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INSTITUTE OF HEALTH SCIENCES

DEPARTMENT OF NUTRITION AND DIETETICS

ANTIOXIDANT ACTIVITY AND INHIBITORY EFFECTS OF BLACK CARROT AGAINST LIPID OXIDATION IN MODEL FOOD SYSTEMS THAT ARE RICH IN MONO AND POLYUNSATURATED FATTY ACIDS

MASTER OF SCIENCE THESIS

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DECLARATION

I hereby declare that this thesis is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of any other degree except where due acknowledgment has been made in the text.

09.05.2016

Dilek ÇİFTCİ

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LIST OF SYMBOLS AND ABBREVIATIONS

LA	Linolenic acid
MUFA	Monounsaturated fatty acid
POV	Peroxide Value
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TPC	Total phenolic content

ABSTRACT

Çiftci D., Antioxidant Activity and Inhibitory Effects of Black Carrot Against Lipid Oxidation in Model Food Systems That are Rich in Mono and Polyunsaturated Fatty Acids, Yeditepe University Institute of Health Sciences, Nutrition and Dietetics Department, Master of Science Thesis, İstanbul, 2016.

Nuts are crucial part of Mediterranean food partterns, and their contribution into the human diet is thought to provide many health effects. U.S Food and Drug Administration recognized the nuts to affect heart-health. Nuts composition is health beneficial due to their macronutrients and micronutrients such as unsaturated fatty acid, vitamin (vitamin E, folic acid, niacin) and mineral (phosphorus, copper) as well as polyphenol constituents.

The exposure to oxidation is the main problem of the nuts. The oxidation causes the change in food composition. This spoilage of foods also leads to the change in taste and odor. Therefore, the change in composition also affects the health. To promote the health effects, synthetic and natural antioxidants are added to the food products. Using natural and synthetic antioxidants is the most effective method that has been used for preventing the lipid oxidation.

The food model of the almond paste with the black carrot juice addition was studied for 31 days and at 4ºC, 20ºC, 30ºC and 60ºC to increase the antioxidant amount and decrease the lipid oxidation. Therefore, two different formulations were prepared without black carrot juice and with black carrot juice. Almond paste was used as control sample. Almond paste and black carrot juice added almond paste samples were stored as triplicate. During storage, the change in peroxide value and total phenolic content of almond pastes were investigated.

The study demonstrated that the lipid oxidation rate decelerated in case of the black carrot juice addition due to increasing antioxidant amount and the synergistic effect of the antioxidants found in almond paste and black carrot juice. As the temperature increased, the lipid oxidation was higher but the addition of black carrot juice in initial days of the storage provides the antioxidant amounts to get higher due to the activation by temperature.

Keywords; Nuts, Almond Paste, Black Carrot, Antioxidant, Phenolic compounds, Fat oxidation, Storage, Prevent of Nutrition Value.

ABSTRACT (Turkish)

Çiftci D., Antioksidan Aktivitesi Yüksek Olan Siyah Havuç Kullanımının Tekli ve Çoklu Doymamış Yağ Asitlerince Zengin Gıda Sistemlerinde Lipid Oksidasyonunun Önlenmesi Üzerine Etkisi, Yeditepe Üniversitesi Sağlık Bilimleri Enstitüsü, Beslenme ve Diyetetik Anabilim Dalı Yüksek Lisans Tezi, İstanbul, 2016.

Kabuklu yemişler Akdeniz gıda ürünlerinin önemli bir parçası olup, beslenmede yer almasının birçok sağlık etkisini sağladığı düşünülmektedir. Amerikan Gıda ve İlaç Kurumu Kabuklu yemişlerin kalp sağlığını etkilediğini belirtmiş ve yemişlerin tüketiminin önemi vurgulanmıştır. Yemişlerin içeriği doymamış yağ asitleri, vitaminler (E vitamini, folik asit, niyasin), mineraller (fosfor, bakır) ve polifenoller gibi makro ve mikro besinler sayesinde zengindir.

Kabuklu yemişlerin oksidasyona maruz kalması önemli bir problemdir. Oksidasyon gıda kompozisyonunda değişikliklere sebep olur ve böylece tat ve kokuda farklılaşmalar olur. Kompozisyondaki değişim de sağlığı olumsuz etkiler. Sağlık etkilerini arttırmak için, doğal ve sentetik antioksidanlar gıda kompozisyonuna katılır. Doğal ve sentetik antioksidanların kullanımı lipid oksidasyonunu engellemek için kullanılmış en etkili yöntemdir.

Siyah havuç suyu eklenmiş badem ezmesi, antioksidan miktarının artıp lipid oksidasyonun azalması için gıda modeli olarak çalışılmış; 31 gün boyunca 4ºC, 20ºC, 30ºC ve 60ºC sıcaklıklarda depolanmıştır.

Antioksidan miktarının artması ve lipid oksidasyonunun azalması için 31 gün boyunca 4ºC, 20ºC, 30ºC ve 60ºC sıcaklıklarda havuç suyu eklenmiş badem ezmesi gıda modeli olarak çalışılmıştır. Bu yüzden, siyah havuç suyu ile ve siyah havuç suyu olmadan iki farklı formülasyon hazırlanmıştır. Sade badem ezmesi control model olarak kullanılmıştır. Sade badem ezmesi ve siyah havuç sulu badem ezmesi üçer tekrarlı olarak depolanmıştır. Depolanma süresi boyunca peroksit değeri ve toplam fenolik miktarındaki değişimler araştırılmıştır.

Çalışmada siyah havuç suyu eklenmesinin antioksidan miktarındaki artış ve badem ezmesi ile siyah havuç suyundaki antioksidanların sinerijistik etkisi sayesinde lipid oksidasyonunu yavaşlattığı görülmüştür. Sıcaklık arttıkça lipid oksidasyonu artmıştır fakat siyah havuç suyu eklenmesi ile depolanma süresinin ilk günlerinde sıcaklıkla antioksidanların aktivasyonu sayesinde antioksidan miktarı da artmıştır.

Anahtar Kelimeler; Kabuklu yemişler, badem ezmesi, siyah havuç, antioksidan, fenolik bileşikler, yağ oksidasyonu, depolama, besin değerinin korunması

.

1. INTRODUCTION and PURPOSE

Nuts are a crucial part of the diet worldwide for thousands of years. The increasing scientific works caused the interest of conscious consumers in food's protein, mineral and vitamin values much more than their taste, smell and flavor [1]. Nuts are much wealthy nutrients with regard to macronutrient and micronutrient [2].

Nuts (almond, hazelnut, walnut, pistachio, peanut etc.) has become the most valueable products in exporting all over the world, and Turkey as well. Beside these economic values, nuts are playing an important role in human health and nutrition [3]. Nuts do not only possess high oil rate (approximately 65-70%) but also they have rich content of monounsaturated fatty acid (especially oleic acid). Nuts have many health benefits for human health due to the oleic acid they contain. They have been an important ingredient for the chocolate and cake industry by their ability to develop organoleptic features [4]. Meanwhile, they are helping to flavor and the texture by getting into formulations of many products. Therefore, it is important to decrease the losses in quality and nutritive value of nuts.

The exposure to oxidation is the main problem of the nuts. The oxidation causes the change in food composition. This spoilage of foods also leads to the change in taste and smell. Therefore, the change in composition also affects the health. To promote the health effects, synthetic and natural antioxidant compounds are added to the food products. Using natural and synthetic antioxidants is the most effective method that has been used for preventing lipid oxidation. In the last couple of years, the addition of natural supplements to the synthetic supplements is preferred in many products. Natural plant extracts are used as "alternative preservatives" in different fields. Using the plant extracts which have been known with their antioxidant properties, is a way to restrain the loss of nutritional value and increase the qualitative properties and shelf life of the product.

One of the alternatives to extend the shelf life is to store the products. The moisture, temperature, insect infestation, packaging etc. are significant parameters to maintain the nutritional value of products. The storage temperature is the most crucial factor for foods to maintain their nutritional value due to the lipid oxidation. Maintaining the sensory quality and components with favorable nutritional values by reducing the undesired chemical change in the almond is a problematic concern for the food industry. Hence, the optimization of storage condition is substantial.

The main purpose in the study is to specify the effect of the black carrot juice antioxidant value on almond paste's oxidation, by comparing it with the model food in various different storage conditions.

2. LITERATURE REVIEW

2.1. Nuts

Tree nuts are defined as dry fruits with one seed in which the ovary wall becomes hard at maturity. The most popular edible tree nuts are almonds (*Prunus amigdalis*), hazelnuts (*Corylus avellana*), walnuts (*Juglans regia*), and pistachios (*Pistachia vera*), pine nuts (*Pinus pinea*), cashews (*Anacardium occidentale*), pecans (*Carya illinoiensis*), macadamias (*Macadamia integrifolia*), and Brazil nuts (*Bertholletia excelsa*) [5].

Nuts can be consumed as a part of human diet in whole (fresh or roasted) form and also can be added to spreads such as peanut butter and almond paste. They are used as oils or added to commercial products of sauce, pastry, ice cream and baked goods. In last decades, the nuts have been a part of daily diets as an energy source [5].

2.2. Composition of Nuts

Nuts are nutrient rich foods. Although the geographical conditions and cultivars may slightly affect the composition of nuts, they are good sources of carbohydrate, fat, protein, dietary fiber, some vitamins and minerals [3]. The average nutritional composition of some nuts is given in Table 2.1 [5].

Nuts have particularly high content of lipid. Pecans, ground nuts and walnuts include between 55 and 75 % of lipid. Nuts are rich in mono- and polyunsaturated fatty acids [5,6]. Almond, cashew, hazelnut, macadamia, pecan and pistachio have higher monounsaturated fat, while Brazil nut, pine nut and walnut have more polyunsaturated fat.

Fatty acids are composed of long unbranching chains of carbon atoms which has bonds with hydrogen and other groups. They have carboxyl group (-COOH) and carboxyl group gives acidic properties. Saturated fats contain fatty acids with single bonds in chemical structure and they are solid at room temperature. Unsaturated fats contain at least a double bond between carbon atoms (Figure 2.1) and liquid at room temperature. Monounsaturated fats possess one double bond in the fat molecule and polyunsaturated fats have more than one double bond.

Table 2.1. Nutritional composition of nuts per 100 g [5].

