

ABANT İZZET BAYSAL UNIVERSITY
THE GRADUATE SCHOOL OF NATURAL AND APPLIED
SCIENCES



PHYTOCHEMICAL CONTENTS AND ANTIOXIDANT
ACTIVITIES OF SOME BREAD (*Triticum aestivum* L.), DURUM
(*Triticum turgidum* ssp. *durum* Desf.) AND HULLED EINKORN
(*Triticum monococcum* ssp. *monococcum*) WHEATS

MASTER OF SCIENCE

YUNUS ŞAHİN

BOLU, JUNE 2016

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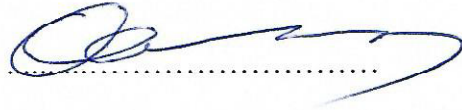
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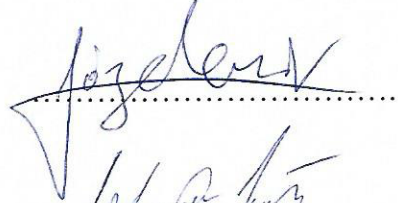
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DEDICATION

I dedicated this thesis to my father, Binali Şahin, my mother, Zeynep Şahin, my brother, Emre Şahin who are my lovely family and my cousin, Bulent Şahin, may his soul rest in peace. You have successfully made me the person i am becoming.



DECLARATION

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

YUNUS SAHİN

A handwritten signature in black ink, appearing to read 'Yunus Sahin', is written over a horizontal line.

ABSTRACT

PHYTOCHEMICAL CONTENTS AND ANTIOXIDANT ACTIVITIES OF SOME BREAD (*Triticum aestivum* L.), DURUM (*Triticum turgidum* ssp. *durum* Desf.), AND HULLED EINKORN (*Triticum monococcum* ssp. *monococcum*) WHEATS

MSC THESIS

YUNUS ŞAHİN

**ABANT İZZET BAYSAL UNIVERSITY GRADUATE SCHOOL OF
NATURAL AND APPLIED SCIENCES**

DEPARTMENT OF BIOLOGY

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(CO-SUPERVISOR: Asst. Prof. Dr. ARZU BİRİNCİ YILDIRIM)

BOLU, JUNE 2016

Wheat (*Triticum* spp.), which is cultivated for centuries in the middle-east, central Asia, Europe, north-Africa, and, then, in America is a leading staple crop around the world. Its marginally grown ancestor, hulled einkorn (*Triticum monococcum* ssp. *monococcum*) possesses desired agronomic, quality characters and, moreover, bioactive compounds, which reduce and prevent chronic diseases such as diabetes, cancer, alzheimer, and cardio-vascular diseases. As more attention has been given to wheat cultivars with strong gluten, protein content, starch composition, and resistance to biotic and abiotic stresses in bread wheat and yellow-colored pasta products in durum wheat health compounds such as fibers, phytochemicals, and bioactives have, however, been undervalued so far. The aim of this study was, then, to examine total phenolics and flavonoids, to quantify their phenolic acids, α -tocopherol by high performance liquid chromatography (HPLC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activities of five bread (*Triticum aestivum* L.), five durum (*Triticum turgidum* ssp. *durum* Desf.), and two hulled einkorn (*Triticum monococcum* ssp. *monococcum*) wheat entries of different origins. Ferulic acid (762.89 and 764.04 $\mu\text{g/g}$ of Pop-1 and Pop-2, respectively), *p*-coumaric (47.64 and 54.09 $\mu\text{g/g}$ of Pop-1 and Pop-2, respectively), and total phenolic content (6.37 and 7.49 $\mu\text{mol GAE/g}$ of Pop-1 and Pop-2, respectively) of einkorn populations were significantly higher than ferulic acid (ranged 148.67-764.04 $\mu\text{g/g}$), *p*-coumaric (ranged 5.06-54.09 $\mu\text{g/g}$), and total phenolic content (ranged 1.60-7.49 $\mu\text{mol GAE/g}$) of bread and durum wheat cultivars. There were no significant differences between bread, durum and hulled einkorn wheat cultivars and populations with respect to total flavonoid content (ranged 0.04 and 0.39 $\mu\text{mol CTE/g}$) and DPPH scavenging activity. Results suggested that possibility of production of hulled einkorn wheat populations and hopefully improved cultivars form them, which were rich in particular health component(s) may provide benefit to the consumers. In addition, higher phenolic content of hulled einkorn may offer novel genetic resources for the improvement of new wheat cultivars and the development of wheat-based functional foods.

KEYWORDS: Anticancer, Antioxidant, Bioactive Compounds, Bread Wheat, Durum Wheat, Hulled Einkorn, Phytochemicals.

ÖZET

BAZI EKMEKLIK (*Triticum aestivum* L.), MAKARNALIK (*Triticum turgidum* ssp. *durum* Desf.) VE KAVUZLU SIYEZ (*Triticum monococcum* ssp. *monococcum*) BUĞDAYLARININ FITOLKİMYASAL İÇERİKLERİ VE ANTIOKSIDAN AKTİVİTELERİ
YÜKSEK LİSANS TEZİ
YUNUS ŞAHİN
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Yüzyıllardır Orta Doğu, Orta Asya, Avrupa, kuzey Afrika ve Amerika’da yetiştirilen buğday (*Triticum* ssp.), dünya çapındaki başlıca ürünlerden biridir. Buğdayın sınırlı miktarda yetiştirilen atalarından olan kavuzlu siyez (*Triticum monococcum* ssp. *monococcum*) arzu edilen agronomik ve kalite özellikleri yanında ayrıca diyabet, kanser, alzheimer ve kalp damar hastalıkları gibi kronik hastalıkları azaltıcı ve önleyici biyoaktif maddeler de bulundurmaktadır. Ekmeklik buğdayda sert glüten, protein içeriği, nişasta oluşumu ve biotik ve abiotik stresslere direnç, makarnalık buğdayda sarı renkli makarna ürünleri dikkate alınmış, ancak, fiber, fitokimyasallar ve biyoaktif maddeler şuna kadar göz ardı edilmiştir. Bu çalışma, yukarıda sayılan nedenlerle, farklı kökene sahip ekmeklik (*Triticum aestivum* L.), makarnalık (*Triticum turgidum* ssp. *durum*) ve kavuzlu siyez (*Triticum monococcum* ssp. *monococcum*) buğdaylarının toplam fenol ve flavonoid değerlerinin ölçülerek değerlendirilmesi yanında bu buğdayların yüksek performanslı sıvı kromatografi (HPLC) aracılığıyla fenolik asitlerinin, α -tocopherol içeriğinin ve 2,2-diphenyl-1-picrylhydrazyl (DPPH) süpürme aktivitesinin ölçülmesi de amaçlanmaktadır. Kavuzlu siyez buğdaylarının ferulik (Pop-1; 762,89 $\mu\text{g/g}$ ve Pop-2; 764,04 $\mu\text{g/g}$), *p*-coumaric (Pop-1; 47,64 ve Pop-2; 54,09 $\mu\text{g/g}$) ve toplam fenolik içerikleri (Pop-1; 7,49 $\mu\text{mol GAE/g}$ ve Pop-2; 6,37 $\mu\text{mol GAE/g}$) olup ekmeklik ve makarnalık buğdaylarının ferulik asit (148,67-764,04 $\mu\text{g/g}$), *p*-coumaric (5,06–54,09 $\mu\text{g/g}$) ve toplam fenol (1,60-7,49 $\mu\text{mol GAE/g}$) içeriğinden istatistiksel olarak daha yüksek bulunmuştur. Toplam flavonoid ve antioksidan aktiviteleri açısından ekmeklik, makarnalık ve kavuzlu siyez tür ve çeşitleri arasında genel olarak anlamlı farklılıklar bulunamamıştır. Sonuçlar kavuzlu siyez üretiminin ve bu buğdaylardan geliştirilebilecek sağlık özellikleri yüksek çeşitlerin tüketici için daha yararlı olabileceğini göstermektedir. Dahası, yüksek fenolik içeriğine sahip kavuzlu siyez buğdayları yeni buğday çeşitlerinin ve fonksiyonel sağlık temelli buğday besinlerinin geliştirilmesi için yeni anaçlar sağlayabilecektir.

ANAHTAR KELİMELER: Antikanser, Antioksidan, Diyabet, Ekmeklik Buğday, Makarnalık Buğday, Kavuzlu Siyez, Fitokimyasallar.

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LIST OF ABBREVIATIONS AND SYMBOLS

ABTS	:2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
AFLP	:Amplified fragment length polymorphisim
ANOVA	:Analysis of variance
ARI	:Anatolian research institute
BE	:Bran equivalent
BHA	:Butylated hydroxanisole
BHT	:Butylated hydroxytoluene
BPC	:Bound phenolic content
CE	:Cathechin equivalent
CRIFC	:Central Research Institute for Agricultural Research
CTE	:Cathecol equivalent
CVD	:Cardio vascular disease
DE	:Diethy ether
DM	:Dry matter
DMPD	:N,N-dimethyl-1,4-diaminobenzene
DMRT	:Duncan Multiple Range Test
DPPH	:2, 2-diphenyl-1-picrylhydrazyl
DW	:Dry weight
EA	: Ethyl acetate
FAOSTAT	:Statistics Data provided by Food and Agriculture Organization of the United Nations
FPC	:Free phenolic content
FRAP	:Ferric reducing ability of plasma
GAE	:Gallic acid equivalent
GC	:Gas chromatography
HPLC	:High performance liquid chromatography
LC	:Liquid chromamotography
LDL-C	:Low density lipoprotein cholestrol
MS	:Mass spectrometry
NMR	:Nuclear magnetic resonance
ORAC	:Oxygen radical absorbance capacity

PAL	:Phenylalanine ammonia lyase
PDA	:Photo-diode array
PDP	:Phytl diphosphate
RFLP	:Restriction fragment length polyphormisim
RNS	:Reactive nitrogen species
ROS	:Reactive oxygen species
SCFA	:Short chain fatty acid
SD	:Standart deviation
SGW	:Stay green wheat
SPSS	:Statistical Package for Social Sciences
TAL	:Tyrosine ammonia lyase
TCA	:Total cultivated area
TCCA	:Total cereal cultivated area
TEAC	:Trolox equivalent antioxidant activity
TFC	:Total flavonoid content
TLC	:Thin-layer chromatography
TPC	:Total phenolic content
Trolox	:6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TSI	:Turkish Statistical Instute
YCP	:Yellow coloured pigment

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1. INTRODUCTION

1.1 Plant introduction and literature review

1.1.1 Plant introduction

Wheat (*Triticum* L.) is a herbaceous annual plant in the grass family Poaceae (Gramineae) and subfamily Triticeae (Hordeae) (Briggle and Reitz, 1963). Wheat includes wild and domesticated (cultivated) species. All domesticated wheat is first classified by Linnaeus in 1753. Wheat forms a polyploid series. In 1918, Sax reported three natural polyploid groups of wheats with chromosome number sets of $2n = 14$ (diploid), 28 (tetraploid) and 42 (hexaploid), respectively. *T. monococcum* ssp. *monococcum* (einkorn wheat) is diploid wheat and derived from *T. boeoticum* (wild einkorn wheat). Bread wheat is *Triticum aestivum* L. Other major species are *T. turgidum* ssp. *durum* and *T. compactum*. All three are products of natural hybridizations among ancestral wheats (Briggle and Reitz, 1963).

Wheat is now extensively grown across the temperate, subtropical, and tropic parts of both hemispheres of the world, from 67 °N in Norway, Finland, and Russia, to 45 °S in Argentina. However, in the subtropics and tropics, the cultivation of wheat is restricted to higher elevations. Main wheat-producing regions across the world are in temperate and southern Russia, the central plains of the United States, southern Canada, Mediterranean basin, north-central China, India, Argentina, and south-western Australia.

Wheat does not need of high temperature and light intensity in the first stages of growth. It can normally grow at 5-10 °C of temperature and above 60% of humidity. Furthermore, it does not require high light intensity in vegetative growth while it needs optimum temperature of 10-15 °C and 65% of humidity for its growth. However, high humidity and light are required before head formation. During pollination, low humidity and high temperature provide good quality seed. In the

case of soil, clay-loam, sufficient humus, potassium, and sandy-loam are preferred to grow wheat species and cultivars (Kün, 1996).

1.1.1.1 Botany – wheat species

Wheat is polyploid and falls into three different polyploidy levels as diploid, tetraploid (durum wheat), and hexaploid (bread wheat). Bread and durum wheat have subgroups of winter, spring, and facultative growth types. Cultivation of those is varied in different geographic regions of Turkey. Winter and facultative wheat cultivars are mainly cultivated in eastern and central Anatolia and Thrace although spring wheat is mainly grown in the southeastern Anatolia and coastal regions under rainfed conditions (Braun et al., 2001).

1.1.1.2 Morphological structure and active constituents in wheat

The most common type of inflorescence in the cereals is the ear (spike). All wheat, barley, and rye have ear inflorescences. An ear is defined in the following way: the flowers (spikelets = partial inflorescences in the case of the grasses) are arranged in rows on the main axis (Figure 1. 1).

A cereal ear (spike) consists of a rachis (central axis) of attached spikelets, each with florets. The rachis (central axis) consists of rachis segments (internodes). The rachis can be of two different kinds:-brittle, that is easily broken into segments (spikelet with a rachis segment) at the nodes, which is particular characteristic of all wild grasses of the sub-family Triticinae. The domesticated wheat such as einkorn and emmer have a moderately brittle rachis, though, that is hardly broken into single segments at threshing, which is typical domesticated plant characteristic, particularly of free-threshing type of wheat (Figure 1.2).

In the Gramineae (Poaceae), ear consists of a group of florets on a much shortened rachis. In wild cereals, the spikelet (with one rachis segment attached) is the unit of dispersal. The spikelet is one- or many-flowered. It is enclosed in two glumes which can have various shapes (compare the single species). A grass floret is

made up of 4 parts; a lemma and a palea, which enclose the ovary (which develops into the grain) and anthers. The lemma can have a long or short extension -an awn.

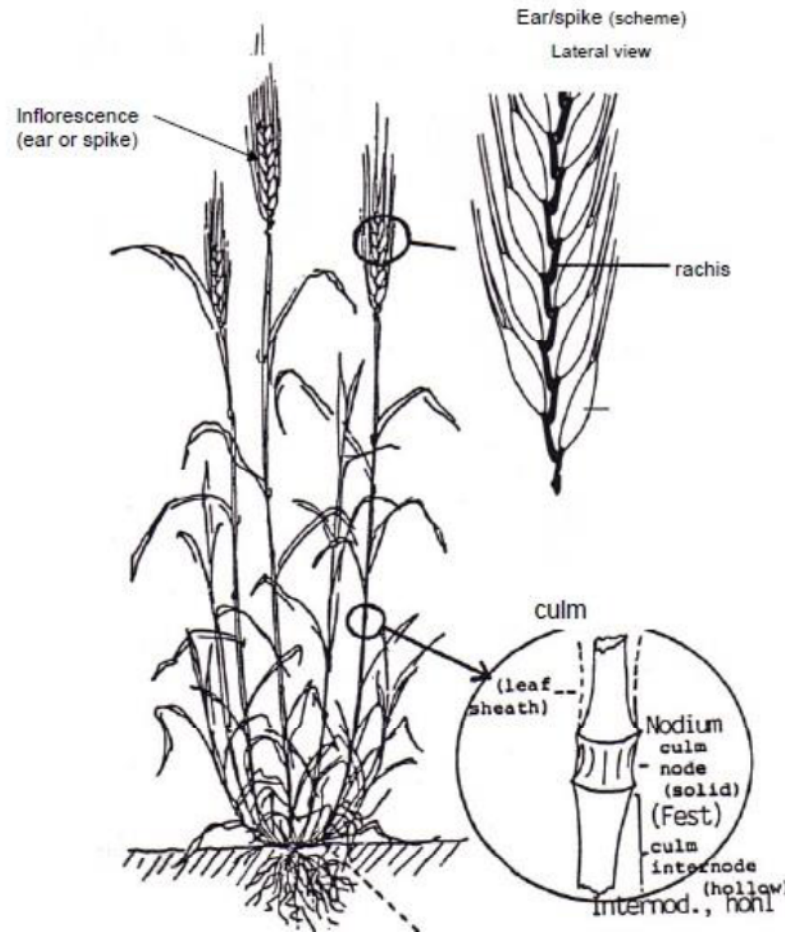


Figure 1.1. Morphology of wheat (Jacomet, 2006).

When the grain is held in the lemma and palea, it represents a hulled (glumed) cereal. With these, the grain needs to be got out from the lemma and palea by processes such as parching in an oven or pounding in a mortar before consumption. When the grain is only loosely held between the lemma and palea, then, the grain is called free-threshing or naked.

The wheat grain or the kernel is the caryopsis. A wheat kernel is about 5-8 mm in length and 2.5-4.5 mm in width, having a longitudinal crease that extends to the center of the kernel. The existence of the crease make difficult to handle the grain. It has been suggested that eliminating the longitudinal crease might improve wheat yield. The wheat kernel is somewhat thicker toward the embryo. At the opposite end hairs of the brush are located (Figure 1.3).

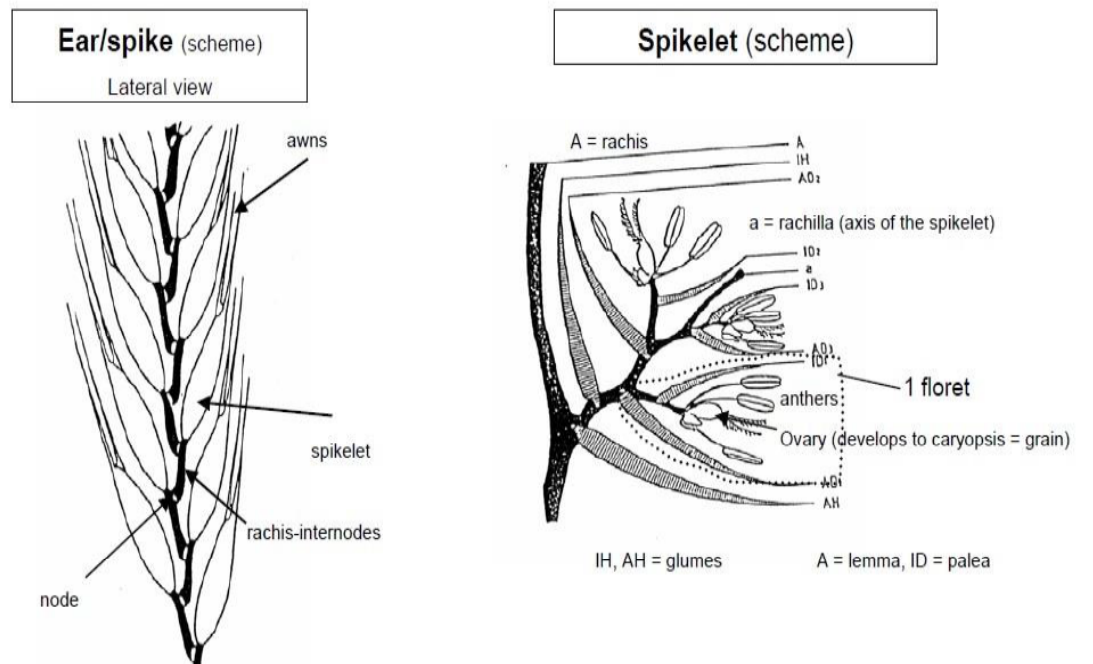


Figure 1.2. Ear scheme of wheat and its sections (Jacomet, 2006).

Wheat is composed of three main parts as embryo, endosperm and bran (Figure 1.3). The wheat germ is the embryo that would eventually develop into the wheat plant. It is rich in lipids (α -linolenic acid), sucrose and monosaccharides, sulfur, amino acids, glutathione, insoluble and soluble fibre, raffinose, flavonoids, vitamin E, B vitamins, minerals and trace elements, phytosterols, betaine and choline, and policosanol. The endosperm consists of large, thin-walled cells, filled mainly with starch and protein. In the sub-aleurone area, especially on the dorsal side of the grain, the dominant cells are elongated in the direction of the endosperm center containing 70-75% of the proteins, which exist in the grain. Starch grains are enclosed in the thin layer of adherent protein and located within a protein matrix which fills the individual cells of the endosperm to varying degrees. The bran is divided into compact cell layers and pigment strand; aluerone layer, hyaline layer, testa, inner and outer layer and pericarp. These layers are rich in soluble and insoluble dietary fibre (xylans, glucans, raffinose, stachyose, fructans), proteins (sulfur amino acids and glutathione), antioxidants, (phenolic acids, carotenoids, lignans, anthocyanins, isoflavonoids) vitamin E, B vitamins, minerals and trace elements, phytic acid, betaine and choline, and enzymes. Furthermore, in cereal grain, bioactive compounds are unevenly distributed within its different parts (Fardet, 2010).

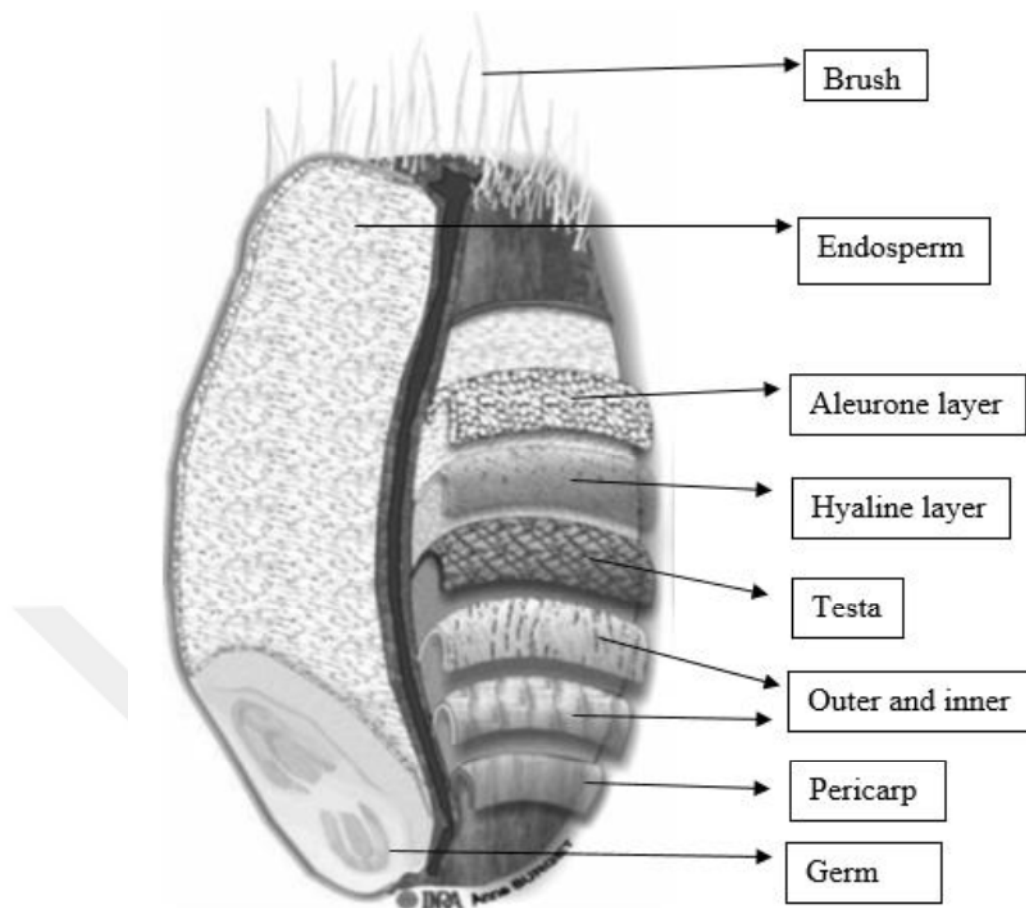


Figure 1.3. Wheat components and their fractions (Fardet, 2010).

1.1.1.3 Origin, diversity, and classification of wheat

The discovery of agriculture caused many changes in human lifestyle like a transition from hunting-gathering to agriculture and sedentary life. Evidence has shown that first agricultural practice emerged from Fertile Crescent region in southeastern Anatolia (Figure 1.4).

Wheat is classified as *Triticum* genus of Triticeae, tribe of Poaceae. It was firstly classified by spikelet features. Hulled, de-hulled, hull color, and grain color were the criteria to classify. Later, spike frequency (loose, semi-loose, non-loose) and chromosome numbers played a role for wheat classification. To classify *Triticum* genus, two main different wheat classifications have been used by researchers; Mac Key's and Dorofeev's classifications. The former is mainly followed by western community and latter is mostly accepted by eastern scientific community. Using two

different classification cause to confusion in scientific community, and, moreover, it is still a challenge in the studies.

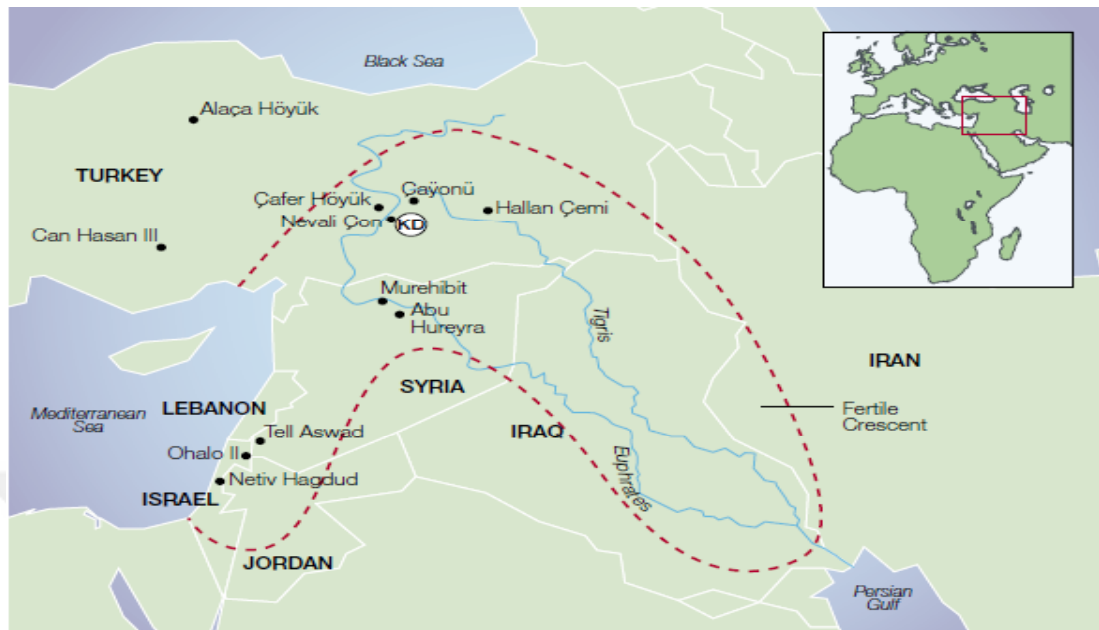


Figure 1.4. The Fertile Crescent has cold rainy winters and dry summers (dashed in red line) (Salamini et al., 2002).

Mac key's classification which has one wild species and nine cultivated tetraploid wheats grouped as under the name of *T. turgidum* L. may not be satisfactory and, as some species having richness of morphological variations and genetic, is underestimated, is turn out to be source of genetic erosion. Dorofeev's concept has not been widely used, though it is well known by researchers, because it was written in Russian language (Hammer et al. 2011). Polyploid changes have been common seen in wheat, comprising diploid ($2n=14$), tetraploid ($4n=28$), hexaploid ($6n=42$). Evolution scheme of wheat species is shown in Figure 1.5 (Miller, 1987).

These wheat groups include wild, feral and domesticated forms, except hexaploid wheat. Domestication is selection or altering key traits, causing changes in morphological ones, for example, wild types transformed into domesticated forms. Feral type is the crosses or hybridized between wild and domesticated genotypes (Salamini et al., 2002).

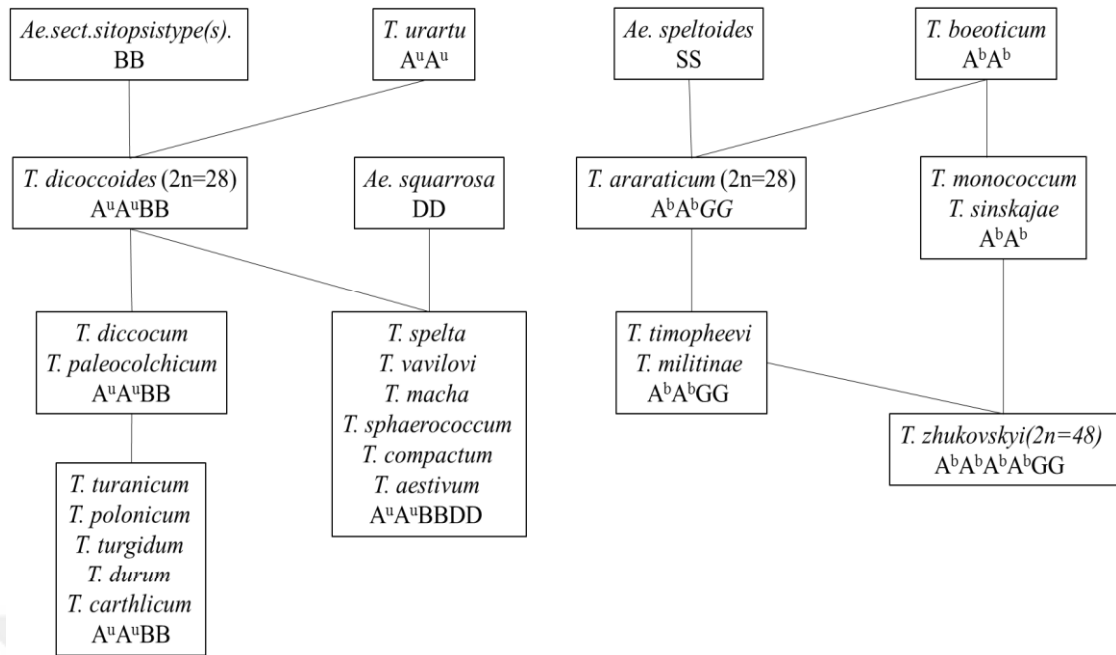


Figure 1.5. The origin of polyploid *Triticum* species. The letter representative donate the genome constitution of corresponding species (Miller, 1987).

1.1.1.3.1 Diploid Wheat (2n=14)

This group has 2n=14 chromosomes, with two genomes and its formula is AA (Figure 1.5). This genome is basic to all polyploidy of wheat. *T. boeoticum* Boiss. (Figure 1.6), *T. urartu* Thum., *T. monococcum* ssp. *monococcum*, *T. sinskajae* are in this group. *T. urartu* Thum. and *T. boeoticum* Boiss. are wild forms of the group and there is no naked seed variety in the group except *T. sinskajae*. *T. boeoticum* Boiss. falls into two ecotypes as ones with a single fertile floret and ones with two fertile florets. To avoid confusion, the former entitled *T. boeoticum* ssp. *aegilopoides*, the latter is *T. boeoticum* ssp. *thaoudar*. *T. boeoticum* and *T. urartu* are genetically set apart due to the sterility of their hybrids, and can be distinguished through isoenzyme and storage protein differences (Miller, 1987). Cultivated *T. monococcum* ssp. *monococum* was most likely artificially selected from *T. boeoticum* ssp. *thaoudar* rather than *T. urartu* (Figure 1.6). *T. sinskajae* is probably a mutant form selected for its free-threshing characteristics (Miller, 1987). In Turkey, it is widespread in the foothills of Ağrı Mountain, the grassland of east and middle Anatolia (Kün, 1996). *T. monococcum* ssp. *monococcum* is first called einkorn,

successfully cultivated and domesticated from its wild diploid progenitor, but not *T. boeoticum* Boiss.

Einkorn is capable of adapted adverse environmental conditions. It has high nutritional value and gives acceptable yields on poor soils. Although it was widely cultivated in the beginning of agriculture, now, it is a relic crop and marginally cultivated in some provinces of Turkey (Bolu, Bilecik, Kastamonu, Çankırı, Sinop, Karabük), Italy, some Balkan countries, and Morocco (Serpen et al., 2008). The site of einkorn domestication was identified by 288 amplified fragment length polymorphism (AFLP) marker loci in einkorn and its wild ancestor.

