ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

INVESTIGATING PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND BIOAVAILABILITY OF RAW/STEAM COOKED BUCKWHEAT, BLACK CHICKPEA AND BROWN LENTIL

M.Sc.THESIS

Bengi Ece YALÇIN

Department of Food Engineering

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

PİŞMEMİŞ VE BUHARDA PİŞİRİLMİŞ KARA BUĞDAY, KARA NOHUT VE KAHVERENGİ MERCİMEĞİN FENOLİK MADDE, ANTİOKSİDAN AKTİVİTESİ VE BİYOYARARLILIKLARININ İNCELENMESİ

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HAZİRAN 2014

Bengi Ece Yalçın, a **M.Sc.** student of ITU **Graduate School of Science Engineering and Technology,** student ID **506111506** successfully defended the **thesis** entitled "**Investigating Phenolic Content, Antioxidant Activity and Bioavailability of Raw/Steam Cooked Buckwheat, Blackchickpea and Brown Lentil**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To quarter century that I lived,

FOREWORD

The thesis which you are reading now, has been studied since September 2013 till May 2014 in the Food Engineering Deparment of Istanbul Technıcal University. I should admit that I could not complete easily if I was all alone.

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May 2014

Bengi Ece YALÇIN
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TABLE OF CONTENTS

Page

ABBREVIATIONS

ABTS : 2,2- azinobis 3-ethylbenzothiazoline-6-sulfonice acid
ANOVA : Analysis of Variance
AO : Antioxidant
BC : Black Chickpea
BL : Brown Lentil
BPRBC : Bound phenolics of raw black chickpea
BPRBL : Bound phenolics of raw brown lentil
BPRBW : Bound phenolics of raw buckwheat
BPSBC : Bound phenolics of steam-cooked black chickpea
BPSBL : Bound phenolics of steam-cooked brown lentil
BPSBW : Bound phenolics of steam-cooked buckwheat
BW : Buckwheat
CUPRAC : Cupric Reducing Antioxidant Capacity
DPPH : 2,2-diphenyl-1-picrylhydrazyl
DW : Dry Weight
FPRBC : Free phenolics of raw black chickpea
FPRBL : Free phenolics of raw brown lentil
FPRBW : Free phenolics of raw buckwheat
FPSBC : Free phenolics of steam-cooked black chickpea
FPSBL : Free phenolics of steam-cooked brown lentil
FPSBW : Free phenolics of steam-cooked buckwheat
GAE : Gallic Acid Equivalent
HPLC : High Performance Liquid Chromatography
IN : Solution Entering the Dialysis Tubing
INRBC : IN of raw black chickpea
INRBL : IN of raw brown lentil
INRBW : IN of raw buckwheat
INSBC : IN of steam-cooked black chickpea
INSBL : IN of steam-cooked brown lentil
INSBW : IN of steam-cooked buckwheat
PG : Post Gastric
PGRBC : PG of raw black chickpea
PGRBL : PG of raw brown lentil
PGRBW : PG of raw buckwheat
PGSBC : PG of steam-cooked black chickpea
PGSBL : PG of steam-cooked brown lentil
PGSBW : PG of steam-cooked buckwheat
OUTRBC : OUT of raw black chickpea
OUTRBL : OUT of raw brown lentil
OUTRBW : OUT of raw buckwheat
OUTSBC : OUT of steam-cooked black chickpea
OUTSBL : OUT of steam-cooked brown lentil

OUTSBW : OUT of steam-cooked buckwheat
SD : Standard Deviation
SPSS : Statistical Package for the Social Science
TEAC : Trolox Equivalent Antioxidant Capacity
TPC : Total Phenolic Content
Trolox : 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

LIST OF TABLES

Page

LIST OF FIGURES

Page

INVESTIGATING PHENOLIC CONTENT, ANTIOXIDANT ACTIVITY AND BIOAVAIBILITY OF RAW/STEAM COOKED BUCKWHEAT, BLACK CHICKPEA AND BROWN LENTIL

SUMMARY

Cereals and legumes are food which are often consumed. As the various chronic diseases increase such as, obesity, cancer and diabetic, consumer attention to healthy diets also increases. There is diverse studies about health beneficial effect of cereals and legumes and it is claimed that they have antioxidant effect on human metabolism.

Cereals belong to the large monocotyledonous grass family (*Gramineae*) which mainly consists of wheat, barley, oats and rice. There is huge production worldwide. They are major energy source for daily diet and contain important minerals and vitamins for human health. Cereal grains have a big role on obesity, cancers, heart, gut, mental, skeleton health and antioxidant protection.

Buckwheat is a whole grain, pseudocereal which have similar macronutrient composition to cereals. It is an important protein source. Because of its beneficial composition and health effects, new product studies have been done.

Legumes are the part of *Leguminnosae* family that contains 13,000 different species with herbs, vines, shrubs and trees. They have wide variation of macro and micronutrients. Recent researches showed therapeutic functionality of them on chronic diseases.

Chickpea and lentil is a cheap source of protein and high energy. Large nutraceutical benefits are subject for new studies. Wild types of this legumes are studied for new functional product development. Black chickpeas and brown lentils are not famous type and products which belong to Turkey local market.

The aim of this study, investigation of phenolic content, antioxidant activity and bioavailability of raw and steam cooked buckwheat, black chickpea and brown lentil and exploring the difference between species and raw/steam cooked conditions.

Raw and steam cooked products were milled and extracted. Differential Scanning Calorimeter (DSC) was used to check the products cooked or not. Total phenolic content was determined with Folin-Ciocalteu method. Flavonoid analysis was done according to methods in literature. Total antioxidant activity analyzed in three different methods as 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), cupric reducing antioxidant capacity (CUPRAC) and 2,2 diphenyl-1-picrylhydrazyl (DPPH)

analysis method. Total recovered phenolics were determined with HPLC-PDA and statistical analysis was done with IBM Social Sciences (SPSS) Statistics Program (21th version).

Steam cooking was done 30 ml for buckwheat (BW), 90 water ml for black chickpea (BC) and brown lentil according to DSC thermographs. Total phenolic content ranged from 44.41 to 265.43 mg GAE/100 mg dry weight. Flavonoid content changed from 368.75 to 1342.76 mg RE/100 g dry weight. Antioxidant activity ranged from 12.85 to 1740.04 mg TEAC/100 g dry weight in different methods. CUPRAC was the most effective method for analyzed raw products.

Bioavailability was experimented with *In Vitro* digestion method. Phenolic, flavonoid content and antioxidant activity was analyzed. The recovery values were calculated to compare the bioavailability of products before and after cooking. Phenolic content ranged from 42.77±7.23 (Steam cooked IN_{BW}) to 315.70±8.86 (Raw OUT_{BW}) mg GAE/100 g DW and recovery of phenolic content gap was between 15.22±3.31% (Steam cooked IN_{BW}) and 191.41±12.54% (Steam cooked PG_{BL}). Flavonoid content was between 2.28±1.29 (Steam cooked IN_{BC}) and 316.50±12.76 (Raw IN_{BC}). The highest recovery belonged to steam cooked PG_{BC} (44.28±13.27 mg RE/100 g DW) and lowest was steam cooked PG_{BC} (0.63±1.06 mg RE/100 g DW). Antioxidant activity was measured by three different methods (ABTS, CUPRAC, and DPPH) as before. CUPRAC was the most efficient method for bioavailability samples.

The highest activity was raw PG_{BL}'s (29.10±1.42 mg TEAC/100 g DW) and activity was not detected for none of the OUT samples with ABTS method. Antioxidant activity data according to CUPRAC method ranged from 73.54±1.98 (Raw IN_{BC}) to 927.38±51.01 mg TEAC/100 g DW (Raw OUT_{BC}) and recovery was between 4.72±0.44% (Raw IN_{BC}) and 75.71±10.21% (Raw OUT_{BL}). The highest score was steam cooked PG_{BC} (19.59±1.51 mg TEAC/100 g DW) and minimum value was raw IN_{BW} according to DPPH method. Minimum and maximum values was analog to bioavailability data set.

Gallic acid, catechin, epicatechin, procatechuic acid and rutin for buckwheat, gallic acid, catechin, rutin, quercetin dihydrate and kaempherol for black chickpea, gallic acid, catechin, epicatechin, coumaric acid and rutin for brown lentil were identified with HPLC-PAD.

PİŞMEMİŞ VE BUHARDA PİŞİRİLMİŞ KARA BUĞDAY, KARA NOHUT VE KAHVERENGİ MERCİMEĞİN FENOLİK MADDE, ANTİOKSİDAN AKTİVİTESİ VE BİYOYARARLILIKLARININ İNCELENMESİ

ÖZET

Sağlıklı beslenmenin önem kazandığı bu günlerde tahıl ürünleri ve baklagillerin gün geçtikçe tüketimi ve bu gıda grubuyla yapılan yeni gıda ürünü tasarımları artmaktadır. Birçok hastalık için koruyucu ve önleyici etkisi olan bu ürünler konusunda yapılan çalışmalar artmaktadır. Serbest radikallerin neden oldukları oksidatif hasar, metabolizma üzerinde mutajenik, kanserojen ve yaşlanma gibi sonuçlar doğurmaktadır. Bu doğrultuda, etkilerini yavaşlatmak veya durdurmak amacıyla antioksidan içerikli besinlerin tüketilmesi önem kazanmaktadır. Birçok sağlık kuruluşu, tüketiciyi sağlıklı beslenmeye yönlendirmekte ve doğal antioksidanlar, sentetik antioksidanlara nazaran daha çok tercih edilmektedir. Bu durum, tam tahıl ve baklagil grubu mensubu ürünlere yönelimi arttırmaktadır.

Tahıl ürünleri, besleyici ve fonksiyonel olmalarının yanı sıra, çalışmalarda antioksidatif özelliklerinin olduğu da belirtilmektedir. Bu besin grubuna ait olan karabuğday, yüksek miktarda protein, ham lif, dengeli aminoasit bileşimine sahip ve B1, B2 ve B6 vitaminlerince zengindir. Bununla birlikte, yapılan araştırmalarda fare ince bağırsağında probiyotiklerin artışına sebep olduğu gözlemlenmiştir. Buğday glütenine hassasiyeti olan çölyak hastalarına uygunluğu yapılan araştırmalarda görülmüştür. Antioksidan özelliklerine bakıldığında ise, metanollü ekstraktlarında başta rutin ve quercetin olmak üzere, kaempferol-3-rutinoside ve az miktarda flavanol rastlanmaktadır.

Baklagiller, değerli besin karakteristiği (Yüksek protein, sindirilebilen/sindirilemeyen karbonhidratlar, polifenoller) ve düşük maliyeti nedeniyle tüketici tarafından tercih edilmektedir. Tohumların bulundurduğu dirençli nişasta, fiber gibi sindirilemeyen lifler nedeniyle tokluk kan şekerini yavaş arttırdığını gösteren bilimsel çalışmalar bulunmaktadır. Bununla birlikte, sağlık üzerine olumlu etkileri olan, doğal antioksidan ve biyoaktif karbonhidratlar gibi fitokimyasalları bünyesinde barındırmaktadır.

Türkiye'nin başlıca üreticilerinden olduğu mercimek, yüksek protein miktarının yanında, çeşitli biyoaktif bileşenler de bulundurmaktadır. Çeşitli türlerindeki renk farklılıkları, içerisindeki antosiyanin varlığına veya miktarına bağlı olarak açıklanmaktadır. Birçok çalışmada, yüksek antioksidan miktarı içerdiği, koroner ve kardiyovasküler hastalıklar üzerindeki önleyici etkisi belirtilmiştir.

Nohut da mercimek gibi protein ve diyet lifi açısından zengin bir baklagildir. Serbest halde biochanin gibi isoflavonları ve ona bağlı bileşikleri diğer bileşenlere oranla daha fazla bulundurmaktadır. Son zamanlarda yabani türdeki ürünler incelenmektedir. Bu türdeki ürünlerde yapılan çalışmalarda, yüksek fenolik içeriğe ve antioksidan aktivitesine rastlanmaktadır.

Bu çalışmada, karabuğday, kahverengi mercimek ve kara nohut ürünleri Türkiye yerel pazarından temin edilmiştir. Amaç sağlıklı beslenme için yeni ürünler olduklarını göstermektir. Çalışma sırasında, fenolik miktarı, antioksidan aktivitesinin, biyoyararlılığının türler arası farklılığı incelendi ve buharda pişirme işleminden sonra meydana gelen farkların irdelendi.

Pişirme işleminin verimi Differantial Scanning Calorimeter (DSC) kullanılarak, termograflara bakılarak incelendi. Buharda pişirme için, karabuğday için 30 ml, kara nohut ve kahverengi mercimek için 90 ml olarak kararlaştırıldı. Pişirilen numuneler bir gün -80^{0}C buzdolabında saklandıktan sonra, sıvı nitrojen ile muamele ederek un haline getirildi ve freze-dryer kullanılarak kurutuldu. Çiğ numuneler de un haline getirilerek, ilgili literatür taraması yapıldıktan sonra ekstraksiyonu yapıldı. Vakumlu uçurma işleminden sonra arda kalan kısım, metanolde çözülerek analiz edildi. 5 gramlık numuneler biyoyararlılık (*In vitro* sindirim metodu) deneyleriyle tamamlandı. Analiz edilecek numuneler bu şekilde hazırlandı.

Toplam fenolik içerik Folin-Ciocalteu metodu kullanılarak analiz edildi. Flavonoid içeriği, ilgili makale temel alınarak incelendi. Toplam antioksidan aktivitesi incelenirken, üç farklı metot kullanıldı. Bu metotlar, Toplam 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), cupric reducing antioxidant capacity (CUPRAC) ve 2,2 diphenyl-1-picrylhydrazyl (DPPH) olarak kararlaştırıldı.

Toplam fenolikler HPLC-PDA kullanılarak belirlendi. İstatistiksel analiz, 0.05 önem düzeyinde Duncan Testi kullanılarak, IBM Social Sciences (SPSS) İstatistik Programı (21. versiyon) aracılığıyla yapıldı.

Çiğ ürünlerde toplam fenolik içerik 44.41'den 263.23 mg GAE/100 g kuru maddeye kadar değişiklik göstermiştir, en fazla karabuğdayda rastlanmıştır. Toplam flavonoid, 368.75'ten 265.23 mg RE/100 g kuru madde aralığında sonuç verdi. Kara nohutun en yüksek flavonoid miktarına sahip olduğu görüldü.

Antioksidan aktivitesi farklı metotlar göz önüne alındığında, 12.85'ten 1740.04 mg TEAC/100 g kuru madde olarak görüldü. En aktifin karabuğday olduğu belirlendi. Analiz edilen numuneler için CUPRAC en uygun antioksidan aktivitesi yöntemi olarak görüldü.

Buharda pişirme işlemi sonrasında, fenolik miktarın (63.87-281.20 mg GAE/100 g kuru madde) arttığı gözlemlendi. Flavonoid miktarında düşüş gözlemlendi. Antioksidan aktivitesi yönteme göre farklılık göstermiştir. Çiğ ürünlerin ekstraktında, en verimli yöntem olarak belirlenen CUPRAC, pişirme işleminden sonra verilerde düşüş gösterdi.

Biyoyararlılık deneyleri, insan mide ve bağırsak ortamı laboratuvar ortamında simüle edilerek gerçekleştirildi. İşlemler sonunda elde edilen PG, IN ve OUT örneklerine

ekstraktlara uygulanan toplam fenolik, toplam flavonoid ve toplam antioksidan analizleri uygulandı. Biyoyararlılığın etkinliği gerikazanım (%) değeri hesaplanarak ölçüldü. Antioksidan aktiviteleri yine üç farklı yöntem (ABTS, CUPRAC ve ABTS) uygulanarak ölçüldü. CUPRAC biyoyararlılık örnekleri için de en uygun antioksidan aktivitesi yöntemi olarak belirlendi.

Fenolik içerik 42.77±7.23'ten (Buharda pişmiş IN_{BW}), 315.70±8.86 mg GAE/100 g kuru maddeye (Çiğ OUT_{BW}) farklılık gösterdi. Geri kazanım ise, 15.22±3.31 (Buharda pişmiş IN_{BW}) - 191.41±12.54% (Buharda pişmiş PG_{BL}) sonucunu verdi. Flavonoid içeriği 2.28±1.29 (Buharda pişmiş IN_{BC}) ve 316.50±12.76 (Çiğ IN_{BC}) aralığında bulundu. En yüksek geri kazanım değeri, buharda pişmiş PG_{BC} (44.28±13.27 mg RE/100 g DW), en düşük değer ise, buharda pişmiş PG_{BC}'ye (0.63±1.06 mg RE/100 g kuru madde) aitti.

ABTS metoduyla ölçülen antioksidan aktivitelerinde en yüksek 29.10±1.42 (Çiğ PG_{BL}) mg TEAC/100 g kuru madde olarak bulunmuştur. Hiçbir OUT örneği için antioksidan aktivitesi saptanmamıştır. IN numuneleri için önemli sayılacak bir fark geri kazanım (%) sonuçlarında bulunmamıştır. BL hariç, diğer ürünlerde pişirme işlemi sonrası PG ve IN geri kazanımlarında düşüş görüşmüştür.

CUPRAC metodu kullanıldığında ise, değerler 73.54±1.98 (Çiğ IN_{BC}) - 927.38±51.01 mg TEAC/100 g kuru madde (Çiğ OUT_{BC}) olarak ölçülmüştür. Geri kazanım oranları ise, 4.72±0.44% (Çiğ IN_{BC}) and 75.71±10.21% (Çiğ OUT_{BL}) olarak hesaplanmıştır.

DPPH metodunda, en yüksek aktivite buharda pişmiş PG_{BC} (19.59±1.51 mg TEAC/100 g DW) ve minimum değere çiğ IN_{BW} olarak bulunmuştur. Geri kazanım sonuçları da bu değerler ile parallellik göstermektedir.

HPLC-PAD ile fenolik yapısı incelendiğinde, karabuğday için, gallic asit, catechin, epicatechin, procatechuic asit ve rutin; kara nohut için, gallic asit, catechin, rutin, quercetin dihydrate ve kaempherol; kahverengi mercimek için ise, gallic asit, catechin, epicatechin, coumaric asit ve rutin tanımlanmıştır.

Literatürde, karabuğday, kahverengi mercimek ve kara nohudun buharda pişirilmiş ve pişmemiş hallerinin biyoyararlılığını, fenolik içeriğini ve antioksidan aktivitesini inceleyen kapsamlı bir çalışma bulunmamaktadır. Bu da araştırmamızın özgünlüğünü ortaya koymaktadır.

1. INTRODUCTION

Cereals are members of monocotyledonous grass family *Gramineae*, and mainly contain wheat, maize, barley, oats, rice, and sorghum (Zhou et al., 2013). For decades cereal grains have been the basic component of human diet directly as the human food besides its use as animal feed worldwide (Awika, 2011; Poutanen, 2012). According to the data of Food and Agriculture Organization (FAO) for 2012 Cereal Production, world total cereal production is 254 million tonnes and China is the biggest producer in the market with 540 million tonnes. Buckwheat (*Fagopyrum esculentum Moench*) is from a subgroup of *Polygonacea* family unlike mostly cereals (Sun and Ho, 2005) and it is suitable for people who got coeliac disease because of its gluten-free position (Inglett et al., 2011).

The term legume includes all of the family *Leguminosae* which contains more than 13,000 different species with herbs, vines, shrubs and trees (Zhou et al., 2013; Sosulski and Sosulski, 2005). Legumes and cereals are regarded as whole grains and they play a dominant role in the diets of humans because of their substantiality, high nutritional value (high protein amount, digestible/indigestible carbohydrates, polyphenols), low cost and long shelf-life (Siva-Cristobal et al., 2010). Algeria is the leader producer with 207.5 million tonnes in the world with respect to FAO 2012 Cereal Production data and soybeans, peanuts, dry beans, peas, broad beans, chickpeas, and lentils are widely produced worldwide.

Many diseases like arthritis, emphysema and atherosclerosis are related to oxidative damage induced by free radicals (FR) which have mutagenic, carcinogenic and aging effects (Lin and Chou, 2009). Accordingly, consumption of foods with high antioxidant levels become an important issue to decrease or prevent negative impacts or FRs. Health organizations recommend a healthy diet for consumers with natural antioxidants which are preferred to synthetic antioxidants (Sun and Ho, 2005).

It is claimed that cereals and legumes have also antioxidant potential as well as being nutritious and functional (Sedej et al., 2011). In a study, four main flavonolglycosides

(rutin, quercetin, kaempferol-3-rutinoside, flavanol) have identified in methanol extracts of buckwheat (Şensoy, 2006). However it is evident that neither pulses nor cereals can be consumed raw. As the common application they are usually soaked in water and then cooked to be digestable. Soaking & cooking was found to create significant losses in total free phenolics by other researchers (Vadivel et al., 2011). While breads which are made with buckwheat flour expressed significantly higher antioxidant activity (AOA) than breads with rice flour (Sakac et al., 2011). So it is not so clear about the cooking effect losses on cereal and legume phenolics and antioxidant activity.

In this study; as an alternative method that can minimize the leaching losses, steam cooking was applied to buckwheat, brown lentils and black chickpea to understand the effect on phenolics, antioxidant activity and bioavailability of phenolics by comparing that of control raw samples. Also another aim is to investigate the different varieties of legumes or cereals for their phenolic contents and antioxidant potential which are traditionally consumed in rural areas of our country.

2. LITERATURE REVIEW

2.1 About buckwheat, brown lentil and black chickpea

2.1.1 Buckwheat

Buckwheat, with the botanical name *Fagopyrum esculentum*, is a plant cultivated for its grain-like seeds, and also used as a cover crop. Despite the name, buckwheat is not related to wheat, as it is not a grass. Because its seeds are eaten, it is referred to as a pseudocereal. The name 'buckwheat' or 'beech wheat' comes from its triangular seeds, which resemble the much larger seeds of the beech nut from the beech tree, and the fact that it is used like wheat.

Common buckwheat was domesticated and first cultivated in inland Southeast Asia, possibly around 6000 BCE, and from there spread to Central Asia and Tibet, and then to the Middle East and Europe.

Buckwheat contains starch (71–78% in groats), depending on hydrothermal treatment, buckwheat groats contain 7–37% of resistant starch. Crude protein is 18%, with biological values above 90%. This can be explained by a high concentration of all essential amino acids especially lysine, threonine, tryptophan, and the sulphur-containing amino acids. It is rich in iron (60–100 ppm), zinc (20–30 ppm) and selenium (20–50 ppb). As phenolic compounds they are reported to involve 10–200 ppm of rutin, 0.1–2% of tannins and catechin-7-O-glucoside in groats. Buckwheat contains a glucoside called rutin, a phytochemical that strengthens capillary walls. It also contains galloylated propelargonidins and procyanidins.

Buckwheat contains D-chiro-inositol, a component of the secondary messenger pathway for insulin signal transduction found to be deficient in Type II diabetes and polycystic ovary syndrome. It is being studied for use in treating Type II diabetes.

