limited the designation due to occupy too much space (Chevremont et al., 2012). Also, UV-C lamps have short life span (approximately 4,000-10,000 hours) and they are fragile. Consequently, it is necessary to design a new sterilization equipment that is more rigid, do not contain toxic substances and have low energy consumption rate (Hamamoto et al., 2007).

The usage of ultraviolet light-emitting diodes (UV-LEDs) has become prominent as a light source of sterilization by UV-lights. UV-LEDs are created by semiconductors

and the wavelength of emitting light depends on the type of material that is used for those semiconductors (Bowker et al., 2011). They do not contain toxic substances such as mercury. Also, their sizes are small that able to minimal designations and sterilization of narrow spaces. They have robust design and do not fragile and have longer lifetime than traditional UV-C lamps. UV-LEDs do not have a warm-up time that consume less energy than UV-C lamps. Lastly, they provide simultaneous or sequential usage in different wavelengths.

UV-LEDs have been widely investigated for water treatment. However, there are limited number of studies related to the use of UV-LEDs for fruit juice pasteurization (Akgün and Ünlütürk, 2017; Lian et al., 2010; Xiang et al., 2020; Cheng et al., 2020, Baykuş et al., 2021). Akgün and Unluturk (2017) investigated the simultaneous application and different configurations of UV-LEDs at four different wavelengths. They achieved 3.76 ± 0.07 log10 CFU/ml reductions on *E.coli* K12 at 280/365 nm after 40 min. Hinds et al. (2020) studied for the inactivation of Bacillus subtilis in model food systems. They achieved complete inactivation (6.82 ± 0.09 log10 CFU/ml) in Peptone Buffered Saline (PBS) at 285 nm after 5 min. Also, 3.58 ± 0.33 log10 CFU/ml reduction on *B.subtilis* in Nutrient Broth (NB) was obtained. In another study, UV-LED irradiation using coupled 280/365 nm for 40 min resulted in >4 log inactivation on *E.coli* K12 in mixed beverage (Baykuş et al., 2021).

Hurdle technology is being investigated in the past 20 years increasingly (Gomez et al., 2011). Hurdle effect has a fundamental importance for the preservation of food since hurdles in a stable product control microbial safety (Leistner, 1995). The limitations of different microbial inactivation techniques could be improved by hurdle technology. Combination of different thermal and non-thermal treatment methods is an effective way to impart an additive or synergistic effect (Aaliya et al., 2021). Using innovative methods together or adding antimicrobial agents can be used as hurdles to ensure microbial safety (Gomez et al., 2011; Ferrario et al., 2020). In present study, filtration method was applied prior to the UV-LED treatment. Then, freshly squeezed ginger juice was added to the formulation of cold-pressed juice as an antimicrobial agent. In that case, it is investigated to increase the inactivation of *E.coli* K12 by using hurdle technology. Also, effects of freshly squeezed ginger juice as an antimicrobial agent was evaluated.

The objectives of this work are;

- Characterization of cold-pressed juice samples via determination of physical and physicochemical properties (pH, brix, color parameters, amount of total phenolic components and antioxidant activity).
- Comparison of physical and physicochemical properties of juice samples by squeezed two different method, cold-pressing and centrifugal juicer.
- Application of UV-LEDs on cold-pressed juice samples with different configurations. Therefore, obtaining the best configuration to reduce natural flora of the cold-pressed juice to ensure microbial safety.
- Assessment of UV-LED application on natural flora of cold-pressed juice.
- Evaluation of the effects of hurdle technology with UV-LEDs on *E.coli* K12 inactivation.

CHAPTER 2

LITERATURE REVIEW

2.1. Fruit Juice Definition

Fruit juices are defined as unfermented but fermentable beverages obtained from fresh, ripe and healthy fruits (Republic of Turkey Ministry of Agriculture and Rural Affairs General Directorate of Protection and Control, 2006).

2.1.1. Value of Fruit Juice

Fruit juice contains almost all fruit properties and nutrient values in it. It is easy to consume and give a chance to have different taste and enhance nutrient value by blending different fruit juices. Consequently, popularity of fruit juices is being raise due to these advantages and their various health benefits. The consumption rate of fruit juices was reported as 6.4 L/person in 2006 (Republic of Turkey Under secretariat of the Prime Ministry for Foreign Trade Export Promotion Center, 2007). Now, it was reached 40 L/person in ABD, 30 L/person in Germany, 30 L/person in Holland and 11 L/person in Turkey (MEYED, 2020). Statistical values shows that the consumption of fruit juice increase day by day around the World.

Fruit juice is an alternative for direct consumption of fruits. The Dietary Guidelines for Americans between 2020-2025 allow for up to half of the recommended daily fruit intake can be replaced by fruit juices (US Department of Health and Human Services, 2020). It is known that the regular consumption of fruit juice up to 500 ml/day contribute better vascular function and reduced blood sugar. Also, fruit juice consumption is associated with reduced risk of stroke (Ruxton and Myers, 2021). They are also consumed in new diets that the detox is aimed (Küçük and Yıbar, 2021). On the other hand, it is simpler to process liquid foods than solid products. Therefore, safety and quality requirements could be satisfied easily (Hakguder, 2009).

2.1.2. Composition and Nutrient Content of Fruit Juice

Fruit juice maintains almost all fruit properties. The major component of fruit juice is water. It is also a source of free sugars, micronutrients, and plant bioactive. One major difference between the fruit and fruit juice is the fibre content of whole fruit. Since the fruit juice contains mainly water, water soluble vitamins, minerals, free amino acids, and phenolic matters could be moved into fruit juice. However, water insoluble matters especially fibres are not transferred into fruit juice (Cemeroğlu, 2004). The range of plant bioactive compounds is extensive. For example, apple juice contains chlorogenic acid, pineapple is a source of ascorbic acid, folate, niacin and thiamin and kiwi contain potassium, polyphenols, and flavonoids as antioxidants (Ruxton and Myers, 2021; Ali et al, 2020). However, present bioactive compounds are worthless without bioactivity. It is a prevalent bias that bioactive compounds are fully taken by body. However, bioavailability of bioactive compounds is also important to take health benefits of them. Consistent studies evidence that the vitamins, minerals, and antioxidants in fruit juices are available to absorbed by body. Therefore, health benefits of bioactive compounds found in fruit juices could be observed.

2.2. Squeezing Techniques

A juice extractor also known as juicer is a machine that has the capability of extracting juice from fruits, leafy greens, and other types of vegetables (Nnamdi et al., 2020). Nowadays, people consider the squeezing technique of fruit juices to maximize the benefit by providing the highest amount of bioactive substance to fruit juices. There are several squeezing techniques, household machines and conventional machines, that are used to extract fruits and vegetables. Many people prepare juices at home to squeeze freshly to avoid the loss in bioactive substances in fruit juice by commercial processing (Kim et al., 2015). Mainly the household juice extractors are classified into four essential types: masticator, centrifuge, twin gear and press juicer (Nnamdi et al., 2020).

2.2.1. Centrifugal Juicing Technique

Centrifugal juice extractors are widely used in household since its practice and fast usage. It extracts the juice from the pulp by rotating at super-sonic speed. Prior to extraction, sharp blades are mashed the fruit or vegetable to obtain the pulp (Nnamdi et al., 2020). The advantageous of the centrifugal juicer is the machine can process juice within seconds. Also, it can chow down the fruit without any pre-processing. Therefore, preparation time to squeeze fruits could be reduced. Also, it is dismountable, and it contributes to the easily cleaning of the machine. On the other hand, there are several disadvantageous properties of this kind of juicers. Centrifugal juicers separate the juice extract from fruit flesh by centrifugal force. However, when the sharp blades spin at high speeds of 8,000-12,000 rpm, heat is generated, and bioactive substances of fruit and vegetable can be affected negatively (Kim et al., 2015, Khaksar et al., 2019). Because, due to the generated heat, oxidation of the juice is observed. Consequently, several unsatisfying properties could be observed like loss of nutrient and reducing juice quality as well as shelf life (Nnamdi et al., 2020).

2.2.2. Cold-Pressing Technique

Cold-press juicers, also known as masticating juicers, are novel juicers that uses a squeezing method rather than grinding and high-speed. It uses a horizontal or vertical auger that rotating at a low speed (approximately 80 rpm) (Kim et al., 2015). The vertically configured cold-press juicers have larger auger, mesh drum and brush holder. Residue is also discharged during extraction (Nnamdi et al., 2020). Cold-press juicers extracts the fruit and vegetables in two steps: first, it crushes the fruits and vegetables and converts them smaller particles. Then, press them to extract its juice very slowly. It minimizes the heat generation due to the slow rotation and preserve the nutritional value of the juices (Khaksar et al., 2019). Also, they perform well on both hard and soft fruits and vegetables, especially for leafy greens. On the other hand, it takes a longer time to extract the juice from fruits and vegetables when compared to the centrifugal juicers. In

addition, market price of cold-press juicers is higher than the that of other juice extractors (Khaksar et al., 2019; Nnamdi et al., 2020).

Figure 2.1. Cold-Press juice extractor and centrifugal juicer

2.3. Quality Problems and Threats

Fruit Juice quality and safety highly depends on the raw material, processing conditions, packaging material and storage conditions. Changing of these parameters can cause the physical, chemical, microbiological and enzymatical deterioration (Souza et al., 2004). Fruit juices are susceptible to microbiological spoilage although their acidic composition. Before the observed outbreaks that are caused by *Salmonella* and *Escherichia coli* O157:H7, it was a generalization that the fruit juices with low-pH value are safe (Cook et al., 1998). Foley et al. (2002) estimated the number of cases for illnesses that associated with the unpasteurized fruit juices as 16000 to 48000 in a year. Fruit juices with low acidic value can also be spoiled by molds, yeasts and aciduric bacteria. Germination of these bacteria could cause some unsatisfied quality problems such as off-flavour, odor and color deterioration of fruit juices (Ray, 2004; Tournas et al., 2006).

Food and Drug Administration (FDA) implemented a regulation for pasteurization of fruit juices to prevent the community from food illnesses related with freshly squeezed fruit juices (FDA, 1998). According to this regulation, Juice processors must evaluate the processing operations using HACCP (Hazard Analysis critical Control Point) principles, and 5-log reduction in the most resistant microorganism must be achieved by this processing (FDA, 1998). Currently, acidic juices (below pH 4.6) containing enteric pathogens such as *E.coli* O157:H7, Salmonella species, and *Cryptosporidium parvum* caused serious foodborne illnesses (FDA, 1998).

On the other hand, some quality losses caused by dissolved oxygen and metal cautions during the squeezing that changes the taste, aroma and color of the fruit juices. For example, browning of fruit juices could be observed which is an unsatisfied result for consumer. Non-enzymatic Maillard reaction is a reason of that undesirable color. This reaction occurs between reducing sugars and amines in the fruit juice, resulting with browning pigments that lead to brownish color of fruit juice (Bates et al., 2001).

2.4. Preservation Techniques

The main goal in the application of a preservation technique to the fruit juice is to eliminate the pathogenic and spoilage microorganisms, also inactivate the enzymes. Preservation techniques that are applied to the fruit juice should have the properties of:

- To inactivate the pathogens and spoilage microorganisms and extending the shelf life of the product. Therefore, food safety should be satisfied.
- Organoleptic properties and nutritional quality should be kept
- Any residues should be observed after processing
- Costing of the processes should be minimized (Raso and Barbosa-Canovas, 2003).

Preservation methods can be classified as two main groups: thermal and non-thermal pasteurization techniques.

2.4.1 Conventional Techniques

The most conventional preservation method is known as thermal pasteurization. It is the best-known method to kill pathogens and reduce to the number of spoilage microorganisms, also to inactivate the enzymes (Aguilar-Rosas et al., 2007). The main objective of the thermal pasteurization is to kill the microorganisms and inactivate the enzymes that causes deterioration and spoilage of the fruit juice (Aguilar-Rosas et al., 2007). Pasteurization describes the mild heat treatment that is applied below 100 °C. Designing of the thermal pasteurization is performed considering by heat resistance of the target microorganism of the product and pH value of the food material (Silvia and Gibbs, 2004). It is known that pathogens of fruit juices such as *E.coli* O157:H7, Salmonella species, and *Cryptosporidium parvum* can be eliminated by conventional pasteurization method (Tandon et al., 2003). However, there are some disadvantages of this process. First, cost is prohibitive due to the required energy and equipment. Then, heating causes off-flavour and organoleptic properties of fruit juices become unsatisfied. Also, the product processed by conventional pasteurization do not meet to demands of the consumer anymore due to the nutritional value degradation by heating (FDA, 1998; Aguilar-Rosas et al., 2007; Choi and Nielsen, 2004).

2.4.2 Nonthermal Techniques

Consumers demand more healthy, nutritive, and fresh-like juice products and thermal pasteurization could not satisfy these properties to the consumer. This demand motivates the researcher to investigate non-thermal alternatives to heat to remove the undesirable effects of conventional pasteurization (Tahiri et al., 2006, Basaran-Akgül et al., 2009).

Some of the alternative nonthermal methods include UV irradiation and ionizing irradiation, addition of microbiocidal agents, pulsed electric field (PEF), high pressure application (HHP), and aseptic packaging (Bates et al., 2001).

Addition of microbiocidal agents is cheap and easy way due to the limited equipment requirements (Moon et al., 2006). Nowadays, using natural antimicrobial agents is also applied to maintain the safety of the food product. It is also giving a chance to design a food with a great diversity of flavours to consumers. Addition of different natural agents with different flavours could enhance the organoleptic properties of the food product (Ferrario et al., 2020).

Some of the nonthermal techniques also has disadvantageous during the application and after the application. For instance, PEF technique which is based on the use of high electric field pulses caused lighter color of orange juice during the storage (El-Hag et al., 2006; Min et al., 2003). Also, yeast inactivation is not efficient by PEF technique (Min et al., 2003; El-Hag et al., 2006; Marselles-Fontanet et al., 2007). On the other hand, industrial application of this method is costly (Tahiri et al., 2006). Application of HHP requires high pressure for inactivation of microorganisms that includes the use of pressure of 100-1000 MPa (Morris et al., 2007). However, it is reported that the investment cost of HHP is prohibitive that makes the industrial application of this technique difficult (Tahiri et al., 2006; L.Parekh et al., 2017). On the other hand, it is reported that the product treated by ionizing radiation has undesirable sensory changes. Especially, taste of the orange juice that is treated by ionizing radiation was inappropriate to consume (Foley et al., 2002).