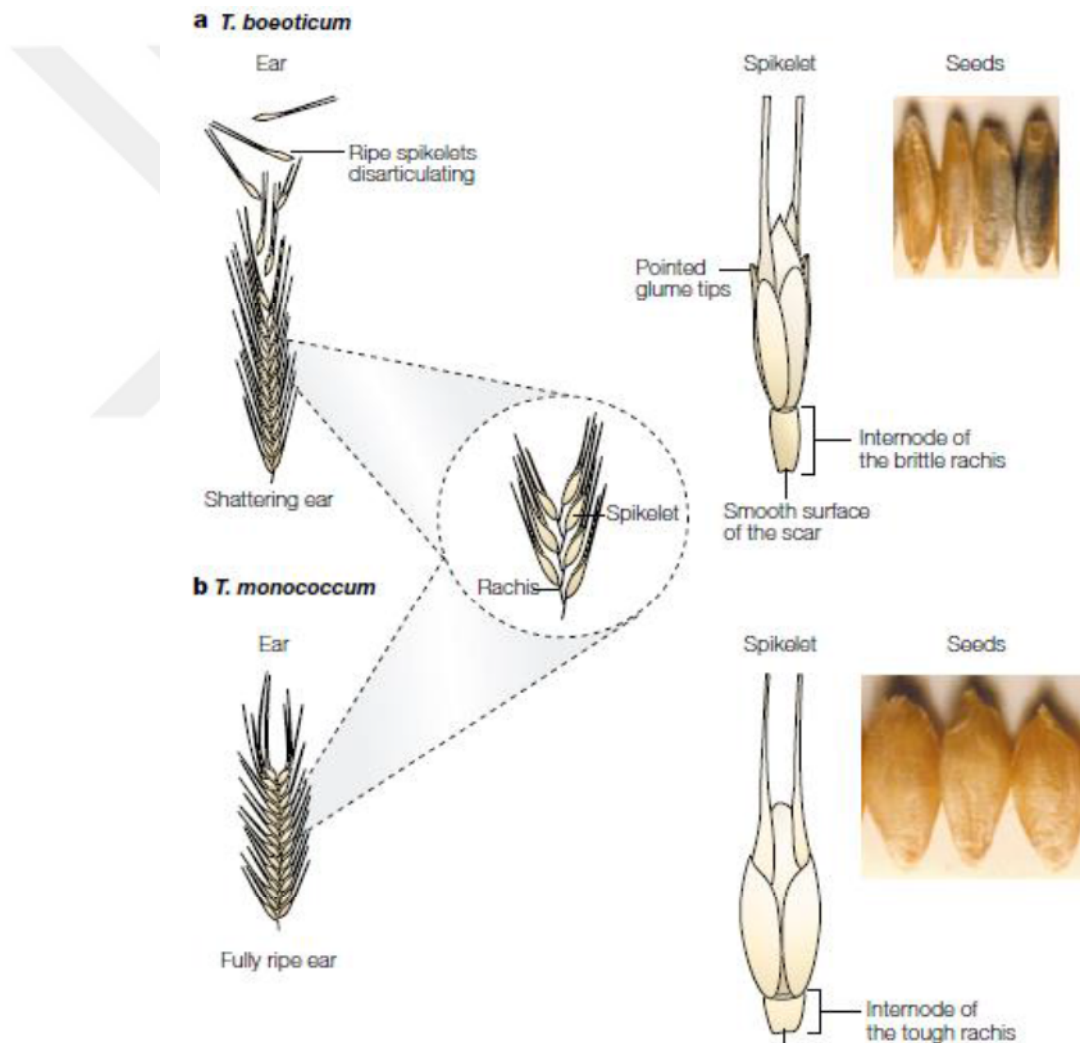


Figure 1.6. The morphological differences which distinguish between wild *T. boeoticum* Boiss. and *T. monococcum* ssp. *monococcum* (Salamini et al., 2002).

The results indicated that wild *T. boeoticum* Boiss. populations from the western foothills of the Karacadag mountain were closer to einkorn. Samples from

Abu Hureyra, Çafar Höyük, Çayönü, and Nevali Çori were also supported the evidence for einkorn domestication. *T. sinskajea*, the naked form of einkorn was isolated from a population collected by Zhukovsky in Turkey in 1970. It is characterized as short statue, soft glume, and compact, awnless ears (Kün, 1996).

1.1.1.3.2 Tetraploid wheat (4n=28)

This group has 4n=28 chromosomes, four genomes and two different formulas as A^uA^uBB and A^bA^bGG (Figure 1.5). It is thought that tetraploid wheat resulted in spontaneous hybridization of diploid wheat and one of Aegilops species. While some of the tetraploid wheat has its progenitor of A genome as *T. urartu*, *T. boeoticum* Boiss. is other progenitor. However, the progenitor of B genome; is challenging. Some tetraploid wheat has A^bA^bGG, where it is the progenitor of G genome is unknown though the progenitor of A genome is *T. boeoticum* Boiss.

T. dicoccoides, wild emmer with two homologous sets of chromosomes (A^uA^uBB) is the wild forms of the group. It has brittle ears that shatter at maturity into spikelets that confer relatively larger seeds (Figure 1.7). It has been found in southeast Anatolia, south of Toros Mountains, Turkey, Egypt, Jordan, and Palestine including regions across Fertile Crescent. Most scientists believe that it is resulted in most probably from spontaneous hybridizations. Two wild diploid kinds of grasses contributed: *T. urartu* (A^uA^u) was the pollen donor and an unknown species closely related to modern *Aegilops speltoides* (SS) was the the female for B genome.

T. dicoccum Schrank. (emmer) is the hulled cultivated form of wild emmer (Figure 1.7). Unlike wild emmer, it has non-brittle rachis. In Turkey, it is marginally cultivated in Kars, Ağrı, Artvin, Bolu and Kastamonu (Serpen et al., 2008).

One of the possible theories is that about 10.000 years ago mankind who cultivated wild emmer selected subconsciously and gave slowly rise to a cultivated emmer. Additionally, about 8,500 BP, natural mutation(s) changed the ears of emmer to a more easily threshed type which later evolved into the free-threshing ears of durum wheat (*T. turgidum* ssp. *durum* Desf, 2n = 4x = 28, genome AABB).

T. turgidum ssp. *durum* Desf. has naked grain and non-brittle rachis (Figure 1.7). Due to its high quality and yield, it is widely accounted for about 5% of world wheat production. Durum wheat is one of the final steps of *Triticum* domestication as well. Present-day modern wheat species which have a single origin are more closely related to wild emmer populations collected from Turkey (including basaltic rocky slopes of Karacadağ), Israel, Jordan, Lebanon, Syria, and Iran (Kün, 1996).

T. timopheevi is another wild form of the tetraploid wheat group. It, having A^bA^bGG genome, was first identified by Zhukovsky, which was collected in Caucasus and East Anotolia. Its A-genome progenitor is *T. boeoticum* Boiss., though, the progenitor of G genome has not been found yet. It is cultivated together with *T. diccicum* Schrank. and highly resistant to stripe rust, it is also, used in breeding studies, though its resistant properties were not transferred to its offsprings (Kün, 1996).

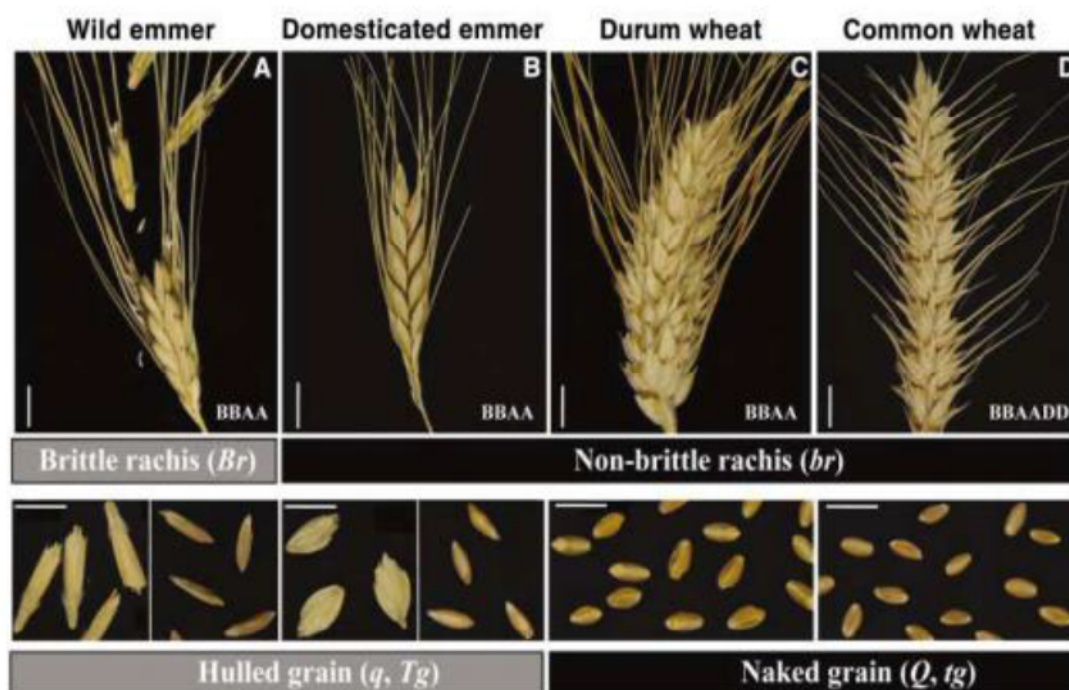


Figure 1.7. Wheat spikes: (A) *T. diccoides*, hulled grain and brittle rachis; (B) *T. diccicum* Schrank hulled and non-brittle rachis; (C) *T. turgidum* ssp. *durum* Desf., naked grain and non-brittle rachis; (D) *T. aestivum* L., naked grain and non-brittle rachis (Peng et al., 2011).

T. turgidum has adapted high rainfall. It is also called as English wheat due to its abundant cultivation in England. In Turkey, it is cultivated together with *T.*

turanicum in the coastal regions of East Black Sea, however, rare in the region of Aegean Sea (Kün, 1996).

T. polonicum has adapted high rainfall like *T. turgidum*. Even though, it firstly appeared in Poland and diffused to other parts of the regions so it is called *T. polonicum*, its origin is South Europe and Toros Mountains. Like *T. turgidum*, it is cultivated together with *T. turanicum*. Even if its thousand kernel weight is greater than 80 kg, its yield and quality are not as much as others (Kün, 1996).

T. carthlicum is found by Vavilov. Its origin is Oltu, Kars, and Caucasus. There are few cultivars in that region but it is not cultivated pure and there is a few economically importance (Kün, 1996).

1.1.1.3.3 Hexaploid wheat (6n=42)

This group has 6n=42 chromosomes, six genomes with the formula of A^uA^uBBDD (Figure 1.5). Hexaploid wheat group is another final step of wheat domestication. Like other wheat groups, this has two forms called as hulled and free-threshing. Moreover, there is no wild type in the group. *T. spelta*, *T. macha*, and *T. vavilovi* involve in hulled species of the group, *T. vulgare*, *T. compactum*, and *T. sphaerococcum* is found in free-threshing species (Kün, 1996).

For the understanding of the origin of present-day hexaploid wheat groups Mcfadden and Sears (1946) proposed a study which shows the progenitors, the tetraploid *T. turgidum* (A^uA^uBB) and the wild grass *Ae. tauschii* (DD), of the hexaploids. It is suspected that tetraploid wheat hybridized with the diploid species at unknown time and place, and it is resulted in hulled spelt-like hexaploid wheat in the past (Figure 1.8), but genetically distinction between European and Iran spelts attracted attention on this issue. In this sense, another study investigating allozyme variation in the A genome reported by Jaaska (1978) concluded that the European spelt originated independently on contrary to the Asian spelt. The European spelt could have emerged by introgressive hybridization of *T. aestivum* with *T. turgidum* ssp. *dicoccon*, whereas the Asian spelt could be the ancestral form of hexaploid wheat. *Aegilops tauschii* (the D genome progenitor) having four morphological

varieties, three of which (*typica*, *anathera*, and *meyeri*) are grouped into *Aegilops tauschii* ssp. *tauschii*, but *Aegilops tauschii* ssp. *strangulata* (Eig) Tzvelev is monotypic (having only one representative). Nishikawa et al. (1980) investigated isoenzyme variation and reported that *T. aestivum* corresponded better to the allelic spectrum in *Ae. tauschii* ssp. *strangulata* than to that in *Ae. tauschii* ssp. *tauschii*. Moreover, Dvorak et al. (1998) supported with similar results by the polymorphism in lengths of restriction fragments (RFLP) analyses. Subspecies *Ae. tauschii* are distributed from eastern Turkey to China and Pakistan whereas *Aegilops tauschii* ssp. *strangulata* occurred in southeastern Caspian Iran and Transcaucasia. The southern Caspian basin is suspected for this hybridization because *Ae. tauschii* ssp. *strangulata* still grows in this area and is considered to be the actual donor.

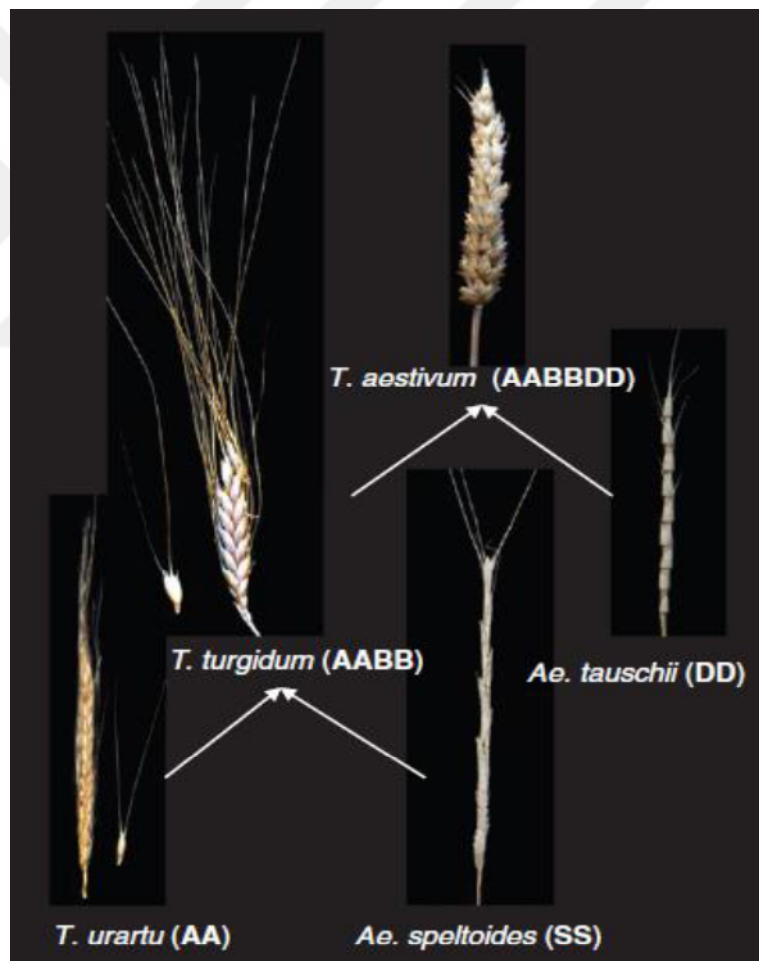


Figure 1.8. Evolution of hexploid wheat (Dvorak, 2013).

Some studies have been reported about the emergence of free-threshing hexaploid wheat but it still needs more researches regarding the scope and the consensus. The most important gene that is involved in the evolution of free-

threshing wheat is the Q factor, which is located on the long arm of chromosome 5A. The Q allele supports the formation of square-headed ears with good threshability. Moreover, it has a pleiotropic character which leads to being favorable to harvesting such as soften glumes, to reduce the length of ears, more spikelets per ear, toughen rachis. Mutation of Q homozygous gene (QQ) in free-threshing hexaploid wheat created morphologically similar with that of *T. spelta*. All free-threshing *T. durum* lines have the Q allele, whereas all hulled *T. dicoccum* or wild *T. dicoccoides* have recessive allele (q). When we use this information Q alleles might be emerged by mutation from hulled tetraploid wheat. Yet, several alleles of *Ae. tauschii* contributed to emerge of free-threshing wheat groups like Tg gene proposed as causing to tenacious glume by inhibiting the Q gene (Salamini et al., 2002). Thus, we can conclude that free-threshing character is not affected by alleles of a single gene, but those of several genes. Morphological characters of hexaploid wheat species are shown in Table 1.1.

T. aestivum ssp. *spelta* is the first cultivated hulled form of hexaploid wheat (Figure 1.9), which is still cultivated in the Middle Europe. Evidence points to that the spelta group is the oldest of the hexaploids, with the aestivum group having arisen from the spelta group by mutation of a single gene, and the *compactum* and *sphaerococcum* groups having arisen from aestivum similarly by the mutation of single genes (Kün, 1996).

T. aestivum ssp. *macha* is a new hulled subspecies which is found firstly in Eastern Anatolia. Its origin is north east Anatolia and Georgia. This subspecies was regarded a long time as *T. diccicum* due to the similarity of their spikes and general appearance. But, its spike is a little bit bigger than that of *T. diccicum* and their chromosome numbers are the basic difference (Kün, 1996).

T. aestivum ssp. *vavilovii* is first found around Van Lake and also a new species of the hexaploid group. It can branch out time to time and has white and black forms. In addition, it has resistance to rust diseases in its habitat, but in others, is not (Kün, 1996).

T. aestivum ssp. *vulgare* (*T. aestivum* L., bread wheat) is naked seed and free-threshing form of hexaploid wheat group, which is economically most important. It

has awned, awnless and short-awned varieties (Figure 1.9). Grain color includes white, red or amber. Glumes color can be white, black and brown. Moreover, bread wheat has spring, winter, and facultative forms (Kün, 1996).

Table 1.1. Taxonomic classification of hexaploid *Triticum* wheat species with specific morphological characters in parenthesis for each corresponding species (Zohary et al. 2000).

Modern grouping (biological species)	Traditional classification
Hexaploid (2n = 42) bread wheat Genomic constitution: AABBDD (the D genome contributor is <i>Aegilops tauschii</i>)	
Collective name: <i>T. aestivum</i> L.	
1. <i>T. aestivum</i> L. ssp. <i>spelta</i> (L.) Thell.	1. Spelt <i>T. spelta</i> L (non-brittle, hulled)
2. <i>T. aestivum</i> L ssp. <i>macha</i> (Dek. & Men.) MK	2. <i>T. macha</i> Dekr. & Men. (non-brittle, hulled)
3.-	3. <i>T. vaviloviiluman.</i> (non-brittle, hulled)
4. <i>T. aestivum</i> L. ssp. <i>aestivum</i>	4. Bread wheat <i>T. aestivum</i> L. [= <i>T. vulgare</i> Host; <i>T. sativum</i> Laro.] (free-threshing)
5. <i>T. aestivum</i> L. ssp. <i>compactum</i> (Host) MK	5. Club wheat <i>T. compactum</i> Host. [= <i>T. aestivo-compactum</i> Schiem.] (free-threshing)
6. <i>T. aestivum</i> L. ssp. <i>sphaerococcum</i> (Percival) Mk	6. Indian dwarf wheat <i>T. sphaerococcum</i> Pere, (free-threshing)
(i) Hexaploid (2n = 42) Zhukovsky's wheat Genomic constitution: AAAAGG Only domesticated forms	
1. <i>Triticum zhukovskyi</i> Men. & Er. (non-brittle, hulled)	

T. aestivum ssp. *compactum* is naked seed form of the hexaploid group (Figure 1.9). It was widely grown in central and east Anatolia and the first cultivar (Ak 702) of Turkey was selected from its populations. It can be yielded in slope lands having arid and weak yield potential. Today, it is still grown in Eskişehir province for good quality bread wheat (Kün, 1996).

T. aestivum. spp. *sphaerococcum* is economically grown in only India and Pakistan. Spike length is the shortest among the hexaploid group. Its flour yield and

protein content is quite high. It is also used in breeding programs for quality improvements (Kün, 1996).

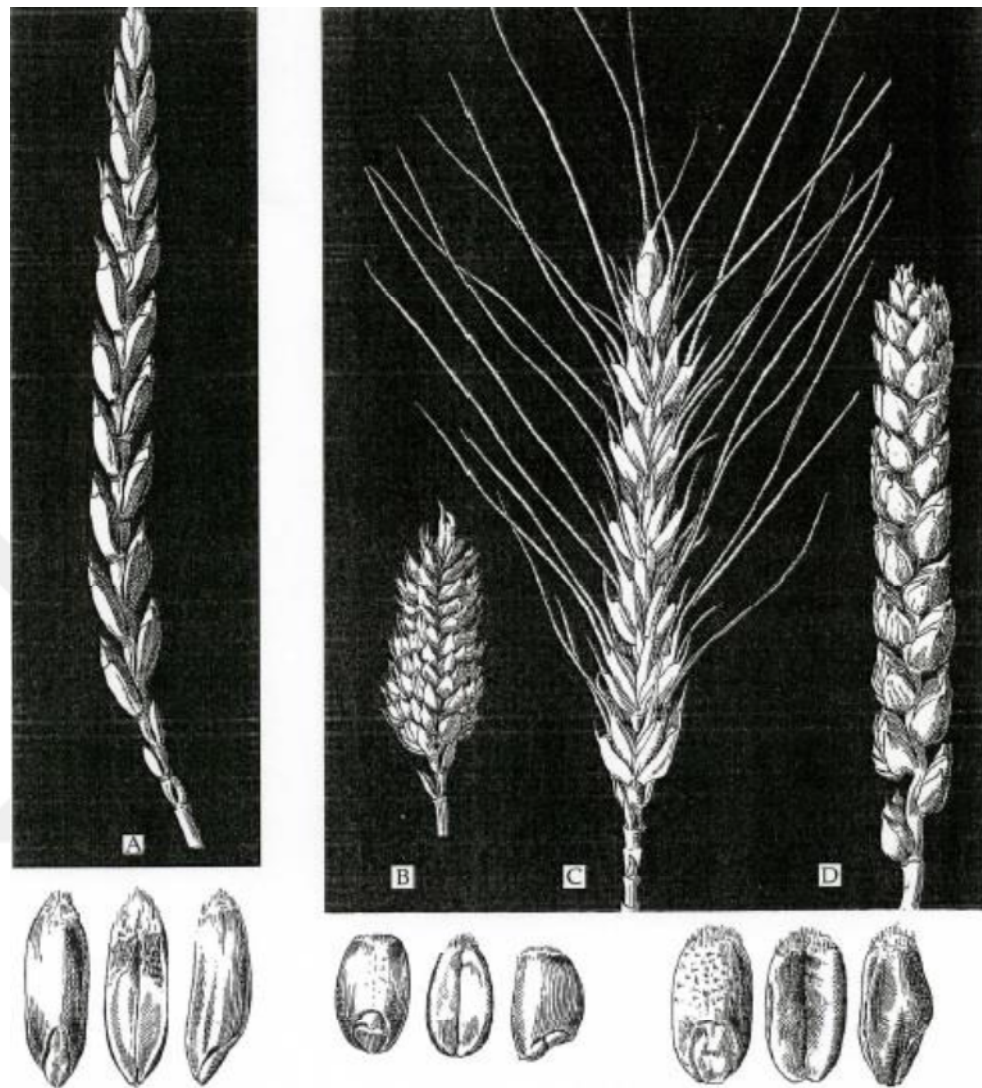


Figure 1.9. Hexaploid wheat species: (A) Ear and grain of *T. aestivum* ssp. *spelta*; (B) Ear and grain of *T. aestivum* ssp. *compactum*; (C) and (D) Ear and grain of awned and awnless *T. aestivum* (bread wheat) varieties (Zohary et al., 2000).

1.1.1.4 Wheat cultivation

Wheat (*Triticum* spp.) is cultivated for centuries in the Middle-East, central Asia, Europe, North-Africa, and America. It is a leading crop around the World. It processes into various products such as bread, pasta, animal feed and so on. Food and Agriculture Organization of United Nation reported that wheat production of Turkey,

some European and Central Asian country have reached between 44,059,346 and 107,826,196 tonnes in 2014 (Figure 1.10) (FAOSTAT, 2014).



Figure 1.10. Wheat production in the world (2014) adapted from (FAOSTAT, 2014).

Turkey has a major role among cereal producing countries in the world. Seventy five percent of Turkey's cropland is devoted to cereals. Wheat, barley, rice and maize are the main cereal species in Turkey. The sown area, production, and yield of wheat in Turkey (2004-2014) are shown in Table 1.2. According to the TÜİK, 9,300,000 hectares of total cereal cultivation area (TCCA) was devoted to the wheat cultivation (% 67) in 2014. Nowadays, TCCAs decreased from 13,832,585 to 11, 726, 526 hectares and, therefore, wheat cultivation areas decreased to 7,919,208 hectares in 2014. But the same percentage area (% 67) was still devoted to the wheat cultivation. Production of wheat has decreased from 34,153,910 to 32,714,157 tonnes, but the yield has increased from 2260 to 2400 kg/hectares between 2004 and 2014. In other words, wheat constantly occupies about 67% of the TCCAs and comprises approximately % 59 of total wheat production in 2014, respectively (TSI, 2016).

Wheat is almost grown in all geographic regions of Turkey, particularly in the Central Anatolia region. Most production of bread wheat is in Central Anatolia Region (36%) while most durum wheat production (46%) is in Southeast Anatolia region (TSI, 2016). Wheat is processed into a wide range of products. For example: simit- sesame seed coated rings of bread-, pide – leavened bread which is about 2-3

cm height and 20-25 cm width, yufka- a thin sheeth of dough used in preperation of sweets and savoury product (i.e. börek), flat bread- produced mainly in villages, bulgur, tarhana- a basic foodstuff made up of wheat flour and yogurt, dough is fermented and then, crumbled into small pieces (Braun et al., 2001).

Table 1.2. Total cereal cultivation area, bread and durum wheat sown area, production, and yield between 2004 and 2014 (TSI, 2016).

Year	Sown Area (hectares)			Production (tonnes)			Yield (kg/hectares)	
	TCCA ¹	Durum	Bread	TCP ²	Durum	Bread	Durum	Bread
2004	13 832 585	2 100 000	7 200 000	34 153 910	5 000 000	16 000 000	2 380	2 220
2005	13 893 241	2 000 000	7 250 000	36 471 600	4 500 000	17 000 000	2 250	2 340
2006	13 041 562	1 510 000	6 980 000	34 642 986	3 500 000	16 510 000	2 311	2 361
2007	12 403 039	1 354 500	6 743 200	29 256 990	2 709 000	14 525 000	2 000	2 150
2008	11 989 973	1 340 000	6 750 000	29 287 281	2 782 000	15 000 000	2 071	2 220
2009	12 067 708	1 335 000	6 765 000	33 577 151	3 740 000	16 860 000	2 800	2 490
2010	12 100 271	1 334 000	6 769 400	32 772 550	3 450 000	16 224 000	2 581	2 391
2011	11 903 435	1 338 000	6 758 000	35 202 073	3 850 000	17 950 000	2 871	2 651
2012	11 293 301	1 190 035	6 339 603	33 377 430	3 300 000	16 800 000	2 770	2 650
2013	11 540 322	1 278 600	6 494 000	37 489 268	4 075 000	17 975 000	3 190	2 770
2014	11 726 526	1 282 463	6 636 744	32 714 157	3 300 000	15 700 000	2 570	2 361

¹TCCA: Total cereal cultivated area.

²TCP: Total cereal production.

1.1.1.5 Biological activity, cardiovascular, diabetes, cancer studies in wheat

1.1.1.5.1 Biological activity

Yilmaz et al. (2015) analysed phenolic acid composition and total phenolic content (TPC) of ten diploid einkorns, one diploid *T. urartu* Tum., two tetraploids, and two hexaploid wheat species and accessions, determined their antioxidant activities via 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing ability of plasma (FRAP), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and N,N-dimethyl-1,4-diaminobenzene (DMPD), and , moreover, evaluated the effect of different extraction solvents buthanol, ethanol, methanol mixture, acetone, and water, and methanol and HCl mixture on antioxidant assays and bioactive compound extractions. They concluded that wild species of wheat had significantly higher phenolic acid (ranged 510-830 mg/kg DM) contents and TPC than other wheat species and accessions. Moreover, acidified methanol and butanol solvents

significantly affected the extraction of hydrophilic bioactives. There was, however, no significant differences between wheat species for DPPH scavenging activity ($p < 0.05$).

Martini et al. (2015) studied genetic and environmental factors on the total antioxidant activity and on the occurrence of certain antioxidant compounds in durum wheat the TPC of ten different durum wheat genotypes which were grown on three consecutive years in Italy. The mean value of TPC was 1061.4 ± 139.8 mg ferulic acid equivalent/kg dry matter (DM) and ranged from 856.6 (the crop year 2011) to 1464.0 mg ferulic acid equivalent/kg DM (the crop year 2010). As for phenolic acids, they were ranged between 752.9 and 1131.60 mg/kg DM. The mean yellow-colored pigments (YCPs) value was 5.47 ± 0.97 mg/kg DM, ranging from 3.91 to 7.47 mg/kg DM. total antioxidant capacity value was 45.34 ± 2.51 mmol trolox equivalent antioxidant capacity (TEAC)/kg DM, ranging from 40.48 to 51.10 mmol TEAC/kg DM. They concluded that crop year, genotype, growing area and their interactions significantly affected the variables and also, The content of Pas and TPC was mostly affected by environment, whereas YCP content and total antioxidant activity level were principally influenced by genetic factors.

Abozed et al. (2014) reported the TPC and DPPH activities of two different wheat, as common and durum, varieties extracted with acetone, methanol, and ethanol. The wheat species cultivated in two different locations and ranged between 1.05-1.11 mg gallic acid equivalent (GAE)/g. The results showed that the extraction solvents and genotype significantly affected the total phenolics and antioxidant activity of whole wheat and bran, and 50% acetone was recommended solvent for extracting phenolic compounds from the tested wheat and bran in the light of study, and, aside from these, bran fraction had more total phenolics and radical scavenging activities than whole wheat.

Žilić et al. (2013) determined that phenolic compounds and the total antioxidant capacity in the grain of ten bread (*T. aestivum* L.) and durum (*T. turgidum ssp. durum* Desf.) wheat genotypes. Wheat genotypes revealed that the cultivars TPC ranged from 0.99 to 1.60 catechin equivalent (CE)/g for bread wheat and ranged from 1.27 to 1.65 CE/g for durum wheat significantly different and total flavonoid content (TFC) of cultivars ranged from 0.027 mg CE/g of DM to 0.042 mg

CE/g of DM. Moreover, ferulic acid (0.175-0.027 $\mu\text{g/g}$) showed similar results and DPPH scavenging activity expressed as IC_{50} (8.82-11.60 mg).

Žilić et al. (2012) determined the distribution of phenolic compounds and YPC in wheat grains and their relation to the total antioxidant capacity (ABTS) of bran and debranned flour. TPC of five bread and durum wheat debranned flour was ranged from 0.53 mg GAE/g to 51.49 mg GAE/g and flavonoids, in five bread and durum varieties, were not detected in whole wheat flour. In contrast, that of the same five different bread and durum varieties in the bran samples was in the range from 173.59 mg CE/kg to 321.86 mg CE/kg. Ferulic acid (14.00-310.00 $\mu\text{g/g}$ for debranned and 9160.92-5502.05 $\mu\text{g/g}$ for bran fraction) and *p*-coumaric (1.00-7.34 $\mu\text{g/g}$ for debranned and 131.00-201.00 $\mu\text{g/g}$ for bran fraction) and ABTS activities (6.78-37.57 mmol Trolox/kg) were also reported as showing significantly differences between cultivars.

(Leoncini et al. 2012) profiled phenolic extracts of six bread wheat (one old and five modern) (*Triticum aestivum* L.) varieties grains and evaluated their antioxidant activities (FRAP and DPPH) and potential antiproliferative. TPC of six different modern and old wheat cultivars ranged from 133.25 to 170.78 mg GAE/100 g. Also, they revealed that bound phenolic content (BPC) of cultivars was greater than free phenolic content (FPC). TFC of the varieties ranged from 20.14 mg CE/100g to 67.69 mg CE/100g and revealed there was significant differences among old and modern varieties ($p < 0.05$). In contrast, DPPH scavenging activities were not significant between varieties with respect to bound phenolic extract, but free and no significant effect on anti-proliferative activities.

In a study performed by Okarter et al. (2010), which indicated the phytochemical content and antioxidant activities (oxygen radical absorbance capacity (ORAC)) of six different cultivars, ferulic acid was predominant substance and showed the total amount of ferulic acid ranged from 310.8 to 496.1 $\mu\text{mol}/100\text{g}$. The ratio of ferulic acids found in the bound phenolic content varied in the range of 87.4% to 97.2%. They also quantified the amount of syringic acid (8.7-14.6 $\mu\text{mol}/100\text{g}$ wheat DM), *p*-coumaric acid (33.5-52.3 $\mu\text{mol}/100\text{g}$ wheat DM), vanillic acid (6.0-8.3 $\mu\text{mol}/100\text{g}$ wheat DM), caffeic acid (4.4-11.6 $\mu\text{mol}/100\text{g}$ DM) alongside ferulic acid. The TPC of two spring and four winter wheats ranged from

841 to 1099 $\mu\text{mol GAE}/100\text{ g}$. Moreover, they profile α -tocopherol ranged from 0.7 to 5.2 $\mu\text{g/g}$.

In another study reported by Serpen et al. (2008), showing 18 ancient wheat (12 emmer, 6 einkorn) and 2 bread wheat varieties grown in different regions of Turkey were examined for their total phenolics and flavonoids, phenolic acids including ferulic and *p*-coumaric acid, lutein, total yellow pigment, and total radical scavenging capacities against ABTS cation. They revealed ferulic acid was predominant compound among phenolic acids followed by *p*-coumaric, *p*-OH benzoic acid and *o*-coumaric. Ferulic acid, *p*-coumaric acid, *p*-OH benzoic acid, and *o*-coumaric ranged between 232.66-775.30, 25.05-54.21, 4.24-9.41, and 1.28-3.80 $\mu\text{g/g}$ whole wheat. Ranged from 2.55 to 8.58 $\mu\text{mol GAE/g}$ of emmer, einkorn (collected from the same region -western Black sea), and bread wheat landraces reported by Serpen et al. (2008). The range of TFC of 12 different emmers was 1.06-2.29 $\mu\text{mol CE/g}$, of 6 different einkorn landraces were 0.80-1.59 $\mu\text{mol CE/g}$ and of 2 bread wheat cultivars were 1.32-1.35 $\mu\text{mol CE/g}$ (Serpen et al., 2008).