High protein buckwheat flour is being studied for possible use as a functional ingredient in foods to reduce plasma cholesterol, body fat, and cholesterol gallstones. The starchy endosperm is white and makes up most or all of buckwheat flour. The seed coat is green or tan, which darkens buckwheat flour. The hull is dark brown or black

and some may be included in buckwheat flour as dark specks. Buckwheat noodles play a major role in the cuisines of Japan (soba) Korea (naengmyeon, makguksu and memil guksu) and the Valtellina region of Northern Italy (pizzoccheri). Buckwheat groats are commonly used in western Asia and Eastern Europe. They are cooked with broth to a texture similar to rice or bulgur. Groats were the most widely used form of buckwheat worldwide during the 20th century, eaten primarily in Estonia, Russia, Ukraine and Poland, called "grechka" in Ukrainian or Russian. The groats can also be sprouted and then eaten raw or cooked. Buckwheat pancakes, sometimes raised with yeast, are eaten in several countries. Farina made from groats are used for breakfast food, porridge, and thickening materials in soups, gravies, and dressings.

Buckwheat contains no gluten and can be eaten by people with coeliac disease or gluten allergies. Many bread-like preparations have been developed. Buckwheat is a good honey plant, producing a dark, strong monofloral honey.

F. esculentum has been also reported to have various biological effects such as anti-atherosclerotic, neuroprotective, photoprotective and antioxidant activity as well as cytotoxicity and inhibitory activity against angiotensin-I converting enzyme and α-amylase. On the other hand, neurodegenerative diseases have become a major health problem, particularly in industrialized countries due to increasing number of elderly population. F. esculentum, known as “karabugday” in Turkey, has become recently popular crop plant, on which cultivation studies have been newly initiated and few phytochemical and bioactivity studies have been done up to date on buckwheat growing in Turkey (Gulpinar et al., 2012).

2.1.2 Black chickpea

Cultivated chickpea (*Cicer arietinum* L.) is noted to be one of the first grain legumes to be domesticated in the old world, and currently ranks as the third most important grain legume in the world after dry bean (Phaseolus vulgaris L.) and dry peas (Pisum sativum L.). Turkey is possibly one of the first domestication areas of chickpea. Turkey is the centre of origin for Cicer, and chickpea is thought to have originated in southeastern Anatolia.

Chickpea, the English name for *Cicer arietinum* L. is also known by the common names Bengal gram (India), Garbanzo (Latin America), Hommes and Hamaz (Arab world), Nohud and Lablabi (Turkey), Pois chiche (French) and Shim bra (Ethiopia).

Commonly described as either '**kabuli**' for the large (29-60 gl I 00 seed) cream coloured seed types, or '**desi**' for the smaller (I5-29 gJ I 00 seed), pigmented, angular-shaped seed types. Chickpeas are grown in semi-arid areas of tropical, sub-tropical and temperate regions of the world. The kabuli type is most often grown in temperate regions and the desi type is produced in the semi-arid tropics. In Turkey black chickpea is cultivated in Malatya- Hekimhan region and used in soups and traditional dishes.

In Mediterranean region the large cream-coloured 'Kabuli' type of seed was most predominately used, and at the eastward, utilisation changed to that of the smaller, dark-coloured 'Desi' type seeds. Maesen (1984) proposed that the south-eastern part of Turkey, near Syria, was the centre of origin of cultivated chickpea since the two closely related species, *C. reticulatum* and *C. echinospermum* occur there (Hannan et al., 2001).

Chickpeas consumption can assist in the treatment of diabetes and high cholesterol. Chickpeas contain 13% protein, 40-55% carbohydrate, and 4-10% oil. Fatty acid composition varies with chickpea type, but is approximately 50% oleic and 40% linoleic acids. Chickpeas are also an excellent source of folate, vitamins B6 and C, and zinc. *Desi* chickpeas have a markedly higher fiber content than Kabulis and hence a very low glycemic index which may make them suitable for people with blood sugar problems.The desi type is used to make Chana Dal, which is a split chickpea with the skin removed.

Chickpeas are the seed of the annual plant *Cicer arietinum* of the pea (*Fabaceae, or leguminosae*) family, widely grown for its nutritious seeds. The oldest records of the cultivated chickpea are from Turkey, where it was grown approximately 7,500 years ago. From there, the crop spread and became a staple food across the Middle East, North Africa and the Indian subcontinent. Today, chickpeas are popular throughout China, India, North and Eastern Africa, Europe, the Americas and Australia. Chickpeas are consumed fresh as a green vegetable, parched, fried, roasted and boiled. They are also consumed as a snack food, sweets and condiments or as a paste such as hummus. They are also ground into flour and used to make soup, bread and sweetmeats. Ancient people associated chickpeas with Venus because they were said to offer medical uses such as increasing sperm and milk, provoking menstruation and urine and helping to treat kidney stones.

Besides nutritional importance, chickpea seed has marked medicinal properties. Seeds enrich the blood and cure skin diseases and inflammation of the ear. They are used as tonic, appetizer, stimulant and aphrodisiac, and they also have anthelmintic properties. Among the food legumes, chickpeas are the most hypocholesterolemic agent and germinated chickpeas are reported to be effective in controlling cholesterol levels in rats. Dietary supplementation with chickpeas for at least 5 weeks resulted in significant reductions in serum total and low-density lipoprotein cholesterols in adult woman and men.

Chickpeas could contribute significantly in the management and/or prevention of degenerative diseases associated with free radical damage, in addition to their traditional role of preventing protein malnutrition. Thus, value-added chickpeas and chickpea-based products could expand into old and new markets alike. (Zia-Ul-Haq et al., 2008).

2.1.3 Brown lentils

Lentils are legumes, seeds of a plant whose botanical name is *Lens ensculenta*. They grow in pods that contain either one or two lentil seeds. Lentils are classified according to whether they are large or small in size with dozens of varieties of each being cultivated. While the most common types are either green or brown, lentils are also available in black, yellow, red and orange colors. The different types offer varying consistencies with the brown and green ones better retaining their shape after cooking, while the others generally become soft and mushy. While the flavor differs slightly among the varieties, they generally feature a hearty dense somewhat nutty flavor.

Lentils are believed to have originated in central Asia, having been consumed since prehistoric times. They are one of the first foods to have ever been cultivated. Brown lentil or with its name in Malatya region as black lentil is belonging to *Lens culinaris Medikus.* It is smaller in size with respect to green lentil and have ball-like structure. Traditionally it is used in dishes such as meatballs, lentil balls, rice pilavs or soups.

Edible seeds are a good source of protein, dietary fiber, folate, iron, and phosphorus. Flour made from lentils is gluten free and may be added to cereal flour to make breads, cakes and baby foods. The seed coat color can be clear, green, tan, gray, brown, or black while the cotyledon is yellow, red, or green. The main market types are red and

green, which together account for an estimated 95% of the world's lentil production (Takeoka et al., 2005).

Lentil, like all other pulses, is an excellent source of proteins, carbohydrates and fiber, and provides many vitamins and minerals. Lentils contain relatively high amounts of lysine and provide a well-balanced amino acid profile when consumed in combination with cereal based foods or foods that are rich in sulphur-containing amino acids (methionine and cysteine) and tryptophan. Lentils are also a good source of dietary carbohydrates and energy. Lentil seeds contain high quantities of alpha-galactosides also known as raffinose family oligosaccharides (RFO). These water-soluble low molecular weight non-reducing sugars are sucrose derivatives, and consist of linear chains of galactosyl residues attached to the glucose moiety of sucrose via a-(1-6) glycosidic linkage (Martinez-Villaluenga et al. 2008).The major RFO of lentil seeds include raffinose (trisaccharide), stachyose (tetrasaccharide) and verbascose (pentasaccharide) non-digestible oligosaccharides including RFO, dietary fiber and resistant starch have been reported to play an important role in promoting human health by acting as prebiotics, which selectively stimulate the growth of beneficial microorganisms in the colon such as *Bifidobacterium* and *Lactobacillus*).Short-chain fatty acids produced as a by-product of the fermentation of the non-digestible oligosaccharides have been reported to play a role in the prevention of colon cancer, the reduction of inflammation and increasing the availability of minerals in the colon (Tahir et al., 2011).

2.2 Significance of Cereals and Legumes on Human Diet

2.2.1 Significance of Cereals in Human Diet

Since the first days of agriculture of grains, cereal grains are major energy sources for human diet and they contribute approximately 70% and 50% of the total calories and protein with the macronutrients (protein, fat, and carbohydrate) they contain (Poutanen, 2012; Zhou et al., 2013). Important minerals, vitamins and other essential micronutrients (20% of magnesium and zinc, 30-40% of carbohydrate and iron, 20-30% of riboflavin and niacin, and over 40% of thiamine) are also supplied with cereal grains (Zhou et al., 2013). Macro and micronutrient distribution of cereal grains are shown in Figure 2.1.

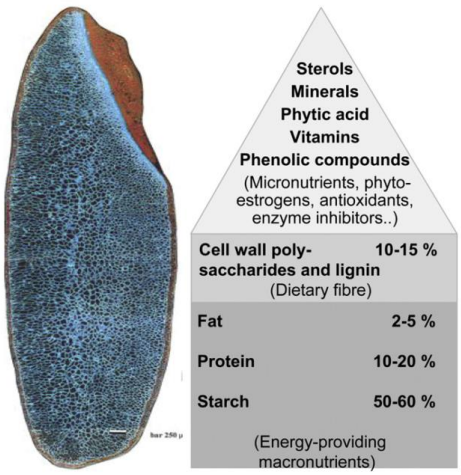

Figure 2.1: Macro and micronutrient distribution of cereal grains (Poutanen, 2012).

In the developing countries, grain-based foods are still basic constituents for the diet and in the absence of grains hunger and undernourishment emerge (Poutanen, 2012). The obesogenic diet have brought diseases, such as type 2 diabetes and cardiovascular diseases and certain types of cancer (Lafiandra et al., 2014; Poutanen, 2012; Zhou et al., 2013). This situation becomes a reason for protective lifestyles such as healthy diets increasing consumption of foods with cereal fiber and whole grain. Cereal-based foods, especially whole grains, has an important role for healthy eating pattern with the sterols, minerals, vitamins, phenolic compounds, phytic acid and dietary fiber that they contain. The cereal composition differs according to grain variety, growing conditions, husbandry and infection (Tester et al., 1995). Whole grain bioactive compounds and their prevention of major problems are given in Table 2.1. Major effects of cereal grains may differ according to their physiochemical properties during milling and food processing (Zhou et al., 2013).

There are less whole grain products than refined products on market and major samples are breads, breakfast cereals and whole-grain cereals such as brown rice or quick-cooking whole grain barley and wheat (Lang and Jebb, 2003). Two or three servings of whole grain cereal is beneficial to get health effects but recommended consumption levels changes from one country to another; for example, in the USA it is between 6-12 servings and in Australia, it is about 4 servings daily (Fardet, 2010).

Table 2.1: Whole-grain cereal bioactive compounds potentially involved in the prevention of major health problems (Fardet, 2010).

Major health problems	**Bioactive compounds**
Body-weight regulation and obesity	Insoluble fibre, fructans, resistant starch, Zn, Ca, tocotrienols, phenolic acids, flavonoids, choline, p-aminobenzoic acid
CVD and heart health	α-Linolenic acid, methionine, oligosaccharides, soluble fibre, resistant starch, phytic acid, Mg, Mn, Cu, Se, K, thiamin, riboflavin, nicotinic acid, pyridoxine, folates, tocopherols, tocotrienols, phylloquinone, β-carotene, lutein, zeaxanthin, phenolic acids, flavonoids, lignans, phytosterols, betaine, choline, inositols, policosanol, p-aminobenzoic acid, γ-oryzanol, avenanthramides, saponins
Type 2 diabetes	Soluble fibre, resistant starch, phytic acid, Mg, Zn, Se, K, Ca, tocopherols, tocotrienols, phenolic acids, flavonoids, betaine, inositols, phytosterols, γ-oryzanol, saponins
Cancers	α-Linolenic acid, oligosaccharides, soluble fibre, insoluble fibre, resistant starch, lignin, phytic acid, Zn, Mn, Cu, Se, P, Ca, riboflavin, nicotinic acid, pyridoxine, folates, tocopherols, tocotrienols, β-carotene, b-cryptoxanthin, phenolic acids, flavonoids, lignans, alkylresorcinols, betaine, choline, inositols, phytosterols, melatonin, p-aminobenzoic acid, saponins
Gut health	α-Linolenic acid, oligosaccharides, soluble fibre, insoluble fibre, resistant starch, riboflavin, pantothenic acid, phenolic acids, policosanol, γ-oryzanol
Mental/brain/nervous system health and neurodegenerative disorders	α-Linolenic acid, methionine, oligosaccharides, Fe, Mg, Zn, Cu, P, Ca, Na, K, thiamin, riboflavin, nicotinic acid, pantothenic acid, pyridoxine, biotin, folates, tocotrienols, phenolic acids, choline, inositols, policosanol, melatonin, γ-oryzanol, saponins
Skeleton health (i.e. bone, tendon, cartilage, collagen, articulation and teeth)	α-Linolenic acid, Fe, Mg, Zn, Mn, Cu, P, Ca, K, nicotinic acid, tocotrienols, phylloquinone, b-cryptoxanthin, flavonoids, lignans, p-aminobenzoic acid
Antioxidant protection (development of diseases in relation to increased oxidative stress)	Reduced glutathione, methionine, cystine, lignins, phytic acid, Mg, Fe, Zn, Mn, Cu, Se, thiamin, riboflavin, tocopherols, tocotrienols, β-carotene, lutein, zeaxanthin, b-cryptoxanthin, phenolic acids, flavonoids, lignans, alkylresorcinols, betaine, choline, policosanol, melatonin, γ-oryzanol, avenanthramides, saponins

Buckwheat is a whole grain, pseudocereal. Pseudocereals have similar macro nutrient composition to cereals but they are broadleaf (nongrass) plants (Asp et al., 2010). Buckwheat is an important protein source with ideal amino acid linkages and it contains starch, vitamin, minerals (Zn, Cu, Mn, Mg) and high amounts of phenolics (Yıldız and Yalçın, 2013). Due to trace amount of prolamine protein, buckwheat identified as gluten-free so, it is convenient for diet of coeliac disease (Sakac et al., 2011).

According to its rich composition, new product development with buckwheat arise. Rufa et al. (2004), observed the effect of tartary buckwheat as a tea on lowering the blood glucose and it seemed that buckwheat had obvious effect on 90.35% of the volunteers. There are also studies which prove the applicability of buckwheat flour for bread, pasta and cookies (Maeda et al., 2004; Sakac et al., 2011).

2.2.2 Significance of Legumes in Human Diet

Legumes are recognized as the second most valuable plant source for human and animal nutrition which are the third largest family with 650 genera and 20,000 species. They are excellent sources of protein, dietary fiber, starch, micronutrients and bioactive compounds with low level of fat with significant quantities of polyphenolic compounds such as flavonoids, isoflavones, phenolic acids, lignans and natural antioxidants (Sasipriya and Siddhuraju, 2012; Boudjou et al., 2013).

Food legumes also contains minerals, vitamins (especially Vitamin B and C) and antinutrients, such as protease inhibitors, phytic acids, saponins, tannins and plant sterols. (Xu and Chang, 2009; Zhou et al., 2013).

In spite of wide variation in the macronutrient composition of legumes, their basic seed structure is the same. Mature seeds contain three major components: the seed coat (testa), embryo and endosperm. Most legume seeds have very little endosperm, as the cotyledons which provide the great majority of the nutritional components of interest to food value (Zhou et al., 2013). Composition of the some important legumes is shown in Table 2.2.

Table 2.2: Proximate Composition of Some Important Food Legumes (Zhou et al., 2013).

Nutrient	Soybean	Chickpea	Peanut	Pea	Lentil	Bean
Water (g)	8.54	11.53	6.50	11.27	10.40	11.02
Protein (g)	36.49	9.30	25.80	24.55	25.80	21.60
Total lipid (g)	19.94	6.04	49.54	1.16	1.06	1.42
Ash (g)	4.87	2.48	2.33	2.65	2.67	3.60
Carbohydrate, by difference (g)	30.16	60.65	16.13	60.37	60.08	62.36
Fiber, total dietary (g)	9.30	17.40	8.50	25.50	30.50	15.20
Iron (mg)	277	105	92	55	56	123
Calcium (mg)	15.70	6.24	4.58	4.43	7.54	5.02
Magnesium (mg)	280	115	168	115	122	171
Phosphorus (mg)	704	366	376	366	451	352
Potassium (mg)	1797	875	705	981	955	1483
Sodium (mg)	2	24	18	15	6	5
Zinc (mg)	4.89	3.43	3.27	3.01	4.78	3.65
Copper (mg)	1.66	0.85	1.14	0.87	0.52	0.84
Manganese (mg)	2.52	2.20	1.93	1.39	1.33	1.06
Selenium (mg)	17.80	8.20	7.20	1.60	8.30	3.20
Nutrient values are per 100 g edible portion						

Beans, broad beans, chickpeas, lentils, peas and soybeans are several examples of well-known and most-consumed legumes in the world and it is presented in Table 2.3 with their production data in 2012 according to FAO database. (Zhou et al., 2013).

Table 2.3: World Production of Legumes in 2012 (FAO, 2012).

Legume	Million Tonnes
Beans, dry	23.6
Beans, green	20.7
Chickpeas	11.6
Peas, dry	9.8
Peas, green	18.4
Lentils	4.6
Soybeans	24.2

The most recent research suggests that regular dietary intake of food legumes can reduce the risk of nutrition related chronic diseases such as obesity, diabetes, heart diseases, cardiovascular diseases and cancers (Sasipriya and Siddhuraju, 2012). The regular intake of legumes can be able to prevent degenerative diseases which are based on free radicals and food legumes are recommended as a good choice for its health promoting benefits (Sasipriya and Siddhuraju, 2012; Xu and Chang, 2009). Recent research have been focusing on the incorporation of wild-type legume grains in the

formulation of supplementary therapeutic foods for various chronic diseases including diabetes, obesity and cardiovascular diseases (Vadivel et al., 2011). On the other hand, there is no general idea about the proportions of total carbohydrate that should be provided by whole grain and legume foods, and processed foods from these sources (Venn and Mann, 2004).

Chickpea is a cheap source of protein and energy with 20–30% protein, approximately 40% carbohydrates, and only 3–6% oil, it also has Ca, Mg, K, P, Fe, Zn and Mn minerals, beneficial carotenoids such as β-carotene and not high amounts of isoflavones (Millan et al., 2006). It is an important food to the affluent populations to reduction and prevention of major food-related health problems. However, more research has to be done for revealing the food and nutraceutical benefit of this important food legume (Akbaba et al., 2012).

Lentil is considered as an annual leguminous crop yielding from one to twelve grains or seeds of variable size, shape, and colour within a pod (type of pulse crop) whose seeds comprise mainly of the condensed type tannin (Amarowicz et al., 2010). It is rich in protein (20−30%), prebiotics (fructooligosaccharides, galactooligosaccharide, resistant starch) and minerals but contains low phytic acid, therefore, bioavailability of lentils folates could have large benefits due to the profiles of other bioactive molecules in lentils (Gupta et al., 2013).

2.3 Phenolic Compounds and Antioxidant Activity

Free radicals are normal species produced during the body's metabolic processes such as phagocytes work by white blood cells acting as a cellular messenger in a biological process called redox signaling, but after distruption of balance between free radicals and antioxidant compounds, highly reactive forms of the free radicals which are reactive oxygen or reactive nitrogen species, are able to initiate chain reaction of oxidation process, which causes damage to the macromolecules such as DNA, lipid, carbohydrates, and proteins. This situation may be a reason for several degenerative diseases. Antioxidants are needed for blocking the damages of free radicals. They are any constituents that can inhibit the oxidation and the capability to inhibit/prevent the oxidative degradation is named as antioxidant activity (Gliszczynska-Swiglo and Oszmianski, 2013; Jomova et al. 2013; Jati and Biesalski, 2013).

Human defense system has the production of its antioxidant substances in the form of enzymes (superoxide dismutases, H_2O_2-removing enzymes) and non-enzyme antioxidants (metal-binding proteins) but under abnormal/diseased conditions, the amount of free radicals exceeds the production of antioxidants. To increase the antioxidant capacity of human system, intake of natural antioxidants with daily diet is one of the best ways. These natural food antioxidants include plant phenolics, antioxidant vitamins, and thiol antioxidants (Gliszczynska-Swiglo and Oszmianski, 2013; Jati and Biesalski, 2013).

Phenolic compounds are a diverse class of chemicals, over 8000 species, containing a hydroxyl group on a benzene ring. Some phenolics are complex molecules derived from the condensation of two or more components from either shikimic acid or polyketide pathways. They are therapeutically useful bioactive substances and many of the phenolic compounds are essential to plant life, for example, by providing a defense against microbial attacks and by making food unpalatable to herbivorous predators (Lee, 2004; Apak, et al., 2011). Main classes of phenolic compounds and their dietary source with main representation is given in Table 2.4.

Phenolic compounds,

- contribute to the color, astringency, bitterness, oxidation reaction, interactions with proteins and aging behavior grapes and wines,
- are markers of floral origin in honeys
- contributing to the bitter and astringent flavor of tea
- the precursors of off-flavors in citrus products
- have browning potentials of apples and grape products
- have role in prevention of chronic diseases and health benefits including anticancer and antiviral activities
- reduce risk of coronary heart disease and stroke.

Table 2.4: Main classes of phenolic compounds, their dietary sources and main representatives (Gliszczynska-Swiglo and Oszmianski, 2013).

Basic Skeleton/Chemical Structure	Class	Dietary Sources	Main Representatives
C6–C1 COOH	Benzoic acids and aldehydes	Berries, cereals, herbs, and spices	4-Hydroxybenzoic, gallic, protocatechuic, salicylic, vanillic, gentisic, and ellagic acids; vanillin
C6–C3	Cinnamic acids	Apple, cherry, plum, berries, tomato, asparagus, white grape, and herbs	p-Coumaric, caffeic, ferulic, sinapic, and chlorogenic acids
C15 (C6–C3–C6)	Flavonoids	Onion, tomato, apple, herbs, tea, citrus fruits	Kaempferol, quercetin, rutin, luteolin, ± catechin, hesperetin, cyanidin, delphinidin
C6–C2–C6	Stilbenes	Grapes and wine	Resveratrol
C18	Betacyanins	Red beet and opuntia	Betanin and isobetanin
Dimers or oligomers	Lignans	Flaxseed, sesame seed, cereals, legumes, berries, and vegetables	Secoisolariciresinol, secoisolariciresinol diglucoside, isolariciresinol, pinoresinol, and matairesinol
Oligomers or polymers	Tannins	Apples, berries, grapes, and red wine	Procyanidins B1, B2, B3, B4, C, gallotannins, and ellagitannins

Table 2.5 summarizes the potential health benefits of phenolics with active compounds in various foods (Lee, 2004).

Table 2.5: Food phenolics that may be prevent diseases (Lee, 2004).

Active Compound	Possible Health Benefit	Food Source
Phenolics Catechins	Linked to low rates of gastrointestinal cancer; may aid immune system, lower cholesterol levels	Green tea, berries
Coumarins	Prevent blood clotting and may have anticancer properties	Parsley, carrots, citrus fruit Parsley,
Flavonoids	Block receptor sites for certain hormones that promote cancer; act as weak antioxidants	Parsley, carrots, citrus fruits, broccoli, cabbage, cucumbers, squash, yams, tomatoes, eggplant, peppers, soy products, berries
Genistein	May block growth of new blood vessels that are essential for tumors to spread; deters proliferation of cancer cells	Soybean products and possibly cabbage-family vegetables
Phenolic acids	May help the body resist cancer by inhibiting nitrosamine and affecting enzyme activity	Parsley, carrots, broccoli, cabbage, tomatoes, eggplant, peppers, citrus fruits, whole grains, berries

2.3.1 Phenolic compounds and antioxidant activity in cereals

The major phenolic compounds present in cereal grains are phenolic acids, flavonoids, and tannins which are concentrated in the bran fraction of cereal grains and exist in free, soluble conjugated, and insoluble bound forms. Ferulic acid is the most common phenolic acid in cereal grains, followed by p-coumaric, synapic, and caffeic acids (Wang et al., 2014). Antioxidant compositions of diffirent cereal types are summarized in Table 2.6.

