

**INVESTIGATION OF THE EFFECT OF  
DIFFERENT PROCESSING TECHNIQUES ON THE  
OVERALL QUALITY AND SHELF LIFE OF  
LOCAL APRICOT VARIETY OF İĞDIR (*Prunus  
armeniaca* L., cv. Şalak)**

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**by  
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## ABSTRACT

### INVESTIGATION OF THE EFFECT OF DIFFERENT PROCESSING TECHNIQUES ON THE OVERALL QUALITY AND SHELF LIFE OF LOCAL APRICOT VARIETY OF İĞDIR (*Prunus armeniaca* L., cv. Şalak)

cv. Şalak apricot is a specific type of product with its unique size, shape and taste. However, its distribution and marketing is very limited due to its perishable nature and restricted shelf life. Since drying is not applicable for cv. Şalak apricot due to its low dry matter content, a novel method which would not alter the fruit quality and maintain the product safety was urged to be developed.

The objective of this thesis was to explore the effect of combined processing (mild heat (MH) treatment+UV-C irradiation+CaCl<sub>2</sub> dipping=MUC) and MAP on the shelf life of cv. Şalak apricots during cold storage. Characteristics of the samples and the effect of MUC on physicochemical properties, enzymatic activities and sensory were determined. At last, samples packed either with MAP or without MAP were stored at 1°C. Microbial quality and physicochemical properties were assessed throughout the storage.

Optimum treatment conditions were 19 min at 3.23 mW/cm<sup>2</sup> for UV-C irradiation; 6% of CaCl<sub>2</sub> at 40°C for 21 min for MH + CaCl<sub>2</sub> dipping. MUC treatment did not alter the physicochemical and sensorial properties, PPO and PME activities. After MUC, coliforms were completely inactivated whereas Total Aerobic Plate Count (TAPC) and Yeast and Mould Count (YMC) were reduced by 2.1-log and 2.67-log, respectively. Microbial shelf life of apricots could be extended from 3-5 days up to 14 days by MUC+MAP. Firmness was also maintained. But, MUC adversely affected the colour. On the other hand, MAP application alone was not sufficient to provide the microbial quality.

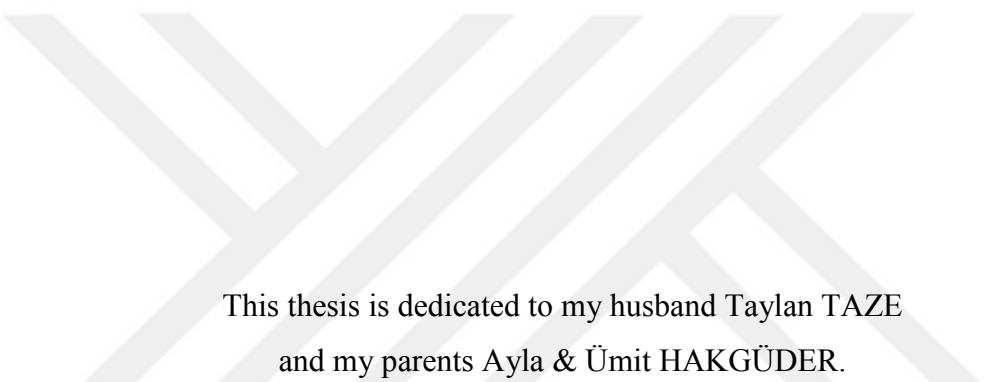
## ÖZET

### İĞDIR'IN YEREL KAYISI ÇEŞİDİNİN (*Prunus armeniaca* L., cv. Şalak) GENEL KALİTESİ VE RAF ÖMRÜ ÜZERİNE FARKLI İŞLEME TEKNİKLERİNİN ETKİSİNİN İNCELENMESİ

Şalak kayısı çeşidi kendine has boyutu, şekli ve tadı ile özel bir üründür. Ancak dayanıksız yapısı ve kısıtlı raf ömründen dolayı dağıtımı ve pazarlaması oldukça sınırlıdır. Düşük kuru madde içeriğinden ötürü kurutmaya elverişli olmadığından, meyve kalitesini değiştirmeyecek ve ürün güvenliğini sağlayacak yeni bir işleme metodu geliştirilmesi gerekmektedir.

Bu tezin amacı birleşik işleme yönteminin (Ilımlı Isıl (MH) işlem+UV-C radyasyon+CaCl<sub>2</sub> daldırma= MUC) ve MAP'in, soğuk depolama süresince Şalak kayısıların raf ömrü üzerine olan etkilerini ortaya koymaktır. Örneklerin karakteristik özellikleri ile MUC işleminin fizikokimyasal özellikler, enzim aktiviteleri ve duyu kalite üzerine etkileri belirlenmiştir. Son olarak, MUC ile işlenmiş örnekler ile hem MAP ile hem de MAP'siz paketlenen kontrol örnekleri 1°C'de saklanmıştır. Saklama süresince mikrobiyal kalite ve fizikokimyasal özellikler değerlendirilmiştir.

En uygun işlem koşulları UV-C için 19 dk, MH için 40°C'de 21 dk, CaCl<sub>2</sub> daldırma için %6 konsantrasyon, 40°C ve 21 dk'dır. MUC işlemi fizikokimyasal ve duyu özellikleri, PPO ve PME enzim aktivitelerini etkilememiştir. MUC'dan sonra başlangıç koliform yükü tamamen inaktive edilmiştir. Bunun yanında toplam canlı sayısı ve maya-küf sayısı sırasıyla 2,1-log ve 2,67-log azaltılmıştır. Kayıların mikrobiyal raf ömrü MUC+MAP işlemi ile 3-5 günden 14 güne kadar arttırılmıştır. Sertlik de korunmuştur. Ancak renk olumsuz etkilenmiştir. Öte yandan tek başına MAP uygulaması mikrobiyal kaliteyi sağlama açısından yeterli olmamıştır.



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# CHAPTER 1

## INTRODUCTION

Apricot (*Prunus armeniaca*) has a significant place in human diet in terms of its vitamin, mineral and phenolic content (Muradođlu et al., 2011; Haciseferođulları et al., 2007).  $\beta$ -carotene which is a precursor of Vitamin A and found in high amounts in apricots makes the fruit valuable for eye-health, bone and teeth development, epithelial tissues and endocrine glands (Haciseferođulları et al., 2007).

Turkey is the leading apricot producer in the world. It totally provides ~20% of the world's apricot and contributes to the 85% of the world's dried apricot production (Haciseferođulları et al., 2007; Akin et al., 2008; Aubert et al., 2010; Kaya et al., 2011; Muradođlu et al., 2011). One of the most important apricot production centres in Turkey is Iđdır province with its 17,782 tons of annual production (Ercisli, 2009; Muradođlu et al., 2011). There are five apricot varieties found in Iđdır province, namely Ađerik, řalak, Tebereze, Ordubat and Ađcanabat. Among the apricot types cv. řalak (*Prunus armeniaca* L., cv. řalak) is the most widely cultivated variety and followed by cv. Tebereze (Özyörük and Gülyüz, 1992; Ercisli, 2009; Kaya et al., 2011). 85% of the apricots grown in this area are cv. řalak variety which is specific to the region (Kaya et al., 2011; Muradođlu et al., 2011). However cv. řalak apricot has a short shelf life after harvesting due to its low dry matter yield and also its climacteric nature.

Traditional processing techniques, namely drying and freezing, are widely used to increase the postharvest shelf life of apricots. But drying is not always a suitable method especially for the food stuff containing low amount of dry substances. Hence fresh consumption was proposed for cv. řalak apricots (Özyörük and Gülyüz, 1992). Nevertheless alternative methods need to be used in order to increase the shelf life of this specific type.

Modern techniques such as UV-C light treatment, edible film coating, modified atmosphere packaging (MAP), microwave treatment, high pressure processing (HPP) and pulsed electric field (PEF) were reported to be applied to many fruits and vegetables to improve the postharvest quality and to prolong the time for marketing (Barret and Lloyd, 2012; Aguayo et al., 2006; Ansorena et al., 2011; Chen et al., 2011;

Yun et al., 2013; Valero et al., 2013; Ramos et al., 2013). Moreover, use of mild heat treatments (35-60°C), addition of CaCl<sub>2</sub> to the dipping solution were also reported in the literature (Abreu et al., 2003; Beirao-da Costa et al., 2006; Beirao-da Costa et al., 2008; Klaiber et al., 2005; Kou et al., 2007; Lara et al., 2006; Lamikanra and Watson, 2007; Rahman et al., 2011). Each method has a particular effect on different quality parameters. For instance, UV-C light treatment aims to decrease the microbial load and hence extends the microbial shelf life of the foods whereas CaCl<sub>2</sub> dipping increases the firmness of the fruit tissue. Mild heat (MH) treatment was also found to reduce the microbial load and prevent fruit decay. Additionally it enhances the tissue firmness via activating PME enzyme and reduces the weight loss during storage. On the other hand, modified atmosphere packaging (MAP) was showed to be effective in preventing decay, delaying ripening and quality maintenance. Furthermore, softening and water loss can be reduced by MAP application.

Besides, there are some advantages and disadvantages of each technique. For example, UV-C light irradiation is advantageous since it is a physical treatment and does not produce toxic products. Furthermore, the cost of the equipment is favourable and it is easy to use. But, penetration of the light is limited and its application in postharvest handling is still restricted. With regards to MH treatment, ease of use, short treatment times and possibility to easily monitor the operation are superior features of the application. However, it may destroy the sensorial quality of the product if applied under harsh conditions. CaCl<sub>2</sub> dipping is a chemical method which enhances fruit firmness. On the other hand, it may alter the flavour of the fresh produce. Although MAP treatment is an effective way to prevent fruit deterioration and to maintain the quality, use of unsuitable levels of CO<sub>2</sub> in the package would result in growth of pathogenic microorganisms. Moreover, the selection of appropriate packaging material is another issue to be handled carefully. It should be also considered that single MAP treatment may not be sufficient to obtain desired characteristics during the storage of the fruits. Therefore combination of various methods is proposed. By this way, one can avoid any intense effects of a single treatment.

In literature there are many studies considering the postharvest quality of strawberry, broccoli, carrot, pepper, kiwifruit, mango, pear, plum, pumpkin, tomato etc. Different modern techniques were applied in order to increase the microbial and sensorial quality, shelf life, and delay ripening. Nevertheless a small number of studies performed using alternative processing methods are related to the postharvest quality of

fresh and dried apricots (Egea et al., 2007a; Egea et al., 2007b; Liu et al., 2009a; Hussain et al., 2011; Hussain et al., 2013; Yun et al., 2013; Hajilou and Fakhimrezaei, 2013; Wei et al., 2014; Yan et al., 2014). Conversely, no study about the quality improvement and shelf life extension of cv. Şalak apricot of Iğdır province is available in literature.

Therefore, the principal aim of this thesis was to develop a processing method based on a hurdle strategy to extend the shelf life of cv. Şalak apricots. For this purpose, the combination of different processing methods including MH treatment, UV-C irradiation and CaCl<sub>2</sub> dipping (MUC) and MAP application were investigated. Their effect on selected physicochemical properties, microbial and sensory quality of cv. Şalak apricots were monitored as well during 28 days of cold storage.

This thesis is composed of eight chapters. The first three chapters cover the introduction, literature review and information about different postharvest treatments used to enhance microbial safety and quality of cv. Şalak apricots. The fourth chapter summarizes some information about the material and methods used throughout this research.

Chapter 5 is related with the evaluation of the effects of UV-C irradiation and mild heating on natural microflora of apricot fruit surfaces. For UV-C irradiation studies two different bench-top systems were used to assess the microbial inactivation efficiency on apricot surfaces. Accordingly optimum treatment conditions were determined for UV-C irradiation process. In the case of MH treatment, different time and temperature combinations were evaluated in terms of colour, firmness and microbial reduction. Treatment conditions which gave the highest possible reduction in the number of naturally occurring microorganisms, on the other hand which would not alter the quality of the apricots were selected.

In Chapter 6, some physicochemical properties of the apricots were determined for sample characterization. Moreover, optimum treatment conditions for CaCl<sub>2</sub> dipping were defined considering the effect of the treatment on firmness value. Afterwards, all the treatments were combined at their optimum conditions. Influence of the application of combined (MUC) treatment on physicochemical and sensory properties, and enzymatic activities of cv. Şalak apricots were evaluated.

In the last part of the thesis (Chapter 7), the effect of MUC treatment and MAP application on the shelf life of cv. Şalak apricots was assessed. For this purpose, control samples were packed both with MAP (Control+MAP) and without MAP (Control-

MAP). Moreover MUC treated samples were stored after MAP application (MUC+MAP). In order to determine the shelf life, the microbial examination of the samples was performed for 28 days with 7-days intervals. Furthermore, physicochemical properties such as weight loss, pH value, Brix, titratable acidity, ripening index, firmness, colour, ascorbic acid content of the samples were also monitored throughout the storage.

Chapter 8 is the conclusion section where all the findings are summarized and additional comments are made for the future studies.



## CHAPTER 2

### LITERATURE REVIEW

#### 2.1. Apricot

Apricot (*Prunus armeniaca* L.) is a stone fruit classified under the *Prunus* species of the Rosaceae family (Hacıseferoğulları et al., 2007; Aubert et al., 2010; Ali et al., 2011). It was believed that apricot came from Armenia since its Latin name was *Armeniaca vulgaris* L. But it was grown in Armenia for a long time (Erdogan-Orhan and Kartal, 2011). Ercisli (2009) also claimed that apricot has been produced in Armenia since 1<sup>st</sup> century A.D. However, it was noted that like peach, apricot is native to China and cultivated over 5,000 years ago in a wide area including, Iran, Turkistan, Afghanistan and Middle Asia (Ercisli, 2009; Aubert et al., 2010). It was speculated that Alexander the Great brought apricot from Persia to Anatolia in 4<sup>th</sup> century BC. After that Anatolia turned into the second homeland for apricot (Ercisli, 2009). Introduction of the fruit into Europe occurred during the Roman period on 1<sup>st</sup> century B.C (Ercisli, 2009; Aubert et al., 2010). Thereafter, it was spread to America on 17<sup>th</sup> century (Ercisli, 2009).

Apricot tree was described as a hardy tree with an average height of 2-10 m (Erdogan-Orhan and Kartal, 2011). Harvest season for apricots starts from June and continues until the middle of August depending on the variety (Özyörük and Gülerüz, 1992; Erdogan-Orhan and Kartal, 2011).

Although apricots are most abundantly consumed as fresh, they are also utilized to produce other products such as jam, jelly, pulp, nectar, juice, dried and frozen apricots owing to its superior nutritional properties (Hacıseferoğulları et al., 2007; Malaslı et al., 2012; Milošević et al., 2014). Nutritional value of apricots, its production volume and apricot varieties found in Iğdır province were discussed in the next sections.

### 2.1.1. Nutritional Value and Health Effects

Sugars, organic acids, phenolic compounds and carotenoids were indicated to play important roles in determining nutritive value of fruits and vegetables (Akin et al., 2008). Apricot is a rich source of polyphenols, especially chlorogenic acid, minerals such as potassium and  $\beta$ -carotene which is a precursor to Vitamin A (Hacıseferoğulları et al., 2007; Bureau et al., 2009; Igual et al., 2012). Vitamin A is reputed to be necessary for the construction of epithelial tissues covering our bodies and organs, eye-health, bone and teeth development and functioning of endocrine glands. Furthermore, this substance is crucial for immune system and body growing functions (Hacıseferoğulları et al., 2007). Besides it was demonstrated that coloured fruits and vegetables were also rich in Vitamin C and folates (Akin et al., 2008).

These phytonutrients are essential for human on account of their antioxidant, antimicrobial, antiallergic properties and ability to prevent chronic diseases, especially certain cancer and eye diseases (Igual et al., 2012; Coşkun et al., 2013). Furthermore Katayama et al. (2011) explored that apricot fruit had the potential to prevent Alzheimer Disease (AD) by virtue of its carotenoid content. AD is characterized by the loss of mental abilities due to the continuous accumulation of amyloid fibrils in the specific areas of the brain. As a result, those specific areas which contribute cognitive functions such as memory and language, are impaired (Katayama et al., 2011). However, it was discovered that apricot carotenoids could exhibit anti-amyloidogenic and fibril-destabilizing effects in vitro. Apricots were designated as the great source of carotenoids (Bureau et al., 2009). Thus apricot looms large in human diet. Its low fat content, sufficient amount of sucrose, glucose and fructose, high levels of antioxidants (lycopene,  $\beta$ -carotene, Vitamin A and E) and minerals (K, P, Mg) are the superior properties of the fruit. Moreover, it is known that apricot has a good anti-anaemia property owing to its high iron (Fe) content (Muradoğlu et al., 2011; Hacıseferoğulları et al., 2007).

Apricot was also showed to have a pharmacological importance since it had antipyretic, antiseptic, emetic and ophthalmic properties along with a laxative effect (Ali et al., 2011).

## 2.1.2. Apricot Production in the World

Important species of *Prunus* genus were indicated to be drupes, i.e. stone fruits, such as apricots, peaches and plums (Bae et al., 2014). In 2013, 4 million tonnes of apricot production was recorded in the whole world (FAO, 2015). Turkey has the biggest share with its almost 811,000 tonnes of apricot production/year in 2013 (Figure 2.1).

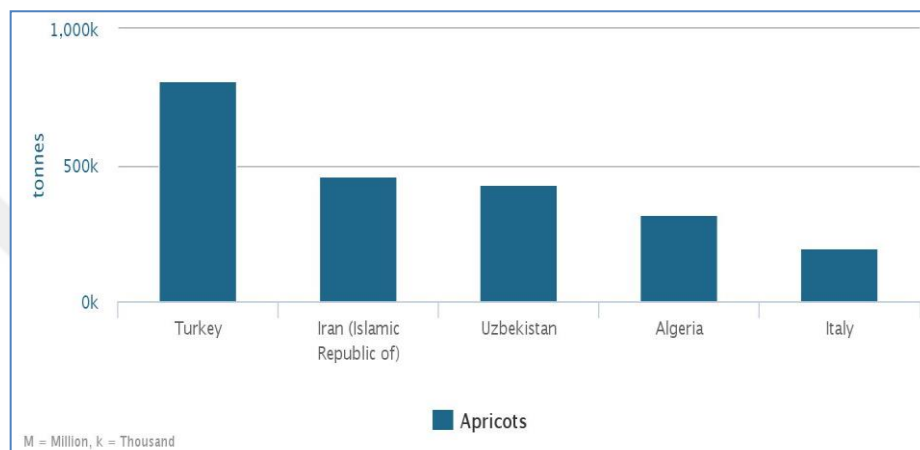


Figure 2.1. Apricot production of top five producers in 2013. (Source: FAO, 2015)

Other most important production centres were demonstrated to be Iran, Uzbekistan, Algeria and Italy (Figure 2.1) (Erdogan-Orhan and Kartal, 2011; Coşkun et al., 2013; Alagöz et al., 2015; FAO, 2015). Furthermore Pakistan was also stated to have an important contribution to the world production (Ali et al., 2011). With regards to Europe, it was speculated that apricot production was mainly took place in France, Greece and Spain except from Italy (Aubert et al., 2010).

## 2.1.3. Apricot Production in Turkey

Turkey is the world's first rate apricot producer with its approximately 685,000 tonnes of contribution to the annual production between years of 2005 and 2013 (FAO, 2015). Despite having fertile soil for the production of apricot throughout the whole country, half of the apricot production of Turkey was reported to occur in the Central Eastern Anatolia Region (Ercisli, 2009; Muradoğlu et al., 2011).



Turkey's most important apricot providing centres are Malatya, Erzincan, Aras valley (Iğdır-Kağızman), İçel (Mut), Elazığ, Sivas, Kahramanmaraş, Kayseri, Niğde, Hatay and Nevşehir provinces (Figure 2.2) (Alım and Kaya, 2005; Ercisli, 2009; Kaya et al., 2013). It was indicated that the first four provinces totally provided 70-75% of the apricot production of the country (Ercisli, 2009). Among them Malatya province takes a significant place in apricot cultivation, production and processing since 50% of the fresh and 90% of the dried apricots are provided from this region (Akin et al., 2008).

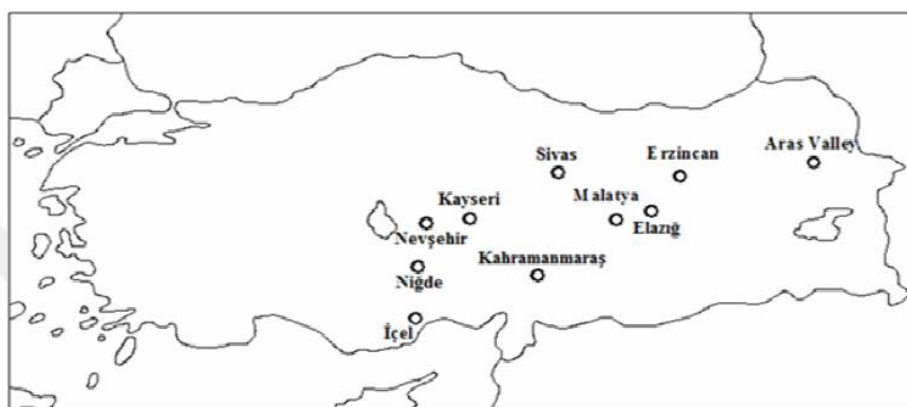


Figure 2.2. Most important apricot production regions in Turkey.  
(Source: Ercisli, 2009)

Besides, Aegean Region and Marmara Region were also encountered in the apricot production centres in Turkey (Topcu and Uzundumlu, 2010; Muradoğlu et al., 2011). However, more than 50% of the annual production was reported to be supplied from East Anatolian Region of the country (Topcu and Uzundumlu, 2010).

#### 2.1.4. Apricot Varieties in Iğdır

Iğdır is located in the east part of Turkey. This microclimate area has favourable conditions for agriculture. Among the fruits grown in this region apricot is the most important one with its 17782 tonnes of annual production volume (Kaya et al., 2011; Muradoğlu et al., 2011). Iğdır province was indicated to contribute to the Turkey's apricot production by almost 2% (Malaslı et al., 2012). Dry and hot summers and cold winters provide quite suitable conditions for apricot farming (Alım and Kaya, 2005).

The most often grown apricot cultivar was reported to be Şalak apricot (Apricos) (*Prunus armeniaca* L., cv. Şalak) which is specific to the region (Kaya et al., 2011).

Muradođlu et al., (2011) and Kaya et al. (2013) denoted that 85% of the apricots in Iđdir region was composed of řalak variety and the rest were Ordubat, Tebereze and Teyvent (Ađcanabat) cultivars.

Among the others, cv. řalak apricot was revealed to have superior characteristics in terms of productivity and fruit quality (Kaya et al., 2013).

## **2.2. Quality and Shelf Life of Apricots**

Sugar level, organic acid content, volatile compound profile, colour, shape, and texture of a fruit determine its sensory attributes (Defilippi et al., 2009). It is widely acknowledged that maturity stage of an apricot at the harvest time identified the fruit quality (Aubert et al., 2010). Since apricot is a climacteric fruit it has a high respiration rate and a rapid ripening process due to pronounced ethylene crisis (Cořkun et al., 2013; Egea et al., 2007b). Ethylene production in climacteric fruits is an auto-induced process (Munoz-Robredo et al., 2012). It was asserted that increases in the ethylene level occurred in a few days which resulted in over-ripened fruits (Pretel et al., 1999). Consequently, apricot fruit suffers from a rapid sensorial and nutritional quality loss once harvested. It was stated that apricots skipped into a degradation process just within 3-5 days after ripening. Similarly Hussain et al. (2011) speculated that shelf life of apricots was limited to 4-5 days under ambient conditions. This particularity brings about serious restrictions for the distribution and marketing of the fruit (Egea et al., 2007b).

In many cases, stone fruits are harvested in pre-climacteric stage where the products are resistant to the mechanical stress. Owing to the fact that apricots are sensitive to mechanical damage and water loss, they are also harvested at this stage (Defilippi et al., 2009). After harvesting fruits are stored under cold storage conditions in order to delay ripening. It was indicated that maximum commercial storage period of apricots was ranging from 2 to 3 weeks depending on the variety (Stanley et al., 2013). However, ripening process progresses rapidly leading to extremely softened and inedible fruit no sooner than the fruit is taken out of the storage (Stanley et al., 2013). Furthermore internal browning after 2-3 weeks of storage was also observed (Wu et al., 2015). Therefore, main factors create customer dissatisfaction were indicated to be the reduction in flavour and textural problems.

Aside from softening, chilling injury is another problem encountered for apricots stored below 7°C (Stanley et al., 2013). The symptoms are characterized as sandy texture due to loss of juiciness and gel breakdown (Stanley et al., 2013). Different preservation techniques, such as canning, freezing, drying and packing in controlled atmospheres, have been used to increase the quality and extend the shelf life of apricots (Iguar et al., 2012; Coşkun et al., 2013).

## **2.3. Preservation Methods**

Various preservation methods are used to extend the shelf life of fruits and vegetables. The main objective of food preservation is to maintain the food quality and safety. Thermal techniques commonly referred to “traditional” methods are the most widely used forms of food preservation. Other techniques include modern physical technologies, application of various chemicals, biological methods and hurdle technology.

### **2.3.1. Traditional Techniques**

Traditional preservation methods are dehydration, fermentation, freezing, refrigeration and thermal treatments. Dehydration and fermentation have been used for centuries. On the other hand, thermal treatments and freezing are relatively newer technologies which were developed in the 20<sup>th</sup> century (Barrett and Lloyd, 2012). Thermal processing is known to effectively reduce the microbial load and inactivate the enzymes which decrease the quality of the products whereas freezing shows its preservation effect by reducing the water activity (Barrett and Lloyd, 2012; Aguilar-Rosas et al., 2007). However, freezing and thawing, together were indicated to cause loss of nutrients, colour, flavour and texture problems by destroying the plant tissue and cellular integrity (Barrett and Lloyd, 2012). Similarly, thermal drying and blanching was also pointed out to be destructive in terms of ascorbic acid, polyphenols, carotenoids and anthocyanins (Rawson et al., 2011).

Although thermal treatments, namely blanching, pasteurization, sterilization and thermal drying, are mostly used processing technologies, they can lead to loss of bioactive compounds, colour and flavour defects (Rawson et al., 2011; Choi and

Nielsen, 2004). Therefore a demand for minimal processing technologies has been arisen (Senorans et al., 2003). As a consequence novel food processing techniques have been established in the past decade.

### **2.3.2. Non-Conventional Techniques**

There are a number of chemical, physical and biological methods to improve the quality and safety of fresh fruits and vegetables. Use of microorganisms and/or their metabolites for this purpose is known as biopreservation (Ramos et al., 2013). Chemical methods include the use of a variety of chemicals namely, chlorine, chlorine dioxide, bromine, iodine, trisodium phosphate, quaternary ammonium compounds, organic acids, calcium-based solutions, ozone, hydrogen peroxide, sulphur dioxide, etc. Some advantages and limitations resulting from the use of chemicals are given in Table 2.1.

Physical methods are also known as minimal food processing techniques. Consumers' increased trend towards healthy, fresh-like and easy to prepare products directed research efforts at these processing techniques. Some of these physical methods include UV radiation, ionizing radiation, pulsed light, pulsed electric field (PEF), high hydrostatic pressure (HHP), cold plasma, ultrasound and ozone processing, and novel packaging practices (Rawson et al., 2011; Ramos et al., 2013). Table 2.2 indicates the advantages and limitations of these methods.

Besides these all, hurdle technology or combined methods have been also used to improve microbial inactivation efficiency and better preserve the quality. One can avoid the adverse impacts of a single technology applied at severe conditions by utilizing combined methods consisting of the application of mild treatments (Senorans et al., 2003).

Table 2.1. Advantages and Limitations of Different Chemical Methods.  
(Source: Ramos et al., 2013)

Methods	Advantages	Limitations
Chlorine	<ul style="list-style-type: none"> <li>• Low cost</li> <li>• Easily available</li> </ul>	<ul style="list-style-type: none"> <li>• Corrosive and liberation of chlorine vapours</li> <li>• Activity affected by pH , temperature, light, air</li> </ul>
Bromine	<ul style="list-style-type: none"> <li>• Possible synergy with chlorine</li> </ul>	<ul style="list-style-type: none"> <li>• Unknown by-products and potential health effects</li> </ul>
Iodine	<ul style="list-style-type: none"> <li>• Less corrosive at low temperature</li> <li>• Broad spectrum</li> </ul>	<ul style="list-style-type: none"> <li>• Corrosive above 50°C and stains commodities</li> <li>• No direct contact use</li> </ul>
Organic acids (Lactic, citric, acetic, tartaric, ascorbic acid)	<ul style="list-style-type: none"> <li>• Easy to use</li> <li>• Economical</li> <li>• No toxicity</li> <li>• Allowed for organic compounds</li> </ul>	<ul style="list-style-type: none"> <li>• Affects sensory quality</li> <li>• Lower antimicrobial efficacy</li> <li>• Low pH use only</li> <li>• Impact on waste water quality</li> </ul>
Hydrogen peroxide	<ul style="list-style-type: none"> <li>• No residue production</li> <li>• Not corrosive at permitted levels</li> </ul>	<ul style="list-style-type: none"> <li>• Low antimicrobial effect at permitted levels</li> <li>• Negative impact on overall quality</li> </ul>
Calcium based solutions	<ul style="list-style-type: none"> <li>• Increase the calcium content of the product</li> <li>• Delays ripening of fruit and vegetables</li> <li>• Reduces decay</li> </ul>	<ul style="list-style-type: none"> <li>• Bitterness and off-flavours associated with CaCl<sub>2</sub></li> </ul>
Ozone	<ul style="list-style-type: none"> <li>• High antimicrobial activity</li> <li>• Extends shelf life</li> </ul>	<ul style="list-style-type: none"> <li>• Deterioration of flavour and colour</li> <li>• Toxic, reactive and corrosive</li> </ul>
Sulphur dioxide	<ul style="list-style-type: none"> <li>• Inhibits microbial growth</li> <li>• Antioxidant property</li> </ul>	<ul style="list-style-type: none"> <li>• Induces asthma</li> <li>• Organoleptic alterations</li> </ul>

Table 2.2. Advantages and Limitations of Some Physical Methods.  
(Source: Ramos et al., 2013)

Methods	Advantages	Limitations
Modified Atmosphere Packaging (MAP)	<ul style="list-style-type: none"> <li>• Extends storage life, provides high quality product</li> <li>• Delays ripening</li> </ul>	<ul style="list-style-type: none"> <li>• Produces high amounts of CO<sub>2</sub>, off-flavours</li> <li>• Temperature control necessary</li> </ul>
Ultraviolet light (UV)	<ul style="list-style-type: none"> <li>• No residual toxicity, easy to use</li> <li>• Reduce deterioration, induces the synthesis of health-promoting compounds</li> </ul>	<ul style="list-style-type: none"> <li>• Difficulties in measuring UV dose</li> <li>• Increase respiration rate, low penetration depth, limited app. on solid food and opaque surfaces</li> </ul>
Pulsed Light (PL)	<ul style="list-style-type: none"> <li>• Rapid and effective microbial inactivation</li> <li>• Few residual compounds, medium cost</li> </ul>	<ul style="list-style-type: none"> <li>• Affected by food composition</li> <li>• Possible adverse chemical effects</li> </ul>
High Pressure Processing (HPP)	<ul style="list-style-type: none"> <li>• Microbial and enzymatic inactivation</li> <li>• No flavour and nutrient degradation</li> <li>• No evidence of toxicity</li> </ul>	<ul style="list-style-type: none"> <li>• Expensive</li> <li>• Affects porous integrity</li> <li>• Foods should have 40% free water for antimicrobial activity</li> </ul>
Ultrasound	<ul style="list-style-type: none"> <li>• Reduction of process time and temperature</li> <li>• Enhances the penetration of solutions</li> </ul>	<ul style="list-style-type: none"> <li>• Needs to be combined with other processes</li> <li>• Penetration affected by solids and air in product</li> </ul>
Cold Plasma	<ul style="list-style-type: none"> <li>• No residue, can be used on vegetables</li> <li>• No shadow effect</li> <li>• Low impact on the internal product matrix</li> </ul>	<ul style="list-style-type: none"> <li>• Mechanism of inactivation is unclear</li> <li>• Physicochemical changes occur in the product</li> <li>• Inactivation is affected by the type of mos.</li> </ul>

## CHAPTER 3

# NOVEL POSTHARVEST TREATMENTS TO ENHANCE MICROBIAL SAFETY AND QUALITY OF cv. ŞALAK APRICOTS

### 3.1. CaCl<sub>2</sub> Dipping

Calcium (Ca<sup>2+</sup>) has a regulatory function in plants (Aghdam et al., 2012). It was reported that Ca<sup>2+</sup> plays an important role in cellular signalling responses, cell wall integrity, membrane function and fruit quality maintenance in terms of prevention of softening, decay and other physiological disorders such as internal breakdown (Aghdam et al., 2012). Awang et al. (2013) expressed that Ca<sup>2+</sup> prevented postharvest mechanical damages by strengthening the cell wall. Moreover postharvest use of calcium based solutions such as calcium lactate and calcium propionate was also proposed for its antimicrobial properties (Saftner et al., 2003; Ramos et al., 2013).

Calcium chloride (CaCl<sub>2</sub>) dipping is used as a postharvest treatment in order to maintain the quality, prevent softening, reduce the rate of rotteness and prolong the storage life of fruits with high senescence index (Ramano Rao et al., 2011; Beirao-da-Costa et al., 2008).

#### 3.1.1. Mechanism of Action

Ca<sup>2+</sup> is known to support tissue integrity via crosslinking the pectic substances using its divalent ionic property (Aghdam et al., 2012). Negatively charged homogalacturonan chains come together in presence of Ca<sup>2+</sup> to create 'egg box' structure (Figure 3.1) (Chen et al., 2011; Aghdam et al., 2012). Thereby activity of cell wall degrading enzymes can be decreased by preventing the access of the enzymes to their substrates (Aghdam et al., 2012; Awang et al., 2013).

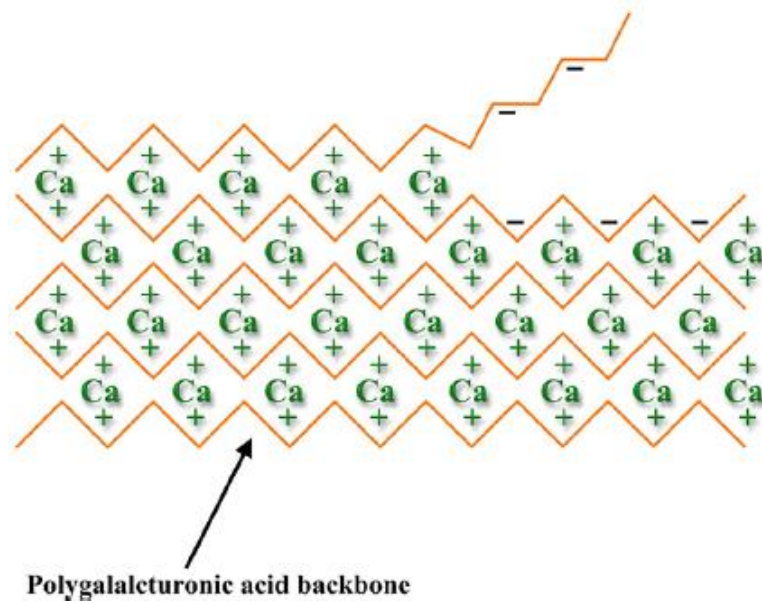


Figure 3.1. The 'egg-box' model.  
(Source: Aghdam et al., 2012)

Moreover, Ca<sup>2+</sup> was shown to delay fruit ripening (Kou et al., 2014). It was speculated that there was a relation between ethylene and Ca<sup>2+</sup> signalling pathways (Aghdam et al., 2012). Cheour and Souiden (2015) declared that Ca<sup>2+</sup> might influence the structure and function of cell walls and membranes and cell catabolism.