Moore et al. (2005) reveal ED_{50} values of eight different soft wheat varieties. Varieties ranged from 23.2 to 27.42 mg of grain equivalent/mL showed significant differences between durum wheat varieties. In addition, Moore et al. (2005) determined soluble-free, soluble-conjugated, and insoluble-bound ferulic acid profile of eight soft wheat cultivars grown in Maryland. The levels of soluble-free ferulic, vanilic, syringic, and *p*-coumaric were 31.95-47.22, 4.37-6.13, 5.79-13.01, and 0.90-1.58 $\mu\text{g/g}$ wheat, respectively, insoluble-bound ferulic acids, vanilic, syringic, and *p*-coumaric were 406.7-587.7, 2.9-5.0, 2.7-4.8, and 9.5-13.1 $\mu\text{g/g}$. Furthermore ferulic acid was mainly found in bound form and it accounts for about 85% among the phenolic acids. Moore et al. (2005) also showed that ferulic acid content varied between 455.92 to 621.47 $\mu\text{g/g}$, and vanilic, syringic, and *p*-coumaric were 8.44-12.68, 8.86-17.77, and 10.40-14.10 $\mu\text{g/g}$ wheat, respectively.

Iqbal et al. (2007) investigated antioxidant activity, TPC and TFC, chelating activity of bran extracts from five wheat varieties indigenous to Pakistan TPC of five hard winter red wheat varieties in bran fraction ranged from 2.12 mg GAE/g to 3.01 mg GAE/g. Iqbal et al. (2007) also revealed TFC of bran of five different hard winter wheat varieties. They were between 259.93 μg epicatechin equivalent/g and

303.76 μg epicatechin equivalent/g. Chelating activity, DPPH radical scavenging activity, ABTS radical cation scavenging activity were evident as ethylenediaminetetracetate equivalent 597–716 mg/g bran, 51-79%, Trolox equivalent 27–36 mmol/g, respectively. Tocopherols also revealed in the range of 22.15-26.06 ppm.

Adom et al. (2003) reported that TPC values of some durum and bread wheat varieties ranged between 7.09-8.59 μmol GAE/g. They concluded that phenolic compound was mainly found as in the bound form. (Lv et al. 2012) determined TPC of ten soft red winter wheat flour extract in the range of 1.66 mg GAE/g-2.00 mg GAE/g. Kim and Kim (2016), while studied the antioxidant and phytochemical content in immature and mature wheat kernels (cultivated in Korea) reported the TFC of mature ones was 2.34 mg CE/g and the other was 4.73 mg CE/g.

Swieca et al. (2014) evaluated some physical and technological (eg., protein and starch content), nutritional, and nutraceutical determinants. TFC of six different spelt wheat varieties ranged from 0.61 mg quercetin equivalent/g DM to 2.12 mg quercetin equivalent/g. The varieties were significantly different from each other for TFC, though TPC was not significant among varieties.

Kim et al. (2013) investigated the changes in antioxidant and antiproliferation activities of bread wheat, rye, barley, and oat before and after germination. They reported TFC of crops, for pre-germination, in the range between 0.28 to 0.90 mg catechin equivalent/g and, for post-germination, in the range between 0.57 to 0.92 mg CE/g (for wheat it was between 0.28 and 0.63, respectively). The highest TPC was in post-germination as 3.97 mg GAE/g, which was 1.61-fold higher than 2.46 mg GAE/g in pre-germination among crops. The DPPH free radical scavenging activity (expressed as IC_{50}) was the greatest in the post-germinated barley at 1.67 mg/mL. The highest ascorbic acid equivalent antioxidant capacity (7.43 mg ascorbic acid equivalent/g sample) was obtained from post germinated barley, while its pre-germination capacity was 5.44 mg ascorbic acid equivalent/g sample.

Dinelli et al. (2011) profiled the phenolic content of 16 old and 6 modern Italian wheat varieties, cropped in the same location and growing season. He reported TFC ranged between 872-1715 μmol CE/100g in grains were significantly

different and bound phenolic was accounted for 72% of TPC. As regards to TFC, the free fraction ranged from 50.7 to 106.1 $\mu\text{mol CE}/100\text{ g}$ of grain and the bound fraction from 78.3 to 148.9 $\mu\text{mol CE}/100\text{ g}$ of grain ($p < 0.05$).

Lacko-Bartošová et al. (2013) evaluated the free flavonoid content and the antioxidant properties of wheat white flour, wheat grain flour, and bran in integrated farming systems. The results revealed that antioxidant activity of wheat fractions ranged between 23.26%, 49.57%, and 76.47%, respectively. The free flavonoid content and antioxidant activity of fractions were not affected by farming systems.

In a study reported by Benincasa et al. (2015), bound and free ferulic acid and *p*-coumaric contents of one einkorn, three emmer, two spelt, one durum, and one soft wheat species and cultivars ranged , for bound ferulic acid, from 303.10 $\mu\text{g/g}$ to 225.7 $\mu\text{g/g}$ and from 0.91 $\mu\text{g/g}$ to 0.33 $\mu\text{g/g}$ for free ferulic acid. Hernandez et al. (2011) reported phenolic acid, including ferulic acid and *p*-coumaric acid, the content of nineteen different cultivars composed of bread and durum wheat, ranged higher for ferulic acid from 439.0 $\mu\text{g/g}$ to 1450.0 $\mu\text{g/g}$ and from 10.1 $\mu\text{g/g}$ to 29.40 $\mu\text{g/g}$.

Brandolini et al. (2013) reported the content of phenolic acids in different *Triticum* species as conjugated and bound forms, which were managed in different years (2008-2011). The conjugated phenolic acids ranged between 36.0 to 52.6 mg/kg DM. Soluble-conjugated ferulic acid, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, and syringaldehyde concentrations ranged as 21.0-34.1, 2.2-4.0, 4.8-7.0, 2.7-4.6, 1.5-3.7, and 0.8-1.4 mg/kg DM for cultivated species in 2008 and 36.1-25.0, 1.6-3.7, 7.6-6.0, 7.2-4.4, 1.6-4.6, and 0.9-1.5 mg/kg DM for cultivated species in 2011, respectively. Bound-insoluble ferulic acid, *p*-hydroxybenzoic, vanillic, syringic, *p*-coumaric, and caffeic acid concentrations were 420-679, 1.0-2.8, 5.6-3.1, 1.9-4.9, 13.7-27.9, and 0.3-2.4 mg/kg for those cultivated in 2008 and 495-578, 1.0-2.4, 2.6-5.4, 2.4-4.9, 14.32.8, and 1.1-5.8 mg/kg for those cultivated in 2011. Phytochemical component differences existed between bound and conjugated composition.

Wang et al. (2013) indicated that distribution of phenolic acids in wheat flour produced from five milling different extraction rate ranging from 60% to 100% in four cultivars sown in two locations in different years (2008-2009). The

concentration of free phenolic acids was 17.0 $\mu\text{g/g}$ of DM. Syringic and ferulic accounted for 55.9% and 34.6% of total amount, respectively. The amount of bound phenolic acids was 262.0 $\mu\text{g/g}$, which was accounted for 52.0% of ferulic acid, while caffeic and *p*-coumaric was accounted for 24.3% and 18.1%.

Chen et al. (2013) reported that the phytochemical content and components of wheat fractions including whole wheat, bran, and aleurone. Wheat samples were first fractionated by abrasive dehulling and then their TPC, oxygen radical absorbance capacity (ORAC), and DPPH were calculated. The method they used failed to separate α -tocopherol from α -tocotrienol. Hence they expressed the results as α -tocopherol + α -tocotrienol for whole wheat, bran, and aleurone, which ranged from 3.0, 3.8, and 6.6 $\mu\text{g/g}$, respectively.

Lu et al. (2015) studied the effect of growing environment, genotype, and their interactions on phytochemical compositions and radical scavenging properties of ten soft winter wheat brans. Wheat samples in the study were cultivated in four different regions. Range for α -tocopherol was between 2.58 $\mu\text{g/g}$ and 10.56 $\mu\text{g/g}$ and δ -tocopherol was between 0.05 $\mu\text{g/g}$ and 0.24 $\mu\text{g/g}$. They also revealed that environment which wheat cultivars were grown had the strongest influence on phytochemical compositions investigated in the study: TPC, total soluble ferulic acid, DPPH, and ABTS.

Labuschagne et al. (2014) determined that tocopherol and tocotrienol concentrations in white flour of 10 South African wheat cultivars grown in 3 environments. Across these three locations, the α -tocopherol concentration varied between 0.48 and 1.21 mg/kg, the β -tocopherols between 0.48 and 1.33 mg/kg, the α -tocotrienol between 0.15 and 0.46 mg/kg, and the β -tocotrienol between 3.63 and 8.73 mg/kg. In the same study, they showed that α and β -tocopherol and α and β – tocotrienol contents were strongly influenced by genotype.

Lu et al. (2014) reported that phytochemical compositions including ferulic acid, tocopherols, and carotenoids, and antiproliferative activities of bran samples of 10 soft winter wheat varieties grown in Maryland. Ferulic acid was the predominant phenolic acid in all 10 wheat bran samples with concentration ranging between 1.1

and 1.7 mg/g. A significant amount of α -tocopherol (2.3–5.3 mg/g) was found in all bran samples along with a minor quantity of δ -tocopherol (0.1 mg/g).

In a study, Hejtmánková et al. (2010), with two einkorn, two emmer, and three spring wheat cultivars (ranged between 5.69–8.57 mg/kg DM) reported significant differences among species and cultivars. Einkorn, emmer, and soft wheat cultivars (Abdel-Aal and Rabalski, 2008), ranged for α -tocopherol between 5.50–11.90 μ g/g of α -tocopherol. (Brandolini et al., 2015) reported similarly that α -tocopherol content of einkorn, emmer, durum, spelt, and bread wheat ranged between 9.90 to 15.00 mg/kg DM and found significant differences among species.

1.1.1.5.2 Cardiovascular Disease

Cardiovascular disease (CVD) is the one of the major cause of deaths in Turkey and includes heart, stroke, and blood vessels diseases. Some studies have shown that fibre consumption from whole grains and bran are inversely associated with a 21% lower risk of CVD in 3588 elderly men and women over 65 years of age (Mozaffarian et al., 2003). Supplementation of wheat fibre (21 g/day), as well as a high fibre diet was shown to reduce cardiovascular risk in 42 patients with dysmetabolic cardiovascular syndrome by significantly decreasing systolic and diastolic blood pressure, reducing fasting glucose, total cholesterol, low-density and high-density lipoprotein cholesterol. Fibre, vitamin E, vitamin B 6, minerals (calcium, magnesium, potassium, phosphorus, selenium, manganese, zinc and iron), antioxidants and phytoestrogens which existed in bran and germ layers were believed to be important. A study showed whether a whole-grain wheat-based diet may increase short chain fatty acid (SCFA) (derived from dietary fiber fermentation by gut microbiota) concentration, and identified possible associations between SCFAs and metabolic changes observed after the nutritional intervention. A 12 week whole-grain wheat-based diet increased fasting plasma propionate. This increase was correlated with the cereal fiber intake and was associated with lower postprandial insulin concentrations (Vetrani et al., 2015). In another study conducted on whether a replacement diet, thereby, substituting all other cereal grains, with products made with organic Khorasan wheat cultivar, an ancient wheat, could provide additive

protective effects in reducing lipid, oxidative and inflammatory risk factors. Consumption of products made with Khorasan wheat resulted in a significant amelioration in total cholesterol (−6.8%), low-density lipoprotein cholesterol (LDL-C) (−8.1%), glucose (−8%), and insulin (−24.6%) from baseline levels, independently of age, sex, traditional risk factors, medication and diet quality. Moreover, replacement of diet with nutrition product made from ancient wheat provided additional protection in the patient (Whittaker et al., 2015).

1.1.1.5.3 Diabetes

There are three main forms of diabetes: Type 1, Type 2, and Gestational. Type 1 or insulin dependent diabetes is due to autoimmune destruction of the pancreatic β -cells, which produce insulin. Type 2 or non-insulin dependent diabetes is due to a combination of defective insulin secretion and insulin resistance, which is characterized by elevated levels of insulin in the blood and, which may progress to loss of β -cell function. Gestational diabetes is similar to Type 2 diabetes where the hormones of pregnancy cause insulin resistance. Rare forms of diabetes not categorized into Type 1, 2, or Gestational diabetes include genetic defects in β cells (autosomal or mitochondrial), genetic abnormalities related to insulin resistance (with or without lipodystrophy or abnormal fat deposition), diseases of the pancreas (e.g. chronic pancreatitis, cystic fibrosis), hormonal defects and chemical or drug related diabetes. Type 1 diabetes requires regular monitoring of blood glucose levels and is treated with replacement of insulin by means of injections several times a day, along with a balanced eating plan and exercise. Type 2 diabetes is treated firstly with a lifestyle change, increasing physical exercise, decreasing carbohydrate intake and weight reduction.

Some studies have been carried out with respect to consumption of wheat and diabetes. Liu (2003) indicated a 27% reduced risk of Type 2 diabetes when consuming three serves of wholegrain foods daily. Stay-green wheat (*Triticum aestivum* L.) (SGW), which is called “Lvfheng 1”, is a mutant type of the common wheat, with delayed leaf senescence because of an extended duration of the active photosynthesis during the grain-filling period Zhang et al. (2013). In a study

indicated by Ji et al. (2015) revealed that SGW components exerting a glycemic control and a serum lipid regulation effect may be due to their free radical scavenging capacities to reduce the risk of type 2 diabetes mellitus in experimental diabetic rats. The effects of a 12-week intervention with either a whole-grain-based or a refined cereal-based diet on postprandial glucose, insulin and lipid metabolism in individuals with metabolic syndrome whole-grain cereals on postprandial glucose, insulin and lipid metabolism were conducted by Giacco et al. (2014). They found a twelve week whole-grain cereal-based diet, compared to refined cereals, reduced postprandial insulin and triglycerides responses having implications for type 2 diabetes risk and cardiovascular disease.

1.1.1.5.4 Cancer

Cancer is one of the leading killers in Turkey. In 2014, cardiovascular diseases were followed by cancer (benign and malignant tumors), which caused deaths of 77587. Compared to 2013, these figures have risen from 76534 mortalities (TSI 2016). Environment, diet, and genetics play a significant role on cancer risk. Recent studies have shown that certain food bioactive compounds have reasonable anti-carcinogenic properties. These include phytoestrogens, phytosterols, and phenols. (Islam and Gallaher, 2015) examined the influence of wheat on colon cancer risk in early and late post initiation stage by carcinogen-treated rats, which were fed with red and white wheat. They found that both red and white wheat significantly reduced morphological [aberrant crypt foci (ACF)] and biochemical (b-catenin accumulated crypts) markers in both early and late post initiation stages and, wheat diets, also, reduced dysplasia markers (sialomucin-expressing ACF and mucin depleted foci) during the late post initiation stage. Furthermore, they concluded that red wheat flour reduced risk markers more than white wheat flour and effect of different wheat types varied for these markers. (Lu et al. (2014) conducted a study on anti-proliferative activities of bran fraction of ten Maryland-grown soft winter wheat cultivars. They found that the Jamestown wheat bran, one of bread wheat cultivars, exhibited inhibition of HT-29 cancer cells, by 28.58% at 50 mg bran equivalents (BE)/mL but not other cultivars. Hence, it was possible to conclude that not only wheat species but also wheat cultivars could show different anti-cancerogenic

activity. Furthermore, in the same study, Caco-2 cells treated with 10.0 and 50.0 mg BE/mL concentrations of wheat bran extracts. At 10.0 mg BE/mL concentration, the Jamestown wheat bran inhibited 31% of the cells. When treated at 50 mg BE/mL concentration, Jamestown wheat bran demonstrated an inhibition potential: 78% of cells were inhibited, as well others to some extent, too. A study indicated by Zhang et al. (2015) on fermented wheat germ extract produced with *Lactobacillus plantarum* dy-1 decreased tumor weight and increased cell apoptosis rate of mice, which were treated with wheat germ, than control group.

1.2 Bioactive Compounds

1.2.1 Phenols

Phenols has a vast number of molecules in secondary plant metabolites, which provide essential functions in the reproduction and the growth of the plants; acting as defense mechanisms against pathogens, parasites, and predators, as well as contributing to the color of plants. In addition to their roles in plants, phenolic compounds play a role in our diet. As the hydroxyl group is always attached to a benzene ring, they are aromatic alcohols. Simple phenols consist of an aromatic ring in which a hydrogen is replaced by a hydroxyl group (Figure 1.11). They are categorized as phenolic acids, flavonoids, stilbenes, coumarins, and tannins (Figure 1.12).

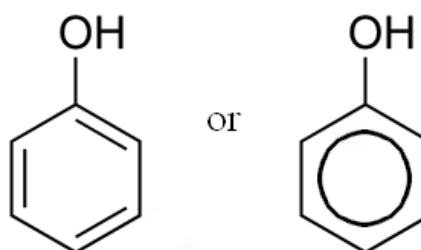


Figure 1.11. Basic structure of phenol compounds.

Phenolic acids are produced in the phenylpropanoid pathway in plants (Figure 1.13) and they are mostly found in the bran layers of wheat (Verma et al., 2009). The phenolic compounds in wheat grain composed of one aromatic ring such as vanilic

acid, sinapic acid, *p*-coumaric acid, ferulic acid, alkylresorcinols, and vitamin E. Phenolic compounds show antioxidant activity by different multi-faceted antioxidant mechanisms. The hydroxyl group of the phenolic ring donates one electron to the radical molecule, which is followed by a proton transfer. That is, the phenol gives electron and thus it is oxidized. Ferulic acid occurs in the trans isomer form and is bounded by ester binding to cell wall polysaccharides in wheat (Adom et al., 2003).

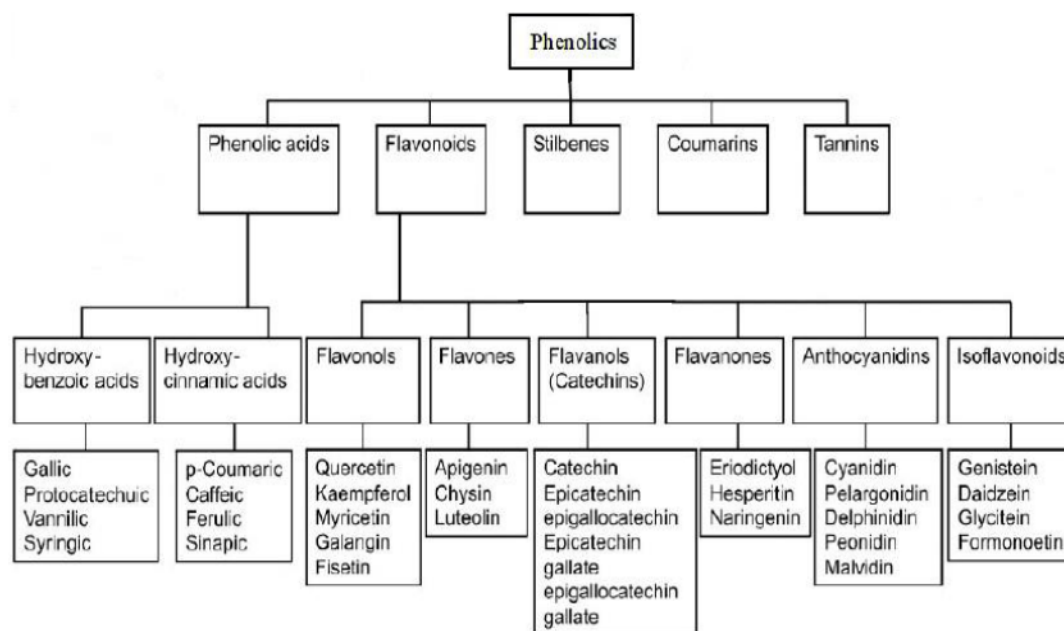


Figure 1.12. Classification of phytochemicals.

The antioxidant potential of ferulic acid is mainly attributed to the electron donation and hydrogen atom transfer to free radicals (Graf, 1992). Its ability to inhibit lipid peroxidation by superoxide scavenging is of greater magnitude than that of cinnamic acid but less than that of caffeic acid (Toda et al., 1991), moreover, oxidation of LDL, the main cholesterol carrier in blood, is greater than that of ascorbic acid (Castelluccio et al., 1996).

The phenylpropanoid pathway is illustrated in detail in Figure 1.13. To explain it briefly, one ammonia molecule is removed from phenylalanine or tyrosine by non-oxidative deamination catalyzed by phenylalanine ammonia lyase (PAL) or tyrosine ammonia lyase (TAL), producing *trans*-cinnamic acid. *Trans*-cinnamic acid is hydroxylated by cinnamic acid hydroxylase to *p*-coumaric acid and from that point on other phenolic compounds such as simple phenolic acids, lignins, flavonoids and coumarins are metabolized by several condensation and modification reactions.

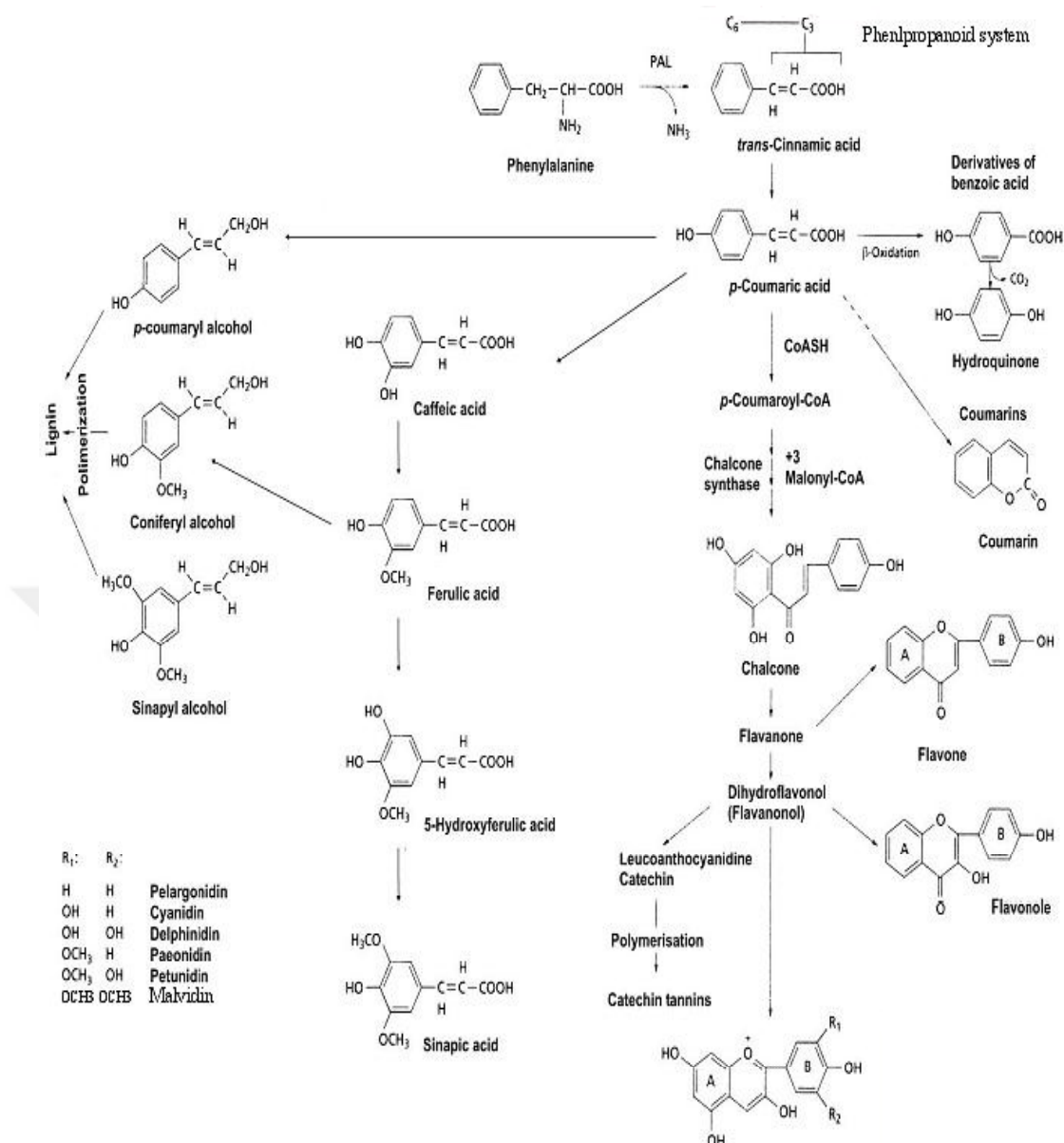


Figure 1.13. Phenylpropanoid pathway.

1.2.2 Flavonoids

Flavonoids are the first class of polyphenols, which are water-soluble pigments located in the vacuoles. These compounds are widely distributed in the plant cells and perform many functions. For example, they are involved in UV filtration, symbiotic nitrogen fixation, floral pigmentation, chemical messengers, physiological regulators, and so on.

Differences in the generic structure of the heterocycle C ring classify them as flavonols, flavones, flavanols (catechins), flavanones, anthocyanidins, and

isoflavonoids (Figure 1.14). Flavonols (quercetin, kaempferol, and myricetin), flavones (luteolin and apigenin), flavanols (catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate), flavanones (naringenin), anthocyanidins, and isoflavonoids (genistein) are common flavonoids in the diet. Flavonoids are most frequently found in nature as conjugates in glycosylated or esterified forms but might occur as aglycones, especially as a result of the effects of food processing.

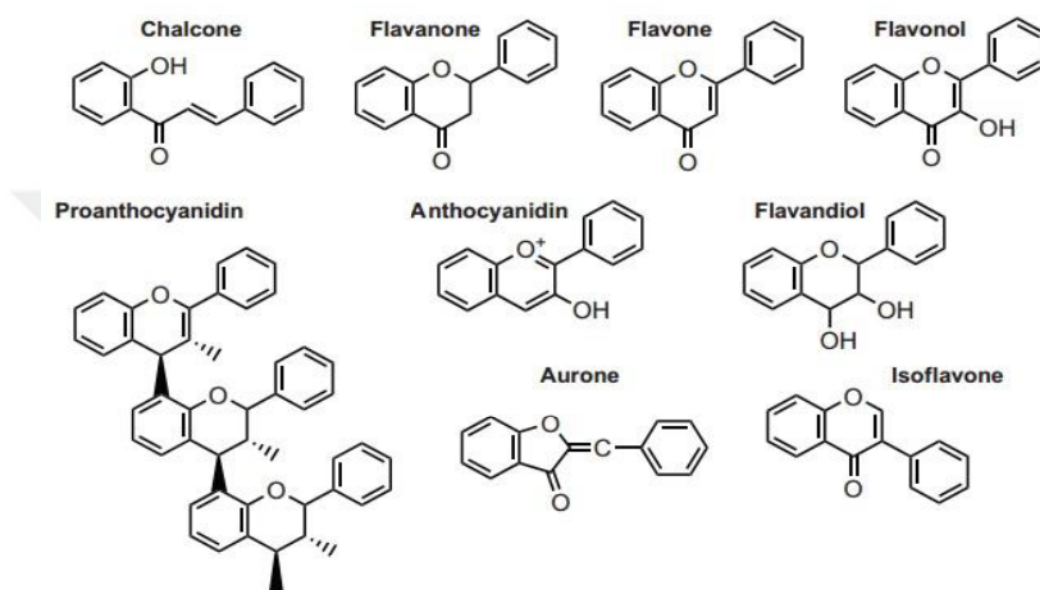


Figure 1.14. Some flavonoids and their molecule structure.

Flavonoids are synthesized, like all phenolics, by the phenylpropanoid pathway (Figure 1.13), converting phenylalanine into 4-coumaroyl-CoA, thus entering the flavonoid biosynthesis pathway. They are very popular because of their health benefit. Some of their activities are anti-cancer, antioxidant, anti-viral, and anti-inflammatory. For example, quercetin, which is one of the flavonoids, has the ability to relieve high fever, eczema, asthma and sinusitis. In addition, some studies have revealed that heart disease is inversely related to intake of flavonoids (Kabera et al., 2014).

1.2.3 Vitamin E

Vitamin E is the collective name for a set of eight related compounds or vitamers: α -, β -, γ -, and δ -tocopherols and the corresponding four tocotrienols.

Vitamin E is lipophilic and, therefore, is primarily found in the wheat germ. Despite the fact that all the different forms of vitamin E have similar antioxidant activity, α -tocopherol is preferentially maintained in plasma. This is due to (i) the specific binding to the α -tocopherol transfer protein and (ii) the extensive hepatic metabolism of the other vitamers (Traber and Atkinson, 2007).

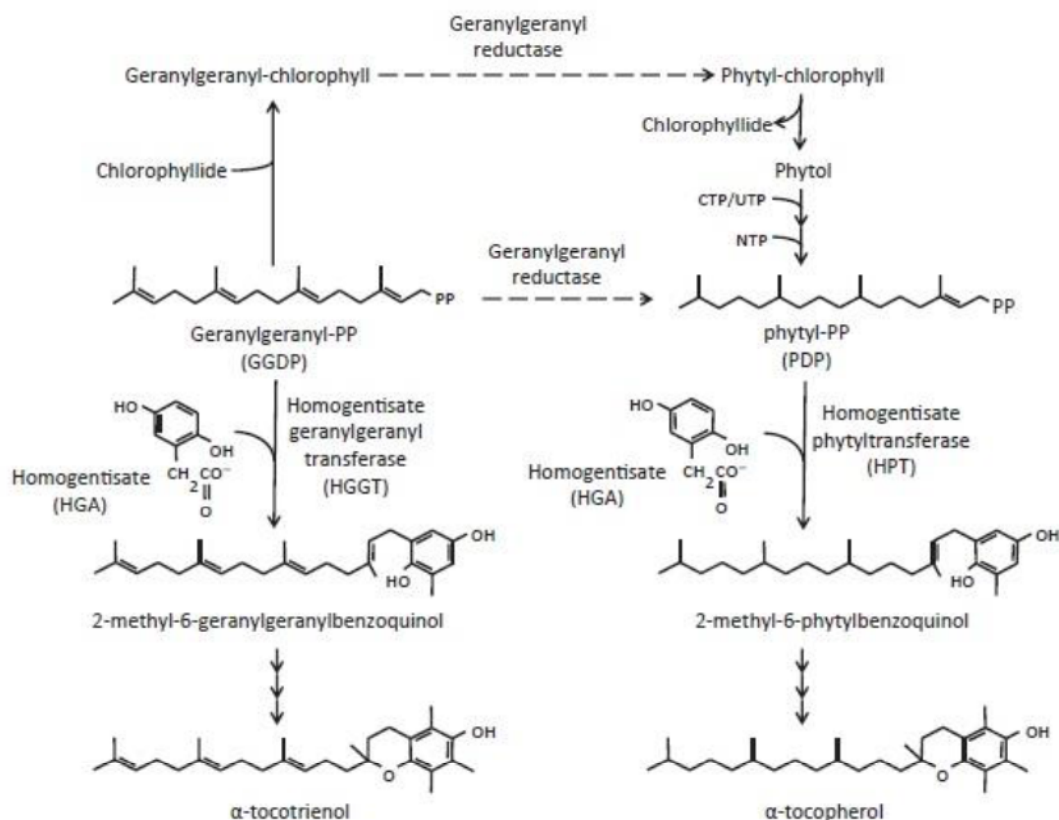


Figure 1.15: Biosynthesis of tocopherol and tocotrienol (Yang et al., 2011).

The biosynthesis of tocopherols occurs in plastids of plant cells. The initial step in tocopherol biosynthesis is the condensation of homogentisate and phytol diphosphate (PDP) to form 2-methyl-6-phytylbenzoquinol (Figure 1.15). This reaction is catalyzed by homogentisate phytoltransferase (HPT). For the synthesis of α -tocopherol, the initial HPT-catalyzed condensation reaction is followed by methylation, cyclization to form the chromanol head group and a second methylation (Yang et al., 2011).

The α -tocopherol molecule consists of a chroman head, which is responsible for the antioxidant function, and a phytol chain that intercalates with the phospholipids of the cell membrane. The free hydroxyl group on the aromatic ring is responsible for the antioxidant properties. The hydrogen from this group is donated

to the free radical, resulting in a relatively stable free radical form of vitamin E (Sies and Murphy, 1991). In this way, vitamin E molecules can interrupt free-radical chain reactions. Vitamin E also has protective effects on glutathione-dependent enzymes (Haaften et al., 2003). The function of vitamin E in the human body has been recently reviewed, the major function appeared to be as radical scavenger protecting the polyunsaturated fatty acids from oxidation, thereby, maintaining the integrity of the cell membrane.