2.5. Ultraviolet (UV) Light

UV-C light processing is another nonthermal processing method that has been used to ensure water disinfection for many years. This process is reported as an effective method on the inactivation of bacteria, protozoa, algae, and viruses (Begum et al., 2009). FDA approved UV-C Light treatment method as a germicidal agent for disinfection of fruit juices (FDA, 2000).

2.5.1. Description of UV Light

Ultraviolet light covers the range of the wavelength between 100 to 400nm in an electromagnetic spectrum (Barbosa-Canovas et al., 2005; Kouchma et al., 2009). The spectrum can be divided into four groups based on wavelengths (Fig 2.1). The subgroups are:

- UV-A (320 to 400 nm)
- UV-B (280 to 315 nm)
- UV-C (200 to 280 nm)
- UV-V (100 to 200 nm)

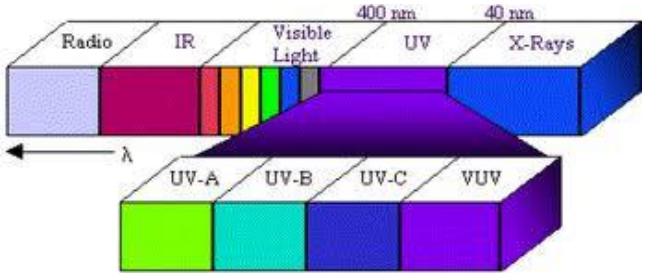

Figure 2.2. Electromagnetic spectrum
(Source: Aqualight 2018)

UV light irradiation at the range of 250-270 nm shows the highest germicidal effect for most microorganisms (Tran and Farid, 2004; Guerrero-Beltran and Canovas, 2005). Koutchma (2009) and Oteiza et al. (2010) declared that the highest inactivation efficiency is obtained at 253.7 nm due to the maximum absorption of UV photons by DNA or RNA of microorganisms at this specific wavelength.

Mercury lamps are used to UV-C light treatments for many years. These lamps are divided into three types. These are low pressure (LP), low pressure high output (LPHO) and medium pressure (MP). Low and medium pressure mercury vapor lamps are used in UV light treatment (Koutchma, 2009).

2.5.2. Mechanism of UV Light

Bacteria are very susceptible to UV irradiation due to their small size, short generation time and inadequate UV-protection pigments. UV sensitivity of bacteria and the inactivation mechanism changes depends on the applied UV range (UV-A, UV-B, UV-C). UV light at different ranges absorbed by different cell components such as DNA, proteins, and lipids.

The main inactivation mechanism of UV-C light is related to the absorption of UV photons by the genetic material of the microorganisms. This genetic material could be pyrimidine bases of DNA (thymine and cytosine) or RNA (uracil and cytosine) (Bolton et al., 2003; Koutchma, 2003). The incident light causes a mutation on the structure of the genetic material by the formation of pyrimidine dimers without any intermediate steps (Figure 2.2). Consequently, transcription and replication are inhibited. This brings about the cell inactivation (Oguma et al., 2002; Chatterley and Linden, 2010).

Figure 2.3. Effect of UV-C light on DNA

Inactivation of UV-A is based on the lipid and protein photooxidation by O2. The photooxidation of lipids and proteins increases the amount of reactive oxygen species (ROS) (e.g. H2O2, O2’ and OH) in the cell. Then, these ROS damage the lipids, proteins, and DNA and cause the cell inactivation (Santos et al., 2003). UV-B irradiation also causes the oxidative stress on lipids and proteins and contributes to the DNA damage (Santos et al., 2003).

2.5.3. Applications of UV Light Treatment

UV-C light is used in different areas for many years. Antimicrobial property of the UV-C light is well known and used for the decontamination in hospitals, pharmaceutical industry, and public buildings. Also, it is used to disinfection of potable water (Begum et al., 2009). UV-C is also used as an alternative pasteurization and shelf-life extension method for beverages. There are many applications of different fruit juices for UV-C irradiation such as apple juice, orange juice, mango juice, pineapple juice, grape juice, blend juices (orange and carrot, guava and passionfruit nectar), and etc. (Caminiti et al., 2012; Tandon et al., 2002, Choi and Nielsen, 2005; Kaya and Unluturk, 2015; Unluturk and Atılgan, 2015; Santhirasegaram et al., 2015; Hanisah, 2009; Guevara et al., 2012). It is also used for surface disinfection of foods (Romo, 2004; Manzocco et al., 2011; Chan et al., 2009).

UV-B radiation is utilized for medical purposes such as sterilization of medical equipment(Argyraki et al., 2016; Takada et al., 2017). UV-A in the range of 365 nm wavelength has high penetration ability that posed a bactericidal effect (Koutchma and Popovic, 2019). However, there are few studies indicated the usability of UV-A for water and fruit juice disinfection (Mori et al., 2007; Yagi et al., 2007; Hamamoto et al., 2007). Nowadays, coupling UV-A with UV-B and UV-C can be used in disinfection of water and foods (Chevremont et al., 2012; Akgün and Unluturk, 2017; Popovic and Koutchma, 2020; Baykuş et al., 2021).

2.5.4. Advantages and Disadvantages of UV Light Treatment

One of the most attractive advantages of UV Light is the low cost of operation. Then, it is effective for most kind of microorganisms (Bintsis et al., 2000). Also, the equipment occupies less than the ones used in other methods. It is a physical method and do not cause any chemical residues (EPA, 1999; Canitez, 2002).

However, UV light is insufficient in highly absorptive media in the case of presenting organic matters and suspended particles that decrease the penetration.

Consequently, the effectiveness of UV light decreases which is the disadvantageous of that method (EPA, 1999).

2.6 UV-LEDs

UV light emitting diodes (UV-LEDs) is an alternative source of ultraviolet light and its usage is relatively new. Fabrication, applications and advantageous of this new technology over the traditional one will be explained below.

2.6.1 Fabrication

UV-LEDs are two-terminal semiconductors that are named as p-type and n-type semiconductors that emit light when a specified voltage is applied across two layers. Then, electrons move into positive charge holes between these two materials (Figure 2.3.)

Emitted wavelength light depends on the semiconductor that is used in UV-LED structure (i.e. indium gallium nitride (GaN) for light in the visible range, and aluminum gallium nitride and aluminum nitride for UV range) (Bowker et al., 2011).

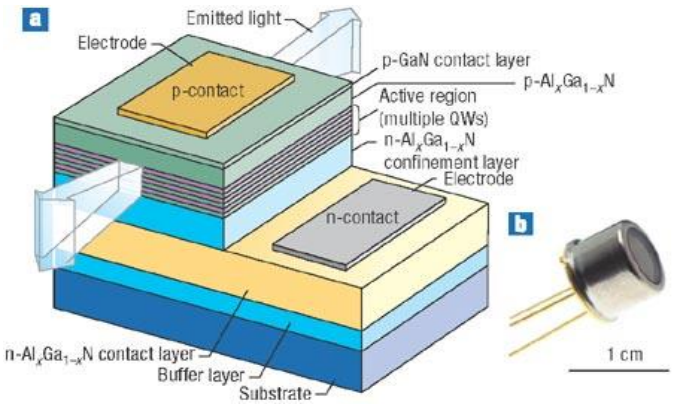

Figure 2.4. UV-LEDs Structure

2.6.2. Application of UV-LEDs

In literature there are limited numbers of studies associated with the use of UV-LEDs for food safety. There are several studies about water disinfection in literature but most of the studies are available for UV-A range (Chatterley and Linden, 2009). However, UV-C LEDs are also used for water disinfection (Li et al., 2017; Chevremont et al., 2012a; Chevremont et al., 2012b; Bowker et al., 2011; Kheyrandish et al., 2017).

Food applications of UV-LEDs are available for both liquid food and solid food. The surface disinfection of chicken, sliced cheese and cabbage was evaluated (Haughton et al., 2012; Kim et al., 2015; Aihara et al., 2014). Also, fruit juice applications studies are available for apple juice, orange juice and mixed beverages prepared by using fruit juices (Akgün and Unluturk, 2017; Lian et al., 2010; Baykuş et al., 2021; Popovic and Koutchma, 2021).

2.6.3. Comparison of UV-LEDs and Conventional UV Lamps

UV-LEDs are considered as potential competitors and alternative to the UV lamps (Kebbi et al. 2020). Table 2.1. shows the difference between UV-LEDs and conventional mercury-vapor UV lamps. UV-LEDs are environmentally friendly, do not contain toxic substances, they are mercury-free unlike conventional mercury-vapor lamps (Mori et al., 2007). In addition, conventional lamps are fragile, large, and less energy efficient. These lamps occupy a lot of space that limits the designation of sterilizers. Also, life span of that conventional lamps is short; almost 4000-10000 hours (Chevremont et al., 2012a). Moreover, low pressure mercury lamps are known to emit light nearly the monochromatic UV light at wavelength of 254 nm. However, UV-LEDs can emit the light at different wavelengths according to the used semiconductors in fabrication (Crawford et al., 2005).

Table 2.1. Comparison of UV-LEDs and conventional UV lamps

UV-LEDs	**Conventional Mercury-Vapor UV Lamps**
Do not contain toxic substances-environmentally friendly	Contain mercury
Robust design	Fragile
Long lifetime	Short lifetime
Provide simultaneous usage in different wavelengths (UV-A, UV-B and UV-C)	Emit monochromatic light at 254 nm (UV-C)

UV-LEDs have robust design, compact size, higher energy efficiency, longer lifetime that is approximately 100000 hours since they do not need a warm-up time (Chevremont et al., 2012b). Furthermore, UV-LEDs allow to design minimal sterilization equipments due to its small size. Also, it could help to sterilize narrow spaces due to their compact size (Mori et al., 2007). On the other hand, UV-LEDs give a chance to use different wavelengths to enhance the germicidal effect (Chatterley and Linden, 2010).

2.7. Hurdle Technology

Hurdle technology is mainly the combination of preservation methods. The microbial stability and safety of most foods based on a several combinations of hurdles that should not be overcome by microorganisms present in the food. The hurdle effect is fundamental for food preservation since the hurdles control the microbial spoilage, food poisoning and in some situations, desired fermentation processes (Leistner, 1995). Hurdle technology is used to improve the preservation of food, enhance the total quality, as well as improve the economic properties of the food. In addition, hurdle technology shows synergistic effect while using different methods to inactivate the microorganisms (Khan et al., 2016).

There are several combinations of conventional and emerging technologies to enhance the microbial inactivation. Radio frequency, microwave heating, irradiation, high pressure processing, thermal treatment, antimicrobial agents are used as combined with each other (Khan et al., 2016).

In order to enhance the microbial reduction by UV light, researchers combined it with other decontamination technologies. UV-C light was combined with several antimicrobial agents (fumaric acid, cinnamaldehyde, citral, vanillin), mild heat, and ultrasound (Beristain-Bauza et al., 2018; Gabriel, 2015; Kim et al., 2009; Kaya and Unluturk, 2019; Ferrario et al., 2020).

CHAPTER 3

MATERIALS AND METHODS

3.1. Preparation of Mixed Fruit Juice Beverage

A mixed fruit juice beverage was formulated to use in this study. Nutrition values and the taste of fruits, also their taste completion with each other was considered while studying formulization. In this part, fruit juice formulation and fruit juice extraction were described. Formulation was performed by change in amounts of used fruit juices. Fruits were extracted by two different techniques: centrifugal juicer and cold-pressed juicer.

3.1.1. Formulation

The mixed fruit juice beverage was prepared using pineapple, green apple, and kiwi. Choosing of fruits for the formulation was made by considering the latest trends and consumer demands. In addition, the health benefits and taste of these fruits were also taken into account. Pineapple (*Smooth Cayenne*), green apple (*Granny Smith*) and kiwi (*Hayward*) were purchased from a local supermarket in Izmir, Turkey.

Three types of formulations were prepared according to the amount of pineapple juice, green apple juice and kiwi juice (Table 1.1). The amount of fruit juice in the formulations was obtained by considering the pH value, as it is important in the processing of fruit juice and flavor. Fruit juice formulations are given by considering the percentages of different juices per unit volume. The initial formulation contained 60% pineapple juice, 35% green apple juice and 5% kiwi juice. Second one included

40% pineapple, 40% green apple and 20% kiwi juices. Third formulation had 50% pineapple, 35% green apple and 15% kiwi juices.

Table 3.1 Mixed fruit juice formulations

Fruit	**Formulation 1 (645)**	**Formulation 2 (831)**	**Formulation 3 (569)**
Pineapple (*Smooth Cayenne*)	60%	40%	50%
Green Apple (*Granny Smith*)	35%	40%	35%
Kiwi (*Hayward*)	5%	20%	15%

3.1.2. Fruit Juice Extraction

Juices from pineapple, green apple and kiwi were obtained using two different kinds of household tabletop juice extractor such as cold press juicer (CPJ) and centrifugal juicer (CDJ) (Figure 3.1). First, the unnecessary parts of the fruits such as leaves and outer skin were removed and the fruits were washed under tap water. Immediately after washing, the fruits were divided into two by weight. Then the juice from fruits was extracted by means of both a cold press juicer (Kuvings B6000S, Istanbul, Turkey) and a centrifugal juicer (Arçelik Roboligo, Istanbul, Turkey). Pineapple, green apple and kiwi fruits were squeezed separately and a short cleaning was done with water between the squeezing. In further step, juices were filtered separately to remove big particles. The mixed fruit juice beverages were prepared according to the formulation given in Table 3.1. Preparation steps of the mixed fruit juice beverage is given in Figure 3.1.