Nuts (100 g)	Energy (kJ)	Fat (g)	Protein (g)	Fiber (g)	SFA (g)	MUFA (g)	PUFA (g)	LA (g)	ALA(g)
Almonds	2418	50.6	21.3	8.8	3.9	32.2	12.2	12.2	0.00
Brazil nuts	2743	66.4	14.3	8.5	15.1	24.5	20.6	20.5	0.05
Cashews nuts	2314	46.4	18.2	5.9	9.2	27.3	7.8	7.7	0.15
Hazelnuts	2629	46.4	15.0	10.4	4.5	45.7	7.9	7.8	0.09
Macadamia nuts	3004	75.8	7.9	6.0	12.1	58.9	1.5	1.3	0.21
Peanuts	2220	49.2	25.8	8.5	6.8	24.4	15.6	15.6	0.00
Pecans	2889	72.0	9.2	8.4	6.2	40.8	21.6	20.6	1.00
Pine nuts	2816	68.4	13.7	3.7	4.9	18.8	34.1	33.2	0.16
Pistachio	2332	44.4	20.6	9.0	5.4	23.3	13.5	13.2	0.25
Walnuts	2738	65.2	15.2	6.4	6.1	8.9	47.2	38.1	9.08

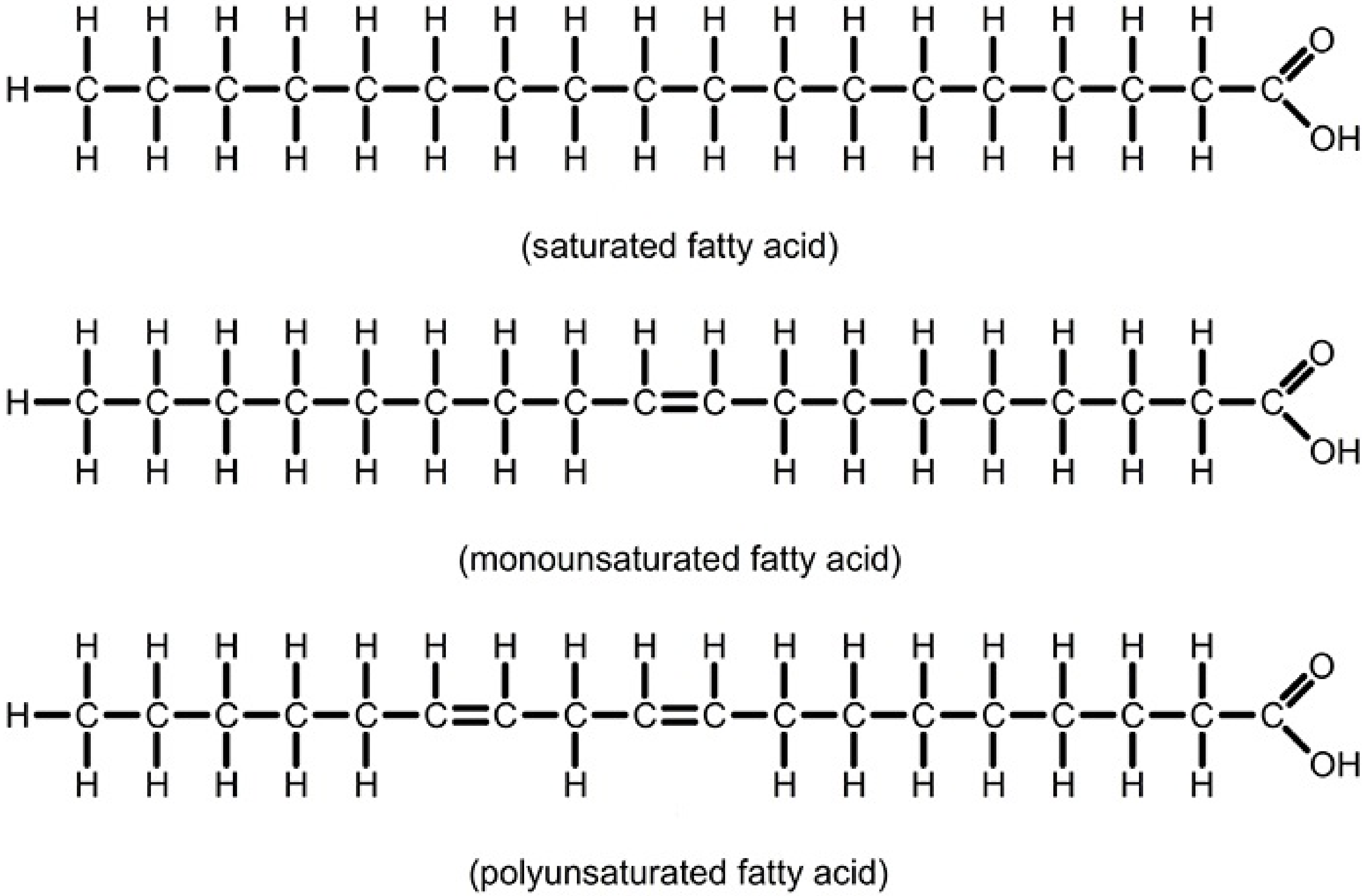

Figure 2.1. Saturated and unsaturated fatty acids [7].

Foods with low saturated fatty acid and high mono-unsaturated fatty acid content have benefits on human health [8]. The fatty acid composition of nuts have health benefits due to low content of saturated fatty acid (4-16%) and almost half of total fat content is composed of unsaturated fats.

2.3. Health Benefits of Nuts

Nuts are rich in mono- and polyunsaturated fatty acids [5,6]. It has been stated that nuts may help to decrease diabetic risk and control the glycemic index because of their macro and micro content of nutrition [9].

They are one of the main sources of vitamin B, which has regulatory effect for the carbohydrate, protein and oil metabolism in human body. Vitamin B2 and vitamin B6 which are hematinic and useful for mental health have a serious importance on adolescence children's nutrition [5].

Vitamin E has an antioxidant feature and with that, it blocks hemolism, provides endurance of the cells and prevents aenemia [5].

Nuts are the best natural source for calcium (takes a role in processing bones and teeth), iron (takes a role in hematinic) and zinc (takes a role in developing growth hormone and sex hormones). Nuts - which has been necessary for impulsing the nerves and working of muscle tissue - have high measure of calcium and low measure of sodium. By the way, they help bones develop and balance the blood pressure [1,3]. Nuts have also rich content of phytosterol and polyphenol compounds. While phytosterols exert both structural and hormonal (estrogen-like) activities polyohenols show as antioxidant and anti-inflammatory effects thus they help to lower cholesterol levels and improve cardiovascular outcomes [10-13].

2.4. Almond

Almond tree, *Prunus dulcis*, is quite similar to peach, plum and apricot tree but its edible part is not fruit, germ or seed [14]. Table 2.2 indicates the average nutritional composition of almonds per 100 g.

Almond may contribute in weight loss and may help reduce belly fat. It may slightly boost metabolism. It has protective effect against type 2 diabetes. A low calorie diet with 62% weight loss may be obtained with three ounces of almond per day. It may also strengther the immune system and digestive system [15].

Almond contributes to the health due to its bioactive compounds and nutritional value [16]. It lowers LDL with no effect on HDL by replacement of half regular fat intake [17]. Almond seed, shell and skin extracts have the potential for free radical scavenging. Furthermore, almond helps to decrease the risk of colon cancer [18] and to lower the risk of diabetes [16]. Almond may help to prevent birth defect in pregnancy due to folic acid content. Almond milk has less calories and may take place on a diet to support weight loss.

2.5. Use of Almonds in the Food Industry

Almond is one of the most widespread tree nuts and takes the first place in the production worldwide. The production of almond in 2006 was more than 1.1 million tons. 63.5%, of this amount was in California, 18.6% in Spain, 10.5 in Italy, 4.2% in Greece and 4.0% in Turkey [20]. The production of almond worldwide is depicted in Table 2.3 and the annual almond production and consumption in Turkey is given in Table 2.4.

Turkey has a wide genetic variation of almond. Almond trees in different ecological conditions provide various product options [21]. Almonds are used in different consumption areas in Turkey; cookies, candy, chocolate, and cake industry, also used in the cosmetic and pharmaceutical industry as almond oil [22].

The regions where the almond is grown are Aegean, West Marmara, Southeastern Anatolia and Mediterranean Regions in Turkey. The average yield for almond production is 17 kg for each tree.

Almonds may be consumed on their own, raw or roasted. It may be made into almond flour, milk, butter, oil and flour. Almond is gluten free thus it can take place in gluten free diets instead of wheat flour. It is also important for vegetarian diet [15].

Almonds are primarily consumed as snack food and limited amounts of almond is added to confectionary and the cosmetic products in Turkey. The annual consumption of almond for a person in Turkey is 0.9 kilogram. The market price increase has caused the lower consumption of almond as snack food. The almond has been sold in packaged form especially in coastal regions (Aegean, Mediterranean and Marmara) [23].

Table 2.2. Nutritional composition of almond per 100 g [19].

Nutrient	**Unit**	**Value per 100 g**
Proximates		
Water	G	4.41
Energy	kcal	579
Protein	G	21.15
Total lipid (fat)	G	49.93
Carbonhydrate	G	21.55
Fiber	G	12.5
Sugar, total	G	4.35
Minerals		
Calcium, Ca	mg	269
Iron, Fe	mg	3.71
Magnesium, Mg	mg	270
Phosphorus, P	mg	481
Potassium, K	mg	733
Sodium, Na	mg	1
Zinc, Zn	mg	3.12
Vitamins		
Vitamin C	mg	0.0
Thiamin	mg	0.205
Riboflavin	mg	1.138
Niacin	mg	3.618
Vitamin B-6	mg	0.137
Folate, DFE	µg	44
Vitamin A, IU	IU	2
Vitamin E (alpha-tocopherol)	mg	25.63
Lipids		
Fatty acids, total saturated	G	3.802
Fatty acids, total monounsaturated	G	31.551
Fatty acids, total polyunsaturated	G	12.329
Cholesterol	µg	0

Table 2.3. Almond production in the world 00 tonnes) [24].

Countries	2000	2005	2006	2007	2008	2009	2010	2011	2012	%
USA	533	703	846	1213	1410	1162	1414	731	720	37.2
Spain	225	218	313	188	180	271	223	212	215	11.1
Iran	90	109	105	115	127	158	158	92	100	5.2
Italy	105	118	113	113	119	107	108	105	90	4.6
Syria	62	229	107	76	83	97	73	130	86	4.5
Turkey	47	45	43	51	53	55	55	70	75	3.9
Tunusia	60	43	56	58	52	60	52	61	70	3.6
Greece	60	48	51	46	35	40	33	30	29	1.5
Other	51	378	412	438	462	562	548	533	550	28.4
World	320	1891	2046	2296	2519	2512	2664	1964	1935	100

Table 2.4. Almond production and consumption in Turkey [25].

Year	Supply=Use (tons)	Usable production (tons)	Imports (ton)	Domestic Use (ton)	Human Consumption (ton)
2010/2011	77,431	54,401	23,030	63,998	62,718
2009/2010	75,892	53,857	22,035	66,417	65,088
2008/2009	71,498	51,824	19,674	62,149	60,906
2007/2008	59,969	49,839	10,130	53,316	52,250
2006/2007	48,818	42,506	6,312	46,794	45,858
2005/2006	49,186	44,190	4,996	47,114	46,172
2004/2005	43,188	36,334	6,854	41,052	40,231
2003/2004	45,495	40,262	5,233	44,185	43,301
2002/2003	43,593	40,262	3,331	42,974	42,115
2001/2002	46,873	41,244	5,629	45,866	44,949
2000/2001	53,193	46,154	7,039	51,964	50,925

2.6. Definition of Shelf Life and Elongation of Shelf Life

All food products are made of biological raw matters and all biological contents spoil by their nature and this spoilage cannot be inevitable. Therefore, all food producers want to diminish the spoilage in most possible way with storage, package, process and formulation [26,27]. Spoilage can happen by the reaction or decay between protein, oil and carbohydrate. The speed of chemical reactions depend on food's water activity, pH and heat; light and ambient oxygen. The chemical reactions may cause changes in food's color, taste and flavor [26].

Shelf life is the duration of storage in specified suitable conditions without physical, microbiological and any other important change in product as presented in market [28]. The shelf of a product is affected by the conditions of environment and the initial quality of the product [29].

Nuts are qualified healthy foods because of their mono fatty acid content but these fatty acids may be exposed to decomposition by lipid oxidation [30]. Unsaturated bonds in chemical composition of fatty acids are highly susceptible to lipid oxidation and lipid oxidation mechanism is affected by some factors as temperature, oxygen, fatty acid composition, water activity and irradiation.