1.3 Plant Extraction, Antioxidant Activity, and HPLC Analyses of the Extracts

1.3.1 Plant Extraction

Secondary metabolites are organic molecules that have no direct function in the normal growth and development of the plants. While primary metabolites have a key role in the survival of the species, playing an active function in the photosynthesis and respiration, absence of secondary metabolites does not result in an immediate death (Taiz and Zeiger, 2012). Plant secondary metabolites are usually classified according to their biosynthetic pathways. Three main groups of secondary metabolites are generally considered: terpenes, phenolic compounds and nitrogen containing compounds (Bourgau et al., 2001). Phenolic compounds (phenolics, flavonoids, tannins, quinones etc) have possessing one or more aromatic rings with one or more hydroxyl groups. Plant phenolics generally serve as a defense against ultraviolet radiation or aggression by pathogens, parasites and predators. Phenolics can be classified into two groups: the flavonoids and the non-flavonoids (Dai and Mumper, 2010). Terpenoids (steroids, carotenoids, volatile oils, diterpenoids etc) are one of most diverse classes of the secondary products. Terpenoids are the largest and most diverse family of natural products.

Due to their large biological activities, plant secondary metabolites have been used for centuries in traditional medicine. Nowadays, they correspond to valuable compounds such as pharmaceuticals, cosmetics, fine chemicals, or more recently nutraceuticals (Kabera et al., 2014). Nitrogen containing compounds (purines,

alkaloids, chlorophylls etc.) present the group of secondary metabolites that primarily composed of basic nitrogen atoms and widely used in medicine. They can also be highly toxic. Various natural alkaloids (taxol, reserpine and morphine) and their derivatives have been used to treat various diseases (Harborne, 1998).

For the separation of these medicinally active compounds in the plants, selective solvents and standard extraction procedure should be used. Bioactive phytochemicals from the plant materials are influenced by their chemical nature, sample particle size, employed extraction method, the type of solvents, extraction time, and temperature.

1.3.2 Antioxidant Activity

For many years, plants and their products have been used as therapeutic resources since human has existed on earth. According to the World Health Organization (WHO), 75% of people still rely on plant-based traditional medicine (Starbuck, 1999). In recent years, there has been growing interest in use of natural products from plants due to inefficient of conventional drugs by side effect or ineffective therapy (WHO, 2002). The number of higher plant species is estimated 250.000-500.000. It is estimated that a small percentage of plant species (6%) has been investigated as phytochemically and only a smaller percentage has been poorly studied for their pharmacological properties (Fabricant and Farnsworth, 2001). Plants have been rich sources of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defenses against infection (Cox, 1994). Bioassays are adaptable for the purpose of screening and testing plant extracts. Bioassays are used to determine the biological activity (e.g. antibacterial, antifungal, antioxidant, anticancer etc.) of botanical products (McLaughlin et al., 1998).

Antioxidants are substances capable to stabilize or deactivate free radicals and prevent them from causing cell damage. Antioxidants are absolutely critical for maintaining optimal cellular and systemic health and well-being (Moon and Shibamoto, 2009).

Oxygen is highly reactive atom that is capable of damaging molecules commonly called free radicals. Oxygen free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS) are products of normal physiologic events. They have a dual role as being both deleterious and beneficial to living systems. They become dangerous when produced in the body along with toxins and wastes. ROS is continuously produced during the body's normal use of oxygen such as respiration. In other words, the body obtained energy by the oxidation of carbohydrates, fats and proteins through both aerobic and anaerobic process which leads the formation of free radicals (Gülçin et al., 2006). Free radicals are highly unstable molecules that have one or more unpaired electrons. They are capable of attacking healthy cells of the body and causing them to lose their structure and function. Free radicals can cause “oxidative stress,” a process that can trigger cell damage. Oxidative stress is thought to play a role in a variety of diseases including cancer, aging, cardiovascular diseases, diabetes, Alzheimer’s disease, Parkinson’s disease, and eye diseases such as cataracts (Aruoma, 1994).

Antioxidant compounds have been identified as free radical or active oxygen scavengers which retard the progress of many chronic diseases as well as lipid peroxidation. All aerobic organisms have antioxidant defenses, including antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxide) and antioxidant food constituents to neutralize free radicals and protect the body from different diseases. The most commonly used antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate, and tert-butyl hydroquinone at the present time. However, BHA and BHT have been restricted due to their side effects such as carcinogenicity. Therefore, there is a growing interest on naturally occurring antioxidants from especially plants instead of synthetic ones in recent years (Gülçin et al., 2006).

A wide variety of phenolic compounds are synthesized in plant tissues during normal growth and development. Plant phenolics contain polyphenolic compounds, flavonoids, chlorophylls, carotenoids, tocopherol derivatives and lignan. The basic structural feature of phenolic compounds is an aromatic ring bearing one or more hydroxygroups (Liyana-Pathirana and Shahidi, 2006; Gülçin et al., 2006). It is well known phenolic compounds exhibited have a wide range of biological effects,

including antibacterial, antiviral, anti-inflammatory, anti-allergic, antithrombotic, and vasodilatory activity. Their broad therapeutic effects can be largely attributed to their antioxidant properties (Cook, 1996; Liyana-Pathirana and Shahidi, 2006).

1.3.3 HPLC Analysis of Wheat Extracts for Phenolic Compounds

In earlier times, many separation techniques were used for phenolic compounds. These were thin-layer chromatography (TLC), preparative TLC, column chromatography, and flash column chromatography. TLC is the most widely used one for monitoring reactions. Although TLC can be used to help determine the number of components in a mixture and purity of a compound, the quantity of material in TLC cannot be estimated visually. However, liquid chromatographic techniques (liquid chromatography (LC), High-Performance Liquid Chromatography (HPLC)) and hyphenated (online-combination) techniques (HPLC- photo-diode array (PDA), LC-mass spectrometry (MS), gas chromatography (GC)-MS, LC-nuclear magnetic resonance (NMR), LC-MS/MS) work for the separation of chemicals by qualitatively and quantitatively. However, HPLC is the most popular technique for the separation of phenolic compounds, both on preparative and analytical scales. Improvements in instrumentation, packing materials, and column technology are introduced all the time, which makes the technique more and more attractive (Andersen and Markham, 2006).

LC was invented by a Russian botanist, Mikhail S. Tswett. His studies concentrated on splitting up compounds, extracted from plants using a solvent, in a column. As the sample moves through the column by gravity, different colored bands could be observed separating because some components were moving faster than other bands. He explained these separated, different-colored bands to the different compounds. He had created an analytical separation of these compounds based on the differing strength of each compound's chemical attraction to the particles. The compounds were more strongly attracted to the particles, while other compounds more strongly attracted to the solvent. This process can be described as the compounds contained in the sample distribute, or partition differently between the moving solvent, called the mobile phase, and the particles, called the stationary

phase. This causes each compound to move at a different speed, thus creating a separation of the compounds.

The basic component of HPLC is shown in Figure 1.16. A reservoir holds the solvent called the mobile phase because it moves. A high-pressure pump transporting the solvent is used to create specified flow rate of the mobile phase. An injector is able to inject the sample into the mobile phase stream that carries the sample into the HPLC column by flowing continuously. The column contains the chromatographic packing material, contributing to separation. This packing material is called the stationary phase because it is held in place by the column hardware. A detector is needed to see the separated compound, wired to computer data station that records the electrical signal and generates the chromatogram.

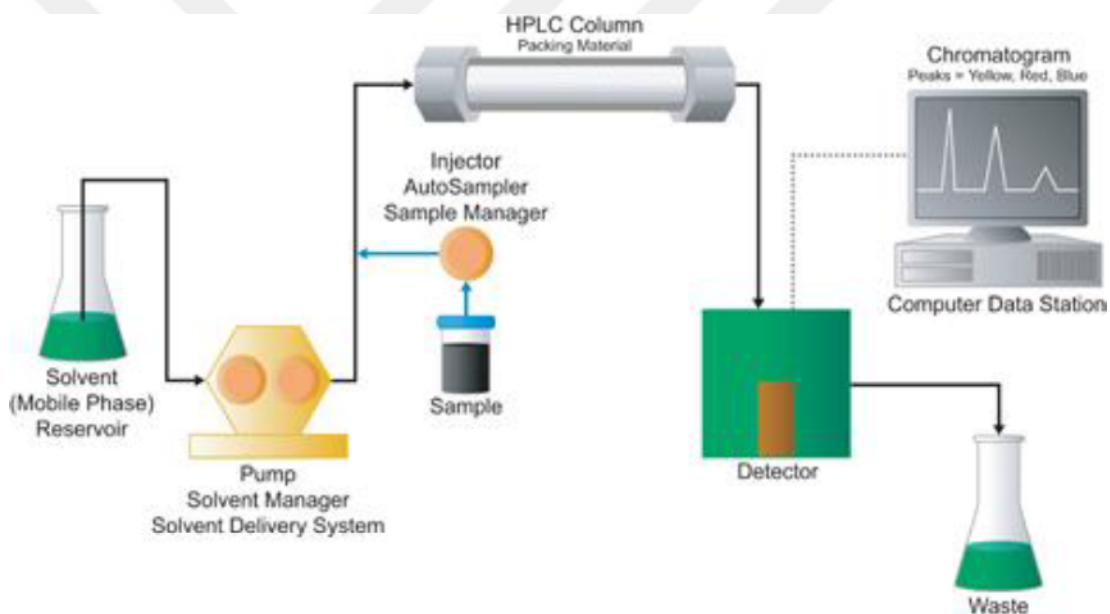


Figure 1.16. Component of HPLC.

Chromatogram is a representation of chemically separated compounds based on their corresponding concentration in the sample (Figure 1.17).

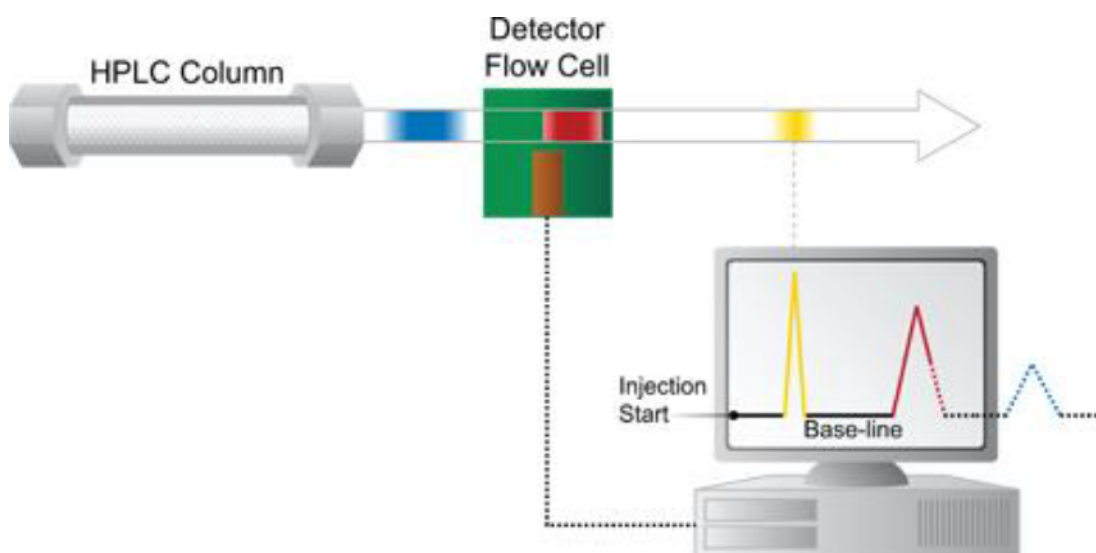


Figure 1.17. Creation of peaks converted from electrical signal.

Each elutes at a specific location, measured by the elapsed time between the moment of injection and the time when the peak maximum elutes. By comparing each peak's retention time with that of injected reference standards in the same mobile and stationary phase, a researcher may be able to identify each compound.

HPLC separation of compounds is based on three primary characteristic comprising polarity, electrical charge, and molecular charge. The structure of a molecule and physicochemical characteristic are determined by its specific orders, called functional groups, of its atoms and bonds between them. These specific arrangements determine if the molecule is polar and non-polar. Based on polarity, the relative retention of different kind of molecules is mostly determined by nature and location of this specific arrangement. Molecules having same or similar polarity tend to attract each other. On the contrary, dissimilar polarity tends to show weaker attraction. This becomes the basis for chromatographic separation based on polarity. Then, a compound in a sample showing similar polarity to stationary phase will be delayed, so that they strongly attract each other. Otherwise, one having a similar polarity with mobile phase will move faster.

Based on polarity separation, it is grouped as normal-phase and reversed-phase HPLC. In normal phase, as it is shown in Figure 1.18, the mobile phase is non-polar solvent and the stationary phase is polar like silica. On the other hands, in reversed phase, the stationary phase is non-polar like C18, the mobile phase is polar one (Figure 1.19).

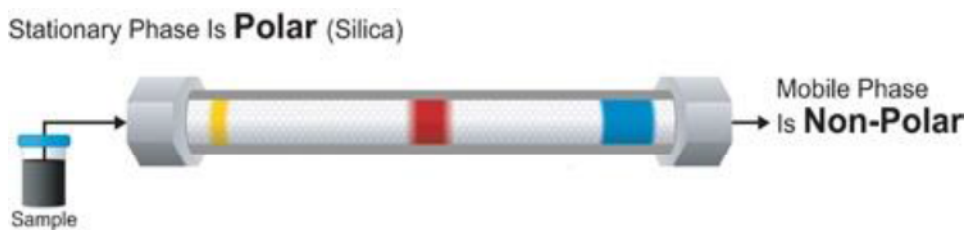


Figure 1.18. Normal-phase chromatography. Stationary phase is polar, thus retaining polar yellow dye showing strong attraction to stationary phase, but mobile phase is non-polar, thus moving blue dye showing strong attraction to mobile phase faster.

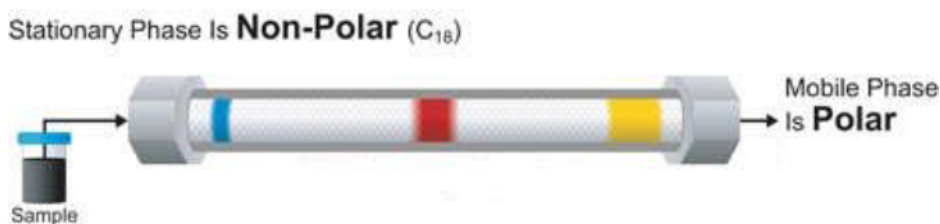


Figure 1.19. Reversed-phase chromatography. Stationary phase is non-polar, thus retaining polar blue dye showing strong attraction to stationary phase, but mobile phase is polar, thus moving yellow dye showing strong attraction to mobile phase faster.

Today, since it is more reproducible and has much application, reversed-phase HPLC is more refers to use of HPLC methods. This typically ensures the proper interaction of analytes with the non-polar, hydrophobic particle surface. A C18-bonded silica is the most popular type of reversed-phase HPLC packing.

For separations based on polarity, the compounds having similar polarity is attracted to the same one and opposites may be repelled. In ion-exchange chromatography based on electrical charge, the same polarity may repel while opposites are attracted to each other. Stationary phases for ion-exchange separations are determined by the nature and strength of the acidic or basic functions on their surfaces and the types of ions that they attract and retain. *Cation* exchange is used to retain and separate positively charged ions on a negative surface. Conversely, anion exchange is used to retain and separate negatively charged ions on a positive surface.

For separation based on size, biomolecules can be separated based upon their size rather than polarity, or electrical charge. Smaller molecules enter more of the pores on their passage through the bed. Larger molecules may only penetrate pores above a certain size so they spend less time in the bed. Mobile phases are chosen for two reasons: first, they are good solvents for the analytes; and, second, they may

prevent any interactions between the analytes and the stationary phase surface based on polarity or charge. In this sense, the larger molecules elute first, while since smaller molecules move into and out of more of the pore, they travel slower and elute later.



2. AIM AND SCOPE OF THE STUDY

Wheat has healthy compounds in the human diet, such as fiber, phytochemicals, and antioxidants etc. (Andersson et al., 2013). Fiber eases digestion and, therefore, decreases the intestinal cancer risks (Huang et al., 2015). Phytochemicals inhibit radical oxidation with DNA or other cell components and protects the plant cells from the deleterious compounds. Bioactive phenolic acids, tocopherols (vitamin E), and carotenoid compounds in wheat showing antioxidant activity (Dinelli et al., 2011; Giambanelli et al., 2013), prevent or reduce the risk for chronic diseases: type 2 diabetes, ischemic heart disease, colon and breast cancers (Dembinska-Kiec et al., 2008; Jangam & Badole, 2014; Lv et al., 2012; Visioli et al., 2000).

During domestication and evolutionary development, many different species have arisen and as more attention has been given to wheat cultivars with strong gluten, free-threshing property, protein content, starch composition, and resistance to biotic and abiotic stresses in bread wheat (Shewry et al., 2013; Vida et al., 2014), and yellow-colored pasta product in durum wheat (Blanco et al., 2011), health compounds such as fibers, phytochemicals, and bioactive compounds have been undervalued. Ancient wheat like einkorn (*T. monococcum* L.) has raised interest for a varied diet. Furthermore, phytochemical contents like phenolic acids, tocopherols, carotenoids etc. with their antioxidative and additional beneficial health aspects play an important role while talking about enhanced health awareness. Information about total phenols and flavonoid contents, phenolic acids, and tocopherols in cereals including common wheat were available and contents of phenolic acids widely range. Less is, however, known about the concentration, composition of total phenols, total flavonoids, phenolic acids and tocopherol content and antioxidant activity in ancient wheat populations than bread and durum wheat species and populations / cultivars. Thus, the aim of this study was

1. To screen and compare the biological activity of the methanolic extracts of bread, durum, and einkorn wheat species and populations / cultivars using antioxidant assay and quantitatively determine total phenol and flavonoid contents.

2. To analyse the phenolic acids (ferulic acid and *p*-coumaric acid) and α -tocopherol contents in the seeds of bread, durum, and einkorn wheat species and populations / cultivars by HPLC.



3. MATERIALS AND METODS

3.1 Plant Extraction, Antioxidant Activity and HPLC Analyses of the Extracts

3.1.1 Plant Material and Extraction

Five bread (*Triticum aestivum* L. cultivars: Akbaşak 073-44, Bayraktar, Gerek 79, Seval, Demir-2000), five durum (*T. turgidum* ssp. *durum* Desf., cultivars: C-1252, Altin 40-98, Ankara-98, Kiziltan-91, Altıntaş) cultivars, and two einkorn (*Triticum monococcum* spp. *monococcum*) populations from Seben-Bolu and İhsangazi-Kastamonu were studied (Table 3.1). Bread and durum wheat cultivars were bred and provided by Central Research Institute for Agricultural Research (CRIFC), Ankara and Anatolian Research Institute (ARI), Eskişehir. Two einkorn populations were provided by Bolu Quality and Feed Industry Corporation, Turkey.

Table 3.1. Species, genomes, names, and locations of wheat samples.

Common Name	Species and Subspecies	Genome	Population or Cultivar	Location
Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	AA	Pop-1	İhsangazi/Kastamonu
Einkorn	<i>T. monococcum</i> ssp. <i>monococcum</i>	AA	Pop-2	Seben/Bolu
Bread wheat	<i>T. aestivum</i> L.	AABBDD	Akbaşak 073-44	CRIFC, Ankara
Bread wheat	<i>T. aestivum</i> L.	AABBDD	Bayraktar	CRIFC, Ankara
Bread wheat	<i>T. aestivum</i> L.	AABBDD	Gerek-79	ARI, Eskişehir
Bread wheat	<i>T. aestivum</i> L.	AABBDD	Seval	CRIFC, Ankara
Bread wheat	<i>T. aestivum</i> L.	AABBDD	Demir-2000	CRIFC, Ankara

Table 3.1. Species, genomes, names, and locations of wheat samples (continued).

Durum wheat	<i>T. turgidum ssp. durum</i> Desf.	AABB	C-1252	CRIFC, Ankara
Durum wheat	<i>T. turgidum ssp. durum</i> Desf.	AABB	Altıntaş	ARI, Eskişehir
Durum wheat	<i>T. turgidum ssp. durum</i> Desf.	AABB	Altın 40-98	CRIFC, Ankara
Durum wheat	<i>T. turgidum ssp. durum</i> Desf.	AABB	Ankara -98	CRIFC, Ankara
Durum wheat	<i>T. turgidum ssp. durum</i> Desf.	AABB	Kızıltan-91	CRIFC, Ankara

Different extracts were prepared from all wheat grains (bread, durum, and eirnkorn wheat). Prior to extraction, all wheat grains were ground into powder. Powdered wheat materials have been extracted by different solvents using different extraction methods.

3.1.1.1 Free Phenolic Extraction

30 g of powdered wheat grains have been extracted with 300 ml of methanol in soxhlet extractor at 60 °C for 24 hours and then filtered (Figure 3.1). Filtrated methanol solvents were evaporated under vacuum using rotary evaporator at 40 °C. Prepared extracts were used for antioxidant activity studies (Total phenol content, total flavonoid content and DPPH● radical scavenging activity), and for HPLC analyses.

3.1.1.2 Alkaline Hydrolysis Extraction

Powdered wheat grains were extracted for bound phenolic acids by the method of Dai and Mumper (2010), with some slight modification. 100 ml of 2M NaOH were added to the Erlenmeyer flask with the residue after the extraction of free phenolic compounds of wheat grains. Erlenmeyer flask was then sealed and stirred about 4h at room temperature (25 °C). After the solution was adjusted pH 2 by 6M HCl, the mixtures were extracted with ethyl acetate (EA) and diethyl ether (DE)

(1:1, v/v) until a white color was observed. Subsequently, the organic solvent (EA/DE) was evaporated at 30 °C to dryness under nitrogen gas and stored at -20 °C (Dai and Mumper, 2010) (Figure 3.2). The extracts were stored at -20 °C until HPLC analyses.



Figure 3.1. Soxhlet apparatus a) Distillation flask, b) Sample, c) Thimble-holder, d) Where solvent is chilled.

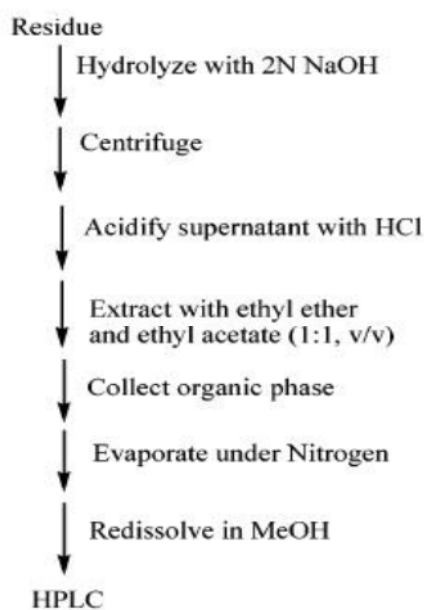


Figure 3.2. Alkaline hydrolysis used in the study.

3.1.1.3 Extraction for α -tocopherol

For α -tocopherol extraction, approximately 0.4 g of powdered wheat grain has been weighted into the test tubes. 200 μ L catechol (0.2 g/mL) and 5 mL of 0.5 M methanolic KOH was added to each wheat sample and stirred. Test tubes were subsequently placed in a water bath for 15 min at 80 °C and were shaken on the vortex every 5 min. Then, 1 mL distilled water and 5 mL hexane were added into the solutions before they were prepared. The mixtures were stirred for 1 min and 3 mL of hexane aliquot of each sample was transferred into the flasks and hexane was evaporated (Hejtmánková, Lachman et al. 2010). When it was used for HPLC analysis, each residue was then dissolved in 0.5 mL methanol.

3.1.2 Antioxidant Activity Assay

3.1.2.1 Free Radical Scavenging Activity (DPPH)

Free radical scavenging activity of the methanolic extracts of each wheat grains was determined spectrophotometrically by monitoring the disappearance of the free radicals at 517 nm according to the method described by Brand-Williams et al. (1995). This spectrophotometric assay uses the stable radical, 2,2-diphenyl-1-picrylhydrazil (DPPH●, Sigma-Aldrich Chemie, Steinheim, Germany) as a reagent.

Methanolic extracts of wheat were weighted as 0.01 g and were dissolved in 10 mL of methanol. Finally, stock solutions of the extracts were composed as 1000 μ g/mL concentration. The extract solutions were diluted to 200, 100, 50, 25, and 12.5 μ g/mL with methanol from stock extract solutions. Ascorbic acid (200, 100, 50, 25 and 12.5 μ g/ml) was used as a standard to express the inhibition capacity of each wheat methanolic extracts as ascorbic acid.

0.1 mM DPPH● solution was daily prepared by methanol. 1.5 mL of the diluted extracts or ascorbic acid (200, 100, 50, 25 and 12.5 μ g/ml) was mixed with 0.5 mL of 0.1 mM DPPH● solution. 1.5 mL methanol and 0.5 mL of the 0.1 mM

DPPH was mixed and was used as a positive control. These solutions were vortexed and incubated at room temperature in dark for 30 minutes. After 30 minutes, the decrease in the absorbance of these solutions was monitored at 517 nm by using a UV-Visible Spectrophotometer (Hitachi U-1900, UV-VIS Spectrophotometer 200V) against blank sample (methanol) (Milardović, Iveković et al. 2006). The experiments were performed in triplicates. The DPPH● scavenging capacity of the extracts was calculated using the following equation:

DPPH● Scavenging Effect (% inhibition) = $[(A_0 - A_1/A_0) \times 100]$ (Gülçin, Oktay et al., 2003), where

A₀ is the absorbance of the positive control

A₁ is the absorbance of tested extracts.

3.1.2.2 Total Phenolic Assay (Folin-Ciocalteu Method)

The amount of total phenolics in the methanolic extracts of wheat was determined according to Folin-Ciocalteu procedure with some modifications (Slinkard and Singleton 1977).

0.01 g each methanolic extracts of wheat was weighted and dissolved by 2 mL distilled water for the preparation of extract solution. These extract solutions were stock solution (5 mg/mL). Later, these solutions were diluted as 1 mg/mL.

Gallic acid was a reference phenol. 0.1 g dry gallic acid was dissolved in 100 mL distilled water and diluted as 500, 250, 200, 150, 100, 50 and 0 µg/mL gallic acid. These gallic acid dilutions were used to prepare a calibration curve and then determine phenol concentration of wheat extracts.

To prepare sodium carbonate (Na₂CO₃) solution, 20 g anhydrous sodium carbonate was dissolved in 100 mL distilled water.

To determine total phenol content of all wheat, 20 µL from each gallic acid dilutions, methanol extracts, or blank (water) was added into separate centrifuge. 1.58 mL distilled water and 100 µL Folin-ciocalteu reagent were added and mixed

well. After 2 minutes, 300 μL of Na_2CO_3 solution was added and shaken very well. The solutions were allowed at 20°C for 2 hours and the absorbance of each solutions were measured at 765 nm against the blank (all reagents without sample) using the spectrophotometer (Hitachi U-1900, UV-VIS Spectrophotometer 200V, JAPAN). After the created calibration curve with standard gallic acid, the total phenolic concentration in the samples was determined. The total phenol content of methanolic extracts from all wheat was expressed as $\mu\text{mol GAE/g DM}$ of extract. All analysis was performed in triplicate.

μmol values of extracts were calculated as follows;

$n = \frac{\text{the amount of TPC of extracts}}{\text{molecular mass of gallic acid}}$

Molecular mass of gallic acid = 170.12 g/mol

3.1.2.3 Total Flavonoid Assay (Aluminum chloride (AlCl_3) Colorimetric Method)

The amount of total flavonoid in the methanolic extracts of wheat was measured by aluminum chloride (AlCl_3) colorimetric assay with some modifications (Marinova et al., 2005). Pyrocatechol was used as a reference flavonoid.

0.005 g of each methanolic extracts of all wheat was weighed and dissolved in 2 mL ethanol (2500 $\mu\text{g/mL}$). Later, the stock extract solutions were diluted to 1250 and 500 $\mu\text{g/mL}$ concentrations. Different standard pyrocatechol solutions was prepared to obtain standard calibration curve of pyrocatechol. To this, 0.1 g pyrocatechol was weighed and dissolved in 100 mL ethanol. Then, this stock solution was diluted as 100, 80, 60, 40 and 20 $\mu\text{g/mL}$.

To determine total flavonoid content of all wheat, 500 μL extract solution, standard solution of pyrocatechol or ethanol (for blank) was added into 10 mL test tube containing 2 mL distilled water. Then, 150 μL of 5% NaNO_2 was added to each test tubes. After 5 minutes, 150 μL of 10 % AlCl_3 was added. At 6 min, 1000 μL of 1M NaOH was added to the mixture. Immediately, 1200 μL distilled water was added to the reaction tube and thoroughly mixed. Absorbance of the each solution

was determined at 510 nm versus a blank. Samples were analyzed in three replications. The total flavonoid content of wheat methanolic extracts were expressed as μmol pyrocatechol equivalents (CTE)/ g dried weight of extracts.

μmol values of extracts were calculated as follows;

$n = \frac{\text{the amount of TFC of extracts}}{\text{molecular mass of pyrocatechol}}$

Molecular mass of pyrocatechol = 110.10 g/mol

3.1.3 HPLC Analysis of Extracts for Phenolic Compounds

In the present study, all the phenolic compounds were chosen as HPLC grade

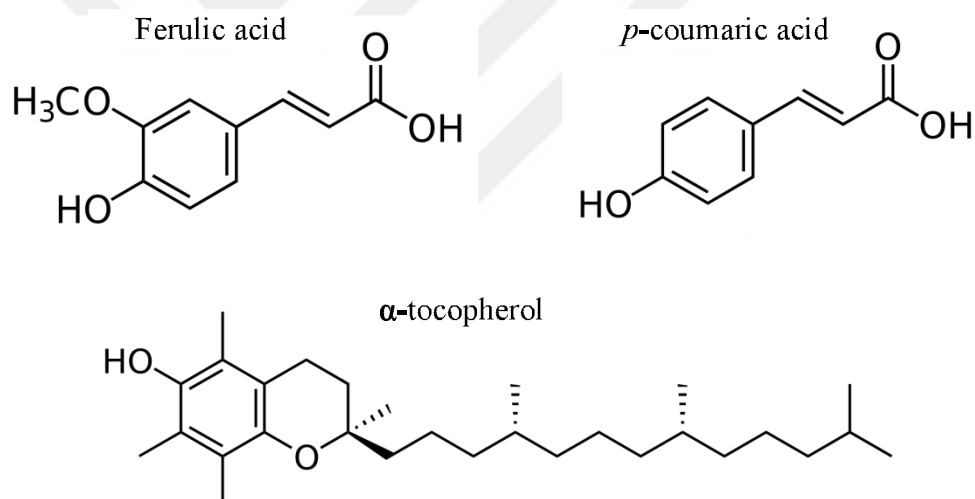


Figure 3.3. Phenolic compounds used as standard in HPLC analyses.

(>98%) and bought from Sigma-Aldrich Chemical Company. Totally, three standard compounds were used in analysis of all prepared extracts by HPLC technique (Figure 3.3).

Extracts were analysed using HPLC system (Dionex, Olten, Switzerland[®]) equipped with 3000 Pump and Diode Array Detector, ACC-3000 Autosampler. Compounds were separated on a reverse phase Ace 5 C18 Column (150 mm × 4.6 mm) by using two different methods with some modifications (Verma et al. 2009).

For ferulic and *p*-coumaric acid analyses, the mobil phase was composed of solvent (A) ultra-distilled water and solvent (B) Acetonitrile, both containing 0.1% formic acid (v/v). The gradient program was started with 100% A at 0 min and changed to obtain 85%, 50% and 100% A at 10, 15 and 20 min, respectively. Flow rate was 0.8 mL/min with 25°C oven and injection volume was 10 μ L. The wavelength was 280 nm for ferulic acid and 280 nm for *p*-coumaric acid.

For α -tocopherol analysis, elution was carried out isocratically. The constant solvent mixture was composed of 96% methanol and 4% ultra-distilled water (0-20 min). Flow rate was 0.8 mL/min with 36°C oven and injection volume was 10 μ L. The chromatograms were obtained at 280 nm. Mobile phases and ultrapure water were filtered through a 0.45 μ m hydrophilic polypropylene membrane filter prior to HPLC injection. Spectra data were recorded from 200 to 400 nm during the entire run.

3.2 Statistical analysis

An experiment in randomized complete block design with three replications was run for each character of all wheat samples. The significance level accepted for all characters was 0.05. Mean differences were separated by Duncan's Multiple Range Test (DMRT) using SPSS version 18.0 (SPSS Inc. Chicago, IL). All data, were reported as mean $\pm$ standard deviations of three replications.

4. RESULTS AND DISCUSSIONS

Main goal of this study was to investigate total phenolic content, total flavonoid content, and antioxidant activity among wheat species as well as cultivars and populations in addition to the detection of their ferulic acid, *p*-coumaric acid, and α -tocopherol content.

4.1 Total phenolic content

Results for total, free, and bound phenolic content analysis were expressed as $\mu\text{mol GAE/g DM}$ for each sample. The standard calibration curve of gallic acid shown in Figure 4.1 was prepared between 0.025-1000 $\mu\text{g/ml}$ and the equation obtained from the curve was used to calculate the absorbance values of the samples measured by T60V spectrophotometer. Highly significant differences existed among wheat species / cultivars ($F=31.60$, $p=0.00$) in ANOVA which was determined by F test. In contrast, there was no significant difference between blocks (treatments) ($F=1.69$, $p=0.22$), which meant all blocks were performed accurately (Table 4.1).