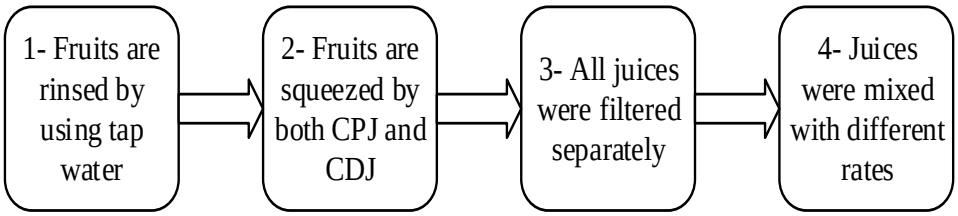

Figure 3.1 Preparation of fruit juice formulations

3.2. Sensory Analysis

Sensory evaluation was carried out with 19 semi-trained panelists and by using 9-point hedonic scale technique. The panelists were between the ages ranging from 12 to 50. All panelists were familiar with the sensorial attributes of all ingredients used in the mixed fruit juice formulation and acquainted with the scoring technique. Consumer acceptance test was applied to determine the most accepted choice among the formulated mixed fruit juices. Based on a 9-point hedonic scale, the panelists evaluated the attributes – appearance, odor, text, flavor and overall acceptance. The results of the sensory evaluation were assessed using analysis of variance (ANOVA) and Tukey's pairwise comparison test ($p < 0.05$). An internal consistency test was applied to calculate Cronbach's alpha (CA) coefficient using Minitab 16 (Minitab Inc., State College, PA, USA). CA measures the similarity between evaluation profiles from different panelists. An evaluation profile corresponds to assessments made by a panelist on a given attribute, overall products (Pinto et al., 2014). The formulation which was scored the highest in the sensory evaluation was used in the following studies. The results of the sensory analysis were evaluated with a radar graph plotted in Excel® (2010).

3.3. Physical, Chemical, Optical and Phytochemical Measurements

Some physical, chemical, and optical properties were measured after the fruit juice is squeezed by cold-pressed juice extractor and centrifugal juice extractor. In that means pH, total soluble solid content (Brix), absorption coefficient, turbidity, fruit juice

yield, color were measured. In addition, the total amount of phenolic content and total antioxidants were measured as phytochemical properties.

3.3.1. pH

Determination of pH values of fruit juice was applied by using a bench top pH meter (HANNA Instruments, USA) at room temperature (25 °C). Calibration was handled by using two kinds of buffers that are adjusted to pH 7 and pH 4 (Kaya, 2011)

3.3.2. Total Soluble Solid Content (Brix)

Total soluble solid content measurements were carried out using a bench-top refractometer (Mettler-Toledo RE40D, AEA Investors Inc., ABD) and the data were reported as Brix. Measurements were done at room temperature (25 °C) and the refractometer was calibrated with water before the measurement.

3.3.3. Absorption Coefficient

The absorbances of fruit juice samples were measured by using a Schimadzu UV-visible Spectrophotometer (UV-2450, Japan) at three different wavelengths 240nm, 280nm and 365nm. Different dilution factors were applied to sample (1:10, 1:25, 1:50, 1:100, 1:250, 1:500 and 1:1000). Absorption coefficients at each wavelength was estimated from the slope of the graph by plotting absorbance values against sample concentrations (Hakgüder, 2009).

3.3.4. Turbidity

Turbidity measurement was done by using HACH 2100AN IS Turbidimeter. Fourty-fourty five mL sample was poured into the glass cuvettes of the turbidimeter,

and measurement was performed. The results were given as Nephelometric Turbidity Unit (NTU) (Hakgüder, 2009).

3.3.5. Fruit Juice Yield

Equal amount of (50g) fruits were weighed to squeeze using both cold-pressed juice extractor and centrifugal juice extractor. After squeezing, volume of the juice was evaluated individually by a volumetric flask. After that, the performance of yield was calculated as percentage for cold-pressed juice and centrifugal juice.

3.3.6. Color

Color parameters awere measured using a chromameter (Konica Minolta CR 400, Japan). CIE color parameters such as L* (Lightness-darkness), a* (redness-greenness), b* (yellowness-blueness) were determined at the end of the measurement. The three-dimensional color space image of L*, a*, and b* values was shown in Figure 3.2. These parameters represent the brightness-darkness, redness-greenness, and yellowness-blueness, respectively. The total color change and Browning Index were also calculated by means of the Equations 3.1 and 3.2:

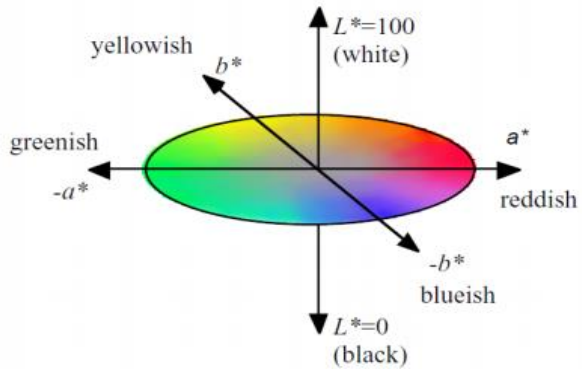

Figure 3.2. CIELAB color space

(Molino et al, 2013)

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (3.2)$$

$$BI = 100 * \frac{\frac{(a* + 1.75L*)}{5.645L* + a* - 3.012b*} - 0.31}{0.172} \quad (3.3)$$

3.3.7. Total Phenolic Content

Total phenolic content of cold-pressed juice (CPJ) and centrifugal juice (CDJ) was determined by using Folin-Ciocalteu method described by Pala and Toklucu (2011). CPJ and CDJ samples were firstly diluted in the ratio of 1:20 with a mixture of MeOH: H2O (3:2). Three hundred μL of diluted CPJ and CDJ mixed with 1.5-mL of Folin–Ciocalteu reagent (MERCK, Germany, catalog no. 1090010100) that was diluted with 1:10 with distilled water in a glass tube. It was followed by the addition of 1.2-mL of sodium carbonate (75 g/L). The mixture was thoroughly mixed by using vortex mixer (ZX3, VELP Scientifica S.r.l., Usmate, Italy) and allowed to stand for a further 90 min in dark and at room temperature. The absorbance was measured at 765 nm using a UV–Visible spectrophotometer (Shimadzu, UV-2450, Japan). The solvent used to dilute the CPJ and CDJ samples (MeOH: H2O (3:2)) was used to set the read zero before measurement. A control sample was prepared like the previous samples, except that 300 μL of distilled water was added instead of the CPJ or CDJ samples. The absorbance of control sample then subtracted from the absorbance of CPJ and CDJ samples. The comparison was made with a calibrated curve generated with different gallic acid (Sigma-Aldrich, Germany) concentrations prepared using with 1000mg/L gallic acid stock solution. The curve was plotted by using different gallic acid concentrations from 1 mg/l to 80 mg/L. The same procedure with the samples was applied as in the preparation of the gallic acid standard curve. The concentrations of total phenolic content in CPJ and CDJ were expressed as mg gallic acid equivalents (GAE) per L according to the equation obtained from gallic acid standard curve demonstrated in appendix A.

3.3.8. Total Antioxidant Activity

3.3.8.1. DPPH Method

Free-radical scavenging activity (RSA) of cold pressed juice (CPJ) and centrifugal juice (CDJ) was obtained by the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the method described by Odriozola-Serrano, Soliva Fortuny, and Martin-Belloso (2009). In this method, antioxidant activity was measured by their scavenging capacity and activity was proportional to the DPPH inhibition. DPPH solution (25 mg/L) was prepared using methanol as solvent. The DPPH solution was prepared freshly at each time prior to perform analysis. The solution in the amount of 3.9 mL was added to a glass tube followed by 10 μL CPJ or CDJ and 90 μL deionized water addition. All samples were incubated for 60 min in the dark at room temperature. Following that, absorbance values were recorded at 515 nm. Blank was prepared by the same procedure with samples by using deionized water. 3.9 mL DPPH solution was added to the glass tube followed by 100 μL deionized water. Blank was also incubated for 60 min in the dark at room temperature. Then, absorbance values were recorded at 515 nm. Methanol was used to set auto zero prior to measurement. Antioxidant capacities of the sample were then calculated in percentage by means of Equation 3.3:

$$\text{DPPH inhibition (\%)} = 100 - \frac{As}{Ab}\ x\ 100 \qquad (3.3.)$$

In which A_s and A_b indicates absorbance values of sample and blank, respectively.

3.4. Microbiological Studies

3.4.1. Microbiological Enumeration

The background flora of fruit juices that were extracted with both cold pressed juicer and centrifugal juicer was determined. Total aerobic mesophilic bacteria count (TAC) and yeast and mold counts (YMC) were checked by surface plating on Plate Count Agar (PCA), and Potato Dextrose Agar (PDA) acidified to 4.0 with 10% tartaric acid (Merck, Darmstadt, Germany), respectively. The PCA and PDA plates were incubated at 30°C for 3 days and 25°C for 5 days, respectively (Hakguder, 2009).

Enumeration of the viable microorganisms of UV-LED treated CPJ samples from different dilutions prepared with 0.1% peptone water were surface-plated in duplicate on Tryptic Soy Agar (TSA, Merck, Darmstadt, Germany) containing 0.1% dihydrostreptomycin. All plates were incubated at 37 ◦C for 24 h and then counted.

3.4.2. Stock Culture Preparation

Since Koutchma et al. (2004) explored that the sensitivities of *E.coli* K12 and *E.coli* O157: H7 were not significantly different from each other, *E.coli* K12 (ATCC 25253) was selected as a pertinent pathogen in this study. The *E. coli* K12 (ATCC 25253) strain was cultured from −80°C lyophilized vials, enriched in a test tube containing nutrient broth (NB, Merck, Darmstadt, Germany) and incubated overnight (18–24 h) at 37 °C. The *E. coli* K12 (ATCC 25253) culture was first adapted to pH 4.0 by using CPJ following the procedure described by Kaya and Unluturk (2019). 0.1 mL of *E.coli* K12 from stock culture was first inoculated into 100 mL CPJ (pH 4.0) and incubated in an orbital shaker (Thermo Electron Corp., OH) at 30°C and 100 rpm for 24h. Acid-adapted cells was transferred onto TSA slants in every hour to keep tabs on the adaptation of the bacteria. After 24h, the maximum viability was observed on the colonies which were transferred onto TSA after 3 hours. These acid-adapted cultures were stocked to use in further studies. Stock cultures were prepared by transferring

acid-adapted cells into cryovials containing 60% NB and 40% glycerol and they stored at -80 °C until used.

3.4.3. Inoculation of *E. coli* K12 into Cold Pressed Mixed Fruit Juice Beverage

The *E.coli* K12 which is a surrogate of *E.coli O157:H7* was inoculated into CPJ and used in the study. Initially, CPJ was pasteurized at 70°C for 120 s. Pasteurization was applied by using a water bath (Memmert, WNB) and thin glass tubes that could be closed by metal lid. First of all, come up time to 70°C by using a thermocouple was determined and this time was added to the pasteurization time. Consequently, cold-pressed mixed fruit juice samples was kept on 70°C for 120 s. At the end of that time, glass tubes were cooled immediately by a cold-water bath. Then, all cold-pressed mixed fruit juice samples were collected in a sterilized glass bottle. After that, 0.1 mL acid-adapted stock culture was inoculated into a test tube that contain 10 mL NB for enrichment. The inoculated culture was incubated overnight at 37 °C to reach the subculture at stationary phase. Then 1 µL from this culture medium was inoculated into 3 mL of CPJ to obtain a final microbial concentration of 6-7 $\log_{10}$ CFU/mL. Sample amount (3 mL) was determined to ensure that the sample depth was 0.15 cm. Sample depth was calculated by the ratio of the sample volume and surface area of Petri dish. An adequate stirring was applied during treatment to ensure equal UV dose distribution through the sample. Also, the edge effect was avoided by using the smallest possible sample volume (Bolton and Linden, 2003).

3.5. UV-LED Irradiation

The inoculated CPJ samples were treated using a static UV-LED unit (Figure 3.1.). Briefly, UV-LED unit was designed with four UV-LEDs that could be demountable. Also, these LEDs could be used together or separately, and the electronic

circuit was connected to a customized four channel power supply. Each UV-LED was operated with a constant forward current of 20 mA. UV-LED lamps (8.33 mm diameter, SETI Sensor Electronic Technology Inc., Columbia, SC, USA) which emit light at 280 nm and 365 nm, were used in this study. In order to investigate the effect of UV-LEDs multiple and single wavelengths, various combinations were applied, and the study was conducted in simultaneous exposure and sequential exposure.

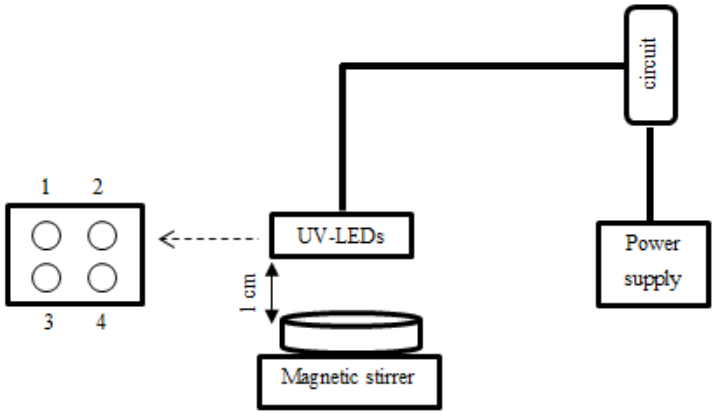

Figure 3.3. Static UV-LED unit

3.5.1. Simultaneous Exposure Treatment

The forward voltage and optical output power of each UV-LED at 20 mA was 5.8 and 5.50 V, and 0.60 and 0.40 mW for the 280 and 365 nm UV-LEDs, respectively. The inoculated sample (3-mL) was placed in a 55-mm sterilized Petri dish. UV-LEDs were fixed facedown, and Petri dish was 1 cm below the UV source. The medium depth was fixed at 0.15 cm to avoid edge effects caused by stirring and provide homogenous light distribution (Unluturk et al., 2010). The samples were exposed to multiple wavelength combination of 280/365 nm (2 lamp emitting at 280 nm and 2 lamp emitting at 365 nm). In simultaneous application, two UV-LEDs with different wavelength were turned on at the same time to irradiate the CPJ. On the other hand,

inoculated CPJ samples were exposed to single wavelength by using four UV-LED lamps emitting light at 280 nm. Both treatments were applied for 40 minutes. In applications using 280 nm and 365 nm UV-LED lamps, UV doses were calculated from the product of incident UV intensity and exposure time (Akgün and Ünlütürk, 2017). At this part of the study, application time was determined as 40 min according to the previous studies (Akgün and Unluturk, 2017, Baykuş et al., 2021). Referenced studies reported that the optimum treatment parameter was 280/365 nm for 40 min exposure time.