Whole grain cereals contain a much wider range of compounds with potential antioxidant effects than do refined cereals. These include vitamin E (mainly in the germ), folates, minerals (iron, zinc), trace elements (selenium, copper, and manganese), carotenoids, phytic acid, lignin and other compounds such as betaine, choline, sulfur amino acids, alkylresorcinols and lignans found mainly in the bran fraction (Apak, 2004). Main bioactive compounds in wheat fractions are shown in Figure 2.2. Whole grain consumption appears to reduce the risk of chronic diseases such as cancer and cardiovascular diseases and the majority of the beneficial components in grain are stated in the bran layer, including phenolic acids which may function as an antioxidant. In cereal grains, the main portion of the phenolic acids covalently bounded to plant cell-wall polysaccharides and lignin. Hydrocinnamic acids are linked via ester bonds to arabinoxylans in the primary plant cell walls and ferulic acid and p-coumaric acid may also be esterified to lignin (Lee, 2004).

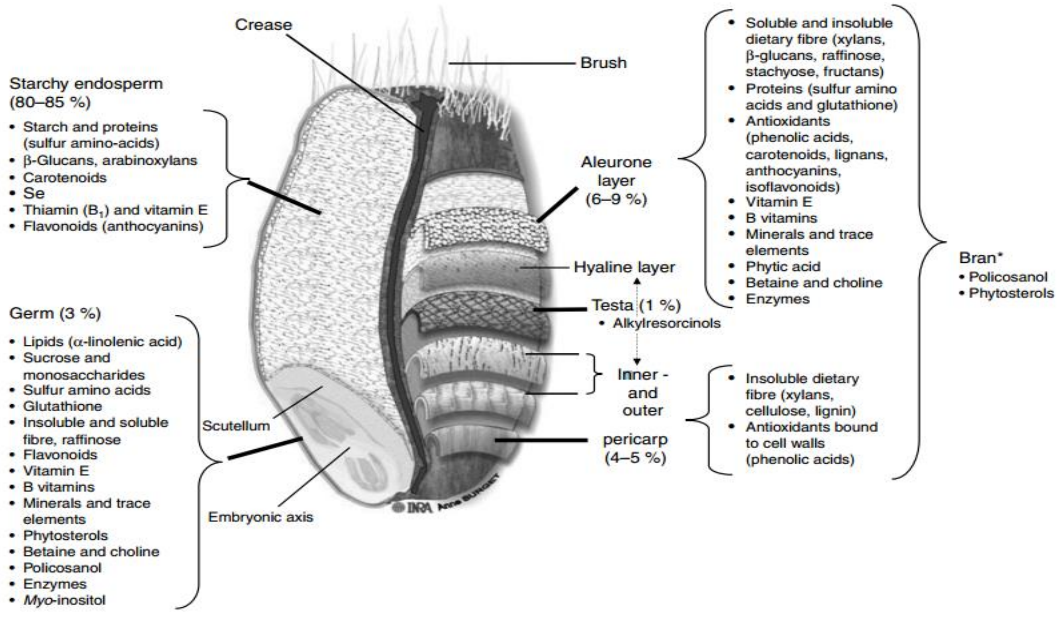

Figure 2.2: The three wheat fraction (bran, germ and endosperm) with their main bioactive compounds (Fardet, 2010).

Table 2.6: Antioxidant composition of different types of cereal (Apak et al., 2011).

Cereals	Antioxidant Compounds	Major Component
Wheat	Vanillic acid, p-hydroxybenzoic acid, protocatechuic acid, syringic acid, p-coumaric acid, caffeic acid, sinapic acid, tocols (β-tocopherol, and α-tocopherol), lysophosphatidylcholine	Ferulic acid
Toasted wheat	Choline, betain	
Corn	p-coumaric acid, syringic acid, vanillic acid, protocatechuic acid, caffeic acid, sinapic acid, α-tocopherol	Ferulic acid
Rice	Vitamin E, γ-oryzanol (Gamma oryzanol is a mixture of substances derived from rice bran oil, including sterols and ferulic acid), tocols (γ-tocotrienols, γ-tocopherol, and α-tocopherol), phosphatidylcholine, sterols (β-sitosterol) similar cysteine and methionine	*trans*-Ferulic acid
Black rice	Cyanidin-3-glucoside, peonidin-3-glucoside	
Oat	Phytic acid, avenanthramides (alkoloids containing phenolic groups), tocols (α-tocotrienols, and α-tocopherol), phenolic acids (vanillic acid, and p-hydroxybenzoic acid), phosphatidylcholine, similar cysteine, methionine, phytic acid	Ferulic acid and caffeic acid
Barley	Benzoic and cinnamic acid derivatives (ferulic acid), proanthocyanidins, quinines, flavonols, chalcones, flavones, flavanones, amino phenolic compounds, similar cysteine and methionine	Ferulic acid *p*-coumaric acid
Rye	Isoferulic acid, coumaric acid, syringic acid, p-hydroxybenzoic acid, caffeic acid, sinapic acid, dimer 8-O-4-di ferulic acid, phosphatidylinositol, tocols (β-tocopherol, and α-tocopherol), similar cysteine and methionine	Ferulic acid
Sorghum	Tannins, anthocyanins (apigeninidin, luteolinidin), apigenin, luteolin, vanillic acid, p-hydroxybenzoic acid, naringenin, carotenoids (lutein, zeaxanthin, β-carotene), α-tocopherol, lysophospholipid	*p*-coumaric acid and ferulic acid
Millet	Flavones (C-glycosylvitexin, vitexin, and glycosylorientin), tocols (α-tocotrienols, and α-tocopherol), lysophosphatidylcholine, and phosphatidylcholine	Ferulic acid, *p*-coumaric acid, cinnamic acid and gentisic acid

Flight and Clifton (2006) reported that, corn had the highest total phenolic content (15.55±0.60 mmol of gallic acid equiv/g of grain) of the grains analysed, followed by wheat (7.99±0.39), oats (6.53±0.19) and rice (5.56±0.17). The major portion of phenolics in grains existed in the bound form (85% in corn, 75% in oats and wheat and 62% in rice). Ferulic acid was the major phenolic compound, with free, soluble-conjugated and bound ferulic acids present in the ratio of 0.1:1:100. Corn had the highest total antioxidant activity (181.42±0.86 mmol of vitamin C equiv/g of grain), followed by wheat (76.70±1.38), oats (74.67±1.49) and rice (55.77±1.62). Extraction and hydrolysis method, processes that applied to the grains may effect the results. For example, refined and whole wheat differ in amounts of constituents as shown in Table 2.7.

Table 2.7: Compositional differences between whole and refined wheat (Flight and Clifton, 2006).

Component	Whole wheat	Refined wheat
Bran (%)	14	<0.1
Germ (%)	2.5	<0.1
Total dietary fibre (%)	13	3
Insoluble dietary fibre (%)	11.5	1.9
Soluble dietary fibre (%)	1.1	1.0
Protein (%)	14	14
Fat (%)	2.7	1.4
Starch and sugar (%)	70	83
Total minerals (%)	1.8	0.6
Selected minerals (µg/g)		
Zinc	29	8
Iron	35	13
Selenium	0.06	0.02
Selected vitamins (mg/g)		
Vitamin B6	7.5	1.4
Folic acid	0.57	0.11
Phenolic compounds (µg/g)		
Ferulic acid	5	0.4
β-tocotrienol	32.8	5.7
Phytate phosphorus (mg/g)	2.9	0.1

The chemistry of the common buckwheat phenolic seed and product differs from other cereal products. In buckwheat, the content of ferulic and hydroxycinnamic acids is low and branaleurone fraction of buckwheat contains bound syringic, p-hydroxybenzoic, vanillic, and p-coumaric acids. These acids can be liberated by alkaline/acid

hydrolysis. The concentration of flavonoids in buckwheat is affected by location, growth conditions and variety. The content of rutin in buckwheat seed may be from 12.6 to 51.1 mg/100 g. The content of hyperin and quercetin in buckwheat hulls is 5.0 and 2.5 mg/100 g (Shahidi and Naczk, 2003).

Hung and Morita (2008), investigated phenolic compounds and the antioxidant capacity of whole buckwheat grains which were milled into 16 flour fractions using the gradual milling system. The phenolic compounds in buckwheat were primarily in free form, whereas the flavonoids existed in grain in insoluble bound forms. Ferulic acid and rutin amounts increased from 2.5 and 2.5μg/g flour of the phenolics less rich fraction to 609.5 and 389.9 μg/g flour of the phenolics rich fraction of grain. The higher phenolic contents in the phenolic rich fractions exhibited the stronger antioxidant capacity than the phenolics less rich fractions.

In a study that subjected about gluten-free pasta, a decrease of total free phenolic compounds from farm to fork (from flour to cooked spaghetti) of about 74.5%, with a range between 55.3 and 100%, for individual compounds was observed. The decrease in bound phenols was 80.9%, with a range between 46.2 and 100% and the spaghetti-making process and the cooking caused losses of 46.1 and 49.4% of total phenolic compounds (Verardo et al., 2011).

2.3.2 Phenolic compounds and antioxidant activity in legumes

Legume grains are rich sources of polyphenolics and natural antioxidants, especially in their hulls. The seed coat of legume grains contain numerous types of phenolics, which play a significant protective role against oxidative damage in human metabolism (Vadivel et al., 2011). Legumes, such as soybeans, stock isoflavones which have a protective effect against cancer, cardiovascular diseases and osteoporosis They also provide catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate (Lee, 2004).

Beneficial physiological effects of legume phenolics in humans and deleterious effects in animal nutrition have been extensively investigated. For example, faba bean hull and its phenolics are known to suppress enzymes in the digestive tract of monogastric animals, primarily poultry and pigs, thereby reducing the bioavailability of macronutrients (Boudjou et al, 2013). Vadivel et al. (2011), reported an important effect of 10 different immature underutilized legume seeds from South India of on type

II diabetes related enzyme inhibition. Due to recent studies, saponins that legume related, protect humans against cardiovascular diseases. Grass peas are rich in polyhphenols which affects antioxidant activity (Fratianni et al., 2014).

Chickpea (Cicer arietinum L.) as a legume, contains flavonols (quercetin, kaempferol andmyricetin), flavonoids (flavonols, flavanones and isoflavones) and nonflavonoids (hydroxybenzoic, hydroxycinnamic acids) (Fratianni et al., 2014). Their antioxidant activity could be effective for the expansion of consumption. According to a study on five pigmented chickpeas, the total phenolic content (TPC) and antioxidant activity varied from 1.23 to 1.51mg GAE/g sample and from 5011 to 5756 mmol TE/100 g sample respectively (Heiras-Palazuelos et al., 2013).

Lentils have the highest total phenolic and tannin content compared with other common pulses. The total phenolic content of lentils are correlated with total antioxidant activity, suggesting that their phenolic compounds are major contributors to antioxidant activity. The cotyledon of lentils contains mainly nonflavonoid phenolic compounds (hydroxybenzoic and hydroxycinnamic acids) and flavonoids present in the seed coat of lentils (Fratianni et al., 2014).

Oomah et al. (2011), investigated antioxidant activity in relation to phenolic contents of green and red lentils and yellow peas which extracted with four solvents, aqueous acetone, ethanol, hot water and water. Aqueous acetone extracted the highest level of total phenolics at about 87 mg of catechin equivalent per gram of sample from lentil hulls followed by hot water, water and aqueous ethanol. Red lentil hull with maximum concentration of phenolic compounds exhibited the strongest antioxidant activity of 260 mg (1040 μM) trolox equivalent/g hull.

2.4 Effect of Cooking on Phenolic Compounds

Cooking is a process that starch granules gelatinize and it depends on available water, time and temperature (Venn and Menn, 2004). Most of the common cooking methods have been reported to diminish the phenolic content and its health promoting properties in various dietary sources (Vadivel et al., 2011). The use of traditional processing such as cooking may help to decrease the tannin and phytic acid contents (Fardet, 2010).

2.4.1 Effect of cooking on phenolic compounds of cereals

The antioxidant capacity of whole buckwheat and its products was affected by hydrothermal treatment. The roasted buckwheat groats and the hydrothermal treated hulls were studied and approximately twice higher antioxidant capacity of the hulls was noted when compared to the groats. The concentration of flavonoids was related to the antioxidant capacity of buckwheat products measured by cyclic voltammetry (Zielinska et al., 2007).

In a research (Zhang et al., 2010), the effects of roasting, pressured-steam heating and microwave heating on total phenolics, total flavonoids, and antioxidant properties of whole-meal flour from tartary buckwheat (Fagopyrum tataricum Gaertn.) were investigated. It showed that thermal treatment of buckwheat flour caused a decrease in total phenolics, total flavonoids and antioxidative activities and the differences in trend of the antioxidant activities due to the thermal treatment were positively correlated with the content of phenolics. Therefore, it can be understood that processing method should be selected carefully for the exploration of tartary buckwheat products.

In a study, buckwheat flour was thus subjected to steam jet-cooking and the performance in cake-baking was evaluated as a fat replacer. Steam jet-cooking caused structural breakdown and starch gelatinization of buckwheat flour, because of increasing its water hydration properties. When buckwheat flour was thermo mechanically modified by steam jet-cooking, it was successfully incorporated into cake formulations for shortening up to 20% by weight, producing low-fat cakes with comparable volume and textural properties to the control (Min et al, 2011).

Verardo et al. (2011), has investigated using buckwheat for the production of gluten-free pasta. The results have shown a decrease of total free phenolic compounds from farm to fork (from flour to cooked spaghetti) of about 74.5% and the decrease in bound phenols was 80.9%. Total phenolic compounds present in dried spaghetti, 11.6% and were dissolved in water after cooking (Verardo et al., 2011).

2.4.2 Effect of cooking on phenolic compounds of legumes

In a research, ten different immature indigenous edible legume seeds collected from various South India were found to contain high levels of total free phenolics. According to effect of different processing methods, soaking & cooking has exhibited a significant loss of total free phenolics, antioxidant and starch digestive enzyme

inhibition properties; open pan roasting has not shown any significant decrease of total free phenolics but affected the antioxidant and health relevant functionality characteristics; sprouting & oil-frying significantly increased the total free phenolic content, antioxidant activity and type II diabetes related enzyme inhibition properties. A suitable mild processing method could be chosen for the consumption of wild type legume seeds in order to increase the dietary intake of phenolic compounds with potential health benefits (Vadivel et al., 2011).

Xu and Chang (2008), investigated the effects of soaking, boiling and steaming processes on the total phenolic components and antioxidant activity in green pea, yellow pea, chickpea and lentil and as compared to original unprocessed legumes, all processing steps caused significant decreases in total phenolic content, DPPH free radical scavenging activity, in all tested legumes. Steaming treatments resulted in a greater retention of TPC, DPPH, and ORAC values as compared to boiling treatments. They also reported that boiling has caused more solid loss than steaming and steam processing exhibited several advantages in retaining the integrity of the legume appearance, texture of the cooked product, shortening process time and greater retention of antioxidant components and activities.

The chickpea seed is rich with essential phosphorus compounds, provitamin A and vitamin B1. After cooking, the reduction in the levels of nutrients and content of saponins have been significant (Bavec and Bavec, 2006). Chickpea lines with colored seed coat contain high levels of polyphenolic compounds with high levels of antioxidant activity. However, common processing procedures, such as soaking and cooking, may decrease the levels of these bioactive compounds and subsequent overall antioxidant activity. Segev et al. (2011) examined the effects of soaking, cooking and steaming processes in relation to total phenolic content (TPC), total flavonoid content (TFC) and ferric reducing ability of plasma antioxidant activity (FRAP AA) of colored chickpea seeds. There was significantly reduction of TPC, TFC and FRAP AA in all processing steps. Steaming was superior to cooking in terms of protecting polyphenol and antioxidant activity.

2.5 Bioavailability and Studies on Cereals and Legumes

The bioavailability is a measure of the degree of both absorption and utilization and it affected by intrinsic and extrinsic factors. The individual needs for the phenolics due

to nutritional and health status sex, and age are intrinsic factors. Components in the diet are the extrinsic factors and could have both improver and inhibitory effects (Frolich, 2001).

The prediction of average daily intake of polyphenols by humans is difficult because of the lack of comprehensive and uniform nutrient compounds related to polyphenol content in food, species and variety differences, as well as the of cultivation and technological conditions during plant growth and processing. Major sources of polyphenols are beverages such as coffee, tea, wine, fruit and vegetable juices as well as legumes. Absorption, metabolism and distribution affects biological properties of polyphenols. Polyphenol absorption is mainly limited by its solubility and mainly in the small intestine, although4, absorption of some flavonoids from the stomach was also observed (Gliszczynska-Swiglo and Oszmianski, 2013).

Bioactive phenolic compounds of cereal grains are generally located in the bran fraction and covalently bound to indigestible polysaccharides. They have very low bioavailability because the complex bran matrix blocks access to the necessary enzymes which contribute to their release in the human gastrointestinal system. For example, the bound phenolic acids have very low bioavailability because of the bran matrix severely hindering their access to the necessary enzymes such as ferulate esterases, xylanases). The bioavailability of bound phenolic acids in corn bran is much lower than that in wheat bran because corn bran has more complex cell wall structure. Releasing these phenolic compounds from bran matrices and/or increasing their accessibility have been demonstrated to be effective in enhancing their bioavailability and for this, various processing technologies have been studied (Wang et al., 2014). Food processing is an option to increase the bioavailability of nutrients, by inactivating antinutritional factors, growth inhibitors (Xu Chang, 2008).

There is few studies about bioavailability of cereal and legume phenolics. Mineral micronutrient bioavailability of legume is observed recently. It is seen that bioavailability can be increased by using natural low-phytic acid lentil varieties, preferably peeled before cooking, which reduced total phytic acid by more than 50%, likewise it can be increased by breeding (Vega et al., 2012). Inhibitory effects of phenolic compounds on mineral bioavailability are observed in several researches and seed coat removal suggested as a solution (DellaValle et al., 2013).

Hemery et al. (2010), studied on the effect of particle size on bioaccessibility of para-coumeric acid, sinapic acid and ferulic acid in nine different bran-rich breads with *In vitro* bioavailability method. The highest amounts of bioaccessible phenolic acids were observed for two of the fractions obtained by electrostatic separation of ground bran. Only the free and conjugated phenolic acids forms have been found to be bioaccessible, and the bioaccessibility of sinapic acid has been much higher than that of ferulic acid, because of the higher solubility of sinapic acid. The use of bran fractionation to reduce the particle size or to include only some parts of the bran in foods can increase nutritional value of grain based products.

3. MATERIALS AND METHODS

3.1 Materials

Buckwheat, black chickpea and brown lentil were obtained from local markets in Turkey. Phenolic acids standards (gallic acid, p-hydroxybenzoic acid, cafeic acid, p-coumaric acid, syringic acid, sinapic acid, protocatechuic acid, 2,3,4-Trihydroxybenzoic acid, 3,4-Dihydroxybenzaldehyde) and the other flavanoids (+)-catechin hydrate, (-)-epicatechin, epigallocatechin gallate, (-)- epigallocatechin, quercetin, Biochanin A, myricetin, (±)- naringenin were obtained from Sigma-Aldrich Chemie GmbH company. The other standard epicatechingallate were from HWI Analytik GmbH. Ferulic acid, vanilic acid and anthocyanins such as delphinidin, cyanidin, pelargonidin, malvidin, cyanidin 3-O-glucoside=kuromanin, cyanidin 3-O-galactoside=idein, cyanidin 3-O-rutinoside=keracyanin, pelargonidin 3-O-glucoside=callistephin, malvidin 3-galactoside, malvidin 3-O-glucoside=oenin and dialysis tubing membrane were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). MN-640 filter paper was obtained from Macherey-Nagel GmbH (Düren, Germany).

3.2 Chemicals

Ammonium acetate (NH_4Ac), Copper (II) chloride ($CuCl_2$), diethyl ether, dipotassium hydrogen phospate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), potassium persulfate ($K_2S_2O_8$), sodium carbonate (Na_2CO_3) and trifluoroacetic acid (99%) were obtained from Merck KGaA (Darmstadt, Germany).

Acetic acid, acetonitrile (99.8%), bile salts, diethyl ether, DPPH, ethanol (≥99.8%), ethyl acetate, Folin-Ciocalteu phenol reagent, gallic acid (≥98%), hexane (≥97%), hydrochloric acid (37%), Methanol (HPLC gradient), neocuproine (Nc), pancreatin enzyme, pepsin enzyme, sodium bicarbonate ($NaHCO_3$) and sodium chloride were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylicacid were obtained from fluka

Chemie (Buchs, Switzerland). ABTS (2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid diammonium salt were obtained from Applichem GmbH (Darmstadt, Germany).

3.3 Extraction

Different methods were applied for cereal (buckwheat, BW) product and legume (black chickpea, BC; brown lentil, BL) products. Procedures were decided according to related scientific articles with optimum results. Before extraction process, all products were grounded.

3.3.1 Extraction of buckwheat

Phenolic compounds of buckwheat were extracted into free and bound phenolics according to the methods of Hung and Morita (2008) with some modifications. Buckwheat flour was weighed 1 g. Free phenolic compounds were extracted with 10 ml of 80% chilled ethanol with continuous shaking in ultrasonic bath for 20 minutes. After centrifugation at 2500 g for 10 minutes, supernatant was collected and the residue re-extracted twice under the same conditions. Then supernatants were evaporated to dryness under reduced pressure. Concentrated slurry was dissolved in 10 ml methanol and stored at -20^0C.

Residue is hydrolyzed with 20 ml of 2 N NaOH at 60^0C with continuous shaking in ultrasonic bath for 90 minutes. The hydrolysate was acidified to pH 2 with 6 N HCl and extracted five times with 2.5 ml hexane at a hexane to water phase ration of 1:1 to remove free fatty acids and other lipid contaminants. Sample was centrifuged at 5^0C, 5000 rpm for 3 minutes in each repetition. The liberated phenolic acids were extracted six times with 5 ml ethyl acetate-water mixture with ratio of 1:1 and supernatants were collected, evaporated to dryness. Concentrated slurry was dissolved in 10 ml methanol and stored at -20^0C.

3.3.2 Extraction of black chickpea and brown lentil

The free and bound phenolics of BC and BL were extracted according to method of Fares and Menga (2012) with some modifications. 1 g of sample was weighed and homogenized in 7 ml methanol/10% acetic acid (85:15; v/v), ultrasonicated for 30 minutes and fulfilled to 10 ml with distilled water. 1 ml was filtered for HPLC analysis for free phenolic compounds. 12 ml distilled water and 5 ml 10 M NaOH were added.

Tubes were sealed and stirred overnight at 20^0C with magnetic stirrer. pH acidified to 2 with HCl (37%) and phenolics were extracted with 15 ml cold diethyl ether/ethyl acetate (1:1; v/v) for three times. After supernatants were collected, evaporated to dryness and dissolved in 10 ml methanol. After alkaline hydrolysis, acid hydrolysis was performed by adding 2.5 ml HCl (37%) with an incubation in ultrasonic bath at 80^0C for 30 minutes. The samples were cooled and the same steps after alkaline hydrolysis were followed.