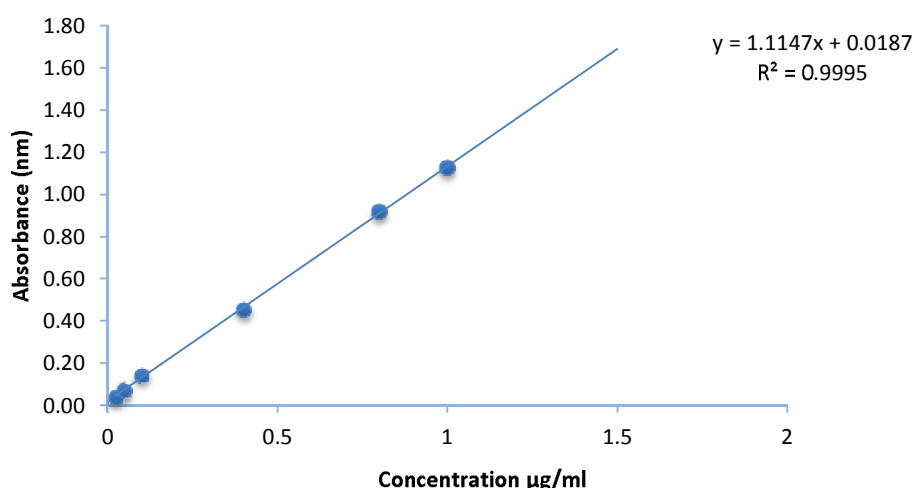


Figure 4.1. Standard calibration curve of Gallic Acid.

Table 4.1. Analysis of variance, which was run in a three replicate randomized block design for free phenolic content of wheat species / cultivars.

Source of variation	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	0.031	31.60	0.00
Blocks (Replications)	2	0.002	1.69	0.22
Error	22	0.001		
Total	35			

DMRT for FPC, and BPC differentiated species, population, and cultivars. Phenolic contents of wheat cultivars and populations were determined as free and bound.

While the TPCs ranged between 0.29 ± 0.02 and 0.75 ± 0.03 $\mu\text{mol GAE/g}$ in free phenolic compound (FPC) extracts of wheat samples, bound phenolic compounds (BPCs) ranged between 1.60 ± 0.30 and 7.49 ± 0.08 $\mu\text{mol GAE/g}$ in BPC extract of wheat samples. When these two different extractions were compared for their phenolic content, bound phenolic compound extracts have possessed higher TPCs for all used wheat samples. On the other hand, the BPC extract contained at least 3 times higher TPC than FPC extracts. Among FPC extracts, Kızıltan-91 has possessed the highest TPC with a value of 0.52 ± 0.03 $\mu\text{mol GAE/g}$. The next high phenolic content was found in Pop-1 and Altin 40-98 as 0.63 ± 0.03 and 0.63 ± 0.05 $\mu\text{mol GAE/g}$, respectively. The lowest one was in Gerek-79 with a value of 0.29 ± 0.02 $\mu\text{mol GAE/g}$. In the present study, although the amount of TPCs in the free phenolic content extracts was found significantly higher, there was no significant difference among einkorn, bread, and durum wheat, but within each species for cultivars and/or populations ($p < 0.05$).

There was significant differences among wheat species for BPCs ($F=355.784$, $p=0.00$) as determined by randomized block design (Table 4.2). In contrast, there was no significant difference between blocks ($F=0.085$, $p=0.776$), which means all blocks were performed accurately. In the light of these findings DMRT test results revealed as follows.

Table 4.2. Analysis of variance for bound phenolic content of wheat species and populations / cultivars in a three replicate randomized block.

Source of variation	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	5.486	355.784	0.000
Blocks (treatments)	2	0.001	0.085	0.776
Error	11	0.015		
Total	36			

Among BPC extracts, the highest amount of TPC was found in Pop-1 with value of $7.49 \pm 0.08 \mu\text{mol GAE/g}$ dry extract, and followed by Pop-2 ($6.37 \pm 0.06 \mu\text{mol GAE/g}$) (Figure 4.2 and Table 4.3). The lowest amount of TPC was found in Bayraktar with value of $1.60 \pm 0.30 \mu\text{mol GAE/g}$ dry extracts. Other tested extracts contained nearly similar amount of TPC ranging between 3.34 and $2.73 \mu\text{mol GAE/g}$.

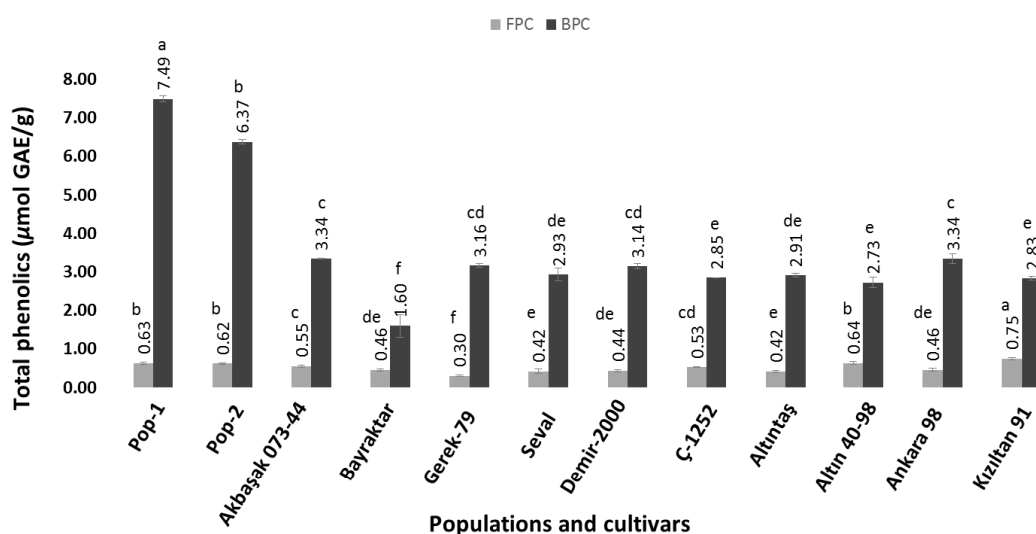


Figure 4.2. Free and bound phenolic contents of wheat species and cultivars / populations (mean $\pm$ SD). The vertical bars represent the standard deviation of each data point. Bars without common letters are significantly different ($p < 0.05$).

Yilmaz et al. (2015) studied the TPC of ten diploid einkorns, another diploid *T. urartu* Tum, two tetraploids, and two hexaploid wheat species and accessions. They reported that the amount of TPCs ranged between 570 and 1012 mg GAE/kg DM. They also reported that there was a significant difference between wild and modern wheat species and accessions. In an other study, researchers studied the TPC of ten different durum wheat genotypes grown on three consecutive years in Italy (Martini et al., 2015). They found that the amount of TPC was 1464.0 mg ferulic acid

equivalent /kg DM in 2010 and 856.6 mg ferulic acid equivalent /kg DM in 2011. In addition, they reported that bound phenolic content was higher than free phenolic content, representing the same result in this study.

Table 4.3. BPCs and FPCs values, which is expressed as both μg GAE/g and μmol GAE/g. Bars without common letters are significantly different ($p < 0.05$).

Cultivars / Populations	Bound phenolics		Free phenolics	
	μg GAE/g	μmol GAE/g	μg GAE/g	μmol GAE/g
Pop-1	1274.72 ^a	7.49 ^a	107.70 ^b	0.63 ^b
Pop-2	1083.00 ^b	6.37 ^b	105.75 ^b	0.62 ^b
Akbaşak 073-44	566.43 ^c	3.33 ^c	94.03 ^c	0.55 ^c
Bayraktar	273.23 ^f	1.60 ^f	77.57 ^{de}	0.46 ^{de}
Gerek-79	532.91 ^{cd}	3.16 ^{cd}	54.45 ^f	0.30 ^f
Seval	498.29 ^{de}	2.93 ^{de}	71.29 ^e	0.42 ^e
Demir-2000	534.73 ^{cd}	3.14 ^{cd}	75.20 ^{de}	0.44 ^{de}
Ç-1252	482.49 ^e	2.85 ^e	89.94 ^{cd}	0.51 ^{cd}
Altıntaş	482.57 ^{de}	2.91 ^{de}	83.68 ^e	0.42 ^e
Altın 40-98	438.19 ^e	2.73 ^e	132.95 ^b	0.63 ^b
Ankara-98	568.84 ^c	3.34 ^c	78.41 ^{de}	0.47 ^e
Kızıltan-91	470.26 ^e	2.83 ^e	138.77 ^a	0.75 ^a

Abozed et al. (2014) investigated the TPC of acetone, methanol and ethanol extracts of common and durum wheat cultivated in two different locations. They reported that the TPC of methanolic extracts of wheat ranged between 1.05-1.11 mg GAE/g as dry extract. Similarly, in our study, we found that the amount of TPC was 1.27 and 1.08 mg GAE/g dry extract in the BPC extracts of Pop-1 and Pop-2 einkorn, respectively. Ciccoritti et al. (2013) have reported that the TPC of wheat ranged from 2.55 to 8.58 μmol GAE/g dry extracts. In an other study, they have reported that some durum and bread wheat contained high amount of TPC ranged between 7.09 and 8.59 μmol GAE/ g dry extract (Adom et al., 2003). In the present study, we found nearly the same amount of TPC in BPC extract of Pop-1 wheat (7.49 μmol GAE/g dry extract). Žilić et al. (2013) investigated the TPC of ten bread and durum wheat cultivars and reported that the TPC ranged from 0.99 to 1.60 μmol CE/g for bread wheat and from 1.27 to 1.65 μmol CE/g for durum wheat. It is difficult to compare their results with our results because they expressed the TPC as mg CE/g dry extracts. In the work of Okarter et al. (2010), two spring and four winter wheats were investigated. They reported that the amount of TPC ranged from 2.55 to 4.99

$\mu\text{mol GAE/g DM}$ for FPC extracts and from 5.82 to 6.62 $\mu\text{mol GAE/g DM}$ for BPC extracts. While the content of free phenolic was higher than that of our results, the content of bound phenolic was lower than our results. The differences in all these results may be explained by growing conditions which have a certain effect on the biosynthesis and accumulation of phenolic compounds. Besides, different genotypes or different extraction solvents may cause differences in phenolic content of wheat.

4.2 Total flavonoid content

The total flavonoid content (TFC) of each wheat sample was expressed as both μmol and $\mu\text{g CTE/g}$ dry extract (Table 4.5 and Figure 4.3). Calibration curve of standard catechol solution was plotted by using a dose range of 0.0125-0.4 $\mu\text{g/ml}$ in order to determine the total flavonoid content of the extracts. The standard calibration curve of catechol was shown in Figure 4.3.

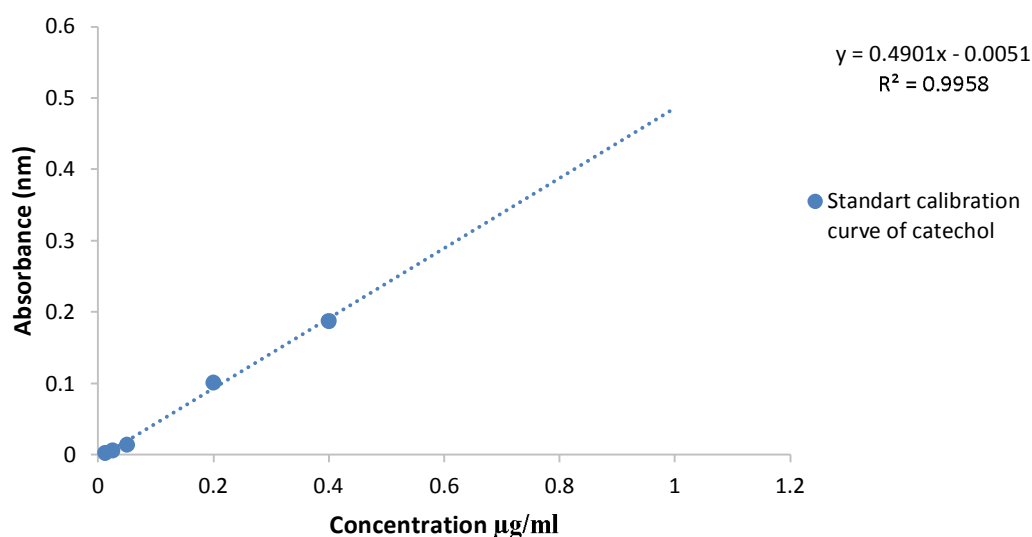


Figure 4.3. Standard calibration curve of catechol.

There was significant differences among wheat species ($F=100.457$, $p=0.00$) (Table 4.4). In contrast, there was no significant difference among blocks (treatments) ($F=2.373$, $p=0.117$), which means all blocks were performed accurately. In the light of these findings DMRT results revealed as follows.

TFC of wheat populations and cultivars was in the range of $0.04 \pm 0.02 - 0.39 \pm 0.01 \mu\text{mol CTE/g}$ dried extract. TFC in the studied wheat extracts indicated that

Altintas contained the highest flavonoid content as $0.39 \pm 0.01 \mu\text{mol CTE/g}$ dried extract and followed by C-1252 ($0.35 \pm 0.03 \mu\text{mol CTE/g}$ dried extract) and Pop-2 extracts ($0.33 \pm 0.04 \mu\text{mol CTE/g}$ dried extract). The TFC of the remained extracts was in the order of Pop-1 > Bayraktar > Ankara-98 > Akbasak 073-44 > Altin > Seval > Demir-2000 > Kiziltan-91 > Gerek-79. Moreover, Gerek-79 contained the lowest flavonoid contents ($0.04 \pm 0.02 \mu\text{mol CTE/g}$ dried extract) and almost 10 times lower than the highest extract (Table 4.5). While there were no significant differences among einkorn, bread wheat and durum wheat, cultivars and/or populations within each species were different from each other ($P < 0.05$).

Table 4.4. Analysis of variance, which was run in a three replicate randomized block design for total flavonoid content of wheat species and cultivars / populations.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	0.037	100.457	0.000
Blocks (treatments)	2	0.001	2.373	0.117
Error	22	0.000		
Total	36			

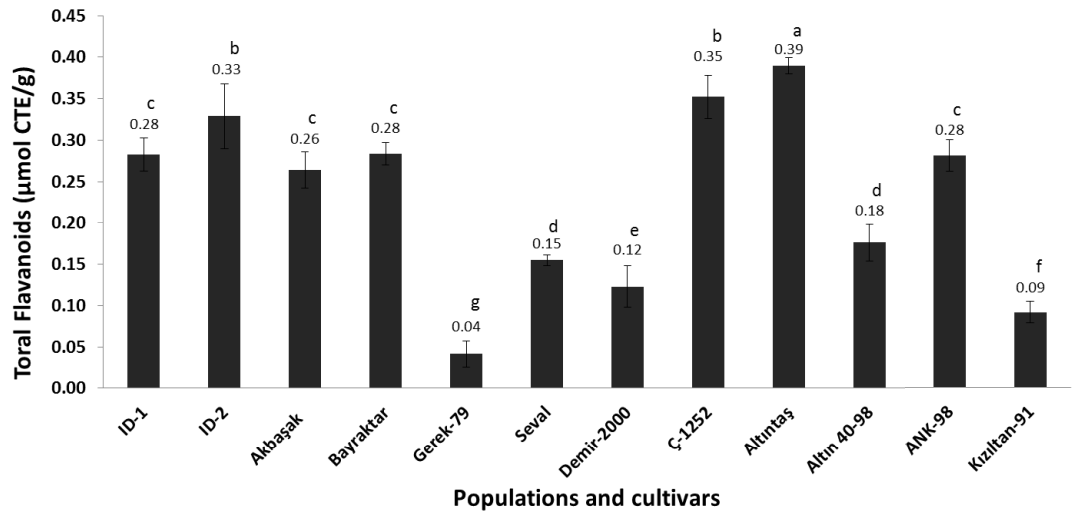


Figure 4.4. Total flavonoid content of wheat cultivars and populations (mean $\pm$ SD). The vertical bars represent the standard deviation of each data point. Bars with no common letters are significantly different ($p < 0.05$).

Table 4.5. TFC values, which is expressed as both $\mu\text{g CTE/g}$ and $\mu\text{mol CTE/g}$. Bars with no common letters are significantly different ($p<0.05$).

Cultivars / Populations	$\mu\text{g GAE/g}$	$\mu\text{mol GAE/g}$
Pop-1	25.98 ^c	0.28 ^c
Pop-2	30.62 ^b	0.33 ^b
Akbaşak 073-44	32.76	0.26 ^c
Bayraktar	35.26 ^c	0.28 ^c
Gerek-79	4.56 ^g	0.04 ^g
Seval	15.96 ^d	0.15 ^d
Demir-2000	24.56 ^e	0.12 ^e
Ç-1252	42.44 ^b	0.35 ^b
Altıntaş	59.91 ^a	0.39 ^a
Altın 40-98	19.41 ^d	0.18 ^d
Ankara -98	35.85 ^c	0.28 ^c
Kızıltan-91	23.05 ^f	0.09 ^f

The phytochemical content of immature and mature wheat kernels cultivated in Korea was investigated (Kim and Kim, 2016). While no differences in the amount of free flavonoids were observed among two wheat kernels, significant differences in bound flavonoids existed. Overall, TPC in mature wheat (2.34 mg CE/g dried extract) was lower than that of immature wheat (4.73 mg CE/g dried extract). Kim et al. (2013) also investigated wheat and reported that the amount of TFC was 0.28 mg catechin equivalent/g for grains at post-germination and 0.63 mg catechin equivalent/g for those at pre-germination. In another study, six different modern and old bread wheat varieties were studied and TFC ranged from 20.14 to 67.69 mg CE/100g and revealed a significant difference among old and modern varieties (Leoncini et al., 2012). Dinelli et al. (2011), while also studied some old and modern cultivars of common wheat, found that old wheat varieties showed higher flavonoid content than that of modern wheats. They also reported that wheats contained higher amount of bound flavonoids than free flavonoids. While the bound flavonoid content in wheat cultivars ranged from 0.78 to 1.49 $\mu\text{mol CE/100g}$ of grain, free flavonoid content ranged from 0.51 to 1.06 $\mu\text{mol CE/100g}$ of grain. In the study of Serpen et al. (2008), obtained ranges for flavonoids in emmer (1.06-2.29 $\mu\text{mol/g}$), einkorn landraces (0.80-1.59 $\mu\text{mol/g}$) and bread wheat cultivars (1.32-1.35 $\mu\text{mol/g}$) were similar to those reported by Dinelli et al. (2011). Žilić et al. (2012), who studied flavonoid contents in whole wheat flour and bread samples, reported that flavonoids in five bread and durum varieties were not detected in whole wheat flour. However, that the bran samples of the same five different bread and durum varieties was in the

range from 173.59 to 321.86 $\mu\text{g CE/g DM}$. There was no correlation between total flavonoid contents of our present study and the results of the Žilić et al. (2012). Consequently, obtained flavonoid contents in these reserchs were higher than those in our study. Differences in these studies might be attributed to the differences in the standards, populations, and cultivars. In addition to those, harvesting time and environmental conditons for maturing may affect the concentration of flavonoids in wheat species and populations (Dinelli et al., 2011).

4.3 DPPH scavenging activitiy

The free radical scavenging effectiveness of various antioxidant ingredients is extensively assessed by DPPH assay. The decreasing ability on the DPPH radical is determined by reduction in its absorbance at 517 nm prompted by antioxidants. The reduction in absorbance of DPPH radical triggered by antioxidants is due to the reaction between antioxidant compounds and radicals.

In DPPH assay here indicated that there was no significant DPPH radical scavenging activity of wheat populations and cultivar. When they were used from 12.5 to 100 $\mu\text{g/ml}$ concentrations, DPPH radical scavenging capacity has only changed from 0.06% to 21.10%. At 100 $\mu\text{g/ml}$ concentration. Altın 40-98 showed DPPH scavenging activity over 20%. Even high amount (200 $\mu\text{g/ml}$) was used the highest DPPH radical scavenging activity was as 22.34% in Altın 40-98 (Table 4.6).

When the extracts were compared, at minimum concentration (12.5 $\mu\text{g/ml}$), Altıntaş (0.06%) and Seval (0.15%) showed the lowest scavenging activity while Pop-2 (2.79%), Gerek-79 (2.41%), and Kızıltan-91 (2.39%) showed the. Indeed, these extracts have no strong scavenging activity. At 25 $\mu\text{g/ml}$, Demir-2000 (5.53%) and Seval (5.37%) had the highest, Altıntaş (0.28%) had the lowest scavenging activity. At the concentration of 50 $\mu\text{g/ml}$, Kızıltan-91 (6.62%), Demir-2000 (5.72%) and Pop-2 (5.54%) showed highest scavenge activity, respectively, but Altintas (0.42%) have the lowest one. At 100 $\mu\text{g/ml}$ concentration, Altın 40-98 (21.10%) had highest Altıntaş (2.22%) the lowest one. At 200 $\mu\text{g/ml}$ concentration, Altın 40-98 (22.34%) and Kızıltan-91 (21.43%) showed the highest Bayraktar (9.68%) and Altıntaş (10.69%) the lowest radical scavenging activity. Overall, there was no

significant difference among einkorn, bread, and durum wheat cultivars and populations, although we found significantly different DPPH radical scavenging activity among doses.

Table 4.6. The percentage inhibition of radical scavenging activity of cultivars and populations at different concentration.

Samples	% of DPPH inhibition ¹				
	12.5µg/mL	25 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL
Ascorbic acid	96.29	96.86	96.57	96.57	96.57
Pop-1	0.35 ^{cd}	2.51 ^c	4.35 ^{ab}	9.44 ^{cde}	13.89 ^{bc}
Pop-2	2.79 ^a	3.87 ^b	5.54 ^a	9.47 ^{cde}	16.43 ^b
Akbaşak 073-44	0.61 ^{bc}	0.76 ^f	1.60 ^{bc}	4.30 ^{fg}	11.62 ^{cde}
Bayraktar	0.66 ^{bc}	1.54 ^e	1.51 ^{bc}	4.97 ^{efg}	9.68 ^e
Gerek-79	2.41 ^a	2.35 ^c	4.28 ^{ab}	7.94 ^{def}	15.52 ^b
Seval	0.15 ^d	5.37 ^a	4.04 ^{ab}	9.56 ^{cde}	12.47 ^{cd}
Demir-2000	0.91 ^b	5.53 ^a	5.72 ^a	11.85 ^{bcd}	16.11 ^b
Ç-1252	0.75 ^{bc}	2.55 ^c	2.55 ^{bc}	8.41 ^{def}	16.62 ^b
Altıntaş	0.06 ^d	0.28 ^g	0.42 ^c	2.22 ^g	10.69 ^{de}
Altın 40-98	0.38 ^{cd}	1.97 ^d	2.26 ^{bc}	21.10 ^a	22.34 ^a
Ankara -98	0.43 ^{cd}	0.87 ^f	3.98 ^{ab}	13.64 ^{bc}	16.52 ^b
Kızıltan-91	2.39 ^a	3.73 ^b	6.62 ^a	16.31 ^b	21.43 ^a

¹Mean values with the same letter within the column are not significantly different from each other at P<0.05.

The comparison of DPPH radical scavenging activity of *T. aestivum*, *T. turgidum* ssp. *durum*, *T. turgidum* ssp. *dicoccum*, and *T. monococcum* reported by Ciccoritti et al. (2013), however, data ranged between 87 ± 3 and 93 ± 8 there represented as ED₅₀ (mg of wheat milled grain required to obtain 50% DPPH scavenging on dry basis) showed that there was no significant difference among wheat species (p<0.05). In another study reported by Yilmaz et al. (2015), *T. monococcum*, ssp. *monococcum*, *T. aestivum* L., and *T. turgidum* ssp. *durum* Desf., which were extracted with 3 different solvents revealed the same result as our study.

On the other hand, there was a significant difference among cultivars and landraces of emmer, einkorn, and bread wheat for antioxidant activities by ABTS^{•+} assay reported by Serpen et al. (2008), ($p < 0.05$). ED₅₀ values of eight different soft wheat varieties ranged from 23.2 to 27.42 mg of grain equivalent/mL and significantly differed among durum wheat varieties (Moore et al., 2005).

4.4 HPLC Analysis of Phenolic Compounds

4.4.1 Ferulic acid and *p*-coumaric acid

Wheat phytochemicals are mainly composed of phenolic acids, which highly contribute to the antioxidant properties. Ferulic and *p*-coumaric acid are the most abundant chemicals in the wheat kernels (Martini et al., 2015). In this study, phenolic acids; ferulic acid, and *p*-coumaric acid were determined within wheat species, cultivars, and populations.

Analysis of variance indicated that there was significant differences among wheat species ($F=233.779$, $p=0.00$) (Table 4.7). In contrast, there was no significant difference among treatments ($F=2.407$, $p=0.113$), which means all treatments were performed accurately. In the light of these findings DMRT revealed the following.

Table 4.7. Analysis of variance, which was run in a three replicate randomized block design for ferulic acid content of wheat species and cultivars / populations.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	88193.103	233.779	0.000
Blocks (treatments)	2	908.181	2.407	0.113
Error	22	377.250		
Total	36			

Ferulic acid content of wheat species, cultivars, and populations (Table 4.3) were expressed as total (free+ bound) ferulic acid content. HPLC results showed that einkorn wheats contained higher ferulic acid than bread and durum wheat cultivars and populations. In other words, Pop-1 ($764.04 \pm 2.40 \mu\text{g/g}$) had the highest ferulic

acid content, followed by Pop-2 ($762.89 \pm 14.17 \mu\text{g/g}$). Moreover, Demir-2000 ($554.82 \pm 8.55 \mu\text{g/g}$) had the highest amount of ferulic acid among bread wheat cultivars. Ankara-98 ($480.11 \pm 31.30 \mu\text{g/g}$) possessed the highest amount of ferulic acid among durum wheat cultivars and were followed by Akbaşak 073-44 ($478.76 \pm 23.90 \mu\text{g/g}$), Altıntaş ($476.20 \pm 16.26 \mu\text{g/g}$), Seval ($460.08 \pm 1.11 \mu\text{g/g}$), Ç-1252 ($439.73 \pm 17.09 \mu\text{g/g}$), Altın 40/98 ($397.80 \pm 12.18 \mu\text{g/g}$), Kızıltan-91 ($396.96 \pm 29.95 \mu\text{g/g}$), and Bayraktar ($148.67 \pm 13.99 \mu\text{g/g}$). There were significant differences among einkorn and bread and durum wheat species and among their cultivars ($p < 0.05$) while there were no differences between bread and durum wheat species and cultivars ($p < 0.05$). Einkorn had 1.8-fold more ferulic acid than bread and durum wheat had.

ANOVA indicated that there was significant differences between wheat species ($F=228.234$, $p=0.00$) for *p*-coumaric content (Table 4.8). In contrast, there was no significant difference between treatment ($F=0.190$, $p=0.671$), which means all treatment was performed accurately. In the light of these findings DMRT revealed the following.

Table 4.8. Analysis of variance, which was run in a three replicate randomized block design for *p*-coumaric content of wheat species cultivars / populations.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars)	2	0.346	0.190	0.671
Species or cultivars	11	414.776	228.234	0.000
Error	11	1.817		
Total	36			

The highest *p*-coumaric acid content among wheat species, cultivars, and populations was present in Pop-1 ($54.09 \pm 1.76 \mu\text{g/g}$) and Pop-2 ($47.64 \pm 2.31 \mu\text{g/g}$), ($p < 0.05$), (Figure 4.9). Furthermore, Demir-2000 ($33.45 \pm 1.23 \mu\text{g/g}$) had the highest *p*-coumaric acid among bread wheat cultivars and was followed by Gerek-79 ($29.33 \pm 0.06 \mu\text{g/g}$), Altın 40-98 ($23.83 \pm 0.73 \mu\text{g/g}$) durum wheat cultivar had the highest ferulic acid. The rest of cultivars are Ç-1252 ($22.32 \pm 1.22 \mu\text{g/g}$), Kızıltan-91 ($18.08 \pm 1.55 \mu\text{g/g}$), Akbaşak 073-44 ($15.28 \pm 0.35 \mu\text{g/g}$), Ankara -98 ($11.32 \pm 1.59 \mu\text{g/g}$), and Bayraktar ($5.06 \pm 0.01 \mu\text{g/g}$). Furthermore, einkorn had 2.5 times higher *p*-coumaric acid than bread and durum wheat species, cultivars, and populations. While

significant differences were observed between einkorn populations and cultivars of bread / durum wheat, there was no significant difference between durum and bread wheat cultivars ($p < 0.05$).

Table 4.9. Average concentration ($\mu\text{g/g}$) of bioactive compounds in einkorn, bread, and durum wheat cultivars and populations.

Cultivar/ Population	ferulic acid ¹	p-coumaric ¹	α -tocopherol ¹
Pop-1	764.04 $\pm$ 2.40 ^a	54.09 $\pm$ 1.76 ^a	1.41 $\pm$ 0.07 ^c
Pop-2	762.89 $\pm$ 14.17 ^a	47.64 $\pm$ 2.31 ^b	3.16 $\pm$ 0.70 ^{bc}
Akbaşak 073-44	478.76 $\pm$ 23.90 ^d	15.28 $\pm$ 0.35 ^g	1.59 $\pm$ 0.70 ^c
Bayraktar	148.67 $\pm$ 13.99 ^g	5.06 $\pm$ 0.01 ⁱ	3.81 $\pm$ 1.40 ^b
Gerek-79	476.50 $\pm$ 6.52 ^c	29.38 $\pm$ 0.06 ^d	4.01 $\pm$ 1.19 ^b
Seval	460.08 $\pm$ 1.11 ^{de}	26.45 $\pm$ 1.82 ^{de}	3.27 $\pm$ 0.12 ^{bc}
Demir-2000	554.82 $\pm$ 8.55 ^b	33.45 $\pm$ 1.23 ^c	9.51 $\pm$ 0.49 ^a
Ç-1252	439.73 $\pm$ 17.09 ^e	22.32 $\pm$ 1.22 ^f	7.68 $\pm$ 0.70 ^a
Altıntaş	467.20 $\pm$ 16.26 ^{de}	15.30 $\pm$ 0.11 ^g	3.09 $\pm$ 0.70 ^{bc}
Altın 40-98	397.80 $\pm$ 12.18 ^f	23.83 $\pm$ 0.73 ^{ef}	3.51 $\pm$ 0.70 ^{bc}
Ankara -98	480.11 $\pm$ 31.30 ^d	11.32 $\pm$ 1.59 ^h	4.38 $\pm$ 1.40 ^b
Kızıltan-91	396.96 $\pm$ 29.95 ^f	18.08 $\pm$ 1.55 ^g	1.66 $\pm$ 0.70 ^c

¹Data represented as mean $\pm$ SD, mean values with the same letter within the column are not significantly different from each other at $P < 0.05$.

Similar values, which were obtained in ten diploid einkorns, another diploid *T. urartu* Tum., two tetraploids, and two hexaploid wheat species and accessions ranged between 471.1-724.9 mg/kg DM in ferulic acid content and 10.8-102.1 mg/kg DM in *p*-coumaric acid content (Yilmaz et al. 2015). It was reported that (Benincasa et al. 2015), bound and free ferulic acid and *p*-coumaric acid contents of one einkorn, three emmer, two spelt, one durum, and one soft wheat species and cultivars ranged from 225.7 $\mu\text{g/g}$ to 303.10 $\mu\text{g/g}$ for bound ferulic acid and from 0.33 $\mu\text{g/g}$ to 0.91 $\mu\text{g/g}$ for free ferulic acid. When compared ferulic acid content for einkorn species,

the content of einkorn species in the literature were lower than the ones reported in our study. Moreover, the *p*-coumaric acid content in our study ranged from 14.2 $\mu\text{g/g}$ to 310.3 $\mu\text{g/g}$. Even it showed the similar results einkorn species content was still almost 2 times higher than other species and cultivars. Although the results exhibited significant differences among wheat species, some similarities to the results in our study still existed (Brandolini et al., 2013). Similar results in ferulic and *p*-coumaric acid content of Maryland soft red winter wheat existed. They ranged between 455.92 to 621.47 $\mu\text{g/g}$ for ferulic acid and 25.05 to 54.21 $\mu\text{g/g}$ for *p*-coumaric acid. These results were lower than those of our einkorn populations (Moore et al., 2005). Hernandez et al. (2011) reported phenolic acids including ferulic acid and *p*-coumaric acid, the ferulic acid content of nineteen different cultivars (bread and durum wheat) ranged from 439.00 $\mu\text{g/g}$ to 1450.00 $\mu\text{g/g}$ and from 10.10 $\mu\text{g/g}$ to 29.40 $\mu\text{g/g}$, respectively. Although *p*-coumaric acid content were similar, except for einkorn reported by us, some of ferulic acid values were higher than the ones found in our study. Furthermore, ferulic acid content in this study were similar to those of emmer and einkorn species reported by Serpen et al. (2008) and ranged between 232.66-775.30 $\mu\text{g/g}$. *p*-coumaric acid content were higher than those of 25.05-54.21 $\mu\text{g/g}$ in our study. However, ferulic acid content of einkorn populations in our study were higher about 2.5 times than those in einkorn landraces.