3.5.2. Sequential Exposure Treatment

Sequential applications were performed in three different combinations. These combinations are presented in Table 3.1. In sequential application, one UV-LED turned on first for a certain time, then it was turned off and the other was turned on for a while for irradiation. The UV dose was calculated from the product of incident UV intensity and exposure time. Exposure time was determined according to the previous studies. studies Akgün and Unluturk (2017) and Baykuş et al. (2021) reported that the optimum parameter for *E.coli* inactivation in fruit juice was 280/365 nm for 40min exposure time. Thus, sequential applications were determined to supply total exposure time as 40min. Also, total exposure time of 60 min for sequential application was applied to investigate the time dependency of the sequential processing.

Table 3.2. Presentation of sequential application parameters

First application		Second application		Total exposure time
Wavelength	Exposure time	Wavelength	Exposure time	
280 nm	20 min	365 nm	20 min	40 min
365 nm	20 min	280 nm	20 min	40 min
280 nm	30 min	365 nm	30 min	60 min

3.5.3. UV Intensity and Dose Calculation by Using Ferrioxalate Actinometrical Method

In this study, actinometrical method was not performed. However, UV dosages was calculated based on the data that was achieved before by means of actinometry. Therefore, this method is given in that thesis.

UV dose was calculated from the product of incident UV intensity and exposure time. UV intensity emitted by each LED was measured by ferrioxalate actinometry (Chevremont et al,, 2012). Potassium ferrioxalate was chosen as a chemical actinometer since it is suitable for wavelength ranges from UV to visible regions (Jankowski et al., 1999). Three millimeters of 12 mM potassium ferrioxalate solution were irradiated with each LED at 280 and 365 nm for 20, 30 and 40 min. At the end of each irradiation, 0.5 mL phenanthroline buffer solution (0.1%: 225 g $CH_3COONa{\cdot}3H2O$, 1 g of phenanthroline in 1 L of H_2SO_4 0.5%) was added to the solution and mixture was stirred in the dark for 30 min. The Fe^{2+} ions produced during the photolysis of potassium ferrioxalate solution formed a red complex with phenanthroline. The absorbance of this mixture was measured by a spectrophotometer. The number of Fe^{2+} ions in the solution can then be determined from the following equation:

$$n_{Fe^{2+}} = (N * V_1 * V_3 * DO_{510})/(V_2 * l * \varepsilon * 10^3) \quad (3.4)$$

where N is the Avogadro number, V_1 the volume of the irradiated solution (mL), V_2 the volume of V_1 taken for analysis (mL), V_3 the final volume after dilution of V_2 (mL), DO_{510} the absorbance of the complex measured at 510 nm in the V_3 solution, ε the coefficient of molar extinction of the complex phenanthroline-Fe^{2+} (ε complex, 510 nm=11,100 $mol^{-1}.L.cm^{-1}$) and l the optical path length (cm).

The number of Fe^{2+} ions was used to calculate incident photon flux using the following formula:

$$P_{0,\lambda} = n_{Fe^{2+}}/(\Phi_{AC} * \Delta t) \quad (3.5)$$

where φ_{AC} =1.25 is the quantum yield of the actinometer at the wavelength of 280 and 365 nm and t the irradiation time (s).

The incident photon flux was used to achieve power emitted by each LED:

$$P_e = (h * c)/\lambda)/P_{0,\lambda} \quad (3.6)$$

where h (J.s) is the Planck's constant, c is the celerity of light ($m.s^{-1}$) and λ the wavelength (m).

In order to calculate intensity (W/cm^2), the luminous flux was divided by irradiated surface. Then, average UV intensity passed through the juice samples was calculated according to Beer-Lambert law (Unluturk et al. 2008):

$$I_{avg} = I_0 * \frac{1-e^{-A_e*L}}{A_e*l} \quad (3.7)$$

I_0 is the incident intensity of the sample surface (mW/cm^2), A_e is the absorbance coefficient of the samples (cm^{-1}) and l is the path length of the samples (cm). UV dosage (mJ/cm^2 or $mW.s.cm^{-1}$) applied to the samples was calculated by multiplying average UV intensity of the sample with UV exposure time:

$$D = I_{avg} * t \quad (3.8)$$

3.5.4. Hurdle Application with Filtration and Antimicrobial Ingredient

A study has been carried out to increase the inactivation efficiency of UV-LED treatment, in which 280/365 nm wavelengths were used together, which gave better results compared to other wavelengths. For this reason, the hurdle technology approach was used to increase the inactivation provided by UV-LEDs. In this study, UV-LED

treatment was combined with the microfiltration process and the addition of an antimicrobial component (ginger) to the formulation. Filtration by using 0.45 μm filter was used prior to UV-LED treatment conducted at 280/365nm wavelengths. In previous part of the study, wavelength at 280/365 nm for 40 min exposure parameter was applied to the cold-pressed mixed beverage. However it is questioned if the inactivation of *E.coli* K12 could be increased by hurdle technology. Therefore, filtration and antimicrobial agent addition was investigated to answer this question.

Filtration was performed by means of a filtration setup comprise of a vacuum pump connected with a Nuch Erlenmeyer and a funnel (Figure 3.4.) Initially, 3 μL culture medium is inoculated to 12 ml CPJ to obtain microbial concentration of 6-7 $\log_{10}$ CFU/mL. Inoculated CPJ was filtered by means of the filtration setup with different filters that has 0.45 μm and 2-4 μm pore sizes. Then, filtered part was treated with UV-LEDs as described in 3.5.1. Also, ginger was added to CPJ to investigate if the antimicrobial property of ginger could increase the inactivation of UV-LEDS. Again 3 μL culture medium was inoculated in 12 ml CPJ+ginger and it was filtered by means of filtration setup that has 0.45 μm filter. After filtration, 3 mL filtered CPJ+ginger was added to the 55-mm Petri dish and treated with UV-LEDs as described in 3.5.1.

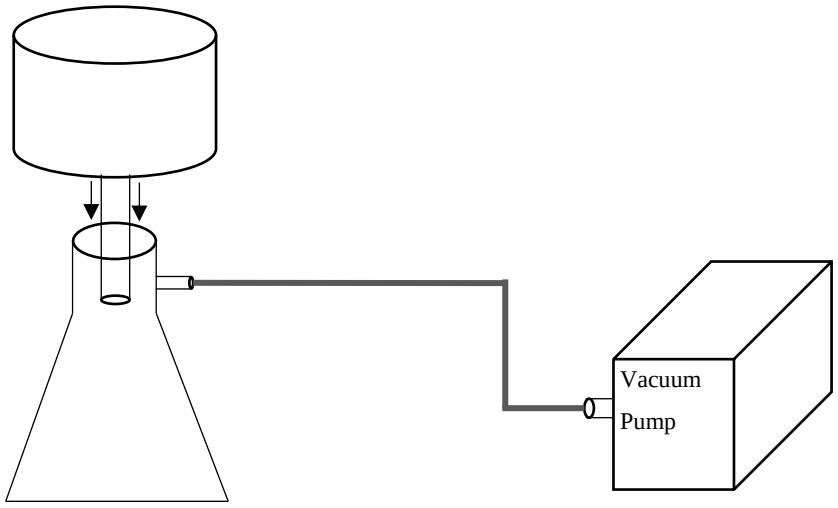

Figure 3.4. Filtration setup

3.6. Statistical Analysis

All the experiments and analysis were performed in triplicate. The results of microbiological, physicochemical and optical measurements were expressed as their means and standard deviations. The experimental data were evaluated using Minitab 17 (Minitab Inc., State College, PA, ABD) for analysis of variance (ANOVA) to determine how significantly the independent variables (UV-LED processing and extraction method) affect the dependent variables (physical, chemical, optical and microbiological properties of cold-pressed and centrifugal mixed beverage). The means of data were compared by Tukey's pairwise comparison test at a 95% confidence interval. Differences of data were significant for p-value is equal or less than 0.05 ($p \leq 0.05$).

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1. Sensory Analysis

Sensory analysis was performed to assess consumer acceptance of formulated juices. The most accepted fruit juice formulation was determined at the end of the study. The average scores of the consumer acceptance test varied from 5.5 ± 1.10 ('neither like nor dislike') to 7.9 ± 1.3 ('like moderately'). Results were evaluated by using a radar graph (Figure 4.1). The sample (645) containing 60% pineapple, 35% green apple, and 5% kiwi were most preferred. The scores of that sample were 7.35 ± 1.27, 7.9 ± 1.25, 7.65 ± 1.31, 7.75 ± 0.97, and 7.7 ± 1.08 for the attributes of appearance, odor, texture, flavor, and overall acceptance, respectively. Only the appearance characteristic of sample 645 differed significantly from other samples ($p < 0.05$). No significant difference was observed between the different sensory properties of the samples. Additionally, the acceptance index (IA), a ratio of the average score to the maximum score of the sample, was calculated to determine the most liked formulation (Table 4.1). Oliveira, Marques, Kwiatkowski, Monteiro and Clemente (2013) indicated that the scores of IA must be equal to or higher than 70%. In our study, the most preferred product formulations had IA higher than 70%. The IA results supported the radar plot data and ensured that the most accepted formulation was 645.

Table 4.1. Index of acceptance

Code of Formulation:	**645**	**831**	**569**
Average score	7.7	7.2	7.25
Maximum score	9	9	9
Index of Acceptance (IA)	85.6%	80.0%	80.6%

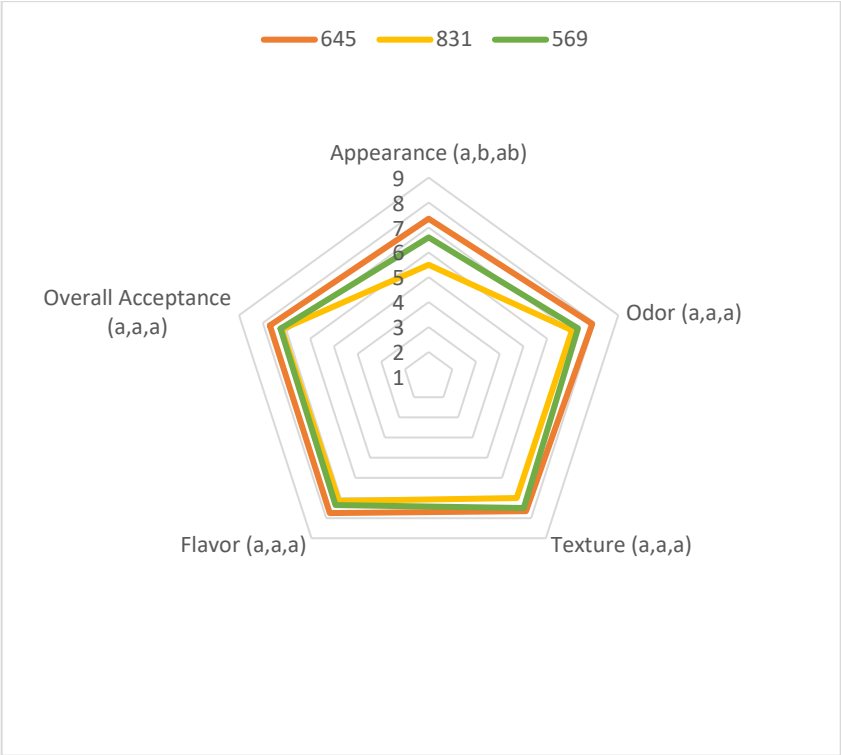

Figure 4.1. Sensory assessment of formulated fruit juices using radar graph.

It is also essential to establish the reliability of factors extracted from scales. The reliability of the scores that panelists voted should be evaluated. In other words, the reliability of the scales was revealed. Cronbach's alpha (CA) is a numerical coefficient that can determine the scale's reliability (Reynaldo and Santos, 1999). According to Nunnally and Bernstein (1994), CA values greater than 0.7 are considered acceptable, indicating panelists presenting similar evaluation profiles. The results of our sensory analysis were deemed to be satisfactory and reliable since the CA was calculated as 0.8. In conclusion, formulation 645 was highly preferred, and this formulation was used throughout the study.

4.2. Physical, Chemical, and Optical Properties of Cold Pressed Juice

Physical, chemical, and optical properties of cold-pressed fruit juice, including pH, total soluble solid (Brix°), color parameters, browning index, turbidity, and

absorption coefficient, were determined and presented in Table 4.2. Also, due to the scarcity of data about cold-pressed mixed beverages, results were compared to those found in the literature that studied juices from other fruits.

Table 4.2. Physical, chemical, and optical properties of cold-pressed juice

pH	3.77 ± 0.01		
Brix (%)	13.15 ± 0.07		
Color	**L***	**a***	**b***
	30.26 ± 0.1	-3.94 ± 0.05	8.60 ± 0.15
ΔE*	31.70 ± 0.02		
Browning index (BI)	22.17 ± 0.37		
Turbidity (NTU)	1621.5 ± 2.12		
Absorption Coefficient	**254nm**	**280nm**	**365nm**
	9.58	7.12	9.34
Total phenolic component (mg GAE/L)	867.25 ± 0.01		
DPPH inhibition (%)	29.70 ± 0.02		

Results were presented as "means ± standard error". Least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p < 0.05$). Abbreviations: NTU (Nephelometric Turbidity Unit).

4.2.1. Physical and Chemical Properties

Since the cold-pressed mixed juice beverage was formalized by using pineapple, green apple, and kiwi juices, the pH value of this juice drink was similar to those of fruit juices. Pineapple is an acidic fruit with a pH of about 3.71 (Sairi et al, 2004). Also, most apple juices have a pH between 3.0 to 4.5 (Jolicoeur, 2011). Kiwi juice is also an acidic fruit with a pH between 2.75 and 3.27 (Wang et al., 2017). The pH of a cold-pressed mixed juice beverage was 3.77 ± 0.01 by the pH of the fruit juices it contains. The yield of cold-pressed juices for pineapple, green apple, and kiwi were 92%, 66%, and 32%, respectively (Figure 4.1.). The highest performance for the amount of yield was obtained for pineapple. The cold-pressed juice's total soluble solid content (ºBrix) was 13.15 ± 0.07 at 20˚C. According to the Turkish Food Codex, fruit juices from apple and pineapple must have a ºBrix value of at least 11.2 and 12.8, respectively. Cold-pressed mixed juice beverage prepared by mixing three different juices had a Brix value higher

than legal limits. That means the brix value of cold-pressed fruit juice beverage is proper according to Turkish Food Codex.