### 3.1.2. Applications

There are many applications of calcium based solutions as a post-harvest dipping treatment. The studies in the literature mainly pointed out the firmness improvement, shelf life extension, storage quality and ripening processes of different fruits and vegetables after the treatment (Irfan et al., 2013; Cheour and Souiden, 2015). For instance, a study conducted by Ramano Rao et al. (2011) revealed that CaCl<sub>2</sub> treated sweet peppers had a longer shelf life, retarded respiration and metabolic activity, and delayed softening. Furthermore, inhibition of ascorbic acid oxidase (AAO) enzyme which allows the retention of vitamin C and prevention of browning was achieved using this chemical (Ramano Rao et al., 2011). On the other hand, Beirao-da-Costa et al. (2008) conjoined the effect of CaCl<sub>2</sub> and mild heat treatments on kiwifruit. They observed that solely CaCl<sub>2</sub> inhibited PME enzyme activity yielding lower firmness. However, it was reported that application of CaCl<sub>2</sub> along with heat increased the formation of calcium pectates which involves in the firmness increment (Beirao-da-



Costa et al., 2008). In relation to this, Lidster and Porritt (1978) indicated that dipping temperature had an influence on the Ca absorption of the fruit tissues.

Another study aimed to investigate the effect of calcium on physicochemical properties and firmness of apricots showed that  $\text{CaCl}_2$  treatment might improve the resistance of apricots to ripening (Liu et al., 2009a). Similarly, Hajilou and Fakhimrezaei (2013) conducted a research on the determination of the effect of postharvest  $\text{CaCl}_2$  dipping and salicylic acid (SA) on the shelf life and quality of apricots. They found that respiration rate of apricots were reduced, metabolic activities were delayed and oxidation of ascorbic acid was slowed down by  $\text{CaCl}_2$  application (Hajilou and Fakhimrezaei, 2013). Moreover, fruit decay in strawberries was reported to be retarded using 1%  $\text{CaCl}_2$  due to improved tissue stability and enhanced resistance against fungal attacks (Chen et al., 2011).

Kou et al. (2014) applied  $\text{CaCl}_2$  to broccoli microgreens at pre-harvest stage. They observed that pre-harvest  $\text{CaCl}_2$  application was resulted in an increased biomass, extended shelf life and enhanced quality during post-harvest stage. Saftner et al. (2003) also could prolong the shelf life of fresh-cut honeydew chunks after both  $\text{CaCl}_2$  and Ca propionate treatment. Additionally, fruit quality was enhanced (Saftner et al., 2003). Similarly, Manganaris et al. (2007) evaluated the effect of  $\text{CaCl}_2$  on quality of peaches.  $\text{CaCl}_2$  dipping at a concentration of 62.5 mM was found to have a potential to be used for peaches in order to maintain the quality (Manganaris et al., 2007).

### **3.1.3. Advantages and Disadvantages**

Pre- and postharvest application of calcium was denoted to reduce senescence without any detrimental effect on consumers' acceptability (Ramano Rao et al., 2011). Besides, calcium was showed to be able to cross link with carboxyl groups of pectins which results in strengthening of the plant cell walls (Beirao-da-Costa et al., 2008; Kou et al., 2014). It was also indicated that postharvest  $\text{CaCl}_2$  application could better preserve ascorbic acid content in apricots (Hajilou and Fakhimrezaei, 2013).

However, it was found that calcium inhibited the beneficial effect of heat treatment by increasing the lipase activity in cantaloupe melon (Lamikanra and Watson, 2007). Other disadvantages of this method were implied as flavour defects and its limited effectiveness on microorganisms (Ramos et al., 2013). Furthermore, it was

expressed that excessive amount of Ca could bring about cytosolic Ca concentration differences in the fruit tissue which leads to cell damages (Kou et al., 2014). Chen et al. (2011) also speculated that phytotoxicity caused by increased levels of Ca in the tissue contribute to the instability of the cell wall.

## **3.2. Mild Heat Treatment**

Heat treatments effectively inhibit enzymatic reactions and reduce microbial loads. Nonetheless, adverse impacts of heat on flavour, texture and other quality parameters of the products discourage its use (Abreu et al., 2003). Therefore application of mild heat (MH) is of great interest. This technique avoids negative effects of heat treatment.

Use of heat treatments as a post-harvest operation is urged since it is a clean and safe way to control pathogenic and spoilage microorganisms during the storage of fresh fruits (Sivakumar and Fallik, 2013). Kou et al. (2007) also mentioned about the potential of the use of MH treatments in order to preserve fresh characteristics of fruits and vegetables.

Different temperatures between 35-60°C and treatment times ranging from a few seconds up to few days were indicated to be used for mild heat applications (Rodoni et al., 2016). On the other hand, heating process can be performed using either microwave, infrared, hot vapour, hot air or hot water (Sivakumar and Fallik, 2013; Rodoni et al., 2016).

### **3.2.1. Mechanism of Action**

It was indicated that the mechanism of MH treatment to maintain the quality was based on the synthesis of heat shock proteins (Kou et al., 2007). On the other hand, it was reported that normal protein synthesis was blocked (Beirao-da Costa et al., 2006). For instance activity of phenylalanine ammonia lyase (PAL) which is the key enzyme for phenolic compound synthesis was said to be inhibited by the treatment (Kou et al., 2007; Ansorena et al., 2011). Thereby, browning reactions can be prevented. Furthermore, it was declared that prevention of browning might be also due to the inhibition of PPO enzyme (Beirao-da Costa et al., 2006).

Maintenance of fruit texture after MH treatment was found to be associated with the activation of PME enzyme (Beirao-da Costa et al., 2006). The enzyme acts on the pectin molecules and forms available sites for  $\text{Ca}^{2+}$  ions. Consequently, cell wall becomes resistant to softening. Additionally, heating was indicated to cause melting of the natural wax layer on the fruit. Plasticized wax would in turn fill the microcracks on the fruit surface. Hence, water loss and subsequent softening can be prevented (Sivakumar and Fallik, 2013). Besides, Lamikanra and Watson (2007) suggested that increased tissue firmness after MH treatment was due to the interference of the cleavage of pectin segments by pectin degrading enzymes. It was pointed out that pectin strands localized closer to each other as a result of the loss of neutral sugar side chains during MH treatment. Therefore, degradation of the structure would be prohibited (Lamikanra and Watson, 2007).

MH treatments are also effective in preventing fruit decay. It was showed that decay control mechanism was based on the inhibition of spore germination and mycelial growth (Sivakumar and Fallik, 2013). Moreover, accumulation of antimicrobial compounds (phytoalexins) and induced synthesis of enzymes which catalyse the hydrolysis of fungal cell walls such as chitinases and  $\beta$ -1;3 glucanases, were revealed to be related to the induced defence mechanisms as a result of MH treatment (Sivakumar and Fallik, 2013).

### **3.2.2. Applications**

Beirao-da-Costa et al. (2006) evaluated the effect of maturity stage and mild heat treatment on the kiwifruit quality. It was stated that quality of the kiwifruit at early stages of ripening was improved by mild heat treatment below 45°C. The effect of mild heat treatment with respect to preserving pear quality attributes was also studied (Abreu et al., 2003). The treatments at 30-40°C for 40-150 minutes were indicated to be effective in reducing or preserving the browning and increasing the firmness (Abreu et al., 2003). Mild heat application in order to extend the shelf life of cantaloupe melon was assessed by Lamikanra and Watson (2007). Reduced respiration and increased firmness were observed after heat treatment.

Klaiber et al. (2005) investigated the effect of washing with warm tap water (50°C) or chlorinated water (4°C, 50°C) on microbial and sensorial quality of carrots.

They suggested the use of warm tap water for minimally processed carrots as an alternative in order to maintain quality and prevent microbial deterioration. Similarly, Rahman et al. (2011) conducted a study on carrots. They tested the antimicrobial effectiveness of alkaline electrolyzed water and citric acid with MH. It was found that the combined treatment could ensure a prolonged shelf life without changing the sensorial quality of the carrots (Rahman et al., 2011). In another study, MH treatment was applied to fresh-cut table grapes by both hot water dipping (45°C for 8 min) and hot air (55°C for 5 min) (Kou et al., 2007). It was indicated that hot water dipping could prevent fruit decay in fresh-cut table grapes.

### **3.2.3. Advantages and Disadvantages**

MH treatment is a physical process which does not leave any residue unlike to chemical treatments. Short application time is another advantage of the method. Moreover, one can easily monitor the operation. It was also noted that MH treatment allows pathogen control and prevents chilling injuries (Sivakumar and Fallik, 2013). However, severe conditions may result in undesired changes in the sensorial quality of the products (Abreu et al., 2003).

### **3.3. UV-C Irradiation**

Ultraviolet light is a component of electromagnetic spectrum and covers the wavelength range of 100-400 nm (Figure 3.2) (Koutchma, 2014a). UV portion of the spectrum can be subdivided into four groups: UV-A (315-400 nm), related to the tanning; UV-B (280-315 nm), responsible for skin burning and further exposure leads to skin cancer; UV-C (200-280 nm), known as germicidal range since it has inactivation efficiency on bacteria and viruses; Vacuum UV (100-200 nm), can be transmitted only in the vacuum otherwise absorbed by almost all substances (Koutchma et al., 2009; Ribeiro et al., 2012; Koutchma, 2014a).

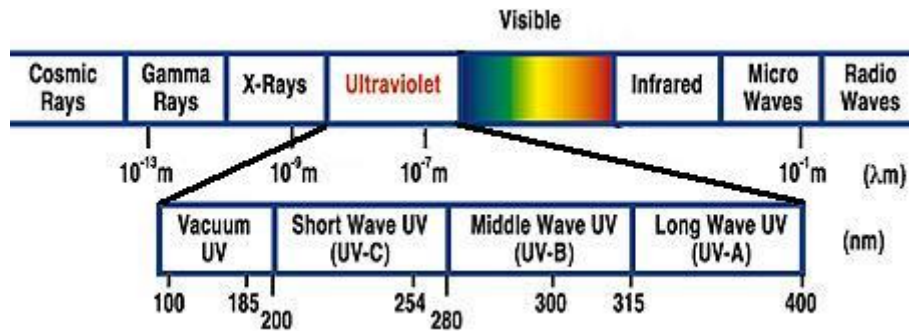


Figure 3.2. Electromagnetic spectrum and its subdivisions.  
(Source: UVLP, 2016)

UV light is known as non-ionizing irradiation since it does not give rise to ionization of the molecules in contrast to X-rays and gamma rays (Boziaris, 2014). Its use for solid foods was approved by U.S. Food and Drug Administration (FDA) in 2002 (Manzocco and Nicoli, 2015). It was also reported that EC novel food regulation permitted its use without authorization (Manzocco and Nicoli, 2015).

UV light irradiation is based on the use of UV light as a non-thermal processing method to reduce microorganisms, induce biological stress and defence mechanisms in plants (Jiang et al., 2010). UV-C irradiation (190-280nm wavelength) serves these purposes (Gonzalez-Aguilar et al., 2007). It was proven to be beneficial to decrease respiration rate, control rot development, delay senescence and ripening in fruits and vegetables (Jiang et al., 2010).

### 3.3.1. Mechanism of Action

UV-C light is known to show its maximum germicidal effect at 254 nm (Sun, 2014). The germicidal effect of UV-C light is due to its absorption by the genetic material and consequent mutations occurred in DNA (Yun et al., 2013). UV-C light causes the microbial inactivation via rendering the cells incapable of reproducing themselves (Koutchma et al., 2009).

Incoming UV-C light photons are absorbed by the genetic material, either DNA or RNA (Koutchma et al., 2009). Electrons of the hydrogen bonds between paired nucleotides become energized which leads to the breakage of the bond and subsequently to the formation of cytotoxic and mutagenic lesions (Koutchma, 2014a). These mutagenic lesions are pyrimidine dimers, mainly formed by covalent linkages between two adjacent thymine bases (Koutchma, 2014a; Hakguder Taze et al., 2015) (Figure

3.3). Dimers were indicated to prevent cell transcription and replication, consequently inactivate the microorganisms (Hakguder Taze et al., 2015).

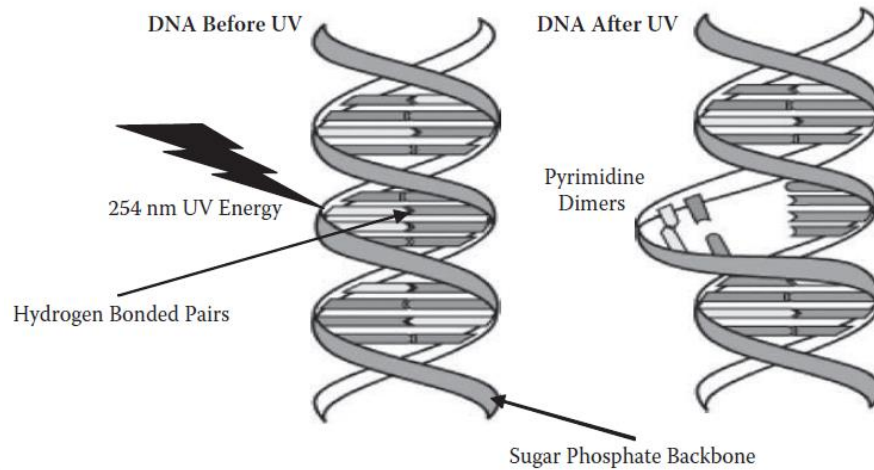


Figure 3.3. UV-C radiation effect on the genetic material.  
(Source: Koutchma et al., 2009)

Besides these, UV-C irradiation is also used for extending the postharvest shelf life of fruits and vegetables. Gonzalez-Aguilar et al. (2007) reported that UV-C induced the pathogen resistance of the plants by activating the plant defence mechanisms. Tiecher et al. (2013) reported that UV-C could prevent rotteness in fruits via inducing the activity of phenylalanine ammonia lyase, chitinase, peroxidase and  $\beta$ -1,3 glucanase enzymes. Moreover, another mechanism related to the extended storability of the products was indicated to be the changed hormonal signalling (Tiecher et al., 2013).

On the other hand, hormetic effect of UV-C light is caused by the stimulation of vital processes inside the cell following the exposure to sub-lethal radiation (Lingegowdaru, 2007). Short UV exposure of fruits was indicated to activate the synthesis of antifungal compounds and delay ripening process which in turn extends the shelf life (Kalia and Parshad, 2015).

### 3.3.2. Applications of UV-C Light on Food Surfaces

In literature some applications are available related to the use of UV-C light as a means of postharvest quality improvement. For instance, Gonzalez-Aguilar et al. (2007) and Gonzalez-Aguilar et al. (2001) evaluated the impact of UV-C light on enzymes

associated with the defence mechanism and postharvest shelf life of mango fruit. They concluded that UV-C irradiation was able to prevent deterioration and maintain the quality of mango fruit by reducing decay and increasing the accumulation of flavonoids. Erkan et al. (2008) and Jiang et al. (2010) also conducted a similar study in order to determine the UV-C light effect on antioxidant capacity, antioxidant enzyme activity, decay and texture in strawberry fruit and mushrooms. Lu et al. (2016) also examined the effect of UV-C treatment on the browning and antioxidant properties of mushrooms. In another study UV-C light was found to promote the accumulation of lycopene in tomatoes (Liu et al., 2009b). Liu et al. (2012) also studied UV-C light treatment of tomatoes to measure the changes in phenolic content and antioxidant activity. On the other hand some researchers utilized UV-C light as a pre-harvest treatment of tomatoes in order to observe its effect on ripening and pathogen resistance (Obande et al., 2011). Moreover, effect of postharvest UV-C irradiation on tomato ripening, ethylene production and cell wall degrading enzymes was investigated (Tiecher et al., 2013; Bu et al., 2013).

One of the latest studies was about evaluation of the ability of UV-C light to reduce *E. coli* O157:H7 and *Salmonella* spp. on apricot fruit (Yun et al., 2013). Yan et al. (2014) also investigated the UV-C inactivation of *E. coli* inoculated onto the apricot surfaces. Likewise, UV-C inactivation rate of *Penicillium expansum* inoculated onto different fruit surfaces was determined by Syamaladevi et al. (2015). Rocha et al. (2015), on the other hand, studied the effect of postharvest UV-C light treatment on prevention of soft-rot in potato seed tubers caused by *P. carotovorum*.

### **3.3.3. Advantages and Disadvantages**

UV-C light irradiation is a physical process and does not form toxic by-products (Manzocco and Nicoli, 2015). Moreover its use is easy and does not require expertise. It can be safely used with some little precautions (Manzocco and Nicoli, 2015). Another advantage is the cost of the system which is favourable in terms of energy and maintenance (Koutchma et al., 2016)

Although UV-C light treatment is widely used in water and air disinfection as well as surface decontamination, its use is still limited in postharvest technology. Scaling studies for industrial applications were reported to be necessary since devices

for disinfection of solids are limited (Ribeiro et al., 2012). Other disadvantages are the limited penetration ability of UV-C light and presence of crevices which can protect microorganisms to be irradiated on the surfaces (Sun, 2014).

### **3.4. Modified Atmosphere Packaging (MAP)**

Modified atmosphere packaging (MAP) is a technology which is based on the changing the gas composition in the package surrounding the food commodity (Zhang et al., 2014). For this purpose various polymeric packaging materials which have vapour-barrier and gas-barrier properties including polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and polyethylene terephthalate (PET) are used (Zhang et al., 2014; Peano et al., 2014). Considering mechanical damages and also commercial reasons the thickness of the films must be between 15 to 100  $\mu\text{m}$  (Peano et al., 2014).

In MAP technology, the amount of oxygen in the package is reduced to 3-5% (Ramos et al., 2013). On the other hand, the rest of the gas composition is replaced by increased amount of  $\text{CO}_2$  (up to 10%) and  $\text{N}_2$  (Kalia and Parshad, 2015). The modification of the atmosphere can be generated by both active and passive ways (Saxena et al., 2008; Ramos et al., 2013).

In active modification, the air in the packaging material is displaced by vacuum application. Afterwards, the desired gas composition is flushed into the package (Oliveira et al., 2015). Nitrogen is generally used as a supplementary gas to achieve the desired level of  $\text{O}_2$  and  $\text{CO}_2$  and to prevent the collapse of the packaging material (Zhang et al., 2014).

On the other hand, passive modification is generated by the respiration of the product itself (Zhang et al., 2014). For this purpose a specific packaging material which allows the diffusion of respiratory gases through the film is selected (Oliveira et al., 2015). However, this process was reported to be slow and difficult to control (Kuzucu and Önder, 2010).



### **3.4.1. Mechanism of Action**

It was indicated that reduction of spoilage in MAP treated products was related to the increased CO<sub>2</sub> levels (Choi et al., 2015). Furthermore, high CO<sub>2</sub> was also pointed out to reduce the ethylene sensitivity of the fresh products (Kuzucu and Önder, 2010). Ethylene biosynthetic pathway was declared to be affected by high CO<sub>2</sub> concentration (Ali et al., 2004). On the other hand, delay of ripening was found to be attributed to the reduced O<sub>2</sub> levels (Choi et al., 2015).

MAP was also revealed to slow down the softening due to the suppression of cell wall degrading enzyme activities by both high levels of CO<sub>2</sub> and low temperature storage (Ali et al., 2004).

In another study, MAP was speculated to prevent weight loss and fruit shrivelling due to the generation of a fruit surrounding medium having a higher relative humidity (Selcuk and Erkan, 2014).

### **3.4.2. Applications**

There are many examples of MAP treatment of fruits in the literature. Different fruits have been used for this purpose. Some of the examples from literature were presented below.

Peano et al. (2014) evaluated the effect of MAP treatment using different packaging materials on the storage quality of apricots. MAP was found to be successful to maintain the postharvest quality of the fruits during storage at 1°C (Peano et al., 2014). Similarly, Muftuoğlu et al. (2012) investigated the quality of edible film coated 'Kabaası' apricots after being packed by passive and active modified atmosphere conditions with different packaging materials. MAP was found to be very effective in preserving the fruit quality of both coated and uncoated apricots during 28 days of storage at 4°C. Moreover Mohsen (2011) and Kuzucu and Önder (2010) discussed the effect of MAP application on the storage quality of apricots.

Ali et al. (2004) studied the changes in cell wall degrading enzyme activities and pectin structure of carambola fruit during low temperature storage after being packed by MAP. In another study low dose gamma irradiation was combined with MAP for the shelf life extension of strawberries (Jouki and Khazaei, 2014). On the other hand,

Selcuk and Erkan (2014) determined the quality attributes of MAP treated pomegranates. Shelf life extension of minimally processed jackfruit after MAP application was also examined.

### **3.4.3. Advantages and Disadvantages**

It was well documented that MAP treatment could retard the onset of ripening and related processes, keep the quality of stored fruits and hence extend their shelf life. However, use of increased amount of CO<sub>2</sub> may allow the growth of pathogenic microorganisms and also cause spoilage of the fruits (Ramos et al., 2013). Furthermore, accumulation of water in the packaging material due to the transpiration of the fruit causes microbial growth (Castellanos et al., 2016). Thereby selection of appropriate packaging material is essential to achieve successful results.

Also, Siracusa (2012) showed that contact of the packaging material with the food commodity might change the barrier properties of the material. This may bring about dissatisfaction with quality attributes of the fruits during the storage period.

Aside from these, MAP and cold storage was demonstrated to be insufficient for minimally processed products (Saxena et al., 2008). Therefore, additional treatments would be necessary in order to maintain the quality.

### **3.5. Hurdle Technology**

Hurdle technology means use of different preservation methods which enhance each other in combination (Ramos et al., 2013). One can avoid severe effects of a single treatment by the help of this approach since it consists of the sequential use of mild treatments (Ramos et al., 2013). Success of the application of combined methods were reported to depend on the type of treatments, physiology and type of the target microorganisms, surface properties of the product to be treated, exposure time, pH and temperature (Ramos et al., 2013).

### 3.5.1. Applications

In literature it was observed that many researchers investigated the combined effect of different techniques such as mild heat treatment, chemical applications, use of organic acids, MAP, gamma irradiation, UV-C radiation etc. A brief summary of the studies were given below.

Sulaiman and Silva (2013) studied the combined effect of pressure and mild temperatures on PPO activity in strawberry puree. In another study chemical treatment including the application of calcium chloride and citric acid, and modified atmosphere packaging was combined in order to evaluate the quality changes in fresh-cut papaya (Waghmare and Annapure, 2013). Additionally Ahari Mostafavi et al. (2013) combined gamma irradiation with biocontrol agent for the preservation of apple quality. Combined effect of aqueous chlorine dioxide and ultrasonic treatments on postharvest quality of plum fruit was also investigated by Chen and Zhu (2011).

Other research studies include the utilization of alkaline water, citric acid and mild heat processing for the processing of carrots (Rahman et al., 2011); combined treatment of rice bran protein film packaging with aqueous chlorine dioxide and UV-C radiation for processing of Goha strawberries (Shin et al., 2012); use of edible coatings in combination to mild heat shocks for the preservation of broccoli (Ansorena et al., 2011); investigation of the combined effect of salicylic acid, hot water and calcium dipping on the quality of strawberry (Shafiee et al., 2010); combined use of mild heat application and refrigeration for better postharvest storage life of pepper sticks (Rodoni et al., 2016); combined effect of UV-C and mild heat treatment on microbial inactivation of pathogens including *Escherichia coli* O157:H7 and *Salmonella* Typhimurium in powdered red pepper (Cheon et al., 2015); combined application of UV-C, MAP and low temperature storage to improve the microbial and physicochemical quality of cherry tomatoes (Choi et al., 2015).

### 3.5.2. Advantages

It was reported that combined preservation methods decreased the nutritional losses and preserved quality attributes of the food products (Khan et al., 2016). Moreover, it avoids the use of intense treatment conditions (Khan et al., 2016). Hence,

negative impacts of some treatments could be overcome by means of combined technologies (Khan et al., 2016).



## CHAPTER 4

### MATERIALS AND METHODS

#### 4.1. Raw Material

cv. Şalak apricots supplied from Iğdır province were immediately transported to a cold storage located in Bornova (Iceberg Taze Saklama Merkezi, İzmir). All the samples were kept at cold conditions until the end of the study. Fruits which were not overripe, free from any defects and any noticeable contamination, and had a uniform size, shape and colour were selected for the experiments.

#### 4.2. Measurement of Physical, Chemical and Sensorial Properties

##### 4.2.1. Fruit Weight, Length and Width

Thirty fruits were randomly selected and used for measurement of weight, length (L) and width (W) by means of a precision balance (Mettler Toledo, Switzerland) and a digital calliper (TORQ 150 mm, Taiwan), respectively.

##### 4.2.2. Moisture Content

Moisture content of the samples was assessed by oven drying method (Chassagne-Berces et al., 2010; Leong and Oey, 2012). Apricot samples were ground using a household blender in order to facilitate the drying. Five grams of raw samples were dried at 70°C using a vacuum drying oven until reaching a constant weight. The difference between two consecutive measurements was not greater than 0.005 g.

### 4.2.3. Water Activity

Hygrolab (Rotronic AG, Switzerland) water activity meter was used for the measurement. Apricots were ground using a blender. Then ground apricot samples were immediately filled into the sample container up to 2/3 of its volume. Water activity ( $a_w$ ) of the samples was determined at 25°C.

### 4.2.4. pH

pH value of the samples were measured using a pH meter (WTW GmbH, Germany). Fruits were squeezed by a household juice processor. At least 50 mL of clear juice was used for the analysis.

### 4.2.5. Titratable Acidity

Sample extract was prepared as it was described in Section 4.2.4. Then 5 g of sample juice pipetted into a 250 mL Erlenmeyer flask. The sample was diluted with 50 mL of deionized water. Then it was titrated against standardized 0.1 N NaOH until the pH reaches 8.1 which is phenolphthalein end point. Results were calculated using the formula (4.1) and expressed as percentage of malic acid per 100 g of sample (Ali et al., 2011; Aubert et al., 2010; Bureau et al., 2009).

$$TA\% = \frac{(\text{mL NaOH used}) \times (f) \times (0.1\text{N NaOH}) \times E}{m} \times 100 \quad (4.1)$$

f; represents the factor of 0.1 N NaOH solution, E; indicates milliequivalent factor which should be selected considering Table 4.1, m; is the weight (g) of the sample.

Table 4.1. Milliequivalent Factors for Different Food Commodities.  
(Source: Mitcham et al., 1996)

Commodity	Predominant Acid	Milliequivalent Factor
Stone fruit, apples, kiwifruit	Malic acid	0.067
Citrus	Citric acid	0.064
Grapes	Tartaric acid	0.075

#### 4.2.6. Soluble Solid Content

Soluble solid content (Brix°) was determined using a refractometer (Mettler-Toledo RE40D, Switzerland). Whole fruits were homogenized and filtered to get a mixture of juice from all regions of the fruits. 0.1 mL of the sample was used to measure Brix value at 20°C.

#### 4.2.7. Ripening Index (RI)

RI was calculated considering the ratio of Brix value and titratable acidity (Valero et al., 2003). RI is important in terms of the determination of the right harvest time. If the fruits are harvested too early, then the desired fruit flavour may not exist. At the same time, the fruit will be unable to ripen properly (Dhat and Mahajan, 2007). On the other hand, if they are harvested at their ripe stage, the fruits will have better characteristics but then, they will be very prone to damage and shelf life will be limited (Missang et al., 2011).

#### 4.2.8. Colour

Colour parameters (CIE  $L^*$   $a^*$   $b^*$ ) of the samples were measured using Konica Minolta CR 400 chromameter (Konica Inc., Japan). Here,  $L^*$  represents brightness-darkness (0 = black, 100 = white),  $a^*$  indicates redness-greenness ( $-a^*$  = greenness,  $+a^*$  = redness), where  $b^*$  shows the yellowness-blueness ( $-b^*$  = blueness,  $+b^*$  = yellowness) of the sample. Three measurements were made at different locations on each sample and averaged. Colour parameters were evaluated both before and after the processing. In order to find out the effect of the treatment on colour of the samples, total colour difference ( $\Delta E$ ) was estimated according to the formula (4.2).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (4.2)$$

#### 4.2.9. Firmness

Texture Profile Analysis (TPA) is widely used for both solid and semi-solid food samples. Test is based on the two-bite procedure in order to mimic the biting action of the mouth (Rosenthal, 2010). After the measurement, one can simply extract the data belong to five primary characteristics, namely hardness, cohesiveness, adhesiveness, elasticity, and fracturability (Fig 4.1). Other properties can be also derived from these properties.

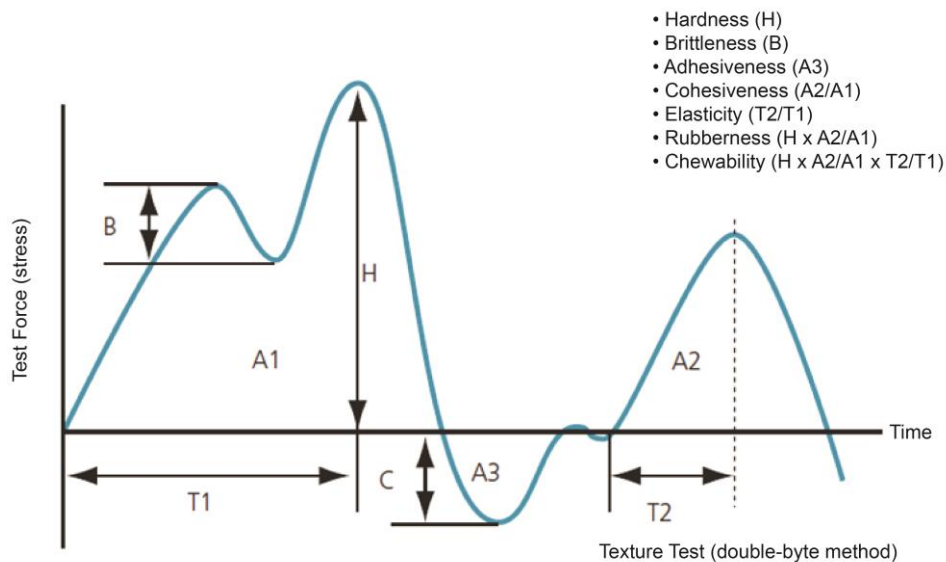


Figure 4.1. Typical TPA graph.  
 (Source: FQ&S, 2016)

Firmness is defined as the peak force of the first compression of the sample (Liu et al., 2009a). TA-XT Plus texture analyser (Figure 4.2) (Stable Micro Systems Ltd., UK) equipped with a 2 mm cylindrical probe was used to assess firmness on two opposite sides of the apricots. Penetration depth was 5 mm and penetration rate was 1 mm/s (Li et al., 2014).



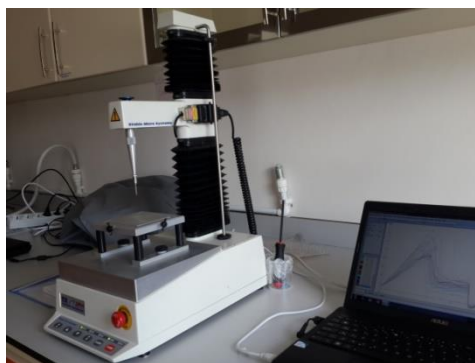


Figure 4.2. Texture analyser.

## **4.2.10. Ascorbic Acid Content**

### **4.2.10.1. Ascorbic Acid Extraction**

Extraction of ascorbic acid from the fruit was carried out according to the method given by Gundogdu et al. (2011) with some modifications. 30 g of cold apricot samples were directly weighed into 60 mL of 3% cold metaphosphoric acid solution in order to prevent oxidation. Then it was homogenized using a household blender which was placed in an ice bath. Following the homogenization the mixture was centrifuged at 6000 rpm for 15 min at 4°C. Afterwards the supernatant was collected and used for further analysis.

### **4.2.10.2. Ascorbic Acid Assay**

Ascorbic acid contents of the extracts were assessed by colorimetric method using a commercially available test kit (catalogue No. 10409677035, R-Biopharm, Roche, Germany).

The method is based on the measurement of the light absorbance of MTT-formazan at 578 nm. MTT-formazan is formed from tetrazolium salt MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] by the effect of L-ascorbic acid and some other reducing substances found in the sample extract in presence of an electron carrier, PMS (5-methylphenazinium methosulfate), at pH 3.5.

According to the method, 1 mL of sodium phosphate/citrate buffer + MTT solution warmed up to 37°C was pipetted into the sample cuvette and sample blank cuvette. After that the solutions were equally mixed with 1.35 mL of ultrapure water and 0.25 mL of sample extract. Different from sample cuvette, ascorbic acid found in the sample blank mixture was removed by adding an ascorbic acid oxidase (AAO) spatula into the sample blank cuvette. The solution mixture and the spatula were allowed to incubate at 37°C for 6 min with mixing the solution every after 2 min for 5s in order to provide an efficient oxygenation. Meanwhile sample cuvette was left to incubate at the same conditions by preventing oxidation of L-ascorbic acid. After incubation the first absorbance measurements ( $A_1$ ) were taken for both sample and sample blank. The reaction between MTT and L-ascorbic acid was started by adding 0.1 mL of PMS solution to the cuvettes. Cuvettes were incubated at 37°C for 15 min in dark conditions. Afterwards absorbance values ( $A_2$ ) were immediately read for both cuvettes. All the absorbance values were read against water. Absorbance differences were determined as follows:

$$\Delta A = (A_2 - A_1)_{\text{sample}} - (A_2 - A_1)_{\text{sample blank}} \quad (4.3)$$

The concentration of ascorbic acid found in the sample was calculated using the formula (4.4).

$$c = \frac{\left(\frac{V \times MW}{\epsilon \times d \times v \times 1000} \times \Delta A\right) [\text{g/L}]}{W} \times 100 [\text{g}/100\text{g}] \quad (4.4)$$

Here,  $V$ ; is the final volume of reaction mixture (2.7 mL),  $MW$ ; is the molecular weight of L-ascorbic acid (176.13 g/mol),  $\epsilon$ ; is extinction coefficient for MTT-formazan at 578 nm (16.9 L mmol<sup>-1</sup> cm<sup>-1</sup>),  $d$ ; is the light path (1 cm),  $v$ ; is the sample volume (0.25 mL),  $W$ ; is the weight of sample in g per 1 L sample solution.

## **4.2.11. Total Phenolic Content (TPC)**

### **4.2.11.1. Extraction of Phenolics**

Extraction of phenolic compounds was carried out considering the methods given by Upadhyaya et al., (2015) and, Kim and Lee, (2002). According to the procedure 10 g of apricot samples were mixed with 30 mL of 80% aqueous methanol which contained 1% of ascorbic acid. The mixture was homogenized using a blender placed in an ice bath. Homogenized sample was transferred to a 250-mL Erlenmeyer flask and put into the ultrasonic bath. The mixture was sonicated for 15 min at room temperature. After the sonication the content was centrifuged at 10000 rpm for 10 min at 4°C. Supernatant was collected and the pellet was resuspended in 30 mL of 80% aqueous methanol for the second extraction. The same steps were applied and the volume of final supernatant (extract) was recorded.

### **4.2.11.2. TPC Assay**

TPC of prepared extracts were assessed using the method given by Karav and Ekşi (2012). 0.5 mL of properly diluted sample extract was mixed with 2.5 mL of 0.2 M Folin-Ciocalteu reagent. The reaction mixture was allowed to stand for 4 min and then 2 mL of 7.5% sodium carbonate was added. The tube contents were incubated for 2 h in dark conditions. Finally absorbance values were measured at 765 nm. TPC of the samples were estimated using the standard curve given in Appendix A. The results were expressed as mg Gallic acid equivalents (GAE) per 100 g of sample.

## **4.2.12. Sensory Analysis**

Triangle test which is a discriminative sensory test was applied to determine if there was a significant difference between treated samples and untreated control samples. 6 trained people were used to evaluate the samples. Panellists were given a set of three samples. Two of them were the same and the third one was different. Each sample was represented with a 3-digit number. The order of the presentation was

random and could have been any of six combinations (AAB, ABA, BAA, ABB, BAB, BBA). Panellists were informed that two of the samples were identical and they were required to find the odd one. If in case of any difference could not be detected, they were asked to guess. Test was repeated three times. Totally 18 responses were obtained. The results were judged using the table given in Appendix B.

### **4.3. Measurement of Enzymatic Activities**

The effect of processing on the activity of quality degrading enzymes, i.e., PPO and PME, was determined.