4.4.2 α -tocopherol content

Vitamin E (tocopherols and tocotrienols) compounds play an important role in the antioxidation of cell membrane and lipoproteins. They also have many beneficial functions to human health, such as preventing cancer, cardiovascular diseases, a protective effect lowering LDL-C by inhibiting cholesterol biosynthesis, and expressing an antioxidant activity (Jacobsson et al., 2004).

ANOVA determined that there was significant differences among wheat species ($F=355.784$, $p=0.00$) for α -tocopherol content. In contrast, there was no significant difference among treatments ($F=0.085$, $p=0.776$) (Table 4.10), which meant all treatments were performed accurately. Duncan Multiple Range Test discriminated the results as follows.

In the present study, the highest amount of α -tocopherol was found in Demir-2000 ($9.51 \pm 0.49 \mu\text{g/g}$) and was followed by Ç-1252 ($7.68 \pm 0.70 \mu\text{g/g}$). The amount of α -tocopherol in other extracts was found in the following order; Ankara -98 ($4.38 \pm 1.40 \mu\text{g/g}$), Gerek-79 ($4.01 \pm 1.19 \mu\text{g/g}$), Bayraktar ($3.81 \pm 1.40 \mu\text{g/g}$), Altın 40/98 ($3.51 \pm 0.70 \mu\text{g/g}$), Seval ($3.27 \pm 0.21 \mu\text{g/g}$), Pop-2 ($3.16 \pm 0.70 \mu\text{g/g}$), Altıntaş ($3.09 \pm 0.70 \mu\text{g/g}$), Kızıltan-91 ($1.66 \pm 0.70 \mu\text{g/g}$), Akbaşak ($1.59 \pm 0.70 \mu\text{g/g}$), and Pop-1 ($5.06 \pm 0.01 \mu\text{g/g}$ of whole wheat) (Table 4.9 and Figure 4.5). No difference has been observed among einkorn, durum, and bread wheat species, but cultivars and populations ($p < 0.05$).

Table 4.10. Analysis of variance, which was run in a three replicate randomized block design for α -tocopherol content of wheat species cultivars / populations.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars)	11	11.689	15.038	0.000
Blocks (replications)	2	0.027	0.034	0.856
Error	11	0.777		
Total	36			

The highest α -tocopherol in this study was Demir-2000 ($9.51 \pm 0.49 \mu\text{g/g}$), followed by Ç-1252 ($7.68 \pm 0.70 \mu\text{g/g}$), Ankara-98 ($4.38 \pm 1.40 \mu\text{g/g}$), Gerek-79 ($4.01 \pm 1.19 \mu\text{g/g}$), Bayraktar ($3.81 \pm 1.40 \mu\text{g/g}$), Altın 40/98 ($3.51 \pm 0.70 \mu\text{g/g}$), Seval ($3.27 \pm 0.21 \mu\text{g/g}$), Pop-2 ($3.16 \pm 0.70 \mu\text{g/g}$), Altıntaş ($3.09 \pm 0.70 \mu\text{g/g}$), Kızıltan-91 ($1.66 \pm 0.70 \mu\text{g/g}$), Akbaşak 073-44 ($1.59 \pm 0.70 \mu\text{g/g}$), and Pop-1 ($5.06 \pm 0.01 \mu\text{g/g}$ of whole wheat) (Table 4.3 and Figure 4.5). No difference existed among einkorn, durum, and bread wheat species, but among cultivars and populations ($p < 0.05$).

In a study (Hejtmánková et al., 2010), with two einkorn, two emmer, and three spring wheat cultivars (ranged between 5.69-8.57 mg/kg DM) reported significant differences among species and cultivars, which was similar to ours ($p < 0.05$). Demir-2000 and Ç-1252 showed similar results to hard wheat cultivars. Einkorn, emmer, and soft wheat cultivars (Abdel-Aal and Rabalski, 2008), ranged for α -tocopherol between 5.50-11.90 $\mu\text{g/g}$. Other cultivars and populations, on the other hand, had lower values than those. (Brandolini et al., 2015) reported the α -tocopherol content of einkorn, emmer, durum, spelt, and bread wheat ranged between 9.90 to

15.00 mg/kg DM and found significant differences among species contrary to our study.

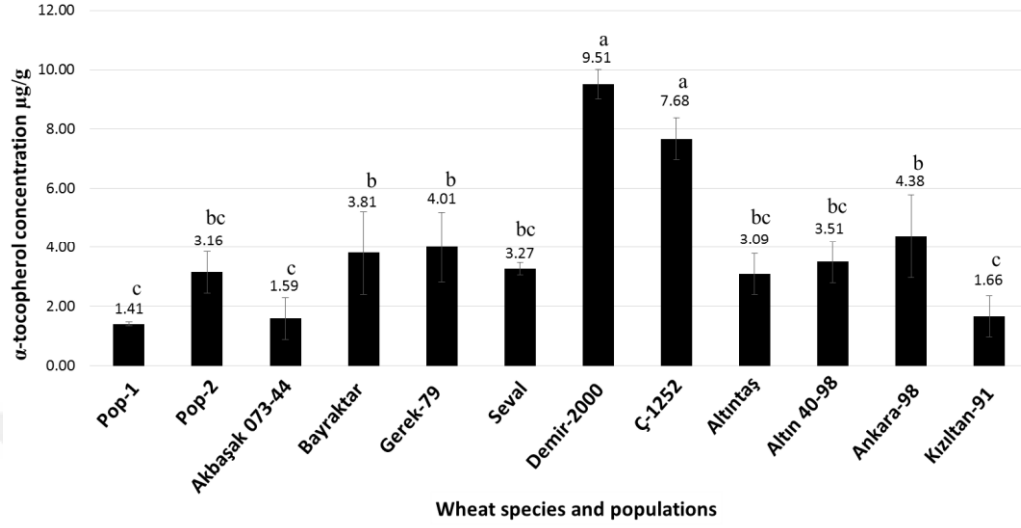


Figure 4.5. α -tocopherol content of wheat species and population. Mean values with the same letter within the column are not significantly different from each other at $P < 0.05$.

5. CONCLUSION AND RECOMMENDATIONS

In this study, total phenolic, flavonoid content, antioxidant activity, phenolic acids, and α -tocopherol content of wheat species, cultivars, and populations were investigated. The results and the study seemed satisfactory based on the data collected and knowledge gained. According to data collected in the study, total phenol content of einkorns were significantly higher than bread and durum wheat cultivars ($p < 0.05$), there was, however, no significant differences between bread and durum wheat cultivars ($p < 0.05$). Differences within bread and durum wheat cultivars were evident. However, total flavonoid content variation among einkorns, bread, and durum wheat were adverse, which there was no differences among einkorns, bread and durum wheat species and populations. Antioxidant activity of wheat species and populations were significantly different within einkorns, bread, and durum wheat species and populations. Ferulic acid, *p*-coumaric acid and α -tocopherol content of wheat species and cultivars were also significantly different within or among wheat species. The contents of einkorns were significantly higher than bread and durum wheat cultivars.

There are some challenges to compare the results of this study with the previous studies due to the range of cultivation and environmental effect, wheat cultivars used, varying testing methods, and extraction solvents. Although the use of only one solvent may be effective, the solubility of individual phenolics differs and the results may not be representative of the true phenolic content of any wheat cultivar. In addition, solvents may not have the capability to remove the insoluble bound phenolics, hence the antioxidant capacity of this phenolics would be significantly underestimated. Environment and genotype are also two strong factors, so even comparisons of the phenolic contents of the same wheat cultivar grown in different locations, or years, become difficult to assess. Whether any of these antioxidant assays are applicable as surrogates of physiological or biochemical reactions that occur within the body is still a matter of debate. Therefore, differences between previous studies and our study can be explained by differences in species, harvest conditions, storing conditions, and extraction methods.

Preference of consumers in preventing and controlling chronic diseases by cereal based diets may successfully be carried out by cultivating new wheat cultivars rich in bioactive compounds. In this study, einkorn populations had significantly higher ferulic, *p*-coumaric and TPCs than modern bread and durum wheat cultivars ($p < 0.05$). Results, here, suggested the possibility of production of einkorn wheat populations, and hopefully possibility of cultivars rich in particular health beneficial components may provide benefit to the consumers. In addition, higher TPC of einkorn may offer novel wheat genetic resources for the improvement of new wheat cultivars and the development of wheat-based functional foods. In this sense, the genes that are responsible for the phytochemical content of hulled einkorn wheat populations can be transformed into bread and durum wheat species and cultivars.

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APPENDICES

APPENDICES

Appendix A IC_{50} Calibration Graphs of Wheat Species and Populations

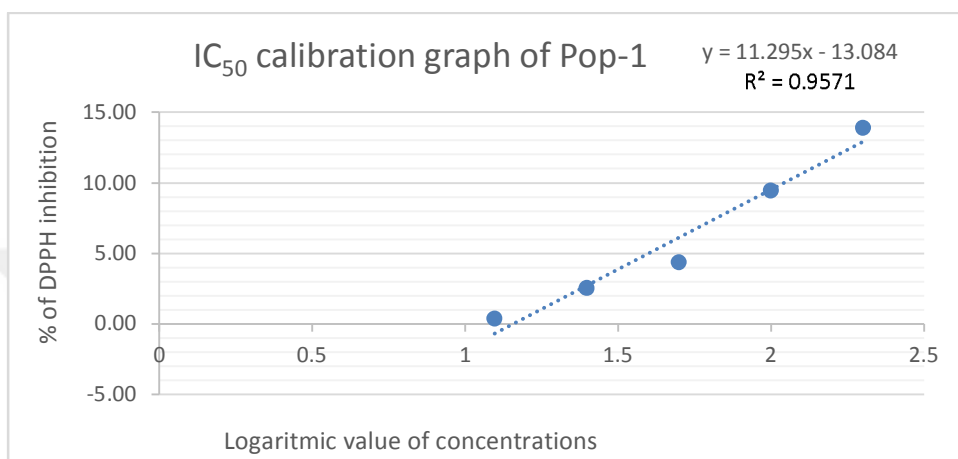


Figure A.1. IC_{50} calibration graph of Pop-1.

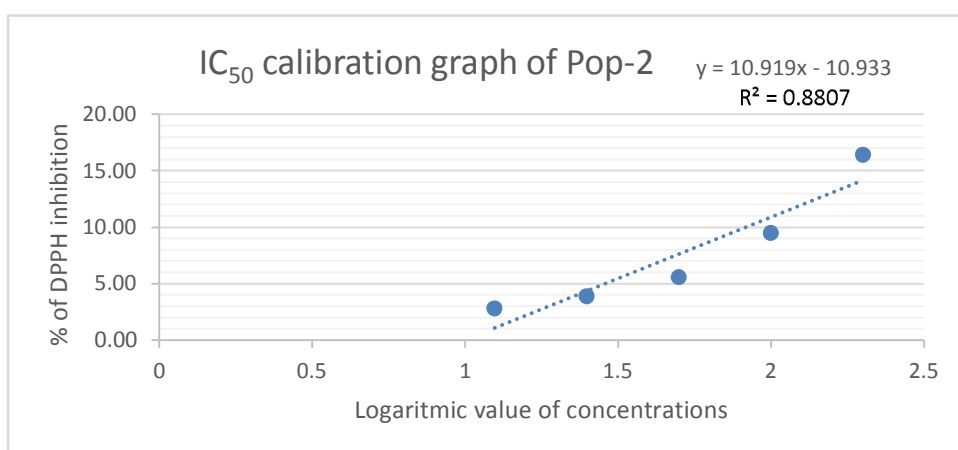


Figure A.2. IC_{50} calibration graph of Pop-2.

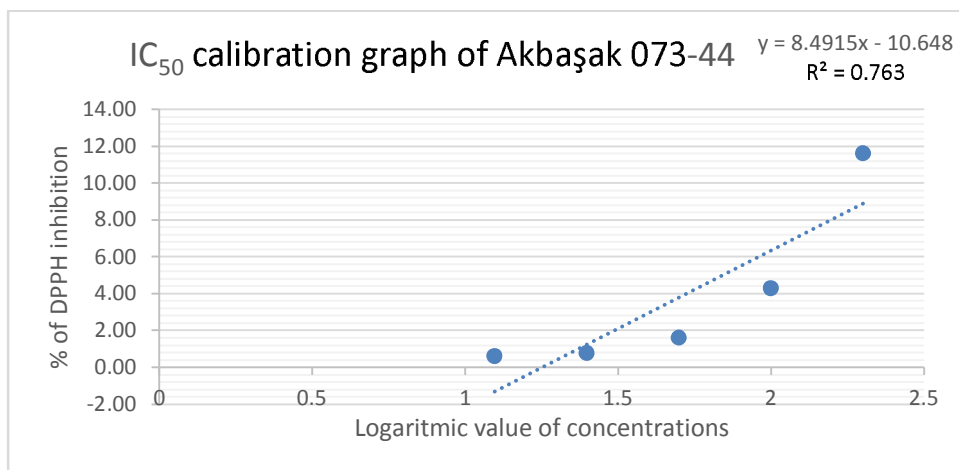


Figure A.3. IC₅₀ calibration graph of Akbaşak 073-44.

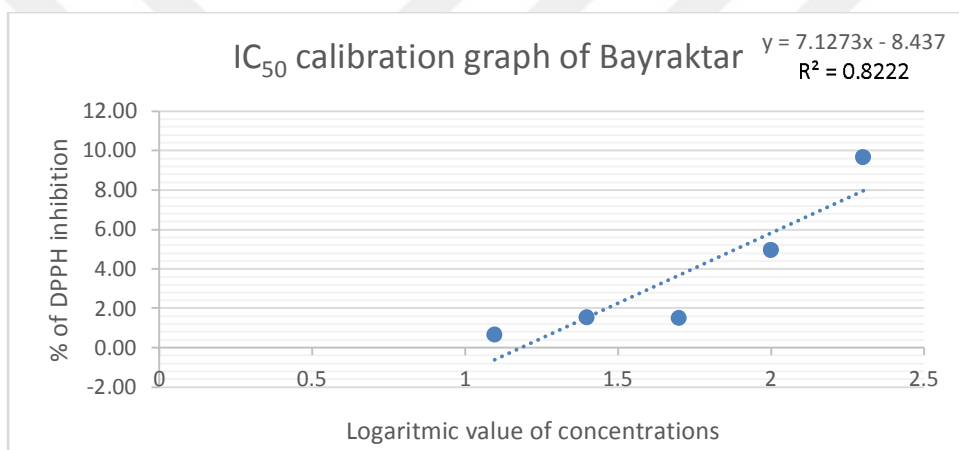


Figure A.4. IC₅₀ calibration graph of Bayraktar.

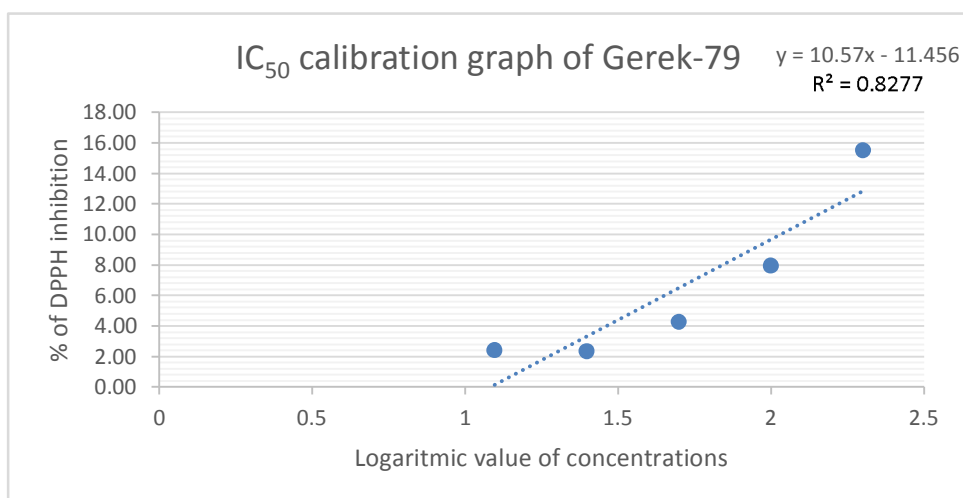


Figure A.5. IC₅₀ calibration graph of Gerek-79.

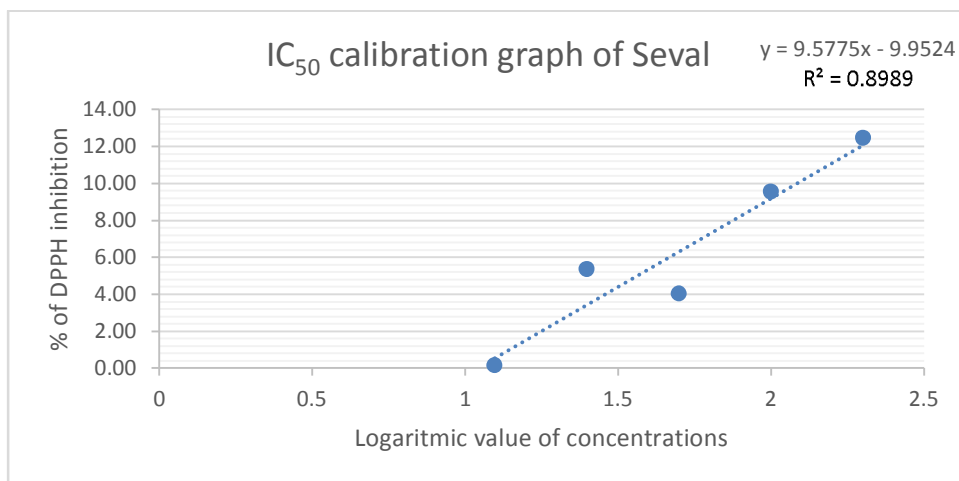


Figure A.6. IC₅₀ calibration graph of Seval.

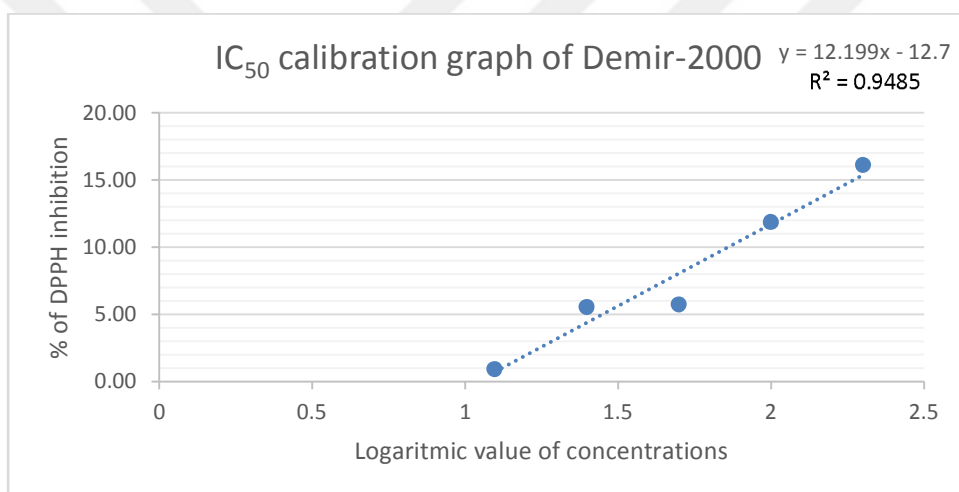


Figure A.7. IC₅₀ calibration graph of Demir-2000.

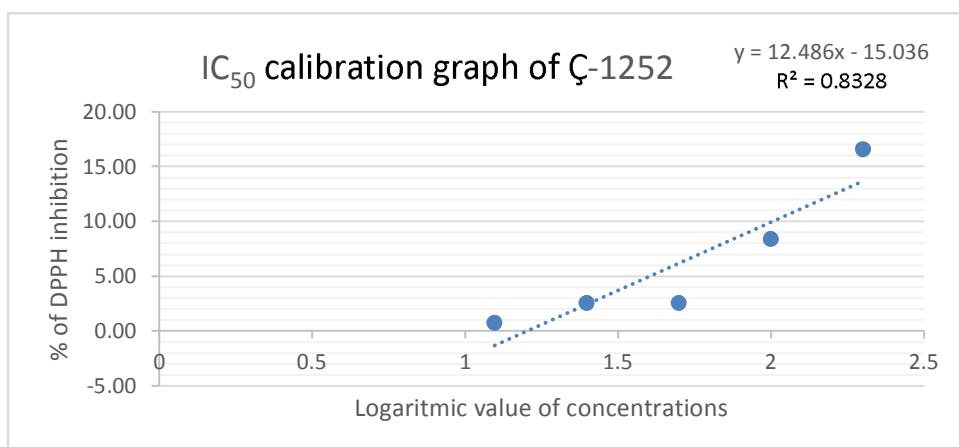


Figure A.8. IC₅₀ calibration graph of Ç-1252.

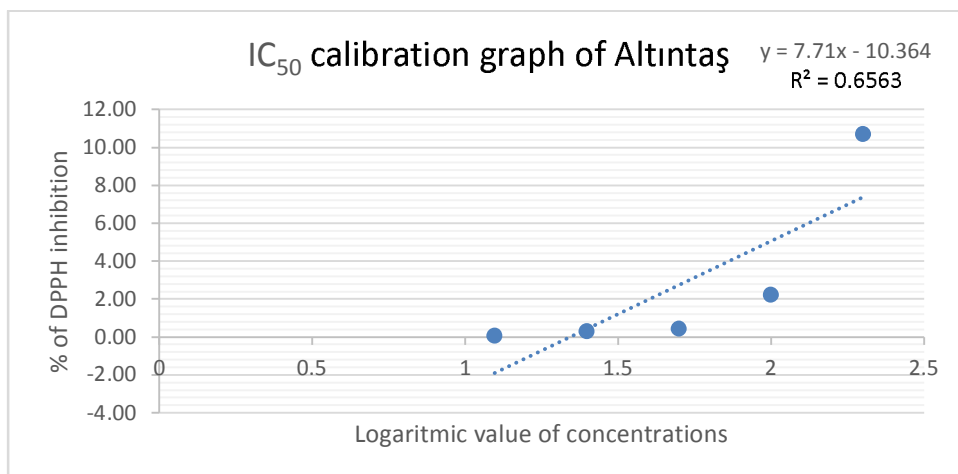


Figure A.9. IC₅₀ calibration graph of Altıntaş.

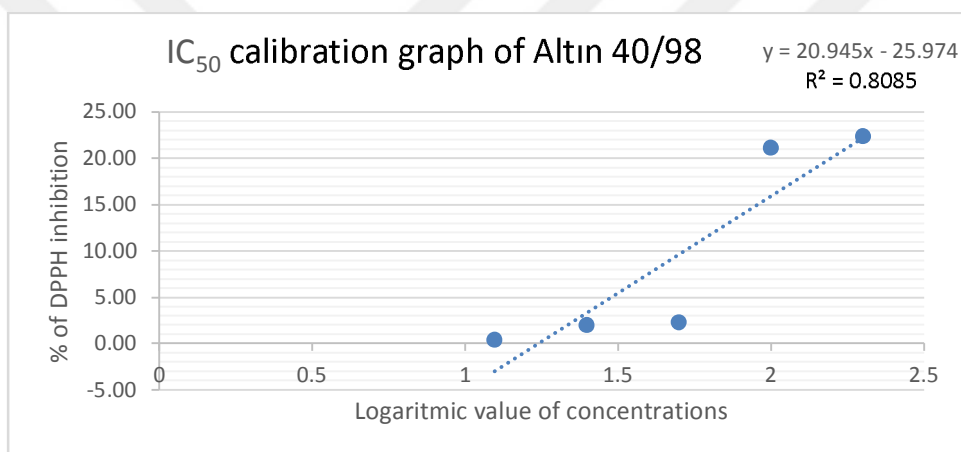


Figure A.10. IC₅₀ calibration graph of Altın 40/59.

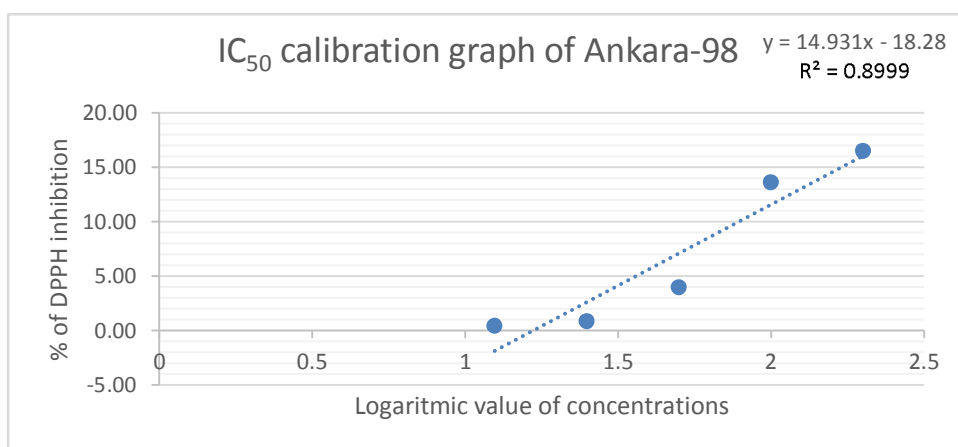


Figure A.11. IC₅₀ calibration graph of Ankara 98.

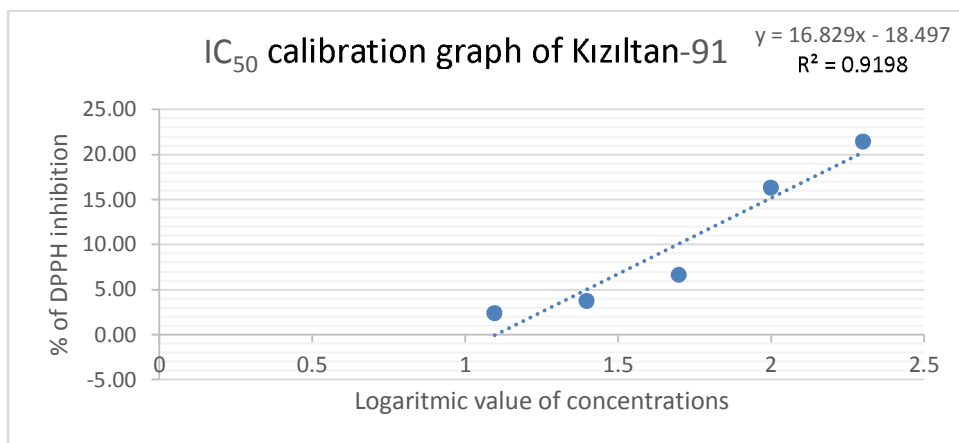


Figure A.12. IC₅₀ calibration graph of Kızıltan-91.

Appendix B HPLC retention times of Wheat Species and Populations

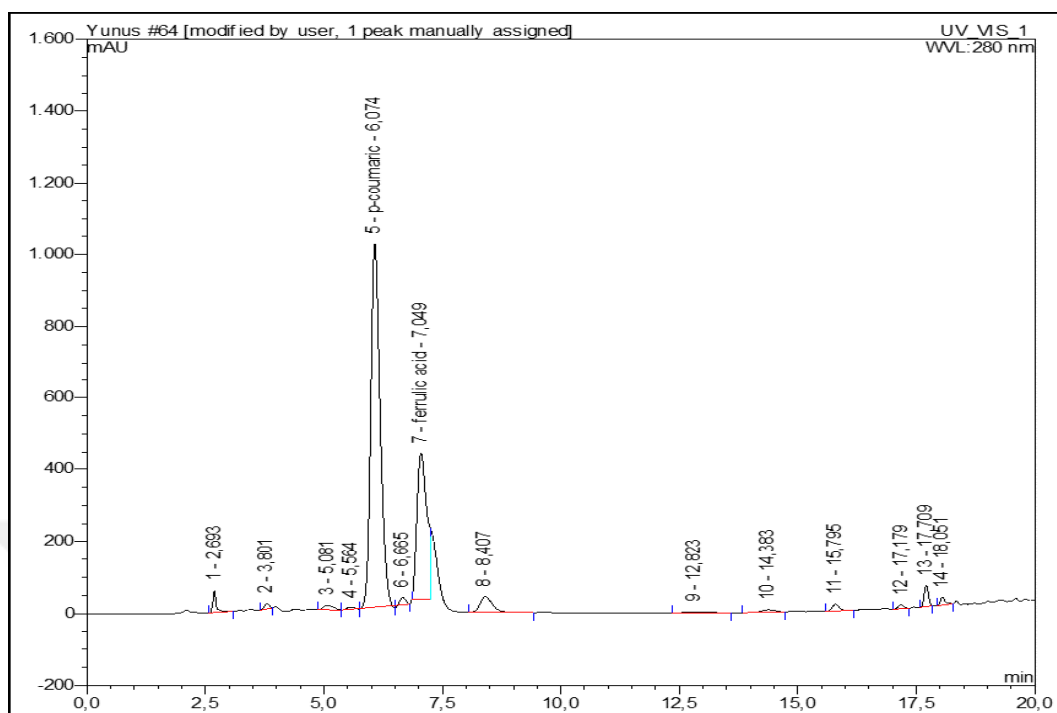


Figure B.1. Ferulic acid and *p*-coumaric acid retention time of Pop-1.

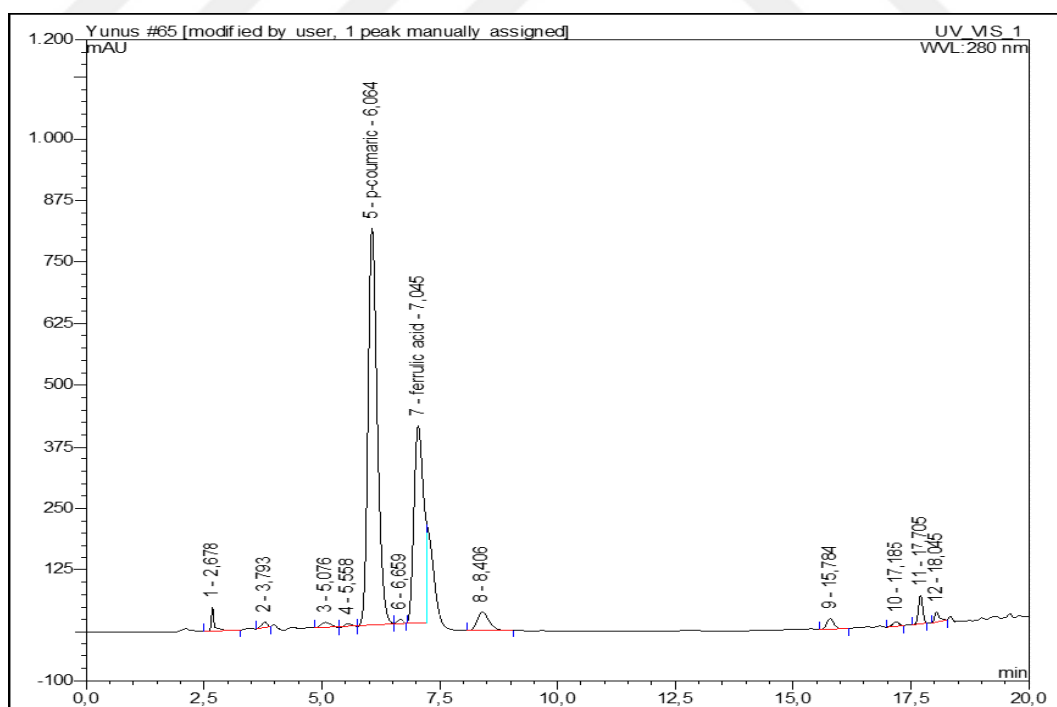


Figure B.2. Ferulic acid and *p*-coumaric acid retention time of Pop-2.

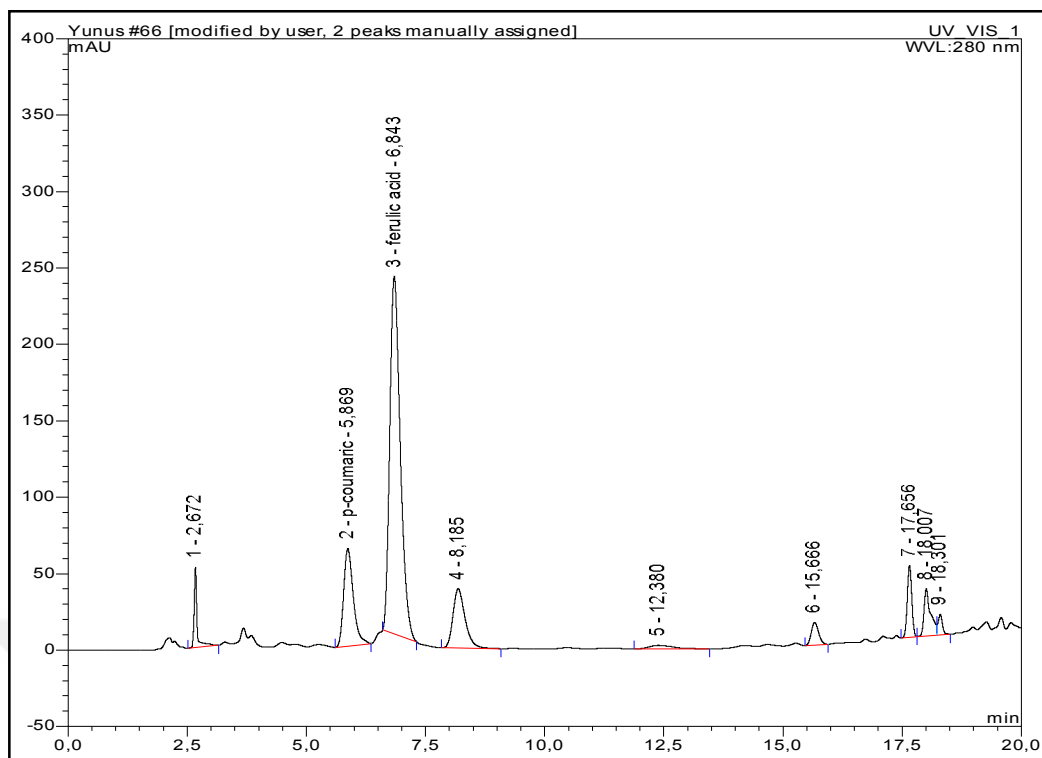


Figure B.3. Ferulic acid and *p*-coumaric acid retention time of Akbaşak 073-44.