3.4 Cooking Trials for Buckwheat, Black Chickpea and Brown Lentil

50 g of sample of each product was soaked in 250 ml of tap water at room temperature for 4 hours (Rehinan et al., 2004). Presoaked samples were cooked in Arzum AR852 Bebbe Steam Cooker with 30, 60 and 90 ml water usage. After cooking process, samples stored at -80^0C for a day. Cooked products were treated with liquid nitrogen and grounded. Afterwards, freeze drying process was completed (Main drying: 26 hours, -30^0C and 0.37 mbar; final drying: an hour, -10^0C, and 2.5 mbar).

It was decided that samples were cooked or not, by investigation of Differential Scanning Calorimeter (DSC) (Model Q10, TA Instruments Inc., New Castle, DE, USA) thermographs of samples and Universal Analysis 2000 Version 4.5A (TA Instruments Inc., New Castle, DE, USA) software was utilized for determining transition onset temperature (T_0), transition peak temperature (T_p), and transition enthalpy (ΔH). The analysis was done according to study of Kim et al. (2006) with some modifications. Approximately 3 mg of samples were weighed into aluminum pans (TA Instruments, Inc., New Castle, DE, USA) and 12 µl of distilled water were added by using a microsyringe. The pans were hermetically sealed and allowed to equilibrate for 2 hours at room temperature. After equilibrium, samples were heated 20 to 180^0C at a rate of 10^0C/min and a sealed empty pan was used as a reference. Gelatinization was evaluated by searching for the presence of any enthalpy according to pure starch systems around 60^0C.

3.5 *In Vitro* Gastrointestinal Digestion Method for Bioavailability

Bioavailability procedure was based on study of McDougall et al. (2005) with some modifications. This method comprises of two steps as gastric fraction and small intestine fraction. 5 g of sample was weighed into a glass beaker, stomach solution was

added and pH was adjusted to 2 with 5 N HCl to simulate gastric conditions. Beakers were sealed with parafilm and continuously shook at 100 rpm in water bath at 37^0C for 2 hours. After gastric digestion, 2 ml sample was taken from post gastric (PG) fraction to Eppendorf Tubes. 4.5 ml of pancreatin and bile salt mixture was added. A piece of cellulose dialysis tube which was washed with distilled water and the bottom of the tube was linked tightly. After dialysis tube was filled with 20 ml $NaHCO_3$ solution to neutralize acidity of the sample, the top of the tube was tied. Dialysis tube was put into the beaker, was sealed with parafilm and placed in the heated water bath at 37^0C for 2 h with continuous shaking, again. This section was the simulation of small intestine. After digestion, the solution outs out of the dialysis tube, which was did not penetrate the serum, was taken in a falcon tube as OUT sample. The solution in the dialysis tube was taken as the IN sample which represented the constituents that entered the serum. After PG, IN and OUT samples were centrifuged at 18000 rpm at 4^0C for 5 minutes, they were stored at -20^0C for analysis. In vitro digestion procedure was applied to all products in duplicate.

3.6 Determination of Total Phenolic Content

The free and bound phenolics in samples were determined according to the method of Hung and Morita (2008). The convenient dilutions of free and bound phenolic extracts (0.5 ml) were oxidized with Folin–Ciocalteu's reagent (0.5 ml) in a centrifuge tube. The reaction was neutralized with saturated Na_2CO_3 solution (1 ml), followed by adjusting the volume to 10 ml with distilled water. The contents in the tubes were thoroughly mixed and allowed to stand at ambient temperature for 45 min until the characteristic blue color developed. Absorbance of the clear supernatants was measured at 725 nm using a spectrophotometer (Shimadzu UV-1700 UV-Vis). The content of total phenolics in each extract was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample and reported as mean value ± SD.

3.7 Determination of Total Flavonoid Content

Total flavonoids of extracts were analyzed with the basis of Dewanto et al. (2002). 1.25 ml distilled water added to 0.25 ml of sample. 75 µl of 5% $NaNO_2$ solvent was added and waited for 6 minutes. Afterwards, 150 µl of 10% $AlCl_3.6H_2O$ solvent was

put into tube. After waiting 5 minutes, 0.5 ml of 1 M NaOH solvent was added and total volume adjusted to 2.5 ml with distilled water. Tube was shook for 10 seconds and absorbance was measured at 510 nm without waiting against a reagent blank. Standard curve was prepared with using rutin and expressed as milligrams of rutin equivalent (RE) per gram of sample and reported as mean value ± SD.

3.8 Determination of Total Antioxidant Activity

Total antioxidant activity of BW, BC and BL samples were analyzed with three different methods. Experiments were made in triplicate and mean values were reported. Trolox with 75% MeOH was used for the standard curve.

3.8.1 ABTS (2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid) analysis method

The method was based on Miller and Rice-Evans (1997) and Toor et al. (2006). 100 µl of extract was put into tube and 1 ml of ABTS+ solution was added. After vortex for 15 seconds and waiting for 45 seconds, absorbance was measured at 734 nm against distilled water.

3.8.2 CUPRAC (Cupric reducing antioxidant capacity) analysis method

The samples were analyzed with CUPRAC method which was based on study of Apak et al. (2004). 1 ml of $CuCl_2$ solution ($1.0x10^{-2}$ M) was added to 100 µl of extract. 1 ml neocuproine alcoholic solution ($7.5x10^{-3}$ M), 1 ml NH_4Ac buffer solution at pH 7.0 and 1 ml distilled water were added respectively. After 30 minutes, the absorbance was measured for each tube at 450 nm wavelength.

3.8.3 DPPH (2,2 diphenyl-1-picrylhydrazyl) radical scavenging method

DPPH method was based on the study of Kumaran and Karunakaran (2006) and Rai et al. (2006). First of all, 2ml of 0.1 mM DPPH solution was added to 100 µl of extract. Samples were stored in dark for 30 minutes. Absorbance was measured at 517 nm wavelength against methanol.

3.9 HPLC Analysis of Major Phenolic Compounds

HPLC analysis were carried out by using the method adapted from Capanoglu et al. (2008). Standard calibration curves were prepared by using gallic, pHBA(-P-hydroxy benzoic acid), cafeic acid, vanilic acid, coumaric acid, ferulic acid, syringic acid,

sinapic acid, protocatechuic acid, quercetin, myricetin, (+)- catechin hydrate, (-) epicatechin, epicatechin gallate, (-)- epigallocatechin gallate, (-) epigallocatechin, delphinidin chloride, cyanidin chloride, cyanidin 3-O-glucoside, cyanidin 3-O-galactoside, cyanidin 3-O-rutinoside, pelargonidin chloride, pelargonidin 3-O-glucoside, malvidin chloride, malvidin 3-galactoside, malvidin 3-O-glucoside, biochanin A, 2,3,4-trihydroxybenzoic acid, 3,4-dihydroxybenzaldehyde (97%). All of the samples and standard solutions were filtered through a 0.45-μm membrane filter and 2 ml of the filtered sample was placed into vials and analyzed in a Waters W600 HPLC system with PDA (Waters 996) detector. Luna C18 column (Phenomenex) was used as the stationary phase.

The mobile phase was including solvent A, Milli-Q water with 0.1% (v/v) TFA and solvent B, acetonitrile with 0.1% (v/v) TFA, acetonitrile with 0.1% (v/v) TFA. A Linear gradient was used as follows: at 0 min, 95% solvent A and 5% solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B; and at 54 min returns to initial conditions. The flow rate was 1 ml/min. Detections were done at 280, 312, 360, and 512 nm wavelengths. Identification was based on the retention times and characteristic UV spectra and quantification was done by external standard curves. Calibration curves, retention times and maximum wavelength of standards are given in Appendix A.

3.10 Statistical Analyses

The results were analyzed statistically by IBM Social Sciences (SPSS) Statistics Program (21th version) by using one way analysis of variance (ANOVA) at 0.05 significant level and Duncan's New Multiple Range Test was applied as post hoc tests. The differences between all samples, PG, IN and OUT fractions were evaluated statistically. Duncan's New Multiple Range Test was applied to exact values to observe the differences between total phenolic and flavonoid contents and antioxidant activity ($p<0.05$). Each analysis was performed in triplicate. The results were reported as mean value ± standard deviation. Statistical analysis results of samples is given at Appendix B.

4. RESULTS AND DISCUSSION

The main goal of this study was the investigation of total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant activity (TAC) diversity between raw or steam-cooked buckwheat, black chickpea and brown lentil in first step. Secondly, changes were examined after steam-cooking and bioavailability. Also phenolic profiles and quantity was determined by HPLC/PDA to calculate recovery values after digestion to compare their bioavailability.

All of the results were expressed by using standards and reported as mg equivalents/100 g dry weight (DW). Each analysis was performed in triplicate for each sample. Statistical analysis was done with SPSS.

4.1 Cooking Trials and DSC Results

Transition onset temperature (T_0), transition peak temperature (T_p) and transition enthalpy (ΔH) of pure isolated starch gelatinization was determined to evaluate the gelatinization temperature of BW, BC and BL. Results of pure starch systems are shown in Table 4.1.

Table 4.1: Transition onset temperature (T_0), transition peak temperature (T_p) and transition enthalpy (ΔH) values of pure starch of BW, BC and BL.

Product	**T_0 (^{0}C)**	**T_p (^{0}C)**	**ΔH (J/g)**
BW	52.41	53.22	0.9187
BC	63.87	69.65	4.251
BL	60.94	66.66	4.703

Gelatinization of BW, BC, BL with 30, 60, 90 ml water usage for steam cooking and raw material was evaluated by searching for the presence of enthalpy according to pure starch systems around 60^0C. T_p and ΔH of samples are given in Table 4.2.

Table 4.2: Transition peak temperature (T_p) and transition enthalpy (ΔH) values of raw and steam cooked (with 30, 60, 90 ml water) products.

Product	T_p (^{0}C)	ΔH (J/g)
BW		
Raw	52.66	0.3629
30	50.41	0.0631
BC		
Raw	69.96	0.3467
60	66.82	0.1939
90	71.34	0.0229
BL		
Raw	69.82	1.4560
60	61.25	0.1509
90	60.73	0.0836

The results showed that 30 ml water for buckwheat and 90 ml water for black chickpea and brown lentil is adequate to steam cook. As gelatinization (transition) temperature was increasing, steam cooking time and water requirement also increased.

Gelatinization temperature of raw buckwheat was ranged from 60^{0}C to 85^{0}C in a study that Zhou et al. (2009) did. T_p was between 70.61 and 73.26^{0}C for different chickpea cultivars (Kaur and Singh, 2006). Barbara and Boye (2013) indicated the gelatinization temperature as 69.70^{0}C for lentil flours.

4.2 Total Phenolic, Total Flavonoid Content and Antioxidant Activity of Raw Products

4.2.1 Total phenolic content

Results for total phenolic content analysis were expressed as mg gallic acid equivalents (GAE)/100 g DW for each sample. The standard calibration curve of gallic acid shown in Figure 4.1 was prepared between 0.01-0.15 mg/ml and the equation obtained from the curve was used to calculate the absorbance values of the samples measured by UV-Vis spectrophotometer.

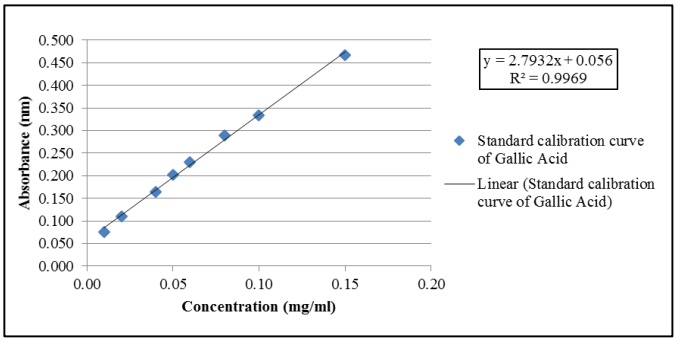

Figure 4.1: Standard calibration curve of Gallic Acid.

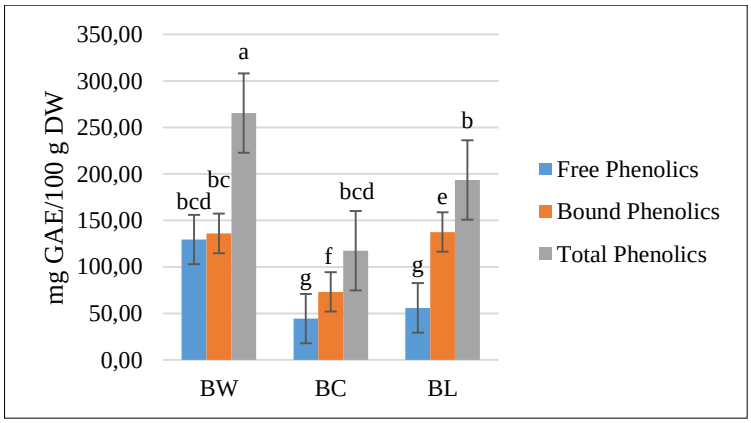

Figure 4.2: Free, bound and total phenolics of samples.

Results and statistical evaluation of total phenolic content analysis are showed in Figure 4.2 and Table 4.3. In BC and BL, bounded phenolics were greater than free phenolics, BW had same amount of phenolics in free and bounded forms. According to table, buckwheat had the highest total phenolic content value and brown lentil, black chickpea followed. BC and BL was similar, statistically. There was significant change in total phenolic content between samples ($p<0.05$) but there was not in groups.

Table 4.3: Phenolic content of raw samples.

Samples	**Phenolic Content (GAE/100 mg DW)**		
	Free	**Bound**	**Total**
BW	129.43 ± 6.83^{bcd}	136.00 ± 1.84^{bc}	265.43 ± 6.90^{a}
BC	44.41 ± 10.33^{g}	60.60 ± 10.33^{f}	105.013 ± 17.43^{bcd}
BL	55.91 ± 4.59^{g}	87.35 ± 0.46^{e}	143.26 ± 19.82^{b}

Quettier-Deleu et al. (2000), studied on phenolic compounds of buckwheat hulls and flours. Amount of total phenolics was denoted as 313.0 mg/100 g DW for flours and 333.0 mg/100 g DW for hulls. In an another research, TPC of wheat and buckwheat flour was claimed as ranging 40 to 191.3 mg GAE/100 g (Sedej et al., 2011).

Heiras-Palazuelos et al. (2013), investigated total phenolic content of six different pigmented chickpea. TPC of free extracts was between 28.0 and 37.0 mg GAE/100 g DW and TPC of bound extracts ranged from 107.0 to 123.0 mg GAE/100 g DW.

In a study which was about differences of phenolic contents of different originated lentils and chickpeas, TPC was stated between 147.0-183.0 for chickpeas and 109.8-159.4 mg GAE/100 g for lentils (Fratianni et al., 2014). Lentils had more phenolic content rather than chickpeas (Zhao et al., 2014).

4.2.2 Total flavonoid content

Results for total flavonoid content analysis were expressed as mg rutin equivalents (RE)/100 g DW for each sample. The standard calibration curve of rutin shown in Figure 4.3 was prepared between 0.05-0.6 mg/ml and the equation obtained from the curve was used to calculate the absorbance values of the samples measured by UV-Vis spectrophotometer.

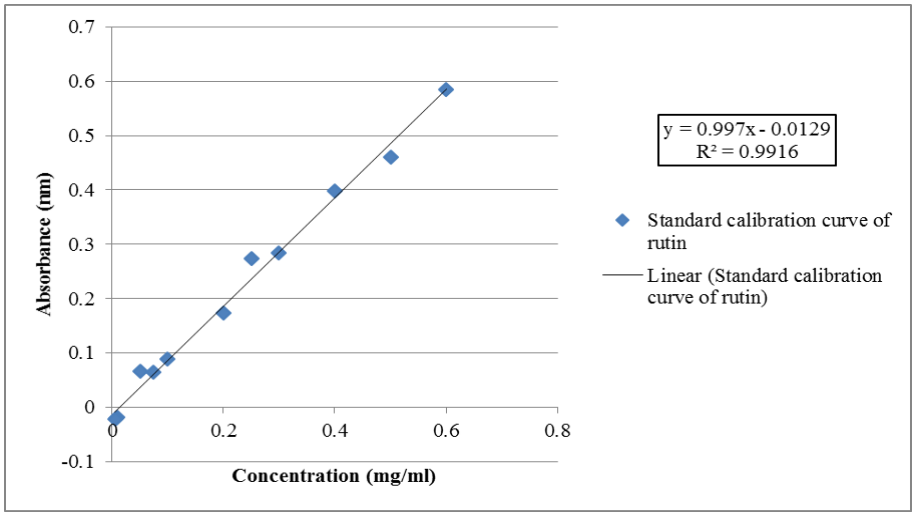

Figure 4.3: Calibration curve of rutin.

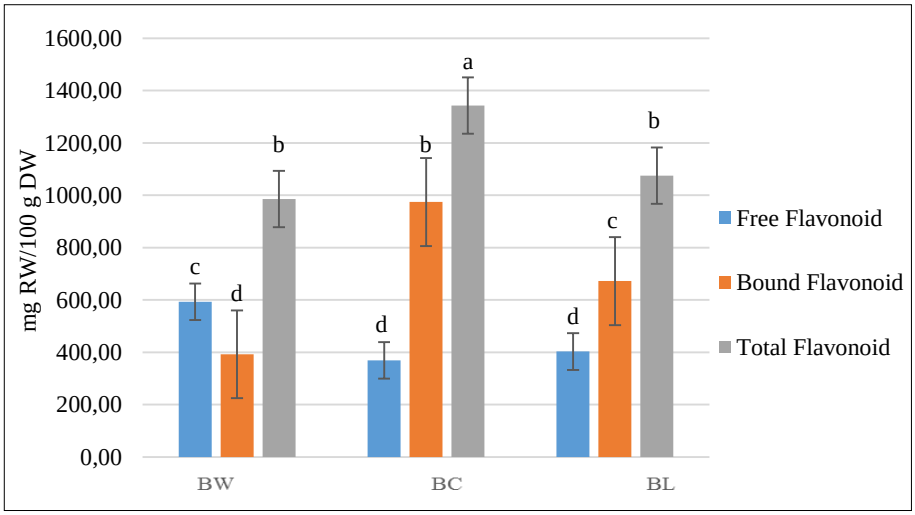

Figure 4.4: Flavonoid content of raw samples.

Results of total flavonoid content analysis are showed in Figure 4.4 and Table 4.4. According to numbers, free flavonoid content of BC and BL were similar and BW had the highest amount of free phenolics. BC had the highest bounded, total flavonoid value and ranking went on with BL and BW which had same amounts. On the other hand, there was significant change statistically in total flavonoid content between samples ($p<0.05$).

Table 4.4: Flavonoid content of raw samples.

Samples	Flavonoid Content (mg RE/100 g DW)		
	Free	Bound	Total
BW	593.10±4.41^{c}	392.11±12.69^{d}	985.21±100.94^{b}
BC	368.75±30.42^{d}	974.01±8.66^{b}	1342.76±187.56^{a}
BL	402.53±39.34^{d}	671.76±97.44^{c}	1074.29±238.29^{b}

In a study about nutritional and flavonoid content of different cultivars, flavonoid content was between 67 and 2250 RE mg/100 g DW (Qin et al., 2010). Total flavonoid of chickpea and lentil was stated as 300 and 2590 mg RE/100 g DW in a research on 10 different legumes (Ren et al., 2012). Differences between literature and our results can be explained with the species and pigment content our products.

4.2.3 Antioxidant Activity

Results for total antioxidant activity analysis were expressed as mg Trolox (TEAC) equivalents (RE)/100 g DW for each sample. The samples were analyzed with three different methods as ABTS, CUPRAC and DPPH. Results of three different methods were summarized in Figure 4.5, they were explained in detail in subtitles. CUPRAC was the most effective method for cereal and legume products as Figure 4.5 showed.

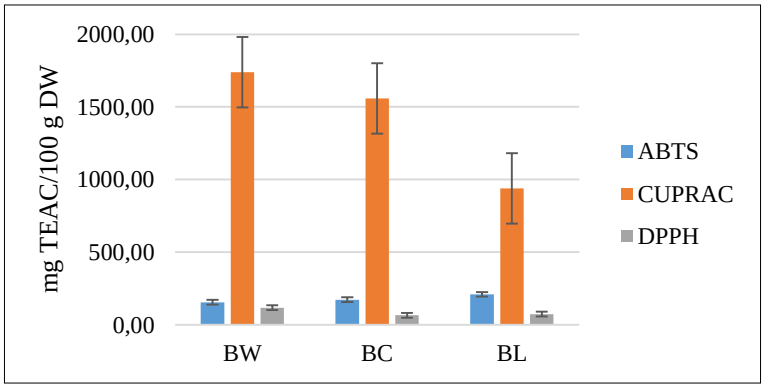

Figure 4.5: Total antioxidant activities of raw samples.

4.2.3.1 Total antioxidant activity by ABTS method

Standard calibration curve for ABTS was prepared by using Trolox as shown in Figure 4.6 and the results were expressed as mg TEAC/g standard for each sample. The standard calibration curve was obtained between 0.01-0.08 mg/ml and the equation was used to evaluate the antioxidant activity of the samples measured by spectrophotometer.

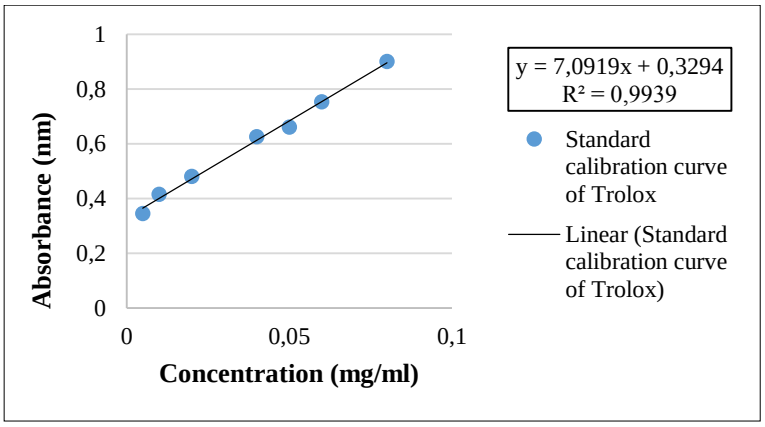

Figure 4.6: Calibration curve of Trolox for ABTS method.

Antioxidant activity of bound extracts was greater than free extracts except for BW. Free antioxidant activity of BC and BL extracts were similar statistically. The highest total antioxidant activity referred to BL; BC, BW followed. There was significant change statistically in total antioxidant activity between samples ($p<0.05$). Results are shown in Figure 4.7 and summarized in Table 4.5.