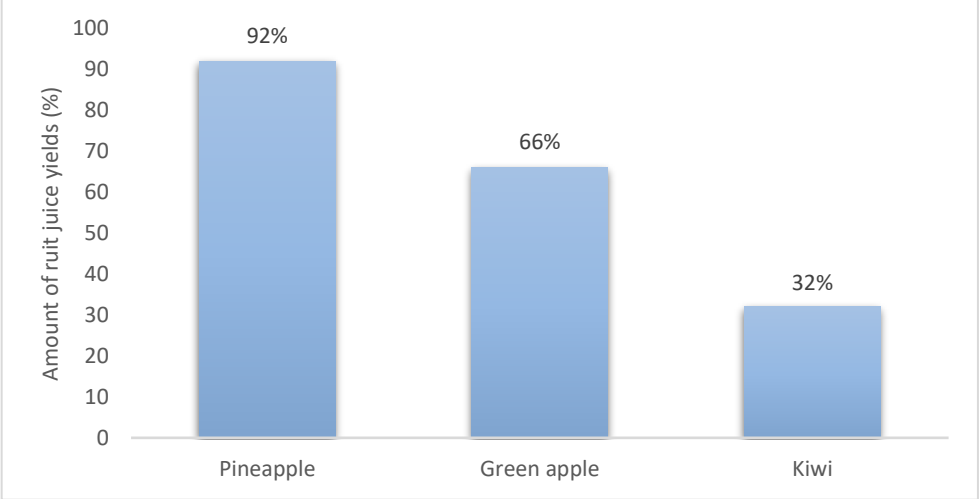

Figure 4.2. Amount of fruit juice yields extracted by cold-pressed juicer

4.2.2. Optical Properties

Color is considered an essential property for the food matrix since it is an indicator of food quality (Wang, 2021). This study used pineapple, green apple, and kiwi to make cold-pressed mixed fruit juice beverages. Indeed, color measurements were performed only for cold-pressed mixed juice beverages, and individual measurements of juice samples for pineapple, green apple, and kiwi were not performed. However, literature values were presented to evaluate the color property of the cold-pressed mixed beverage. Shamsudin et al (2014) reported that the L* value of the pineapple juice was 39.51 ± 0.47. Costa et al. (2013) found the L* value of pineapple pulp as 82.44 ± 0.36 (diluted with water). Also, Assawarachan and Noomhorm (2010) reported that the L* value of pineapple juice was 36.28 ± 0.08. Besides, the L* value of apple juice was obtained as 58.19 ± 0.42, 25.40 (Noci et al., 2008), 49.3 ± 0.4 (Ferrario and Guerrero, 2016) and 19.77 ± 0.03 (Abid et al., 2013). Patrignani et al. (2019) determined the L* value of kiwi juice as 33.40. Wang et al. (2020) established the L* value of kiwi juice as 107.10 ± 3.32. Also, 35.9 (Tomadoni et

al., 2015) was reported. L* value of cold-pressed mixed beverage was 30.26 ± 0.01. Since the beverage was mixed with pineapple, green apple, and kiwi juices, the L* value was expected to be linear with the L* values of the components. Turbidity of the cold-pressed juice was obtained as 1621.5 ± 2.12 NTU. Turbidity of the cold-pressed juice was extensively occurred by suspended particles of the juice sample. The cold-pressed juice was freshly squeezed with no enzymatic clarification or filtration. Therefore, the cold-pressed juice could be considered a pulpy fruit juice. Vaillant et al. (2008) discussed the relationship between suspended insoluble solids content and turbidity. They revealed that turbidity could be a suitable parameter for measuring insoluble solids. It was concluded that the cold-pressed juice contains suspended insoluble solids that cause turbid structure and could be accepted as pulpy juice.

The absorption coefficient is an important parameter in evaluating UV irradiation's effectiveness, as it influences the UV light intensity and its ability to penetrate liquid materials. The absorption coefficient of cold-pressed juice was obtained at 254 nm, 280 nm, and 365 nm, which were the processing parameters in this study. The absorption coefficient was calculated from the slope of absorbance values plotted against the dilution factors. As it shown in Table 4.2, the values were 9.58 ± 0.015 cm^{-1}, 7.12 ± 0.07 cm^{-1} and 9.34 ± 0.009 cm^{-1} at 254 nm, 280 nm and 365 nm, respectively. The absorption coefficient is affected by the absorption of the juice. Juice with low absorption has a low absorption coefficient. Lower UV absorption allows more penetration of UV light through the juice. Thus, more components such as microorganisms or nutrients in the juice will be exposed to UV light (Sew et al., 2014). Results show that the cold-pressed mixed beverage absorbed the highest UV light at 254 nm. Then the lowest amount of UV light was absorbed at 280 nm. Suspended particles and dissolved particles could affect UV absorption. UV absorption increases when the number of suspended particles increases. Thus, clear juices have lower absorption of UV light (Sew et al. 2014, Akgün and Unluturk, 2017). On the other hand, color and pH values significantly affect the absorption coefficient (Sew et al., 2014). Pierscianowski et al. (2021) evaluated the cold-pressed kale juice property and UV-C treatment. They reported that the absorption coefficient of cold-pressed kale juice at 254 nm was 109 ± 4. Also, L* of cold-pressed kale juice was 14.60 ± 1.82. Compared to this study, the absorption coefficient was lower, and the L* value of cold-pressed mixed beverages was higher than those. The difference in L* value and colors of juice samples probably caused this absorption coefficient.

4.2.3. Total Phenolic Content

Phenolic compounds are secondary metabolites that are present in fruits and vegetables. They are essential antioxidant groups founds in fruits and vegetables. They can scavenge the free radicals and act as antioxidants (Wootton-Beard et al., 2011). The total phenolic content of the cold-pressed mixed beverage was determined as 867.25 ± 0.01 mg GAE/100 mL. The total phenolic content was determined to right after the extraction to prevent any beverage oxidation. Wang et al. (2021) obtained the total phenolic part of several cold-pressed fruit and vegetable juices. The highest phenolic content was 1201.3 μg of GAE/g in green kale juice. The difference in the amount of total phenolic content with the present study is probably caused by different fruit and vegetable content. In other words, the accumulation of phenolic compounds is also differentiated by the genotype, color, and processing technique. (Wang et al., 2021). Since there is no study about the amount of total phenolic content of cold-pressed mixed beverage or its ingredients, a specific discussion could not be performed.

4.2.4. Total Antioxidant Activity

Due to the complex nature of biological samples, it is difficult to determine a universal method to obtain the antioxidant activity (Wang et al., 2021). In this study, DPPH scavenging method was used to obtain total antioxidant capacity of cold-pressed mixed beverage. Cold-pressed mixed beverages' DPPH radical scavenging activity was 29.70 ± 0.02 %. Kim et al. (2015) reported that the DPPH scavenging activity of cold-pressed tomato juice was 55.6 ± 0.2 %, higher than the cold-pressed mixed beverage. In another study, 19 different cold-pressed fruit and vegetables were discussed, and the highest DPPH activity was found in organic green kale juice (Wang et al., 2021). Also, they revealed that the most increased antioxidant activity was found in phytochemical-rich vegetables. In other words, the differences between DPPH scavenging activity of different cold-pressed juice are probably caused by the difference in the phytochemical content of fruits and vegetables.

4.3. Physical, Chemical, Optical, and Phytochemical Properties of Centrifugal Juice

Physical, chemical, and optical properties of centrifugal fruit juice, including pH, total soluble solid (Brix°), color parameters, browning index, and turbidity, were determined and presented in Table 4.3. The centrifugal mixed beverage absorption coefficient was not obtained because UV-LEDs did not treat the centrifugal juice. The absorption coefficient is a value that is important for UV irradiation, as described in 4.2.2. Also, due to the scarcity of data about centrifugal mixed beverages, results were compared to those found in the literature that studied juices from other fruits.

Table 4.3. Physical, chemical, and optical properties of centrifugal juice

pH	3.75 ± 0.01		
Brix (%)	13.85 ± 0.07		
Color	**L***	**a***	**b***
	27.25 ± 0.04	-2.65 ± 0.03	6.26 ± 0.08
ΔE*	28.08 ± 0.06		
Browning index (BI)	17.84 ± 0.37		
Turbidity	756 ± 15.56		
Absorption Coefficient	**254nm**	**280nm**	**365nm**
	-	-	-
Total phenolic component (mg GAE/L)	922 ± 0.01		
DPPH inhibition (%)	28.15 ± 0.01		

Results were presented as "means ± standard error". Least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p < 0.05$). Abbreviations: NTU (Nephelometric Turbidity Unit).

4.3.1. Physical and Chemical Properties

Since the centrifugal mixed juice beverage was formalized using pineapple, green apple, and kiwi juices, the pH value of this juice drink was similar to those of fruit juices, as described in 4.2. Since the pH of the centrifugal mixed beverage was 3.75 ± 0.01, the juice was classified as very acid (Gomes et al., 2022). The yield of centrifugal

juices for pineapple, green apple, and kiwi were 47%, 50%, and 31%, respectively (Figure 4.1.). The highest performance for the amount of yield was obtained for green apples. The centrifugal juice's total soluble solid content (ºBrix) was 13.85 ± 0.07 at 20˚C. According to the Turkish Food Codex, fruit juices from apple and pineapple must have a ºBrix value of at least 11.2 and 12.8, respectively. Centrifugal mixed juice beverage prepared by mixing three different juices had a Brix value higher than legal limits as a centrifugal mixed beverage. Thus, it could be claimed that the centrifugal mixed juice beverage was also consumable on the market according to Turkish Food Codex.

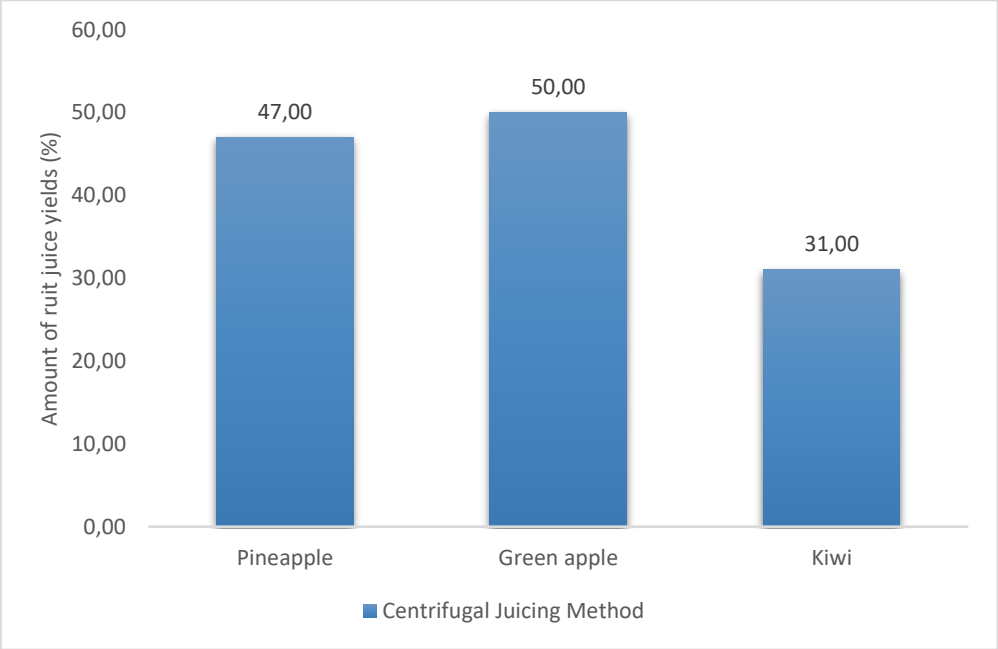

Figure 4.3. Amount of fruit juice yields extracted by centrifugal juicer

4.3.2. Optical Properties

In this study, pineapple, green apple, and kiwi were used to make centrifugal mixed fruit juice beverages. Indeed, color measurements were performed only for centrifugal mixed juice beverages, and individual measurements of juice samples for pineapple, green apple, and kiwi were not performed. On the other hand, there is no

study about extracting pineapple, green apple, ad kiwi juices by cold-press and centrifugal methods separately. Therefore, a complete discussion was performed in 4.2.2. It is expected to observe the linear L* value of centrifugal mixed beverage with literature values. However, L* values presented in the literature also show changes. Probably it is caused by different cultivars of fruits and other kinds of extraction methods (Wang et al., 2021).

Turbidity of the centrifugal juice was obtained as 756 ± 15.56 NTU. Turbidity of the centrifugal juice was extensively occurred by suspended particles of the juice sample. The centrifugal mixed beverage was freshly squeezed with no enzymatic clarification or filtration as a cold-pressed mixed beverage. However, the turbidity of centrifugal mixed beverages was lower than that of the cold-pressed mixed beverage. The different extraction techniques could cause this. Centrifugal juicers have a mesh chamber in which sharp blades rotate at high speed to slice the fruit. Sliced fruits spilled out by rotation, and fewer particles were retained in the juice when compared to the cold-pressed juices (Nnamdi et al., 2020; Kim et al., 2015). Consequently, lower turbidity was obtained than in cold-pressed mixed beverages.

4.3.3. Total Phenolic Content

The centrifugal mixed beverage's total phenolic content (TPC) was 922 ± 0.01 mg GAE/100 mL. The total phenolic content was determined right after the extraction to prevent beverage oxidation. Kim et al. (2015) reported that the TPC of tomato juice extracted by a centrifugal juicer was 47.7 ± 0.1 (mg TAE/L). In another study, the TPC of grape juice extracted by centrifugal juicer was obtained as 90.3 ± 1.4 (mg TAE/L) (Kim et al., 2017). Du et al. (2019) obtained the TPC of apple juice extracted by centrifugal juicer as 307.3 ± 16.7 (mg/ L GAE). Ferreira et al. (2016) studied the TPC of pineapple juice of different cultivars. They obtained different TPC levels for each cultivar, ranging from 71.07 ± 1.20 to 126.95 ± 7.51. The results show that the TPC level of juices is affected by genotype, cultivars, and color (Wang et al., 2021).

4.3.4. Total Antioxidant Activity

DPPH scavenging method was used to investigate the antioxidant activity of centrifugal mixed beverages. The DPPH radical scavenging activity of centrifugal mixed beverage was 28.15 ± 0.01 %. Kim et al. (2015) reported that the DPPH scavenging activity of tomato juice extracted by a centrifugal juicer was 49.8 ± 0.1 %, higher than the centrifugal mixed beverage. Antioxidant activity of pineapple juice with different cultivars was obtained, and it is reported that the antioxidant activity changed with different cultivars (Ferreira et al., 2015). Also, Wang et al. (2019) reported that the highest antioxidant activity was found in phytochemical-rich vegetables. In other words, antioxidant activity changes depending on the difference in phytochemical content of fruits and vegetables.