#### **4.3.1. Polyphenoloxidase (PPO) Enzyme**

##### **4.3.1.1. PPO Extraction**

Method given by Sulaiman et al. (2015) was used with some modifications for the extraction of PPO enzyme. 20 g of thin apricot surface layer (0.85 mm) obtained by a fruit peeler was mixed with 40 mL of 0.2 M Na-phosphate buffer (pH 7.0) containing 0.5% of PEG 6000 (w/v) and 1% Triton X-100 (v/v). 100  $\mu$ L of 1M NaCl was also added to this mixture. Then the mixture was homogenized for 3 min using an ice bath externally. The homogenate was centrifuged at 11000 rpm for 30 min at 4°C. After that the supernatant was collected and combined for further analysis.

##### **4.3.1.2. PPO Activity Assay**

Spectrophotometric method proposed by Sulaiman et al. (2015) was handled for the determination of PPO activity. The sample cuvette contained 3 mL of 0.01M catechol substrate in a 0.07 M (pH 5.8) Na-phosphate buffer and 100  $\mu$ L of undiluted PPO extract from the fruit. The pH of Na-phosphate buffer used in the analysis was 5.8, since that is within the range for optimal enzyme activity. The blank was prepared by mixing 100  $\mu$ L of distilled water with 3 mL 0.01M catechol solution in Na-phosphate buffer (pH 5.8). An increase in absorbance was recorded at 420 nm every 30 s for 20

min. Enzyme activity was calculated from the linear portion of the absorbance against time curve and was expressed as Unit/mL enzyme extract using the formula (4.5) (Cemeroğlu, 2013). 1 unit of enzyme activity is expressed as the amount of enzyme that causes a 0.001 absorbance increase per minute for every 1 mL of extract.

$$\text{Activity, (U/mL enzyme extract)} = [S/0.001] \times [1/V_e] \times [V_{\text{rxn}}] \times \text{df} \quad (4.5)$$

S; slope of the linear portion of the absorbance versus time curve (abs/min),  $V_e$ ; volume of enzyme extract in the reaction mixture (mL),  $V_{\text{rxn}}$ ; total volume of reaction mixture (mL), df; dilution factor.

## **4.3.2. Pectinmethylesterase (PME) Enzyme**

### **4.3.2.1. PME Extraction**

Method given by Pombo et al. (2009) was used with some little modifications. According to the extraction method, 20 g apricot surface layer (0.85 mm) was mixed with 60 mL of 1M NaCl with the addition of 1% PVPP. After that the mixture was homogenized using a blender. The homogenate was stirred for 4 h at 4°C. Finally it was centrifuged at 11000 rpm for 30 min at 4°C. Supernatant was collected and its pH was adjusted to pH 7.5 by NaOH solutions with various concentrations.

### **4.3.2.2. PME Activity Assay**

Activity assay offered by Hagerman and Austin (1986) was slightly modified and used. According to the method 0.5 mL of sample extract of which pH was adjusted to 7.5 was mixed with 2 mL of 0.5% (w/v) apple pectin (prepared in 0.1 M NaCl solution, pH 7.5), 0.35 mL of 3mM potassium phosphate buffer (pH 7.5) and 0.15 mL of 0.01% (w/v) bromothymol blue in 3mM potassium phosphate buffer (pH 7.5). Buffer solution was used as a blank. The assay was carried out at 30°C and a decrease in the absorbance was measured at 620 nm every 15 s for 15 min. The slope of absorbance versus time curve was obtained and the activity was determined using equation (4. 5). 1

unit of enzyme activity was expressed as the amount of enzyme that causes a 0.001 absorbance decrease per minute for every 1 mL of extract.

#### 4.4. Microbiological Analysis

Treated and untreated samples were peeled and peels (0.85 mm thick) were suspended in 0.1% peptone water in a ratio to make 1:10 dilution. Then the mixture was homogenized using a blender. Blender and its reservoir were disinfected before and after homogenization of each sample in order to prevent contamination. Serial 10-fold dilutions were made using 0.1% peptone water. 0.1 mL of the aliquot from adequate dilutions was plated onto the agar media using spread plate technique. A general purpose medium, Tyriptic Soy Agar (TSA, Merck, Germany), was used to enumerate total aerobic plate count (TAPC) found on apricot surfaces. Plates were incubated at 30°C for 3 days. Yeast and mould count (YMC) was determined in Potato Dextrose Agar (PDA, BD Difco Corp, United States) plates acidified with 10% tartaric acid solution to pH 3.5. Plates were incubated at 25°C for 5 days. Coliforms were tested in Violet Red Bile Agar (VRBA, Merck, Germany) plates at 37 °C for 1 day. Microbial loads were expressed as log CFU/g.

##### 4.4.1. Modelling of Survival Data

Survival curves of microorganisms (natural flora) on apricot surfaces after exposed to UV-C irradiation were modelled using GInaFiT, a freeware tool (Geeraerd and Van Impe Inactivation Model Fitting Tool) (Geeraerd et al., 2005) based on the following models: log-linear regression, log-linear + tail, Weibull model, Weibull + tail, double Weibull, Biphasic model, Biphasic + shoulder.

Log-linear regression model assumes equal sensitivity or resistance of all cells against heat and equal chance of death (Bigelow and Esty, 1920). The model is described by the following equation (4.6) (Geeraerd et al., 2005).

$$\begin{aligned} \log(N) &= \log(N(0)) - \frac{t}{D} \\ &= \log(N(0)) - \frac{k_{max} t}{\ln(10)} \end{aligned} \quad (4.6)$$

$N$ ; the microbial cell density (CFU/mL or CFU/g) during time,  $N(0)$ ; the initial concentration of microbial cells (CFU/mL or CFU/g),  $k_{max}$ ; the first order inactivation constant (1/ time unit),  $D$ ; decimal reduction time (time unit).

Although it is assumed that there is a linear relationship between survival numbers of microorganisms and the treatment time, most of the cases survival curves of treated microorganisms exhibit a nonlinear relationship. Log-linear + tail model is used for curves displaying an upward concavity, i.e., tailing, after a log-linear reduction due to the presence of a more resistant subpopulation which cannot be further inactivated in time (Geeraerd et al., 2000). On the other hand, Bevilacqua et al. (2015) reported that existence of a tail might be due to the adaptation of the cells to the lethal process or it might be resulted from the fact that all the cells did not receive the same lethal dose during the treatment. The model is defined as shown in equation (4.7) (Geeraerd et al., 2005).

$$\log(N) = \log\left(\left(10^{\log(N(0))} - 10^{\log(N_{res})}\right) \times e^{-k_{max}t} + \left(10^{\log(N_{res})}\right)\right) \quad (4.7)$$

$N_{res}$ ; represents the residual microbial load (CFU/mL or CFU/g).

It was noted that Weibull model was applied for concave inactivation curves (Mafart et al., 2002). The model is able to describe the cell-dependant resistance to the treatment. It was assumed that each cell had different level of resistance to the applied treatment (Mafart et al., 2002). Weibull model is mathematically described according to the following equation (4.8) (Bevilacqua et al., 2015).

$$\log \frac{(N)}{(N(0))} = - \left(\frac{t}{\delta}\right)^p \quad (4.8)$$

$\delta$ ; indicates the first reduction time and it is similar to  $D$  value,  $p$ ; is the geometrical shape parameter and it has no dimension.

Weibull + tail model can explain the concave curves followed by a residual subpopulation (Albert and Mafart, 2005). The mathematical model used for these types of curves was given in the following equation (4.9).

$$\log(N) = \log \left[ (N(0) - N_{res}) 10^{\left(-\left(\frac{t}{\delta}\right)^p\right)} + N_{res} \right] \quad (4.9)$$

Double Weibull model was derived for curves showing double concave shape (Coreller et al., 2006). Weibull model is commonly used to describe both thermal and non-thermal inactivation patterns of microorganisms (Coroller et al., 2006). However, in double Weibull model it was assumed that the population consisted of two subpopulations having different resistance to the stress. Moreover, it was estimated that the inactivation kinetics of two sub-groups followed a Weibull distribution. Mathematical expression of double Weibull model was shown below (equation 4.10).

$$\log(N) = \log \left[ \left( \frac{10^{N(0)}}{1+10^\alpha} \right) \times \left( 10^{\left(-\left(\frac{t}{\delta_1}\right)^{p+\alpha}\right)} \right) + \left( 10^{\left(-\left(\frac{t}{\delta_2}\right)^p\right)} \right) \right] \quad (4.10)$$

Biphasic model for biphasic inactivation curves observed in thermal and nonthermal inactivation due to lethal water activity or pH values (Cerf, 1977; Geeraerd et al., 2005). It means that inactivation curve has two different slopes ( $k_1$  and  $k_2$ ) (Bevilacqua et al., 2015). The model is defined below (equation 4.11).

$$\log(N) = \log(N(0)) + \log \left( (f \times e^{-k_{max1}t} + (1-f) \times e^{-k_{max2}t}) \right) \quad (4.11)$$

Moreover, biphasic + shoulder model was also applied to the data. It was noted that this model was used for biphasic inactivation kinetics preceded by a shoulder (Geeraerd et al., 2005). This type of inactivation kinetic was reported to be uncommon. Goodness of fit for each inactivation curve was judged considering determination coefficient ( $R^2$ ), adjusted determination coefficient (adj- $R^2$ ) and root mean sum of squared error (RMSE).



## 4.5. Mild Heat (MH) Treatment

### 4.5.1. Application

Mild Heat (MH) was applied to apricot samples in a temperature-controlled water bath (Haake, Germany) as shown in Figure 4.3.



Figure 4.3. Temperature controlled water bath.

Since apricot is a highly perishable fruit, hot water dipping can damage the structure of the fruit and cause loss of internal solutes. Hence, apricots were packed before mild heat treatment as recommended by Beirao-da-Costa et al. (2006). Thereby the sole effect of temperature on the microbial load could be revealed rather than the washing effect of dipping. Four apricots for each run were vacuum packed using a handheld vacuum packaging equipment (Super Vacuum, PRC) (Figure 4.4).



a)



b)

Figure 4.4. Packaging before MH treatment; a) Handheld vacuum packaging equipment  
b) Packed apricots.

### **4.5.2. Process Parameters**

Temperature and treatment time were the two factors selected for these experiments. Since no data were found regarding the most appropriate temperature and time combination for mild heat treatment of apricots, the ranges for this study were determined considering the previous studies conducted with cantaloupe melon and kiwi fruit (Lamikanra and Watson, 2007; Beirao-da-Costa et al., 2008; Beirao-da-Costa et al., 2006). Factor levels for the temperature and the treatment time were selected to be in the range of 40 and 60 °C, and 10 and 60 min, respectively. Control samples were unprocessed apricots.

### **4.5.3. Experimental Design**

Response Surface Methodology (RSM) was used to optimize the treatment conditions. Therefore a D-optimal design was generated using Design Expert Programme (7.0.0) (Stat-Ease, Inc., USA). D-optimal design was selected to reduce the number of experiments. The design contains the best subset of all possible experiments (Triefenbach, 2008). Temperature-treatment time combinations generated by the programme were given in Table 4.2. There were totally 16 runs. Response variables were survival number (log CFU/g), firmness (N) and colour parameters;  $L^*$ ,  $a^*$ ,  $b^*$ . Microbiological analyses were performed considering the method given in section 4.4. The goal was to keep firmness,  $L^*$  value and  $b^*$  value at their maximum level. However, survival number and  $a^*$  value were preferred to be stayed at the minimum level since high numbers of microorganisms and saturated redness are not desired in terms of fresh fruit quality.

Table 4.2. Temperature - Treatment Time Combinations for MH Treatment.

Number of Runs	Temperature (°C)	Treatment Time (min)
1	40	24
2	40	60
3	40	60
4	41	10
5	41	10
6	44	41
7	46	10
8	47.5	26
9	50	60
10	50	60
11	51	38
12	53	15
13	60	10
14	60	10
15	60	60
16	60	60

#### 4.5.4. Statistical Analysis

All the microbiological analyses and physical measurements were repeated three times. The data were expressed as mean value  $\pm$  standard deviation. The data obtained after MH treatment were statistically analysed by Design Expert programme. The source of variation occurred in the data was investigated by means of analysis of variances (ANOVA). ANOVA tables were generated in order to determine the effective factors on the responses. Subsequently, optimum treatment conditions were chosen considering 3D plots given by the software.

## **4.6. CaCl<sub>2</sub> Dipping**

### **4.6.1. Application**

Whole apricot samples were dipped into CaCl<sub>2</sub> for different time periods. CaCl<sub>2</sub> solutions were prepared in the glass beaker at different concentrations. The temperature of the solution was kept constant using a thermostatically controlled water bath externally. Apricot to solution ratio was 1:4 (w/v). After the dipping process, fruits were air dried for 20 min. Then they were used for further analyses.

### **4.6.2. Process Parameters**

Process parameters were CaCl<sub>2</sub> concentration (0-3%) and dipping time (2-21 min). Different concentrations of CaCl<sub>2</sub> and different dipping times (min) were evaluated at 40°C. Although temperature was not considered as a process factor, the effect of dipping at 25°C was also assessed using the highest CaCl<sub>2</sub> concentration (3%) and the highest dipping time (21 min). The processing parameters were determined according to the similar studies found in literature (Irfan et al., 2013; Zheng et al., 2014; Lamikanra and Watson, 2007; Liu et al., 2009a; Chen et al., 2011; Beirao-da-Costa et al., 2008).

### **4.6.3. Experimental Design**

Response Surface Methodology (RSM) was used for the optimization study. A D-optimal design was created by Design Expert (7.0.0) (Stat-Ease, Inc., USA). Factors were CaCl<sub>2</sub> concentration (0-3%) and the treatment time (2-21 min). Different combinations of the factors were given in Table 4.3. There were totally 16 runs. Response variable was firmness (N). The optimization purpose was to find the treatment conditions that would yield the highest firmness value.

Besides, the effect of dipping process of apricots in 6% of CaCl<sub>2</sub> at 25°C and 40°C for 21 and 30 min were also assessed in view of the results of the previous design.

Table 4.3. Concentration - Treatment Time Combinations for CaCl<sub>2</sub> Dipping.

Number of Runs	Concentration (%)	Treatment Time (min)
1	0	2
2	3	2
3	0	7.55
4	1.70	12.70
5	1	2
6	2	2.30
7	0	21
8	1.70	12.70
9	3	21
10	3	21
11	1.5	21
12	3	10.36
13	0.2	14.32
14	0	2
15	3	2
16	0	21

#### 4.6.4. Statistical Analysis

All the measurements were carried out in triplicate. The data were presented as mean values± standard deviation. Statistical analysis was conducted by Design Expert software. ANOVA table was constructed to determine the factor effects. Moreover, one way analysis of variance and a Tukey's test was applied using Minitab 16 (Minitab Inc, US/Canada) in order to find out the CaCl<sub>2</sub> concentration that would result in a significant difference on the firmness value (p<0.05).

#### 4.7. UV-C Light Irradiation

Whole apricot samples were used for UV-C irradiation process without subjecting them into any pre-treatment. For this purpose two different bench top UV-C units were utilized. The details of the systems were given in the next sections.

The effect of UV-C light on the inactivation of microorganisms (i.e. natural flora on the surface of apricots) was examined by determining total aerobic plate count (TAPC), coliforms, yeast and mould count (YMC).

#### 4.7.1. Bench Top UV-C Systems

##### 4.7.1.1. Two-Lamp UV-C Lightening System

UV-C inactivation studies were performed in order to determine the logarithmic reduction levels of natural microorganisms on the surface of apricots by using bench scale collimated beam apparatus which was described by Bolton et al. (2003). The system consists of two identical 15 W low pressure mercury vapour lamps (UVP XX-15, UVP Inc., CA, USA) emitting UV-C light at 254 nm. About 30% of the light is emitted in the UV-C range. The shortwave UV-C intensity measured at 2 inches is  $1,670\mu\text{W}/\text{cm}^2$ . The system configuration was shown in Figure 4.5.

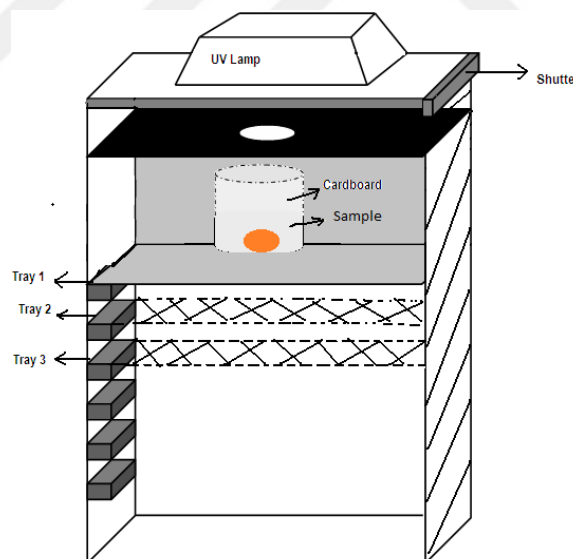


Figure 4.5. Two-lamp UV-C system.  
(Source: Hakgüder, 2009)

In this system, lamps were mounted on the top. A manually controlled shutter just below the lamps was used to regulate the UV-C light passing through the hole located under the shutter. In order to prevent the light scattering, a cardboard was used. Moreover a tray system was assembled in the system to adjust the incident light

intensity. Three apricot samples were processed for each treatment. A layer of aluminium film was placed under the fruit to ensure that the bottom of the fruits expose to UV-C irradiation. Also, fruits were manually rotated by 180° at half time of the estimated exposure time to provide uniform radiation so that all parts of the fruit had equal chance to receive the light from the lamp. The system was closed by a front door for safety reasons. Before the treatment lamps were switched on about 30 minutes to provide complete activation.

#### 4.7.1.2. Four-Lamp UV-C Lightening System

Configuration of custom made four-lamp system designed and constructed for apricot applications was shown in Figure 4.6.

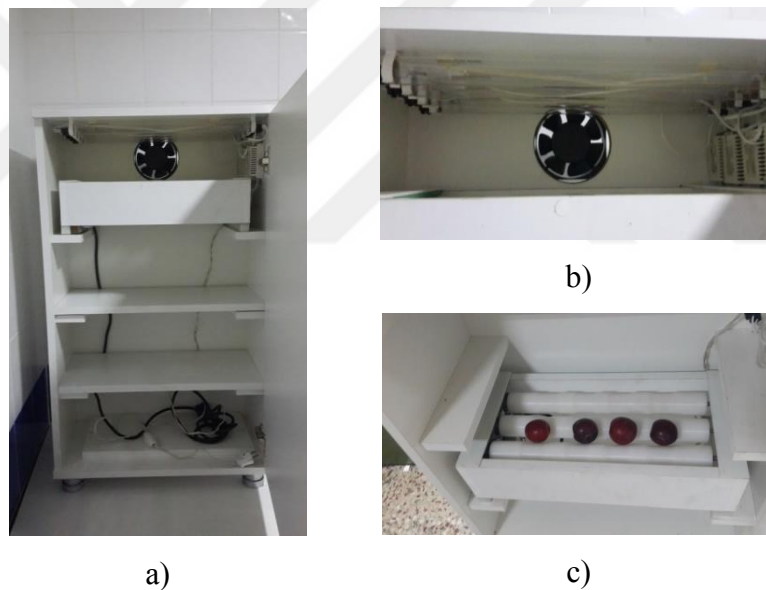


Figure 4.6. Four-lamp system; a) Tray system b) UV lamps and a cooling fan c) Rotating roller bearing.

A tray system was designed to allow an application of different UV light intensity levels (Figure 4.6.a). The distance between the lamps and the rotating roller bearing was 10.8 cm when the first tray was used. Moreover, the system consisted of four identical low pressure mercury vapour UV-C lamps emitting light at 254 nm (UVP XX-15, UVP Inc., CA, USA) (Figure 4.6.b). The lamps are of 15 W, 50.2 cm in length (L) with a 2.5 cm diameter quartz sleeve. Since working with four lamps may cause a temperature rise in the chamber, a cooling fan was also installed on the back side of the

cabin (Figure 4.6.b). Eight apricot samples were processed at the same time. A rotating roller bearing (dimensions of 45x22x14 cm) moving fruits upward and downward at a speed of 2 rpm was designed to provide a uniform radiation among fruit surfaces (Figure 4.6.c). In order to prevent the direct contact of UV light to human skin a cover was used to close the front part of the system.

#### 4.7.2. UV Dose (Fluence) Measurement

UV dose ( $D$ ) is the product of light intensity ( $I$ ,  $\text{mW}/\text{cm}^2$ ) and time ( $t$ , s) as shown in the following formula (4.12) (Yun et al., 2013);

$$D = I \times t \quad (4.12)$$

##### 4.7.2.1. Intensity Measurement

Incident light intensity ( $I$ ) at the level of the sample was directly measured by a radiometer (UVX-25, UVP Inc., CA, USA) (Figure 4.7) and expressed as  $\text{mW}/\text{cm}^2$ .



Figure 4.7. UV radiometer.  
(Source: UVP, 2016)



#### 4.7.2.2. UV-C Light Intensity Distribution in Four-Lamp UV-C Lighting System

Samples were located on different places along the rotating roller bearing axis in the four lamp system. In multiple-lamp systems samples might be exposed to different doses. It is of great importance to provide same UV dose for all samples during the UV-C radiation in such a multiple-lamp system. Determination of the light intensity distribution is necessary to better design the applications and to control UV dose variations. Therefore a mathematical model (Multiple Point Source Summation (MPSS) Method) was applied to find the incident light intensity distribution in the treatment chamber. On the other hand, instrumental measurements of incident light intensity ( $I$ ) were also performed. Light intensity at 15 different locations on the axis of the rotating roller bearing (Figure 4.8) was directly measured with the radiometer and expressed as  $\text{mW}/\text{cm}^2$ . Simulated results were compared with the experimental measurements.

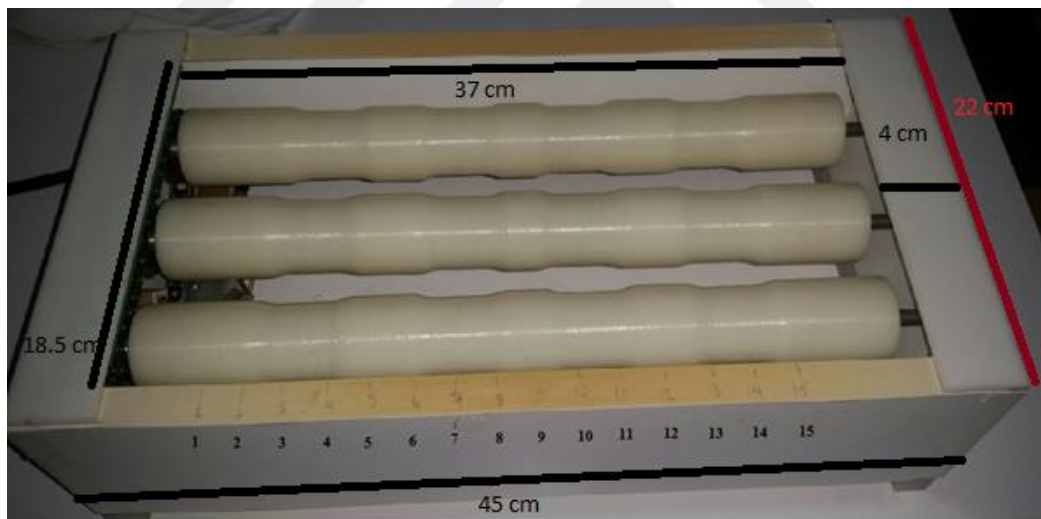


Figure 4.8. Intensity measurements on the rotating roller bearing.

##### 4.7.2.2.1. Multiple Point Source Summation (MPSS) Method

Incident light intensity distribution along the lamp axis and through the radial direction was calculated using finite-line source model (MPSS) reported by Blatchley III (1997) and Unluturk et al. (2004). The parameters used in this model were shown in Figure 4.9.

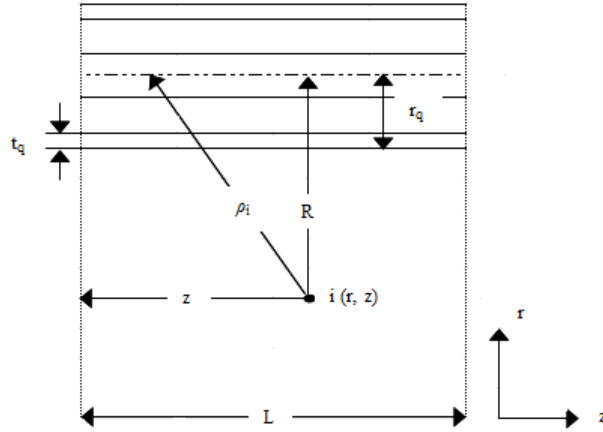


Figure 4.9. Schematic drawing of the parameters used in MPSS model.

This model assumes that the lamp is made up of “n” number of point sources emitting UV-C light at an equal intensity level (Unluturk et al., 2004). The intensity received by a receptor site at any location in the reactor is estimated as the sum of the light energy emitted from all point sources (Equation 4.13). Receptor was assumed to be a point or a small sphere as per stated by Unluturk et al. (2004).

$$I(r, z) = \sum_{i=1}^n \frac{P_{\lambda}}{4\pi\rho_i^2} \exp \left[ -(\sigma_q t_q + \sigma_a (R - r_q)) \frac{\rho_i}{R} \right] \quad (4.13)$$

$P_{\lambda}$ ; Lamp output power at wavelength  $\lambda$  (W)

$\rho_i$ ; Distance from  $i$ th point source to receptor site (cm)

$\sigma_q$ ; Absorbance coefficient for quartz jacket ( $\text{cm}^{-1}$ )

$t_q$ ; Quartz jacket thickness (cm)

$\sigma_a$ ; Absorbance coefficient for air ( $\text{cm}^{-1}$ )

$R$ ; Radial distance from lamp axis to receptor site (cm)

$r_q$ ; Quartz jacket outside radius (cm)

According to the formula, absorption of the emitted light by the air and quartz jacket was neglected. Moreover, UV-C light intensity provided by other light sources was also not considered. The distance between the lamp axis and rotating roller bearing was divided into 100 points whereas axial direction was considered to be composed of 1001 points in order to partition the discrete region and create a mesh for mathematical solution.

### **4.7.3. Process Parameters**

The only process variable was exposure time. Factor levels were between 4 and 20 min. Incident light intensity was kept constant at its maximum level since it was known that light penetration was limited. Therefore the tray in the two lamp and four lamp UV-C lighting systems was kept at the highest level in order to maximize the light intensity. The distance between the sample and the light source, as well as the incident light intensity measured at this distance in the two lamp UV-C lighting system was 23 cm and  $0.29 \text{ mW/cm}^2$ , respectively. On the other hand, the incident light intensity was measured as  $3.23 \pm 0.48 \text{ mW/cm}^2$  at a distance of 10.8 cm in four lamp UV-C lighting system.

### **4.7.4. Experimental Design**

A D-optimal design was created for the experiments conducted in both systems. D-optimal design was used to reduce the number of experiments as it was explained in section 4.5.3. Factor levels given by the design were 4, 6, 8, 10, 12, 16, 18, 20 min. There were totally 13 runs. However, experiments were repeated at least three times. Response variable was survival number (log CFU/g).

Additionally, 25 and 30 min of exposure times were also tested in four-lamp system. The goal of optimization study was to minimize the survival number, i.e., to provide high microbial inactivation on the surface of apricot samples.

### **4.7.5. Statistical Analysis**

Design Expert software was utilized to statistically evaluate the microbiological data. Analysis of variances (ANOVA) was performed in order to explain the changes occurred in the data. ANOVA tables were obtained. A numerical optimization was also performed to find the optimum exposure time which would result in the lowest survival number, i.e. the highest inactivation level of microorganisms naturally found on the surface of apricot samples. Furthermore Tukey's test was applied to observe any

significant changes in the mean values of survival numbers after different exposure times.

#### **4.8. Hurdle Technology**

CaCl<sub>2</sub> application, MH treatment and UV-C irradiation were combined after finding their optimum processing conditions providing the highest microorganism inactivation rate on the surface of apricot samples. CaCl<sub>2</sub> dipping and MH treatment were applied together at predefined conditions using aforementioned methods. After being processed by CaCl<sub>2</sub>+MH, apricots were subjected to UV-C light for optimum exposure time (MUC). Effect of combined treatments (MUC) on the microbial and sensorial quality, firmness, colour, TPC, PPO and PME enzyme activities were assessed.

Modified Atmosphere Packaging (MAP) and storage conditions were also considered in the concept of hurdle technology. The details of packaging and storage were given in the next sections.

#### **4.9. Modified Atmosphere Packaging (MAP)**

Samples treated by combined methods (MUC) and untreated control samples were packed for the shelf life study using MAP technique.

A bench-scale packaging equipment (Multivac P200, Germany) as shown in Figure 4.10 was used for MAP. The concentration and composition of gas mixture used in modified atmosphere packaging was 3%O<sub>2</sub>, 4% CO<sub>2</sub>, 93% N<sub>2</sub> as selected by considering the literature data (Batu, 2009).



Figure 4.10. Table-top MAP equipment.  
(Source: Cheftools, 2016)

Treated (MUC) and untreated apricots were packed using MAP. For this purpose almost 8 apricot samples were filled into the double layer (Polyamide/Polyethylene) film bags (15 x 30 cm) which had a thickness of 80  $\mu\text{m}$ . The oxygen and carbondioxide permeability of the packaging material was 87.75 mL  $\text{m}^2$  day atm and 175.97 mL  $\text{m}^2$  day atm at 23  $^{\circ}\text{C}$ , respectively. On the other hand water vapour permeability was 11.82 g  $\text{m}^2$  day mmHg at 37.8  $^{\circ}\text{C}$  and 90% of relative humidity. The atmosphere existed in the bag was removed by vacuum application. Afterwards specific gas mixture was automatically injected into the bags for 5 s at a rate of 8  $\text{m}^3/\text{h}$ .

In order to reveal the effect of MAP on the shelf life of apricot samples, untreated control samples were packed under MAP (Control + MAP) and without MAP (Control - MAP). In order to exclude the MAP effect, 3 mm-diameter holes were made on each corner of the packaging material as per suggested by Choi et al. (2015).

Storage was performed with 3 different treatments including control samples namely, MUC + MAP, Control + MAP, Control – MAP. Totally 24 packages were prepared for the shelf life study (3 treatments x 4 weeks x 2 replications).

#### **4.10. Shelf Life Study**

As a part of hurdle technology, MUC + MAP, Control + MAP, Control – MAP samples were stored at 1  $^{\circ}\text{C}$  for 28 days. The changes in some physicochemical attributes and microbial quality of the apricots were assessed during the storage to determine their shelf life. The sampling has been made at intervals of 7-days during the storage period. The shelf life of these apricots was calculated by counting the days

required for them to attain the last stage of ripening, but up to the stage when they still remain acceptable for marketing (Ramano Rao et al., 2011).

#### **4.10.1. Microbiological Quality**

The microbial shelf life of the products was monitored on the day of 0, 7, 14, 21 and 28 by evaluating the total aerobic plate counts, yeast and mould counts, and coliforms. The microbiological analyses were conducted as described in the section 4.4.

#### **4.10.2. Physicochemical Quality**

Firmness, ascorbic acid content, colour, TA %, Brix °, RI, pH values of the samples were determined during the course of the shelf life study according to the aforementioned methods. Moreover weight loss was calculated by the following formula (4.14).

$$\text{Weight Loss \%} = \frac{W_0 - W}{W_0} \times 100 \quad (4.14)$$

$W_0$ ; is the initial weight of the apricots and  $W$ ; is the weight of the apricots measured on the sampling day.

#### **4.10.3. Statistical Analysis**

All the measurements were repeated two times. Mean values of microbial and physicochemical data were used for statistical analysis. Results were statistically evaluated by Tukey's test using Minitab 16 (Minitab Inc, US/Canada). Existence of a significant difference between the resulting mean values of the measuring parameters for each treatment was investigated for each sampling day by pairwise comparison.

## CHAPTER 5

# THE EFFECTS OF UV-C IRRADIATION AND MILD HEATING ON NATURAL MICROFLORA OF APRICOT FRUIT SURFACES

### 5.1. Introduction

Apricots are highly perishable products. Especially, the marketability of cv. Şalak apricots is limited due to its high water content. It was reported that aging process of the fruits occurs rapidly after harvesting (Hashemi et al., 2017). Moreover, favourable conditions of apricots for microbial invasion restrict their shelf life (Wei et al., 2014). Microbial growth was indicated to be the major cause for fruit losses at the postharvest stage (Villalobos et al., 2017).

Different techniques, such as ionizing irradiation, edible film coating, UV-C radiation and mild heating (Wei et al., 2014; Cheon et al., 2015; Hashemi et al., 2017) were used for the postharvest microbial quality maintenance of fruits and vegetables. It was reported that UV-C irradiation had an inactivation effect on various microorganisms and viruses (Cheon et al., 2015). Additionally, UV-C processing does not cause the formation of toxic by-products. It is also favourable from an economic point of view. Combination of UV-C treatment with mild heating was revealed to increase the inactivation efficiency and maintain the sensory and nutritional quality of the products (Cheon et al., 2015). Furthermore, adverse impacts of heating can be avoided using mild conditions in combination to UV-C treatment.

Considering these, the objectives of this study were: to determine the effect of UV-C irradiation and mild heat (MH) treatment on natural flora of cv. Şalak apricot surfaces and to find the optimum conditions for both UV-C irradiation and MH which would result in the highest microbial reduction without altering the fruit quality.

## 5.2. Materials and Methods

### 5.2.1. Apricots

Apricots which were free from any noticeable contamination and defect were selected and used for further analyses (details were given in the section 4.1).

### 5.2.2. UV-C Treatments

#### 5.2.2.1. Two-Lamp UV-C Lightening System

The two-lamp UV-C lightening system was well described in section 4.7.1.1.

##### 5.2.2.1.1. Measurement of UV Dose

Incident light intensity was measured as  $0.29 \text{ mW/cm}^2$  in two-lamp system by means of a radiometer. UV dose received by the apricot samples were calculated as it was described in the section 4.7.2. Applied UV dosages were ranging between 0 and  $348 \text{ mJ/cm}^2$  (Table 5.1).

Table 5.1. Applied UV Doses in Two-Lamp System.

Exposure Time (s)	Incident Light Intensity (I, $\text{mW/cm}^2$ )	UV Dose ( $\text{mJ/cm}^2$ )
0	0.29	0
240	0.29	69.6
360	0.29	104.4
480	0.29	139.2
600	0.29	174
720	0.29	208.8
960	0.29	278.4
1080	0.29	313.2
1200	0.29	348



### **5.2.2.1.2. UV-C Treatment of Apricots**

Application procedure was explained in section 4.7.1.1. Briefly, apricot samples were placed under the light source and the light was directed onto the samples in order to prevent scattering. All the surfaces of the fruits were exposed to same UV dose by manual rotation of the samples at definite time intervals. Different exposure times ranging between 4-20 min were assessed in terms of microbial inactivation. Process parameters and experimental design were given in section 4.7.4. Samples were microbiologically analysed after the treatment.

### **5.2.2.2. Four-Lamp UV-C Lightening System**

Details of the four-lamp system were well documented in section 4.7.1.2.

#### **5.2.2.2.1. UV-C Intensity Distribution**

UV-C light intensity distribution in the four-lamp system was found both instrumentally and mathematically considering the method given in section 4.7.2.2. Instrumental measurements were performed at different points along the rotating roller bearing by a radiometer as indicated in section 4.7.2.1. For mathematical calculations multiple point source summation method (MPSS method) explained in section 4.7.2.2.1 was used.

#### **5.2.2.2.2. UV-C Treatment of Apricots**

Apricots were subjected to UV-C light in four-lamp system using a rotational roller bearing in order to provide a rotational motion of the samples during the treatment. The details can be found in section 4.7.1.2. Process parameter was exposure time (4-20 min). Experimental design was explained in section 4.7.4. After the treatment, microbial inactivation was investigated by means of microbial analysis.

### **5.2.2.2.3. Modelling Inactivation Data**

Microbial inactivation data were modelled using a freeware tool, GInaFiT, of which details were reported in section 4.4.1. Different models were utilized for this purpose. The model which could best describe the data was chosen considering the goodness of fit parameters such as the determination coefficients and RMSE values (section 4.4.1).

### **5.2.3. Mild Heat (MH) Treatment**

Mild heat (MH) treatment was performed in a temperature controlled water bath as previously described in section 4.5.1. Apricot samples were dipped into the water at different temperatures for different treatment times. Process parameters and experimental design was given in section 4.5.2 and 4.5.3, respectively. Colour and firmness measurements were performed using the methods given in section 4.2.8 and 4.2.9, respectively. Moreover, the effect of treatment on microbial counts was also examined.