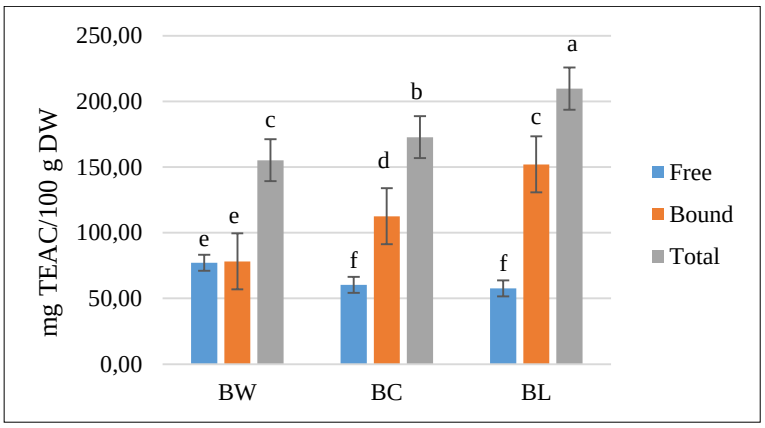

Figure 4.7: Antioxidant activity of raw samples with ABTS method.

Antioxidant activity of buckwheat by ABTS method was resulted as 129.90 mg TEAC/100 g DW (Zielinska et al., 2007). It was 50.06 TEAC/100 g DW for chickpea and between 350.41 and 370.43 mg TEAC/100 g DW for lentils (Han and Baik, 2008).

Table 4.5: Antioxidant activity with ABTS method of raw samples.

Samples	ABTS (mg TEAC/100 g DW)		
	Free	**Bound**	**Total**
BW	77.10±0.49^{e}	78.14±0.38^{e}	155.24±0.79^{c}
BC	60.22±3.28^{f}	112.58±7.23^{d}	172.80±6.84^{b}
BL	57.73±1.57^{f}	151.98±5.59^{c}	209.71±6.81^{a}

4.2.3.2 Total antioxidant activity by CUPRAC method

Standard calibration curve for CUPRAC was prepared by using Trolox as shown in Figure 4.6 and the results were expressed as mg TEAC/g standard for each sample. The standard calibration curve was obtained between 0.005-1 mg/ml and the equation was used to evaluate the antioxidant activity of the samples measured by spectrophotometer.

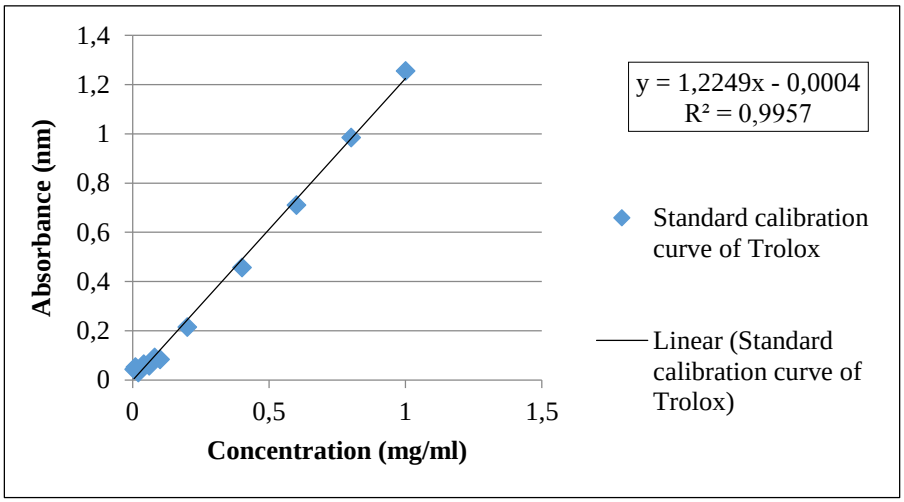

Figure 4.8: Calibration curve of Trolox for CUPRAC method.

Antioxidant activity of bound extracts was greater than free extracts. The highest free total antioxidant activity by CUPRAC was BW's. BC had the highest value in bounded extracts and greatest total antioxidant activity referred to BW and BC, BL followed. There was significant change statistically in total phenolic content between samples ($p<0.05$). Results are shown in Table 4.6.

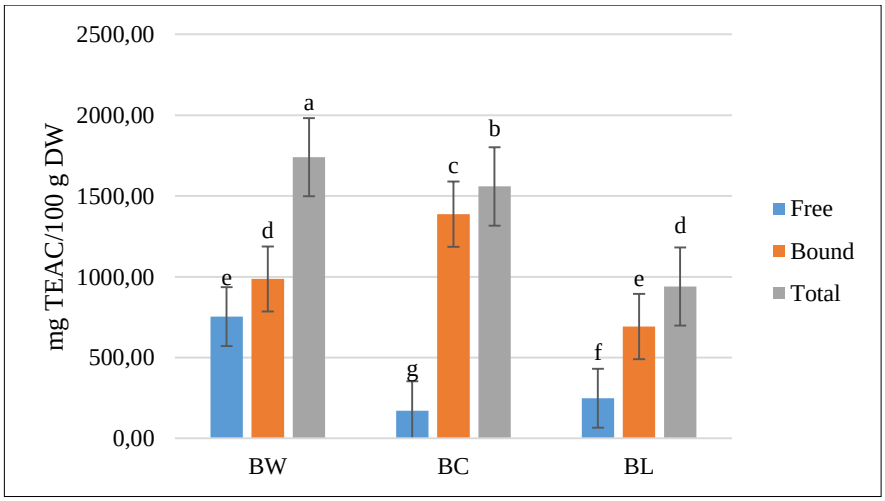

Figure 4.9: Antioxidant activity of raw samples with CUPRAC method.

Table 4.6: Antioxidant activity with CUPRAC method of raw samples.

Samples	CUPRAC (mg TEAC/100 g DW)		
	Free	Bound	Total
BW	753.35±28.33^{e}	986.69±71.11^{d}	1740.04±99.17^{a}
BC	171.56±12.21^{g}	1387.71±22.47^{c}	1559.27±39.20^{b}
BL	247.72±17.19^{f}	692.08±55.44^{e}	939.81±60.57^{d}

Gorinstein et al. (2008), analyzed the antioxidant activity of cereals and pseudocereals with different methods. According to records, antioxidant activity result by CUPRAC method was 228.77 mg TEAC/100 g DW for buckwheat, 112.25 TEAC/100 g DW for soybean. There was not enough literature knowledge for CUPRAC method on legumes.

4.2.3.3 Total antioxidant activity by DPPH method

Standard calibration curve for DPPH was prepared by using Trolox as shown in Figure 4.10 and the results were expressed as mg TEAC/g standard for each sample. The standard calibration curve was obtained between 0.005-1 mg/ml and the equation was used to evaluate the antioxidant activity of the samples measured by spectrophotometer.

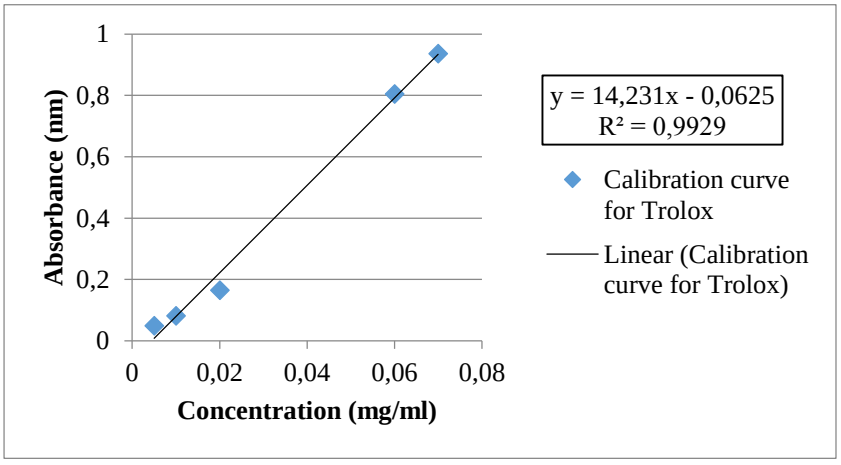

Figure 4.10: Calibration curve of Trolox for DPPH method.

Antioxidant activity of bound extracts was greater than free extracts and Bounded BC-BL had the similar activity. The highest total antioxidant activity by DPPH referred to BW; BL, BC followed. There was significant change statistically in total phenolic content between samples ($p<0.05$). Results are shown in Table 4.7 and Figure 4.11.

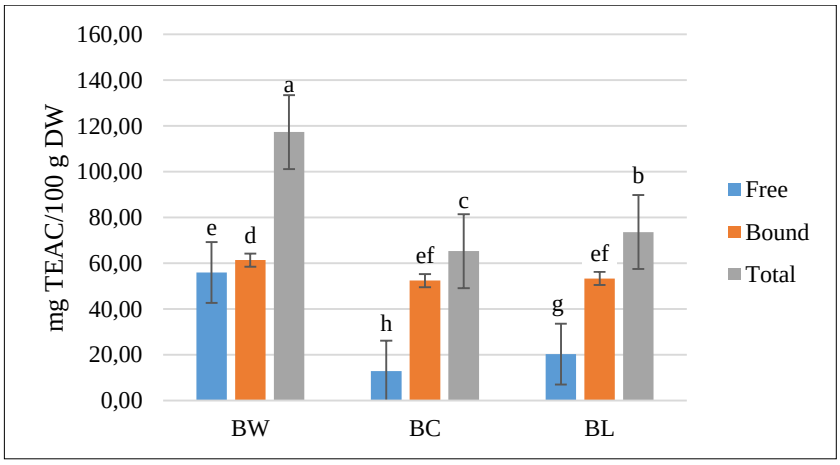

Figure 4.11: Antioxidant activity of raw samples with DPPH method.

Table 4.7: Antioxidant activity with DPPH method of raw samples.

Samples	DPPH (mg TEAC/100 g DW)		
	Free	**Bound**	**Total**
BW	55.96±26.31^{e}	61.37±28.69^{d}	117.33±2.69^{a}
BC	12.85±9.57^{h}	52.44±25.19^{ef}	65.29±1.89^{c}
BL	20.29±6.14^{g}	53.39±24.83^{ef}	73. 68±4.49^{b}

Inglett et al. (2011), found that, antioxidant activity of buckwheat by DPPH method was between 230.28 and 251.18 mg TEAC/100 g DW. According to a study about solvent affect to antioxidant activity, data for chickpea ranged from 11.83 to 93.06 and for lentil from 35.88 to 769.34 mg TEAC/100 g DW (Xu and Chang, 2007).

4.3 Effect of Steam-Cooking on Total Phenolic, Flavonoid Content and Antioxidant Activity

4.3.1 Total phenolic content

According to Table 4.8, BW had the highest free, bounded and total phenolic content. BC and BL was similar for free and bounded phenolics, statistically ($p<0.05$).

Table 4.8: Phenolic contents of steam-cooked products.

Samples	Total Phenolic Content (GAE/100 mg DW)		
	Free	**Bound**	**Total**
BW	166.56±9.39^{b}	114.64±14.25^{d}	281.20±12.58^{a}
BC	68.84±7.62^{e}	73.01±3.71^{e}	141.86±9.98^{c}
BL	63.87±5.52^{e}	68.83±17.48^{e}	132.70±19.12^{c}

After steam cooking, free BW and BC extracts were grown. There was no important change in free BL extracts. BW also showed increase in bound extracts. On the other hand, bound BC did not increase critically and bound BL decreased. In total values,

BW and BC had higher values than raw material but BL had not. Figure 4.12 and Table 4.9 show the experimental results.

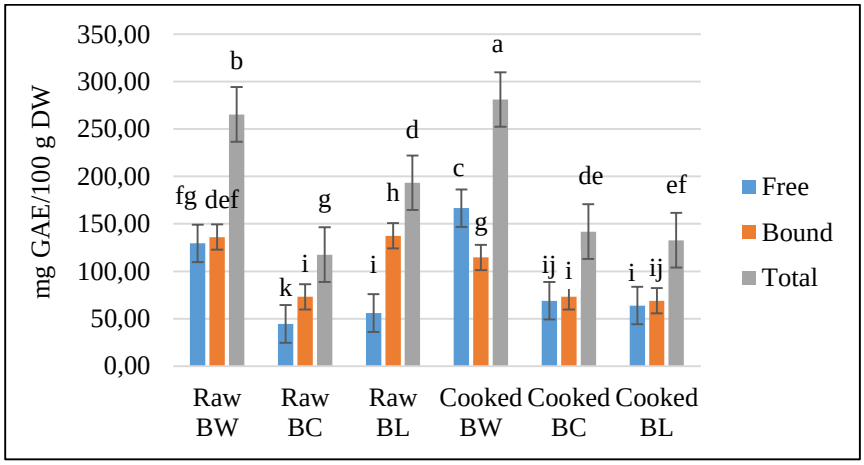

Figure 4.12: Phenolic content of raw and steam cooked products.

Table 4.9: Phenolic content of raw and steam-cooked products.

	Samples	Phenolic Content (GAE/100 mg DW)		
		Free	**Bound**	**Total**
RAW	**BW**	129.43±6.83^{fg}	136.00±1.84^{def}	265.43±6.90^{b}
	BC	44.41±10.33^{k}	60.60±10.33^{i}	105.013±17.43^{g}
	BL	55.91±4.59^{j}	87.35±0.46^{h}	143.26±19.82^{d}
STEAM COOKED	**BW**	166.56±9.39^{c}	114.64±14.25^{g}	281.20±12.58^{a}
	BC	68.84±7.62^{ij}	73.01±3.71^{i}	141.86±9.98^{de}
	BL	63.87±5.52^{ij}	68.83±17.48^{ij}	132.70±19.12^{ef}

Literature review showed that, increasing of the cooking time, and temperature increased the losses. Roasting decreased the phenolic content of buckwheat (Zhang et al., 2010). Reduction of total phenolic content was seen in spaghetti with buckwheat flour by boiling (Verardo et al., 2011). Vadivel et al. (2011), studied on total phenolic content of various wild legumes with three different cooking methods. Soaking-cooking and open-pan roasting caused a decrease of TPC but, sprouting-oil frying process enhanced the content. No reduction for chickpea in TPC was obtained during steaming in a research which was done by Segev et al. (2011). Lentil *cv. Pardina* and *cv. Crimson* was developed their TPC after their seeds were cooked (Han and Baik, 2008).

4.3.2 Total flavonoid content

BW had the highest free flavonoid content and BC-BL were similar. All of the products had close value of bounded flavonoids. BW had the greatest total flavonoid

content and BC-BL were similar. Flavonoid contents of cooked products were shown in Table 4.10.

Table 4.10: Flavonoid content of steam-cooked products.

Samples	Total Flavonoid Content Flavonoid Content (mg RE/100 g DW)		
	Free	Bound	Total
BW	318.98±59.79^{c}	263.23±49.39^{d}	582.21±66.82^{a}
BC	130.12±25.49^{e}	234.43±33.17^{d}	364.55±36.69^{b}
BL	100.33±35.44^{e}	225.22±57.39^{d}	325.55±71.94^{bc}

After steam cooking, there was decrease in all sample for flavonoid content. After cooking process, free and bound BC and BL decreased to similar amounts ($p<0.05$). Experimental data is shown in Table 4.11 and Figure 4.13.

Table 4.11: Flavonoid content of raw and steam-cooked products.

	Samples	Flavonoid Content (mg RE/100 g DW)		
		Free	Bound	Total
RAW	**BW**	593.10±4.41^{e}	392.11±12.69^{fg}	985.21±100.94^{c}
	BC	368.75±30.42^{fgh}	974.01±8.66^{c}	1342.76±187.56^{a}
	BL	402.53±39.34^{f}	671.76±97.44^{d}	1074.29±238.29^{b}
STEAM COOKED	**BW**	318.98±59.79^{hi}	263.23±49.39^{ij}	582.21±66.82^{e}
	BC	130.12±25.49^{k}	234.43±33.17^{j}	364.55±36.69^{fgh}
	BL	100.33±35.44^{k}	225.22±57.39^{j}	325.55±71.94^{ghi}

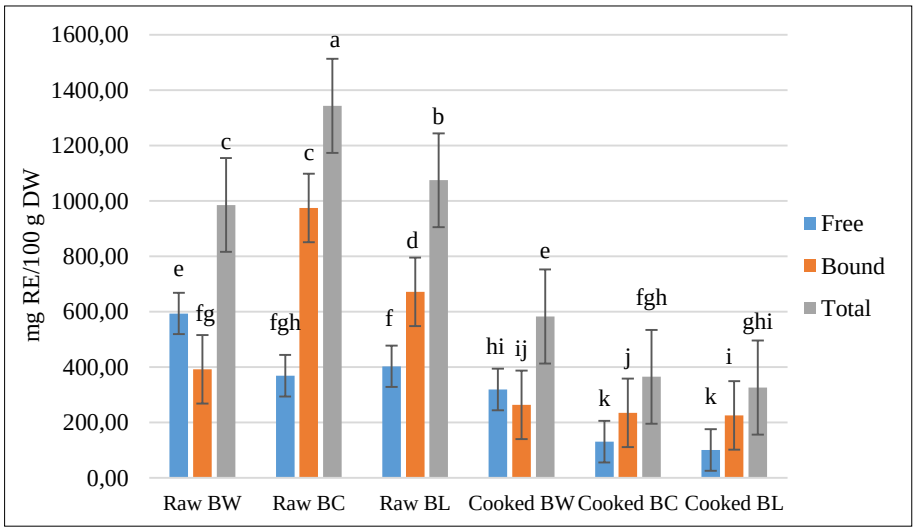

Figure 4.13: Flavonoid content of raw and steam-cooked products.

Zhang et al. (2010), investigated effect of the different thermal processes on antioxidant properties of buckwheat. Flavonoids in buckwheat decreased obviously with three methods but the least loss was pressured steam heating. In a research that subjected to evaluate the nutritional and bioactive microconstituents of cooked dry

Mediterranean Legumes, stated TFC as 604.0 for chickpea, 2109.9 for large lentil and 1841.9 µg RE/g fresh weight for small lentil (Kalogeropoulos et al., 2010).

4.3.3 Total Antioxidant Activity

Cooking process effected on antioxidant activities. In a study that Zhang et al. (2010) had done, antioxidant activity of buckwheat flour (analyzed with Fe-induced Fenton assay, pyrogallol autoxidation assay, liposome peroxidation assay) decreased with steam-heating.

In a case about antioxidant activities of cool season legumes, antioxidant activities of legumes had shown a reduction after steam cooking with DPPH and oxygen radical absorbance capacity (ORAC) method (Xu and Chang, 2008).

4.3.3.1 Total antioxidant activity by ABTS method

BW and BC had the highest antioxidant activity. BL was the most active sample in bounded extracts. All of the samples had the different total antioxidant activities and BL was the highest. Antioxidant activity of steam-cooked samples were shown in Table 4.12.

Table 4.12: Antioxidant activity with ABTS method of steam-cooked samples.

Samples	ABTS (mg TEAC/100 g DW)		
	Free	**Bound**	**Total**
BW	89.99±0.46^{e}	89.87±0.90^{e}	179.85±1.22^{c}
BC	81.78±7.83^{ef}	123.21±13.87^{c}	204.99±19.29^{a}
BL	76.70±1.71^{f}	153.67±11.30^{d}	230.37±11.14^{b}

All of the products showed higher antioxidant activity with ABTS method, except for Bounded BL, after steam cooking. Bounded extracts of BL remained same ($p<0.05$). Results are shown in Table 4.13 and Figure 4.14.

Table 4.13: Antioxidant activity with ABTS method of raw and steam-cooked samples.

	Samples	ABTS (mg TEAC/100 g DW)		
		Free	**Bound**	**Total**
RAW	**BW**	77.10±0.49^{h}	78.14±0.38^{h}	155.24±0.79^{e}
	BC	60.22±3.28^{i}	112.58±7.23^{f}	172.80±6.84^{d}
	BL	57.73±1.57^{i}	151.98±5.59^{e}	209.71±6.81^{c}
STEAM COOKED	**BW**	89.99±0.46^{g}	89.87±0.90^{g}	179.85±1.22^{d}
	BC	81.78±7.83^{gh}	123.21±13.87^{d}	204.99±19.29^{b}
	BL	76.70±1.71^{h}	153.67±11.30^{e}	230.37±11.14^{a}

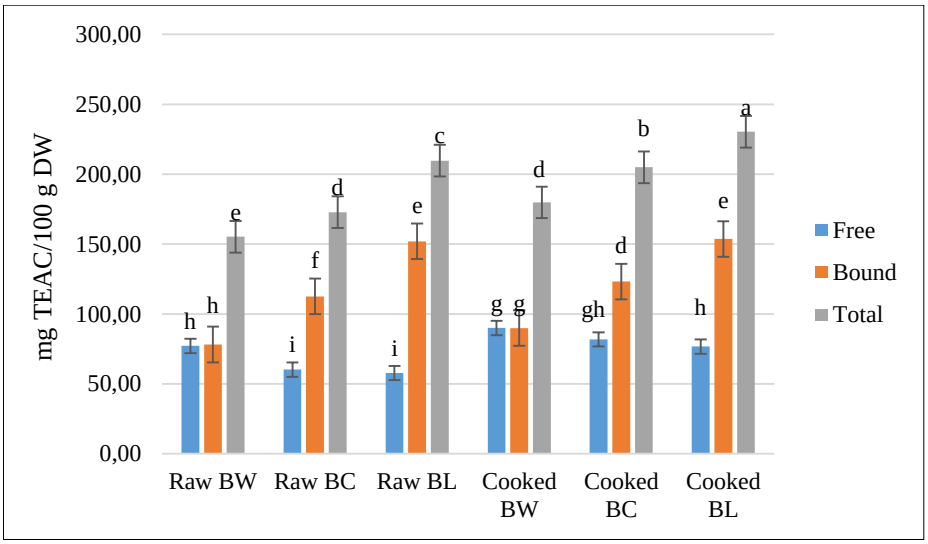

Figure 4.14: Antioxidant activity with ABTS method of raw and steam-cooked samples.

Han and Baik (2008), investigated the some processing techniques on some legumes. The antioxidant activity of raw, decorticated, cooked and soaked seeds with ABTS method, were compared to each other. Cooking had no significant effect on AOX activity for lentil *cv. Crimson* and chickpea and lentil had higher activity than chickpeas.

4.3.3.2 Total antioxidant activity by CUPRAC method

Results of antioxidant activity analysis by CUPRAC method showed that BW was more active than other free extracts. The highest value belonged to BC in bound extracts and BW-BL showed similar activity. BW and BC had the highest antioxidant activity by CUPRAC method ($p<0.05$).

Table 4.14: Antioxidant activity with CUPRAC method of steam-cooked samples.

Samples	CUPRAC (mg TEAC/100 g DW)		
	Free	Bound	Total
BW	803.18±77.85^{c}	629.88±47.93^{d}	1433.05±73.82^{a}
BC	220.70±26.82^{e}	1192.32±55.27^{b}	1413.02±78.28^{a}
BL	252.43±14.51^{e}	563.13±153.99^{d}	815.57±155.01^{c}

After steam cooking, there was negligible change in free extracts. Antioxidant activities of bound extracts decreased. In case, total antioxidant activity by CUPRAC method decreased either. Experimental data was displayed in Table 4.15 and Figure 4.15.

Table 4.15: Antioxidant activity with CUPRAC method of raw and steam-cooked samples.