Weight loss associated with decrease in moisture causes the change of quality negatively. Optimum water activity is 0.585 for whole almond product, 0.565 for crust and 0.745 for the inner part. Additionally, the moisture of almond vary between 9.5 % and 12.0% [28]. Pastry cook sells almond paste pastries directly to consumer with no hermetically sealed packages and almond are usually stored at room temperature. However, almond paste has a short shelf life.

2.7. Lipid Oxidation Mechanism in Foods

Oxidative rancidity is the major cause of deterioration in foods [31,32]. It causes undesired changes in sensory quality of foods. It may also have adverse health effects. Nuts are sensitive to lipid oxidation due to their rich unsaturated fatty acids contents [33]. Certain fats can resist the lipid oxidation in a specific extent wheras other fats are more susceptible based on the number of double bonds, the antioxidant presence and other factors.

The oxidation of oils takes place by means of a chain reaction which is a type of reaction that is characterized by extreme speed. A chain reaction takes place in three stages known as Initiation, propagation and termination [34].

Lipid oxidation is chain reactions which are initiated due to hydrogen dissociation or an oxygen radical addition, causing the oxidative damage of polyunsaturated fatty acids (PUFA). Polyunsaturated fatty acids are more sensitive than saturated fatty acids. In the initiation step, which is slow to ocur, a hydrogen atom is removed from an unsaturated triglyceride molecule with the production of a free radical which is an unstable molecular group and it reacts with other molecules in order to be more stable. Initiation step begins by initiators such as light, heat, transition metals and enzymes.

Initiation $$RH \rightarrow R. + .H \quad (2.1)$$

In the propagation step, the free radical R· group may react with oxygen and cause the formation of a peroxy radical $RO_2\cdot$. $RO_2\cdot$ radical may react with other lipid molecule and leads to formation of a hydroperoxide RO_2H, and other R· lipid radical.

Propagation $$R. + O_2 \rightarrow RO_2. \quad (2.2)$$

$$RO_2. + RH \rightarrow RO_2H + R. \quad (2.3)$$

In the termination phase, two radical groups react and generates the final stable products that ends the formation of free radicals. Termination phase also occurs in case of addition of antioxidants or free radical scavengers reacting with free radicals generated during propagation. The reaction mechanism is depicted in Figure 2.2.

Termination $$R. + R. \rightarrow RR \quad (2.4)$$

$$R. + RO_2. \rightarrow RO_2R \quad (2.5)$$

$$RO_2. + RO_2. \rightarrow RO_2R + O_2 \quad (2.6)$$

Energy in the form of heat and light, catalyst, double bond, enzyme, chemical oxidant, natural ontioxidant, fatty acid, monoglyceride, diglyceride, types of oxygen are factors affecting the lipid oxidation.

Lipid oxidation must be minimized in processing and after purchase. After processing, the consumer wants to minimize the oxygen availability and to decrease the rate of reaction [35] thus several precautions may be taken to lower the rate of oxidative rancidity in foods include:

- Storage of food products should be kept in dark environment,
- Prevention of the light transmission to the sensitive food products,
- Use of selectively light – absorbent packaging materials [36].

2.8. Mechanism of Antioxidants

Antioxidants are the chemicals that protect both foods and consumer health against free radicals that are produced as a result of lipid oxidation. They are synthetically or naturally occurring compounds they are able to delay the initiation or decelarate the rate of lipid oxidation. The antioxidant molecules inhibit the accumulation of free radicals by acting as oxygen scavengers (removing oxygen from the oil) or they capture free radicals [37]. The reaction mechanism how the antioxidant AH may be reacted with the fatty acid free radical or with the peroxy free radical is given as:

$$AH+R\cdot \rightarrow RH+A\cdot \qquad (2.7)$$

$$AH+ROO\cdot \rightarrow ROOH+A\cdot \qquad (2.8)$$

They appear to enter termination reactions such as:

$$A\cdot + A\cdot \rightarrow A-A \qquad (2.9)$$

$$A\cdot + ROO\cdot \rightarrow ROO-A \qquad (2.10)$$

Antioxidants also inhibit specific enzymes that degrade lipid peroxide molecules into oxygen and water [37].

An antioxidant molecule must react with free radicals before the reaction of free radicals with lipid molecules. The products of the reaction of antioxidants and free radicals must not cause the formation of new free radicals. The antioxidant molecule must have lipid soluble property.

Antioxidants are primarily used to increase the shelf life of foods and prevent the formation of off smell bitterness. The categories of food antioxidants are given in Figure 2.2.

An ideal antioxidant [38]:

- Should not cause of any health problem,
- Should not change of the taste, smell and flavor of the food,
- Should be affective in low concentrations,
- Should be easily mixed with food,
- Should be cheap,
- Should keep its activity after baking and cooking processes.

Antioxidants can be classified as antioxidant vitamins, antioxidant minerals, antioxidant proteins, antioxidant enzymes and antioxidant phytonutrients. Carotenoids, ellagic acid, flavonoids, resveratrol, glucosinolates, phytoestrogens are some examples of phytonutrients.

Phenolics are the most active natural antioxidants, they can free radicals, create chelate with metals and inactivate lipoxygenase enzyme [39,40]. Many foods contain phenolic chemicals which have been the most powerful antioxidants and make contribution to defense of metabolism against oxidative damages.

Tocopherols are the other group of compounds that have antioxidant effects against lipid oxid. Tocopherols -that have been found to be protective to cancer and heart diseases & decrease lipid oxidation in human blood. The antioxidant activity of tocopherols shows an alteration to substract concentration, compound, ambient temperature and situation of oxidation. Also fruits and vegetables [41-44], hazelnut, almond and walnut are the good source of tocopherols thick shelled dried fruits and oiled seeds [45,46,12,3] are very valuable food groups in terms of containing natural antioxidants.

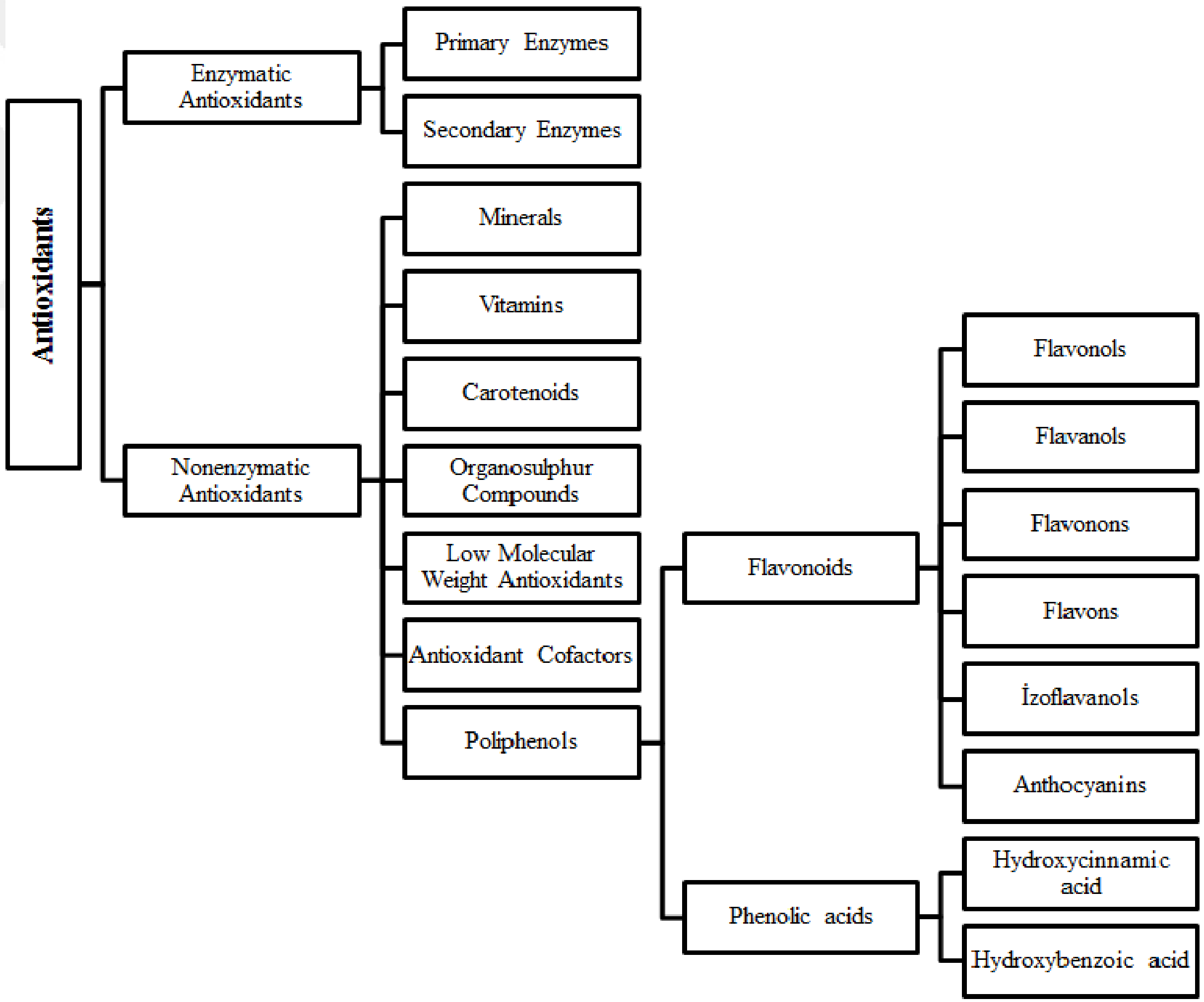

Figure 2.2. Antioxidants [59].