4.4. Comparison of Quality Parameters of Cold Pressed and Centrifugal Juice Samples

The juice yield of both the cold pressing method (CPJ) and centrifugal juicing method (CDJ) are compared in Table 4.4. The juice yield was obtained from 50 g of kiwi, green apple, and pineapple separately. CPJ juice yield was higher than CDJ juice yield. Compared to the CDJ technique, the kiwi, green apple, and pineapple juice yields obtained by the CPJ method were higher by 3.23%, 32%, and 95.74%, respectively. Especially for pineapple and green apple, the cold pressing method provided a significantly higher amount of juice. Since the pineapple and green apple had more rigid structure, pressing might be more effective for these fruits (Nnamdi et al., 2020, Kim et al., 2015). The results of yields show similarity with the studies in the literature. Donaldson (2020) evaluated the yield of six different types of juice for carrot, apple, celery, spinach, and their combination. In that study, the yield of apple juice by using the cold pressing method (vertical, single auger) was higher than that of the centrifugal juicing process (centrifugal, pulp ejecting). In another study, tomato juice was extracted using a low-speed masticating juicer (cold-pressing) and a high-speed centrifugal juicer.

Results showed that the juice yield of a low-speed masticating juicer (79.9 ± 1.6 %) was remarkably higher than that of a high-speed centrifugal juicer (54.8 ± 1.3 %) (Kim et al. 2015). The reason for that difference is probably caused by the blade of the centrifugal juicer. Since the edge of the centrifugal juicer rotates very fast, many fruit or vegetables were deflected without being extracted (Kim et al., 2015). Finally, the total soluble solid content of dregs of fruit or vegetables remains higher than cold pressing. Consequently, extracted parts remain lower than the cold-press method.

Table 4.4. The juice yield of extraction methods

	Pineapple	**Green apple**	**Kiwi**
Cold Pressing Method (ml)	46[a]	33[a]	16[a]
Centrifugal Juicing Method (ml)	23.5[b]	25[b]	15.5[a]

Results were presented as "means± standard error". Least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p < 0.05$).

Table 4.5 shows the physical properties of cold-pressed mixed beverage (CPJ) and centrifugal mixed beverage (CDJ). Homogeneity of CPJ and CDJ was also evaluated. This evaluation was performed for individual fruit juices that CPJ and CDJ includes. Green apple juice prepared by the CDJ method was separated into two layers just after squeezing, while the yield of green apple extracted by the CPJ method was not divided into two layers. Pineapple juice and kiwi juice extracted by CPJ were also more homogenous than that of yield by CDJ. Layer separation decreases the overall quality of fruit juices. This is a non-satisfying property for the consumer (Kim et al. 2015). The size of the pulps probably causes that difference in the fruit juice. CDJ has rotating blades that cut the fruit into small particles. These tiny particles then cause phase separation during storage. More distinct phase separation and foam formation were observed mainly in green apple juice obtained by squeezing with CDJ. On the other hand, CPJ squeezes fruits employing pressure without any blade, thus causing highly homogeneous fruit juice. Kim et al. (2015) examined the tomato juice prepared by centrifugal juicer and cold press juicer by optical microscope. They revealed that the juice prepared by centrifugal juicer has more fine air bubbles in the upper layer. The bubbles are formed by denaturation of proteins or pectic substances at the grinding step

of high-speed rotation of a flat blade disk in a centrifugal juicer. The bubbles allow for incorporating insoluble components of tomato juices such as tissues and fiber, causing layer separation.

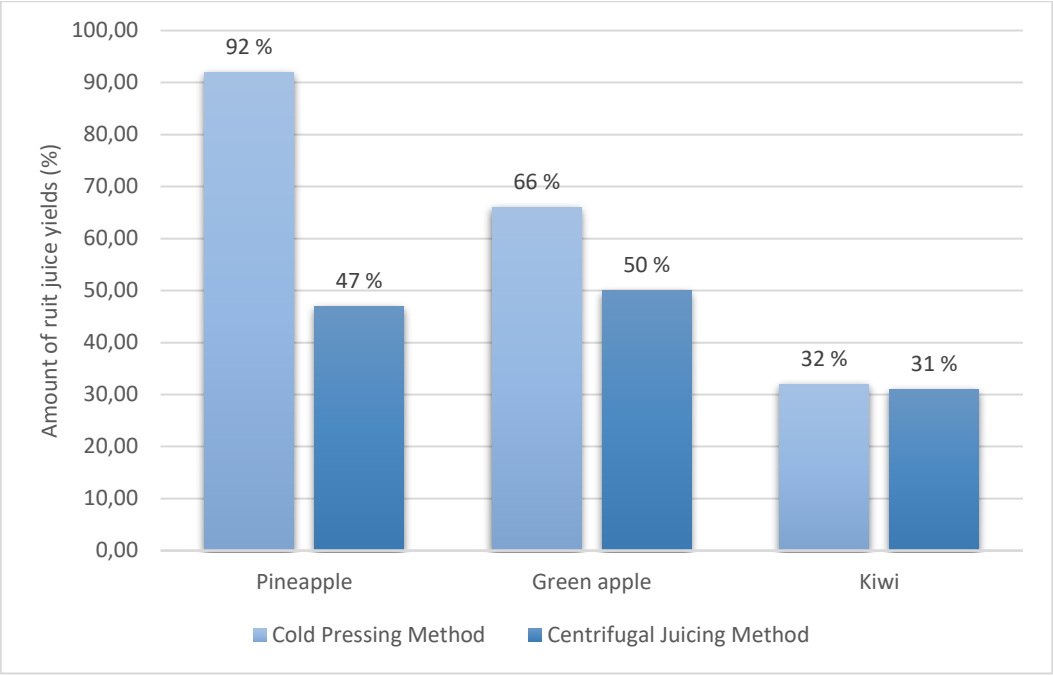

Figure 4.4. Amount of fruit juice yields extracted by cold-pressed and centrifugal juicer

Another reason for the difference in homogeneity is probably the different enzyme activity yielded by two different methods. On the other hand, Donaldson (2020) obtained the enzyme activity of apple juice produced by CPJ and CDJ. Enzyme activity that was acquired by the CPJ method was higher. The enzyme activity could indicate poorer extraction or increased denaturation of enzymes during the extraction of juice (Donaldson, 2020). Since the enzyme activity of CPJ was higher, that probably means the fruit was extracted better without any denaturation or cell disruption. Thus, more elevated enzyme activity indicates cell structure maintained by the CPJ method. Therefore, homogenous and good-quality juice was observed. Fruit juices prepared by centrifugal juices were separated into two layers caused by damaged cells attached easily to the air bubbles (Kim et al., 2015).

After obtaining the cold-pressed mixed beverage and centrifuged mixed beverage, some physical properties were discussed since these physical attributes are essential for UV irradiation (Ferrario and Guerrero, 2016). Turbidity, pH, and total soluble content (˚Brix) of juice samples were compared (Table 4.5). The pH of cold-pressed mixed beverages and centrifuged mixed beverages was not significantly different. The Brix value of centrifuged mixed beverage was higher than that of cold-pressed mixed beverage. However, the difference between them was not increased. To obtain the cause of the difference, the Brix value of juices may be determined individually. Kim et al. (2015) reported that the Brix value of cold-pressed tomato juice was higher than that of centrifuged tomato juice. Also, they revealed that the extraction method did not affect the pH of tomato juices which is similar to the present study.

Table 4.5. Comparison of physical-chemical and optical properties of CPJ and CDJ fruit juice

Quality attribute		**CPJ**	**CDJ**
Color	L	30.26 ± 0.1^{a}	27.25 ± 0.04^{b}
	a*	-3.94 ± 0.05^{b}	-2.65 ± 0.03^{a}
	b*	8.60 ± 0.15^{a}	6.26 ± 0.08^{b}
Browning index (BI)		22.17 ± 0.62^{a}	17.84 ± 0.37^{b}
Turbidity (NTU)		1621.5 ± 2.12^{a}	756 ± 15.56^{b}
pH		3.77 ± 0.01^{a}	3.75 ± 0.01^{a}
Brix (%)		13.15 ± 0.07^{b}	13.85 ± 0.07^{a}

Results were presented as "means ± standard error". Least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p < 0.05$). Abbreviations: NTU (Nephelometric Turbidity Unit).

The L* value of CPJ fruit juice was higher than CDJ fruit juice, which means CPJ fruit juice's lightness was higher. However, the browning index of CDJ fruit juice was lower than that of CPJ fruit juice. That difference may be caused by the a* and b* values. These values could indicate the existence of browning elements (Baykuş et al., 2021). a* value of CDJ fruit juice was higher, showing the browning. However, the b*value of CPJ fruit juice was higher, which could be the reason for the higher browning index. The higher browning index may be due to higher enzyme activity and a higher percentage of suspended particles resulting from more excellent soluble fiber

content. In particular, green apple juice (35%), which was squeezed with CPJ and then added to the juice mix, contributed to higher suspended particles in the resulting product. On the other hand, the a* value of CDJ fruit juice was higher than the CPJ fruit juice. This is probably due to the heat the juice was exposed to during squeezing in the CDJ method. This exposure probably caused a higher a* value. Since air bubbles were formed by the CDJ method, the contact with air probably increased and accelerated the juice's oxidation. This oxidation caused a lower L* value and higher a* deal for CDJ (Kim et al., 2015). Also, a higher b* value indicates more yellowness for CPJ. Pineapple that was used in the cold-pressed mixed beverage probably increased the yellowness. CPJ method could maintain the cell structure and prevent forming separated layers. Thus, more homogenous, and brilliant colors could be observed by CPJ (Kim et al., 2015, Wang et al., 2021). Furthermore, a higher b* value caused higher BI for the CPJ method, while the L* value of CDJ was lower than that of the CPJ method.

Total phenolic content and total antioxidant activity of cold-pressed mixed beverage and centrifugal mixed beverage were compared. The beverage, extracted by cold-press and centrifugal juicer, was rich in bioactive substances. Pineapple exhibits high levels of phenolic compounds and vitamin C. Also, carotenoid content is responsible for about 35% of the total pigments of the fruit (Ferreira et al., 2016, Freitas et al., 2014). Green apple is a good source of polyphenols, procyanidins, and quercetin glycosides, showing solid antioxidant activity (Rembialkowska et al., 2017). Kiwi fruit also contains a significant level of ascorbic acid which acts as an antioxidant (Cassano et al., 2007). However, the juicing method could affect the number of bioactive components. Thus, cold-pressing, and centrifugal extraction were discussed from the amount of total phenolic content and total antioxidant capacity.

The results shown in Figure 4.4 represent the total antioxidant capacity and the phenolic content of both CPJ and CDJ. Antioxidant activity of CDJ and CPJ were slightly different them each other. However, the difference was not significant. Similarly, Khaksar et al. (2019) reported no significant differences between different types of extraction methods in terms of the bioactive components. Contrastingly, Kim et al. (2017) obtained significantly higher antioxidant activity of grape juice for cold-pressed grape juice than extracted by centrifugal juicer. Also, Wang et al. (2021) reported the most increased DPPH activity for cold-pressed green kale juice among the cold-pressing, centrifugal juicer, and blender. In another study, the DPPH activity of tomato juice was obtained for extraction from a cold-pressing and centrifugal juicer.

Tomato juice extracted from cold-pressing was significantly higher than from centrifugal juicer (Kim et al., 2015). However, an important factor should be considered to make a good discussion. Processing time is vital since the heat generation by centrifugal juicer increases over time. In this study, a low amount of fruit was extracted, and the extraction time was short (approximately 20 to 30s). If the extraction time were longer, probably more heat generation would be observed, which could be a reason for the lower antioxidant capacity for mixed beverages extracted from centrifugal juicers (Khaksar et al. 2015).

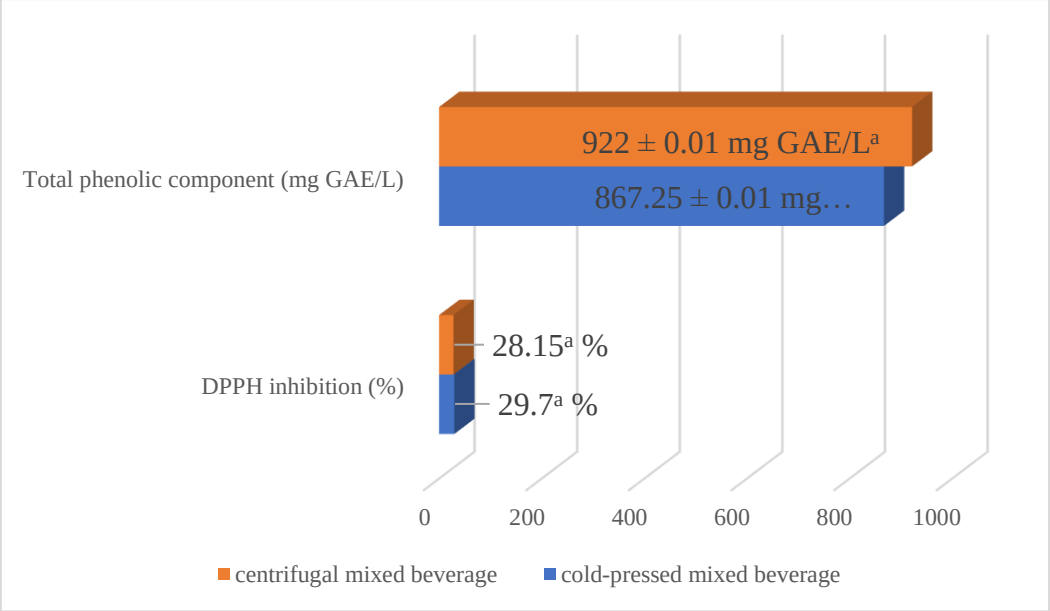

Figure 4.5. Phytochemical properties of cold-pressed mixed beverage and centrifugal mixed beverage.