	Samples	CUPRAC (mg TEAC/100 g DW)		
		Free	Bound	Total
RAW	BW	753.35±28.33^{fg}	986.69±71.11^{e}	1740.04±99.17^{a}
	BC	171.56±12.21^{j}	1387.71±22.47^{c}	1559.27±39.20^{b}
	BL	247.72±17.19^{j}	692.08±55.44^{gh}	939.81±60.57^{e}
STEAM COOKED	BW	803.18±77.85^{f}	629.88±47.93^{hi}	1433.05±73.82^{c}
	BC	220.70±26.82^{j}	1192.32±55.27^{d}	1413.02±78.28^{c}
	BL	252.43±14.51^{j}	563.13±153.99^{i}	815.57±155.01^{f}

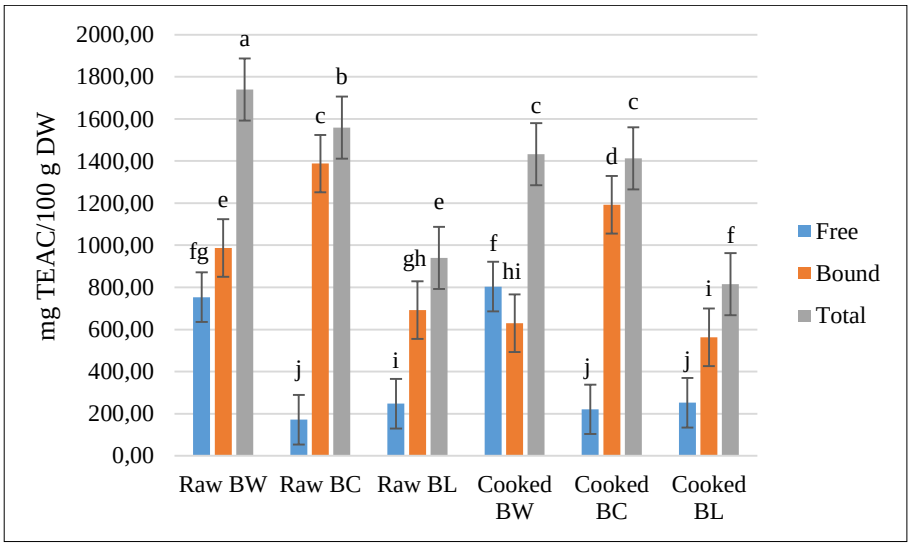

Figure 4.15: Antioxidant activity with CUPRAC method of raw and steam-cooked samples.

4.3.3.3 Total antioxidant activity by DPPH method

BW has the highest antioxidant activity in free extracts and it was similar in bounded extracts. BW was the most active one according to total values and BC-BL was similar, statistically ($p<0.05$). Data for antioxidant activity of cooked products was shown in Table 4.16.

Table 4.16: Antioxidant activity with DPPH method of steam-cooked samples.

Samples	DPPH (mg TEAC/100 g DW)		
	Free	Bound	Total
BW	53.66±5.92^{c}	38.04±3.43^{d}	91.70±5.49^{a}
BC	16.33±3.43^{f}	33.04±2.83^{d}	49.38±4.84^{b}
BL	23.38±17.94^{e}	38.29±2.97^{d}	61.67±17.77^{b}

There is no remarkable change in antioxidant activity by DPPH method in free extracts ($p<0.05$). The activity decreased in bound extracts. Total activity showed lower values BW and BL. Change in total antioxidant of BC, did not show an important difference. Statistical comparison of raw and steam-cooked products were shown in Table 4.17.

Table 4.17: Antioxidant activity with DPPH method of raw and steam-cooked samples.

	Samples	DPPH (mg TEAC/100 g DW)		
		Free	Bound	Total
RAW	BW	55.96±26.31^{ef}	61.37±28.69^{de}	117.33±2.69^{a}
	BC	12.85±9.57^{j}	52.44±25.19^{f}	65.29±1.89^{d}
	BL	20.29±6.14^{hi}	53.39±24.83^{f}	73. 68±4.49^{c}
STEAM COOKED	BW	53.66±5.92^{f}	38.04±3.43^{g}	91.70±5.49^{b}
	BC	16.33±3.43^{ij}	33.04±2.83^{g}	49.38±4.84^{de}
	BL	23.38±17.94^{h}	38.29±2.97^{g}	61.67±17.77^{de}

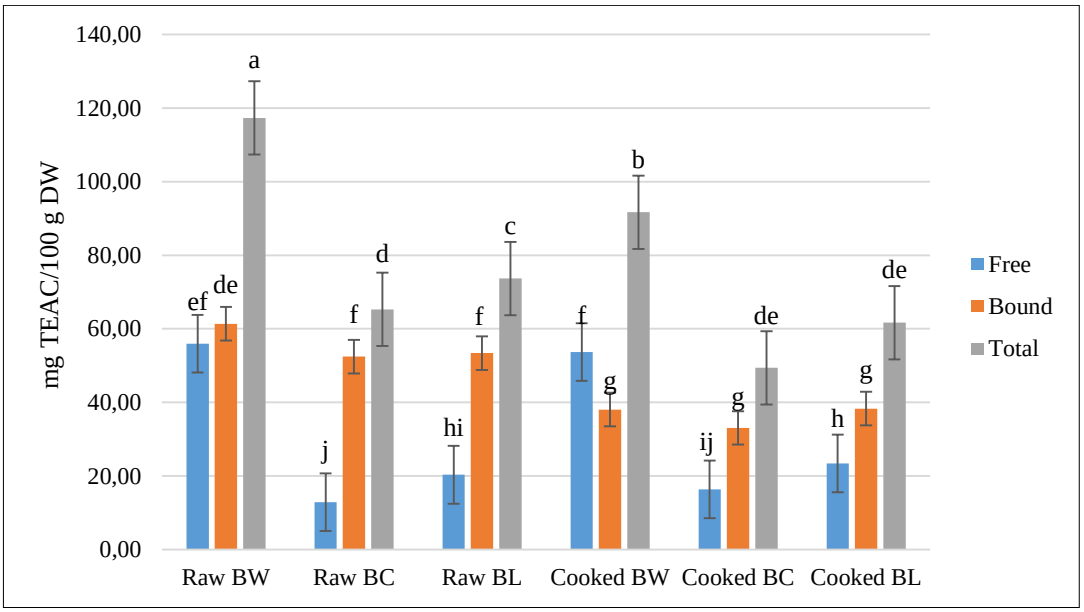

Figure 4.16: Antioxidant activity with DPPH method of raw and steam-cooked samples.

In a research about wild legumes, DPPH values of soaked and cooked seeds showed reduction when were compared to results of raw seeds (Vadivel et al., 2011). After hydrothermal treatment (steaming and heating), antioxidant activity of buckwheat, with DPPH method, was decreased approximately 70% (Zielinska et al., 2007).

4.4 Changes in Total Phenolic, Flavonoid Contents and Antioxidant Activities after *In Vitro* Digestion

4.4.1 Total phenolic content

Results of *In Vitro* digestion of phenolics were evaluated statistically ($p<0.05$). PG and OUT values were higher than IN values, generally. Raw OUT_{BW} was the highest. There was no significant difference between raw BW and BL OUT values and PG values of raw BC and BL, IN values between steam cooked BW, BC, BL products. Results are shown in Table 4.18 and Figure 4.17.

Table 4.18: Total phenolic contents of samples after *in vitro* digestion.

	Samples	Phenolic Content (GAE/100 mg DW)			
		INITIAL	PG	IN	OUT
RAW	BW	265.43±6.90	163.04±33.61^{de}	45.68±9.27^{f}	315.74±8.86^{a}
	BC	105.013±17.43	214.76±16.58^{cd}	52.77±2.53^{f}	217.92±24.87^{cd}
	BL	143.26±19.82	223.52±56.31^{cd}	229.03±14.70^{cd}	295.60±18.60^{ab}
STEAM COOKED	BW	281.20±12.58	248.75±17.96^{bc}	42.77±7.23^{f}	231.58±40.25^{cd}
	BC	141.86±9.98	198.74±7.14^{de}	51.43±6.99^{f}	196.96±55.75^{de}
	BL	132.70±19.12	259.44±11.40^{bc}	58.09±10.19^{f}	221.95±6.99^{cd}

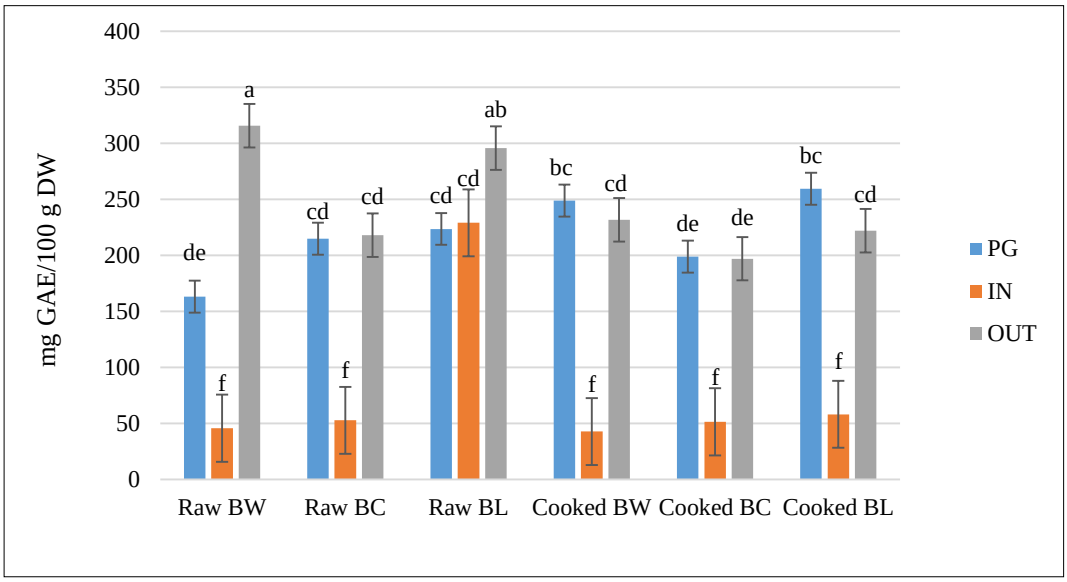

Figure 4.17: Total phenolic contents of samples after *in vitro* digestion.

In vitro digested wheat brans showed higher phenolic content than raw extracts. This situation was explained as pH changes released the bound phenolics from polysaccharides' cell wall thus making them more available for measurement and more bioavailable after gastrointestinal digestion (Liyanapathirana and Shahidi, 2004). In a study on in vitro gastrointestinal digestion of pea products, enzymatically digested seed flours had higher TPC than raw and cooked material (Stanisavljevic et al., 2014).

The best distribution was belong to PG value of steam cooked BL, PG and OUT value of BC with no significant difference. PG recovery values (%) ranged from 61.42 to 191.41. Steam cooking increased the recovery of PG_{BW} and PG_{BL}. On the other hand, IN_{BL} decreased after cooking process, there were no significant change for other products. OUT_{BW} and OUT_{BC} values also decreased after steam cooking. Results are shown in Table 4.19 and Figure 4.18.

Table 4.19: Total phenolic content distribution (recovery %) between PG, IN and OUT fractions.

	Samples	Recovery of Phenolic Content (%)			
		INITIAL	PG	IN	OUT
RAW	BW	100	61.42±14.92^{fg}	17.21±3.50^{hi}	118.95±13.95^{cd}
	BC	100	182.75±40.96^{a}	44.90±2.56^{gh}	185.44±56.00^{a}
	BL	100	115.58±30.00^{cd}	118.43±16.21^{cd}	152.85±11.06^{b}
STEAM COOKED	BW	100	92.88±8.03^{de}	15.22±3.31^{i}	82.12±4.28^{ef}
	BC	100	138.99±20.44^{bc}	35.98±2.47^{ghi}	137.69±25.09^{bc}
	BL	100	191.41±12.54^{a}	42.81±8.97^{ghi}	164.10±61.85^{ab}

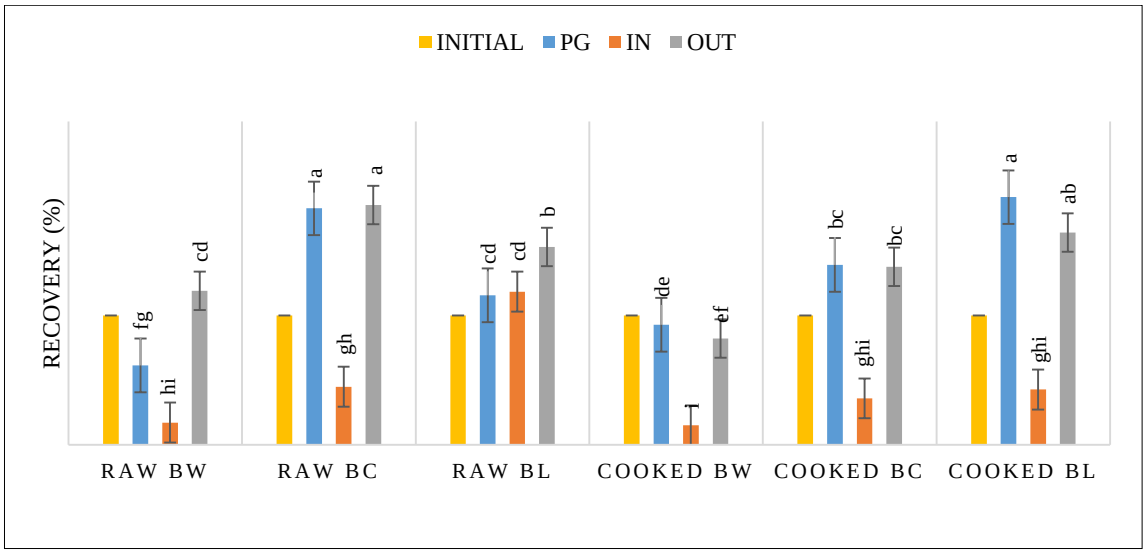

Figure 4.18: Total phenolic content distribution (recovery %) between PG, IN and OUT fractions.

4.4.2 Total flavonoid content

Raw IN_{BC} had the highest flavonoid content in all of the samples. According to PG values, steam cooked PG_{BW} was greater than others. There were no significant difference between cooked IN products and they were less than raw products. Important difference was not observed between OUT data of raw and steam cooked samples. Experimental data is displayed in Table 4.20 and Figure 4.19.

Table 4.20: Total flavonoid contents of samples after *in vitro* digestion.

	Samples	Flavonoid Content (mg RE/100 g DW)			
		INITIAL	PG	IN	OUT
RAW	BW	985.21±100.94	82.60±10.29^{defg}	161.11±16.40^{bc}	140.56±0^{bcde}
	BC	1342.76±187.56	71.38±5.53^{efgh}	316.50±12.76^{a}	100.35±8.11^{cdef}
	BL	1074.29±238.29	36.62±6.25^{fgh}	16.65±0.52^{fg}	138.70±37.87^{bcde}
STEAM COOKED	BW	582.21±66.82	197.34±21.10^{b}	11.80±1.93^{g}	156.51±18.44^{bc}
	BC	364.55±36.69	154.12±45.94^{bc}	2.28±1.29^{g}	144.36±32.75^{bcd}
	BL	325.55±71.94	12.69±18.89^{g}	6.63±3.78^{g}	104.22±22.02^{cdef}

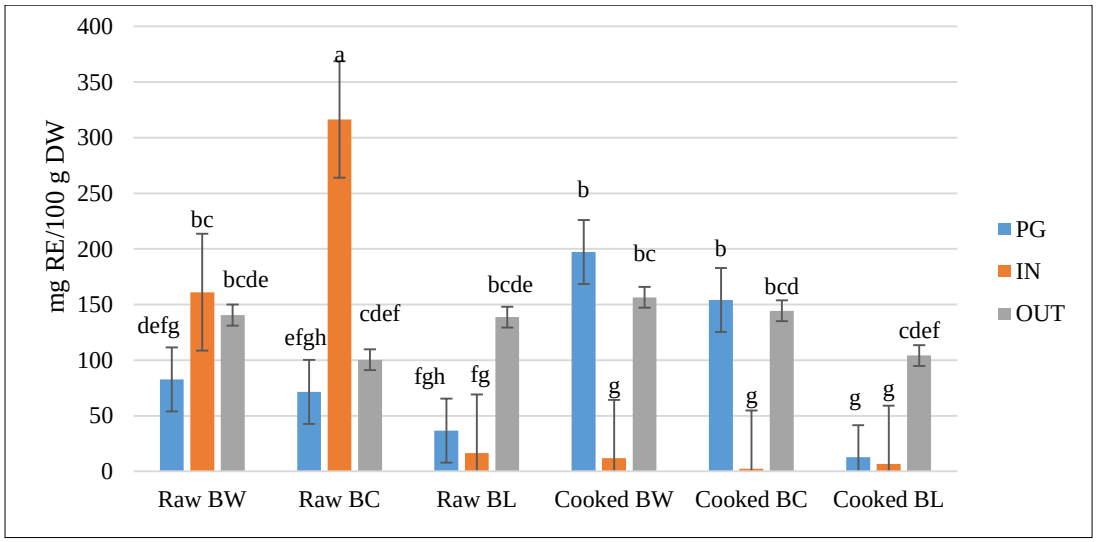

Figure 4.19: Total flavonoid contents of samples after *in vitro* digestion.

After *in vitro* gastrointestinal digestion, there was a decrease in flavonoid content both raw and steam-cooked products. Similar results were seen in study on bioavailability of buckwheat bread. As same as total flavonoid content had reduction with enzymatic digestion (Gawlik-Dziki et al., 2009). Akillioglu and Karakaya (2010) stated that soaked and cooked samples of pinto beans showed a reduction of TFC after *in vitro* digestion.

Recovery increased in PG and OUT values, but decreased in IN values. The highest score was steam cooked PG_{BC}. PG_{BW} and PG_{BC} developed after cooking. However, PG_{BL} was not different significantly. Recovery of IN_{BW} and IN_{BC} of raw samples were greater than cooked samples but there were no important distinction for IN_{BL}. Cooking process advanced the recovery for OUT for three of the samples. Recovery results are shown in Table 4.21 and Figure 4.20.

Table 4.21: Total flavonoid content distribution (recovery %) between PG, IN and OUT fractions.

	Samples	Recovery of Flavonoid Content (%)			
		INITIAL	PG	IN	OUT
RAW	**BW**	100	8.38 ± 2.26^{fghi}	16.35 ± 31.65^{ef}	14.27 ± 3.16^{efg}
	BC	100	5.32 ± 3.45^{ghi}	23.57 ± 15.21^{de}	7.47 ± 3.39^{fghi}
	BL	100	3.41 ± 3.27^{hi}	1.55 ± 1.08^{i}	12.91 ± 5.80^{fgh}
STEAM COOKED	**BW**	100	33.90 ± 5.37^{abc}	2.03 ± 0.59^{i}	26.88 ± 5.37^{cd}
	BC	100	42.28 ± 13.27^{a}	0.63 ± 1.06^{i}	39.60 ± 10.87^{ab}
	BL	100	3.90 ± 10.06^{hi}	2.04 ± 1.68^{i}	32.01 ± 15.78^{bcd}

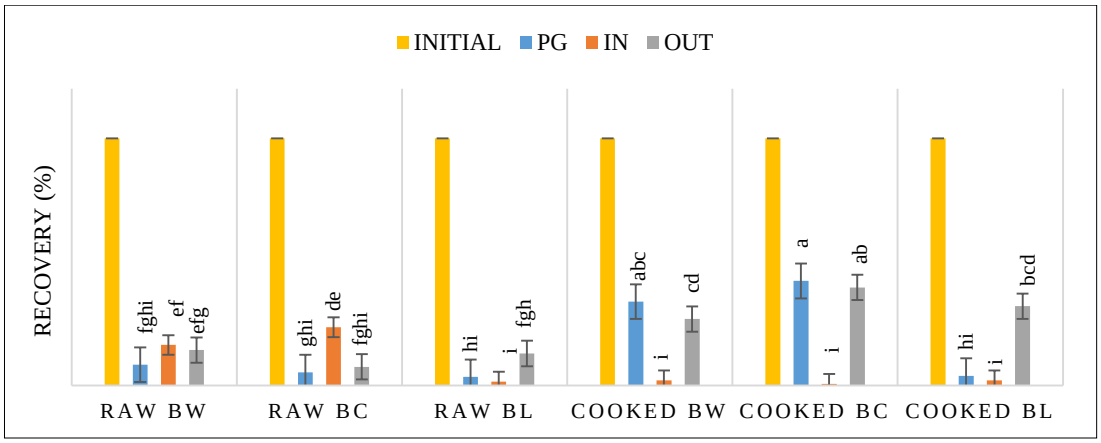

Figure 4.20: Total flavonoid content distribution (recovery %) between PG, IN and OUT fractions.

4.4.3 Total Antioxidant Activity

Results differed according to ABTS (0-29.10±1.42 mg TEAC/100 g DW), CUPRAC (73.54±1.98- 927.38±51.01 mg TEAC/100 g DW) and DPPH (2.30±0.08-19.59±1.51 mg TEAC/100 g DW) method that used. CUPRAC was the most effective method for analyzing antioxidant activity of these three products.

Recovery values were calculated to compare the bioavailability of products before and after steam cooking.

Reference for total antioxidant activity with CUPRAC method of *in vitro* digested materials could not be find from literature. Studies were done with mostly DPPH method.

4.4.3.1 Total antioxidant activity by ABTS method

Raw PG_{BL} showed the best antioxidant activity in all products. The activity slowed down after steam cooking in PG samples. There were no significant difference between products according to type or raw/steam cooked. Antioxidant activity was not detected by ABTS method in none of OUT samples. Results are summarized in Table 4.22 and Figure 4.21.

Table 4.22: Total antioxidant activity by ABTS method of samples after *in vitro* digestion.

	Samples	ABTS (mg TEAC/100 g DW)			
		INITIAL	PG	IN	OUT
RAW	BW	155.24±0.79	27.56±0.56^{b}	10.80±0.08^{f}	0^{g}
	BC	172.80±6.84	23.58±0.16^{c}	11.09±0.39^{f}	0^{g}
	BL	209.71±6.81	29.10±1.42^{a}	10.32±0.03^{f}	0^{g}
STEAM COOKED	BW	179.85±1.22	14.56±1.89^{e}	10.41±0.07^{f}	0^{g}
	BC	204.99±19.29	16.32±3.05^{d}	10.53±0.03^{f}	0^{g}
	BL	230.37±11.14	26.48±1.18^{b}	10.14±0.45^{f}	0^{g}

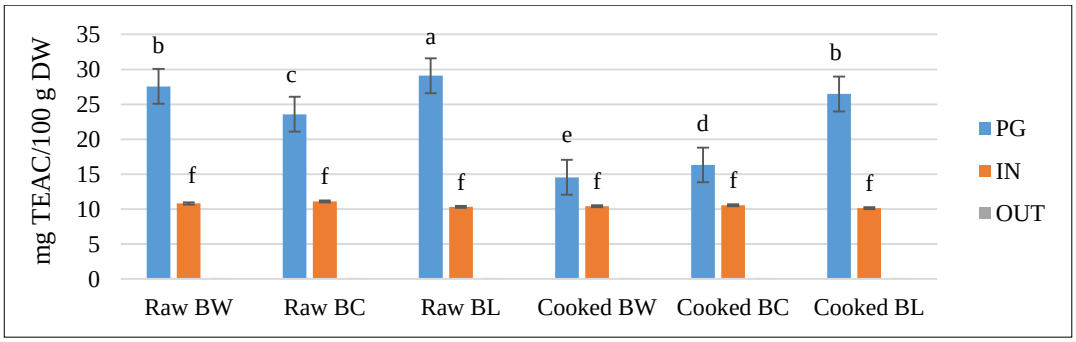

Figure 4.21: Total antioxidant activity by ABTS method of samples after *in vitro* digestion.