### **5.2.4. Microbial Analysis**

Microbiological analysis was carried out according to the method which was described in Chapter 4, section 4.4. Briefly, the natural microorganisms found on apricot surfaces, i.e. total mesophilic aerobic bacteria (i.e., total aerobic plate count, (TAPC)), coliform bacteria, yeasts and moulds (i.e., yeast and mould count, (YMC)) were determined by enumerating on the TSA, VRBA and PDA plates by using a spread plating technique. TSA, VRBA and PDA plates were incubated at 30°C for 3 days, 37°C for 1 day and 25°C for 5 days, respectively. Microbial loads were expressed as log CFU/g.

### **5.2.5. Statistical Analysis**

Microbial data obtained after UV-C treatments were statistically evaluated and ANOVA tables were generated as it was described in section 4.7.5. Moreover, existence of a significant difference between mean values of TAPC, YMC and coliform counts were also evaluated by Tukey's test. Optimum treatment time was determined considering the statistical results.

Besides, statistical analyses of microbial data, firmness and colour values obtained after MH treatment were also performed and optimum treatment conditions were selected as it was described in section 4.5.4.

## **5.3. Results and Discussion**

### **5.3.1. UV-C Treatment by Means of Two-Lamp UV-C Lightening System**

Apricot samples were microbiologically analysed prior to any UV-C treatment. According to the microbial count results, control samples were containing  $5.86 \pm 0.25$  log CFU/g of mesophilic aerobic bacteria (TAPC). Whereas yeast and mould count (YMC) was  $5.70 \pm 0.62$  log CFU/g. On the other hand, average number of coliforms was obtained as  $4.54 \pm 1.73$  log CFU/g. It is obvious that a high standard deviation caused by the sample variations. Janisiewicz and Buyer (2010) stated that natural (or resident) micro flora of nectarine fruit surfaces were not studied well. Therefore, the knowledge about the microbial ecology of these types of fruit surfaces is very limited. There is a lack of information about the native flora of cv. Şalak apricot surfaces. The total mesophilic aerobic counts obtained in this study were higher than the work conducted by Türkyılmaz et al. (2013). They reported the total mesophilic aerobic bacteria in apricots (*Prunus armenica* L., var. Hacıhaliloğlu) as 3.45 log CFU/g. This value was lower compared to our study because different apricot varieties were used in two studies. Although no literature information is available about native flora count on Şalak apricot cultivar fruit surfaces, it can be concluded that total aerobic counts and yeast and mould count of apricot samples are compatible with the results of the study performed

by Allende et al. (2006). They found approximately 5.2 log CFU/g total aerobic count and 4.8 log CFU/g yeast count on the surface of 'Red Oak Leaf' lettuce.

Figure 5.1 shows the changes in the total aerobic plate count (TAPC) on the apricot surfaces exposed to UV-C at different exposure times.

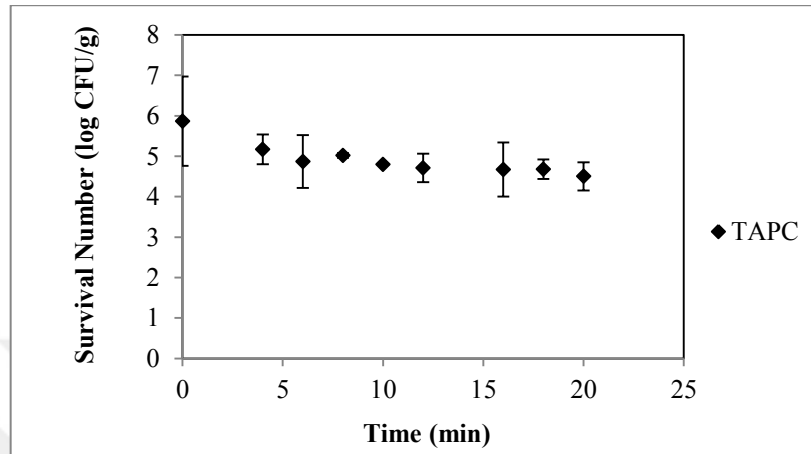


Figure 5.1. The total aerobic plate count (TAPC) on the apricot surfaces exposed to UV-C irradiation by two-lamp system.

It is clear that UV-C light illumination was not sufficiently effective on TAPC. Although TAPC of samples displayed a decreasing trend, it can be observed that survival number declined at a decreased rate and stayed almost constant after 12 min of UV-C light exposure (Figure 5.1).

The logarithmic reductions of TAPC, YMC and coliform obtained after the UV treatment were given in Table 5.2. According to the results, TAPC was reduced by 1.36-log after 20 min of UV-C light exposure ( $348 \text{ mJ/cm}^2$ ) (Table 5.2). Although the highest reduction (1.36 log CFU/g at  $348 \text{ mJ/cm}^2$  UV dose) in TAPC seemed to be obtained after 20 min of UV-C exposure, pairwise comparison test indicated that increase in exposure time did not significantly change the log survival number of TAPC (Table 5.2).

Table 5.2. Log-Survival and Log-Reduction Numbers Obtained in Two-Lamp System.

ExposureTime (s)	Log Survival (TAPC)	Log Reduction (TAPC)	Log Survival (YMC)	Log Reduction (YMC)	Log Survival (Coliforms)	Log Reduction (Coliforms)
0	5.86±1.10 <sup>a</sup>	0	5.70±0.66 <sup>a</sup>	0	4.54±1.73	0
240	5.17±0.36 <sup>a</sup>	0.69	5.40±0.28 <sup>a</sup>	0.3	ND	4.54
360	4.87±0.65 <sup>a</sup>	0.99	5.38±0.06 <sup>a</sup>	0.32	ND	4.54
480	5.02±0.06 <sup>a</sup>	0.84	5.43±0.04 <sup>a</sup>	0.27	ND	4.54
600	4.79±0.01 <sup>a</sup>	1.07	5.10±0.33 <sup>a</sup>	0.6	ND	4.54
720	4.71±0.35 <sup>a</sup>	1.15	5.42±0.09 <sup>a</sup>	0.28	ND	4.54
960	4.67±0.67 <sup>a</sup>	1.19	5.06±0.61 <sup>a</sup>	0.64	ND	4.54
1080	4.68±0.24 <sup>a</sup>	1.18	5.01±0.01 <sup>a</sup>	0.69	ND	4.54
1200	4.50±0.35 <sup>a</sup>	1.36	5.14±0.15 <sup>a</sup>	0.56	ND	4.54

<sup>a</sup>: Means having same letter in the same column denote insignificant difference at  $p > 0.05$ .

ND: Not detected.

The lack of a significant difference in the number of survivals in response to increased UV dose might be due to the surface properties of the samples as explained by Guan et al. (2012). It was noted that microorganisms found on uneven surfaces such as mushrooms, could survive from the lethal effect of the treatment by protecting themselves from UV-C light exposure (Guan et al., 2012). Furthermore, variations in the initial load of the samples might affect the results. Although all the analyses were replicated at least three times and three apricot samples were microbiologically analysed for each treatment, there was still a discrepancy in the microbial data. Additionally, manual rotation of the apricots during the treatment might not be sufficient to provide an equal UV-C dose among fruit surfaces.

Conversely, no coliform growth was detected after UV-C treatment (Table 5.2). The difference between log-reductions of TAPC and coliforms can be explained by sensitivity of different microorganisms to UV-C light which may be related to the amount of thymine bases found in their genetic material (Koutchma et al., 2009). Cantwell and Hofmann (2008) speculated that coliforms were sensitive to UV-C irradiation. Unluturk et al. (2010) reported that gram negative bacteria (such as coliforms) were more sensitive to UV-C light than gram positive bacteria due to their cell wall property. Gram positive bacteria were indicated to have a thick layer of peptidoglycan which does not allow the UV light to penetrate inside the cell (Unluturk et al., 2010). However, gram negative bacteria can not resist UV-C light due to their thin peptidoglycan layer (Unluturk et al., 2010). Low level of reduction in TAPC might be also due to the existence of a mixed microbial population on the fruit surface. The

mixed microbial culture and high numbers of colonies on the surfaces exhibit a shielding effect (Hakguder Taze et al., 2015). Allende et al. (2006) also studied the inactivation of native flora in ‘Red Oak Leaf’ lettuce and they were able to decrease lactic acid bacteria population just by 1 log after 711 mJ/cm<sup>2</sup> of UV dose. It was reported that inactivation of natural flora was much more difficult than that of inoculated microorganisms (El-Hag et al., 2006; Hakguder Taze et al., 2015). For instance, El-Hag et al. (2006) achieved 1-log more reduction in the number of inoculated microorganisms in orange juice as compared to the reduction of naturally grown microorganisms after the juice samples were treated under the same PEF conditions. In another study, similar PEF treatment conditions resulted in almost 3-log higher inactivation of *E. coli* inoculated into the apple juice than that of naturally grown microorganisms (El-Hag et al., 2010). It was speculated that inactivation of natural micro flora is difficult than inoculated microorganisms due to the differences in their growth phases and their resistance to environmental conditions (El-Hag et al., 2006; El-Hag et al., 2010). The resistance of natural flora was also explained as wild type microorganisms had a DNA repair mechanism that could excise pyrimidine dimers (Allende et al., 2006).

On the other hand, survival number of yeasts and moulds seems not to be significantly affected by UV-C irradiation (Figure 5.2). Microbial reduction was only 0.56-log CFU/g even if the samples were exposed to 20 min of UV-C light treatment (Table 5.2).

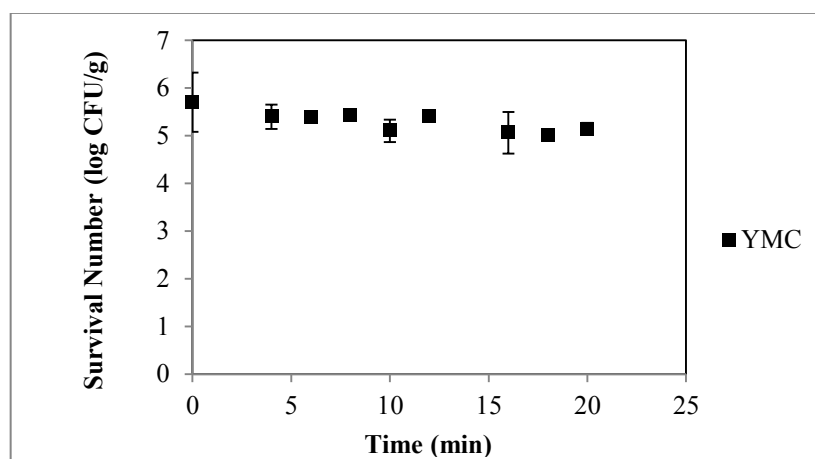


Figure 5.2. The yeast and mould count (YMC) on the apricot surfaces exposed to UV-C irradiation by a two-lamp system.

It means that UV-C light treatment was much less efficient in inactivating yeasts and moulds naturally occurring on apricot surfaces. Yeasts were reported to be more resistant to UV-C light than bacteria due to their big size, differences in genetic material and also in cell wall composition and thickness (Hakguder Taze et al., 2015).

There is a fluctuation in the log survival data of YMC given in Figure 5.2. This might be arisen from the variation observed in the natural flora counts on different sample surfaces. Use of more than three samples for one treatment time was highly recommended so as to prevent the variation in microbiological data. But then this brought about requirements for changes in the system configuration. It was concluded that a system that was able to process many apricots at the same time was required for UV-C treatment. Hence, a new system was designed and constructed for further studies.

Statistical analysis revealed that exposure time was a very significant factor ( $p=0.0001$ ) on the response. Also model was found to be very significant with a p value of 0.0003 (Figure 5.3).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	0.82	2	0.41	21.20	0.0003	significant
A-Exposure time	0.75	1	0.75	38.42	0.0001	
A <sup>2</sup>	0.093	1	0.093	4.81	0.0531	
Residual	0.19	10	0.019			
Lack of Fit	0.067	5	0.013	0.53	0.7472	not significant
Pure Error	0.13	5	0.025			
Cor Total	1.02	12				

Figure 5.3. ANOVA table for UV-C experiments with two-lamp system.

Interaction between exposure time and the survival number of TAPC signified that longer exposure times did not improve the microbial reduction (Figure 5.4).

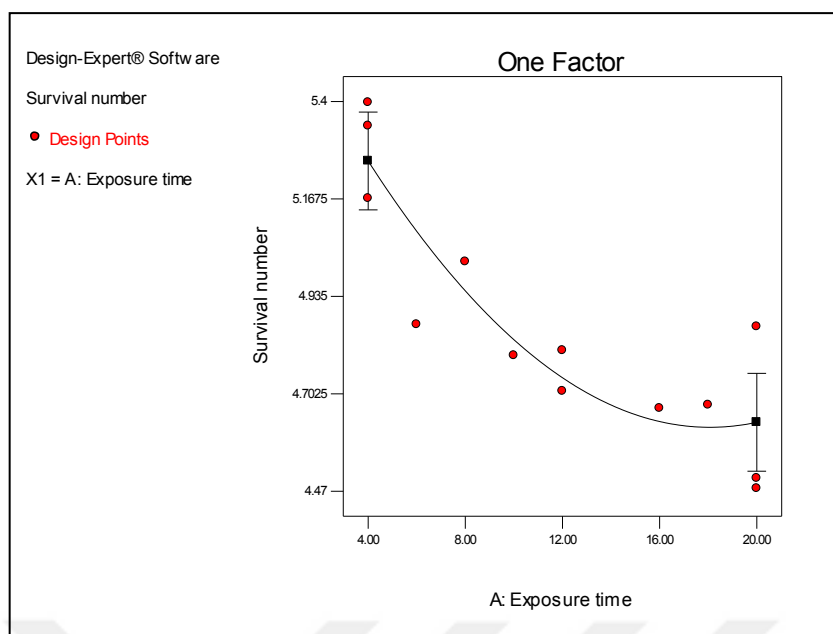


Figure 5.4. Interaction between exposure time and survival number of TAPC.

According to Figure 5.4 the survival number reached a plateau region after 12 min of exposure. Thereby improvement in the microbial reduction was not possible by applying longer exposure times. Moreover long treatment times are unfavourable with respect to industrial applications. Additionally, pairwise comparison test results referred to insignificant changes in the survival numbers obtained after different exposure times (Table 5.2). It means current system was unable to effectively decontaminate apricot surfaces. Considering these facts, the new system consisted of 4 lamps was designed to increase UV light intensity and consequently the UV dose. It was well documented that the rate of decrease in the number of survivals was associated with the applied UV dose (Koutchma et al., 2009). The results of the inactivation study performed by four-lamp system were given in the upcoming sections.

### 5.3.2. UV-C Treatment by Means of Four-Lamp UV-C Lightening System

Incident UV light intensity ( $I$ ,  $\text{mW}/\text{cm}^2$ ) was measured at fifteen different points on both front and back side of the rotating roller bearing axis in order to find the light intensity distribution (Figure 5.5). Average intensity ( $I_{\text{avg}}$ ) was calculated as  $3.23 \pm 0.48 \text{ mW}/\text{cm}^2$ .



<b>Roller 3</b>														
2.07	2.34	2.64	2.94	3.13	3.38	3.42	3.51	3.53	3.55	3.45	3.45	3.34	3.23	2.97
<b>Roller 2</b>														
2.13	2.5	2.85	3.18	3.38	3.55	3.65	3.73	3.76	3.71	3.71	3.66	3.52	3.32	3.19
<b>Roller 1</b>														

Figure 5.5. Measured intensity values along the rotating roller bearing.

Besides the instrumental measurements, radial and longitudinal UV light intensity profiles were estimated using MPSS model as previously described. Figure 5.6 and Figure 5.7 refer to the radial and axial distributions of light energy at selected distances, respectively. It is obvious that light intensity reached its maximum level at the nearest point to the lamp whereas it showed a decreasing trend as the receptor point moved radially away from the light source (Figure 5.6). As can be seen from Figure 5.6, UV intensity through the radial direction ranged from 1.65 mW/cm<sup>2</sup> to 17.19 mW/cm<sup>2</sup> at the surface of the rotating roller bearing.

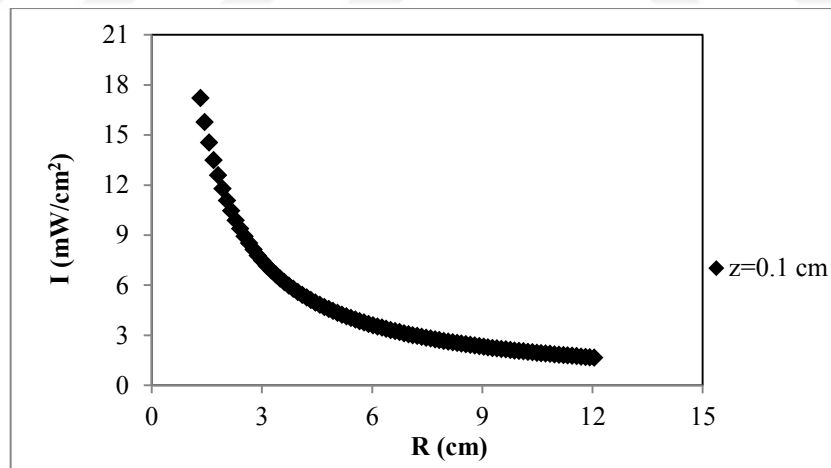


Figure 5.6. Radial UV intensity profile.

On the other hand, Figure 5.7 indicated that light intensity along lamp axis was lower at both ends of the tube, whereas it stayed almost constant at the centre of the lamp similar to other studies (Unluturk et al., 2004; Grimes et al., 2010; Reda, 2011). The maximum and minimum light intensity was calculated as 5.55 mW/cm<sup>2</sup> and 3.07 mW/cm<sup>2</sup>, respectively (Figure 5.7).

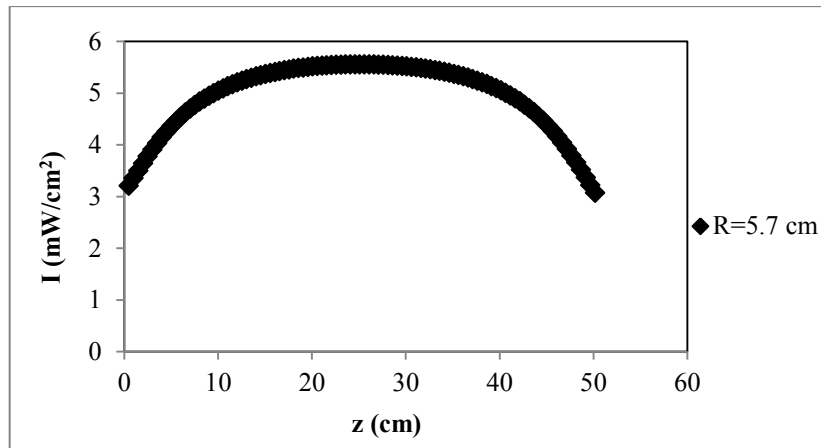


Figure 5.7. Axial UV intensity profile.

Considering the light intensity distribution along the lamp axis, it can be concluded that apricots which were placed near to the both ends of the rotating roller bearing received lower UV doses.

According to the instrumental measurements and numerical solutions for longitudinal light intensity distribution, MPSS model estimated higher intensity values compared experimental measurements (Figure 5.8). On the other hand, the light intensity profile estimated from MPSS model showed good agreement with experimental data (Figure 5.8). The prediction can be improved by taking the absorption of UV light in the air and the quartz sleeve covering the UV lamp into account (Unluturk et al., 2004). Moreover, the UV-C lamp output power was assumed to be 30% of the total lamp power which may have also contribution in over-estimating UV intensity data. Eliminating the errors in the modelling step can increase the prediction ability of MPSS model.

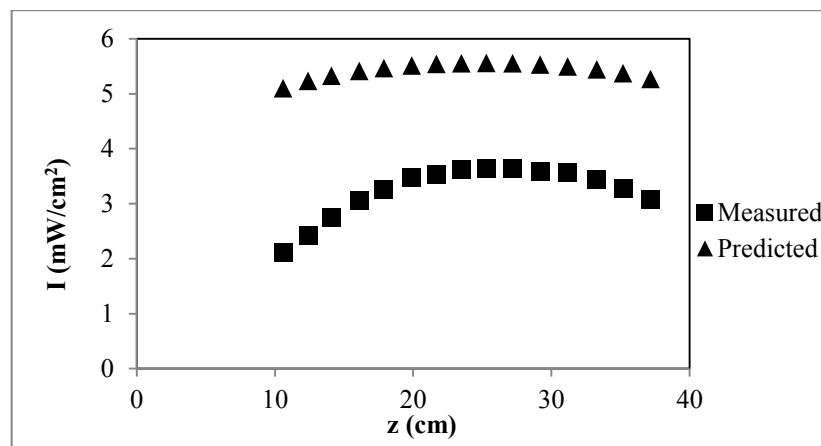


Figure 5.8. Comparison of predicted and measured light intensity along the lamp axis.

Estimated UV dose values in four-lamp system were ranging between 0 and 3876 mJ/cm<sup>2</sup> (Table 5.3).

Table 5.3. Estimated UV Doses in Four-Lamp System.

Exposure Time (s)	Average Light Intensity ( $I_{avg}$ , mW/cm <sup>2</sup> )	UV Dose (mJ/cm <sup>2</sup> )
0	3.23	0
240	3.23	775.2
360	3.23	1162.8
480	3.23	1550.4
600	3.23	1938
720	3.23	2325.6
960	3.23	3100.8
1080	3.23	3488.4
1200	3.23	3876

Previous results obtained with two-lamp system pointed out that the maximum UV dose was 348 mJ/cm<sup>2</sup> after 20 min of light exposure. As it can be seen from Table 5.3, new bench top UV-C system was very effective on increasing the UV dose value. Almost ten times higher UV dosage was reached after 20 min of exposure (3876 mJ/cm<sup>2</sup>). It was expected that higher UV doses would result in better inactivation of naturally occurring microorganisms.

According to the microbial count results, control samples were containing 6.10±0.80 log CFU/g of TAPC, 5.81±0.17 log CFU/g of YMC, and 4.74±0.53 log CFU/g of coliforms. However, samples displayed variations in microbial counts. This might be due to the discrepancy between the levels of infection of the fruits by microorganisms during growth and after harvesting (Guerreiro et al., 2016; Lamikanra, 2002).

To the best of our knowledge, no literature data was available about the resident microflora of fresh Şalak apricot cultivar. Hussain et al. (2011) obtained 5-log of TAPC and 5.1-log of YMC on dried apricot surfaces. Similarly Huang et al. (2013) enumerated 5.31-log of TAPC and 4.68-log of YMC in apricot nectar which was extracted from untreated apricot samples. It was stated that resident microflora of fruits were not studied well (Janisiewicz and Buyer, 2010). Therefore, the knowledge about the microbial ecology of fruit surfaces is very limited. Janisiewicz and Buyer (2010) reported that main genera identified on nectarine surfaces were *Curtobacterium* (21.31%), followed by *Pseudomonas* (19.99%), *Microbacterium* (13.57%), *Clavibacter*

(6.96%), *Pantoea* (6.59%) and *Enterobacter* (4.26%). Similar results with some exceptions were obtained with plum fruit in another study (Janisiewicz et al., 2013). Moreover, they indicated that *Pseudomonas syringae* and *Pseudomonas agglomerans* which have biocontrol activity against fruit decay could be found on nectarines throughout the entire growing season (Janisiewicz et al., 2013). *Curtobacterium* and *Pseudomonas* were demonstrated to be the most dominated ones in both studies. However, isolation and identification of the microorganisms were not addressed in this study.

Figure 5.9 shows the changes in log survival number of TAPC against exposure time.

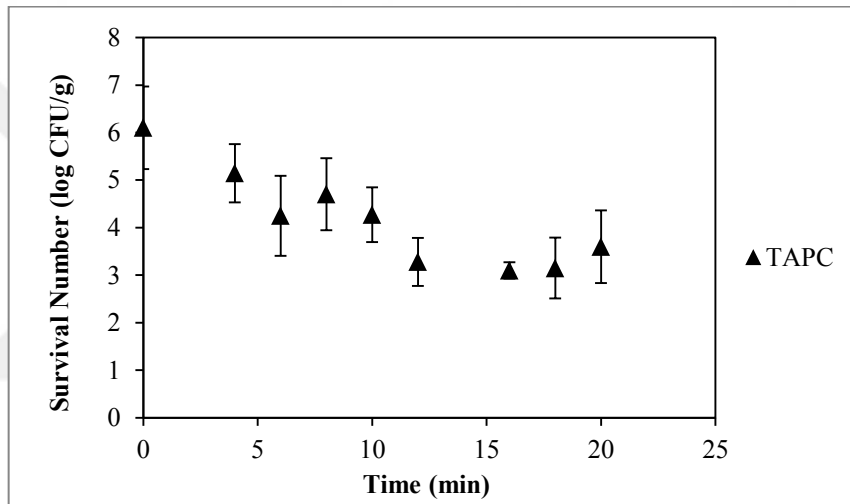


Figure 5.9. The total aerobic plate count (TAPC) on the apricot surfaces exposed to UV-C irradiation by a four-lamp system.

According to Figure 5.9, initial TAPC of 6.10-log CFU/g decreased up to 3.10 log CFU/g as the exposure time increased. However, some deviations occurred in the survival data. In order to clearly visualize the survival numbers Table 5.4 was given below.

Table 5.4. Log-Survival and Log-Reduction Numbers Obtained in Four-Lamp System.

ExposureTime (s)	Log-Survival (TAPC)	Log-Reduction (TAPC)	Log-Survival (YMC)	Log-Reduction (YMC)	Log-Survival (Coliforms)	Log-Reduction (Coliforms)
0	6.10±0.87 <sup>a</sup>	0	5.81±0.19 <sup>a</sup>	0	4.74±0.53	0
240	5.15±0.61 <sup>ab</sup>	0.95	3.44±0.41 <sup>b</sup>	2.37	ND	4.74
360	4.25±0.84 <sup>ab</sup>	1.85	3.57±0.45 <sup>b</sup>	2.24	ND	4.74
480	4.71±0.76 <sup>ab</sup>	1.39	3.96±0.92 <sup>b</sup>	1.85	ND	4.74
600	4.27±0.58 <sup>ab</sup>	1.83	4.08±0.54 <sup>b</sup>	1.73	ND	4.74
720	3.28±0.51 <sup>b</sup>	2.82	3.57±0.50 <sup>b</sup>	2.24	ND	4.74
960	3.10±0.17 <sup>b</sup>	3	3.56±0.49 <sup>b</sup>	2.25	ND	4.74
1080	3.15±0.64 <sup>b</sup>	2.95	3.86±0.22 <sup>b</sup>	1.95	ND	4.74
1200	3.60±0.76 <sup>b</sup>	2.5	3.45±0.49 <sup>b</sup>	2.36	ND	4.74

<sup>a-b</sup>: Means having different letters in the same column denote significant difference at  $p < 0.05$ .

ND: Not detected.

Despite the fact that maximum log reduction in TAPC was found as 3-log after 16 min of UV-C light exposure (at 3100.8 mJ/cm<sup>2</sup> UV dose), pairwise comparison indicated no significant differences in the survival numbers after 12 min and 16 min of UV exposure (Table 5.4). Although an increase in the exposure time was anticipated to yield higher log reductions, extending the treatment time did not improve the number of reductions in TAPC. Variation in the initial microbial loads of each apricot sample might cause this observation. Moreover, presence of UV resistant flora can also give rise to the tailing effect (Figure 5.9). Tailing phenomenon was explained as the continuation of the microorganisms to exist in the medium even after being exposed to higher UV-C doses (Koutchma et al., 2009).

Inactivation curve obtained for YMC was shown in Figure 5.10. Similar reduction trend as encountered for TAPC was observed for YMC (Figure 5.10). Unlike TAPC results, maximum reduction of YMC was 2.37-log just after 4 min of exposure. According to this, it can be concluded that inactivation of YMC was faster than TAPC. However, survival number of YMC could not be further decreased after longer exposure times. Applying higher doses did not significantly affect the number of survivals (Table 5.4). It means that UV-C light treatment was less efficient in inactivating yeasts and moulds naturally occurring on apricot surfaces.

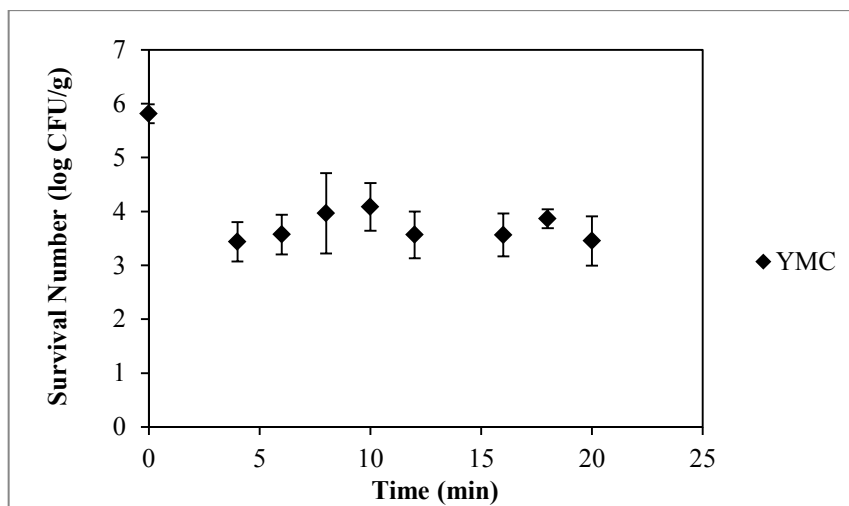


Figure 5.10. The yeast and mould count (YMC) on the apricot surfaces exposed to UV-C irradiation by a four-lamp system.

As it was mentioned before, samples were initially containing almost 4.74-log CFU/g of coliforms. Nonetheless, growth of coliforms was not attained in none of the samples after UV-C treatment (Table 5.4).

In order to describe the inactivation curve for both TAPC and YMC, different models were applied to the data as described before. Log linear + tail model proposed by Geeraerd et al. (2000) best fit the data with the lowest RMSE and the highest  $R^2$  and adj- $R^2$  values (Table 5.5).

Table 5.5. Comparison of RMSE,  $R^2$  and Adj- $R^2$  Values.

MODEL	TAPC			YMC		
	RMSE	$R^2$	Adj- $R^2$	RMSE	$R^2$	adj- $R^2$
Log Linear	0.5304	0.7543	0.7236	0.6341	0.2897	0.2009
Log Linear+Tail	0.3393	0.9126	0.8869	0.2486	0.9045	0.8772
Weibull	0.4615	0.8373	0.7908	0.3419	0.8194	0.7677
Weibull+Tail	0.3660	0.9123	0.8684	0.5146	0.6492	0.4738
Double Weibull	0.4462	0.8696	0.8044	0.3602	0.8281	0.7421
Biphasic	0.3665	0.9120	0.8680	0.2688	0.9057	0.8585
Biphasic+Shoulder	0.4015	0.9120	0.8417	0.2923	0.9057	0.8302

Although the first order kinetics model was widely used to describe the microbial inactivation behaviour of microorganisms subjected to different processing by various techniques, most of the studies proved that non-thermal inactivation of microorganisms might not be explained by linear models (Hakguder Taze et al., 2015).

There are many other studies in literature which report survival curves with tails and shoulders (Baysal et al., 2013). It was showed that non-linear survival curves were commonly used for describing the behaviour of microbial populations undergone to UV-C treatment (Koutchma, 2014b). Tailing observed in non-linear survival curves was found to be attributed not only to the differences in resistance of the cells to UV-C irradiation, but also the adaptation of microorganisms to UV-C light (Koutchma, 2014b).

Baysal et al. (2013) showed that UV-C inactivation of *Alicyclobacillus acidoterrestris* spores in both apple juice and grape juice obeying to log linear plus tailing model. This novel model is used to explain both the linear part and the tailing occurred in the survival data due to the existence of a subpopulation that is more resistant to treatment or that may not have undergone the same lethal dose, not completely deactivated or showing high variability in the number (Geeraerd et al., 2000). Hereinbefore, light intensity distribution in the four-lamp UV system indicated variations in applied UV doses among apricots. Thereby, microorganisms which were not exposed to same UV dose might have survived UV-C treatment causing a tailing in the survival curve. Moreover, variations in the initial microbial loads of fruit samples could bring about the underestimation of the real effect of the treatment. It is also possible to explain the tailing phenomenon by existence of a mixed population on the apricot surfaces having different resistances to the UV-C light.

Similar to the previous results obtained using two-lamp system, exposure time was found to be a significant factor on the survival number with a p-value of 0.0004 (Figure 5.11). Also model was significant ( $p= 0.0009$ ). Moreover, interaction between the factor and the response variable indicated the same pattern as it was obtained in two-lamp system.

Response	1 Survival number					
ANOVA for Response Surface Quadratic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	6.24	2	3.12	15.42	0.0009	significant
A-Exposure time	5.44	1	5.44	26.90	0.0004	
A <sup>2</sup>	0.94	1	0.94	4.63	0.0570	
Residual	2.02	10	0.20			
Lack of Fit	0.71	5	0.14	0.54	0.7396	not significant
Pure Error	1.31	5	0.26			
Cor Total	8.26	12				

Figure 5.11. ANOVA table for UV-C experiments with four-lamp system.

Optimization of the exposure time was required to reach the minimum level of survivals. Considering the microbiological results, treatment times of 25 min and 30 min were also tested for more microbial reduction. 2.24-log and 2.88-log reductions in TAPC were obtained for 25 and 30 min, respectively. On the other hand, YMC was reduced by 2.20-log and 1.72-log after 25 and 30 min of UV-C exposure, respectively. However, application of longer exposure times did not significantly alter the survival numbers ( $p > 0.05$ ) as compared to the other results given in Table 5.4. As a conclusion, the treatment times above 20 min would not be very effective to reach the target log reduction on apricot surfaces. Besides, optimization solution given by Design Expert programme also referred to 19 min of exposure time in order to minimize the survival number (Figure 5.12).

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Exposure time	is in range	0	30	1	1	3
Survival number	minimize	3	6.101	1	1	3
<b>Solutions</b>						
Number	Exposure time	Survival number	Desirability			
1	<u>19.37</u>	<u>3.37126</u>	<u>0.880</u>	<u>Selected</u>		
2	19.41	3.37127	0.880			

Figure 5.12. Optimum exposure time given by statistical analysis.

It was concluded that, handling samples containing varying amount of initial microbial loads was the biggest challenge in this study. Although eight fruits were used



for each treatment times, and the average values of many replicates were taken into account to explain the results, there was discrepancy in the log survival data. This situation interferes with the interpretation of the results. Another problem was the variations arisen in light intensity measurements. Hereinbefore light intensity measurements were taken from fifteen different places on the surface of the rotating roller bearing. However, it was realized that the samples did not achieve same amount of UV dose due to the inequality of the intensity values among different places. Similar case was reported by Yan et al. (2014). It was denoted that ensuring dose uniformity among the fruit samples was the main problem in UV-C treatment of fruits due to their irregular shapes and overlapping each other while they were being processed (Yan et al., 2014). Therefore researchers set up a commercial UV-C chamber and evaluated the dose uniformity (Yan et al., 2014). Similar to our findings, they discovered that UV intensity was low at the end of the chamber. Film dosimeters were also used to measure the radiation dose received by each apricot. Results were so interesting that there were large variations in UV dose values among six locations tested on each fruit. However it was explained that the reason for the variation was not the size of the fruit. Depending on the location in the treatment chamber some areas of the fruits were indicated to receive even 10 times higher UV doses. It was stated that especially tip area of the apricots had the lowest dose. They could reach only 0.5-0.7 log CFU/g inactivation of surrogate *E. coli* ATCC 25922 by applying a UV dose of 127 mJ/cm<sup>2</sup> (Yan et al., 2014). It was also highlighted that an improved method was needed to rotate the fruit during UV-C processing in order to provide a uniform radiation (Yan et al., 2014). Although our new system was a promising design to be able to process the foodstuff having irregular shape by UV-C light, it was speculated that the dose distribution among fruit surfaces was not estimated accurately. It was possible that all the fruit surfaces were not exposed to the same amount of UV dose due to their irregular shapes. This might be another reason for the deviation observed in the data.