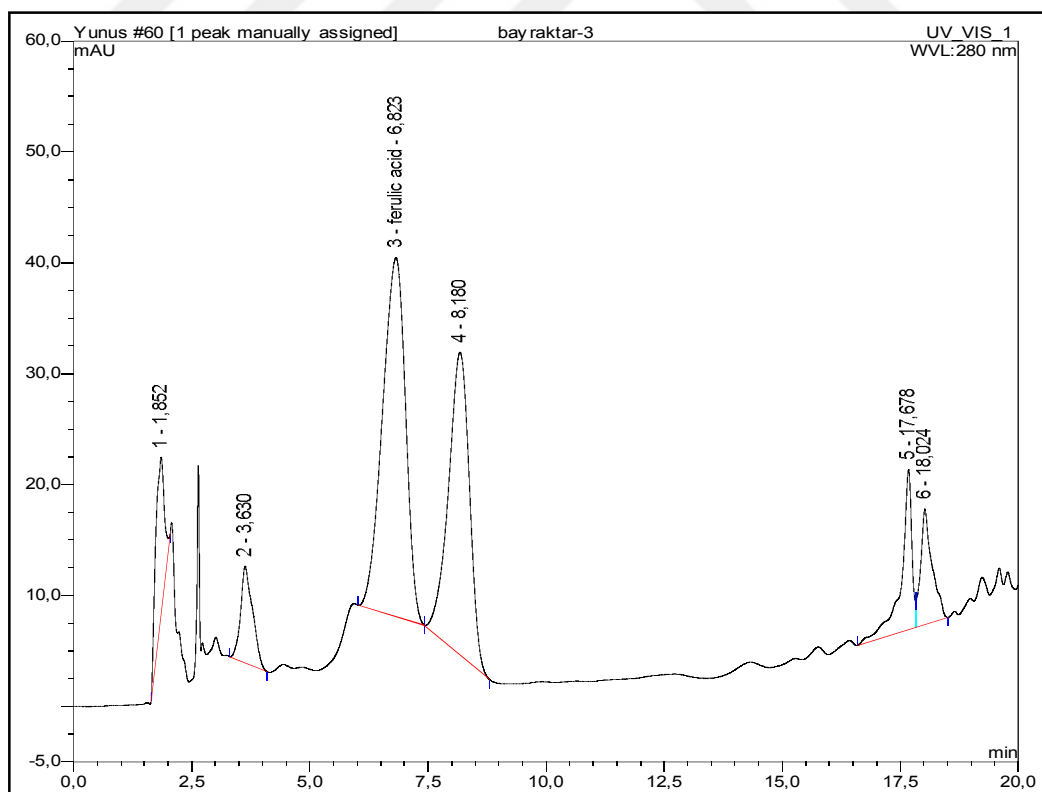


Figure B.4. Ferulic acid and *p*-coumaric acid retention time of Bayraktar.

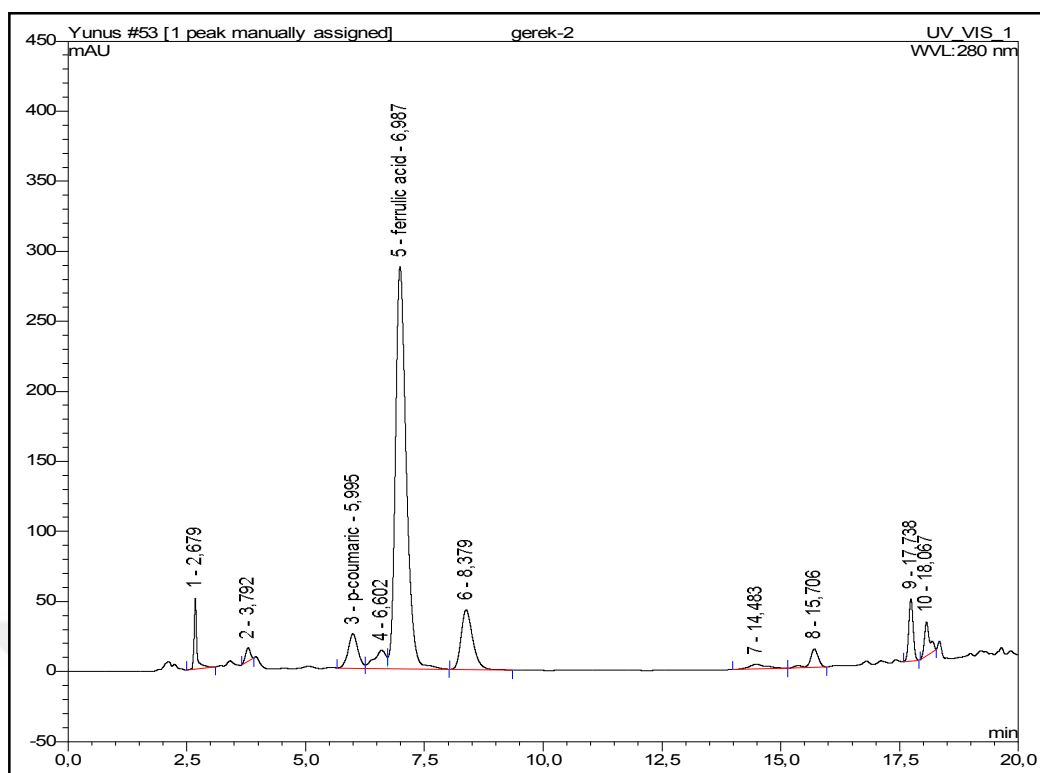


Figure B.5. Ferulic acid and *p*-coumaric acid retention time of Gerek-79.

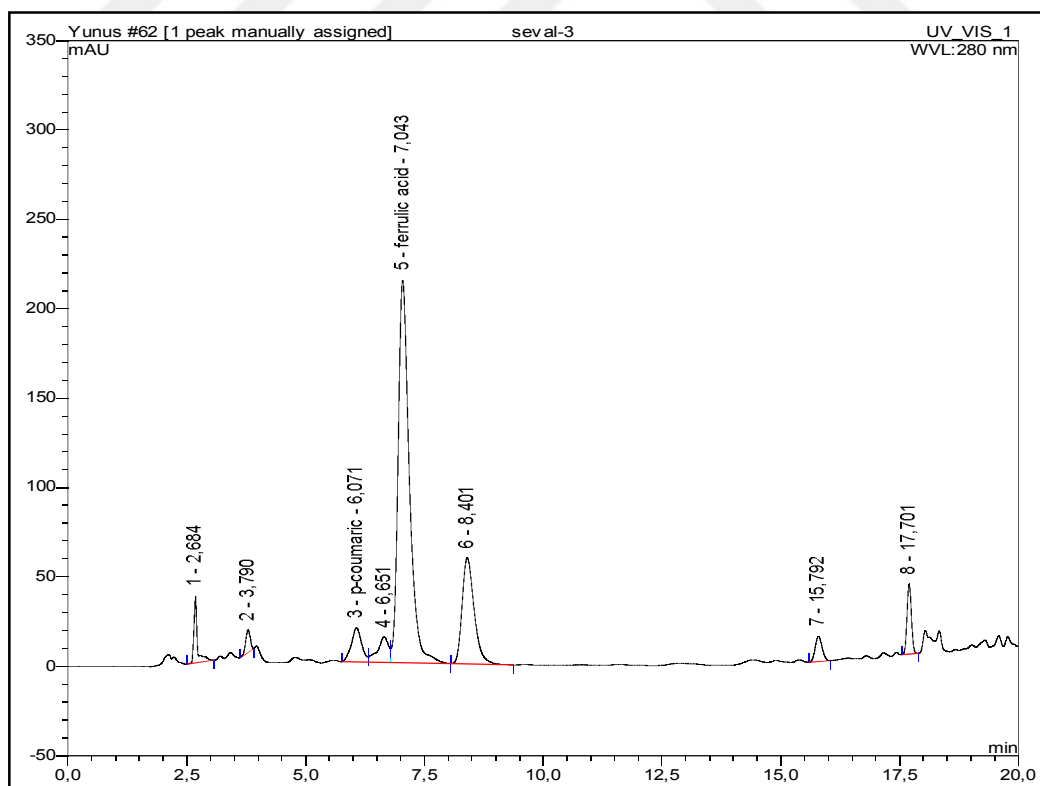


Figure B.6. Ferulic acid and *p*-coumaric acid retention time of Seval.

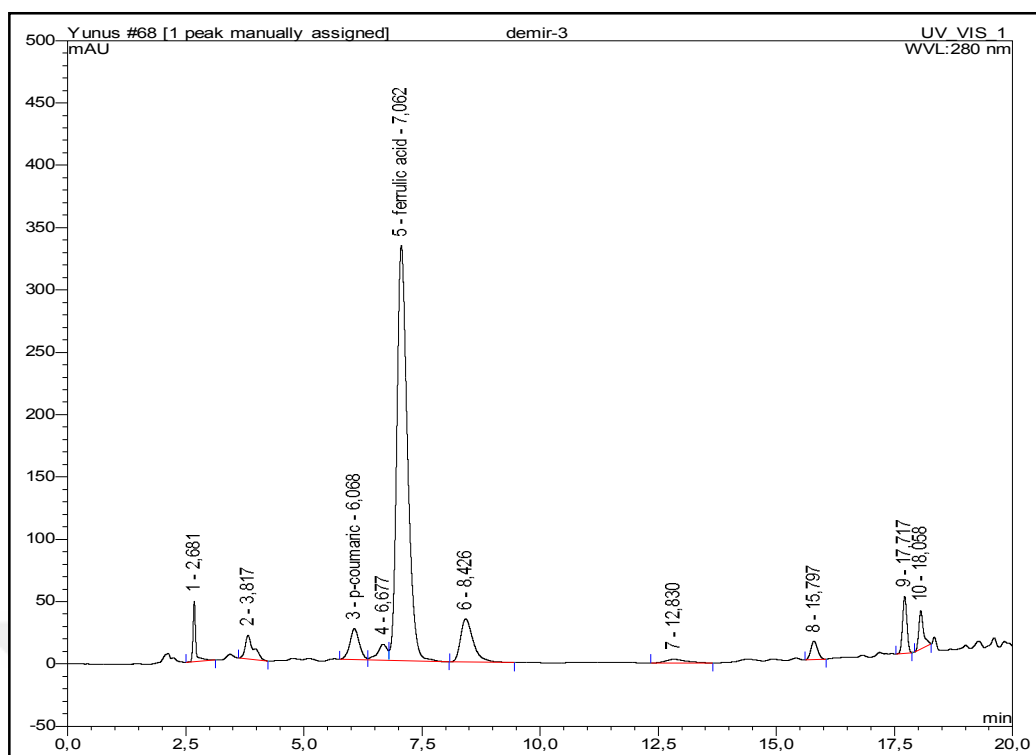


Figure B.7. Ferulic acid and *p*-coumaric acid retention time of Demir-2000.

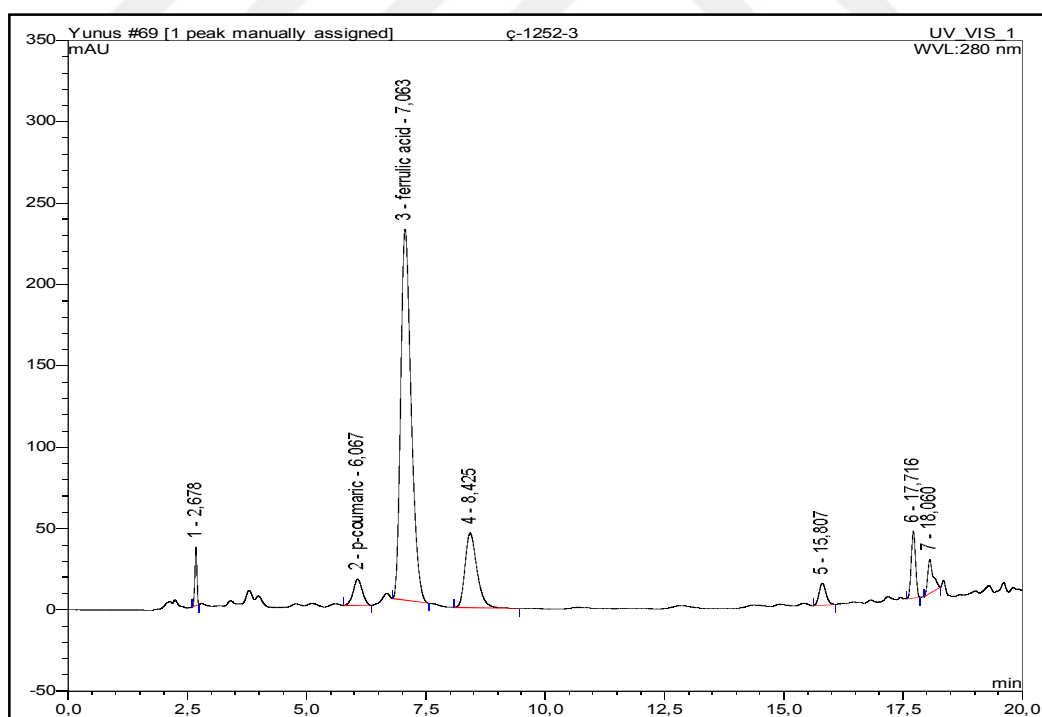


Figure B.8. Ferulic acid and *p*-coumaric acid retention time of Ç-1252.

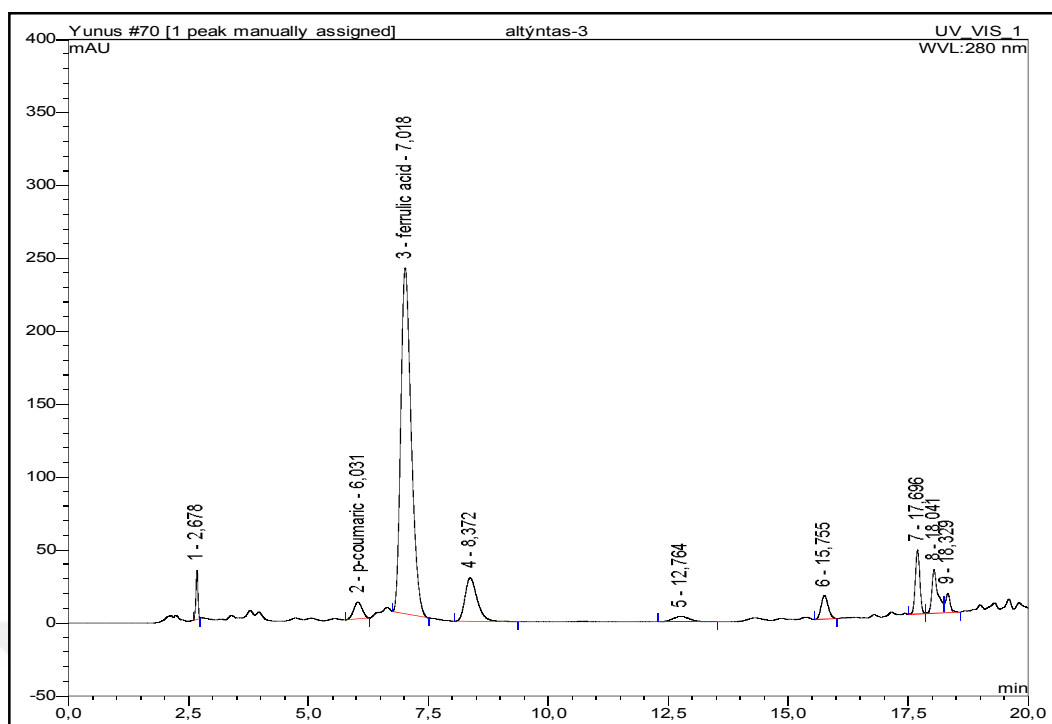


Figure B.9. Ferulic acid and *p*-coumaric acid retention time of Altıntaş.

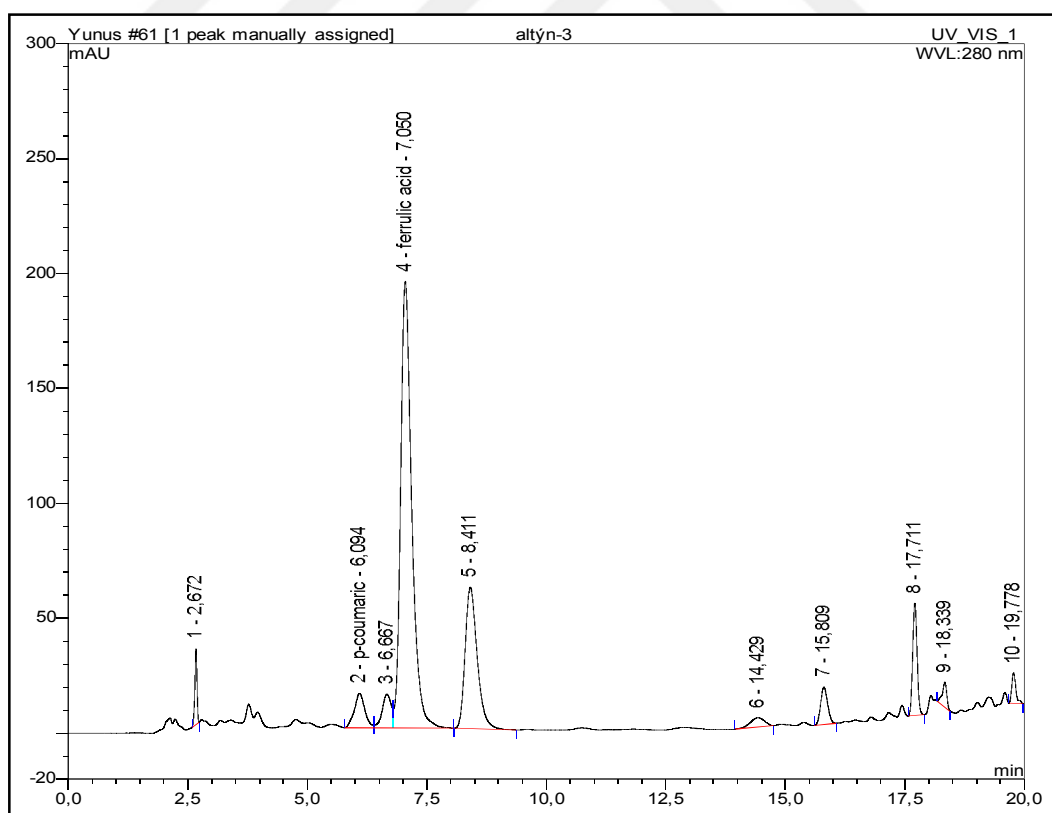


Figure B.10. Ferulic acid and *p*-coumaric acid retention time of Altın 40/98.

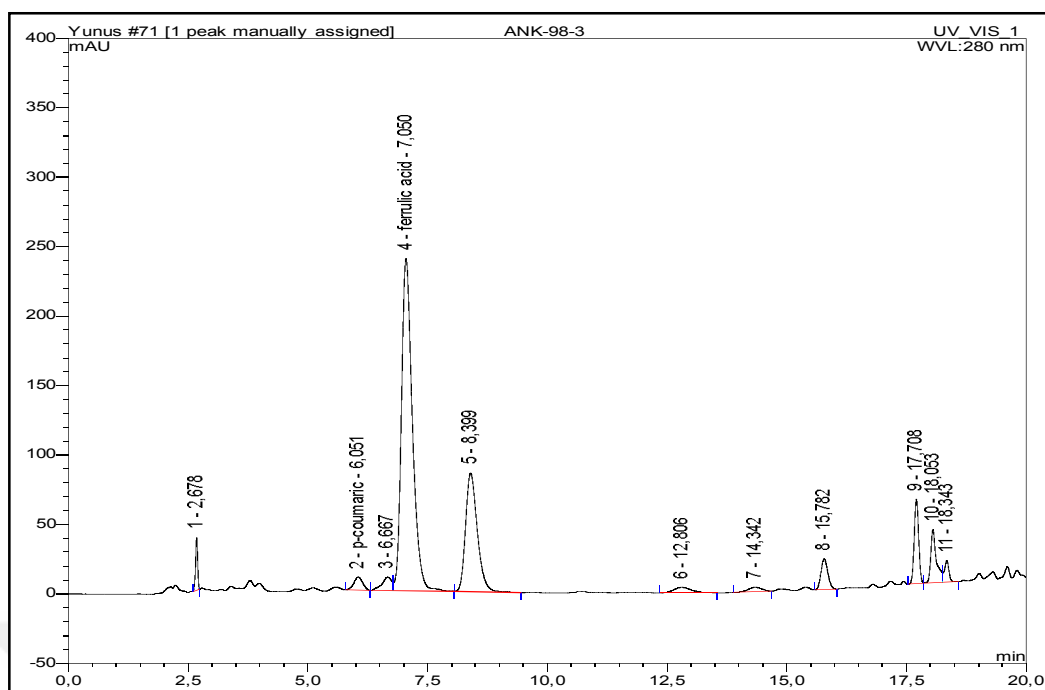


Figure B.11 Ferulic acid and *p*-coumaric acid retention time of Ankara-98.

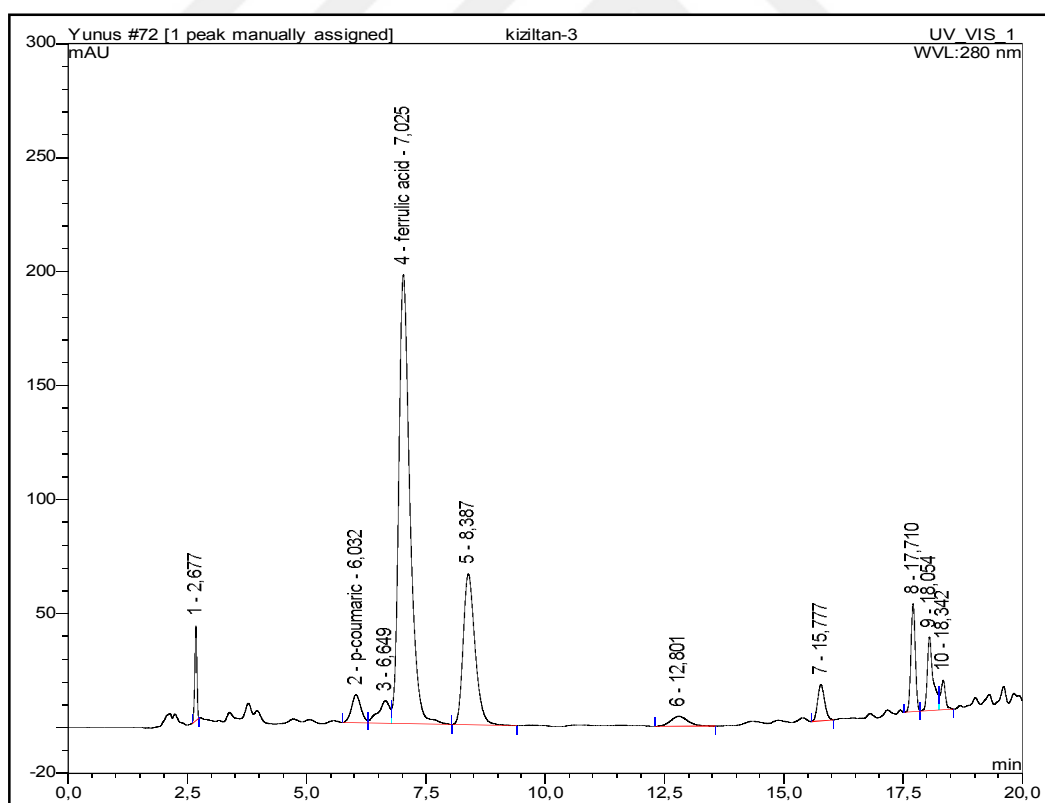


Figure B.12. Ferulic acid and *p*-coumaric acid retention time of Kızıltan-91.

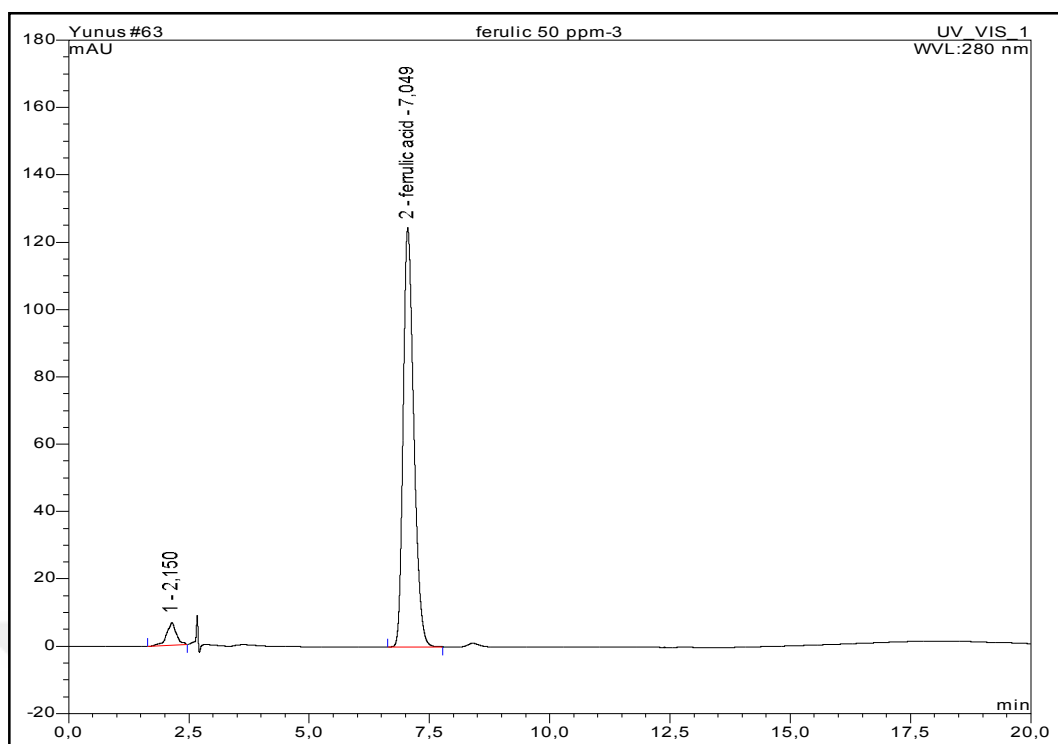


Figure B.13. Ferulic acid retention time.

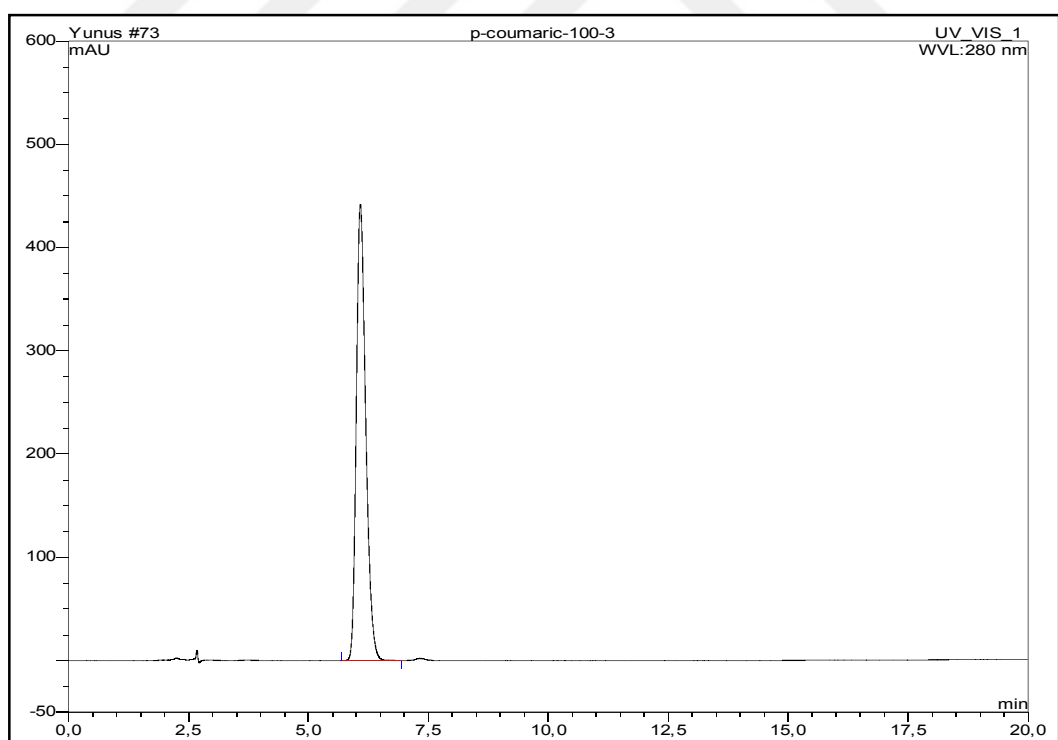


Figure B.14. *p*-coumaric retention time.

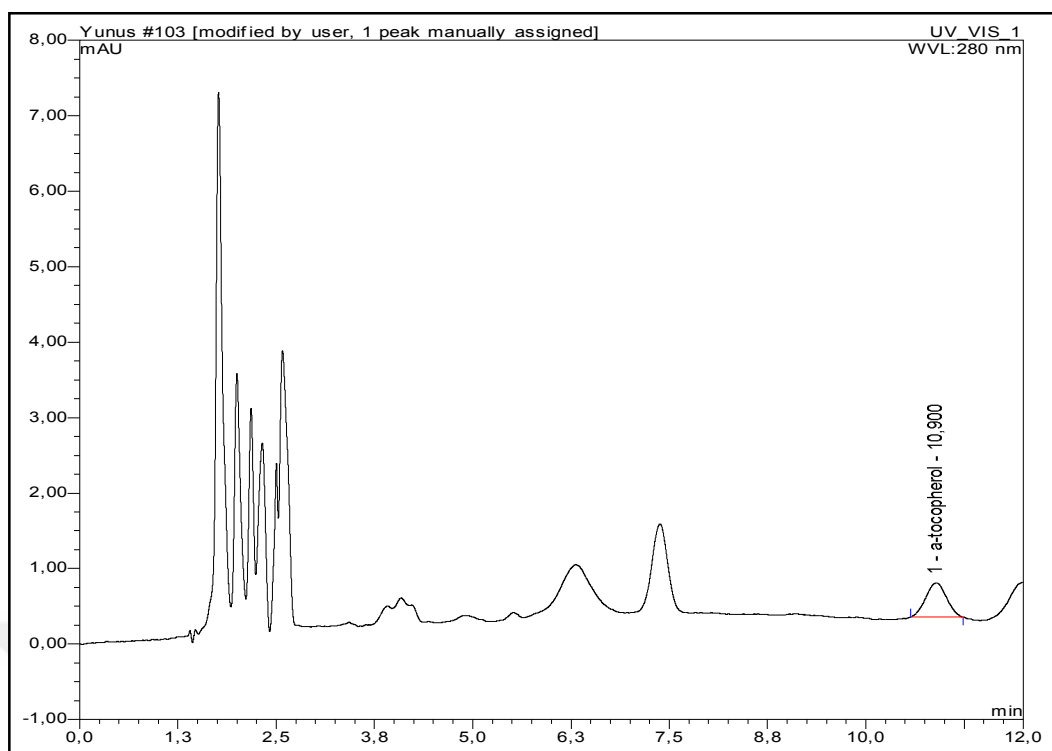


Figure B.15. α -tocopherol retention time of Pop-1.