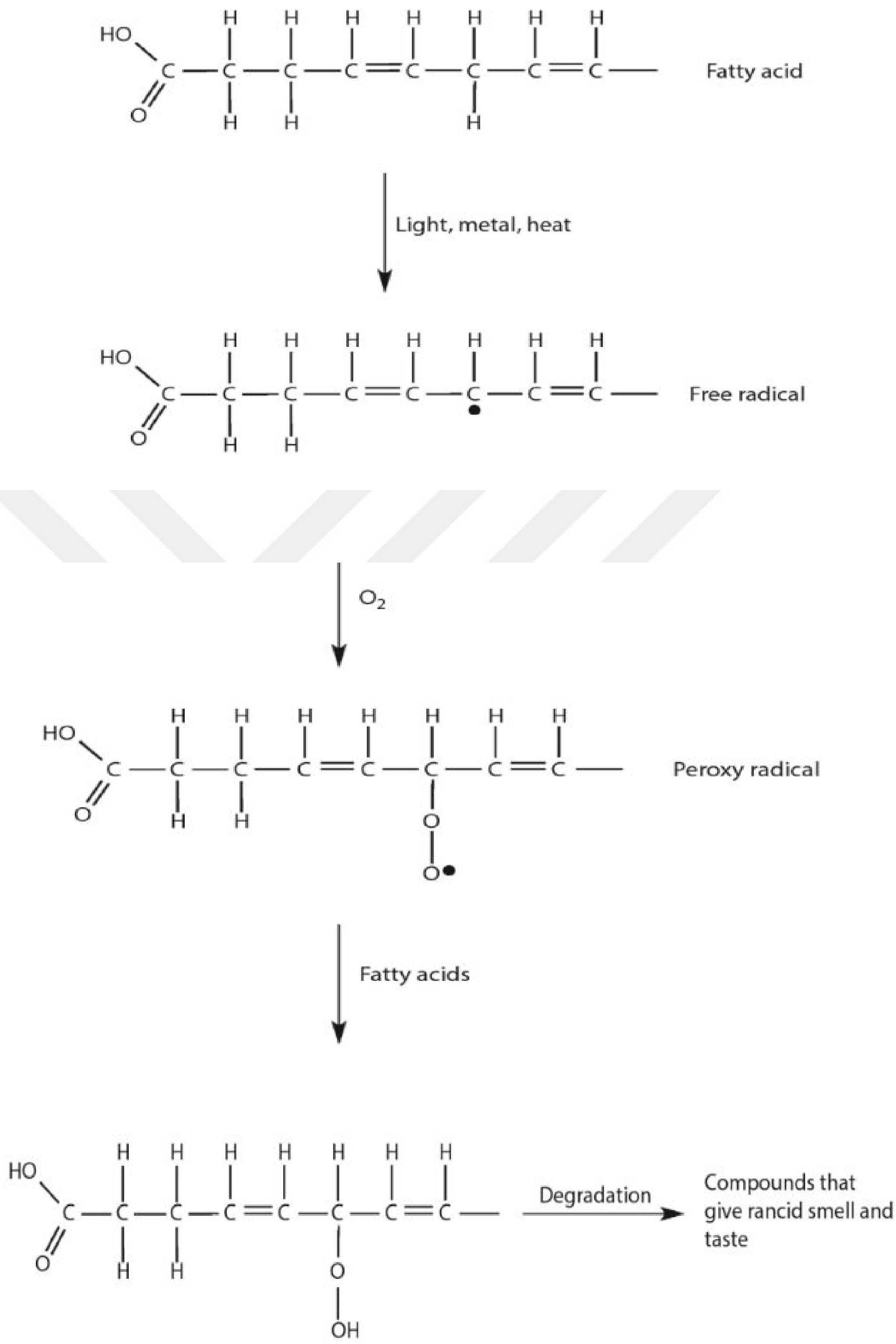

Figure 2.3. Lipid oxidation mechanism [36].

Anthocynanins provides red, blue and purple colours to fruits, vegetable and flowers. Anthocynanins may help to reduce risks of atherosclerosis, cancer, diabetes and neurodegenerative disorders [47]. Anthocyanins are used in some diseases for healing effects on blood circulation disorders and eye diseases have been proved [48]. Increased demand for safe and natural colorants has promoted the demand for natural colorants as alternative to synthetic additives.

In some circumstances, antioxidant mixtures or substances with synergistic effect must be used instead of only one type of antioxidant. The use of antioxidant combinations may contribute different properties, allow for better control and accuracy, and provide synergistic effect.

The mechanism of the synergistic effect between different antioxidants is not known exactly. There are some hypotheses about the synergistic effect. Primary antioxidants may be regenerated, oxidation initialization may be reduced by metal ion complexing, high energy form of oxygen may be scavenged, lipid peroxides may be reduced to stable non-radical products [49-51].

Many foods contain natural antioxidants. In the food industry foods are rich in antioxidant are used as food addditivies to retard or prevent oxidation of lipids in foods. Certain spices and herbs used in foods have antioxidant effect because of the high content of phenolic compounds besides their contribution in flavor [52]. The oxidative rancidity in food products is also reduced by the addition of natural antioxidants in fruit products.

In parallel with development of food and drug industry, consumer's demand for the healthier food products has increased. Foods which are rich in different nutrients gained much attention. The unsaturated fatty acids and the foods rich in unsaturated fatty acids are one of the foods known for their health benefits.

The latest studies show that oxidative stress take a big role in cause aging, some of the cancer types, heart diseases, cataract, Parkinson's, Alzheimer's, diabetics and likely chronic diseases [53-57]. The most practical and effective way to protect from oxidative stress is to consume foods that contain antioxidant [58].

Black carrot is root vegetable and has high amount of bioactive compounds such as anthocyanins and dietary fibers. Black carrot has a high potential of antioxidant activity due to its high the anthocynanin contents [60]. The chemical composition of black carrot is shown in Table 2.5.

Table 2.5. Nutritional composition of black carrot per 100 g [61].

Component	Unit	Mean	Minimum	Maximum
Energy	kcal	42	41	43
Energy	kJ	175	171	179
Water	g	87.66	87.29	88.02
Ash	g	0.84	0.76	0.92
Protein	g	0.87	0.75	1.00
Nitrogen	g	0.14	0.12	0.16
Fat, total	g	0.14	0.12	0.16
Carbohydrate	g	8.01	8.00	8.02
Fiber, total dietary	g	2.48	2.35	2.61
Fiber, water-soluble	g	0.90	0.84	0.97
Saccharide	g	0.00	0.00	0.00
Glucose	g	1.85	1.85	1.85
Fructose	g	0.14	0.14	0.14
Lactose	g	0.00	0.00	0.00
Maltose	g	0.00	0.00	0.00
Salt	mg	206	196	215
Iron, Fe	mg	0.26	0.24	0.28
Phosphorus, P	mg	29	21	38
Calcium, Ca	mg	33	31	35
Magnesium, Mg	mg	17	17	18
Potassium, K	mg	256	240	273
Sodium, Na	mg	82	78	86
Zinc, Zn	mg	0.15	0.14	0.17
Thiamin	mg	0.029	0.026	0.032
Riboflavin	mg	0.029	0.022	0.035
Niacin, preformed	mg	1.121	1.114	1.308
Vitamin B-6, total	mg	0.072	0.064	0.079
Beta-carotene	µg	0	0	0
Lycopene	µg	0	0	0
Lutein	µg	0	0	0

Black carrot is low in calorie, dietary fiber, vitamin K, vitamin C, potassium, and manganese and carotenoids. It has anti-carcinogenic effect against colon cancer, anti-inflammatory properties, eye health protective property and it may improve vascular health [62,63].

In the food industry black-carrot juice is used as an antioxidant and a natural coloring food additive. Black carrot juice concentrate has 12 times more antioxidants and 40% more beta carotene than regular orange carrot juice. The total anthocyanin amount in black carrot juice is almost double of carrot anthocyanin amount [64]. Due to antioxidant content, black carrot juice has the potential of antimicrobial and anticancer properties. Anthocynanins in black carrot shows extremely strong antioxidant effect, even more than Vitamin E analogues. Black carrot has more antioxidant effect than yellow and orange carrot types [62,63].

3. MATERIALS AND METHODS

3.1. Materials

In this project, almonds were used and supplied from Balıkesir. Kernels of almonds were placed in vacuumed bags stored.

Black carrots were used as a source of natural antioxidant and supplied from Konya. They were washed, cleaned, applied shock freezing and then, stored in vacuumed bags at -25ºC.

3.2. Reagents and Solutions

The chemicals potassium iodide, sodium thiosulphate, petroleum ether, hexane, methanol, acetic acid, chloroform, gallic acid, Folin-Ciocalteou reagent and starch (soluble) were purchased from Sigma-Aldrich.

3.3. Methods

3.3.1. Formulation of food models

The compositions of plain almond pastes and almond paste with black carrot juice are given in Table 3.1 [28].

Two different of almond paste samples were prepared. To prepare the control samples, firstly the raw almond kernels were blanched and dried on the cotton piece of cloth. Their thin hulls were skinned and they were grinded in mixer. Confectioner's sugar and water, were added in the mixer. They were mixed together until they get homogeneous. The mixture was kneaded, shaped as a roll and cut 1 cm sized rounds.

To prepare with black carrot juice samples, firstly the raw almond kernels were blanched and dried on the cotton piece of cloth. Their thin hulls were skinned and they were grinded in mixer. Confectioner's sugar and black carrot juice, were added in the mixer.

They were mixed together until they get homogeneous. The mixture was kneaded, shaped as a roll and cut 1 cm sized rounds.

Both samples were stored in hermetically sealed containers at 4 ºC, 20 ºC, 30 ºC and 60 ºC for 31 days.

Table 3.1. Formulations of almond paste types

Almond paste (%)		Black carrot juice concentration (%)	
Almond flour	50	Almond flour	50
Sugar	40	Sugar	40
Water	10	Black carrot juice	10

3.3.2. Preparation of almond paste extract

Prepared almond paste (10 gr) was transferred to dark-colored flasks and mixed with 200 ml of solvents with petroleum ether and stored at room temperature. After 24 hours, mixture were filtered through filter paper and residue was extracted with equal 200 ml solvent. After 24 hours, the total petroleum ether was evaporated at 40ºC using rotary evaporator. The obtained almond extract were kept in sterile sample tubes and stored in a refrigerator at 4°C [65].

3.3.3. Determination of total ash

Total ash is determined by the mass formed by burning the specimen at 525°C in a muffle furnace until white ash is formed. The almond paste samples were prepared about 3 g and were placed crucibles. Crucibles were set in the muffle furnace until the ash was appeared after 12 hour. The crucibles were removed from the muffle furnace and placed in desiccator for cooling about 1 hour. Crucibles were weighed and results were calculated as % ash content. The total ash is determined according to AOAC Methods 900.02.

$$\%\text{Ash}(\text{wet basis})=\frac{m_{ash}}{m_{wet}}\times 100 \quad (3.1)$$

$$\%\text{Ash}(\text{dry basis})=\frac{m_{ash}}{m_{dry}}\times 100 \quad (3.2)$$

3.3.4. Determination of moisture content

The moisture concent is determined according to AOAC method 925.45 The black carrot juice added almond paste samples were weighed about 5 g was placed on a desiccated, tared petri dish and recorded as weighed of sample in a vacuum oven at 103°C until it reaches a constant weight for 6 hours.

After drying petri dishes were cooled in desiccator at room temperature 25^{o}C until 2 hours. After cooling, samples were weighed again and recorded as the dry weight. This analysis were triplicated and results expressed as % moisture.

$$\%Moisture(wet\ basis)=\frac{m_{moisture}}{m_{wet}}\times 100 \tag{3.3}$$

$$\%Moisture(dry\ basis)=\frac{m_{moisture}}{m_{dry}}\times 100 \tag{3.4}$$

3.3.5. Determination of the fat

It was identified by using Soxhlet method. 10 g grinded almond samples were weighed on Soxhlet cartridge and subjected to extraction with n-hexane for 6 hours at 60°C. n-hexane in the extract was suspended under vacuum, in rotary evaporator at 60°C. A trace of solvent left was evaporated by leaving the sample at 103±2°C for 20 minutes. The sample was cooled in desiccator and after, oil measure was determined in percentages by looking the weighing difference.

For the oils that will used in peroxide and total phenolic matter determination, cold extraction method was used and petroleum ether in the extract was removed in rotary evaporator at a lower degree (60°C). Residue after vacuuming was evaporated by applying nitrogen [66].

3.3.6. Determination of the peroxide value

It is a measure of the amount of active oxygen in oils and it represents the active oxygen amount in 1 g oil in micrograms. The peroxide value is determined according to AOCS Official Method Cd8-53. Being measured with 1.00 ±0.01 g sensitivity, the specimen is kept in a 250 mL Erlenmeyer flask with a ground glass joint and the oil is dissolved by adding 30 mL acetic acid-chloroform mixture (3:2 v/v). Then, after adding 0.5 mL saturated KI, it is constantly shaken at high speed for 1 min. Immediately afterwards, 30 mL distilled water is added and the specimen is titrated with 0.01 N sodium thiosulfate solution. The peroxide value is calculated using the formula given in Equation 3.1.

$$\text{Peroxide Value}\left(\frac{\text{mEq}}{\text{kg}}\right)=\frac{(\text{S-B})\times \text{N}}{\text{W}}\times 1000 \tag{3.5}$$

where S = volume of titrant (ml) for sample

B = volume of titrant (ml) for blank

N = normality of sodium thiosulfate solution (mEq/ml)

W = sample mass (g)

3.3.7. Determination of total phenolic content

Total phenolic determination of the almond extract was based on the prosedure were using the Folin-Ciocalteou reagent and also were using as a spectrophotometric method [67].