Gumienna et al. (2009), studied on *in vitro* digestion of lentils and according to results, there were no significant difference between sample before digestion and digestion for 4 hours in stomach for antioxidant activity with ABTS method. AOX activity enchanted in small intestine digestion after 2 hours and 4 hours. On the other hand, our experimental results did not show an increase. This can be explained with method difference and digestion duration.

When values were divided by total activity, results changed. The highest becomes PG_{BW}. The decline that after steam cooking in PG's were same. Recovery of IN_{BW} and IN_{BC} were higher when they were raw; IN_{BL} did not effected. Distribution equaled to zero for all of the samples for OUT values. Results are in Table 4.23 and Figure 4.22.

Table 4.23: Total antioxidant activity (by ABTS method) distribution (recovery %) between PG, IN and OUT fractions.

	Samples	Recovery of Antioxidant Activity by ABTS Method (%)			
		INITIAL	PG	IN	OUT
RAW	**BW**	100	17.75 ± 0.37^{a}	6.96 ± 0.05^{e}	0^{i}
	BC	100	13.64 ± 0.87^{b}	6.42 ± 0.02^{ef}	0^{i}
	BL	100	13.87 ± 0.08^{b}	4.92 ± 0.19^{h}	0^{i}
STEAM COOKED	**BW**	100	8.09 ± 1.19^{d}	5.79 ± 0.05^{fg}	0^{i}
	BC	100	7.96 ± 1.95^{d}	5.14 ± 0.02^{gh}	0^{i}
	BL	100	11.49 ± 0.54^{c}	4.40 ± 0.19^{h}	0^{i}

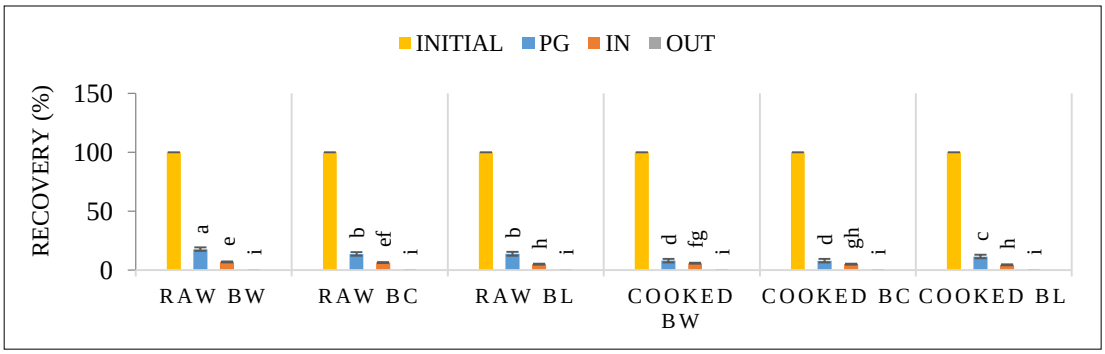

Figure 4.22: Total antioxidant activity (by ABTS method) distribution (recovery %) between PG, IN and OUT fractions.

4.4.3.2 Total antioxidant activity by CUPRAC method

The highest antioxidant activity was observed in raw OUT_{BC}. There was no pattern for PG values. PG_{BW} increased, but there were no significant change for PG_{BC} and PG_{BL} after cooking. IN values were similar for raw products and AOX activity increased when they were steam cooked. Diminution of activity was appeared after process for OUT results. Data sets are given in Table 4.24 and Figure 4.23.

Table 4.24: Total antioxidant activity by CUPRAC method of samples after *in vitro* digestion.

	Samples	CUPRAC (mg TEAC/100 g DW)			
		INITIAL	PG	IN	OUT
RAW	BW	1740.04±99.17	396.65±5.09^{e}	96.59±19.58^{h}	782.30±3.89^{b}
	BC	1559.27±39.20	372.37±63.49^{e}	73.54±1.98^{h}	927.38±51.01^{a}
	BL	939.81±60.57	271.06±11.85^{fg}	82.63±3.47^{h}	711.54±41.60^{b}
STEAM COOKED	BW	1433.05±73.82	607.65±23.99^{c}	579.53±110.69^{cd}	152.82±7.10^{h}
	BC	1413.02±78.28	328.34±31.87^{ef}	517.03±68.00^{d}	138.93±5.34^{h}
	BL	815.57±155.01	247.29±7.02^{g}	604.98±132.91^{c}	108.26±15.10^{h}

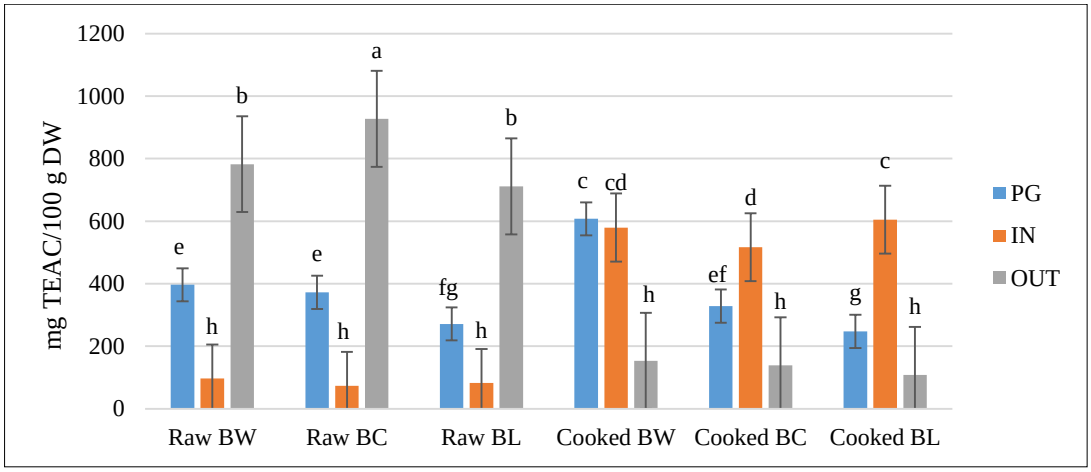

Figure 4.23: Total antioxidant activity by CUPRAC method of samples after *in vitro* digestion.

Recovery of AOX activity was highest for steam cooked IN_{BC} and raw OUT_{BL}. Recovery pattern was similar for PG, IN, OUT outcomes when it was compared to Table 4.24. Results are shown in Table 4.25 and Figure 4.24.

Table 4.25: Total antioxidant activity (by CUPRAC method) distribution (recovery %) between PG, IN and OUT fractions.

	Samples	Recovery of AOX Activity by CUPRAC Method (%)			
		INITIAL	PG	IN	OUT
RAW	BW	100	22.80±0.63^{f}	5.55±1.25^{gh}	44.96±1.92^{c}
	BC	100	23.88±4.17^{f}	4.72±0.44^{h}	59.48±3.90^{b}
	BL	100	28.84±1.79^{f}	8.79±3.31^{gh}	75.71±10.21^{a}
STEAM COOKED	BW	100	42.40±2.92^{cd}	40.44±9.55^{cd}	10.66±0.74^{gh}
	BC	100	23.24±2.36^{f}	36.59±5.74^{de}	9.69±0.67^{gh}
	BL	100	30.32±1.21^{ef}	74.18±20.18^{a}	13.27±2.29^{g}

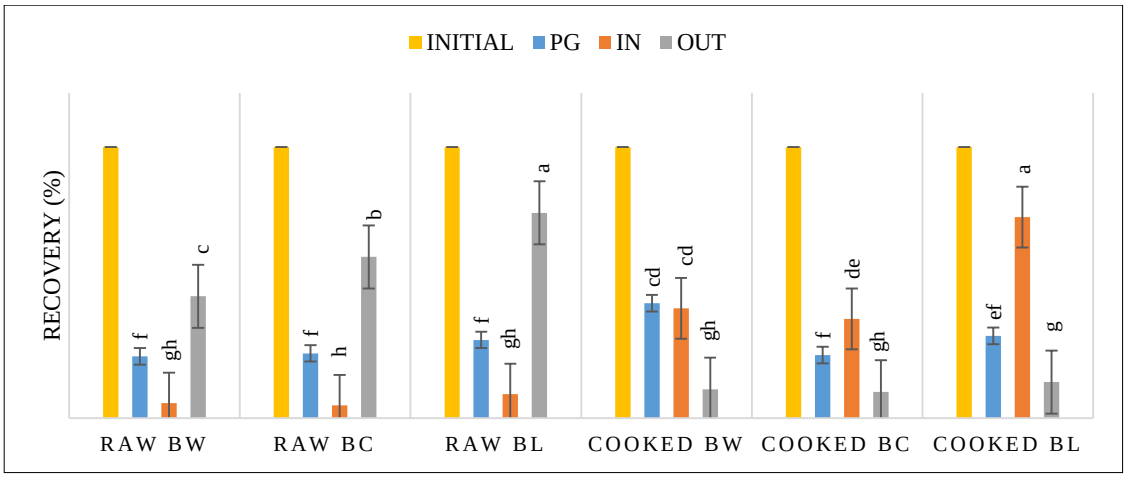

Figure 4.24: Total antioxidant activity (by CUPRAC method) distribution (recovery %) between PG, IN and OUT fractions.

4.4.3.3 Total antioxidant activity by DPPH method

PG values higher than IN and OUT values for antioxidant activity by DPPH method. The highest scores were steam cooked PG_{BC} and PG_{BL}. After processing, activity increased according to IN results. On the other hand, OUT_{BL} decreased but OUT_{BW} and OUT_{BC} was similar as it is shown in Table 4.26 and Figure 4.25.

Table 4.26: Total antioxidant activity by DPPH method of samples after *in vitro* digestion.

	Samples	DPPH (mg TEAC/100 g DW)			
		INITIAL	PG	IN	OUT
RAW	BW	117.33±2.69	15.72±0.56^{b}	2.30±0.08^{g}	4.62±0.26^{ef}
	BC	65.29±1.89	12.92±1.42^{c}	3.33±0.03^{fg}	4.40±0.39^{ef}
	BL	73. 68±4.49	13.32±0.16^{c}	2.33±0.39^{g}	7.75±0.57^{d}
STEAM COOKED	BW	91.70±5.49	15.50±0.84^{b}	5.03±0.42^{ef}	5.33±1.03^{ef}
	BC	49.38±4.84	19.59±1.51^{a}	5.22±0.27^{ef}	3.98±0.91^{efg}
	BL	61.67±17.77	18.21±1.63^{a}	4.52±0.26^{ef}	5.89±0.75^{e}

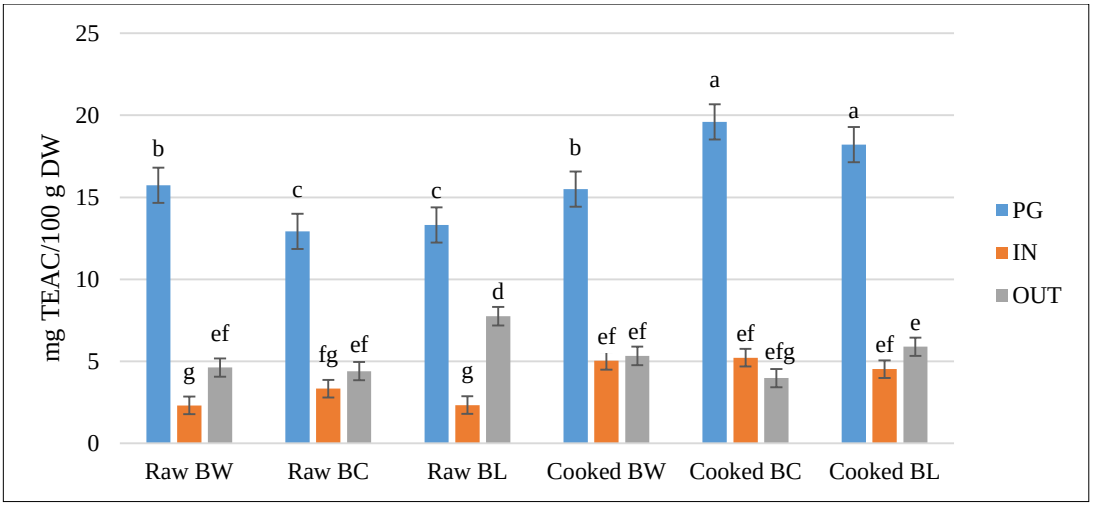

Figure 4.25: Total antioxidant activity by DPPH method of samples after *in vitro* digestion.

Recovery of AOX activity by DPPH method was increased for PG and IN values after cooking for all of the products. There were no significant difference between OUT_{Raw} and $OUT_{SteamCooked}$. Results are in Table 4.27 and Figure 4.26.

Wheat extracts which affected by *in vitro* digestion (Liyanapathirana and Shahidi, 2004) and *in vitro* digestion of wheat bread with buckwheat addition (Gawlik-Dziki et al., 2009) were investigated for their antioxidant activity with DPPH method. AOX activity showed an augmentation after digestion step when was compared with unprocessed materials. Antioxidant activity of seeds of different pea samples also showed an increase after enzymatically digestion. Disparity between references and experimental data can be explained with different *in vitro* digestion systems and different products.

Table 4.27: Total antioxidant activity (by DPPH method) distribution (recovery %) between PG, IN and OUT fractions.

	Samples	Recovery of Antioxidant Activity by DPPH Method (%)			
		INITIAL	PG	IN	OUT
RAW	BW	100	13.40±0.39^{d}	1.96±0.45^{j}	3.93±0.32^{hij}
	BC	100	19.79±3.38^{c}	5.10±0.38^{ghi}	6.74±6.70^{fgh}
	BL	100	18.08±4.37^{c}	3.16±0.77^{ij}	10.52±1.08^{de}
STEAM COOKED	BW	100	16.91±1.26^{c}	5.48±0.54^{ghi}	5.81±1.64^{ghi}
	BC	100	39.68±3.94^{a}	10.57±0.81^{de}	8.05±2.97^{efg}
	BL	100	29.53±4.31^{b}	7.34±0.93^{fg}	9.55±2.34^{ef}

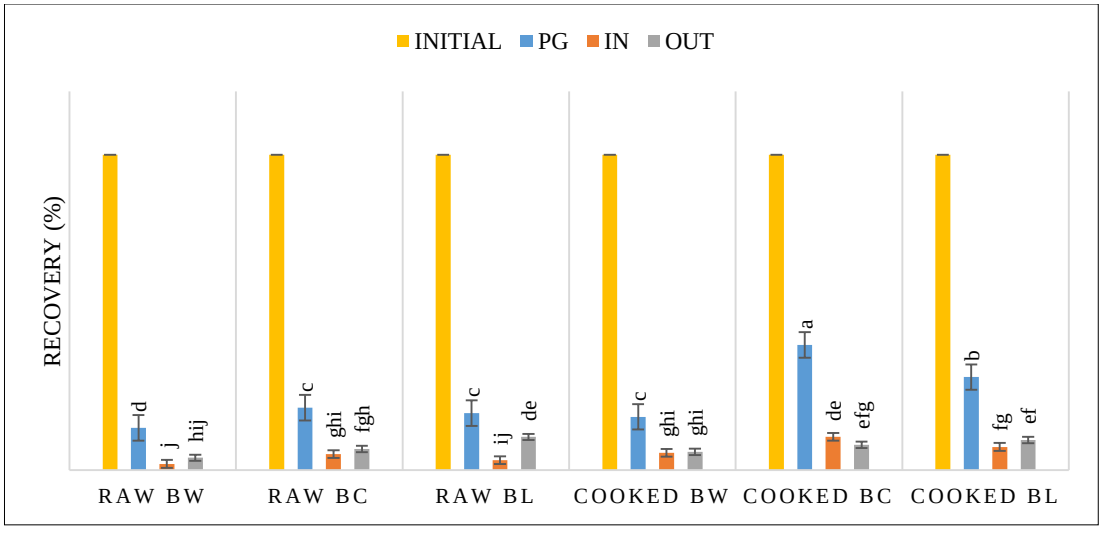

Figure 4.26: Total antioxidant activity (by DPPH method) distribution (recovery %) between PG, IN and OUT fractions.

4.5 The Relations between Total Phenolic and Total Antioxidant Activity Methods

The relations between total phenolic content analysis, total flavonoid analysis and all of the total antioxidant activity methods (CUPRAC, DPPH and ABTS) were evaluated by basic linear regression analysis. The correlation coefficients are given in Table 4.28.

According to the Table 4.28, there was an important relation between total phenolic content and all of total antioxidant activity methods, CUPRAC (r=0.648), DPPH (r=0.856) but, except for ABTS (r=0.367). On the other hand, all of them were statistically significant ($p<0.01$). Flavonoid had weak involvement with ABTS (0.251) but strongly related with CUPRAC (0.611) and DPPH (0.618). The relation between CUPRAC and DPPH, CUPRAC and ABTS were also statistically significant ($p<0.01$). There was weak relation (0.566) between ABTS and DPPH, but according to statistical evaluation it was significant again. CUPRAC and DPPH was related significantly (0.793; $p<0.01$).

Table 4.28: Regression analysis for total phenolic content and total antioxidant activity methods.

Methods	Folin	Flavonoid	ABTS	CUPRAC	DPPH
Folin	-	-	-	-	-
Flavonoid	0.378*	-	-	-	-
ABTS	0.367*	0.251*	-	-	-
CUPRAC	0.648*	0.611*	0.630*	-	-
DPPH	0.856*	0.618*	0.520*	0.793*	-

* The regression variance analysis for the results is significant, statistically ($p<0.01$).

4.6 Evaluation of Phenolic Compounds by HPLC-PDA

Phenolic compounds were evaluated by HPLC-PDA after steam cooking and GI digestion. Identification was made with retention times and maximum wavelengths. There were no significant peak for none of the samples at 520 nm wavelength.

Gallic acid, Catechin, Epicatechin, Procatechuic Acid and Rutin were determined in BW samples. Differences were observed between free and bound extracts. Free phenolic compounds of raw buckwheat (FPRBW) were shown an increase except for Procatechuic Acid and Rutin after cooking process. Rutin and Gallic Acid content were decreased after steam cooking for bound phenolics of raw buckwheat (BPSBW). Identified phenolic components were given in Table 4.29.

Table 4.29: Major phenolic components that identified in raw and steam cooked buckwheat samples.

Compound (µg/ml)	FPRBW	BPRBW	FPSBW	BPSBW
Gallic acid	2.74±0.03	15.21±11.93	4.12±3.25	1.99±0.40
Catechin	2.81±0.26	ND	3.57±0.58	2.61±0.54
Epicatechin	ND	ND	2.57±0.61	4.93±0.92
Procatechuic acid	1.79±0.11	ND	1.24±0.93	ND
Rutin	13.55±1.20	ND	7.66±5.69	2.92±1.39

There were unidentified peaks between 18.02-20.863, 25.97, 32.58 and 42.46 minutes retention time in FPRBW. Aleksenko (2013), studied on antioxidant activity and phenolic compounds of buckwheat. According to research, the main phenolic compounds in buckwheat extracts were rutin, catechin and epicatechin, 1-O-caffeoyl-O-rutinoside (m/z 487), and epicatechin-O-3,4-dimethyl- gallate. Retention times differed because of the method which was applied. After all, catechin deriatives, swertiamacroside, epicatechin deriatives, hyperin, vitexin were determined by RP-HPLC-ESI-TOF-MS (Verardo et al., 2011).

Cooking process made an increase on PG digestion of gallic acid. There were no difference IN values and a decrease of OUT. Catechin and Rutin were diminished for all PG, IN and OUT values after steam-cooking. Results were shown in Table 4.30.

Table 4.30: Major phenolic components that identified in GI digested raw and steam cooked buckwheat samples.

Compound (µg/ml)	PGRBW	INRBW	OUTRBW	PGSBW	INSBW	OUTSBW
Gallic acid	5.74±0.25	5.04±1.83	42.09±5.49	14.99±11.19	5.48±2.88	35.68±7.35
Catechin	10.11±0.26	40.48±2.83	195.25±2.05	4.64±0.60	26.18±9.14	169.04±14.3
Epicatechin	2.96±1.03	ND	ND	ND	ND	ND
Procatechuic acid	ND	ND	ND	ND	ND	1.50±0.35
Rutin	13.24±0.15	ND	10.12±0.76	8.50±7.25	ND	8.01±1.96

Gallic acid and quercetin dihydrate content increased after cooking process. Free catechin content reduced meanwhile, bounded increased. Rutin and quercetin dehydrate in free phenolic content of raw BC, catechin in bounded phneolic in raw BC, quercetin dehydrate and kaempherol in free phenolic of steam cooked samples were not detected. Phenolic contents summarized in Table 4.31.

Table 4.31: Major phenolic components that identified in raw and steam cooked black chickpea samples.

Compound (µg/ml)	FPRBC	BPRBC	FPSBC	BPSBC
Gallic Acid	3.28±0.44	103.10±76.29	5.76±1.43	191.72±103.23
Catechin	110.68±2.40	ND	80.29±20.50	32.57±19.59
Rutin	ND	3.85±0.08	2.76±0.28	2.51±0.28
Quercetin dihydrate	ND	1.56±0,13	ND	3.53±0.45
Kaempherol	1.05±0.74	0.54±0.04	ND	0.32±0.05

There were undefined peaks at 17-20th, 32nd and 35th retention times. Singh et al. (2014), found shikimic acid, gallic acid, trans-chloregenic acid, tannic acid, syringic acid, rutin, sinapic acid, ferulic acid, myricetin, salicylic acid, quercetin and kaempherol in chickpea. Retention times were similar.

Digestion of gallic acid decreased in post gastrointestinal and intestinal after cooking; OUT values increased. Rutin reduced and kaempherol was not observed in *vitro* digestion system. Results were shown in Table 4.32.

Table 4.32: Major phenolic components that identified in GI digested raw and steam cooked black chickpea samples.

Compound (µg/ml)	PGRBC	INRBC	OUTRBC	PGSBC	INSBC	OUTSBC
Gallic Acid	16.85±2.60	7.58±0.77	45.49±12.55	1.62±0.80	3.68±2.61	64.50±19.3
Catechin	10.52±1.85	122.04±7.17	276.45±64.4	208.96±98.9	ND	347.88±34.8
Rutin	8.26±0.48	3.44±1.31	10.54±2.85	7.85±0.00	2.50±0.28	3.64±0.83
Quercetin dihydrate	1.20±0.38	ND	ND	ND	ND	ND
Kaempherol	ND	ND	ND	ND	ND	ND

Gallic acid, catechin, epicatechin, coumaric acid and rutin were identified in brown lentil. Cooking caused a decrease in free gallic acid and rutin as bounded gallic acid increased. Catechin was observed after cooking process.

Table 4.33: Major phenolic components that identified in raw and steam cooked brown lentil samples.

Compound (µg/ml)	FPRBL	BPRBL	FPSBL	BPSBL
Gallic Acid	21.39±19.39	3.68±0.06	2.99±0.46	258.82±19.37
Catechin	ND	ND	9.77±0.95	25.26±13.73
Epicatechin	9.12±6.72	ND	14.20±0.51	6.72±2.31
Coumaric Acid	3.44±3.18	0.59±0.11	4.92±0.22	ND
Rutin	3.33±0.00	ND	1.90±0,11	5.13±0.76
Quercetin dihydrate	ND	18.23±0.84	ND	25.73±9.67

There were unidentified peaks in 30th minute retention time at 312 nm wavelength which might be a phenolic acid. Catechin and epicatechin deriatives, procyanidin dimers, quercetin diglycoside, and trans-p-coumaric acid were the dominant phenolics

in green lentils (Amarowicz et al., 2010). According to another study, 24 compounds identified in red lentil extract using an HPLC-ESI-MS method and quercetin diglycoside, catechin, digallate procyanidin, and p-hydroxybenzoic were the dominant phenolics in the extract (Amarowicz et al., 2009). Phenolic profiles varied in different type of lentils.