### 5.3.3. Mild Heat (MH) Treatment

#### 5.3.3.1. Microbial Reduction

Apricot samples used in this study were initially containing  $7.51\pm 0.32$  log CFU/g of TAPC,  $5.80\pm 0.31$  log CFU/g of YMC and  $5.17\pm 0.22$  log CFU/g of coliforms, respectively.

Log-reductions in the number of mesophilic aerobic bacteria (TAPC) and yeasts and moulds (YMC) achieved after mild heat treatment were shown in Table 5.6. Reductions in the number of coliforms were not given since no coliform growth was observed after any treatment.

Table 5.6. Log-Reductions after MH Application.

Treatment time (min)	Temperature (°C)	Log Survival (TAPC)	Log reduction (TAPC)	Log Survival (YMC)	Log reduction (YMC)
24	40	$5.77\pm 0.02$	1.74	$3.80\pm 0.14$	2.00
60	40	$5.32\pm 0.12$	2.19	$3.74\pm 0.28$	2.06
10	41	$5.90\pm 0.31$	1.61	$4.89\pm 0.10$	0.91
41	44	$5.48\pm 0.13$	2.03	$5.09\pm 0.13$	0.71
10	46	$6.38\pm 0.05$	1.13	$4.06\pm 0.09$	1.74
26	47.5	$5.03\pm 0.02$	2.48	$4.12\pm 0.08$	1.68
60	50	$3.72\pm 0.09$	3.79	ND	5.80
38	51	$3.93\pm 0.00$	3.58	ND	5.80
15	53	$4.31\pm 0.13$	3.20	ND	5.80
10	60	$3.68\pm 0.39$	3.83	ND	5.80
60	60	$3.64\pm 0.34$	3.87	ND	5.80

Values are mean±standard deviation of two replicates.

The highest log reduction in TAPC was attained as 3.87-log when the apricots were treated at 60°C for 60 min (Table 5.6). However, maximum reduction of YMC was found as 5.8-log since yeast and mould colonies were not detected after processing at temperatures above 50°C. Rahman et al. (2011) washed shredded carrots with warm water at 50°C for 3 min and they obtained 1.32-log and 1.08-log reductions in TPC and YMC, respectively. In another study it was noted that dipping uncut carrots into warm tap water (50°C) reduced the total aerobic bacteria by 2.0-log CFU/g (Klaiber et al., 2005). Although higher reductions observed in this study, increase of temperature above 50°C negatively affected the quality of the apricots. Colour of samples became darker above this temperature. Additionally, apricots no more possessed their characteristic

aroma at around 60°C. It was also noticed that fruits lost their firmness due to hot water effect. Hence, firmness and colour parameters of the samples were also assessed to find the optimum treatment conditions.

### 5.3.3.2. Effect of MH on Firmness and Colour

Firmness and colour parameters of the apricots were measured after MH treatment. The results were given in Table 5.7.

Table 5.7. Firmness and Colour Parameters after MH Processing.

Treatment time (min)	Temperature (°C)	Firmness (N)	L*	a*	b*	ΔE
24	40	5.79±1.35	86.18±8.34	4.50±5.17	60.66±6.87	6.7
60	40	4.80±1.20	84.52±10.33	7.44±7.78	61.73±9.11	6.96
10	41	5.74±0.88	83.31±5.25	5.05±4.91	58.18±3.44	2.91
41	44	5.20±0.78	87.73±7.39	3.86±4.41	44.52±4.01	13.15
10	46	5.66±1.58	84.07±10.36	6.38±5.07	42.56±5.12	13.75
26	47.5	4.57±1.07	80.83±8.42	7.70±10.78	40.95±4.19	15.27
60	50	3.77±0.81	54.71±6.31	11.24±5.06	32.03±5.58	36.4
38	51	3.23±1.09	57.25±3.27	14.42±3.52	36.45±3.33	32.45
15	53	4.92±1.99	71.58±6.73	9.92±7.08	49.27±5.72	12.86
10	60	3.24±1.15	57.50±3.65	15.78±3.70	37.56±5.11	32.03
60	60	4.28±1.59	53.21±3.51	11.54±2.49	32.22±4.10	37.44

Values are mean±standard deviation of at least three replicates.

As can be seen from the table, firmness and lightness ( $L^*$ ) of the apricots were reduced subsequent to MH application above 50°C. Similarly  $b^*$  value displayed a decreasing trend with increased temperatures which indicates reduced yellowness. On the contrary,  $a^*$  value implied an increase at temperatures above 50°C which means improved redness. Moreover,  $\Delta E$  values above 50 °C indicated great differences ( $\Delta E > 6$ ) in the colour of processed apricots (Table 5.7). It was also possible to visually observe the changes occurred in the product (Figure 5.13). Additionally, the smell of the apricots was not acceptable considering the fresh product characteristics. However, application of short treatment time (10 min) at 41 °C reduced the noticeability of the colour difference between processed and unprocessed apricot samples ( $\Delta E < 3$ ).



Figure 5.13. Appearance of apricots a) Before MH b) After MH at temperatures above 50 °C.

Discolouration of the fruits after being processed by MH can be assigned to PPO enzyme activity. PPO activity is known to result in unwanted colour changes and formation of off-flavour in processed foods (Mahmood et al., 2009). Temperature was pointed out to be a significant factor affecting the enzyme activity (Mahmood et al., 2009). Moreover, fruit cultivar was another important factor that influenced the rate of enzymatic action (Barrett, 2002). Demir et al. (2012) reported that maximum enzyme activity was obtained at 30°C for PPO extracted from Igdır apricot. Besides, the enzyme displayed more than 80% of its activity even at 50°C (Demir et al., 2012). In another study, optimum temperature for apricot PPO was found to be 20°C and the enzyme was indicated to retain 24% of its activity for 60 min at 60°C (Mahmood et al., 2009). Similarly, Abreu et al. (2003) addressed the stability of the enzyme at temperatures between 35-50°C. It was denoted that complete inactivation of PPO enzyme was possible at 80°C (Abreu et al., 2003). In our study, temperatures above 50°C ended up with enhanced browning of the apricots which might be due to increased tissue damage at those temperatures as per stated by Abreu et al. (2003). Additionally heat can cause expansion of immobilized air which exists in the intercellular region and consequently release of the trapped air from injured tissues along with the sap leakage (del Valle et al., 1998). Finally it can be concluded that increased free oxygen concentration leads to higher PPO activity.

As it was expressed before, firmness of apricots was also reduced by heating. del Valle et al. (1998) explained the changes of quality parameters after thermal treatment. They suggested that thermal treatment caused degradation of cell membranes which further leads to deprivation of cell turgor pressure and accordingly loss of firmness. Moreover, it was reported that the thermal solubilisation of pectic substances was resulted in reduction of cell adhesion and thinning or dissolution of middle lamella which gives firmness to the tissue (Paniagua et al., 2014; del Valle et al., 1998). It was

well documented that softening of the fruit tissues increased the risk of pathogen development (Paniagua et al., 2014). Thereby slowing down the softening is a critical issue with respect to postharvest fruit quality maintenance. Loss of firmness due to heating effect can be eliminated by use of calcium cations in the dipping water as per recommended by del Valle et al. (1998).

### 5.3.3.3. Statistical Analysis

Statistical analysis was performed in order to determine which factor, i.e., time and temperature, was effective on the responses, i.e., survival number, colour parameters ( $L^*$ ,  $a^*$ ,  $b^*$ ) and firmness, and which treatment-time combination was the optimum point. ANOVA table for survival number was given in Figure 5.14.

Response 1 survival number						
ANOVA for Response Surface Cubic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	14.92	9	1.66	35.72	0.0002	significant
A-Time	0.060	1	0.060	1.29	0.2986	
B-Temperature	1.97	1	1.97	42.42	0.0006	
AB	0.019	1	0.019	0.41	0.5434	
A <sup>2</sup>	0.26	1	0.26	5.50	0.0574	
B <sup>2</sup>	0.014	1	0.014	0.30	0.6065	
A <sup>2</sup> B	0.18	1	0.18	3.95	0.0939	
AB <sup>2</sup>	0.96	1	0.96	20.64	0.0039	
A <sup>3</sup>	0.027	1	0.027	0.58	0.4753	
B <sup>3</sup>	0.13	1	0.13	2.91	0.1389	
Residual	0.28	6	0.046			
Lack of Fit	0.048	1	0.048	1.04	0.3547	not significant
Pure Error	0.23	5	0.046			
Cor Total	15.20	15				

Figure 5.14. ANOVA table for survival number.

Figure 5.14 pointed out that temperature was the most significant factor on survival number. Although time factor seems insignificant with a p-value greater than 0.1, a significant quadratic effect ( $AB^2$ ,  $p=0.0039$ ) could be masking the significance of linear factor effect. Therefore, influence of the factor itself on the response should have been investigated by keeping the other factor at a constant level. In order to see the

effect of factors on survival number, a 3D surface plot was generated as seen in Figure 5.15.

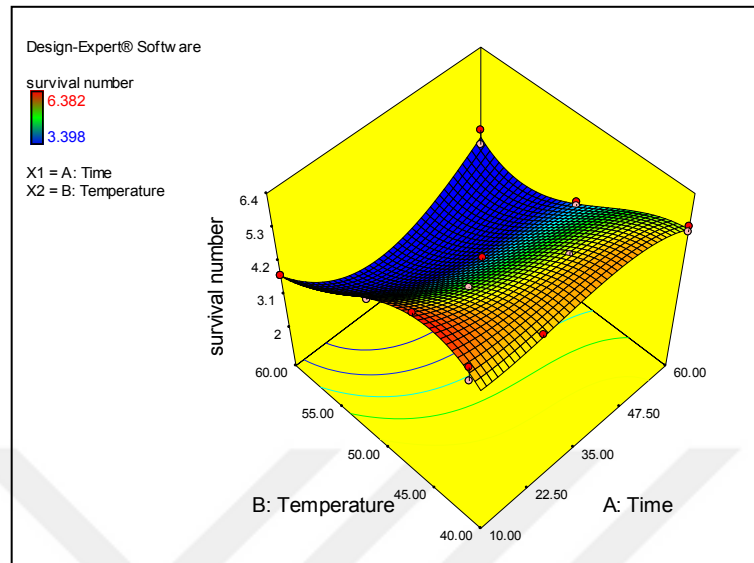


Figure 5.15. 3D plot for survival number.

Significance of the quadratic effect gave a curvature to the surface of the plot. According to Figure 5.15, blue coloured regions of the plot yield the lowest survival number. Regarding this information, time-temperature combination which provides the lowest microbial load can be optimized. On the other hand, it can be said that all possible treatment conditions which would result in the lowest survival number required temperatures above 50°C. As previously mentioned, temperatures above 50°C were not suitable for apricots with respect to quality characteristics. Effect of factors on these parameters was also statistically investigated. ANOVA table for firmness was shown below (Figure 5.16).

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	9.93	3	3.31	11.01	0.0009	significant
A-Time	0.73	1	0.73	2.41	0.1462	
B-Temperature	6.58	1	6.58	21.90	0.0005	
AB	2.23	1	2.23	7.43	0.0184	
Residual	3.61	12	0.30			
Lack of Fit	2.94	7	0.42	3.15	0.1128	not significant
Pure Error	0.67	5	0.13			
Cor Total	13.54	15				

Figure 5.16. ANOVA table for firmness.

It was found that temperature was a significant factor on firmness with a p-value of 0.0005 whereas time factor was not effective on the response (p-value >0.1) (Figure 5.16). However, interaction of two factors (AB) and quadratic effects ( $A^2B$  and  $AB^2$ ) were having significance.

In order to see the changes in firmness value with respect to temperature and time, a 3D surface plot was created (Figure 5.17).

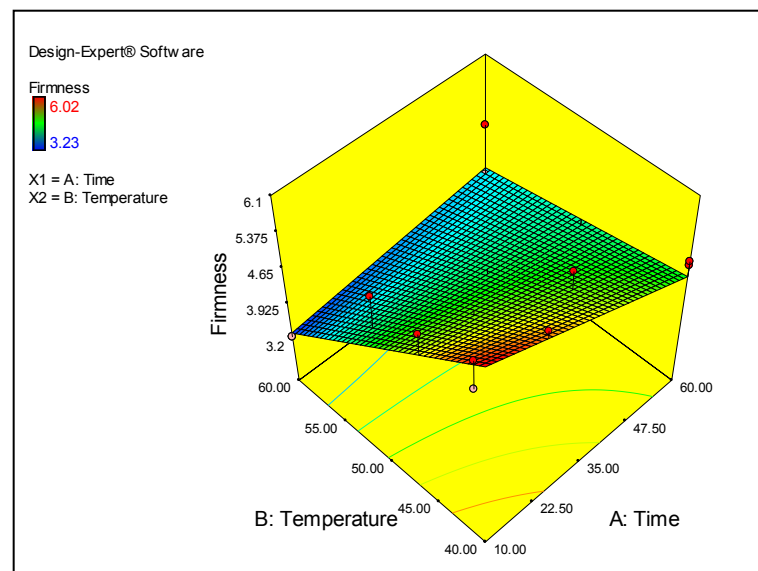


Figure 5.17. 3D plot for firmness.

As mentioned previously, significant quadratic effects give a curvature to the plot. Red-orange coloured regions of the graph indicate the highest firmness values. It is

obvious that temperatures below 50°C need to be chosen in order to maximize the firmness of the product.

MH effect on each colour parameter was individually evaluated. Figure 5.18 shows ANOVA table for L\* value.

Response	3	L*				
ANOVA for Response Surface Cubic Model						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	3104.73	9	344.97	18.04	0.0011	significant
A-Time	34.20	1	34.20	1.79	0.2295	
B-Temperature	368.59	1	368.59	19.28	0.0046	
AB	51.74	1	51.74	2.71	0.1511	
A <sup>2</sup>	3.29	1	3.29	0.17	0.6928	
B <sup>2</sup>	0.36	1	0.36	0.019	0.8956	
A <sup>2</sup> B	27.94	1	27.94	1.46	0.2722	
AB <sup>2</sup>	230.41	1	230.41	12.05	0.0133	
A <sup>3</sup>	8.429E-003	1	8.429E-003	4.409E-004	0.9839	
B <sup>3</sup>	27.17	1	27.17	1.42	0.2782	
Residual	114.71	6	19.12			
Lack of Fit	52.75	1	52.75	4.26	0.0940	not significant
Pure Error	61.96	5	12.39			
Cor Total	3219.44	15				

Figure 5.18. ANOVA table for L\* value.

According to the table, only temperature was found to be an important factor on L\* value (p= 0.0046). Nonetheless AB<sup>2</sup> term also designates a significant effect. This might be masking the real effect of time factor.

Considering 3D plot (Figure 5.19) it can be said that use of 40°C for longer treatment times (above 20 min) gave rise to higher L\* values which indicate increased lightness.



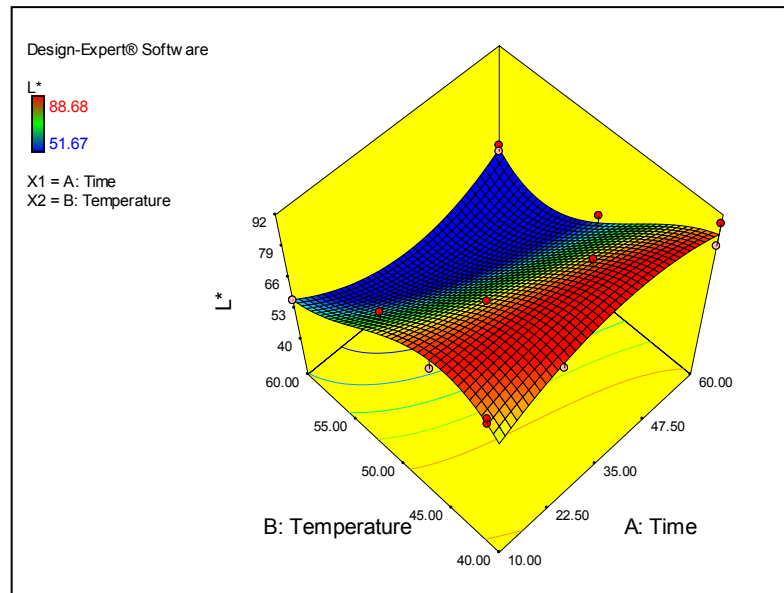


Figure 5.19. 3D Plot for  $L^*$  value.

ANOVA table generated for  $a^*$  value was given in Figure 5.20. Statistical analysis revealed that temperature was the only one factor that has a substantial importance on the response ( $p= 0.0006$ ).

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	159.48	2	79.74	10.13	0.0022	significant
A-Time	0.55	1	0.55	0.070	0.7957	
B-Temperature	158.18	1	158.18	20.09	0.0006	
Residual	102.36	13	7.87			
Lack of Fit	66.43	8	8.30	1.16	0.4569	not significant
Pure Error	35.93	5	7.19			
Cor Total	261.84	15				

Figure 5.20. ANOVA table for  $a^*$  value.

Since no interaction term was significant, one factor plot for the response was provided as seen in Figure 5.21. The graph depicted that the increase of temperature improved the redness of the samples.

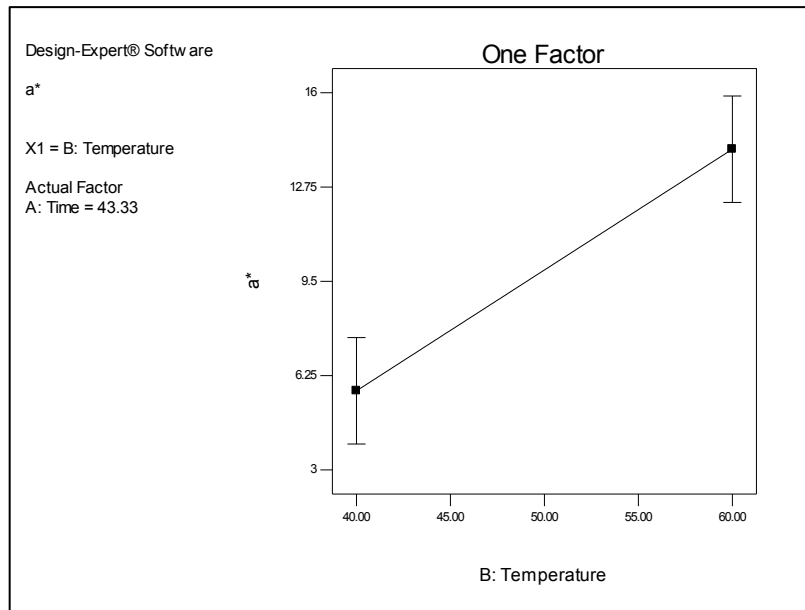


Figure 5.21. One factor plot for a\* value.

Lastly, ANOVA table for b\* value demonstrated that time was the significant factor ( $p=0.0005$ ) on the response (Figure 5.22). Moreover  $B^2$ ,  $AB^2$  and  $B^3$  terms were also found to be significant.

Response	5	b*				
<b>ANOVA for Response Surface Reduced Cubic Model</b>						
Analysis of variance table [Partial sum of squares - Type III]						
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	2016.91	6	336.15	58.49	< 0.0001	significant
A-Time	160.87	1	160.87	27.99	0.0005	
B-Temperature	12.43	1	12.43	2.16	0.1755	
AB	11.12	1	11.12	1.93	0.1977	
B <sup>2</sup>	270.16	1	270.16	47.01	< 0.0001	
AB <sup>2</sup>	60.08	1	60.08	10.45	0.0103	
B <sup>3</sup>	118.81	1	118.81	20.67	0.0014	
Residual	51.73	9	5.75			
Lack of Fit	30.27	4	7.57	1.76	0.2728	not significant
Pure Error	21.45	5	4.29			
Cor Total	2068.64	15				

Figure 5.22. ANOVA table for b\* value.

3D plot pointed out higher b\* values at 40°C for all treatment times between 10 and 60 min (Figure 5.23).

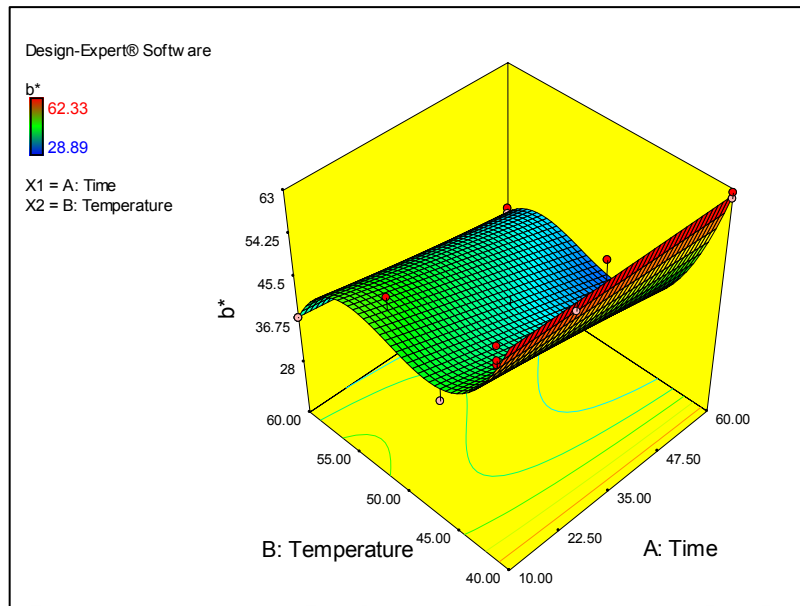


Figure 5.23. 3D plot for  $b^*$  value.

Considering the fresh product characteristics and microbial safety optimum conditions were offered by Design Expert programme. Superficial browning and softening which can occur during MH treatment cause darkening of the product and firmness loss. Therefore the goal was to keep firmness,  $L^*$  value and  $b^*$  value at their maximum level for optimization purpose. However, survival number and  $a^*$  value were set to minimum level for optimization since high numbers of microorganisms and saturated redness are not desired. Eventually, offered conditions were obtained as given in Figure 5.24.

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Time	is in range	10	60	1	1	3
Temperature	is in range	40	60	1	1	3
survival number	minimize	3.398	6.382	1	1	3
Firmness	maximize	3.23	6.02	1	1	3
$L^*$	maximize	51.67	88.68	1	1	3
$a^*$	minimize	3.6	15.78	1	1	3
$b^*$	maximize	28.89	62.33	1	1	3

Solutions								
Number	Time	Temperature	survival number	Firmness	$L^*$	$a^*$	$b^*$	Desirability
1	<u>21.38</u>	<u>40.00</u>	<u>5.8307</u>	<u>5.89328</u>	<u>88.9279</u>	<u>7.35622</u>	<u>57.6616</u>	<u>0.747</u>
2	21.07	40.00	5.82251	5.8827	88.6923	7.35659	57.6332	0.747
3	58.05	40.00	5.66531	5.12075	88.68	7.31192	61.5106	0.729
4	60.00	59.79	3.6361	4.25452	52.9084	12.0275	32.1969	0.204

Figure 5.24. Optimum conditions for MH treatment.

Statistical programme recommended the use of 40 °C for varying treatment times to obtain the desired product characteristics. Although almost 2-log reduction could be achieved at this temperature, 40 °C for 21 min was selected as optimum MH treatment condition for further studies.

#### **5.4. Conclusion**

Results indicated that maximum reduction was only 1.36-log in TAPC in two-lamp system after the samples were exposed to the highest UV dose (348 mJ/cm<sup>2</sup> UV dose at 0.29 mW/cm<sup>2</sup> UV intensity) whereas 0.56-log reduction was attained for YMC at the same condition. On the other hand, coliform growth was not observed after any treatment. Statistical analysis revealed that survival numbers for both TAPC and YMC did not significantly differ between treatments. It is obvious that two-lamp system was insufficient to effectively inactivate native flora of cv. Şalak apricots. Moreover, some ups and downs in the survival data were occurred due to the sample to sample variation. It brought about the need for a system that could process more than 3 samples at the same time. Thereby a new UV-C system which provides higher light intensity was constructed using four UV-C lamps.

The four-lamp UV-C system was able to provide almost 10 times higher UV intensity value (3.23 mW/cm<sup>2</sup>) in comparison to two-lamp system. Moreover four-lamp system was able to process 8 apricots at the same time in order to prevent variations in the resulting survival numbers. A rotating roller bearing was also included in the system for equal light exposure among the samples. Results indicated that the new system could reduce the number of survivals by maximum 3-log for TAPC and 2.37-log for YMC after applying 3100.8 mJ/cm<sup>2</sup> and 775.2 mJ/cm<sup>2</sup> of UV dose for 16 min and 4 min at 3.23 mW/cm<sup>2</sup>, respectively. Similar to the previous findings, coliforms were completely inactivated after any treatment. It was observed that new system could make a significant decrease in the survival numbers. However there were still some deviations in the log-survival data. When the incident light intensity distribution along the lamp axis was investigated, it was realized that light intensity was lower at both ends of the tube. It means that apricots located at the end parts could receive less amount of UV dose. Hence use of the central part of the rotating roller bearing was highly recommended to prevent uneven UV light exposure among apricots. On the other hand,

it was found that inactivation curves for both TAPC and YMC indicated a tailing. Log linear + tailing model was found to best describe the inactivation kinetics for both TAPC and YMC after UV-C treatment. Observation of tailing effect indicated the existence of a resistant subpopulation which was not affected by higher UV doses. Therefore application of longer treatment times (25 and 30 min) did not improve the microbial reduction. Tailing indicates that the inactivation efficiency of UV-C treatment needs to be increased by combination of other methods in a hurdle strategy. Considering the statistical analysis, optimum exposure time which would yield the lowest log-survival number was found to be 19 min.

Effect of MH treatment on the inactivation of indigenous flora was also investigated in this study. Temperatures varying from 40 to 60 °C were applied for different treatment times (10-60 min). It was observed that temperatures above 50 °C reduced the number of native microorganisms by at least 3-log. However, the physical quality of the apricots was adversely affected by high temperatures. Firmness and colour parameters of the samples were also assessed at the same treatment conditions. It was found that the fruit quality could be best preserved at low temperatures. Optimum treatment conditions that would result in the lowest survival number, the highest firmness,  $L^*$  and  $b^*$  value and the lowest  $a^*$  value were determined as 40 °C for 21 min by statistical analysis. Although use of 40 °C was not sufficient to reach the lowest survival numbers, it was required in terms of product quality. Hence, it was concluded that combination of MH with UV-C treatment would yield better inactivation results.

## CHAPTER 6

# EFFECTS OF CALCIUM CHLORIDE (CaCl<sub>2</sub>) DIPPING AND COMBINATION TREATMENTS OF MILD HEATING, UV-C IRRADIATION AND CaCl<sub>2</sub> DIPPING (MUC) ON THE SELECTED PHYSICOCHEMICAL PROPERTIES, SENSORIAL QUALITY AND ENZYMATIC ACTIVITY OF ŞALAK APRICOT CULTIVAR

### 6.1. Introduction

Turkey is the major apricot producer of the world. One of the most important apricot production centres in Turkey is Iğdır province. 85% of the apricots grown in this area are composed of Şalاک variety and this cultivar is specific to the region (Kaya et al., 2011; Muradođlu et al., 2011). Approximately 16% of apricots produced in Turkey were reported to be dried (Alagöz et al., 2015). However, cv. Şalاک apricot of Iğdır is not suitable for drying due to its low dry matter content. Hence fresh consumption was proposed for Şalاک apricot cultivar (Özyörük and Gülerüz, 1992). Information about physical properties of foods was reported to be required in terms of design and engineering of various equipment, post-harvest handling, operations, transport and storage (Milošević et al., 2014). Moreover, these characteristics are important with respect to consumer acceptance and marketability of the product (Milošević et al., 2014).

A treatment that inhibits the rapid softening after harvest would allow increasing the shelf life of cv. Şalاک apricots. UV-C light irradiation which is based on the use of UV light as a non-thermal processing method to reduce microorganisms, induce biological stress and defence mechanisms in plants is one of those novel processing methods (Jiang et al., 2010). In literature some applications related to the use of UV-C light as a means of postharvest quality improvement are available. For instance Gonzalez-Aguilar et al. (2007) and Gonzalez-Aguilar et al. (2001) evaluated the impact

of UV-C light on enzymes associated with the defence mechanism and postharvest shelf life of mango fruit. Erkan et al. (2008) and Jiang et al. (2010) also conducted a similar study in order to determine the UV-C light effect on antioxidant capacity, antioxidant enzyme activity, decay and texture in strawberry fruit and mushrooms. Although UV-C light treatment is widely used in water and air disinfection as well as surface decontamination, its use is still limited in postharvest technology.

Conventional heat treatments were reported to effectively inhibit enzymatic reactions and reduce microbial loads. Nonetheless, adverse impacts of heat on flavour, texture and other quality parameters of the products discourage its use (Abreu et al., 2003). Therefore application of mild heat is of great interest. This technique avoids negative effects of the conventional heat treatment. There are some studies in literature such as the one performed by Beirao-da-Costa et al. (2006) in which the kiwifruit quality after mild heat treatment was evaluated. It was stated that mild heat treatment below 45°C at a firm ripe stage improved the quality characteristics of the fruit. The effect of mild heat treatment with respect to preserving pear quality attributes was also investigated (Abreu et al., 2003).

Use of calcium-based solutions as a postharvest treatment is known to maintain the quality, prevent softening, reduce the rate of rotteness and prolong the storage life of fruits with high senescence index (Ramano Rao et al., 2011; Beirao-da-Costa et al., 2008).

Determination of the effect of processing on nutritional value and product desirability is only possible by measuring physical and chemical properties (Radunic et al., 2015). Thus, the objectives of this study were: to determine some physical and chemical properties of cv. Şalak apricots; to find the optimum treatment time, temperature and concentration for CaCl<sub>2</sub> dipping which could maintain the fruit firmness; to investigate the changes of the colour, firmness, total phenolic content, PPO and PME enzyme activities and sensorial quality of cv. Şalak apricots subjected to mild heat treatment, UV-C irradiation and CaCl<sub>2</sub> dipping combined in a hurdle strategy.

## **6.2. Materials and Methods**

As described in section 4.1, cv. Şalak apricots were selected and used in this study. Fruit weight, length and width were measured as delineated in section 4.2.1.

Moisture content and water activity of the samples was determined as reported in section 4.2.2 and 4.2.3, respectively. pH value of the samples was found regarding the section 4.2.4. Titratable acidity (TA) of the apricots was estimated according to the section 4.2.5. Brix measurement was performed as defined in section 4.2.6. RI of the samples was calculated as previously described in section 4.2.7. Colour parameters were measured as it was expressed in section 4.2.8. Firmness of the apricots was determined according to section 4.2.9. Ascorbic acid content of the fruit samples were found regarding the method 4.2.10. The change in TPC of the apricots after the treatment was detected using the method described in section 4.2.11. PPO and PME enzyme activities were assessed after the treatment using the procedure given in section 4.3. A sensory panel was performed according to the technique defined in section 4.2.12.

### **6.2.1. CaCl<sub>2</sub> Dipping**

CaCl<sub>2</sub> dipping was carried out considering the method and the process parameters given in section 4.6. Briefly, factors were CaCl<sub>2</sub> concentration (0-3%) and dipping time (2-21 min). The response variable was apricot firmness. There were totally 16 runs. Experimental runs were conducted at 40°C. Besides, 30 min of dipping and 6% of CaCl<sub>2</sub> were also assessed. Furthermore the effect of CaCl<sub>2</sub> dipping at a concentration of 6% of CaCl<sub>2</sub> for 21 min was evaluated at 25°C in order to reveal the effect of the dipping temperature on apricot firmness. Effect of treatment on firmness of the apricots was investigated. Optimum treatment conditions were determined.

### **6.2.2. Combined Treatment (MUC)**

The combination of MH treatment, UV-C irradiation and CaCl<sub>2</sub> dipping (MUC) were applied in a hurdle strategy to apricot samples (see section 4.8). For this purpose optimized CaCl<sub>2</sub> concentration (6%) was determined according to the results of the section 6.2.1. The optimum dipping time (21 min) and temperature (40 °C) were adjusted in terms of optimized mild heat treatment conditions. Optimum processing conditions for UV-C treatment (3682.2 mJ/cm<sup>2</sup> UV dose for 19 min at 3.23 mW/cm<sup>2</sup>) was determined in Chapter 5. Effect of hurdle technology on the colour, firmness, TPC,



PME and PPO enzyme activities, and sensorial quality of cv. Şalak apricots was evaluated according to the methods given above.

## 6.3. Results and Discussion

### 6.3.1. Sample Characterization

Some selected physicochemical properties of Şalak apricot cultivar were determined and the data are shown in Table 6.1. Measurements were carried out on apricots collected in 2015 and 2016 summer seasons.

Table 6.1. Physical and Chemical Properties of cv. Şalak Apricot Cultivar.

Property	Year 2015	Year 2016
Length (mm)	54.84±2.15	43.21±2.21
Width (mm)	43.63±2.54	33.47±1.67
L/W	1.26	1.29
Weight (g)	59.32±8.45	27.10±6.64
L*	65.53±1.11	69.15±2.66
a*	4.93±1.54	4.41±2.51
b*	42.34±1.63	41.86±2.41
a*/b*	0.12	0.11
Firmness (N)	4.30±1.47	3.56±0.99
pH	3.91±0.01	4.42±0.06
<sup>1</sup> T.A % (malic acid/100 g fresh apricot)	0.98±0.02	0.50±0.03
Brix %	13.74±0.06	8.90±0.35
<sup>2</sup> RI	14.05±0.06	17.86±0.70
Moisture content %	85.49±0.16	90.15±0.14
<sup>3</sup> a <sub>w</sub>	0.96±0.001	0.98±0.007
<sup>4</sup> AsA (mg/100 g fresh apricot)	16.11±0.99	3.82±0.12

<sup>1</sup> : Titratable (total) acidity, <sup>2</sup> : Ripening index, <sup>3</sup> : Water activity, <sup>4</sup> : Ascorbic acid

Material characteristics of apricots were reported to be substantial for a proper design and construction of an apparatus for collecting and handling of the fruits and processing them after harvesting (Haciseferoğulları et al., 2007). Length (L), width (W)

and weight of the apricots were measured and L/W ratio was calculated in order to determine the size and shape of the samples. According to the results, cv. Şalak apricots harvested in 2015 season were found to be bigger sized with higher L, W and weight measurements than the fruits harvested in 2016. The difference between two seasons might be due to climate and growing conditions such as irrigation and fertilizing. On the other hand Akin et al. (2008) reported the average weight of apricots from Iğdır as  $32.33 \pm 1.47$  g. L/W ratio indicated that fruits had a cylindrical shape with a long fruit section in both seasons. Özyörük and Güteryüz (1992) conducted a research on the characteristics of apricot cultivars grown in Iğdır province and they classified the fruit samples considering their length, width, weight and skin colour. They found that cv. Şalak apricots were very big (average weight of 62.1 g), long-shaped and light orange coloured fruits. Similarly apricot samples used in this study were also regarded as light orange coloured based on the categorization of skin colours (Özyörük and Güteryüz, 1992). Measured colour parameters indicated that lightness-darkness value ( $L^*$ ) pointed out lightness. Decrease of  $L^*$  value was reported to be in relation with the accumulation of carotenoids (Akin et al., 2008). Also an increase in carotenoid concentration would result in high  $a^*$  and  $b^*$  values in the positive side which is an indication of redness and yellowness, respectively. According to this study  $a^*$  and  $b^*$  values were in the positive side representing a slight redness and yellowness of the samples. It was stated that  $a^*/b^*$  ratio was very useful for fruits in order to determine their colour (Barrett et al., 2010). The ratio was said to be negative for green fruits, approximately 0 for yellow fruits and positive for red to orange fruits.  $a^*/b^*$  ratio of the apricots used in this study were found to be almost 0.1. This value indicated the yellowness of the samples.