In order to calculating total phenolics, calibration curve which was made of gallic acid was used and phenolics were calculated over gallic acid. Total phenolics that extracts include was calculated equally to the gallic acid and by using Folin-Ciocalteu method. 20 µl sample aliquot of extract (40 to 200 mg/L) was mixed with 1580 µl distillate water and 100 µl Folin-Ciocalteu reactive was added to the mix. After 2 minutes, 300 µl Na_2CO_3 with 20 % water was added to the mix and they were all mixed in vortex. As a control sample, reactive mix with no extract was used. The mix was incubated for 2 hours at 25ºC and after that, absorbance was measured at 720 nm on a UV visible spectrophotometer. Gallic acid was compared to the calibration curve. Total phenolic content was calculated equally to the gallic acid (mg gallic acid /100 g extract). Three collateral experiments were conducted and results were given as average values [67].

4. RESULTS and DISCUSSION

4.1. Compositional Analysis

The composition of the model foods are given in Table 4.1.

Table 4.1. Composition of model food before storage

Sample	Moisture %	Ash %	Total Fat %
Control	12.0	1.5	25.5
Almond paste- with black carrot	12.5	1.6	25.6

Moisture increases the activation of catalysts and oxygen and enhances the oxidation rate [68]. Therefore, the moisture content of the almond was an important parameter of the study. Almond paste with black carrot analyzed. The moisture content in almond paste increases from 8.0% to 12.0% due to the addition of water in the control model.

The food model of black carrot juice added almond paste has the moisture content of 12.5%, almost the same as the almond paste food model.

The ash content was determined as 1.5% and 1.6% for almond paste and black carrot juice added almond paste, respectively.

Additionally, the initial total fat content of the almond paste and the black carrot juice added almond paste are 25.5% and 25.6%, respectively. The fat contents are almost same.

After storage, the moisture content of the almond paste stored at 4°C, 20°C, 30°C and 60°C were found as 12%, 11.5%, 11.0% and 10.0% , respectively. The moisture content of the black carrot juice added to the almond paste after storage at 4°C, 20°C, 30°C and 60°C were found as 12.5%, 12.0%, 11.6% and 11.0%, respectively. At 4ºC, there was no significant change in moisture content of both model foods. The moisture concents decreased significantly at 20ºC, 30ºC and 60ºC during all storage times due to higher water acitivity.

The moisture content of products stored at different temperatures was compared, and it was found that the moisture level decreases as the storage temperature increases.

The moisture of both almond pastes stored at different times and temperatures were found in the levels consistent with other studies [28]. The inversely proportional relation between the moisture content and temperature is the result of water activity in oxidation and the compositional change.

On the other hand, the loss in moisture content is higher in the almond paste model than black carrot juice added almond paste model. When the final initial moisture content was compared to initial moisture content, the loss is 16.7% in almond paste model and the loss in black carrot juice added almond paste model is 12%.

The higher moisture content obtained in black carrot juice added almond paste may be due to the fiber content in black carrot. Also, the addition of the black carrot juice may decrease the oxidation rate by the effect of the rich of total phenolic content, especially anthocynanins, and it influences water activity by less water migration from the black carrot juice added almond paste food model.

The fat content of the almond paste after storage at 4°C, 20°C, 30°C and 60°C was found as 24.5%, 23.7%, 23.0% and 22.5%, respectively. The fat content of the black carrot juice added almond paste after storage at 4°C, 20°C, 30°C and 60°C was found as 24.8%, 24.0%, 23.5% and 23.2%, respectively (Table 4.2).

The initial temperature for the almond paste and the black carrot juice added to the almond paste were 25.5% and 25.6%, respectively.

The minimum and maximum loss in fat content in proportion to initial fat content is 3.9% and 11.8% for the almond paste. The minimum and the maximum loss in the fat content in proportion to initial fat content are 3.1% and 9.4% for black carrot juice added almond paste (Table 4.3).

In all samples, the fat content decreased at all temperatures, but the fat content loss gets higher as temperature increases.

The fatty acid composition was destructed by the iniation of the lipid oxidation and the amount of fatty acids decreased. The results show that black carrot juice addition decelerates the oxidation. Therefore, the loss in fat content in black carrot juice addition is lower.

Table 4.2. Moisture and fat content after storage for almond paste

	Moisture %	Moisture loss %	Fat %	Fat content loss%
4°C	12.0	Not significant	24.5	3.9
20°C	11.5	4.2	23.7	7.1
30°C	11.0	8.3	23.0	9.8
60°C	10.0	16.7	22.5	11.8

Table 4.3. Moisture and fat content after storage for black carrot juice-almond paste

	Moisture %	Moisture loss %	Fat %	Fat content loss%
4°C	12.5	Not significant	24.8	3.1
20°C	12.0	4.0	24.0	6.3
30°C	11.6	7.2	23.5	8.2
60°C	11.0	12.0	23.2	9.4

As similar with moisture, the temperature and the fat content have an inversely proportional relation as a result of oxidation reactions and the compositional change in fatty acids. The black carrot juice diminishes the activity of the oxidation reaction and the addition of natural antioxidants supports the nutrional value for longer.

4.2. Determination of Total Phenolic Content and Lipid Oxidation

The antioxidant activity of phenolic compounds that plants possess, diminish the effect of free radicals thus the studies of phenolic compounds take great attention [69,70]. They react with free radical in the propagation and termination steps in lipid oxidation mechanism. The antioxidant amount is affected by some factors such as time, presence of metals, light, some enzymes and temperature. Therefore, the storage time was determined as 31 days. Four different temperature was selected as 4°C, 20°C, 30°C and 60°C.

Total phenolic content was determined by Folin – Ciocalteu method and the standard curve was prepared for concentration values of 40 µg/mL, 80 µg/mL, 120 µg/mL, 160 µg/mL and 200 µg/mL as shown in Figure 4.1.

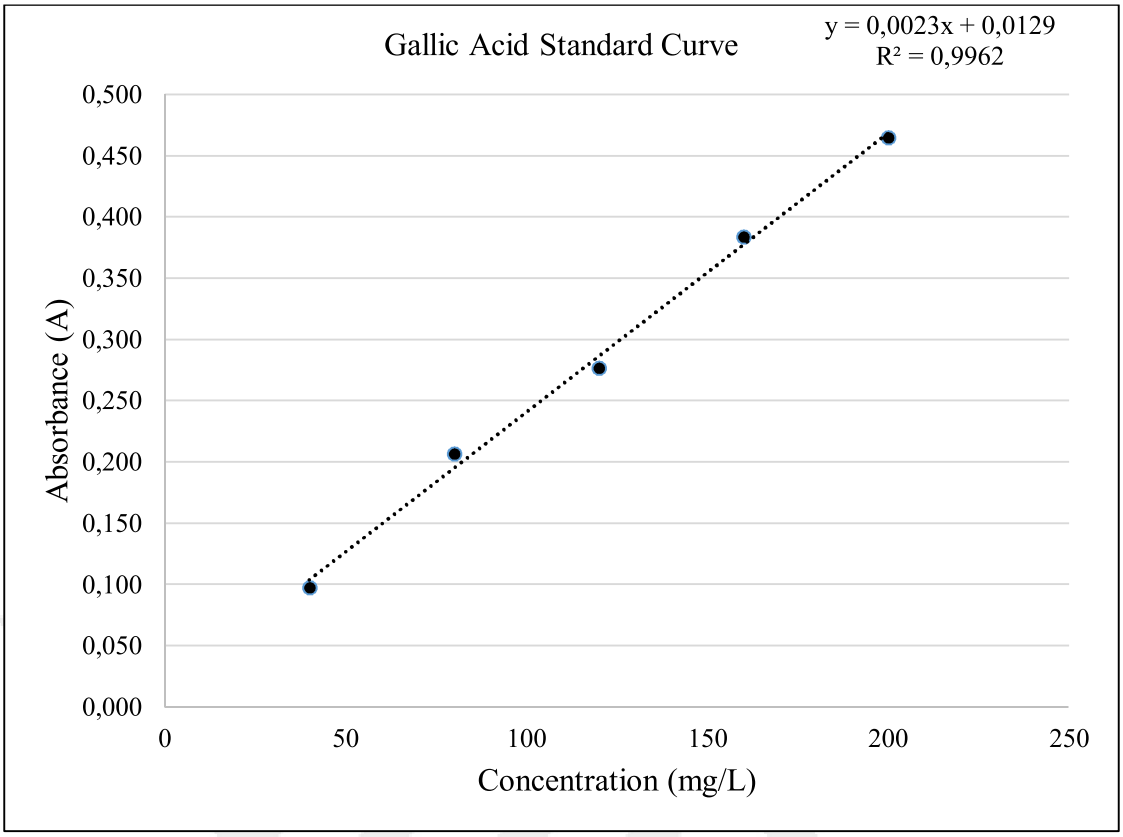

Figure 4.1. Gallic acid standard curve

Using the equation of standard curve, total phenolic content was calculated for 4°C, 20°C, 30°C and 60°C during 31 days. The comparison graphs of phenolic content for almond paste and black carrot added almond paste for each temperature.

Lipid oxidation was determined by peroxide value. According to the consumed sodium thiosulfate amounts in Equation 3.1, the peroxide values were calculated.

The phenolic content and peroxide values were drawn simultaneously for both food models as in Figures 4.2-4.9.

At 4°C, there are not high variations in the total phenolic content. The minimum phenolic content is 69.75 mg/100 g and the maximum phenolic content is 95.90 mg/100 g after storage at the almond paste. The minimum phenolic content is 181.49 mg/100 g and the maximum phenolic content increases to 191.78 mg/100 g for black carrot juice added almond paste.

At 4°C, the peroxide value reaches 0.64 mEq/kg while the addition of black carrot juice decreases this value to 0.37 mEq/kg.