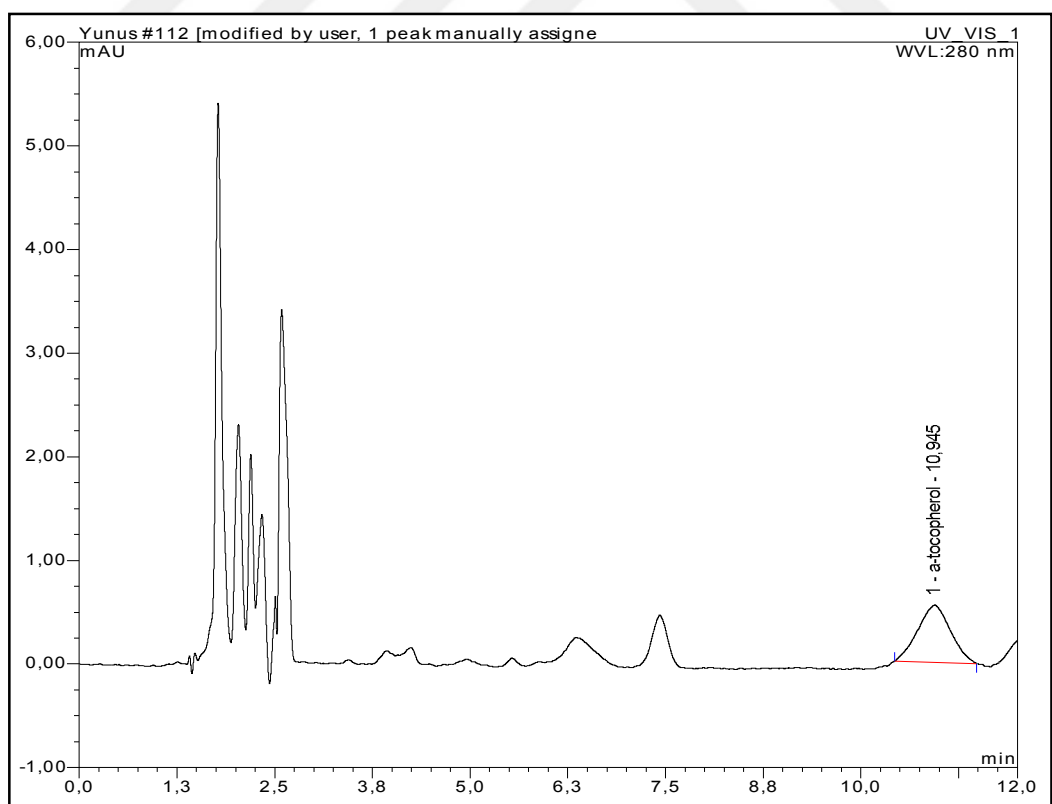


Figure B.16. α -tocopherol retention time of Pop-2

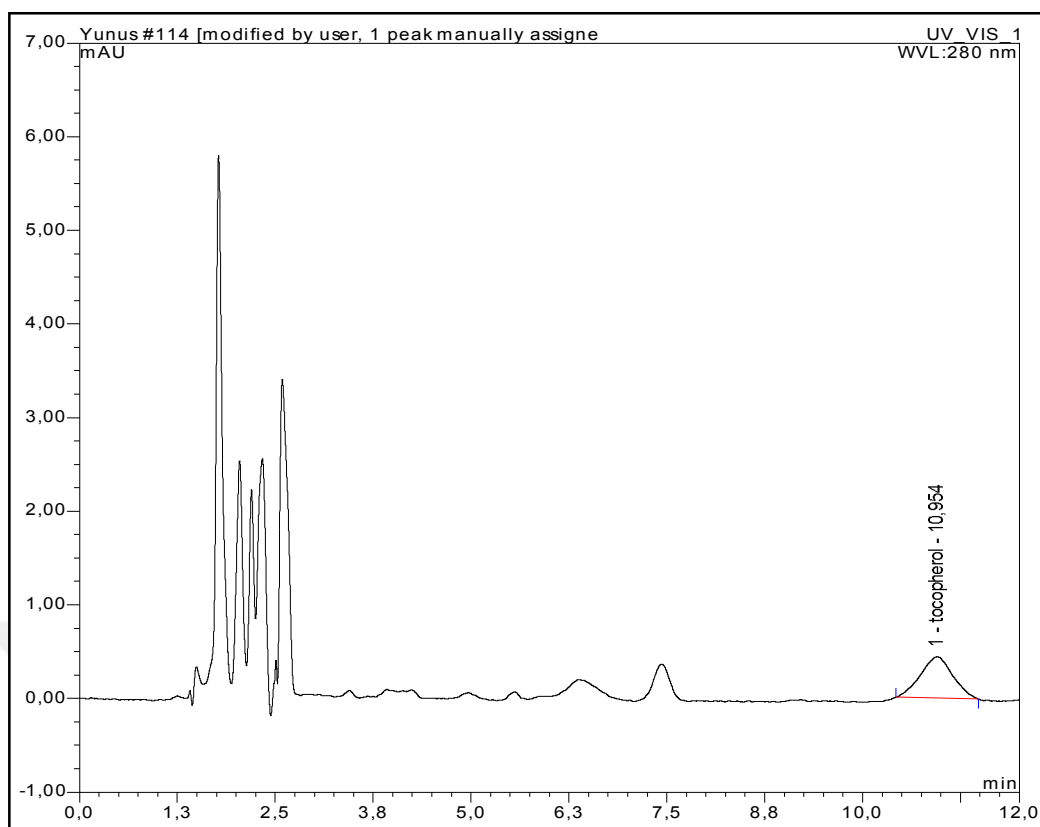


Figure B.17. α -tocopherol retention time of Akbaşak 073-44.

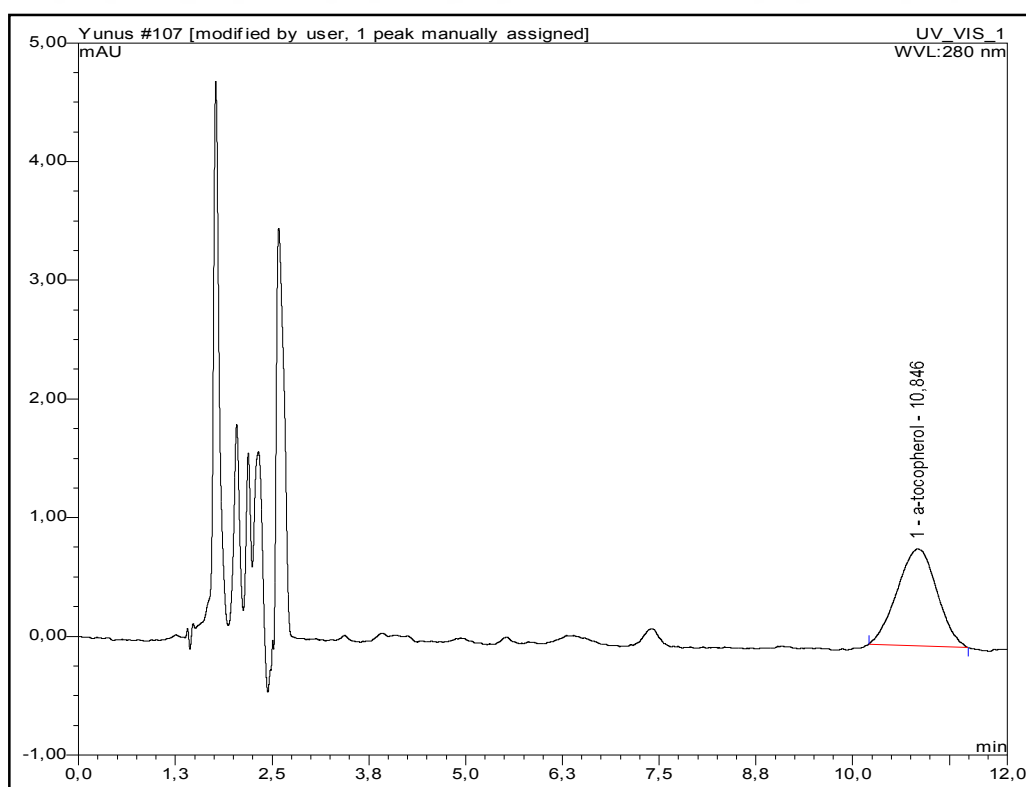


Figure B.18. α -tocopherol retention time of Bayraktar.

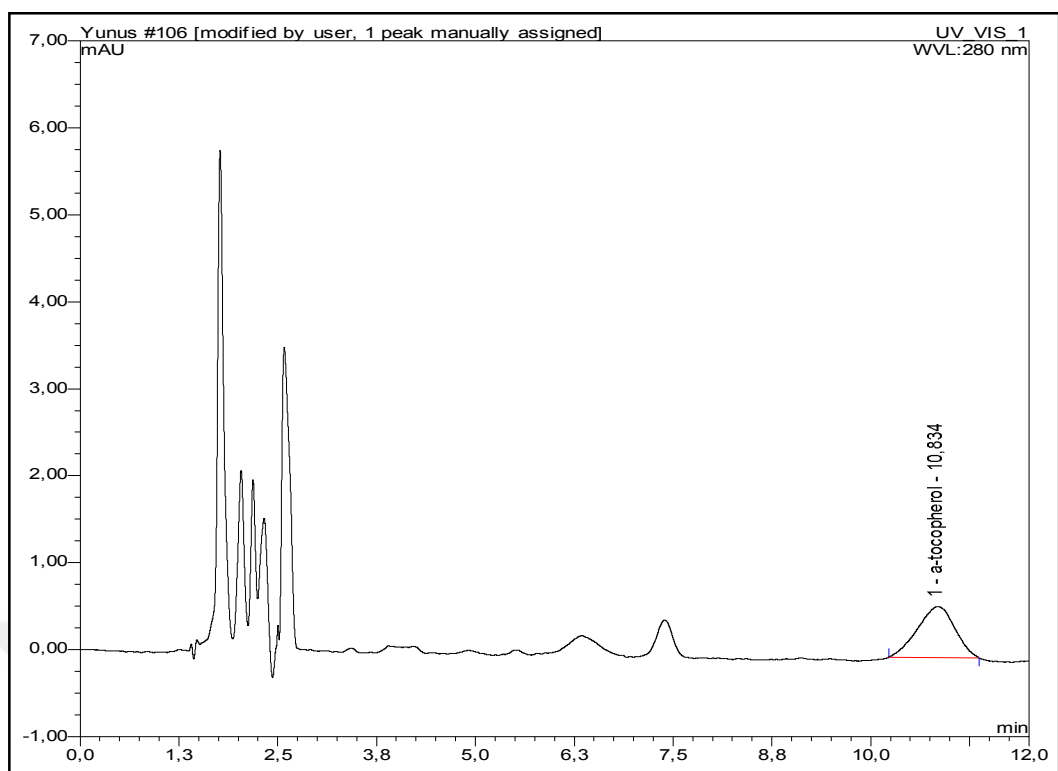


Figure B.19. α -tocopherol retention time of Gerek-79.

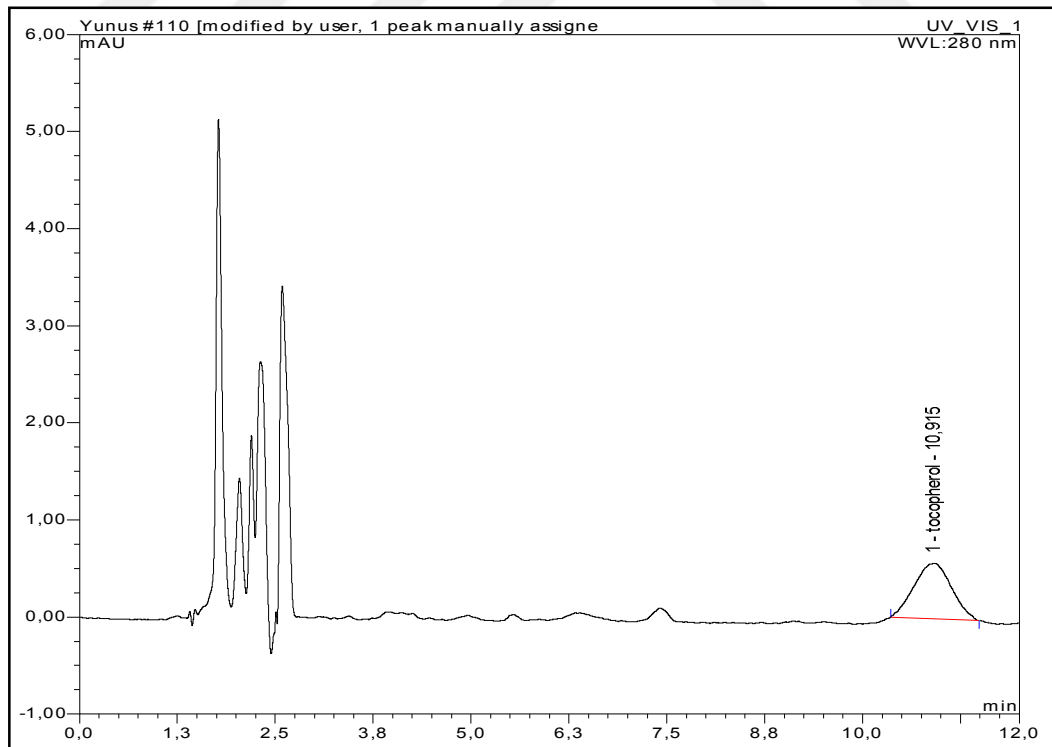


Figure B.20. α -tocopherol retention time of Seval.

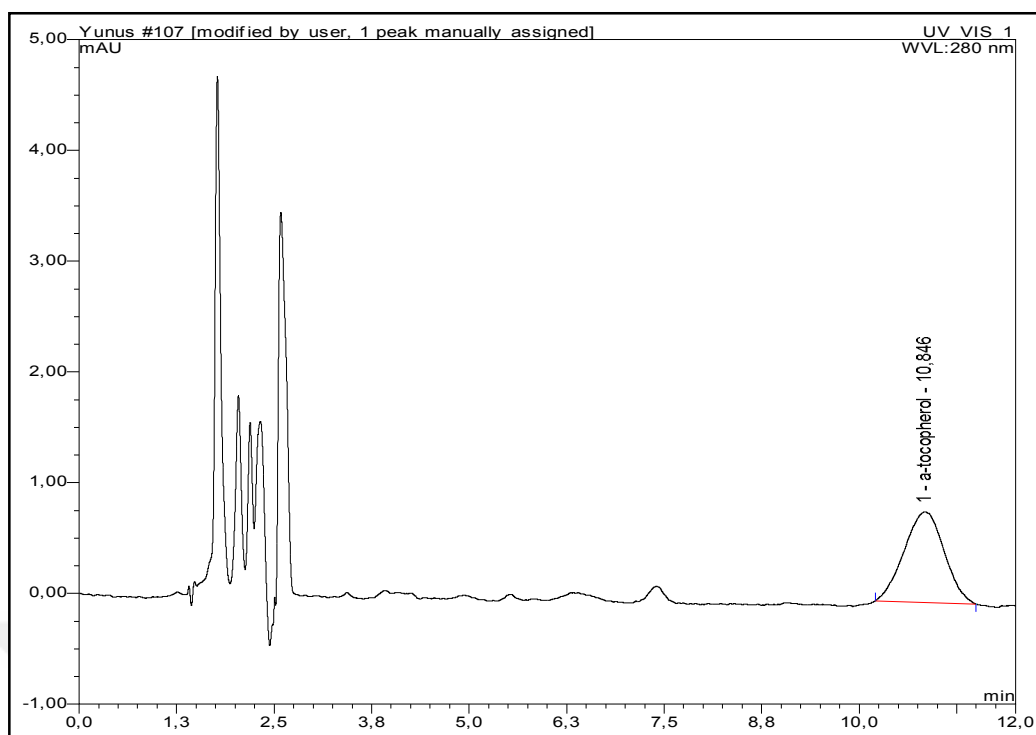


Figure B.21. α -tocopherol retention time of Demir-2000.

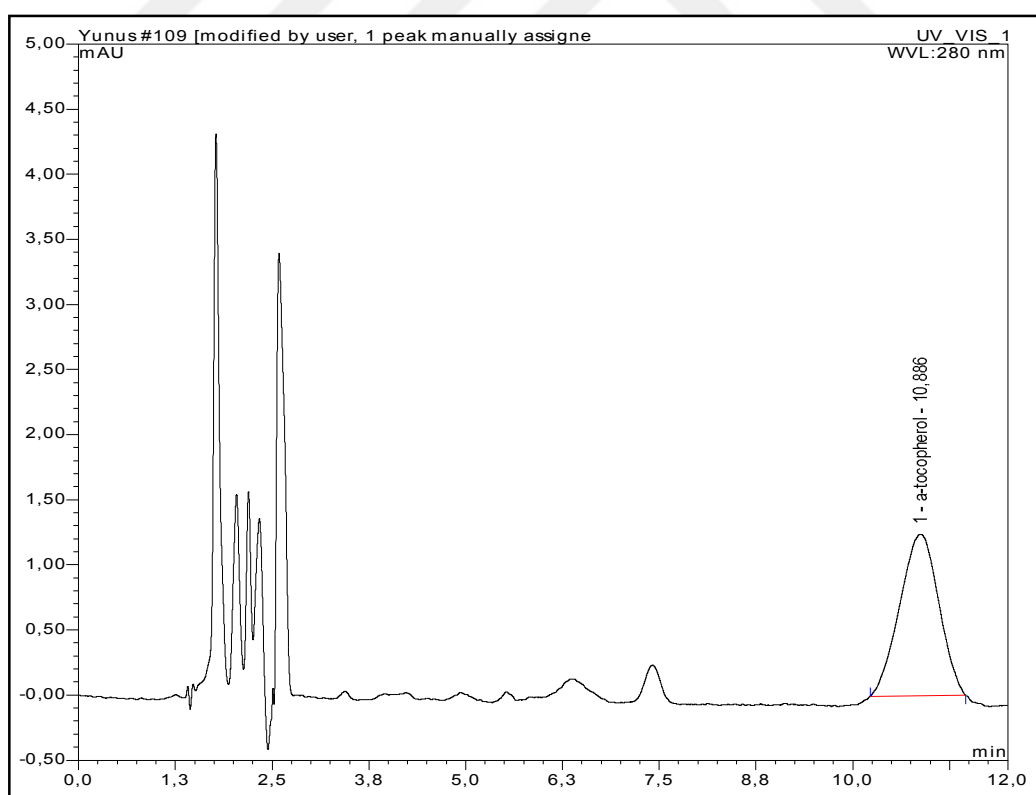


Figure B.22. α -tocopherol retention time of Ç-1252.

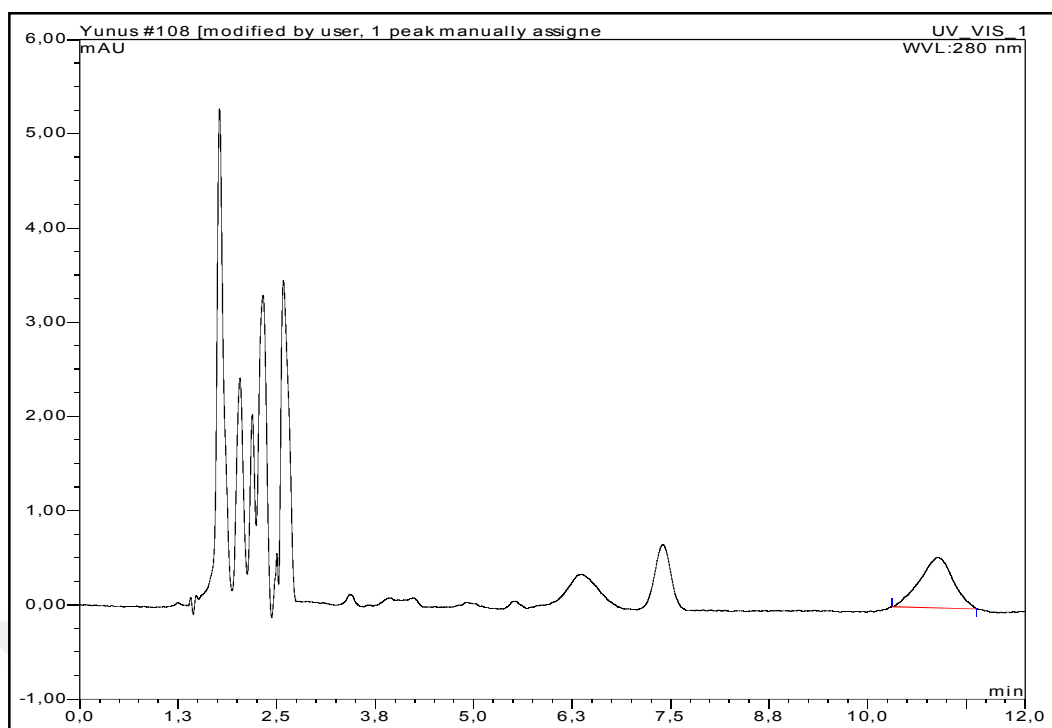


Figure B.23. α -tocopherol retention time of Altıntaş.

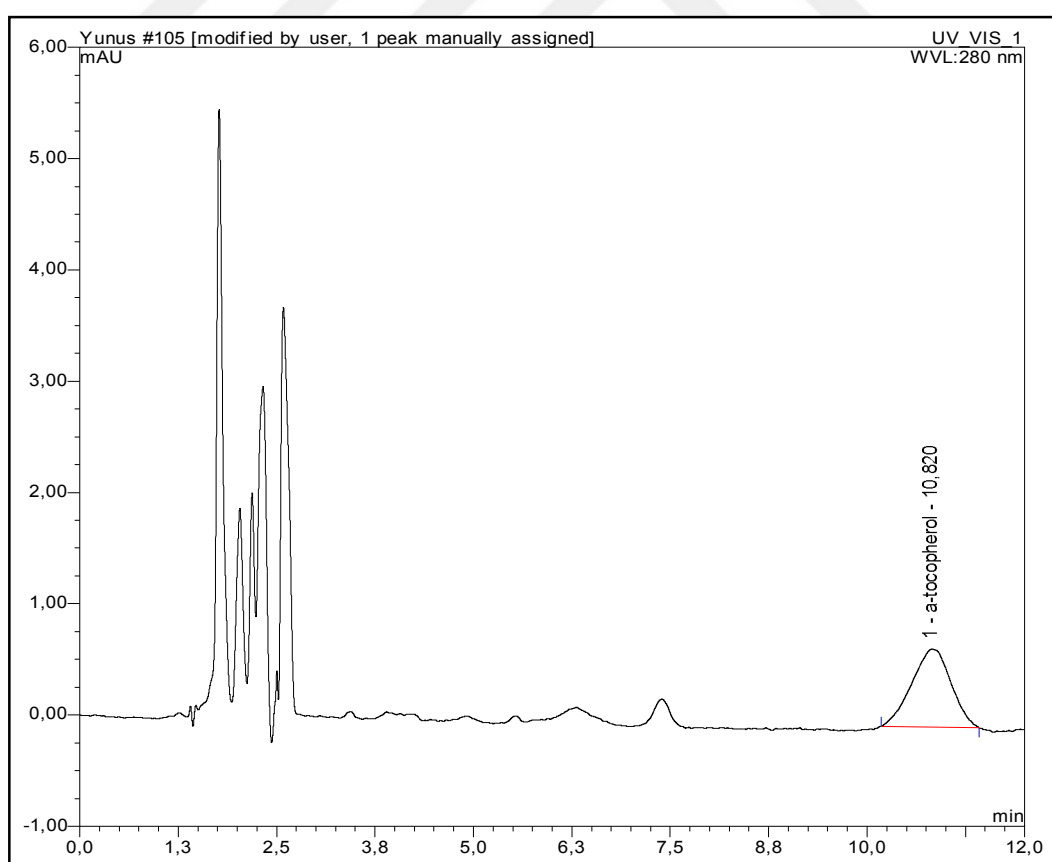


Figure B.24. α -tocopherol retention time of Altın 40/98.

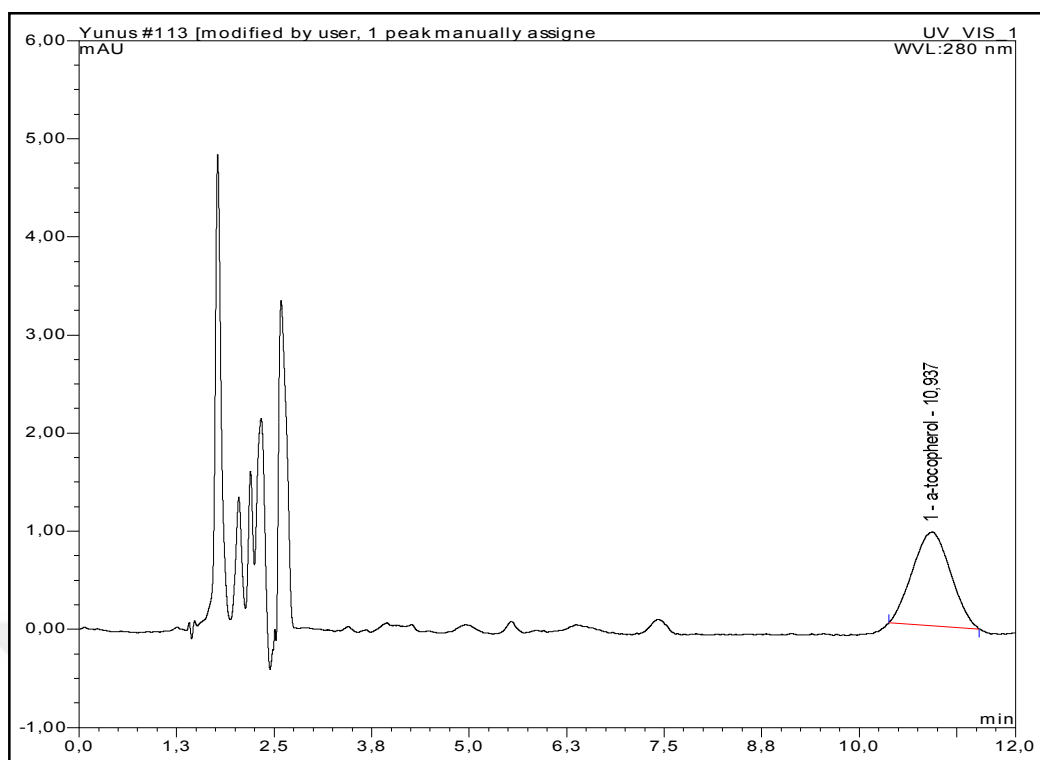


Figure B.25. α -tocopherol retention time of Ankara-98.

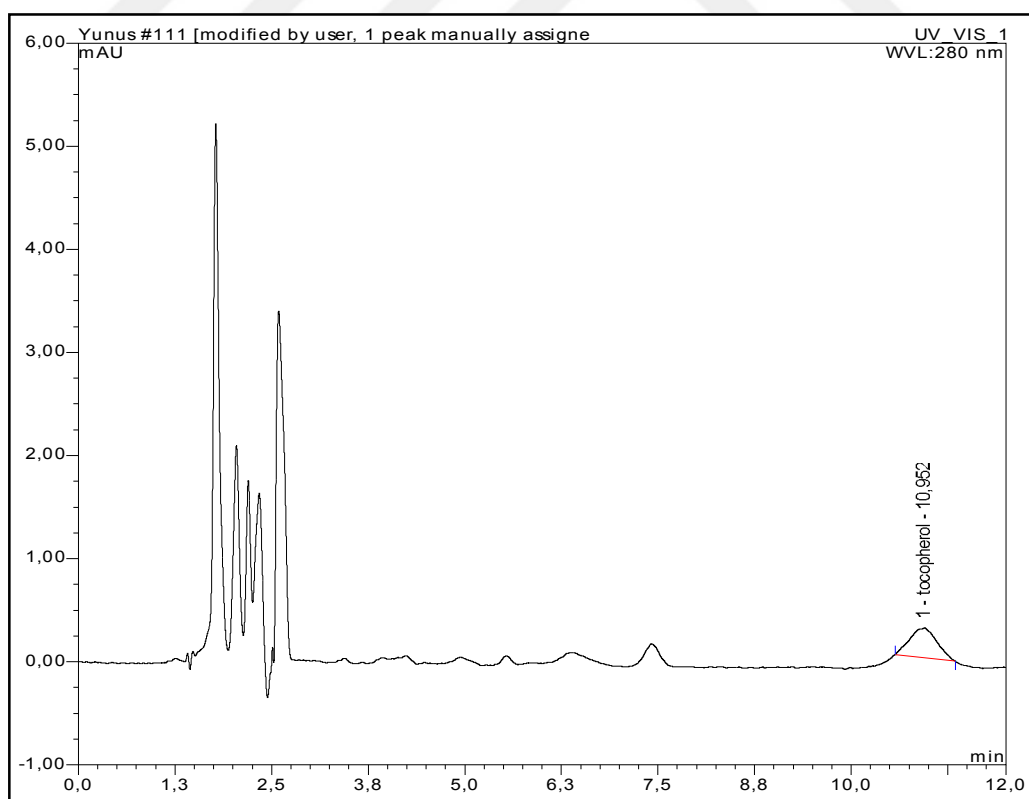


Figure B.26. α -tocopherol retention time of Kızıltan-91.

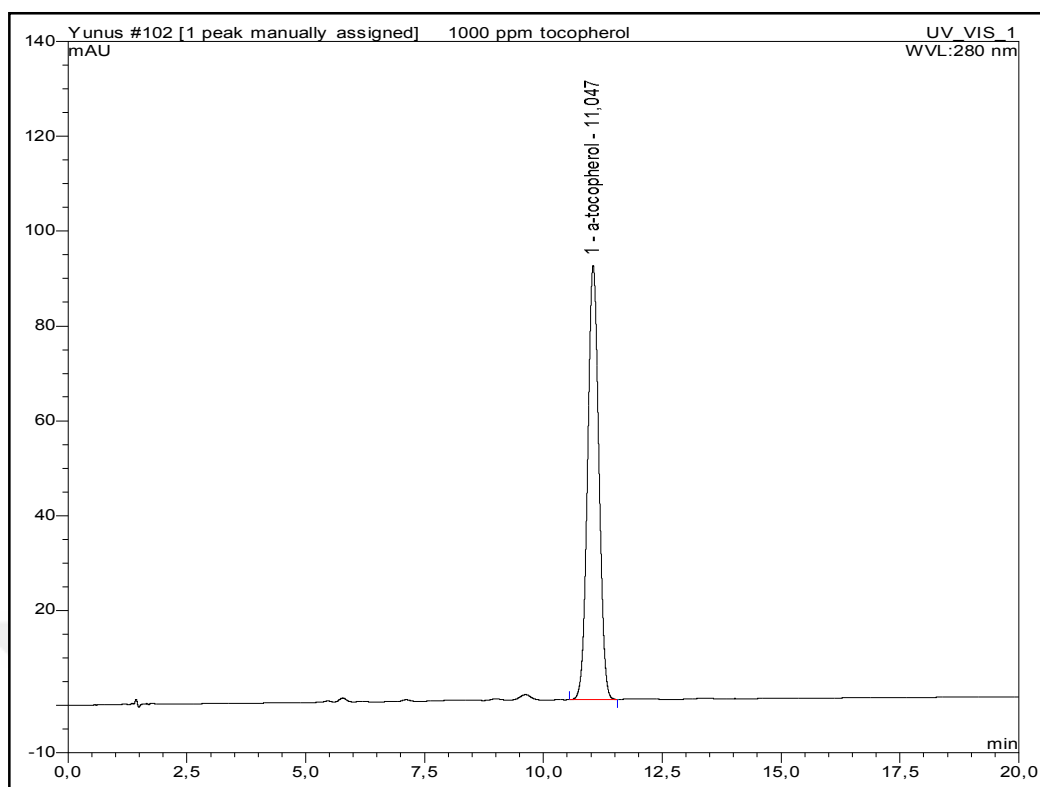


Figure B.27. α -tocopherol retention time.

Appendix C ANOVA table of DPPH assay

Table C.1. Analysis of variance, which was run in a three replicate randomized block design for 12.5 $\mu\text{g/mL}$ extract of DPPH inhibition of wheat species / cultivars.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	1.848	57.882	0.000
Blocks (Replications)	2	0.004	0.117	0.738
Error	22	0.032		
Total	35			

Table C.2. Analysis of variance, which was run in a three replicate randomized block design for 25 $\mu\text{g/mL}$ extract of DPPH inhibition of wheat species / cultivars.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	7.648	37.862	0.000
Blocks (Replications)	2	0.005	0.107	0.438
Error	22	0.022		
Total	35			

Table C.3. Analysis of variance, which was run in a three replicate randomized block design for 50 $\mu\text{g/mL}$ extract of DPPH inhibition of wheat species / cultivars.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	7.288	4.956	0.007
Blocks (Replications)	2	1.462	0.995	0.340
Error	22	1.470		
Total	35			

Table C.4. Analysis of variance, which was run in a three replicate randomized block design for 100 $\mu\text{g/mL}$ extract of DPPH inhibition of wheat species / cultivars.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	83.331	11.663	0.000
Blocks (Replications)	2	18.313	2.563	1.000
Error	22	7.145		
Total	35			

Table C.5. Analysis of variance, which was run in a three replicate randomized block design for 200 $\mu\text{g/mL}$ extract of DPPH inhibition of wheat species / cultivars.

Source	Df	Mean Square	F	Sig.
Species / Populations and Cultivars	11	30.658	22.261	0.000
Blocks (Replications)	2	1.675	1.216	0.294
Error	22	1.377		
Total	35			

CURRICULUM VITAE

PERSONAL INFORMATION

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- 2. Identification and characterization of conserved microRNA in Einkorn (*T. monococcum*) wheat by high yield and simulation analyses.** . A scientific project supported by the Abant İzzet Baysal University Research Foundation, Date: 2015-16, Project no: 2015.03.01.880, Position: Researcher

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SCIENTIFIC ACTIVITY

Poster Presentations

- **Şahin Y**, Yıldım A, Yücesan B, Zencirci N, Erbayram Ş, Gürel E. (2016) “Phytochemical content and antioxidant activity of einkorn (*Triticum monococcum* ssp. *monococcum*), bread (*Triticum aestivum* L.), and durum (*Triticum durum* Desf.) wheat”. The 41st FEBS Congress, **Kuşadası, Aydın, Turkey**.