Muradoğlu et al. (2011) investigated some physicochemical properties of different apricot genotypes cultivated in Iğdır province. They found that cv. Şalak apricot was having a pH value of  $5.27 \pm 0.03$  and TA of  $0.21 \pm 0.01\%$  malic acid. Comparing the results given in Table 6.1, it can be obviously said that apricots harvested in 2015 season were more acidic with a lower pH and higher TA%. However, pH and TA% values of the apricots obtained in 2016 season were similar to the findings of Muradoğlu et al. (2011). It can be concluded that seasonal changes brought about some differences in physicochemical properties of the apricots. Total soluble solid content (Brix) of the apricots were also different in two seasons (Table 6.1). Total soluble solid content (Brix value) of cv. Şalak apricots were reported as 11-14.5% in other studies (Ercisli, 2009; Özyörük and Güteryüz, 1992; Muradoğlu et al., 2011; Akin

et al., 2008). However, brix value of the apricots in 2016 was found to be lower as compared to literature data. As the fruit matures TA value decreases whereas total soluble solid content (Brix) increases. The ratio between Brix and TA values is used to determine the ripening index of the fruits (Valero et al., 2013). According to Table 6.1, RI value of cv. Şalak apricots were found to be similar in both seasons. However Muradoğlu et al. (2011) estimated almost five times higher RI value (68.55) for the apricots that they used. This clear difference in maturity levels can explain the variations between the data.

Although moisture content and water activity of the apricot samples (Table 6.1) were slightly lower in season 2015, those values obtained in season 2016 were comparable with the results of the study performed by Akin et al. (2008). They reported that apricot varieties from Iğdir region had a moisture content of 87.28% and a water activity level of 0.993 (Akin et al., 2008). This difference might be caused from the harvest season, climate, and variety of the apricots used in this study. Moisture content gives information about the dry matter content of the foodstuff. Dry matter content was stated to be the most important parameter with respect to processing and commercial value of the apricots (Akin et al., 2008). Low dry matter content is known to give sensitivity to apricots for being transported and handled. Therefore apricots with low dry matter content, such as Iğdir apricots, are preferred to be consumed freshly rather than to be used for drying processes. According to a study, Malatya apricots suitable for drying process were indicated to have a dry matter yield of at least 22% (Akin et al., 2008).

Fruit firmness was also determined (Table 6.1) and it was found that fruit firmness was slightly lower in season 2016 with respect to season 2015. According to Haciseferoğulları et al. (2007) fruit firmness was changing between 4.04 and 9.75 N for different apricot cultivars. Besides, it was indicated that part of the fruit tested for firmness affected the results. For instance, sap region was reported to be the hardest region for all apricot types tested except for Zerdali type whereas medium region was the softest part (Haciseferoğulları et al., 2007). In conclusion, the firmness values of apricots collected in two seasons were in the similar range reported by Haciseferoğulları et al. (2007).

Ascorbic acid (AsA) contents of the samples were found to be lower in season 2016 as compared to season 2015 (Table 6.1). According to the literature, amount of AsA in fresh apricots were changing between 8 and 20 mg/100 g products (Rasanu et

al., 2005). In another study AsA content was found as 11 - 18.2 mg/100 g apricots for different apricot types grown in Iğdır region (Özyörük and Güteryüz, 1992). On the other hand Akin et al. (2008) showed that Iğdır apricots had an AsA content of 68.4 mg/100 g dry weights. Considering the moisture content of the samples (Table 6.1), AsA content of the apricots were calculated as 111.01 mg/100 g dry weights and 38.78 mg/100 g dry weights for seasons 2015 and 2016, respectively. As a conclusion it can be speculated that results showed variability with respect to the other studies in the literature. Variety of the fruits, soil, climate and harvesting time of fruits might cause this variability as per stated by Rasanu et al., (2005). Kan et al. (2014) showed that dry farming conditions increased the amount of Vitamin C found in apricots. Furthermore, different measurement methods used in different studies might be another factor that affects the results. However, apricots are not considered as the major source of Vitamin C. Thereby results can be regarded as acceptable.

### 6.3.2. Effect of CaCl<sub>2</sub> Dipping on Firmness

An experimental design was created as described in section 4.6 and firmness of the apricots was evaluated after CaCl<sub>2</sub> dipping process. Results were statistically assessed and ANOVA table was generated (Figure 6.1).

Response	1	firmness			
ANOVA for Response Surface Linear Model					
Analysis of variance table [Partial sum of squares - Type III]					
Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	2.07	2	1.04	1.84	0.1974 not significant
A-time	2.07	1	2.07	3.67	0.0775
B-concentration	6.435E-003	1	6.435E-003	0.011	0.9164
Residual	7.31	13	0.56		
Lack of Fit	4.14	8	0.52	0.82	0.6197 not significant
Pure Error	3.17	5	0.63		
Cor Total	9.38	15			

Figure 6.1. ANOVA table for CaCl<sub>2</sub> dipping.

It was observed that CaCl<sub>2</sub> application at concentrations of 0-3% for treatment times of 2-21 min at 40°C did not result in a significant change in the firmness values of

the apricots. ANOVA table revealed that the model was insignificant ( $p$ -value  $>0.05$ ). An insignificant model means that the factors used at their current levels did not affect the response. Variations occurred in the data might be due to the noise. Therefore factor levels needed to be revised. Besides the above mentioned experimental design, 6% of  $\text{CaCl}_2$  and 30 min of dipping time were also assessed at  $40\text{ }^\circ\text{C}$  in terms of firmness enhancement. Firmness values of the apricots were compared before and after the processing (Table 6.2).

Table 6.2. Firmness of Apricots Before and After  $\text{CaCl}_2$  Dipping.

Treatment	Firmness (N)	
	Before	After
21 min 3% $\text{CaCl}_2$	6.04±0.94 <sup>a</sup>	6.48±0.75 <sup>a</sup>
21 min 6% $\text{CaCl}_2$	7.36±1.19 <sup>a</sup>	6.46±1.30 <sup>a</sup>
30 min 3% $\text{CaCl}_2$	6.78±0.78 <sup>a</sup>	6.07±0.90 <sup>a</sup>
30 min 6% $\text{CaCl}_2$	6.47±1.26 <sup>a</sup>	5.51±1.27 <sup>a</sup>

<sup>a</sup>: Means do not share a letter in the same row indicate significant difference at  $p<0.05$ .

Table 6.2 indicated that application of  $\text{CaCl}_2$  dipping did not significantly change the initial firmness values after the processing. Effect of  $\text{CaCl}_2$  concentration and treatment time was also compared (Table 6.3).

Table 6.3. Effect of  $\text{CaCl}_2$  Concentration and Dipping Time on Firmness.

Treatment Time (min)	Firmness (N)	
	3% $\text{CaCl}_2$	6% $\text{CaCl}_2$
21 min	6.48±0.75 <sup>aA</sup>	6.46±1.30 <sup>aA</sup>
30 min	6.07±0.90 <sup>aA</sup>	5.51±1.27 <sup>aA</sup>

<sup>a</sup>: Means do not share a letter in the same row indicate significant difference at  $p<0.05$ .

<sup>A</sup>: Means do not share a letter in the same column indicate significant difference at  $p<0.05$ .

As explained in Table 6.3, neither treatment time nor  $\text{CaCl}_2$  concentration significantly altered the firmness of the apricot samples. Therefore 30 min of application was excluded. Moreover 21 min was determined as the optimum processing time for mild heat (MH) treatment as explained in section 5.3.3. For the ease of application MH treatment and  $\text{CaCl}_2$  was combined and hence 21 min was selected as the optimum dipping time.

On the other hand, samples treated by 3% and 6% of CaCl<sub>2</sub> for 21 min at 40°C were stored for five days at the refrigerator in order to evaluate the effect of treatment on the preservation of the fruit firmness during storage. After the storage period firmness of the apricots were assessed once more. Firmness of the apricots treated by 3% CaCl<sub>2</sub> for 21 min was 2.85±0.86 whereas it was 4.22±0.94 for the apricots treated with 6% of CaCl<sub>2</sub>. It was observed that 6% of CaCl<sub>2</sub> treatment better preserved the texture during refrigerated storage. Thereby 6% of CaCl<sub>2</sub> was chosen for further studies. Manganaris et al. (2007) also noted that firmness of CaCl<sub>2</sub> treated peaches was higher than control samples after refrigerated storage. Penetration of calcium ions into the fruit tissue was showed to be enhanced after 5 days of storage due to the formation of gaps for calcium ions by virtue of ripening (Manganaris et al., 2007).

Lastly, effect of dipping temperature was investigated using both 40 °C and 25 °C for 21 min at a concentration of 6% of CaCl<sub>2</sub>. Apricot firmness was found to be 5.86±0.76 N and 5.67±1.23 N after the fruits were processed at 25 °C and 40 °C, respectively. Statistical analysis of the results referred to an insignificant effect of temperature on the firmness of the apricots right after processing (p=0.47). Although it is known that application of mild heat in combination to CaCl<sub>2</sub> dipping supports the entrance of Ca<sup>+2</sup> ions into the fruit tissue (Lamikanra, 2002; Beirao-da Costa et al., 2008), any improvement in the fruit texture was not observed when the samples were treated at 40°C. Lidster and Porritt (1978) pointed out that calcium uptake was higher when the temperature of the apple sample was considerably higher (38 °C) than that of dipping solution (0 or 21 °C). They also denoted the improved liquid entrance due to decreased internal gas pressure after cooling of the sample by dipping it into the solution (Lidster and Porritt, 1978). However, apricot samples used in this study were at the ambient temperature for CaCl<sub>2</sub> application. This might be the reason for insignificant changes in the firmness of apricots after being processed.

In conclusion, optimum treatment conditions for CaCl<sub>2</sub> dipping was determined as 6% of CaCl<sub>2</sub>, 21 min and 40 °C considering the optimum conditions for MH treatment. Although dipping temperature was found to have no effect on the firmness right after processing, apricots could better maintain their firmness throughout the storage after being processed at these conditions.

### 6.3.3. Effect of the Combination Treatments (MH Treatment, UV-C Irradiation, CaCl<sub>2</sub> Dipping) (MUC) on the Physicochemical Properties of Şalak Apricot Cultivar

#### 6.3.3.1. Colour and Firmness

cv. Şalak apricots were evaluated in terms of colour and firmness before and after being processed by the combination of MH treatment, UV-C irradiation and CaCl<sub>2</sub> dipping (MUC). Results were shown in Table 6.4.

Table 6.4. Effect of MUC on Colour and Firmness of cv. Şalak Apricots.

	L*	a*	b*	Firmness
<b>Control</b>	66.30±4.37 <sup>a</sup>	5.05±2.34 <sup>a</sup>	40.70±3.01 <sup>a</sup>	3.56±0.99 <sup>a</sup>
<b>MUC</b>	65.70±4.00 <sup>a</sup>	4.11±2.51 <sup>a</sup>	40.06±3.20 <sup>a</sup>	3.04±0.30 <sup>a</sup>

<sup>a</sup>: Means do not share a letter in the same column indicate significant difference at p<0.05.

Results indicated that MUC treatment did not significantly alter the colour parameters and firmness of the apricots. As it was explained in section 6.3.2, CaCl<sub>2</sub> treatment facilitated the preservation of fruit firmness during storage. Similarly Wu et al. (2015) demonstrated that firmness of ‘Xiaobai’ apricots did not show a noticeable change after mild heat and CaCl<sub>2</sub> application during the first 3 days of storage. However, application of calcium and mild heat resulted in delayed softening. Furthermore treated apricots were indicated to get higher scores with respect to fruit appearance during the storage time (Wu et al., 2015). In another study Aghdam et al. (2013) stated that use of calcium as a post-harvest treatment slowed down the aging processes of the fruits during storage. It was indicated that firmness of fig fruits was conserved after the treatment. Moreover, mulberry fruit colour was reported to be retained after processing (Aghdam et al., 2013). Cheon et al. (2015) combined the use of UV-C irradiation and mild heat in order to inactivate pathogens found on pulverised red pepper. They also investigated the effect of combined treatment on the colour of the samples. It was reported that colour of the samples did not significantly change after

being processed by either UV-C irradiation, mild heat or combined methods (Cheon et al., 2015).

Although no significant differences were observed between control and treated samples, processed apricots were expected to have better quality during storage. Quality parameters were investigated in detail throughout the shelf life study. The results were given in Chapter 7.

### **6.3.3.2. Total Phenolic Content (TPC)**

Total phenolic content (TPC) of the control and treated samples were assessed in this part of the study. The mean TPC of untreated control samples was found to be  $2035.92 \pm 225.73$  mg GAE/100 g of fresh weight. In literature a great variance was observed in the TPC of different apricot cultivars. Akin et al. (2008) evaluated TPC of different apricot cultivars from Malatya region. They revealed that TPC of different cultivars was changing between 740.7 and 1335.2 mg GAE/100 g of fresh weight (Akin et al., 2008). In another study TPC of apricots grown in Northern areas of Pakistan was determined as ranging between 4591 and 7310 mg GAE/100 g of dry weights (Ali et al., 2011). Sochor et al. (2010) reported the TPC of twenty one genotypes of apricots grown in Czech Republic. It was denoted that TPC of the apricots were between 41 and 170 mg GAE/100 g of fresh weight (Sochor et al., 2010). Similarly, the lowest and the highest TPC of 37 different apricot cultivars from Spain were measured to be 32.6 and 160 mg/100 g of fresh weight, respectively (Ruiz et al., 2005). Drogoudi et al. (2008) investigated the TPC of 29 apricot cultivars originated from Greece and America and their crossbreeds. TPC of the apricots were indicated to be ranging between 30 and 740 mg GAE/100 g of fresh weight (Drogoudi et al., 2008). Campbell et al. (2013) conducted a study using similar methods with Drogoudi et al. (2008). According to the results TPC of the apricots from Northeast USA varied from 44 to 345.1 mg GAE/ 100 g of fresh weight (Campbell et al., 2013). On the other hand the lowest level of TPC for apricots was addressed by Kim et al. (2014). Japanese apricot 'Backaha' was denoted to have  $160.73 \pm 10.11$   $\mu$ g GAE/ 100 g of dry weights (Kim et al., 2014). Considering the literature it can be speculated that variety of the apricots had a great influence on the TPC of the fruits. Ogah et al. (2014) also indicated that amount of polyphenols in Rosaceous plants differed with respect to genetics, fruit location, pre- and post-harvest



factors and conditions related to the climate. Additionally, extraction and measurement methods are the other critical factors that affect the results. For instance Wani et al. (2015) achieved almost three times higher TPC as compared to other studies when they used methanol as an extraction solvent instead of ethanol. However, there is no widely accepted extraction method which can be applied for all food substances as per stated by Dai and Mumper (2010). This may bring about differences among the results. As regards to the information given above, TPC of cv. Şalak apricots was the highest among the TPC of other cultivars. Thereby cv. Şalak apricots can be assumed to be rich in total phenolics.

TPC of cv. Şalak apricots were also measured after they were processed by MUC. TPC was recorded to be  $2153.15 \pm 136.77$  mg GAE/ 100 g of fresh weight after the treatment. As compared to control samples, treated apricots were found to have higher TPC. However, the difference between mean values was not significant ( $p > 0.05$ ). It means that MUC treatment at defined conditions did not notably alter the TPC of cv. Şalak apricots. Wu et al. (2015) also examined the TPC of 'Xiaobai' apricots after the fruits were processed by  $\text{CaCl}_2$  (0.5% for 5 min at 20 °C) and hot water dipping (34 °C for 5 min). No significant difference was observed between control and treated samples with respect to TPC (Wu et al., 2015). It was reported that plants synthesized phenolics in order to protect themselves from excessive exposure to UV radiation (Ogah et al., 2014). Moreover, increase in TPC was pointed out to be associated with the induced phenylalanine ammonia lyase (PAL) enzyme activity due to ethylene hormone (Ahari Mostafavi et al., 2013). Therefore it can be said that MUC treatment most probably did not cause an increase in the PAL enzyme activity. Likewise, ethylene crisis was not stimulated by the processing. Furthermore applied UV-C dose did not affect the biological state of the fruit.

#### **6.3.3.3. Effect of the Combination Treatments (MH Treatment, UV-C Irradiation, $\text{CaCl}_2$ Dipping) (MUC) on Polyphenol Oxidase (PPO) and Pectin Methylesterase (PME) Enzyme Activities of Şalak Apricot Cultivar**

Impact of MUC treatment on polyphenol oxidase (PPO) and pectin methylesterase (PME) enzymes was explored in the context of this study.

It was stated that an optimization was required for the extraction of PPO from the plants having high phenolic content (Wuyts et al., 2006). Use of phenol-adsorbing agents such as polyvinylpyrrolidone (PVP), polyvinylpolypyrrolidone (PVPP) and polyethylene glycol (PEG) was recommended (Wuyts et al., 2006; Palma-Orozco et al., 2011). PVPP is a crosslinked and insoluble form of PVP. Though PVPP can still absorb water and swell rapidly. Moreover, it is easier to remove PVPP from the extraction medium than PVP. PVP, PVPP and PEG were indicated to have high hydrogen bonding affinity and consequently high affinity to polyphenols. Hence they are used to remove polyphenols (Arcuri et al., 2003; Palma-Orozco et al., 2011). However, PVP was revealed to be not useful for preparation of PPO extract since it inhibits the enzyme (Smith, 1979). On the other hand PEG was reported to be the most used organic polymer for this kind of precipitation. Therefore these different polymers were tested in different concentrations (4% PVPP, 4% PVP, 1% PEG, and 0.5% PEG) for the extraction process of PPO from cv. Şalak apricots. In summary, use of 4% of PVP resulted in browning of the extract. Lower level of PVP was reported to cause browning of the extract due to rapid substrate auto-oxidation (Rocha and Morais, 2001). Higher concentrations would be necessary. Increasing the level of PVP might have resulted in higher activity but the economic aspect of it would be limiting. On the other hand high concentration of PVPP (5-10%) was reported to prevent discolouration of extracts (Wuyts et al., 2006). Nonetheless PVPP is an expensive chemical. Moreover, it was shown that PVPP was ineffective at high pH due to the ionization of phenolic hydroxyl groups (Smith, 1979). Optimum pH level was indicated to be 3.5 for PVPP-phenolic binding (Smith, 1979). Besides these, PEG is the cheapest phenol remover among the other polymers used. It was denoted that prevention of modification of the enzyme and browning was difficult in some plant materials (Sojo et al., 1998). Thus usage of PEG as a complement to Triton X detergent was recommended. As regards to Sojo et al. (1998), PEG/phosphate system was found to be the optimum for banana pulp PPO. They claimed that PEG could effectively remove polyphenols and high phosphate concentration (0.1 M) stabilized the enzyme. Thereby the enzyme can remain latent after extraction process. Furthermore extraction using 0.5% PEG gave the highest enzyme activity results. Hence, it was chosen for further PPO activity assays.

Activity of crude PPO enzyme extracted from cv. Şalak apricot peels was assayed and calculated as  $2294 \pm 107.39$  U/ mL (Table 6.5). Demir et al. (2012) examined the purification of PPO enzyme obtained from cv. Şalak apricots. They

reported 567 U/ mL activity of crude enzyme extract. Results of this study implied higher enzyme activity. This might be due to the extraction procedure. Demir et al. (2012) did not use Triton X detergent for the extraction of the enzyme. However, Wuyts et al. (2006) proved that use of Triton X detergent improved the enzyme activity due to the breaking down of plastids where PPOs are located, solubilisation of membrane-bound PPO and stimulation of latent PPO.

Table 6.5. PPO and PME Enzyme Activities of Processed and Unprocessed cv. Şalak Apricots.

Treatment	PPO Activity (U/mL)	PME Activity (U/mL)
Control	2294±107.39	36±0.00
MUC	2604±186	36±0.00

MUC treated samples were also assessed in terms of PPO activity. Enzyme activity was obtained as 2604±186 U/ mL after the treatment. Although it seems that treated samples had higher PPO activity than the control samples (Table 6.5), the difference between mean values was not significant ( $p>0.05$ ). This revealed that PPO enzyme found in cv. Şalak apricot peels could not be inactivated by MUC treatment. Ioannou and Ghoul (2013) reviewed different methods in order to prevent enzymatic browning of fruits and vegetables. Use of calcium salts as a chemical treatment, application of heat as a physical method and alternative non-thermal processing techniques such as PEF, HHP and UV-C treatment were addressed. Calcium was indicated to reinforce the cell walls. Thereby contact of phenolic compounds and the PPO can be avoided by cell compartmentation. On the other hand, heat application was indicated to be the most efficient method to inactivate PPO enzyme. Abreu et al. (2003) designated the stability of the enzyme at temperatures between 35-50°C. It was denoted that complete inactivation of PPO enzyme was possible at 80°C (Abreu et al., 2003; Sulaiman et al., 2015). However, high temperatures required for the enzyme inhibition were reported to destroy product quality. UV-C light as a non-thermal technique was pointed out to cause a reduction in the enzyme activity due to the formation of protein clumps after exposing the light (Ioannou and Ghoul, 2013; Müller et al., 2014). Although UV-C treatment avoids the detrimental effects of heat, inactivation of PPO by means of UV-C light was stated to be difficult. Pombo et al. (2011) reported that activity of PPO enzyme found in strawberry fruit was not affected by UV-C treatment

for the first four hours of storage. On the contrary, increase in the activity of the enzyme was observed after ten hours of storage (Pombo et al., 2011). According to the results of this study, applied processes were not sufficient to reduce the PPO activity. This might be due to the use of low temperatures at which enzyme could stay stable. Arslan et al. (1998) also investigated the inactivation of PPO from Malatya apricots by heat. Even though 40 °C for 40 min was applied, the enzyme was reported to retain its activity. Moreover, UV-C treatment time used in this study might not be sufficient to inactivate this oxidative enzyme. Sampedro and Fan (2014) demonstrated that UV-C light had an insignificant effect on PPO enzyme in apple juice. In another study complete inactivation of PPO in apple juice was achieved after 100 min of UV-C light exposure (Falguera et al., 2011).

Besides these all, PME activity was also evaluated. No difference in PME activities was detected between control and treated samples right after the processing (Table 6.5). Manganaris et al. (2007) could observe the changes in the activity of PME enzyme extracted from untreated and calcium treated whole peach samples only after four weeks of storage. They found that PME activity was higher in calcium treated peaches (Manganaris et al., 2007). Moreover, MUC treatment did not deactivate the PME enzyme in the apricot peels. Similarly, Beirao-da Costa et al. (2008) claimed that mild heat applications did not inactivate PME enzyme in kiwifruit but it induced the enzymatic activity in a non-reversible way. In another study, PME in apricot nectars could not be eliminated by HHP treatment (Huang et al., 2013). PME was reported to be resistant to pressure (Huang et al., 2013). However, most of the PME enzymes were considered as susceptible to thermal inactivation (Huang et al., 2013). Ünal and Şener (2015) studied the characterization of PME from ‘Alyanak’ apricot. They reported that the enzyme could be entirely inactivated at 80°C.

#### **6.3.3.4. Effect of Combination Treatments of Mild Heating, UV-C Irradiation and CaCl<sub>2</sub> Dipping (MUC) on the Sensorial Quality of Şalak Apricot**

According to the results, participants could identify the different sample 8 times over 18 responses. However, critical number of correct responses which is required to reveal the statistically significant difference between the samples was 10 ( $\alpha=5\%$ )

(Appendix B). It shows that participants could not distinguish the control samples from MUC treated apricots.

Furthermore, panellists commented that the taste, colour and firmness of the samples were so similar that they had difficulties in finding the odd sample. However, only once MUC treated sample was found to be softer and darker by 3 panellists in one session. Additionally, one of the participants claimed that she could perceive a strange taste in MUC treated sample. Probably it was due to the  $\text{CaCl}_2$  treatment. It was indicated that use of  $\text{CaCl}_2$  might contribute to the formation of bitterness in the fruits (Beirao-da Costa et al., 2008; Saftner et al., 2003).

Although the reliability of triangle tests was questioned with respect to non-homogeneous food applications, the result of the sensory analysis was found to be compatible with the instrumental measurements. As stated before, the difference between the control and the treated samples could not be verified by the panellists. Similarly instrumental measurements of colour and firmness indicated insignificant changes between control and MUC treated samples.

#### **6.4. Conclusion**

Some physical and chemical properties of cv. Şalâk apricots harvested in 2015 and 2016 seasons were evaluated in order to characterize the samples. Results indicated that physicochemical properties of apricot samples showed some differences between two seasons. In season 2016 apricots were smaller sized with a lower soluble solid content and higher titratable acidity. Moreover, firmness and ascorbic acid content of the samples were found to be lower in season 2016. Seasonal changes were indicated to cause some variations in the data.

Firmness of the apricot samples were also assessed after  $\text{CaCl}_2$  dipping. Apricot is known to be a climacteric fruit which is suffered from a rapid quality loss after harvesting. Therefore  $\text{CaCl}_2$  was applied to the samples as a postharvest technology in order to improve the fruit firmness and storability. Optimum treatment time and concentration for  $\text{CaCl}_2$  dipping were determined. For the ease of application MH treatment was combined with  $\text{CaCl}_2$  dipping. Therefore temperature of the dipping solution was kept at  $40^\circ\text{C}$ . According to the results, 6% of  $\text{CaCl}_2$  application for 21 min yielded significantly higher firmness values after 5 days of refrigerated storage. It was

stated that pectin esterase enzymes were initiated by mild heat treatments applied before dipping (Lamikanra, 2002). The enzymes were reported to provide more binding sites for  $\text{Ca}^{+2}$  ions by reducing the esterification degree of pectin (Lamikanra, 2002; Lionetti et al., 2012). De-esterified homogalacturonan residues were demonstrated to form a network with  $\text{Ca}^{+2}$  ions (Lionetti et al., 2012). Subsequently, firmness was reported to be enhanced.

After the determination of optimum conditions for  $\text{CaCl}_2$  dipping, MH treatment, UV-C irradiation and the dipping process were combined. Effect of combined treatment (MUC) on colour, firmness, PPO and PME enzyme activities, TPC and sensorial quality of the apricots were estimated. Experimental results showed that MUC treatment did not significantly affect the colour and firmness of the apricots. Although firmness of the samples did not exhibit a dramatic increase after the treatment, textural quality of the treated apricots was expected to be better preserved during storage. Moreover, TPC of the samples was not influenced by the combined treatment. Enzymatic activities also did not change after processing. PPO enzyme could not be inactivated after being processed by MUC. Although heat and UV-C irradiation were involved in the process, treatment conditions were not enough to deactivate the enzyme. Conversely, PPO enzyme kept its stability during the process. Furthermore, no significant change was observed in PME enzyme activity after the treatment. It was well documented that PME enzyme is a thermolabile enzyme which can be inactivated at  $80^\circ\text{C}$ . However, current treatment conditions were not able to reduce the enzymatic activity. Sensory analysis revealed that panellists could not distinguish between control and MUC treated samples. Instrumental measurements also supported this finding.

## CHAPTER 7

# THE INFLUENCE OF COMBINATION TREATMENTS OF MILD HEATING, UV-C IRRADIATION AND CaCl<sub>2</sub> DIPPING (MUC) AND MODIFIED ATMOSPHERE PACKAGING (MAP) ON THE SHELF LIFE OF ŞALAK APRICOT CULTIVAR

### 7.1. Introduction

Owing to the superior health-promoting effects, consumers have a tendency towards fresh fruits (Aneja et al., 2014). However, limited shelf life of fresh products is the main problem for both producers and the consumers (Mukhopadhyay et al., 2014). It directly affects the economy in a negative way.

Geographical characteristics of Iğdir province allow growing various fruits in the region (Alım and Kaya, 2005). Apricot is one of the most important fresh produces grown in the area. It was reported that 2% of Turkey's apricot production was supplied from the region (Malaslı et al., 2012). Moreover apricot production was indicated to be the major source of income for local farmers (Alım and Kaya, 2005). However, the fruit has a short shelf life due to its climacteric respiratory activities in the postharvest stage. It suffers from a rapid loss of quality due to the increased sensitivity to physical damage and microbial invasion (Missang et al., 2011). As a result, deterioration of the apricots occurs within 3-5 days after ripening (Egea et al., 2007b).

Moulds are the substantial source of fruit decay observed during storage period (Kalia and Parshad, 2015). Some synthetic fungicides were stated to be used in order to eliminate fungal growth. Nevertheless, the cost of the chemicals, residues on the fruit tissue and occurrence of fungicide-resistant pathogenic mutants are disincentive factors for the utilization of pesticides (Kalia and Parshad, 2015). Therefore, environmentally safe and effective alternative methods urged to be used. Mild heat treatment was distinguished with its ability to maintain both microbial and physical quality of fresh products as an alternative to chemical applications (Kou et al., 2007).

Another quality degrading problem takes place during the storage of fresh fruits is the loss of firmness. Although apricots are harvested at their unripe stage in order to prevent any damages during the transport of the fruits (Missang et al., 2011), softening arises as the time passes due to the ripening process. Ethylene hormone was shown to be responsible for the regulation of the ripening in the fruits. Moreover, calcium was also found to play a vital role in the process (Aghdam et al., 2012). Additionally calcium was said to enhance the resistance of the products towards stress conditions (Aghdam et al., 2012). Postharvest calcium applications were indicated to be effective in preserving the firmness of fresh fruit and vegetables (Saftner et al., 2003). Besides, Saxena et al. (2008) declared that MAP treatments prolong the shelf life via decreasing the rate of ripening and preventing decay. Another emerging technology in order to keep the storage quality of fresh produce is the use of UV-C irradiation. Gonzalez-Aguilar et al. (2007) speculated that UV-C application retarded the ripening process of apples, tomatoes, grapes, oranges and peaches. Furthermore, fruit decay was reported to be decreased by the treatment. This is related to the induced synthesis of antimicrobial compounds and activated plant defense-related enzymes such as phenylalanine ammonia lyase (PAL) and peroxidase (POD) (Gündüz et al., 2015).

The aim of this study was to evaluate the influence of both combined treatment of mild heat, CaCl<sub>2</sub> dipping and UV-C irradiation (MUC), and modified atmosphere packaging on the shelf life of cv. Şalâk apricots stored at 1°C for 28 days. For this purpose, some physical and chemical properties were assessed along with the microbial quality throughout the storage time.

## **7.2. Materials and Methods**

cv. Şalâk apricots of 2016 season were supplied from Iğdir province and fruits that were free from any defects, any noticeable contamination, and had a uniform size, shape and colour were selected as it was mentioned in section 4.1. The combined processing method including mild heat, CaCl<sub>2</sub> dipping and UV-C irradiation (MUC) was applied to the samples according to the details given in section 4.8. Right after the MUC treatment, apricot samples were packed by MAP technology using the selected gas concentration and composition given in section 4.9. Moreover control samples were also packed with and without MAP. In summary, the MUC treated and packaged under



MAP (MUC+MAP) and control samples packaged under MAP (Control+MAP) and without MAP (Control-MAP) were stored at 1°C for 28 days. Then the microbiological shelf life and physicochemical characteristics of Şalak apricot cultivar were assessed. Conditions for the storage of packed samples were provided in section 4.10.

During the shelf life study, the weight loss, colour parameters, total colour difference, firmness, pH, titratable acidity (TA), total soluble solid content (°Brix), ripening index (RI), ascorbic acid content of the samples were monitored and statistically evaluated considering the methods given in the Chapter 4.

Microbiological shelf life of apricot samples were determined according to methods discussed in Chapter 4. Tyriptic Soy Agar (TSA, Merck, Germany) was used to enumerate total aerobic plate count (TAPC) found on apricot surfaces. Yeast and mould count (YMC) were determined using Potato Dextrose Agar (PDA, BD Difco Corp, United States) acidified with 10% tartaric acid solution to pH 3.5. Coliforms were counted by means of a Violet Red Bile Agar (VRBA, Merck, Germany). Microbial loads were expressed as log CFU/g.

## **7.3. Results and Discussion**

### **7.3.1. Effect of MUC Processing and MAP Packaging on Physicochemical Properties of Apricot Samples during Shelf Life Period**

The impact of both MUC treatment and MAP on weight loss, colour, firmness, pH, titratable acidity, total soluble solid content (°Brix), ripening index (RI) and ascorbic acid content of the apricot samples were investigated. Control-MAP samples suffered from a complete decay at the end of the storage time. Therefore the impact of storage on the above mentioned properties could not be evaluated for Control-MAP samples on the 28<sup>th</sup> day of storage. The results were discussed in the upcoming sections.

### 7.3.1.1. Weight Loss

Weight loss of processed and unprocessed samples during the storage period was depicted in Figure 7.1.

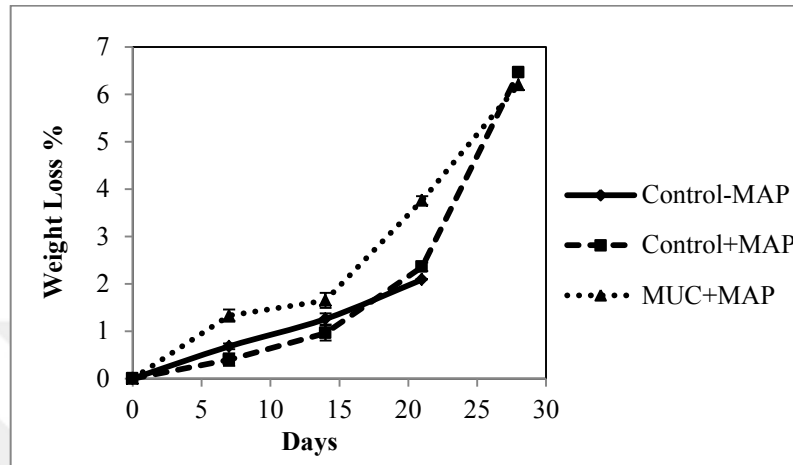


Figure 7.1. Weight loss of processed and unprocessed apricot samples stored at 1°C.

Weight loss of Control+MAP samples was the lowest among the others during the first 14 days of storage whereas MUC+MAP samples had the highest level of fresh weight loss (Figure 7.1). It can be said that MUC treatment could not prevent the weight loss of apricot samples. It might be due to the increased respiration rate and metabolic activity after the treatment. Gonzalez-Aguilar (2004) found that UV-C treated peach samples exhibited higher respiration rate and ethylene production after they were stored at 5°C for 14 and 21 days. On the contrary, Yang et al. (2014) could lower the respiration rate of the peaches during storage at 20°C by means of UV-C treatment. The contradiction between the results was reported to be due to the different storage temperatures (Yang et al., 2014).

According to the results, it is obvious that weight loss was accelerated after the 14<sup>th</sup> day and reached almost the same level for Control+MAP and MUC+MAP samples at the end of the storage. cv. Şalak apricots processed by MUC+MAP underwent  $6.37 \pm 0.09\%$  weight loss after 28 days whereas Control+MAP samples lost  $6.2 \pm 0.09\%$  of its fresh weight after the same period of time. Choi et al. (2015) declared that weight loss greater than 5% adversely affected the marketability of the fresh products. On the other hand, weight loss percentage could not be calculated for Control-MAP samples on the 28<sup>th</sup> day of storage since the apricots were completely decayed. Ali et al. (2004)

indicated that prolonged storage at low temperatures more than 14 days speeded up the aging process of carambola fruit which led to enhanced water removal from the fruit. But they could limit the weight loss by MAP treatment (Ali et al., 2004). In this study use of MAP was found to lower the rate of water loss just for 14 days considering the Control-MAP and Control+MAP samples.

It was denoted that weight loss occurred during storage was related to the postharvest conditions, storage time, respiration and transpiration of the products (Choi et al., 2015). Packaging material was also pointed out to be associated with the weight loss (Kuzucu and Önder, 2010). Mostly used polymeric-based packaging stuffs were known to have low permeability to water vapour (Castellanos et al., 2016). Therefore condensation of the evaporated water was reported to occur in the package during the storage which led to undesired microbial growth (Castellanos et al., 2016).

### 7.3.1.2. Colour

Colour changes of treated and untreated samples were monitored during the 28 days storage period. Change of lightness ( $L^*$ ) value was given in Figure 7.2.

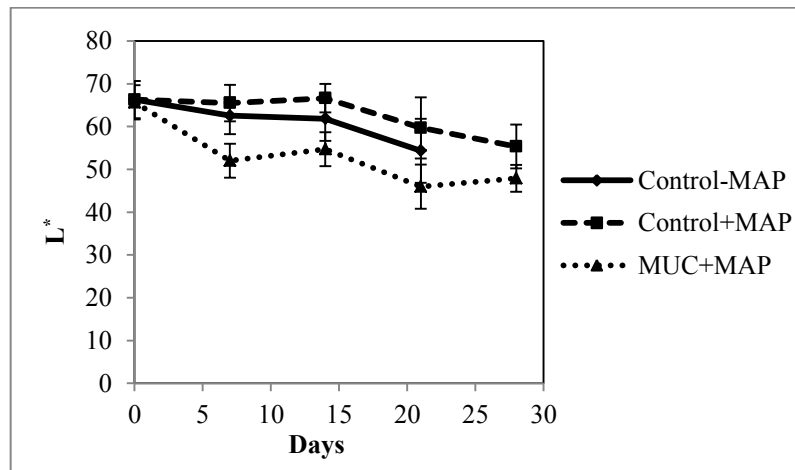


Figure 7.2. Effect of MUC processing and MAP on  $L^*$  value of cv. Şalak apricots throughout the storage at 1°C.