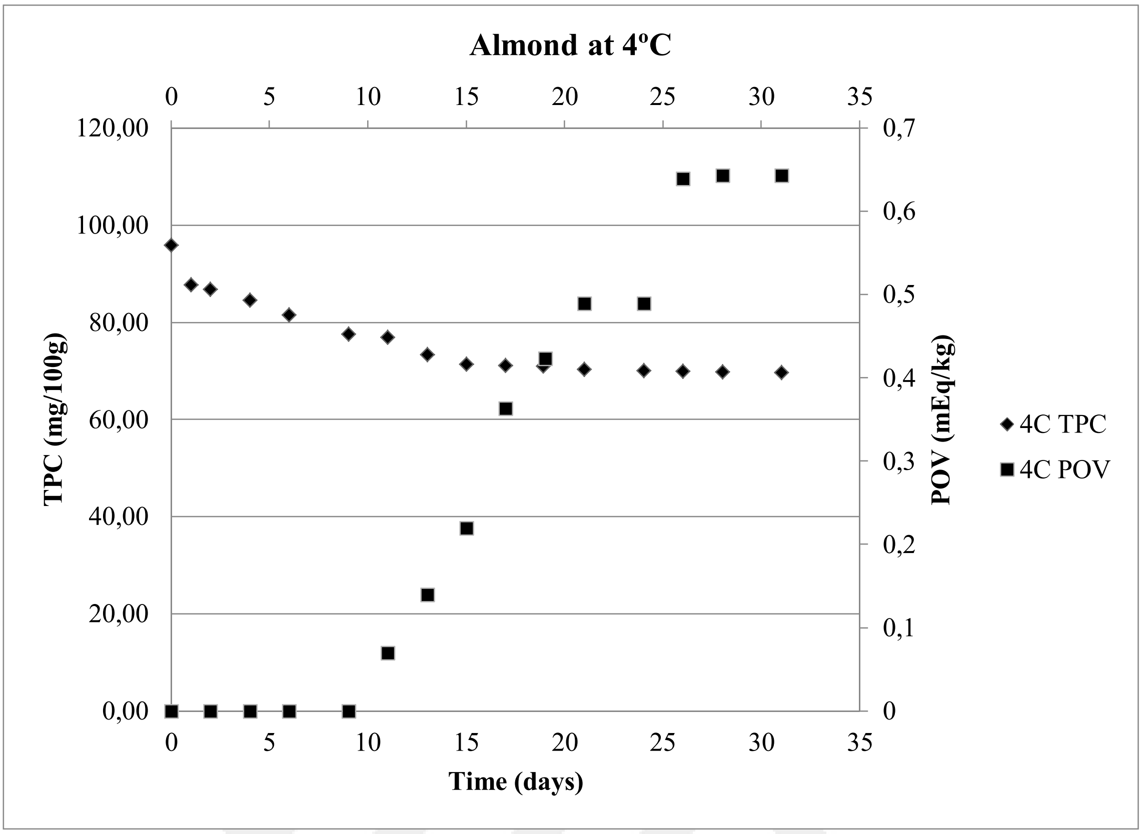

Figure 4.2. Analysis for almond paste at 4ºC

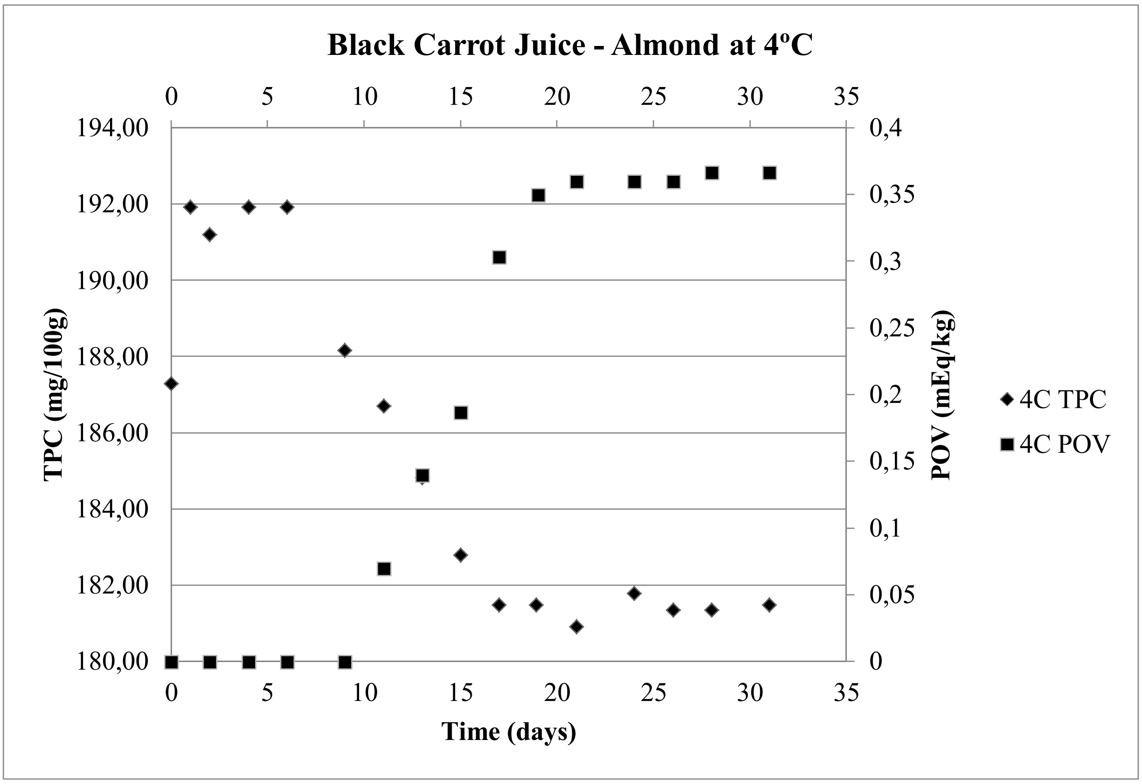

Figure 4.3. Analysis for black carrot juice-almond paste at 4ºC

When the total phenolic content and peroxide value were compared simultaneously, it may be observed clearly that the peroxide value, an indication of the oxidation reaction, increases as the total phenolic content decreases as in Figures 4.2 and 4.3. The decrase in the total phenolic content and the peroxide formation are lower in the black carrot juice added almond paste as seen in Figure 4.3.

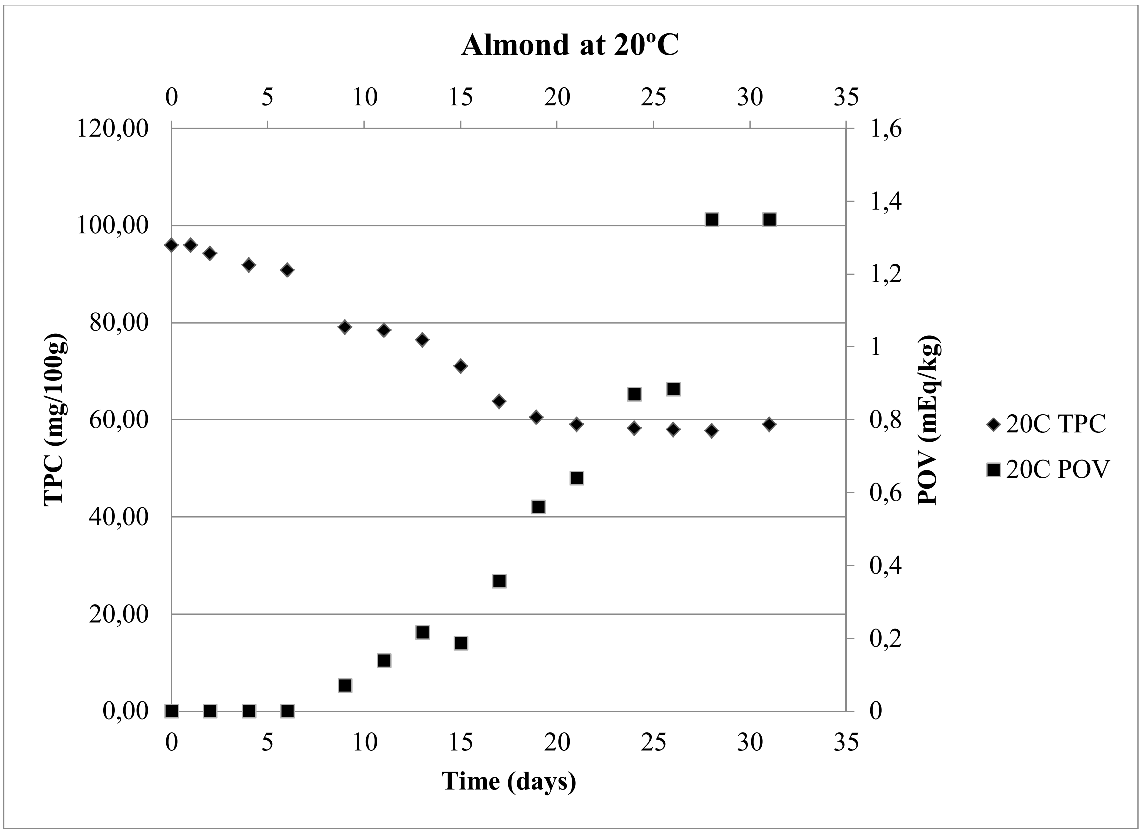

Figure 4.4. Analysis for almond paste at 20ºC

At 20°C, the minimum phenolic content is 59.02 mg/100 g and the maximum phenolic content is 95.90 mg/100 g after storage for almond paste. The minimum phenolic content is 172.36 mg/100 g and the maximum phenolic content increases to 252.80 mg/100 g while the initial phenolic content was 187.29 mg/100 g for black carrot juice added almond paste.

At 20°C, the peroxide value reaches 1.35 mEq/kg while the addition of black carrot juice decreases this value to 0.59 mEq/kg.

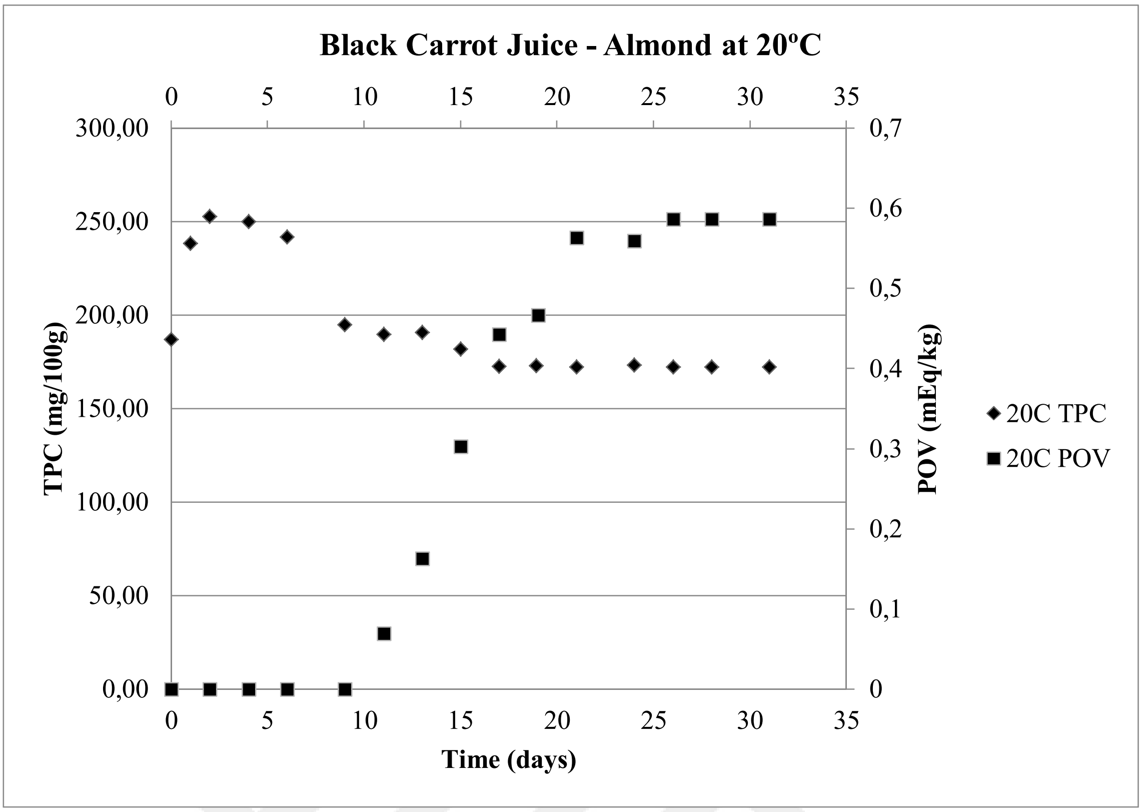

Figure 4.5. Analysis for black carrot juice-almond paste at 20ºC

At 30°C, the minimum phenolic content is 51.50 mg/100 g and the maximum phenolic content is 98.59 mg/100 g after storage while the initial total phenolic content is 95.90 mg/100 g for almond paste. The minimum phenolic content is 170.50 mg/100 g and the maximum phenolic content increases to 252.80 mg/100 g while the initial total phenolic content was 187.29 mg/100 g for the black carrot juice added almond paste.