The total phenolic content (TPC) of CPJ was significantly lower than that of the centrifugal mixed beverage (Figure 4.4). It was probably caused by the extraction method. Cell components are discharged in the centrifugal extraction method due to cell wall destruction (Kim et al., 2015). Since the amount of TPC was obtained immediately after the extraction, the measurement was probably performed before a high oxidation level of discharged bioactive substances from the cell. However, the TPC of cold-pressed grape juice and tomato juice was higher than that extracted by a centrifugal juicer (Kim et al., 2015; Kim et al., 2017). On the other hand, Wang et al. (2021) compared the TPC level of some fruits and vegetables extracted from cold-pressing and

centrifugal juicers. They reported that the highest TPC level was changed depending on the type of fruit and vegetable. The difference between the present study and the literature is probably caused by different reactions of fruits to extraction techniques.

Overall, the antioxidant capacity of CPJ and CDJ did not differ from each other, while the TPC amount was significantly higher for CDJ. The equality of antioxidant capacity was probably obtained by the amount of different bioactive components than polyphenols. It could be the ascorbic acid content of CPJ higher than CDJ. Since the ascorbic acid also acts as an antioxidant, the capacity of antioxidant activity of CPJ was enhanced by this bioactive component. In this study, the ascorbic acid content of CDJ and CPJ was not obtained. However, studies show that the ascorbic acid content of fruit and vegetable juices extracted from cold-press was higher than those extracted from centrifugal juicers (Kim et al., 2015; Kim et al., 2017; Wang et al., 2021). The heat generation probably causes that difference by the centrifugal juicer. In this study, probably the heat is generated by a centrifugal juicer during extraction, although the processing time was short. Since the ascorbic acid is more sensitive than the phenolic compounds, generated heat had a degradation effect on ascorbic acid that CDJ contains more than those phenolic compounds (Mrad et al., 2012, Volf et al., 2014, Sripakdee et al., 2015). Consequently, the antioxidant capacity of CPJ and CDJ was obtained as not significantly different from each other, probably caused by this reason.

4.5. Pasteurization of Cold Pressed Juice

4.5.1. The Effect of Different Configurations of UV-LEDs on the Quality of Cold Pressed Juice

To investigate the usability of UV-LEDs in the pasteurization of the formulated cold-pressed juice, UV-LEDs radiating at 280nm and 365nm in different configurations were used. Cold-pressed juice was exposed to UV lights simultaneously and sequentially with UV-LED lamps emitting at different wavelengths. *E.coli* K12 adapted to pH 3.4 was used to measure UV-LEDs' effectiveness.

4.5.1.1. Simultaneous Application

The inactivation effects of two different wavelengths, including 280 nm and 280/365 nm, on *E.co*li K12 in cold-pressed juice for 40 min were investigated using a static benchtop UV-LEDs system. Simultaneous application is handled by four identical lamps that exposing at 280 nm. Also, exposure at 280 nm with 2 lamps and at 365 nm with 2 lamps were used to investigate the effects of the wavelength combination. Cold-pressed juice was pasteurized as described in 3.4.3 before disclosing UV-LEDs. The achieved logarithmic reductions of *E.coli* K12 in cold-pressed juice samples exposed to UV-LEDs at different wavelengths and treatment times are presented in Table 4.7. Higher inactivation was obtained by 280/365 nm wavelength combination (3.34 ± 0.097 $\log_{10}$ CFU/ml). The results show that 365 nm UV-LED application had synergistic effect on the inactivation. Although the sensitivity of *E.coli* K12 was less than that of 280 nm irradiation, 365 nm contributes the inactivation applied simultaneously as 280/365 nm (Baykuş et al., 2021). The wavelength at 365 nm is UV-A irradiation, whose penetration property is better than UV-B and UV-C bands (Hinds et al., 2019). However, the inactivation potential of UV-A irradiation is not found to be significant when solely applied. To inactivate the bacterium, UV-C irradiation must be absorbed by DNA, causing the formation of pyrimidine dimers that inhibit the transcription and replication of the cell (Akgün and Ünlütürk, 2017; Gayan et al., 2014, Hinds et al., 2020). Germicidal wavelengths are found in the UV-C band that can cause cell damage. Because the peak absorption of UV-light by DNA occurs between 200 - 280 nm (Hinds et al., 2019, Kaya et al., 2017; Koutchma et al., 2010), on the other hand, the peak absorbance of aromatic proteins such as tryptophan and tyrosine occur at 280 nm that support this wavelength to be absorbed more efficiently by the cell (Hinds et al., 2021). UV-A irradiation contributes to the inactivation of the cell via photosensitizing reactions. These reactions cause occurring reactive oxygen species (ROS), singlet oxygen (O_2^-), and hydrogen peroxide (H_2O_2). These materials damage the cell membrane and other cell components. This inhibits protein synthesis and contributes to cell inactivation (Chatterly and Linden, 2010).

On the other hand, direct damage of DNA by UVC rays could be correctable by DNA-repair enzymes. This repair could be by dark repair or photo-reactivation (Oguma et al.,

2001; Song et al., 2016). These mechanisms are based on the reactivation enzymes produced by the cell. That means DNA repair might be prevented by damaging reactivation enzymes. The absorption spectrum of proteins peaks at 280 nm, which helps to destroy the repair enzymes and avoid DNA repair (Song et al., 2016). Akgün and Unluturk (2017) investigated the PPO enzyme inactivation by UV-LEDs. They revealed that UV-LED irradiation coupling UVA and UVC rays showed better inactivation on PPO enzymes. Probably higher penetration property of UVA rays contributes to the enzyme inactivation. Also, substances resulting from UV-A irradiation likely damage the reactivation enzymes that contribute to the higher inactivation of *E.coli* K12 (Akgün and Unluturk, 2017).

Table 4.6. Achieved inactivation by treatments

	Parameters	**Inactivation (log_{10} CFU/ml)**
Simultaneous Applications	280/365 nm simultaneously for 40min	3.34 ± 0.097^{a}
	280nm for 40min (with 4 Lamp)	2.78 ± 0.080^{b}
Sequential Configuration Applications	280nm for 20min followed by 365nm 20min	1.51 ± 0.086^{a}
	365nm for 20min followed by 280nm 20min	1.00 ± 0.205^{b}
	280nm for 30min followed by 365nm for 30min	1.51 ± 0.046^{a}

Results were presented as "means ± standard error". Least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter in their category are significantly different ($p < 0.05$).

Simultaneous application of 280 nm for 40 min (63.09 mJ/cm^2) inactivated *E.coli* K12 cells by 2.78 ± 0.080 log_{10} CFU/ml which was lower than coupled application (280/365 nm). Hinds et al. (2021) studied the inactivation of *Bacillus subtilis* on black peppercorns by UV-LEDs at 280 nm for 20 min exposure. They also applied 300 nm and 365 nm wavelengths without coupling them. The maximum inactivation (0.98 log_{10} CFU/ml) was reached with the application of 280 nm wavelength for 20 min. Similarly, Hinds et al. (2020) studied the inactivation of *B.subtilis* on model food systems. 285

nm, 365 nm, 405 nm, and 285/365 nm applications were evaluated, and complete inactivation (6.82 ± 0.09 $\log_{10}$ CFU/ml) was observed for 285 nm and 285/365 nm for 5 min and 10 min applications. Also, no significant reduction was observed at 365 nm. As these studies also revealed, 365 nm application might contribute to the inactivation of bacteria by different mechanisms.

Table 4.7. UV dosages of treatments

Treatments	UV intensity (mW/cm^2)	Time (min)	UV Dose (mj/cm^2)
280 nm (4 Lamps)	0,026	40	63.09
280 nm (2 Lamps)	0,045	20	54.49
365 nm (2 Lamps)	0,051	20	60.96
280 nm (2 Lamps)	0,045	30	81.74
365 nm (2 Lamps)	0,051	30	91.43

4.5.1.2. Sequential Application

Sequential application of UV-A and UV-C rays were examined to investigate the effect of UV-A on inactivation. As to sequential application, 280 nm followed by 365 nm and 365 nm followed by 280 nm parameters. Exposure times were given in Table 4.7. and related UV dosages were presented in Table 4.8. Total UV dosages for sequential application could not be calculated, but separate applications were established. For *E.coli* K12 inactivation, UV dose at 280 nm for 20 min (2 Lamps) was 54.49 mj/cm^2 and at 365 nm for 30 min was 60.69 mj/cm^2. Note that the investigation was performed to establish the effects of UV-A rays when applied before and after UV-C irradiation instead of the simultaneous application. Therefore, the treatments that are presented in Table 4.7. was performed in that manner. Simultaneous or sequential applications cause additive effects when UVB and UVC rays are used together. The method of application does not change the additive effect. This situation is named a

fundamental mechanism of UV disinfection and the Second Law of Photochemistry (Song et al., 2019). The Second Law of Photochemistry states that "for each photon of light absorbed by a chemical system, only one molecule is activated for a photochemical reaction" (Bolton and Cotton, 2008). Thus, an activated molecule by a photon could not be activated again, and each molecule is activated by a photon independently. Mechanisms of UV-B and UV-C irradiation inactivation are the same, formation of pyrimidine dimers. In that situation, a photochemical reaction damages DNA or RNA by causing the formation of pyrimidine dimers. Finally, inactivation was observed for exposed cells. Eventually, UV-B and UV-C rays induce the same photochemical reaction, which causes only an additive effect instead of any synergistic effect on the inactivation of the cell (Song et al., 2019). For that reason, the impact of UV-A irradiation before and after UV-C irradiation was investigated and UV-LED lamps emitting at 280 nm and 365 nm were used since the inactivation mechanism of and induced reactions by UV-A is different than UV-C rays.

Sequential application of 280 nm for 20 min followed by 365 nm for 20 min inactivate 1.51 ± 0.086 $\log_{10}$ CFU/ml *E.coli* K12. Reversing the sequence to apply 365 nm first and then 280 nm exposure for 20 min cause 1.00 ± 0.205 $\log_{10}$ CFU/ml inactivation. Results show that the sequence of application was important for inactivation performance. UV-C application after UV-A established lower inactivation than that of the reverse situation. Song et al. (2019) applied UV-A and UV-C rays sequentially for the 40s, and they revealed that the application of UV-A before UV-C rays caused a continuous decrease in the number of *E.*coli. Otherwise, *E.coli* counts recovered, and inactivation performance was decreased. They claimed that when UV-A irradiation was applied after UV-C rays, recovery of *E.coli* was observed, and inactivation performance was reduced. However, in that study, exposure times were longer (20 min). Although any *E.coli* K12 was recovered at starting, photosensitizing reactions caused by UVA irradiation started after a while. Consequently, reactive oxygen species and other chemical elements that contribute to the inactivation of *E.coli* occurred (Charletty and Linden, 2010). The application of UVA irradiation after UV-C irradiation increased the inactivation due to the longer treatment time. On the other hand, cold-pressed juice consists of many ingredients and complex chemicals such as nutrients. Because of that, UVA irradiation (Photosensitization) reactions with natural organic matters might be a reason for increasing inactivation after UV-C ray irradiation (Song et al., 2019). Application of 280 nm for 30 min followed by 365 nm for 30 min

was also investigated. 1.51 ± 0.046 log_{10} CFU/ml inactivation on *E.coli* K21 was observed, which is not significantly different from the 20 min application. Probably the application times should be longer than 40 min to obtain higher inactivation of *E.coli* K12 (Akgün and Unluturk 2017; Baykuş et al., 2020).

4.5.2. The Effect of UV-LED Treatment on Natural Flora of Cold Pressed Juice

Cold-pressed juice was exposed to UV-LEDs to examine the effect on natural flora. UV-LEDs irradiated cold-pressed juice at 280/365 nm for 20, 40, and 60 min since the inactivation of *E.coli* K12 was highest at 280/365nm application. Physical properties of cold-pressed mixed beverage that was used in this part of the study was shown in Table 4.3. The number of total aerobic mesophilic bacteria count (TAC) and yeast and molds (YM) was obtained before and after the UV-LED application to establish the log_{10} CFU/ml reduction after treatment. Results are shown in Table 4.9. The highest decrease was obtained at 280/365 nm for 60 min application for both TAC and YM (1.168 ± 0.018 and 1.188 ± 0.007). The number of TAC and YM reductions increased with increasing exposure time. Similarly, Hakguder et al. (2013) indicated that the UV exposure time resulted in lower log survival numbers of TAC and YM. The reduction in TAC and YM was lower than that of *E.coli* K12. It is probably caused by the difference in the cell structure of bacteria and yeast, and molds. It is known that yeast and molds are more resistant to UV irradiation than bacteria. They have larger sizes, thicker cell walls, and lower content of thymine or cytosine basis in their genomes (Tran and Farid, 2004). Therefore, bacteria are more likely to be inactivated since UV passage is more accessible due to the cell structure. On the other hand, bacteria have different cell wall constructions. A higher level of pyrimidine in DNA increases the possibility of cross-linkage of neighboring thymine and cytosine (Torkamani and Niakousari, 2011). In conclusion, natural flora was more resistant to UV light which caused a lower reduction than inoculated microorganisms (Linden and Darby, 1998; Baykuş et al., 2021)

Table 4.8. Number of reductions on natural flora of cold-pressed mixed beverage

UV-LEDs at 280/365nm wavelength combination			
	20min	40min	60min
TAC	0.165 ± 0.168^{c}	0.784 ± 0.082^{b}	1.168 ± 0.018^{a}
YMC	0.412 ± 0.047^{a}	0.718 ± 0.112^{a}	1.188 ± 0.007^{a}

Results were presented as "means ± standard error". Least significant difference was determined by Tukey pairwise comparison test. Means that do not share the same letter are significantly different ($p < 0.05$).

4.5.3. Hurdle Technology for Cold Pressed Juice Pasteurization

Hurdle technology is a set of methods used to increase microorganisms' inactivation (Putnik et al., 2020). All hurdles are not used together, or all hurdles are not combined. In this study, a cold-pressed juice sample was filtered before exposing UV-LEDs to retain the bacteria that might be adsorbed on particles of cold-pressed juice. At this moment, decreasing the initial microbial load of the cold-pressed juice sample was aimed. In another case, freshly squeezed ginger juice was added as a hurdle to increase the reduction of *E.coli* K12 prior to filtration and UV-LED treatments. Ginger is known as a natural antimicrobial (Güceyü et al., 2019). Thus, it was used in this study as a hurdle to increase the reduction on *E.coli* K12. Then, again cold-pressed mixed beverage was filtered before exposure to UV-LEDs. In that part of this study, effects of pre-filtration before exposure to UV-LEDs and antimicrobial effect of ginger and its addition effect on reduction was discussed.