$L^*$  value of treated and untreated samples showed a decreasing trend throughout the storage time (Figure 7.2). However, it can be observed from Figure 7.2 that Control+MAP samples could maintain their lightness for 14 days at 1°C. Likewise Table 7.1 designated that the differences between  $L^*$  values of Control+MAP samples

on the 0<sup>th</sup>, 7<sup>th</sup> and 14<sup>th</sup> days of storage were insignificant. Choi et al. (2015) revealed that MAP application using low O<sub>2</sub> and raised CO<sub>2</sub> concentrations retarded the colour deterioration by reducing the ethylene synthesis. Muftuoğlu et al. (2012) found that ripening process of ‘Kabaası’ apricots was slowed down using MAP. Therefore samples packed by MAP retained their lightness for 28 days as compared to unpacked control samples (Muftuoğlu et al., 2012). Results of Control-MAP samples supported this finding. cv. Şalak apricots which were packed without MAP effect exhibited darkness as compared to the Control+MAP samples (Figure 7.2). Table 7.1 also pointed out that lightness values of Control-MAP samples were significantly lower (p<0.05) than that of Control+MAP samples.

Table 7.1. Change of L\* Value of Treated and Untreated cv. Şalak Apricots Packed with or without MAP during Storage at 1°C.

Colour Parameter	Treatment	Storage Time (day)				
		0	7	14	21	28
L*	Control-MAP	66.30±4.37 <sup>aA</sup>	62.55±4.32 <sup>bB</sup>	61.85±5.17 <sup>bB</sup>	54.36±7.47 <sup>bC</sup>	*
	Control+MAP	66.30±4.37 <sup>aA</sup>	65.51±4.25 <sup>aA</sup>	66.64±3.32 <sup>aA</sup>	59.72±7.16 <sup>aB</sup>	55.34±5.14 <sup>aC</sup>
	MUC+MAP	65.70±4.00 <sup>aA</sup>	52.04±3.97 <sup>cB</sup>	54.73±3.95 <sup>cB</sup>	45.97±5.15 <sup>cC</sup>	47.92±3.12 <sup>bC</sup>

<sup>a-c</sup>: Means having different letters in the same column denote significant difference at p<0.05.

<sup>A-C</sup>: Means having different letters in the same row denote significant difference at p<0.05.

On the other hand, a significant decrease in lightness was observed in MUC+MAP samples after 7 days of storage (Figure 7.2). The lowest L\* values were obtained with MUC+MAP samples during the storage time whereas Control+MAP samples displayed the highest L\* values on each sampling day (Table 7.1). It means that MUC treatment caused darkening of the product during storage at 1°C. Gonzalez-Aguilar et al. (2004) reported that UV-C treatment increased the respiratory activity and ethylene synthesis in peaches. As stated before, increased metabolic activities cause an undesired colour change. Furthermore, lower storage temperatures (2°C) were indicated to cause chilling injury and stimulate PPO and peroxidase (POD) activities which lead to enhanced browning (Ali et al., 2004). It was stated that there was a relation between water loss and the browning occurred due to chilling injury (Ali et al., 2004). Similar to L\* value, a considerable increase was encountered in the weight loss of MUC treated samples on the 7<sup>th</sup> day of storage according to the weight loss data given in section 7.3.1.1. Physiological changes such as water loss are correlated with the membrane damage. A damaged membrane becomes more pervious to water. Moreover, subcellular

compartmentalisation which prevents the physical proximity of the enzymes and their substrates also gets disrupted. Thereby, an impaired membrane allows the enzymes and substrates to get mixed. Consequently, browning reactions are started (Ali et al., 2004). Furthermore, heat treatment can cause expansion of immobilized air which exists in the intercellular region and consequently release of the trapped air from injured tissues along with the sap leakage (del Valle et al. 1998). Finally it can be concluded that increased free oxygen concentration leads to higher PPO activity.

The change of  $a^*$  value of the apricots during the storage was shown in Figure 7.3.

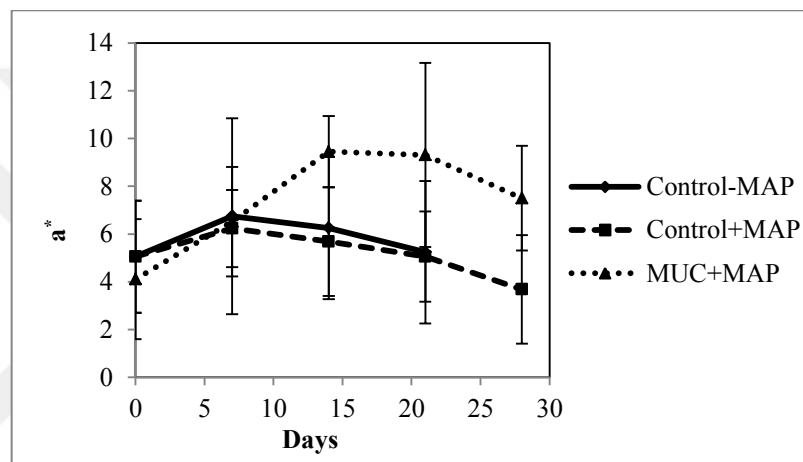


Figure 7.3. Effect of MUC processing and MAP on  $a^*$  value of cv. Şalak apricots throughout the storage.

According to Figure 7.3,  $a^*$  value (redness) of all samples increased and reached almost the same level after 7 days of storage. Weingerl and Unuk (2015) pointed out that increase in colour parameter  $a^*$  was related to the degradation of chlorophylls during storage. Table 7.2 also demonstrated that the mean values of all samples on the 7<sup>th</sup> day were found not to be statistically different. Although redness of Control-MAP and Control+MAP samples started to reduce after the 7<sup>th</sup> day,  $a^*$  value of MUC+MAP samples continued to increase up to the 14<sup>th</sup> day (Figure 7.3). After that redness of MUC+MAP samples did not significantly change until the end of storage period (Table 7.2). Enhanced redness of MUC+MAP samples compared to control samples might be due to the browning. Besides the enzymatic browning, non-enzymatic reactions such as Maillard reaction were also found to be responsible for browning in fruits (Maskan, 2006). Moreover, ripening process might cause the accumulation of carotenoids (Pretel et al., 1999). Carotenoids are known to be the principal pigments for the fruit colour

(Caprioli et al., 2009). Increased carotenoid concentration is distinguished by lower  $L^*$  value and higher  $a^*$  value (Akin et al., 2008).

Table 7.2. Change of  $a^*$  Value of Treated and Untreated cv. Şalak Apricots Packed with or without MAP during Storage at 1°C.

Colour Parameter	Treatment	Storage Time (day)				
		0	7	14	21	28
$a^*$	Control-MAP	5.05±2.34 <sup>aA</sup>	6.75±4.10 <sup>aA</sup>	6.25±2.98 <sup>bA</sup>	5.24±2.98 <sup>bA</sup>	*
	Control+MAP	5.05±2.35 <sup>aAB</sup>	6.23±1.62 <sup>aA</sup>	5.68±2.27 <sup>bA</sup>	5.05±1.89 <sup>bAB</sup>	3.68±2.27 <sup>bB</sup>
	MUC+MAP	4.11±2.51 <sup>aC</sup>	6.52±2.29 <sup>aB</sup>	9.45±1.49 <sup>aA</sup>	9.31±3.85 <sup>aA</sup>	7.50±2.19 <sup>aAB</sup>

<sup>a-b</sup>: Means having different letters in the same column denote significant difference at  $p < 0.05$ .

<sup>A-C</sup>: Means having different letters in the same row denote significant difference at  $p < 0.05$ .

The effect of processing and packaging on  $b^*$  (yellowness) value of cv. Şalak apricots was represented in Figure 7.4.

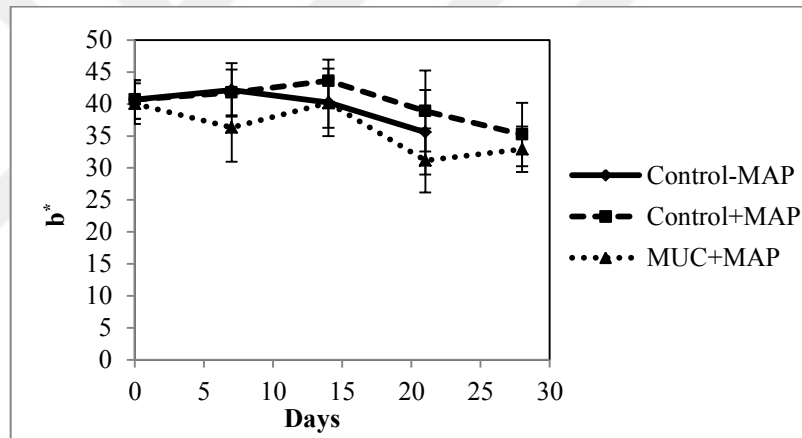


Figure 7.4. Effect of MUC processing and MAP on  $b^*$  value of cv. Şalak apricots throughout the storage.

Considering Figure 7.4, it can be said that yellowness of the apricot samples tended to reduce after the 14<sup>th</sup> day of storage time. Statistical analysis also revealed that  $b^*$  value of Control-MAP samples did not change until the day 14 but there was a significant reduction on the 21<sup>st</sup> day (Table 7.3). Besides, Control+MAP samples reached the highest  $b^*$  value on the 14<sup>th</sup> day as compared to the initial value (Table 7.3). However, a continuous reduction in  $b^*$  value was observed after the 14<sup>th</sup> day. On the other hand, measured  $b^*$  values displayed some variations in case of MUC+MAP application. It was noticed that yellowness was declined on the 7<sup>th</sup> day of storage (Figure 7.4). Nonetheless, a significant increase in  $b^*$  occurred on the 14<sup>th</sup> day (Table 7.3). Colour parameter  $b^*$  was observed to reach its initial value on that day. It might be

resulted from accelerated ripening process due to MUC treatment. Hereafter a significant decrease was appeared (Table 7.3). It was reported that enzymatic reactions were not the only one reason for modifications in fruit colour (Maskan, 2006). Degradation of colour pigments such as carotenoids and chlorophylls due to heating processes were also indicated to be responsible for the colour changes (Maskan, 2006). Reduction in  $b^*$  value means diminished yellowness of the samples. This might be developed from the destruction of carotenoids after the 14<sup>th</sup> day of storage.

Table 7.3. Change of  $b^*$  Value of Treated and Untreated cv. Şalak Apricots Packed with or without MAP during Storage at 1°C.

Colour Parameter	Treatment	Storage Time (day)				
		0	7	14	21	28
$b^*$	Control-MAP	40.70±3.01 <sup>aA</sup>	42.21±4.18 <sup>aA</sup>	40.26±5.30 <sup>bA</sup>	35.58±6.61 <sup>aB</sup>	*
	Control+MAP	40.70±3.02 <sup>aB</sup>	41.81±3.57 <sup>aAB</sup>	43.62±3.33 <sup>aA</sup>	38.90±6.34 <sup>aB</sup>	35.24±4.95 <sup>aC</sup>
	MUC+MAP	40.06±3.20 <sup>aA</sup>	36.34±5.38 <sup>bB</sup>	40.17±3.87 <sup>bA</sup>	31.18±5.00 <sup>bC</sup>	32.93±3.55 <sup>aBC</sup>

<sup>a-b</sup>: Means having different letters in the same column denote significant difference at  $p < 0.05$ .

<sup>A-C</sup>: Means having different letters in the same row denote significant difference at  $p < 0.05$ .

In order to express the colour differences occurred during the whole storage time, total colour difference ( $\Delta E$ ) was calculated (Table 7.4).

Table 7.4. Total Colour Differences throughout the Storage Time.

Colour Parameter	Treatment	Storage Time (day)			
		7	14	21	28
$\Delta E$	Control-MAP	7.18±4.37	7.69±4.93	14.37±8.30	*
	Control+MAP	5.32±2.70	5.39±2.53	10.41±5.53	13.43±5.14
	MUC+MAP	15.47±3.96	12.97±3.56	22.58±7.13	19.74±3.91

$\Delta E$  designates the extent of colour difference between processed and unprocessed products (Huang et al., 2013). The differences were classified as; not noticeable ( $\Delta E = 0$  to 0.5), slightly noticeable ( $\Delta E = 0.5$  to 1.5), noticeable ( $\Delta E = 1.5$  to 3), well visible ( $\Delta E = 3$  to 6), and great ( $\Delta E = 6$  to 12) by Huang et al. (2013). Based on this categorization, Table 7.4 pointed out that total colour differences were above well visible range for all samples regardless of the processing and packaging type. However, total colour difference was the smallest for Control+MAP samples on each sampling day. Appearance of packed fruits during the storage time was given in Figure 7.5.



Figure 7.5. cv. Şalak apricots during 4 weeks of storage at 1°C: 1) Control-MAP, 2) Control+MAP, 3) MUC+MAP; a) 7<sup>th</sup> day, b) 14<sup>th</sup> day, c) 21<sup>st</sup> day d) 28<sup>th</sup> day.

When the modified atmosphere effect was eliminated, apricot samples showed a rapid deterioration (Figure 7.5). Fruits started to decay on the 21<sup>st</sup> day. Therefore some colour changes were observed. These changes were reflected by increased  $\Delta E$  value on the 21<sup>st</sup> day of storage (Table 7.4). As explained before, MUC+MAP samples suffered from browning during the shelf life study. Colour darkening can be clearly observed from Figure 7.5. Moreover  $\Delta E$  value indicated a great difference (Table 7.4) even on the 7<sup>th</sup> day of storage. It can be concluded that MUC treatment obviously altered the fruit



colour. Although treated samples were packed by MAP, reduced O<sub>2</sub> and increased CO<sub>2</sub> levels were not enough to prevent colour degradation.

### 7.3.1.3. Firmness

According to the firmness measurements, Control-MAP samples faced with a rapid softening during the storage at 1°C (Figure 7.6). This might be due to the drop of turgor pressure resulting from the water loss and increased metabolic activity as per stated by Ali et al. (2004). On the other hand, untreated control samples packed with MAP could better preserve their firmness values as shown in Figure 7.6. Caprioli et al. (2009) suggested that providing high CO<sub>2</sub> concentrations in the atmosphere surrounding the fruit could retard the onset of ripening, ethylene synthesis and softening of the peaches and nectarines.

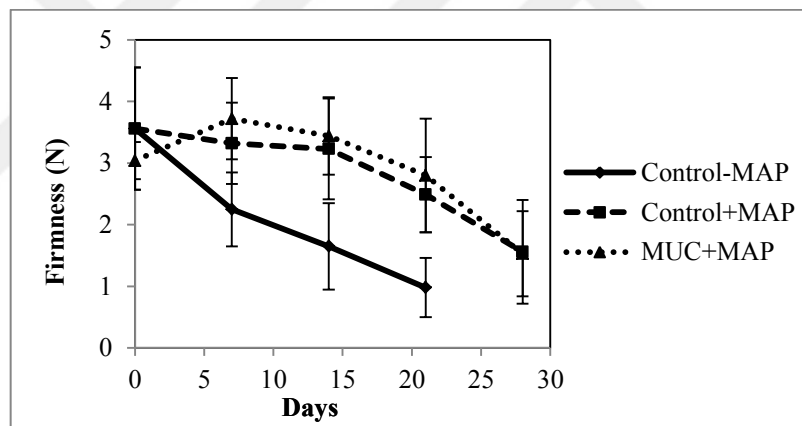


Figure 7.6. Effect of MUC processing and MAP on firmness of cv. Şalak apricots throughout the storage.

In other respects, MUC+MAP samples showed an increase in the firmness on the 7<sup>th</sup> day of storage. This improvement in the firmness can be attributed to the CaCl<sub>2</sub>+MH treatment aside from MAP effect. Beirao-da Costa et al. (2008) also referred the increase in firmness of kiwifruits to occurrence of calcium pectates in the cell wall due to the enhanced calcium linkage in presence of mild heat treatment. Furthermore, it was speculated that calcium diffusion into the fruit tissue could be activated by heating application (Abreu et al., 2003). Consequently firmness can be improved.

After the 7<sup>th</sup> day, Control+MAP samples and MUC+MAP samples exhibited a similar trend with respect to firmness value as depicted in Figure 7.6. Statistical analysis

also proved that firmness of Control+MAP and MUC+MAP samples was not significantly differed on the 14<sup>th</sup>, 21<sup>st</sup> and 28<sup>th</sup> days of storage (Table 7.5).

Table 7.5. Change of Firmness during Storage at 1°C.

Parameter	Treatment	Storage Time (day)				
		0	7	14	21	28
Firmness (N)	Control-MAP	3.56±0.99 <sup>aA</sup>	2.25±0.60 <sup>cB</sup>	1.65±0.70 <sup>bC</sup>	0.98±0.48 <sup>bD</sup>	*
	Control+MAP	3.56±0.99 <sup>aA</sup>	3.32±0.66 <sup>bA</sup>	3.23±0.82 <sup>aA</sup>	2.49±0.61 <sup>aB</sup>	1.56±0.84 <sup>aC</sup>
	MUC+MAP	3.04±0.30 <sup>aBC</sup>	3.72±0.66 <sup>aA</sup>	3.44±0.63 <sup>aAB</sup>	2.80±0.92 <sup>aC</sup>	1.53±0.69 <sup>aD</sup>

<sup>a-c</sup>: Means having different letters in the same column denote significant difference at p<0.05.

<sup>A-D</sup>: Means having different letters in the same row denote significant difference at p<0.05.

### 7.3.1.4. pH, Titratable Acidity, Total Soluble Solid Content (°Brix) and RI

It was observed that pH value of the apricot samples raised after 7 days of storage at 1°C, irrespective of the processing and packaging type (Figure 7.7). Increase of pH value was much higher in Control-MAP samples whereas it was the lowest in MUC+MAP samples. Ibrahim (2016) denoted that increased pH was an indication of decreased acidity of the juices. Furthermore storage temperature, applied processing methods and type of the fruit were asserted as effective on the pH value (Ibrahim, 2016).

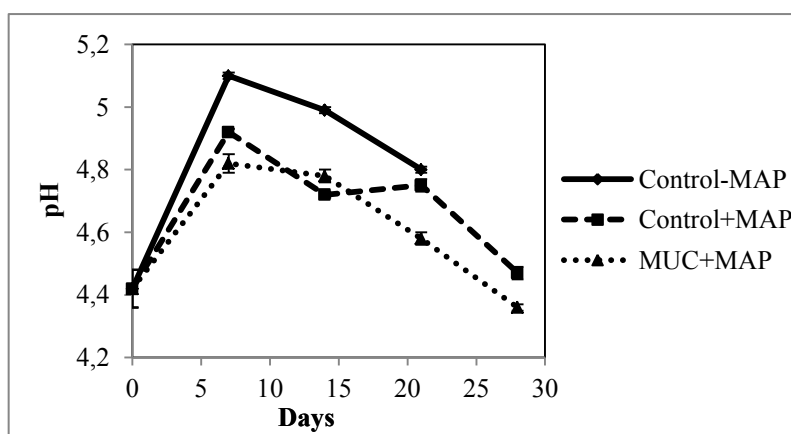


Figure 7.7. Effect of MUC processing and MAP on pH value of cv. Şalak apricots throughout the storage.

It is known that as the fruit matures the acidity decreases. Thereby it can be said that ripening of the apricots progressed during the storage time and ended up with high

pH values on the 7<sup>th</sup> day of storage due to the decreases in total acidity. Later on pH value of the samples started to decline (Figure 7.7). It might be due to the occurrence of some biochemical reactions and microbial proliferation throughout the storage period (Ibrahim, 2016).

Figure 7.8 demonstrated the changes in TA of the samples during the storage time.

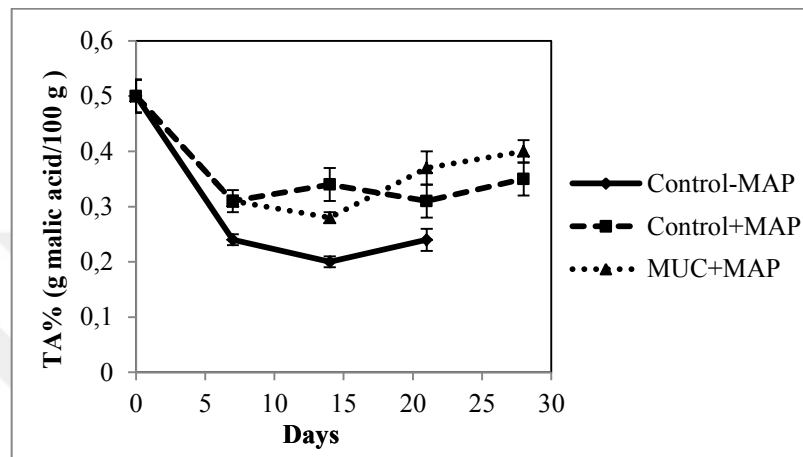


Figure 7.8. Effect of MUC processing and MAP on TA% of cv. Şalak apricots throughout the storage.

TA% of the apricots was decreased as expected during the first 7 days of storage. After that TA% of Control-MAP samples were kept almost constant throughout the storage (Figure 7.8). Similarly Control+MAP samples retained the remaining acidity for the rest of the storage time. However, a significant increase in TA% of the MUC+MAP samples was observed on the last day of storage (Figure 7.8). This might be resulting from the increase of Cl<sup>-</sup> ions (Chen et al., 2011).

It is obvious that Brix values increased on the 7<sup>th</sup> day (Figure 7.9). Slight increases in soluble solid content of apricots were reported in literature (Stanley et al., 2013). It is known that apricots do not contain starch (Kurz et al., 2008). Hence, increase in Brix value was not due to the conversion of starch into simple sugars. However, sugars could have been synthesized from other carbohydrates (Genard and Souty, 1996; Stanley et al., 2013). Moreover, enhanced Brix values might also be caused from the water loss. Ahari Mostafavi et al. (2013) argued that loss of water also caused an increase in the concentration of total soluble solids.

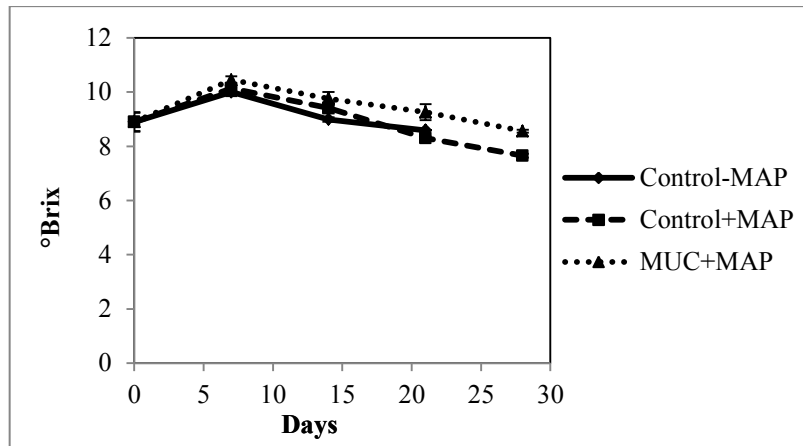


Figure 7.9. Effect of MUC processing and MAP on Brix° of cv. Şalak apricots throughout the storage.

As the time proceeded, Brix values of the samples were reduced (Figure 7.9). All the samples exhibited a similar trend. However, Brix value of MUC+MAP samples seems to be higher than the others at the end of storage. The decrease in Brix value was found to be associated with the increased respiration rate (Peano et al., 2014).

Considering TA% and Brix values, RI of the samples were calculated. Figure 7.10 describes the changes of RI values during the storage period. As stated before, increase of pH due to the decrease in TA% value and enhanced Brix values pointed out the ripening of the apricots.

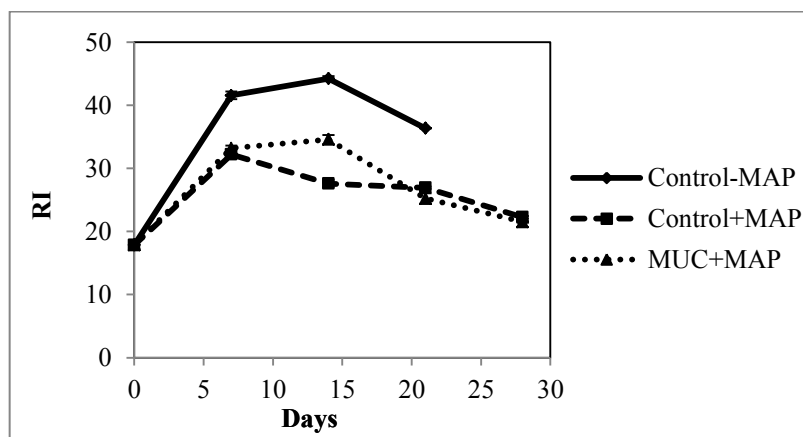


Figure 7.10. Effect of MUC processing and MAP on RI of cv. Şalak apricots throughout the storage.

Apricot is known to be a climacteric fruit. Ripening process of the climacteric fruits is driven by the ethylene hormone which gives rise to increased ripening rate (Valero et al., 2003; Caprioli et al., 2009). Ethylene is a self-stimulated hormone

(Munoz-Robredo et al., 2012). Its production reaches a peak value when physical and chemical alterations arise, such as loss of firmness, reduction in acidity versus enhancement in sugar levels and colour degradation (Pretel et al., 1999; Valero et al., 2003). As it was described until now, some physical and chemical changes occurred in the apricot samples during the storage. It can be said that ripening of apricot samples progressed in the first 7 days of storage. As compared to initial value of each individual parameter, significantly higher pH value and Brix degree, and lower total acidity values due to the metabolic activity were obtained on the 7<sup>th</sup> day of storage (Table 7.6).

Table 7.6. Change of pH, TA%, Brix° and RI during Storage at 1°C.

Parameter	Treatment	Storage Time (day)				
		0	7	14	21	28
pH	Control-MAP	4.42±0.06 <sup>aD</sup>	5.10±0.01 <sup>aA</sup>	4.99±0.01 <sup>aB</sup>	4.80±0.01 <sup>aC</sup>	*
	Control+MAP	4.42±0.06 <sup>aC</sup>	4.92±0.01 <sup>bA</sup>	4.72±0.01 <sup>cB</sup>	4.75±0.02 <sup>bB</sup>	4.47±0.02 <sup>aC</sup>
	MUC+MAP	4.42±0.06 <sup>aC</sup>	4.82±0.03 <sup>cA</sup>	4.78±0.02 <sup>bA</sup>	4.58±0.02 <sup>cB</sup>	4.36±0.01 <sup>bC</sup>
TA%	Control-MAP	0.50±0.03 <sup>aA</sup>	0.24±0.01 <sup>bB</sup>	0.20±0.01 <sup>cB</sup>	0.24±0.02 <sup>bB</sup>	*
	Control+MAP	0.50±0.03 <sup>aA</sup>	0.31±0.02 <sup>aB</sup>	0.34±0.03 <sup>aB</sup>	0.31±0.03 <sup>abB</sup>	0.35±0.03 <sup>aB</sup>
	MUC+MAP	0.50±0.03 <sup>aA</sup>	0.31±0.01 <sup>aCD</sup>	0.28±0.01 <sup>bD</sup>	0.37±0.03 <sup>aBC</sup>	0.40±0.02 <sup>aB</sup>
°Brix	Control-MAP	8.90±0.35 <sup>aB</sup>	10.00±0.15 <sup>bA</sup>	8.99±0.08 <sup>cB</sup>	8.59±0.01 <sup>bB</sup>	*
	Control+MAP	8.90±0.35 <sup>aC</sup>	10.14±0.06 <sup>bA</sup>	9.41±0.20 <sup>bB</sup>	8.30±0.06 <sup>bD</sup>	7.65±0.07 <sup>bE</sup>
	MUC+MAP	8.90±0.35 <sup>aCD</sup>	10.45±0.13 <sup>aA</sup>	9.76±0.24 <sup>aB</sup>	9.26±0.30 <sup>aC</sup>	8.56±0.05 <sup>aD</sup>
RI	Control-MAP	17.86±0.70 <sup>aD</sup>	41.58±0.63 <sup>aB</sup>	44.23±0.38 <sup>aA</sup>	36.38±0.05 <sup>aC</sup>	*
	Control+MAP	17.86±0.70 <sup>aD</sup>	32.21±0.24 <sup>cA</sup>	27.60±0.58 <sup>bB</sup>	26.94±0.18 <sup>bB</sup>	22.26±0.20 <sup>aC</sup>
	MUC+MAP	17.86±0.70 <sup>aE</sup>	33.21±0.41 <sup>bB</sup>	34.60±0.71 <sup>cA</sup>	25.23±0.82 <sup>cC</sup>	21.53±0.13 <sup>bD</sup>

<sup>a-c</sup>: Means having different letters in the same column for each parameter denote significant difference at p<0.05.

<sup>A-E</sup>: Means having different letters in the same row denote significant difference at p<0.05.

However, Control-MAP and MUC+MAP samples showed the highest RI on the 14<sup>th</sup> day (Table 7.6). This might be caused by the variations in Brix and TA values. On the other hand, Tiecher et al. (2013) reported that ethylene synthesis in tomato fruit was induced by UV-C irradiation. Although control samples were indicated to complete climacteric respiration after 7 days-storage, ethylene production was found to be still higher in UV treated tomatoes (Tiecher et al., 2013). High ethylene production was considered as a sign of incomplete ripening (Tiecher et al., 2013). It was also possible that ripening of MUC+MAP samples was completed on the 14<sup>th</sup> day. After the RI

achieved the highest value, it started to decline for all samples due to the reduction in Brix value and increase in the acidity.

### 7.3.1.5. Ascorbic Acid (AsA) Content

AsA content of processed and unprocessed cv. Şalak apricots was monitored during 4 weeks of storage. Results were presented in Figure 7.11.

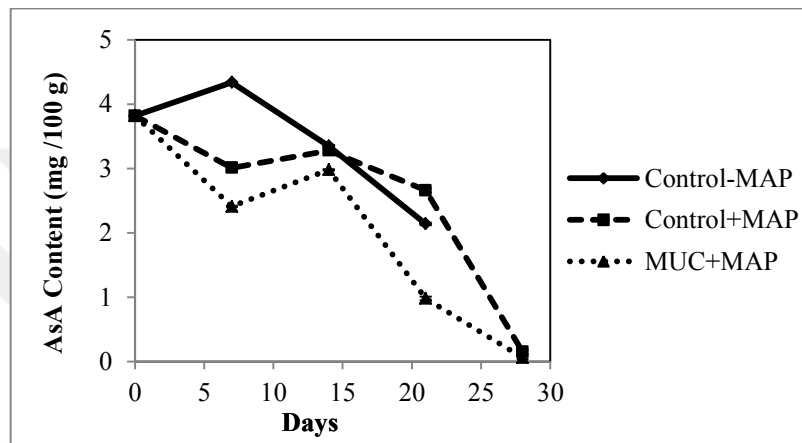


Figure 7.11. Effect of MUC processing and MAP on AsA content of cv. Şalak apricots throughout the storage.

AsA content of apricot samples showed a decreasing trend during the storage period (Figure 7.11). Nevertheless, average AsA contents of the samples were not significantly different from each other on the day 14 (Table 7.7). However, AsA content of MUC+MAP samples was significantly reduced on the 21<sup>st</sup> day and reached the lowest level among the others. Processing of the apricots by UV-C and mild heating might be effective on the ascorbic acid content. Lu et al. (2016) revealed that UV-C treatment adversely affected the AsA content of button mushrooms during storage. Moreover, AsA content of fresh squeezed white grape juice was found to diminish during 14 days-storage due to UV-C effect (Unluturk and Atılğan, 2015). In another study, gas composition was denoted to alter the AsA content (Franck et al., 2003). It was explored that AsA could be regenerated based on the concentrations of O<sub>2</sub> and CO<sub>2</sub> in the atmosphere. Controlled atmosphere conditions i.e., low O<sub>2</sub> and elevated CO<sub>2</sub> concentrations, caused the degradation of AsA throughout the storage whereas pears stored in air indicated to have higher AsA throughout the storage (Franck et al., 2003).

Besides, ascorbic acid oxidase (AAO) enzyme activity remarked to be a determinant factor on AsA content (Ramano Rao et al., 2011). Although calcium dips were stated to have an improving effect on AsA content (Shafiee et al., 2010), MUC+MAP samples suffered from a continuous degradation of AsA during the shelf life (Table 7.7). On the other hand, it can be observed from Table 7.7 that Control+MAP samples could better preserve the AsA content until the last day of storage time. Cold storage might have reduced the rate of ascorbic acid degradation as per stated by Barberis et al. (2012). Furthermore, respiratory activities of Control+MAP samples were not induced by any treatment. Therefore AsA could be maintained during storage. Despite the fact that Control+MAP samples were having higher levels of AsA content during the storage, AsA contents of both Control+MAP samples and MUC+MAP samples were completely diminished on the last day of storage (Table 7.7).

Table 7.7. Change of AsA Content during Storage at 1°C.

Parameter	Treatment	Storage Time (day)				
		0	7	14	21	28
AsA	Control-MAP	3.82±0.12 <sup>aAB</sup>	4.34±0.31 <sup>aA</sup>	3.35±0.02 <sup>aB</sup>	2.14±0.10 <sup>bC</sup>	*
	Control+MAP	3.82±0.12 <sup>aA</sup>	3.01±0.04 <sup>aBC</sup>	3.28±0.17 <sup>aB</sup>	2.66±0.04 <sup>aC</sup>	0.15±0.00 <sup>aD</sup>
	MUC+MAP	3.82±0.12 <sup>aA</sup>	2.41±0.86 <sup>aB</sup>	2.98±0.02 <sup>aAB</sup>	0.98±0.06 <sup>cC</sup>	0.06±0.04 <sup>aC</sup>

<sup>a-c</sup> : Means having different letters in the same column denote significant difference at p<0.05.

<sup>A-D</sup> : Means having different letters in the same row denote significant difference at p<0.05.

AsA is known to be a water soluble antioxidant (Liu et al., 2014). It was reported to be used as a marker for nutritional quality retention for the processed products (Ibrahim, 2016). Water soluble vitamins were indicated to be more sensitive (Yahyaie et al., 2013). Light, heat, metals, pH of the environment, presence of oxygen, enzymes and moisture were declared to be effective on the degradation of AsA (Yahyaie et al., 2013). An immediate AsA degradation process begins after the fruit are harvested and the degeneration consistently progresses throughout the extended storage time (Liu et al., 2014; Ibrahim, 2016). It was also implied that AsA was highly susceptible to oxidative processes and get extracted into the water during the storage period (Ibrahim, 2016). Villa-Rodriguez et al. (2013) also claimed that antioxidant status of the foods could be affected by irradiation processes since irradiation was indicated to break chemical bonds and to form reactive oxygen species (ROS) and free radicals (Villa-Rodriguez et al., 2013). The reason to observe that MUC+MAP samples had the lowest AsA contents during the storage might be the accelerated ripening and

sensitivity of AsA to the treatment. Moreover, higher activity of enzymes such as PPO, POD (peroxidase) and lower AsA capacity could result in a substantial decrease in AsA levels (Picouet et al., 2016).

### 7.3.2. Effect of MUC Processing and MAP Packaging on The Survival Behaviour of Natural Microbial Flora of Apricot Surfaces during Shelf Life Period

In order to demonstrate the effect of postharvest MUC processing and packaging on the microbial quality of cv. Şalak apricots, survival numbers of TAPC, YMC and coliforms were monitored during 4 weeks of storage. Figure 7.12 describes the changes in TAPC during shelf life period.