The phenolic content at 30°C gets higher at the initial days of the storage. This might be due to the synergistic effect of antioxidants found in almond paste and black carrot juice. Another reason might be the activation of the antioxidants by increasing temperature. Additionally, some enzymes may work better and temperature may provide the optimum conditions for antioxidant mechanism. The antioxidant amount increase is higher in black carrot juice addition case.

At 30°C, the peroxide value reaches 3.50 mEq/kg while the addition of black carrot juice decreases this value to 1.58 mEq/kg. At 4°C, 20°C and 30°C, the addition of black carrot juice decrease the peroxide value to more than the half.

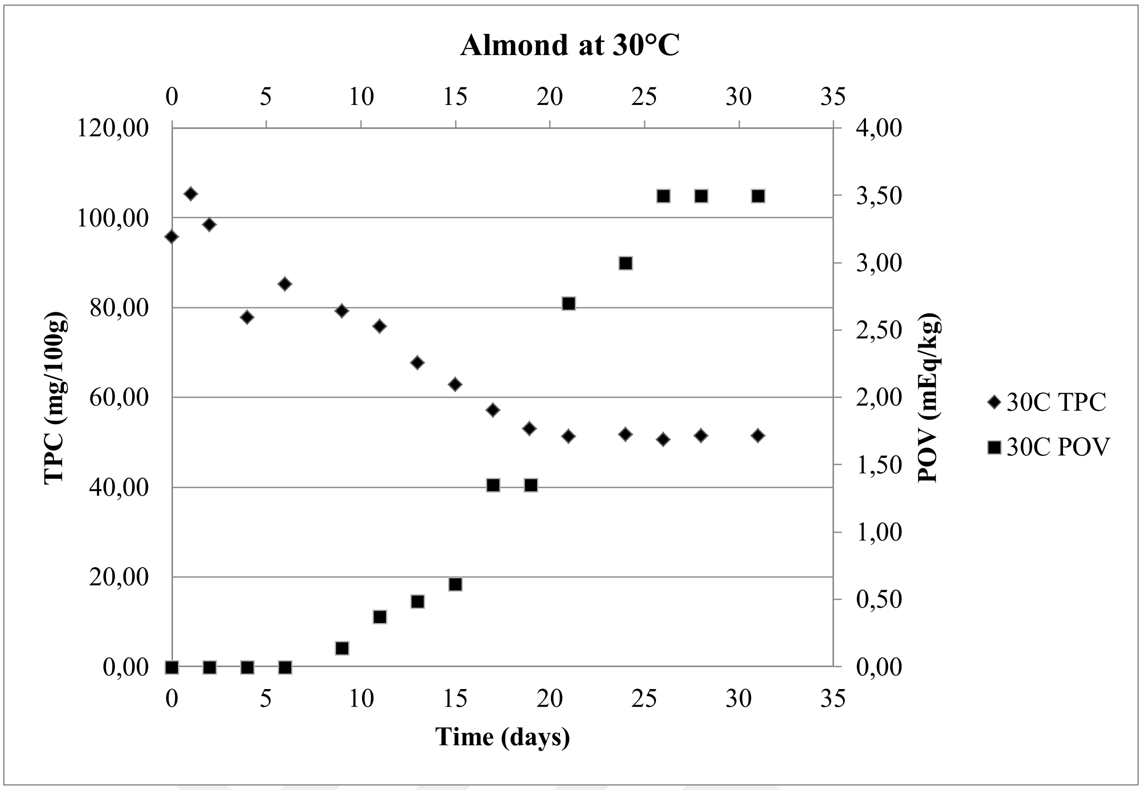

Figure 4.6. Analysis for almond paste at 30ºC

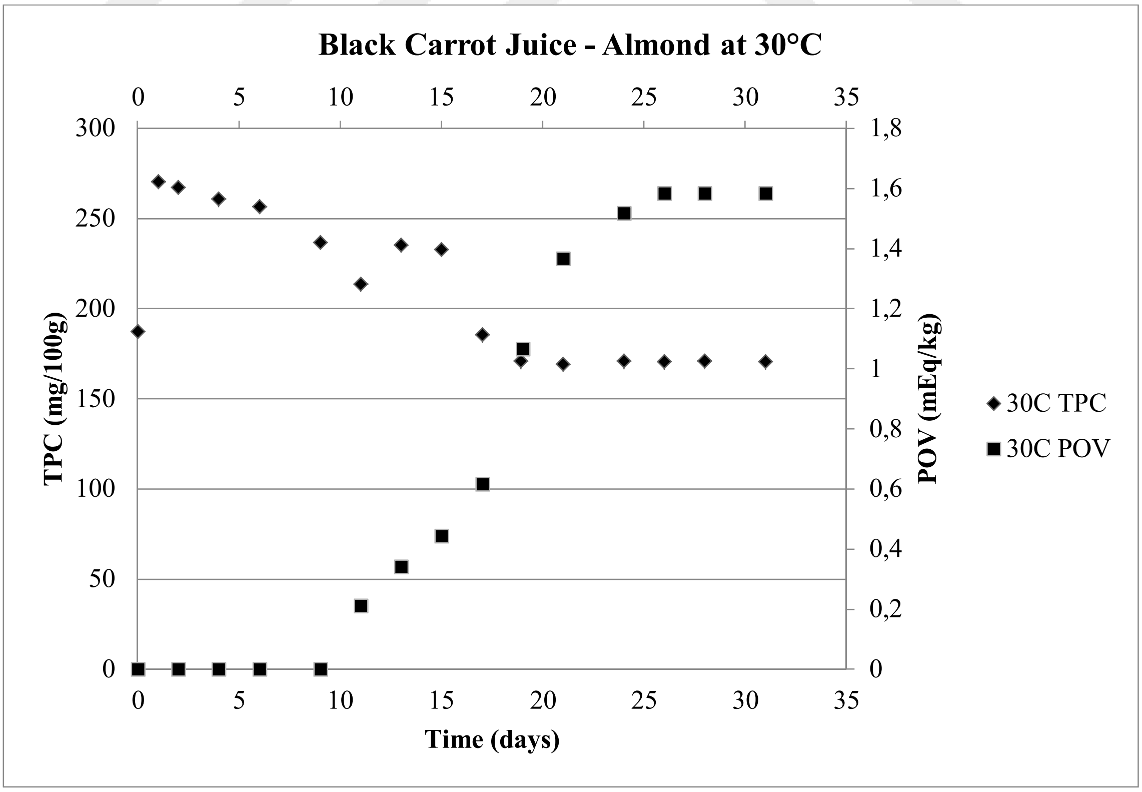

Figure 4.7. Analysis for black carrot juice-almond paste at 30ºC

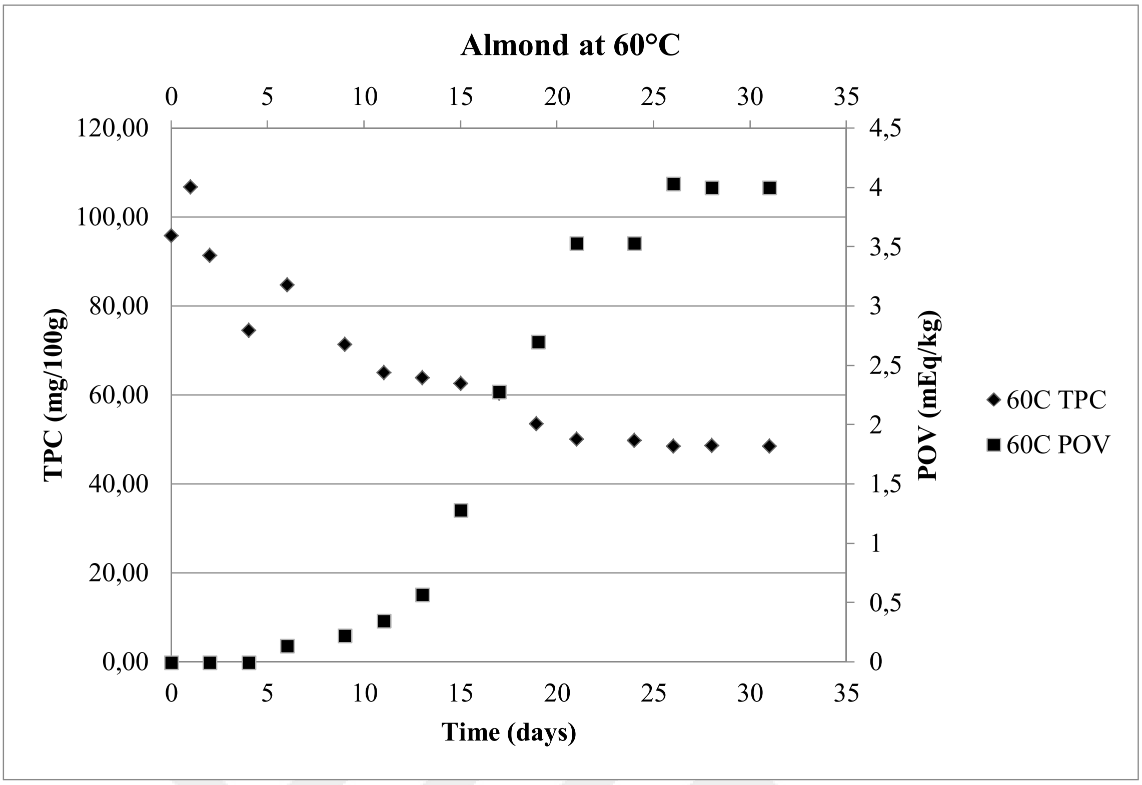

Figure 4.8. Analysis for almond paste at 60ºC

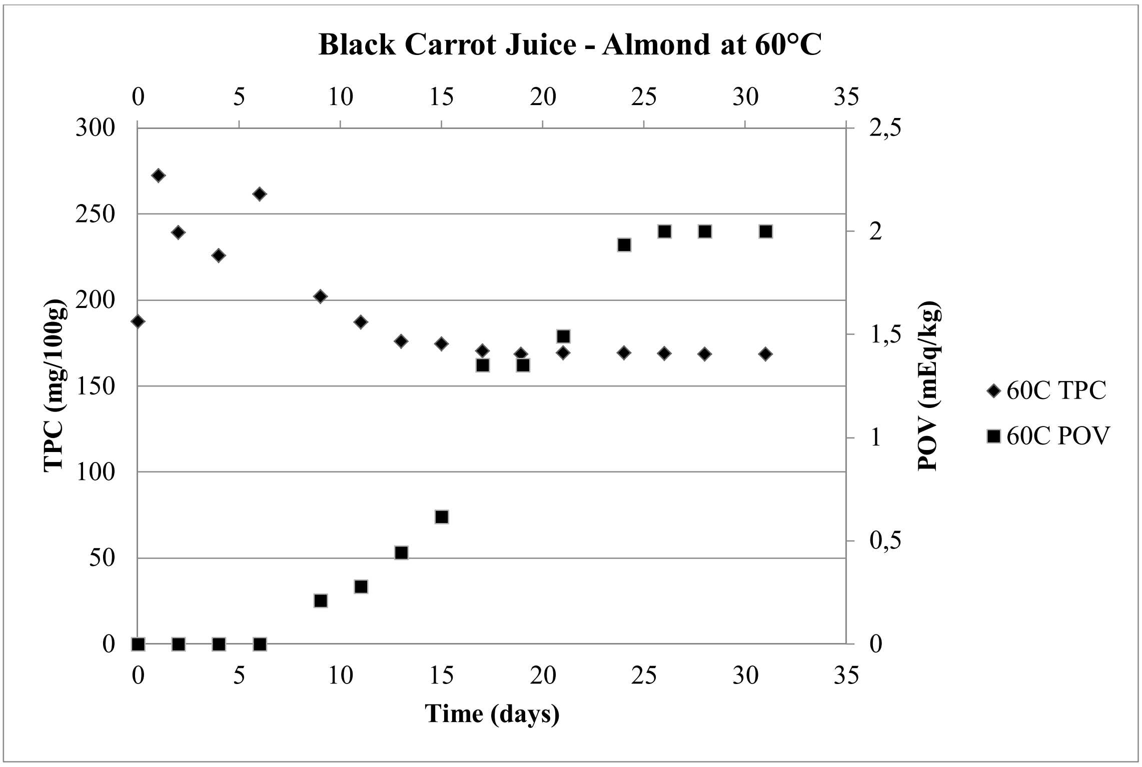

Figure 4.9. Analysis for black carrot juice-almond paste at 60ºC

At 60°C, the minimum phenolic content is 48.60 mg/100 g and the maximum phenolic content is 106.86 mg/100 g after storage while the initial total phenolic content is 95.90 mg/100 g for almond paste. The minimum phenolic content is 168.32 mg/100 g and the maximum phenolic content increases to 272.36 mg/100 g while the initial phenolic content was 187.29 mg/100 g for black carrot juice added almond paste.