The turbidity of cold-pressed mixed beverage used in this part of study was 2212.5 NTU. The value was different than those used in previous experiment. This is caused by the fruits were purchased from the market at different time intervals. Therefore, climate and different seasons cause a difference on the composition of fruits. After filtration of cold-pressed mixed beverage sample, 0.72 ± 0.88 $\log_{10}$ CFU/ml *E.coli* K12 was retained. Probably the number of adsorbed bacteria on particles of cold-pressed juice was not homogenized although equal mixing process. Therefore, the retention amount could not be standardized. Hou et al. (2021) applied three-stage ultra-filtration in another study. After that, total aerobic bacteria were decreased by 3.96 $\log_{10}$ CFU/ml, and the mold and yeast were completely inactivated. Carneiro et al. (2002)

treated pineapple juice with an organic filter with a 0.3 µm pore size membrane, and they showed that total aerobic bacteria, yeast, and molds were all below 1 $\log_{10}$ CFU/ml. Zhao et al. (2016) treated korla pear juice by ultrafiltration (pore size 0.05 µm), and ultrafiltration retained microorganisms with 3.05 and 3.36 $\log_{10}$ CFU/ml reductions of total aerobic count and yeast and mold. In addition, Zhao et al. (2014) treated fresh apple juice by ultrafiltration (pore size 0.05 µm). They obtained the reductions in the numbers of total aerobic count and yeast and mold by 1.79±0.18 and 1.78±0.19 log cycles, respectively. In the present study, filters with a 0.45 µm pore size were used larger than ultrafiltration and microfiltration. To obtain a higher number reduction of bacteria, filters with smaller pore sizes should probably be used. Further, filtration is followed by UV-LED irradiation at 280/365 nm for 40 min since the highest inactivation was achieved at this processing condition before. 2.71 ± 0.45 $\log_{10}$ CFU/ml total inactivation was obtained on *E.coli* K12 after UV-LED treatment.

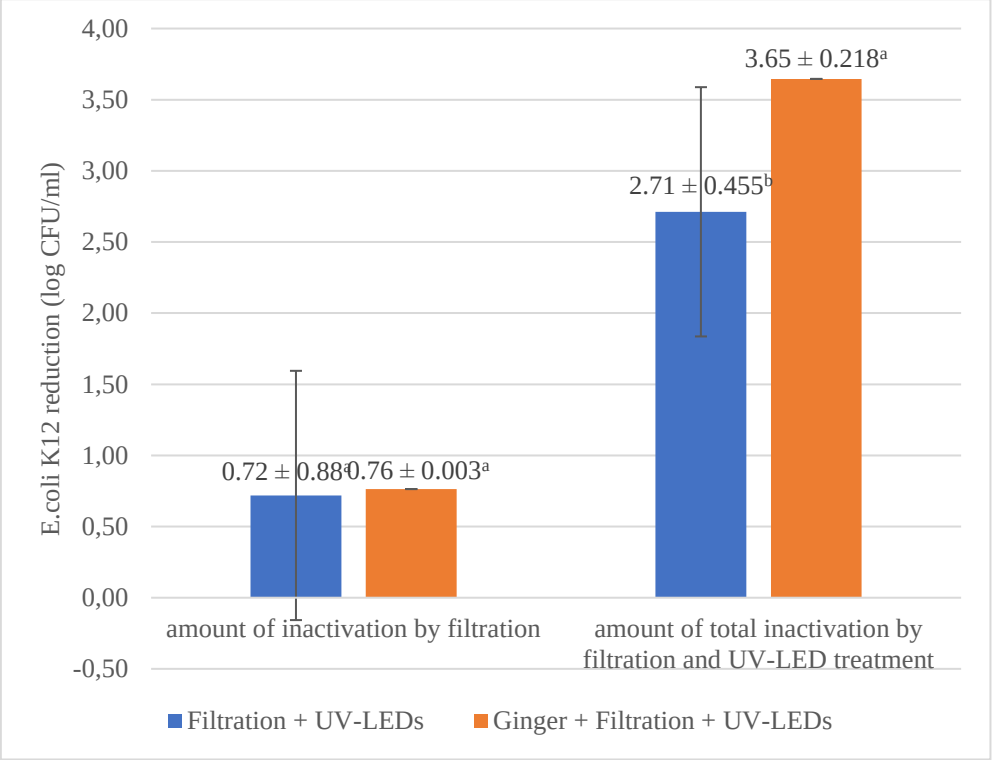

Figure 4.6. Amount of inactivation of *E.coli* K12 by hurdle treatments

Also, cold-pressed juice that freshly squeezed ginger juice added was treated with UV-LEDs at 280/365 nm for 40 min. Before UV-LED treatment, ginger added cold-pressed mixed beverage was filtered by a filter with a 0.45 µm pore size. After

filtration, 0.76 ± 0.003 log_{10} CFU/ml *E.coli* K12 was reduced. Following that, the filtered cold-pressed mixed beverage was exposed to UV-LEDs at 280/365 nm for 40 min. In that case, *E.coli* K12 was reduced by 3.65 ± 0.218 log_{10} CFU/ml by the addition of filtration. Inactivation of *E.coli* K12 was higher when freshly squeezed ginger juice was added. The reason for higher inactivation is probably caused by the antimicrobial effect of ginger (Riaz et al., 2015; Güceyü et al., 2019). Possibly, freshly squeezed ginger juice showed an additive effect on the inactivation of *E.coli* K12. By hurdle processing, Ferrario et al. (2020) treated the turbid fruit juices. They used UV-C plus antimicrobials (encapsulated citral and vanillin), and the addition of that antimicrobials showed an additive effect. They observed the change in log reductions on *E.coli* K12 during refrigerated storage at (4 ± 1 °C), and UV-C treated samples remained unaltered for almost seven days. In another study lemongrass oil was used as a hurdle to treat goat meat. They obtained the complete inactivation of *E.coli* K12 by using 1% lemongrass oil and UV-C with 0.2 mW/cm^2 hurdle treatment (Degala et al., 2018). These studies were similar to the present study by demonstrating the antimicrobial additive as a hurdle supported the inactivation of bacteria.

In this part of the study, two kinds of hurdle was investigated to increase the reduction of *E.coli* K12. When compared these two applications, antimicrobial additive showed higher inactivation ability than only filtration process. Although the number of retained bacteria by filtration of cold-pressed mixed beverage and ginger juice added cold-pressed mixed beverage was not significantly different, total inactivation was significantly higher for ginger added cold-pressed mixed beverage. Lastly, in order to achieve better inactivation results, probably different filter sizes should be used. Also, different dosages of antimicrobial additive (ginger) should be investigated.

CHAPTER 5

CONCLUSION

In this study, the quality parameters of the cold press squeezing method and the traditional centrifugal squeezing method were compared, and the usability of UV-LEDs for pasteurization of cold-pressed mixed beverage was evaluated. Microbial safety and product quality of the juice were evaluated after simultaneous application of UV-LED treatment with wavelengths of 280/365 nm and sequential application of 280 nm and 365 nm. In addition to UV-LED treatment, the inactivation efficiency of the target microorganism (*E.coli* K12) was investigated by using hurdle technologies such as filtering through a 0.45 micron filter and adding ginger juice to the formulation as a natural antimicrobial agent.

Physical, chemical and optical properties of cold-pressed mixed beverage (CPJ) and centrifugal mixed beverages (CDJ) such as pH, TSS (Brix°), color parameters, browning index (BI) and turbidity were determined. It was found that the color parameters (a*, b*, L*), BI, and turbidity of both beverages were significantly different. L* (lightness) of the CPJ was higher than that of CDJ. However, BI of the CPJ sample was higher when compared to that of the CDJ. This was probably the higher b* value that indicates the yellowness. Moreover, a* value that indicates the presence of browning elements was lower for CPJ. Also, the juice yield of two different techniques for the juices extracted from pineapple, green apple and kiwi was assessed. The CPJ method yielded a higher amount of juice than the CDJ, especially the pineapple juice obtained by the CPJ method was quite high. Besides, there was no phase separation and air bubbles observed after the extraction with CPJ method, and the color of the juices extracted by CPJ was quite good. It was concluded that the physical properties of juices extracted by CPJ were more satisfactory than those of CDJ.

As the consumer demands healthy and nutritionally rich juices, it is important to determine the nutritional value of the extracted juices. For that, antioxidant activity and the amount of total phenolic content (TPC) of CPJ and CDJ were determined. TPC of CPJ was lower than that of CDJ while the antioxidant activity of CPJ and CDJ samples

were not significantly different. This could be reasoned by the ascorbic acid content of juice samples. Since the heat generation was assumed to CDJ during the extraction, ascorbic acid was degraded, and the antioxidant activity was equaled with CPJ in overall. However, further work on this assumption is required. Finally, it could be concluded that the antioxidant activity and the amount of TPC of CPJ was not superior to the CDJ.

The effect of different wavelengths of UV-LEDs on the quality of cold-pressed juice was evaluated. For this purpose, simultaneous application of UV-LEDs emitting light at 280 nm and 365 nm were used. The results indicated that UV-LED treatment at a wavelength combination of 280/365 nm for 40 min could inactivate a higher number of *E.coli* K12 cells than at a wavelength of 280 nm and the same exposure time. When using sequential applications of 280 nm and 365 nm, the highest reduction in *E.coli* K12 was achieved by applying a wavelength of 280 nm for 20 min followed by 365 nm for 20 min. However, the highest inactivation was achieved by simultaneous application of 280 nm and 365 nm for 40 min exposure time.

The effect of 280/365 nm combined wavelength application on natural flora of cold pressed juice was investigated. The highest reduction on total aerobic mesophilic bacteria count (TAC) was obtained with 60 min exposure time while the reduction of yeast and mold count (YMC) was not affected by exposure time.

Additionally, the effect of hurdles such as filtration and natural antimicrobial agent on microbial reductions were also evaluated. Freshly squeezed ginger juice was used as an antimicrobial additive and showed higher inactivation efficiency compared to filtration.

In summary, this thesis revealed the difference between the two different fruit juice extraction techniques: cold-press and centrifugal extraction. In conclusion, total antioxidant activity and TPC of juices obtained by two techniques was not different. However, the physical properties, especially homogeneity, yield and color of cold-pressed juices were found to be more satisfactory than the juices obtained by centrifugation. Based on this, pasteurization of the cold-pressed beverage was carried out with UV-LEDs, which have been shown to better preserve the nutritional content and other properties. Although 5 log reduction (legal limit required for juice pasteurization) of target microorganism was not achieved, the combination of 40 min exposure time and the addition of ginger juice with a wavelength of 280/365 nm was shown to provide the highest inactivation (3.67 log) in high turbidity mixed drinks. It

was revealed that the freshly squeezed ginger juice had an additive effect on *E.coli* K12 reduction. As a result, it has been demonstrated that there is a potential for the use of simultaneous UV-LEDs in non-thermal technology systems to be designed for cold press juice pasteurization in the future. However, since it has been shown that the antibacterial activity of UV-LEDs is not sufficient alone in turbid fruit juices, this technology needs to be combined with different barrier approaches. For example, by adding natural products such as ginger, which has antimicrobial activity, to cold press beverage formulations, both the taste and nutritional content will be enriched, and the microbial safety of the product can be ensured.

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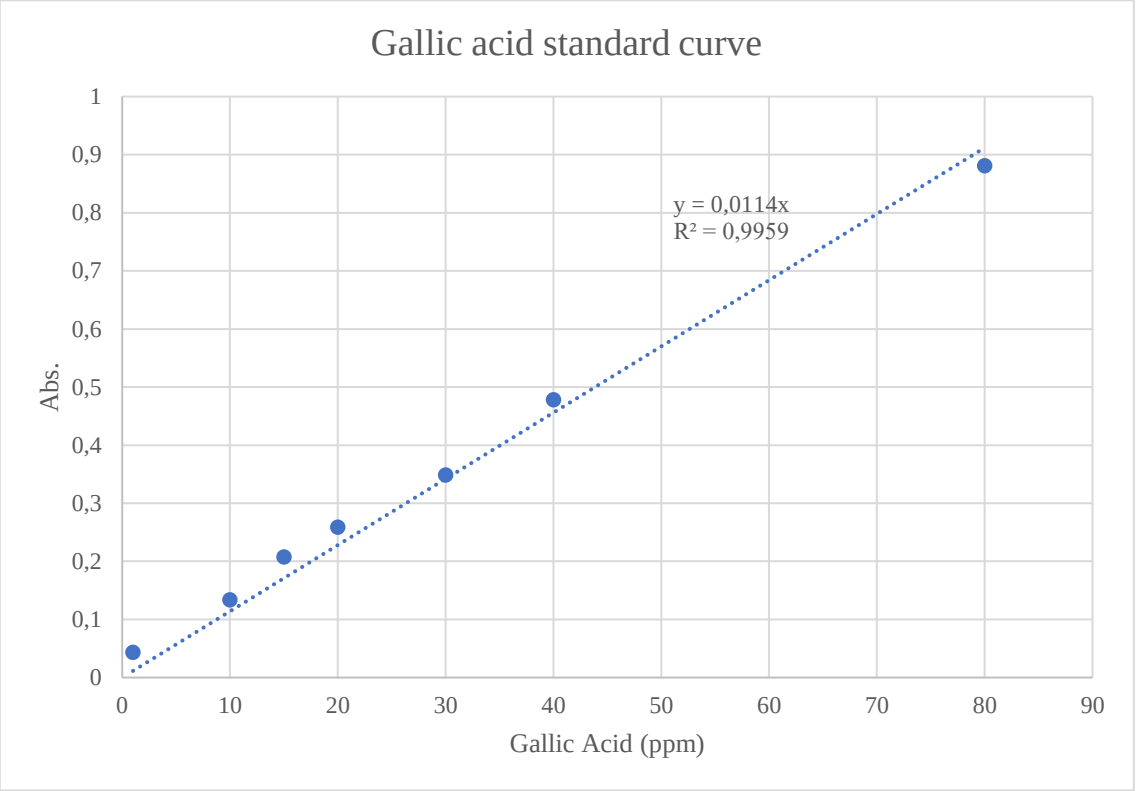
APPENDIX A

GALLIC ACID STANDARD CURVE



Appendix A. Gallic acid standard curve