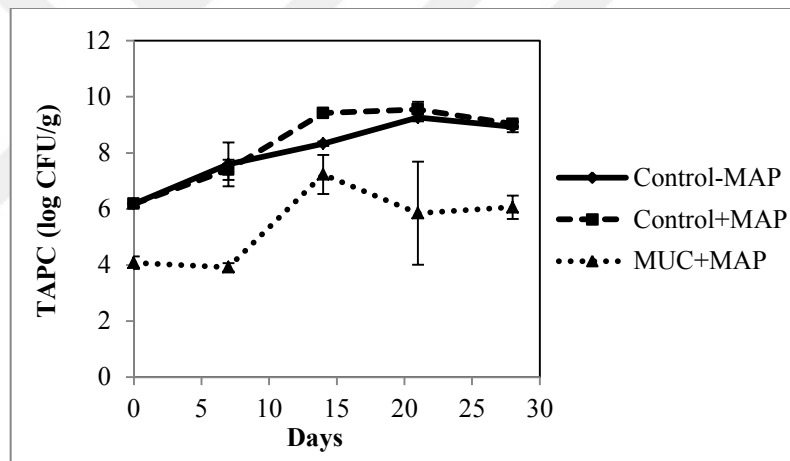


Figure 7.12. Effect of MUC processing and MAP on TAPC of cv. Şalak apricots throughout the storage.

Initial TAPC of control samples was almost 6-log CFU/g (Figure 7.12). To the best of our knowledge no information about the microbial load of cv. Şalak apricots is available in literature. However, initial load of the samples were found to be higher than that of other fruit and vegetables. For instance, Guerreiro et al. (2016) reported  $4.4 \pm 0.24$  log CFU/g of microbial load for cherry tomatoes. Similarly, Klaiber et al., (2005) obtained almost 4-log CFU/g of TAPC for minimally processed carrots. On the other hand, 6.7-log CFU/g of initial count was denoted for grated carrots (Klaiber et al., 2005). The differences might be attributed to the fruit variety, cultivation, harvest and postharvest handling as per stated by Guerreiro et al. (2016).



As can be seen from Figure 7.12, TAPC of control samples exhibited an increasing trend as the time proceeded. However, TAPC of Control-MAP and Control+MAP samples reached a stationary phase after the 14<sup>th</sup> day and did not significantly change until the end of the storage (Table 7.8).

Table 7.8. Change of TAPC during Storage at 1°C.

Treatment	Storage Time (day)				
	0	7	14	21	28
<b>Control-MAP</b>	6.18±0.12 <sup>aC</sup>	7.59±0.78 <sup>aB</sup>	8.33±0.08 <sup>abAB</sup>	9.26±0.13 <sup>aA</sup>	8.93±0.19 <sup>aAB</sup>
<b>TAPC (log CFU/g)</b>					
<b>Control+MAP</b>	6.18±0.12 <sup>aC</sup>	7.40±0.36 <sup>aB</sup>	9.42±0.07 <sup>aA</sup>	9.55±0.27 <sup>aA</sup>	9.03±0.09 <sup>aA</sup>
<b>MUC+MAP</b>	4.08±0.22 <sup>bB</sup>	3.92±0.15 <sup>bB</sup>	7.23±0.70 <sup>bA</sup>	5.85±1.84 <sup>aAB</sup>	6.06±0.42 <sup>bAB</sup>

<sup>a-b</sup>: Means having different letters in the same column denote significant difference at p<0.05.

<sup>A-C</sup>: Means having different letters in the same row denote significant difference at p<0.05.

Initial TAPC of MUC+MAP samples demonstrated that MUC treatment could reduce the initial count by nearly 2-log as compared to control samples. During the first 7 days, MUC+MAP samples did not indicate an increment in the number of survivals. Stability of TAPC for 7 days can be correlated to the MAP application. Saxena et al. (2008) found that MAP with low O<sub>2</sub> and high CO<sub>2</sub> restricted the growth of bacteria rather than yeasts and moulds in fresh-cut jackfruit bulbs. Nevertheless, after 7 days of storage a sharp increase in TAPC was obtained for MUC+MAP samples. The marked increment could be related to the recovery of the cells sublethally injured by UV-C. It was stated that sublethally injured cells were able to repair themselves after the UV-C processing (Cheon et al., 2015; Choi et al., 2015). Allende et al. (2006) also pointed out the repair process of wild type microorganisms. In order to prevent photoreactivation of sublethally injured cells, use of dark coloured packaging materials can be recommended. In spite of the fact that TAPC of MUC+MAP samples attained its peak value on the day 14 (Figure 7.12), the microbial load did not exceed the recommended limit of 7.7-log CFU/g for TAPC (Klaiber et al., 2005) during the shelf life study.

Although MUC+MAP samples were having lower TAPC as compared to others, statistical analysis revealed that TAPC of all samples was not significantly different from each other on the 21<sup>st</sup> day (Table 7.8). This was because of the variation in TAPC of MUC+MAP samples. Several plates exhibited no growth. Thereby, the differences between treatment means became insignificant. Same situation was also reported by

Kou et al. (2007). However, TAPC of MUC+MAP samples was significantly lower than that of others on the last day of storage (Table 7.8).

Influence of MUC treatment and MAP at cold storage on YMC of cv. Şalak apricots was shown in Figure 7.13.

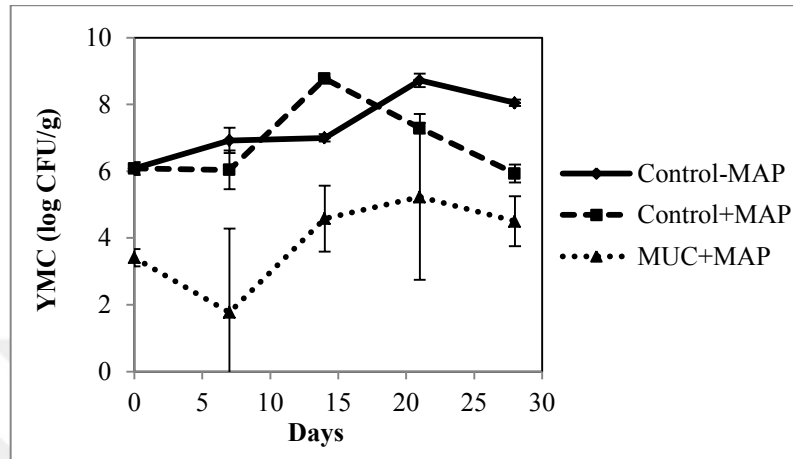


Figure 7.13. Effect of MUC processing and MAP on YMC of cv. Şalak apricots throughout the storage.

Control samples were initially having YMC in the order of almost 6-log CFU/g (Figure 7.13). It is known that fresh produce can harbour great number of microbial populations (Leff and Fierer, 2013). The composition of microbial communities displays varieties among different types of fresh products (Leff and Fierer, 2013). For instance, Mukhopadhyay et al. (2014) found the initial YMC for grape tomatoes as 4-log CFU/g which is lower than that of cv. Şalak apricots. The differences between different products may be arisen from environmental factors such as, pH and water activity, growing conditions, transport and storage conditions, and farming practices (Leff and Fierer, 2013).

Figure 7.13 and Table 7.9 indicated a significant increase in the initial YMC of Control-MAP samples on the 7<sup>th</sup> day of storage. Nevertheless the number of the yeast and mould population was not changed until the day 14. Afterwards YMC was again significantly increased on the 21<sup>st</sup> day and did not change until the end of the storage. This gradual increase in YMC of Control-MAP samples might be due to the changes in physical conditions such as, pH and moisture content, and decrease in nutritional factors necessary for the microbial growth. After the cells adapted themselves to the changed environment they continued to increase and then reached a stationary phase. Furthermore, presence of multiple nutrients in the medium might have caused the

observation of growth in stages (Ray, 2003). In such a case, microorganisms prefer to use the limited nutrient firstly. Subsequent to the consumption of the first nutrient, a short lag phase occurred and then microorganisms start utilizing the other nutrient (Ray, 2003). Finally, growth curve reaches a stationary phase. According to the visual observations the fruits were decayed when the stationary phase was reached on the 21<sup>st</sup> day (Figure 7.5).

Table 7.9. Change of YMC during Storage at 1°C.

	Treatment	Storage Time (day)				
		0	7	14	21	28
YMC (log CFU/g)	Control-MAP	6.08±0.19 <sup>aC</sup>	6.92±0.38 <sup>aB</sup>	7.00±0.11 <sup>aB</sup>	8.72±0.20 <sup>aA</sup>	8.05±0.10 <sup>aA</sup>
	Control+MAP	6.08±0.19 <sup>aC</sup>	6.04±0.58 <sup>aC</sup>	8.77±0.03 <sup>aA</sup>	7.29±0.16 <sup>aB</sup>	5.93±0.27 <sup>bC</sup>
	MUC+MAP	3.41±0.26 <sup>bA</sup>	1.77±2.51 <sup>bA</sup>	4.58±0.99 <sup>bA</sup>	5.23±2.48 <sup>aA</sup>	4.5±0.75 <sup>bA</sup>

<sup>a-b</sup>: Means having different letters in the same column denote significant difference at p<0.05.

<sup>A-C</sup>: Means having different letters in the same row denote significant difference at p<0.05.

In case of Control+MAP samples, YMC was stable for the first 7 days of storage. MAP application limits the oxygen in the package atmosphere. Thereby, microorganisms needed to adapt themselves to the reduced oxygen levels. First 7 days might be considered as the lag phase of growth for YMC of Control+MAP samples. After the adaptation period, the number of cells displayed a substantial increase on the 14<sup>th</sup> day. YMC reached the peak value on this day and then started to decrease due to that the environment became unfavourable for the growth. However, YMC of Control-MAP and Control+MAP fruits did not statistically differ until the last day of the storage (Table 7.9). Although both treatments resulted in the same level of microbial proliferation, fruit decay could not be visually observed in Control+MAP samples throughout the storage time whereas rottenness was detected in Control-MAP samples on the 21<sup>st</sup> day of storage (Figure 7.5). This was due to the MAP effect. Jouki and Khazaei (2014) declared that high CO<sub>2</sub> in the atmosphere restricted spore germination and mycelium growth of *B. cinerea* and other fungi. Furthermore, MAP was designated to prevent decay (Selcuk and Erkan, 2014).

MUC+MAP treatment was found to reduce YMC by almost 2.6-log as compared to the initial load of control samples. This higher level of inactivation might be due to MH treatment since UV-C treatment is known to have a limited effect on YMC. Allende et al. (2006) found no differences in yeast counts of UV-C treated and

untreated ‘Red Oak Leaf’ lettuce samples at the end of storage. It can be said that YMC of MUC+MAP fruits did not significantly change throughout the whole storage period considering the statistical analysis (Table 7.9). Although a decrease was observed on the 7<sup>th</sup> day (Figure 7.13), it was due to the variations occurred in the data. As it was explained earlier, no growth was observed in some of the plates. This brought about some deviations in the data.

Yeast counts were denoted to restrict the shelf life of vegetables packed by MAP (Allende et al., 2006). It was also expressed that 5-log CFU/g limit was considered for YMC in order to maintain sensorial quality of the product (Allende et al., 2006). As can be seen from Table 7.9, YMC of MUC+MAP samples did not exceed 5-log-limit until the 21<sup>st</sup> day. Moreover samples did not show any visible sign of fruit decay (Figure 7.5). Reduced soft-rot could be due to induced strengthening of ultrastructural barriers and synthesis of antimicrobial compounds by heat treatment effect (Sivakumar and Fallik, 2013; Rodoni et al., 2016). Sivakumar and Fallik (2013) stated that MH treatment, MAP application and cold storage were able to reduce decay in many fruits such as peaches, nectarines, tomatoes and strawberries. Similarly Villalobos et al. (2014) also argued that mycelial growth and decay could be prevented by MAP treatment. Furthermore UV-C treatment was also indicated to reduce fungal infections and decay in mangoes and kiwifruits (Gonzalez-Aguilar et al., 2001; Gonzalez-Aguilar et al., 2007; Bal and Kok, 2009).

Coliform counts belong to the treated and untreated samples were given below (Figure 7.14).

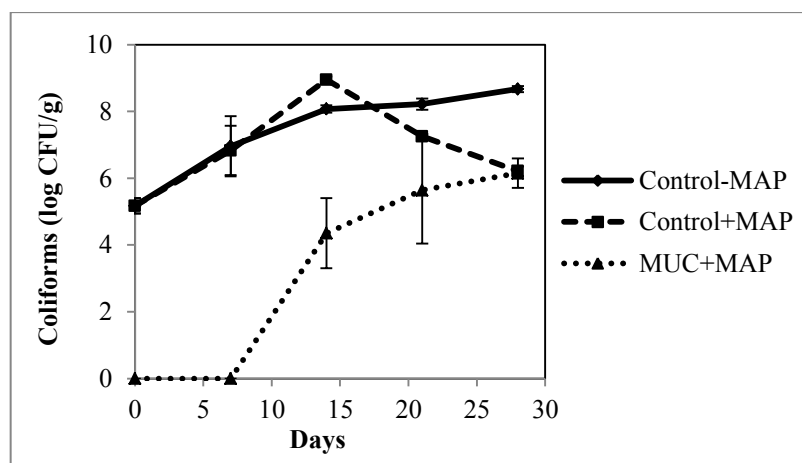


Figure 7.14. Effect of MUC processing and MAP on Coliforms of cv. Şalak apricots throughout the storage.

Initial coliform count of control samples was determined to be almost 5-log CFU/g (Figure 7.14). Growth curve of coliforms found in Control+MAP and Control-MAP samples exhibited a similar trend throughout the storage. Although the number of coliforms increased until the day 14, it started to reduce after the 14<sup>th</sup> day for Control+MAP samples (Figure 7.14). This reduction might be due to the lack of nutrients for microbial growth and accumulation of metabolic wastes (Ray, 2003). On the other hand, statistical analysis revealed that Control+MAP and Control-MAP samples did not significantly differ in coliform counts during 21 days of storage (Table 7.10). However, on the last day of storage coliform count of Control+MAP fruits was significantly lower than that of Control-MAP samples.

Table 7.10. Change of Coliforms during Storage at 1°C.

	Treatment	Storage Time (day)				
		0	7	14	21	28
Coliforms (log CFU/g)	Control-MAP	5.17±0.23 <sup>aC</sup>	6.96±0.90 <sup>aB</sup>	8.07±0.11 <sup>aAB</sup>	8.22±0.17 <sup>aAB</sup>	8.67±0.09 <sup>aA</sup>
	Control+MAP	5.17±0.23 <sup>aC</sup>	6.83±0.74 <sup>aB</sup>	8.95±0.10 <sup>aA</sup>	7.25±0.07 <sup>aB</sup>	6.21±0.14 <sup>bBC</sup>
	MUC+MAP	ND <sup>bB</sup>	ND <sup>bB</sup>	4.35±1.05 <sup>bA</sup>	5.64±1.60 <sup>aA</sup>	6.15±0.44 <sup>bA</sup>

<sup>a-b</sup>: Means having different letters in the same column denote significant difference at p<0.05.

<sup>A-C</sup>: Means having different letters in the same row denote significant difference at p<0.05.

ND: Not detected.

Processing of the apricots by combined MUC treatment resulted in complete reduction of coliforms (Figure 7.14). The treatment was much more effective on the coliforms as compared to TAPC and YMC. Different types of microorganisms have different levels of sensitivity to the processing by UV-C light due to some differences in their genetic material and cell wall properties. For instance bacteria and oocysts of *Cyrtosporidium* and *Giardia* were reported to be more sensitive to UV-C light irradiation than yeasts and moulds (Koutchma et al., 2009; Hakguder Taze et al., 2015). On the other hand gram-positive bacteria were indicated to be more resistant to UV-C irradiation due to their thicker cell walls than gram-negative bacteria (Snider et al., 2009). Coliforms are classified as gram-negative bacteria. However, resident microflora of the foods can be composed of a mixed population including gram-positive and gram-negative bacteria. Thereby UV-C treatment might have resulted in higher reductions in the number of coliforms as compared to TAPC. Moreover, pre-processing by MH

treatment might render the coliforms sublethally injured for further processing by UV-C light. This could also increase the number of inactivated cells.

Figure 7.14 shows that coliform count was not changed during the first 7 days. As stated before, this adaptation (lag) phase might arise from the repairment of sublethally injured cells. Then, microorganisms entered the exponential growth phase and increased their number by almost 4-log on the 14<sup>th</sup> day (Table 7.10). It can be observed from Figure 7.14 that the rate of growth slowed down after the 14<sup>th</sup> day. Coliforms reached the stationary phase. Table 7.10 also indicated that coliform counts did not significantly alter between 14<sup>th</sup> and 28<sup>th</sup> days of storage. Additionally, a gas formation and swelling in the packages was distinguished in both of the Control+MAP and MUC+MAP samples after the 14<sup>th</sup> day of storage. Despite the fact that the number of coliforms was lower than that of Control+MAP samples on the 14<sup>th</sup> day, they reached the same level on the last day of storage. It should be also considered that some big fluctuations occurred in the MUC+MAP samples due to that some of the plates did not exhibit microbial growth whereas some of them provided high numbers of colonies.

#### **7.4. Conclusion**

The effects of both packaging by MAP and MUC treatment were evaluated in terms of physicochemical and microbial quality of cv. Şalâk apricots under cold storage (1°C) for 28 days.

Results showed that weight loss was the highest for MUC+MAP samples indicating the increased respiration rate of the samples due to MUC treatment. However, weight loss percentage of MUC+MAP samples did not exceed 5% limit which was set for the marketability of the fresh products until the 21<sup>st</sup> day of storage. Moreover, MAP application was determined to restrict the water loss for 14 days. However, water accumulation was observed in the package for both of the MUC+MAP and Control+MAP samples due to the low water vapour permeability of the packaging material.

Colour which is an important quality parameter exhibited some changes during the shelf life. It was clear that MUC treatment resulted in the browning of the products with higher  $L^*$  and  $a^*$  values after 7 days of storage. This might be due to the accelerated ripening process and accumulation of carotenoids. On the other hand,

control samples packed using modified atmosphere conditions displayed better preservation of initial lightness throughout the storage time. However, reduction in  $a^*$  value was observed after the 7<sup>th</sup> day of storage. Furthermore yellowness ( $b^*$ ) decreased for all samples after the 14<sup>th</sup> day. Total colour difference index ( $\Delta E$ ) revealed that colour of both control and treated samples were changed in a well visible way regardless of the processing and packaging type. However, the difference was the highest for MUC+MAP treated samples.

In terms of firmness, MUC+MAP treatment ensured the highest firmness values as compared to others during the storage. MAP treatment was also found to be effective in preserving the fruit firmness. Storing the fruits without being treated by MUC and packed by MAP ended up with a rapid softening process.

It was observed that pH of all samples signified an increase after 7 days at cold storage which was in accordance with a decrease in acidity of the fruits. Ripening of the apricots led to the decrease in acidity and an increase in Brix value. Brix value of the samples showed a decrease after the 7<sup>th</sup> day of storage which might be due to the microbial proliferation. Nevertheless MUC+MAP samples had the highest Brix values during the shelf life. This can be attributed to the reduced number of microorganisms by MUC treatment. MAP application limited the ripening process of the fruits for 7 days as RI indicated the lowest value for Control+MAP samples. Although MUC treatment speeded up the process, RI values for MUC+MAP samples were lower than that of Control-MAP samples. MUC+MAP samples finalized the ripening on the 14<sup>th</sup> day with the highest RI value.

AsA content of Control+MAP and MUC+MAP samples followed a similar decreasing trend during the first 7 days whereas Control-MAP samples revealed an increase since they were stored under normal atmosphere conditions. On the other hand, MAP and MUC applications decreased the AsA content up to some extent. In spite of the fact that all the samples reached the same level of AsA content on the 14<sup>th</sup> day, AsA content of MUC+MAP samples was found to be more susceptible to degradation.

It was observed that natural microflora of apricot samples could be lowered using MUC processing. MUC+MAP samples exhibited the lowest TAPC throughout the shelf life and the number of aerobic bacteria was decreased by 2-log. Furthermore TAPC of MUC+MAP samples did not exceed the 7.7-log limit during the whole storage. On the other side, individual MAP application was found not to be effective on the inactivation of TAPC. Besides, MUC treatment was also able to reduce YMC in the

apricot samples. The treatment yielded almost 2.6-log reduction and YMC of the treated samples did not go beyond 5-log limit of better sensorial quality. But YMC of MAP treated control samples attained great numbers during the shelf life. Additionally, fruit decay could be prevented by both MUC and MAP treatments. However, Control-MAP samples suffered from soft-rot after the 14<sup>th</sup> day of storage. In addition to these all, MUC treatment was found to be very effective in inactivating the coliforms. A complete inactivation of coliforms (almost 5-log) was obtained after the processing and the number of coliforms did not change during the first 7 days of storage. After the 14<sup>th</sup> day of storage, swelling in the Control+MAP and MUC+MAP packages was observed due to the excessive growth of the coliforms.

In conclusion, MUC+MAP treatment could extend the microbial shelf life of cv. Şalak apricots up to 14 days under the current treatment and storage conditions. On the other hand, colour was found to be negatively affected by the treatment and the colour difference could be visually detected during the storage. This is an important quality parameter with respect to consumer acceptance. Thereby, processing parameters which would not result in an unwanted colour change during the storage period should be further investigated. Moreover, consumer acceptance test should be applied to the treated samples during the storage period in order to determine if the treated apricots are still acceptable by the real consumers or not. Besides all, the effect of different packaging materials, which can absorb ethylene in order to retard the ripening process and/or which allow the transmission of water vapour in order to prevent the accumulation of water leakage, on the shelf life of cv. Şalak apricots needs to be explored.



## CHAPTER 8

### OVERALL CONCLUSION

The main objective of this Ph.D. study was to develop a processing method which would extend the shelf life of cv. Şalak apricot of Iğdır province. Iğdır province is known to contribute to the national apricot production by almost 2%. Hence, it is an important means of living for local farmers in Iğdır. Moreover, cv. Şalak apricot is a specific type of product with its unique size, shape and taste. However, its distribution and marketing is very limited due to its perishable nature and restricted shelf life. Since drying is not applicable for cv. Şalak apricot due to its low dry matter content, a novel method which would not alter the fruit quality and maintain the product safety was urged to be developed. By this way, marketability of this specific fruit could be enhanced and economical losses could be prevented. It would be also possible to generate income from exportation of cv. Şalak apricots to Europe and other countries. Moreover, consumers would have the chance to reach this specific apricot for longer time through the season. Hence, the applicability of UV-C irradiation processing, Mild Heat (MH) treatment, CaCl<sub>2</sub> dipping and Modified Atmosphere Packaging (MAP) along with cold storage was investigated throughout the Ph.D. thesis.

The first aim of the study was to find an optimum processing condition for UV-C treatment to provide microbial safety in Şalak apricot cultivar. For this purpose the highest possible microbial inactivation in the natural microflora of the apricot samples was aimed and studied using two different bench-top UV-C systems. The first system was containing two identical low pressure mercury vapour lamps emitting UV-C light at 254 nm. Additionally, the second system was equipped with four rollers without grooves with fine tothing allowing a manual rotation of the fruits in order to provide an equal UV-C dose among the fruit samples. Different treatment times (0-20 min) were tested at the highest light intensity level (0.29 mW/cm<sup>2</sup>) by means of a first UV-C system. It was found that only 1.36-log reduction in Total Aerobic Plate Count (TAPC) was achieved after the fruits were exposed to the highest UV-C dose in the first system (348 mJ/cm<sup>2</sup>). Although coliforms were completely inactivated (almost 4.5-log) after UV-C treatment, Yeast and Mould Count (YMC) was found not to be significantly

affected by the treatment. Only 0.56-log reduction was obtained for YMC. Therefore, a new system with four lamps and a rotating roller was designed and constructed for further studies. Light intensity was increased to 3.23 mW/cm<sup>2</sup> by this new system. Furthermore rotating rollers allowed the samples to be exposed the same level of UV-C light irradiation throughout the study. Different treatment times ranging from 0 to 30 min was evaluated in terms of microbial inactivation. Results indicated that inactivation of TAPC was increased to 3-log after 16 min of exposure whereas YMC could be reduced by 2.37-log using the four-lamp system. Similar to the previous findings, no coliform growth was attained after any treatment. Nonetheless, application of treatment times longer than 20 min did not result in further decrease in the number of microorganisms. Additionally some fluctuations in the microbial data were detected. In order to reveal the effect of light intensity distribution on the inactivation data, the light intensity distribution along the lamp axis was estimated by means of a numerical solution. According to the results, the light intensity was maximized in the central parts whereas it reduced on both ends of the lamps. Thereby it was decided that apricots should have been located in the central parts of the rotating rollers for further studies. Moreover, microbial data were mathematically modelled. It was observed that the microbial data agreed well with log linear+tail model which indicated that application of higher UV-C doses would not give rise to improve log reduction in the number of microorganisms. Considering these all, the optimum treatment conditions for UV-C light irradiation was found to be 3.23 mW/cm<sup>2</sup> light intensity and 19 min of exposure time which was supplied by the second UV-C system.

The second aim was to find out optimum conditions for Mild Heat (MH) treatment. For this purpose an experimental design was created. According to that, temperatures between 40-60°C for 10-60 min were investigated considering both microbial inactivation and quality parameters (colour and firmness) of the fruits. Results showed that the highest microbial reductions (at least 3-log) could be obtained at temperatures above 50°C. However, fruits lost their appealing colour, flavour and firmness at those conditions. Therefore, optimum treatment conditions were determined considering the quality characteristics of the fruits. Accordingly, statistical software yielded an optimum condition for MH treatment as 40°C for 21 min.

The third aim of the study was to investigate the optimum conditions for CaCl<sub>2</sub> dipping which would result in the highest firmness value. For this purpose firmness of the samples was investigated in response to CaCl<sub>2</sub> dipping applied at concentrations of

0-6% for 2-21 min at 40°C. As a result, it was observed that fruits could better maintain their firmness value during refrigerated storage after they were dipped into 6% of CaCl<sub>2</sub> solution for 21 min at 40°C. Hence MH treatment and CaCl<sub>2</sub> dipping were combined for the ease of application.

The fourth aim of the thesis was to evaluate the combined effect of MH treatment, CaCl<sub>2</sub> dipping and UV-C treatment (MUC) applied at optimum conditions on the colour, firmness, TPC, PPO and PME enzyme activities and sensorial quality of cv. Şalak apricots. Application of MUC treatment as dipping into CaCl<sub>2</sub> solution at a concentration of 6% for 21 min at 40°C, subsequent exposure to UV-C light for 19 min ( $I=3.23 \text{ mW/cm}^2$ ) resulted in no significant changes in colour and firmness. Moreover, TPC and enzymatic activities were not affected by the combined treatment. Sensory test results also indicated that panellists were unable to detect the differences between treated and untreated samples. Instrumental measurements supported these findings. Additionally, some selected physicochemical properties of cv. Şalak apricots collected in season 2015 and 2016 were also compared. For this purpose, fruit length & width, weight, Brix, titratable acidity, firmness and ascorbic acid content were analysed. According to the results, seasonal changes brought about some differences in specific characteristics. For instance fruits were found to be smaller sized, lower in Brix value and having higher acidity in season 2016. Additionally, ascorbic acid content and firmness was also lower as compared to season 2015. The variations in physicochemical properties caused by the seasonal changes are needed to be taken into account when designing a process for extending shelf life of fruits.

The last aim of this Ph.D. thesis was to evaluate the effect of both MUC treatment and MAP on the shelf life of cv. Şalak apricots during cold storage (1°C) for 28 days. For this purpose MUC treated samples were packed with PE/PA films with a gas composition of 3% O<sub>2</sub> + 4% CO<sub>2</sub> + 93% N<sub>2</sub> (MUC+MAP). On the other hand, untreated control samples were either packed with (Control+MAP) or without MAP (Control-MAP). Weight loss, colour, firmness, Brix, titratable acidity, pH value, ripening index, ascorbic acid content of the fruits were monitored along with microbial examination during the storage period. According to the results, MAP was effective in preventing the weight loss during 14 days of storage. Although MUC treatment was found to increase the weight loss, the ratio did not exceed 5% limit of marketability until the 21<sup>st</sup> day. On the other hand, colour of the MUC treated samples exhibited darkening during the shelf life study. The change in the lightness ( $L^*$ ) and redness ( $a^*$ )

of MUC samples could be both visually and instrumentally observed after 7 days of storage. But MAP treatment could better preserve the initial  $L^*$  value of the untreated samples. However, total colour difference ( $\Delta E$ ) values of the samples referred to well visible changes in the colour parameters of all samples regardless of the processing type. With regards to firmness results MUC+MAP provided the highest firmness throughout the storage. MAP treatment could also preserve the firmness. But, Control-MAP samples suffered from a rapid loss of firmness during the storage. pH value of all samples increased during the first 7 days due to the decrease in acidity values as the ripening proceeded. In accordance with this finding, Brix values were also increased. Nevertheless, it was observed that Brix value of control samples showed a decrease which might be due to the microbial proliferation whereas MUC+MAP had the highest Brix values during the whole storage. According to the ripening index values, MUC+MAP samples concluded the ripening process on the 14<sup>th</sup> day of storage. In respect of ascorbic acid contents, MAP and MUC treatments resulted in the same ascorbic acid levels on the 14<sup>th</sup> day of storage. Lastly and most importantly, MUC treatment was determined to be very effective on preserving the microbial quality of the samples. MUC+MAP samples had the lowest TAPC and YMC during the whole shelf life study. It was also indicated that TAPC and YMC of MUC+MAP samples did not exceed 7.7-log and 5-log limits, respectively. It was speculated that these limits were considered in terms of safety and sensorial quality. Moreover fruit decay could be prevented by both MUC and MAP treatments. However, MAP treatment was not sufficient to prevent microbial growth during 28 days of storage.

In conclusion, MUC+MAP treatment could extend the microbial shelf life of cv. Şalak apricots up to 14 days. However, colour was adversely affected by MUC treatment. Therefore, treatment conditions should be reconsidered for further studies. Instead of dipping the apricots into warm water, washing of the fruits with chlorine and warm water can be considered in order to eliminate the negative impacts of heat on the fruit quality. Also, MH treatment can be done by means of Infrared Radiation or hot air. Besides, other techniques such as hydrogen peroxide, electrolyzed water or chlorine dioxide etc., can be combined with UV-C irradiation. Due to health risks of sulphur dioxide in dried fruits, these combination techniques can be also a good alternative method to reduce the level of sulphur dioxide usage in dried fruits. Furthermore, application of sulphur dioxide at low levels can be integrated into combined processing of fresh cv. Şalak apricots in order to prevent browning.

On the other hand, different packaging materials and different gas compositions in MAP technology can be further evaluated in terms of better quality retention. Moreover, the study can be conducted with apricots harvested in pre-climacteric stage where the products are resistant to the mechanical stress in order to delay ripening. Subsequent storage study can be conducted in real warehouse storage conditions and right on the field production spot. The author also concluded that a consumer acceptance test should be applied during the shelf life period in order to be sure about the acceptability of the treated products by the consumers.

Besides all, the following recommendations are offered for practitioners in the field of apricot processing:

- It is recommended that researchers and R&D engineers use the information provided in this thesis as a basis for evaluating the possibility of the application of alternative techniques in order to prolong the shelf life of cv. Şalâk apricots.
- Preservation of the apricot fruit quality has a great importance for the consumer acceptance. Therefore it is recommended that a particular attention is given to the selection of the processing conditions which would not result in loss of apricot quality.
- According to the fact that UV-C light intensity distribution along the lamp axis referred to the highest light intensity at the central part of the lamp, localization of the UV-C lamps in a commercial UV-C system for apricot processing is of great concern. Thereby, it is highly recommended that UV-C lamps mounted above the conveyors which would carry the fruits are located in such a way that the incident light intensity will be the same for all apricots through the whole processing line.
- It is also suggested that equal UV-C light exposure of apricot surfaces is ensured by using a system that is able to move fruits upward and downward during its transfer through the conveyors.
- Based on the results of this study, it is proposed that properties of packaging material and storage conditions are carefully determined in order to provide a better quality during storage of cv. Şalâk apricots.

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## APPENDIX A

### STANDARD CURVE FOR THE DETERMINATION OF TOTAL PHENOLIC CONTENT

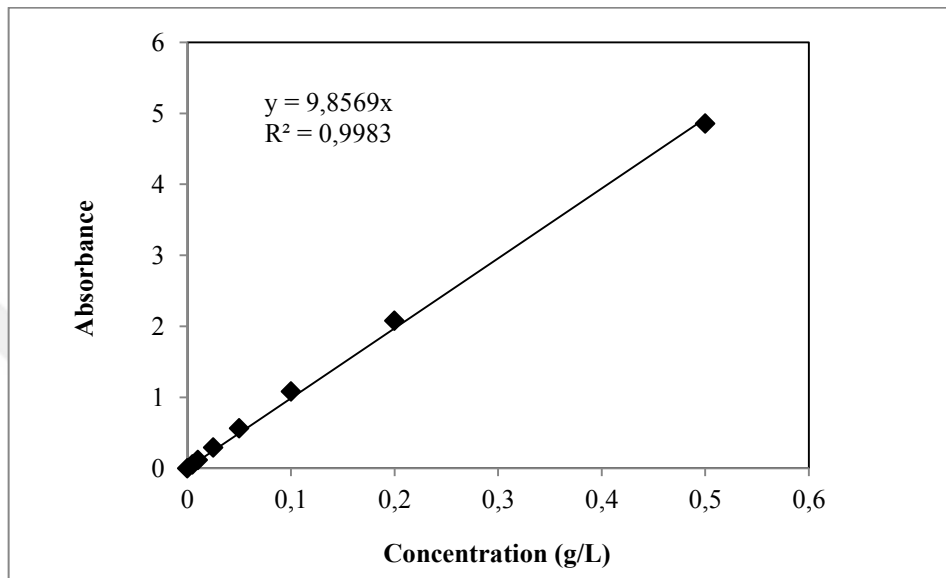


Figure A.1. Standard curve for TPC determination.

## APPENDIX B

### MINIMUM NUMBER OF CORRECT RESPONSES REQUIRED FOR TRIANGLE TEST

Table B.1. Minimum Number of Correct Responses Required for Triangle Test.

(Source: Altuğ Onoğur and Elmacı, 2015)

No. of Responses	Min. No. of Correct Responses Required ( $\alpha=5\%$ )
5	4
6	5
7	5
8	6
9	6
10	7
11	7
12	8
13	8
14	9
15	9
16	9
17	10
18	10
19	11
20	11
21	12
22	12
23	12
24	13
25	13
26	14
27	14
28	15
29	15
30	15

## VITA

Bengi Hakgüder Taze was born in Salihli, Turkey, on October 7, 1984. After she graduated from Sekine Evren Anatolian High School in 2002, she enrolled to Süleyman Demirel University, Department of Food Engineering in fall semester of 2002. She continued her education in that university until the end of year 2003. She was honoured with certificate of achievement during her education in Suleyman Demirel University. In spring semester of 2004, she was admitted as an undergraduate transfer student to Celal Bayar University, Department of Food Engineering. She graduated with high honour degree (ranked second in the department) from Celal Bayar University in July 2006 and obtained her Bachelor of Science degree in the field of Food Engineering. Besides, she also graduated with Bachelor of Science degree from Anadolu University, Department of Business Administration in year 2012. After getting BSc degree in the field of Food Engineering, she was approved to İzmir Institute of Technology, Graduate School of Engineering and Sciences, Food Engineering Master of Science programme in September 2006. Then she started to work as a research assistant in the Department of Food Engineering in 2007. She also conducted teaching assistantship for the courses of Statistical Process Monitoring and Quality Control, and Physical Properties of Foods and Biological Materials in years 2010-2011 and 2012-2013. During her master education she studied UV disinfection of some of the fruit juices. She got her MSc degree in spring semester of 2009. Afterwards, she was approved to İzmir Institute of Technology, PhD programme of Food Engineering in fall semester of 2009. During her PhD she achieved a permanent position in Iğdır University, Department of Food Engineering. She has been working there as a research assistant since January 2014. Aside from oral and poster presentations in her field of education, she had two published research papers namely;

1. Hakguder Taze B., Unluturk S., Buzrul S., Alpas H., 2015. The impact of UV-C irradiation on spoilage microorganisms and colour of orange juice. *Journal of Food Science and Technology*, 52(2): 1000-1007.
2. Göğüş, N<sup>\*</sup>, Hakgüder Taze, B<sup>\*</sup>, Demir, H., Tarı, C., Ünlütürk, S., Lahore, M. F. 2014. Evaluation of orange peel –an industrial waste- for the production of *Aspergillus sojae* polygalacturonase considering both morphology and rheology effect. *Turkish Journal of Biology*, 38:537-548 (\*: Equally contributed to the study).