At 60°C, the peroxide value reaches 4.00 mEq/kg while the addition of black carrot juice decreases this value to 2.00 mEq/kg. At 60°C, the addition of black carrot juice decreases the peroxide value to the half. As a result, the black carrot juice addition is more effective in lower temperatures with respect to 60°C.

At 60°C, the phenolic content decreases and lipid oxidation rate are more rapid due to the temperature. The composition of fatty acids is destructed under high temperature conditions.

Kornsteiner et al. (2006) argued that the total phenolic content of almond varies between 47-239 mg/100 g which is consistent with the study [71]. Additionally, the total phenolic content of the black carrot is 1653.3 mg/100 g. The black carrot juice contains 10% of the mixture in the study. Therefore, the phenolic content in the study is as expected [72].

The initial minimum and the maximum phenolic contents are summarized in Tables 4.4 and 4.5 to visualize the change in total phenolic content by evaluating all the data obtained from total phenolic content for different temperatures.

Table 4.4. The minimum and the maximum total phenolic contents in the almond paste

T (°C)	Initial Phenolic content (mg/100 g)	minimum phenolic content (mg/100 g)	maximum phenolic content (mg/100 g)	$\left(\frac{m_{max}-m_0}{m_{max}}\right)\times100$	$\left(\frac{m_{max}-m_{min}}{m_{max}}\right)\times100$
4	95.90	69.75	95.90	0.0%	27.3%
20	95.90	59.02	95.90	0.0%	38.5%
30	95.90	51.50	98.59	2.7%	47.8%
60	95.90	48.60	106.86	10.3%	54.5%

Table 4.5. The minimum and the maximum total phenolic contents in the black carrot – almond paste

T (°C)	Initial Phenolic content (mg/100 g)	minimum phenolic content (mg/100 g)	maximum phenolic content (mg/100 g)	$\left(\frac{m_{max}-m_0}{m_{max}}\right)\times 100$	$\left(\frac{m_{max}-m_{min}}{m_{max}}\right)\times 100$
4	187.29	181.49	191.78	2.3%	5.4%
20	187.29	172.36	252.80	25.9%	31.8%
30	187.29	170.50	270.47	30.8%	37.0%
60	187.29	168.32	272.36	31.2%	38.2%

The total phenolic content changes with the temperature increase. The maximum variation in the total phenolic contents occurs at 60ºC. The variation with respect to the initial total phenolic content is 10.3% and the variation with respect to minimum total phenolic content is 54.5% for the almond paste. The variation with respect to the initial total phenolic content is 31.2% and the variation with respect to minimum total phenolic content is 38.2% for the almond paste. The difference between the variations with respect to the initial total phenolic content and the minimum phenolic content is lower for the black carrot juice added almond paste due to the lower lipid oxidation.

To analyze the effect of the temperature during storage on total phenolic content, all the total phenolic contents of all temperatures were analyzed. The phenolic content increases as the temperature increases as well during the initial days of the storage but then the variation of the phenolic content have more fluctuations and at the highest temperature, the phenolic content decreases more rapidly at lower temperatures.

The black carrot juice has high phenolic content and it increases the phenolic content of the mixture. At the same time, the increase of the phenolic content in the black carrot juice added almond paste in the initial days are higher rather than almond paste and addition of the black carrot juice get slower the decrease in phenolic content.

The maximum oxidation is the highest at 60°C for both the almond paste and black carrot juice added almond paste, but the addition of the black carrot juice to the almond paste lowers the lipid oxidation. As the temperature increases, the initiation of lipid oxidation starts earlier but the addition of the black carrot juice delays the initiation of the lipid oxidation. It has been found that the phenolic compounds preserve the lipid composition of the food models. Moreover, the synthetic and the natural phenolic compounds may be added to the fat-rich products [58].

The maximum peroxide value is the indication of the maximum oxidation in units of mEq/kg. Therefore, maximum oxidation, initial oxidation time and final oxidation time for the almond and the black juice added almond paste are summarized in Table 4.6.

The maximum oxidation is 0.64 mEq/kg at 4°C, 1.35 mEq/kg at 20°C, 4.0 mEq/kg at 30°C and 4.0 mEq/kg at 60°C with the initiation of oxidation at 11^{th}, 9^{th}, 9^{th} and 9th days for almond paste, respectively. The maximum oxidation is 0.37 mEq/kg at 4°C, 0.59 mEq/kg at 20°C, 1.58 mEq/kg at 30°C and 2.0 mEq/kg at 60°C with the initiation of oxidation at 11^{th}, 9^{th}, 9^{th} and 9th days for black carrot juice added almond paste, respectively.

Table 4.6. Oxidation and oxidation times

	Almond Paste			Almond Paste – Black Carrot		
T (°C)	max oxidation (meq/kg)	Initial oxidation time (day)	Final oxidation time (day)	max oxidation (meq/kg)	Initial oxidation time (day)	Final oxidation time (day)
4	0.64	11	26	0.37	11	28
20	1.35	9	28	0.59	11	26
30	3.50	9	26	1.58	11	26
60	4.00	9	26	2.00	9	26

The addition of the black carrot juice into the almond paste has a crucial role to deccelarate the lipid oxidation due to the synergistic effect of the antioxidants. Especially, tocopherols in almond paste and anthocynanins in the black carrot juice may decrease the oxidation rate and scavenge the free radicals more effectively. On the other hand, the addition of the black carrot juice has a balancing effect in the amounts of the antioxidant because the kernel of the almond has the maximum amount of antioxidant molecules. However, the almond paste is produced by removing the kernel. Black carrot juice addition increases the antioxidant effect. The antioxidants increase at the beginning of the storage. Flavonoids play a role as booster and have recycling effect on the other antioxidant. Anthocynanins as a part of the flavonoids in black carrot may recyle the tocopherols in almond paste and therefore the antioxidants may increase before the beginning of the lipid oxidation.

5. CONCLUSION

The nuts have rich content of nutritive subtances. They are rich in fatty acids which have health effects on hearth health, mental health, diabetes, weight loss, digestive system and immune system.

The fatty acids are sensitive to oxidation and the oxidation may initiate by temperature, light, some enzymes and presence of metals. The phenolics are antioxidant molecules and crucial components that preserve the foods from lipid oxidation that causes chemical changes in the food composition as well as deterioration in flavor and odor. The addition of antioxidants to food products is a method for food preservation. Especially, natural antioxidants are more preferable to synthetic antioxidants. Sythetic anxtiodants may show carcinogenic effect.

Almond is one of the most consumed nuts and has high content of phenolics but the lipid oxidation is also a problem for almond. The study targets the delay of the lipid oxidation by the addition of black carrot juice to almond paste due to the high phenolic content, especially anthocynanins.

The almond paste was the control food model and the black carrot juice added almond paste was second food model. Both models were stored for 31 days at 4°C, 20°C, 30°C and 60°C. The analyses of the moisture, ash, total fat, total phenolic content and peroxide formation were performed.

Moisture content in almond is needed for the activation of catalysts and oxygen. The moisture content decreases as the storage temperature gets higher in almond paste. In case of addition of black carrot juice; the moisture content is higher than almond paste. Therefore, the addition of black carrot juice contributes the moisture content even in high temperatures due to the high phenol content and fiber content. The fiber content of black carrot absorbs the moisture in the food model. Additionally, the increasing amount of phenolics has an influence that regulates water activity and lowers the water migration from the food models. Especially, anthocynanins found in black carrot have stronger effect than tocopherols in almond paste. Thus, the lower lipid oxidation is provided by anthocynanins and the less moisture loss is obtained.

During the storage, the fat content decreases with time and temperature. The addition of the black carrot juice protects the fat content in a specific rate and helps the almond paste preserve fat content. The higher phenolic content lowers the lipid oxidation and the less chemical change in the fats of food models occurs. The fatty acid amount gets lower in lipid oxidation. Another factor that affects the lower fat content loss in black carrot juice added almond paste model may be the fiber content in black carrot juice. Thus, the fiber content might absorb the fat content and prevent the migration of the fat molecules.

During the initial days of the storage, phenolics increases as the temperature increases. When the lipid oxidation begins, the fluctuations at higher temperatures increase and the phenolic content decreases rapidly. In the case of the black carrot juice addition, the phenolic content gets higher and after lipid oxidation, the decrease in phenolic content get slower.

The addition of black carrot juice into almond paste deccelarates lipid oxidation by the synergistic effect of antioxidants found in almond paste and black carrot juice. Especially, tocopherols in the almond paste and the anthocynanins in the black carrot juice may decrease the oxidation rate and scavenge the free radicals more effectively. The kernel of the almond has the maximum amount of antioxidant molecules. However, the almond paste is produced by removing the kernel. Black carrot juice addition increases the the antioxidant amount to improve the shelf life of the product due to the probable recycling effect of anthocynanins on tocopherols.

Above all, the addition of natural antioxidants to food products is an effective option to preserve the foods and their nutritional values. Besides the synthetic antioxidants, natural antioxidants are promoted for health benefits. Almond paste has a significant place in human diet and the black carrot juice addition provides both tasty and health product with longer shelf life.

6. RECOMMENDATIONS

In the study, total phenolic content and peroxide values were determined for black carrot juice added almond paste food model.

As a further study, monounsaturated and polyunsaturated fatty acid contents may be determined before storage and after storage because polyunsaturated fatty acids are more sensitive to lipid oxidation. Some methods may be performed to determine the synergistic effect of the antioxidants.

The black carrot extract may be used instead of its juice not to lose the antioxidant amount and fiber content. The formulations may be prepared according to the suitable moisture level and texture structure. The texture and sensory analysis may be performed to evaluate the food model formulation in order to prepare a new food product for market.

The encapsulation of black carrot juice added almond paste pieces may be done to decrease the antioxidant loss and to prevent lipid oxidation. Another method may be to use the most proper packaging techniques to preserve the nutritive values and to elongate the shelf life.

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