Table 4.34: Major phenolic components that identified in GI digested raw and steam cooked brown lentils samples.

Compound (µg/ml)	**PGRBL**	**INRBL**	**OUTRBL**	**PGSBL**	**INSBL**	**OUTSBL**
Gallic Acid	46.21±0.55	10.91±0.29	ND	1.87±0.07	11.25±2.86	80.63±37.67
Catechin	ND	ND	ND	39.55±2.45	7.67±3.57	126.04±9.78
Epicatechin	ND	16.89±2.71	ND	46.02±0.22	13.52±2.90	ND
Coumaric Acid	12.43±9.69	3.73±0.29	1.37±0,07	ND	1.89±0.82	11.82±0.83
Rutin	ND	ND	7.23±0,31	4.29±0.88	ND	ND
Quercetin dihydrate	ND	ND	ND	ND	1.24±0.26	ND

Although OUT value was increased, PG and IN of gallic acid reduced after cooking. Catechin was observed in gastrointestinal digestion of cooked samples. Epicatechin and rutin was increased in PG and epicatechin and coumaric acid decreased in IN values after cooking.

5. CONCLUSION AND RECOMMENDATION

In this study, phenolic content, flavonoid content and antioxidant activity of raw and steam cooked buckwheat, black chickpea, brown lentil and their bioavailability were investigated. It can be presumed as a useful study according to collected data and knowledge.

The results showed that 30 ml water for buckwheat and 90 ml water for black chickpea and brown lentil is adequate to steam cook. As gelatinization (transition) temperature was increasing, steam cooking time and water requirement also increased.

Steam cooking was done 30 ml for buckwheat (BW), 90 water ml for black chickpea (BC) and brown lentil according to DSC thermographs. Total phenolic content ranged from 44.41 to 265.43 mg GAE/100 mg dry weight. Flavonoid content changed from 368.75 to 1342.76 mg RE/100 g dry weight. Antioxidant activity ranged from 12.85 to 1740.04 mg TEAC/100 g dry weight in different methods. CUPRAC was the most effective method for analyzed raw products.

After steam cooking, there was an increase in phenolic content (63.87-281.20 mg GAE/100 g DW), generally. Flavonoid content decreased and antioxidant activity showed variation according to different methods.

Bioavailability was experimented with *In Vitro* digestion method. Phenolic, flavonoid content and antioxidant activity was analyzed. The recovery values were calculated to compare the bioavailability of products before and after cooking. Phenolic content ranged from 42.77±7.23 (Steam cooked IN_{BW}) to 315.70±8.86 (Raw OUT_{BW}) mg GAE/100 g DW and recovery of phenolic content gap was between 15.22±3.31% (Steam cooked IN_{BW}) and 191.41±12.54% (Steam cooked PG_{BL}). PG values decreased by steam cooking. Flavonoid content was between 2.28±1.29 (Steam cooked IN_{BC}) and 316.50±12.76 (Raw IN_{BC}). The highest recovery belonged to steam cooked PG_{BC} (44.28±13.27 mg RE/100 g DW) and lowest was steam cooked PG_{BC} (0.63±1.06 mg RE/100 g DW). Recoveries of PG and OUT were increased, IN was decreased by

cooking except for BL samples. There were no important difference between PG and IN of raw/cooked products. OUT_{BL} also showed an increase.

Antioxidant activity was measured by three different methods (ABTS, CUPRAC, and DPPH) as before. CUPRAC was the most efficient method for bioavailability samples.

The highest activity was raw PG_{BL}'s (29.10±1.42 mg TEAC/100 g DW) and activity was not detected for none of the OUT samples with ABTS method. There was no significant difference between IN samples. PG and IN recovery values decreased except for BL's.

Antioxidant activity data according to CUPRAC method ranged from 73.54±1.98 (Raw IN_{BC}) to 927.38±51.01 mg TEAC/100 g DW (Raw OUT_{BC}) and recovery was between 4.72±0.44% (Raw IN_{BC}) and 75.71±10.21% (Raw OUT_{BL}). PG_{BW} and IN values of all of the samples were increased, OUT values were decreased after cooking process. There was an inverse ratio between IN and OUT values.

The highest score was steam cooked PG_{BC} (19.59±1.51 mg TEAC/100 g DW) and minimum value was raw IN_{BW} according to DPPH method. AOX activity of PG_{BW} did not show important change, PG_{BC} and PG_{BL} increased with result of similar values after cooking process. IN increased and OUT_{BL} was decreased. Minimum and maximum values was analog to bioavailability data set.

Gallic acid, catechin, epicatechin, procatechuic acid and rutin for buckwheat, gallic acid, catechin, rutin, quercetin dihydrate and kaempherol for black chickpea, gallic acid, catechin, epicatechin, coumaric acid and rutin for brown lentil were identified with HPLC-PAD.

Differences between literature results and our experimental data can be explained with,

- Different species,
- Harvest conditions,
- Storing conditions,
- Difference in extraction method.

Buckwheat, black chickpea and brown lentil are functional foods. In addition to this, consumption of these products can be increased and new product development can be done.

REFERENCES

Akbaba, U., Şahin, Y. and Türkez, H. (2012). The element contents in chickpeas grown under organic and conventional farming regimes using WDXRF analysis for human nutrition and health. *TUBITAK* 36: 279- 287.

Akillioglu, H.G. and Karakaya, S. (2010). Changes in total phenols, total flavonoids, and antioxidant activities of common beans and pinto beans after soaking, cooking, and in vitro digestion process. *Food Science and Biotechnology* 19(3): 633-639.

Aleksenko, S.S. (2012). Antioxidant Activity and Phenolic Compounds of Buckwheat and Barley by the Data of Spectrophotometry and HPLC. *Journal of Analytical Chemistry* 68(5): 458–465.

Amarowicz, R., Estrella, I., Hernandez, T., Duenas, M., Trossynska, A., Kosinska, A. and Pegg, R.B. (2009). Antioxidant Activity of a Red Lentil Extract and Its Fractions. *International Journal of Molecular Sciences* 10: 5513-5527.

Amarowicz, R., Estrella, I., Hernandez, T., Robredo, S., Troszynska, A., Kosinska, A. and Pegg, R.B. (2010). Free radical-scavenging capacity, antioxidant activity, and phenolic composition of green lentil (Lens culinaris). *Food Chemistry* 121: 705- 711.

Apak, R., Guclu, K., Ozyurek, M., Kardemir, S.E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamin C and E using their cupric ion reducing capability in the presence of neocuprione: CUPRAC method. *Journal of Agricultural and Food Chemistry* 52: 7970-7981.

Apak, R., Tutem E., Ozyürek, M. and Guclu, K. (2011). Antioxidant Activity/Capacity Assay Methods Applied to Fruit and Cereals. Tokuşoğlu, Ö. (Eds), In: Fruit and Cereal Bioactives: Sources, Chemistry and Applications, CRC Press, pp: 361-383.

Asp, N., Poutanen, K., Richardson, D.P. and Kamp, J. (2010). Exploiting Bioactivity of European Cereal Grains for Improved Nutrition and Health Benefits. Healthgrain Consortium, pp: 1-4.

Awicka, J. M. (2011). Major Cereal Grains Production and Use aroumd the World. In: Advances in Cereal Science: Implications to Food Processing and Health Promotion. ACS Symposium Series, American Chemical Society, Washington DC, pp: 1-13.

Barbara, C. and Boye, J.I. (2013). In vitro protein digestibility and physico-chemical properties of flours and protein concentrates from two varieties of lentil (Lens culinaris). *Food & Function* 4: 310-321.

Bavec, F. and Bavec, M. (2006). Legumes. In: Organic Production and Use of Alternative Crops. CRC Press, pp: 201- 223.

Boudjou, S., Oomah, B.D., Zaidi, F. and Hosseinian, F. (2013). Phenolics content and antioxidant and anti-inflammatory activities of legume fractions. *Food Chemistry* 138: 1543-1550.

Cabanoglu, E., Beekwilder, J., Boyacıoglu, D., Hall, R., Vos, R. D. (2008). Changes in antioxidant and metabolite profiles during production of tomato paste. *Journal of Agricultural and Food Chemistry.* 56: 964-973.

DellaValle, D.M., Vandenberg, A. and Glahn, R.P. (2013). Seed Coat Removal Improves Iron Bioavailability in Cooked Lentils: Studies Using an in Vitro Digestion/Caco-2 Cell Culture Model. *Journal of Agricultural and Food Chemistry* 61: 8084−8089.

Dewanto, V., Wu, X., Adom, K.K. and Liu, R.H. (2002). Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. *Journal of Agricultural and Food Chemistry* 50: 3010-3014.

FAO/WHO (1998). Carbohydrates in human nutrition. FAO Food and Nutrition Paper-66.

Fardet, A. (2010). New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? *Nutrition Research Reviews*, 23: 65-134.

Fares, C. and Menga, V. (2012). Effects of toasting on the carbohydrate profile and antioxidant properties of chickpea (Cicer arietinum L.) flour added to durum wheat pasta. *Food Chemistry* 131: 1140–1148.

Flight, I. and Clifton, P. (2006). Cereal grains and legumes in the prevention of coronary heart disease and stroke: a review of the literature. *European Journal of Clinical Nutrition* 60, 1145–1159.

Fratianni, F., Cardinale, F., Cozzolino, A., Granese, T., Albanese, D., Matteo, M., Zaccardelli, M., Coppola, R. and Nazzaro, F. (2014). Polyphenol composition and antioxidant activity of different grass pea (Lathyrus sativus), lentils (Lens culinaris), and chickpea (Cicer arietinum) ecotypes of the Campania region (Southern Italy). *Journal of Functional Foods* 7: 551–557.

Frolich, W. (2001). Bioavailability of Minerals from Cereals. In: Handbook of Dietary Fiber. In Human Nutrition. CRC Press, pp: 499- 530.

Gawlik-Dziki, U., Dziki, D., Baraniak, B. and Lin, R. (2009). The effect of simulated digestion in vitro on bioactivity of wheat bread with Tartary buckwheat flavones addition. *LWT - Food Science and Technology* 42: 137–143.

Gliszczynska-Swiglo, A. and Oszmianski, J. (2013).Antioxidant and Prooxidant Activity of Food Components. Grzegorz Bartosz (Eds.), In: Food Oxidants and Antioxidants, CRC press, pp: 375-430.

Gorinstein, S., Lojek, A., Ciz, M., Pawelzik, Delgado-Licon, E., Medina, O.J., Moreno, M., Salas, I.A. and Goshev, I. (2008). Comparison of composition and antioxidant capacity of some cereals and pseudocereals. 43: 629–637.

Gulpinar A.R., Orhan, I.E., Kan, A., Senol, F.S., Celiki S.A. and Kartal, M. (2012). Estimation of in vitro neuroprotective properties and quantification of rutin and fatty acids in buckwheat (Fagopyrum esculentum Moench) cultivated in Turkey. *Food Research International* 46 (2): 536‑543

Gumienna, M., Lasik, M. and Czarnecki, Z. (2009). Influence of Plant Extracts Addition on the Antioxidative Properties of Products Obtained From Green Lentil Seeds during In Vitro Digestion Process. *Polish Journal Of Food And Nutrition Sciences* 59(4): 295-298.

Gupta, D.S., Thavarajah, D., Knutson, P., Thavarajah, P., McGee, R.J., Coyne, C.J. and Kumar, S. (2013). Lentils (Lens culinaris L.), a Rich Source of Folates. *Journal of Agricultural and Food Chemistry* 61: 7794−7799.

Han, H. and Baik, B. (2008). Antioxidant activity and phenolic content of lentils (Lens culinaris), chickpeas (Cicer arietinum L.), peas (Pisum sativum L.) and soybeans (Glycine max), and their quantitative changes during processing. *International Journal of Food Science and Technology* 43: 1971–1978.

Hannan, R., Acikgoz, N. and Robertson, L.D. (2001). Chickpeas (Cicer L.). N. Maxted et al. (eds.), Plant Genetic Resources of Legumes in the Mediterranean, Springer Science+Business Media, Dordrecht.

Heiras-Palazuelos, M.J., Ochoa-Lugo, M.I., Gutierrez-Dorado, R., Lopez-Valenzuela, J.A. et al. (2013). Technological properties, antioxidant activity and total phenolic and flavonoid content of pigmented chickpea (Cicer arietinum L.) cultivars. *International Journal of Food Sciences and Nutrition* 64(1): 69- 76.

Hemery, Y.M., Anson, N.M., Havenaar, R., Haenan, G.R.M.M., Noort, M.W.J. and Rouau, X. (2010). Dry-fractionation of wheat bran increases the bioaccessibility of phenolic acids in breads made from processed bran fractions. *Food Research International* 43: 1429- 1438.

Hung, P.V. and Morita, N. (2008). Distribution of phenolic compounds in the graded flours milled from whole buckwheat grains and their antioxidant capacities. *Food Chemistry* 109: 325- 331.

Inglett, G. E., Chen, D., Berhow, M. and Lee, S. (2011). Antioxidant activity of commercial buckwheat flours and their free and bound phenolic compositions. *Food Chemistry* 125: 923–929.

Jati I, R., Vadivel, V., Nohr, D. and Biesalski H.K. (2013). Antioxidant activity of anthocyanins in common legume grains. In: Watson, R.R. (ed.) "Bioactive foods and chronic disease states". Academic Press, Elsevier Inc, pp: 485- 497.

Johnson, R.C., Thavarajah, D., Combs Jr, G.F. and Thavarajah., P (2013). Lentil (Lens culinaris L.): A prebiotic-rich whole food legume. *Food Research International* 51: 107–113.

Jomova, K., Lawson, M. and Valko, M. (2013). Mechanisms of Antioxidant Activity. Grzegorz Bartosz (Eds.), In: Food Oxidants and Antioxidants, CRC press, pp: 325-442.

Kalogeropoulos, N., Chiou, A., Ioannou, M., Karathanos, V.T., Hassapidou and Andrikopoulos, N.K. (2010). Nutritional evaluation and bioactive microconstituents (phytosterols, tocopherols, polyphenols, triterpenic acids) in cooked dry legumes usually consumed in the Mediterranean countries. *Food Chemistry* 121(3): 682-690.

Kaur, M. and Singh, N. (2006). Relationships between Selected Properties of Seeds, Flours, and Starches from Different Chickpea Cultivars. *International Journal of Food Properties* 9: 597- 608.

Kim, J. H., Tanhehco, E. J., & Ng, P. K. W. (2006). Effect of extrusion conditions on resistant starch formation from pastry wheat flour. *Food Chemistry* 99: 718–723.

Kong, F. and Singh, R. P. (2008). Disintegration of solid foods in human stomach. *Journal of Food Science*, 73: 67-80.

Kumaran, A. and Karunakaran, R.J. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. *Food Chemistry* 97: 109- 114.

Lang R. and Jebb S.A. (2003). Who consumes whole grains, and how much? *Proc Nutr Soc* 62: 123–127.

Lee, H.S. (2004). Phenolic Compounds in Foods. In: Nollet, L.M.L. (eds.) "Handbook of Food Analysis". Marcel Dekker Inc., pp: 657-715.

Lin, Y. and Chou, C. (2009). Effect of heat treatment on total phenolic and anthocyanin contents as well as antioxidant activity of the extract from Aspergillus awamori-fermented black soybeans, a healthy food ingredient. *International Journal of Food Sciences and Nutrition* 60(7): 627–636.

Liyanapathirana, C. and Shahidi, F. (2004). Antioxidant activity of wheat extracts as affected by in vitro digestion. *Biofactors* 21: 325-328.

Maeda, T., Miyake, K., Tahara, M. and Morita, N. (2004). Effect of Buckwheat Substitution for Wheat Flour on Pasta and Cookie. Proceedings of the 9th International Symposium on Buckwheat, Prague, pp: 543-546.

McDougall, G. J., Dobson, P., Smith, P., Blake, A. and Stewart, D. (2005). Assesing potential bioavailability of raspberry anthocyanins using an in vitro digestion system. *Journal of Agricultural and food Chemistry*. 53: 5896-5904.

Millan, T., Clarke, H.J., Siddique, K.H.M., Buhariwalla, H.K., Gaur, P.M., Kumar, J., Gil, J., Kahl, G. and Winter, P. (2006). Chickpea Molecular Breeding. *Euphytica* 147: 81-103.

Miller, N. J. and Rice-Evans, C.A. (1997). Factors influencing the antioxidant activity determined by the $ABTS^{+}$ radical cation assay. *Free Radical Research* 26: 195-199.

Min, B., Lee, S.M., Yoo, S., Inglett, G.E. and Lee, S. (2010). Functional characterization of steam jet-cooked buckwheat flour as a fat replacer in cake-baking. *Journal of the Science of Food* 90: 2208-2213.

Oomah, B.D., ⁎, Caspar, F., Malcolmson, L.J. and Bellido, A. (2011). Phenolics and antioxidant activity of lentil and pea hulls. *Food Research International* 44: 436–441.

Poutanen, K. (2012). Past and future of cereal grains as food for health. *Trends in Food Science & Technology* 25: 58-62.

Qin, P., Wang, Q., Shan, F., Hou, Z. and Ren, G. (2010). Nutritional composition and flavonoids content of flour from different buckwheat cultivars. *International Journal of Food Science and Technology* 45: 951–958.

Quettier-Deleu, C., Gressier, B., Vasseur, J., Dine, T., Brunet, C., Luyckx, M., Cazin, M., Cazin, J., Bailleul, F. and Trotin, F. (2000). Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. *Journal of Ethnopharmacology* 72: 35–42.

Rai, S., Wahile, A., Mukherjee, K., Saha, B.P. and Mukherjee, P.K. (2006). Antioxidant activity of Nelumbo nucifera (sacred lotus) seeds. *Journal of Ethnopharmacology* 104: 322–327.

Rehinan, Z., Rashid, M. and Shah, W.H. (2004). Insoluble dietary fibre components of food legumes as affected by soaking and cooking processes. *Food Chemistry* 85: 245- 249.

Ren, S., Liu, Z and Wang, P. (2012). Proximate composition and flavonoids content and in vitro antioxidant activity of 10 varieties of legume seeds grown in China. *Journal of Medicinal Plants Research* 6 (2): 301-308.

Rufa, L., **Jianzhen, R. and Wei, S.** (2004). An Observation of the Effect of Tartary Buckwheat Tea on Lowering Blood Glucose. Proceedings of the 9th International Symposium on Buckwheat, Prague, pp: 543-546.

Sakac, M., **Torbica, A., Sedej, I. and Hadnadev, M.** (2011). Influence of breadmaking on antioxidant capacity of gluten free breads based on rice and buckwheat flour. *Food Research International* 44: 2806-2813.

Sasipriya, G. and Siddhuraju, P. (2012). Effect of different processing methods on antioxidant activity of underutilized legumes, Entada scandens seed kernel and Canavalia gladiata seeds. *Food and Chemical Toxicology* 50: 2864-2872.

Sedej, I., Saka, M., Mandi, A., Misan, A., Tumbas, V. and Hadnadev, M. (2011). Assessment of antioxidant activity and rheological properties of wheat and buckwheat milling fractions. *Journal of Cereal Science* 54: 347-353.

Segev, A., Badani, H., Galili, L., Hovav, R., Kapulnik, Y. Shomer, I. and Galili, S. (2011). Total Phenolic Content and Antioxidant Activity of Chickpea (Cicer arietinum L.) as Affected by Soaking and Cooking Conditions. *Food and Nutrition Sciences* 2: 724-730.

Shahidi, F. and Naczk, M. (2003). Phenolic Compounds in Cereals, Legumes and Nuts. In: Phenolics in Food and Nutraceuticals. CRC Press.

Silva-Cristobal, L., Osorio-Diaz, P., Tovar, J. ve Bello-Perez, L. A. (2010). Chemical composition, carbohydrate digestibility, and antioxidant capacity of cooked black bean, chickpea, and lentil Mexican varieties. *Journal of Food* 8(1): 7- 14.

Singh, A., Jain, A., Sarma, B.K., Upadhyay, R.S. and Singh, H.B. (2014). Rhizosphere competent microbial consortium mediates rapid changes in phenolic profiles in chickpea during Sclerotium rolfsii infection. *Microbiological Research* 169:3 53–360.

Sosulski, F. and Sosulski, K. (2005). Legumes: Horticulture, Properties and Processing. In: Handbook of Food Science, Technology, and Engineering, Volume 1, Taylor & Francis Group, pp: 1-14.

Sreerama, Y., Sashikala, V.B. and Pratape, V.M. (2010). Variability in the Distribution of Phenolic Compounds in Milled Fractions of Chickpea and Horse Gram: Evaluation of Their Antioxidant Properties. *Journal of Agricultural and Food Chemistry* 58: 8322-8330.

Sun, T. and Ho, C. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry* 90: 743-749.

Şensoy, I., Rosen, R.T., Ho, C. ve Karwe, M.V. (2006**).** Effect of processing on buckwheat phenolics and antioxidant activity. *Food Chemistry* 99: 388–393.

Tahir, M.,, Lindeboom, N., Monica Ba°ga1, M.,Albert Vandenberg, A. and Chibbar R.N. (2011). Composition and correlation between major seed constituents in selected lentil (Lens culinaris. Medik) genotypes. Can. J. Plant Sci. 91: 825-835.

Takeoka G. R., Dao, L.T., Tamura, H. Harden, L.A.. (2005). Delphinidin3-O-(2-O-â-D-Glucopyranosyl-r-L-arabinopyranoside): A Novel Anthocyanin Identified in Beluga Black Lentils, J. Agric. Food Chem. 53, 4932-4937.

Tester, R.F., Morrison, W.R., Ellis, R.H., Piggot, J.R., Batts, G.R., Wheeler, T.R., Morison, J.I.L., Hadley, P. and Ledward,D.A. (1995). Effects of elevated growth temperature and carbon dioxide levels on some physicochemical properties of wheat starch. J. Cereal Sci. 22, 63-71.

Toor, R.K., Savage, G.P. and Lister, C.E. (2006). Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. *Journal of Food Composition and Analysis* 19: 1–10.

Vadivel, V., Stuetz, W., Scherbaum, V. and Biesalski, H.K. (2011). Total free phenolic content and health relevant functionality of Indian wild legume grains: Effect of indigenous processing methods. *Journal of Food Composition and Analysis* 24(7): 935- 943.

Vega, M.P., Fratini, R.M. and Muehlbauer, F.J. (2012). Lentil. In: Genetics, Genomics and Breeding of Cool Season Grain Legumes. Taylor & Francis Group, pp: 98- 150.

Venn, B.J. and Mann, J.I. (2004). Cereal grains, legumes and diabetes. *European Journal of Clinical Nutrition* 58: 1443–1461.

Verardo, V., Arraez-Roman, D., Segura-Carretero, A., Marconi, E., Fernandez-Gutierrez, E. and Caboni̇, M.F. (2011). Determination of Free and Bound Phenolic Compounds in Buckwheat Spaghetti by RP-HPLC-ESI-TOF-MS: Effect of Thermal Processing from Farm to Fork. *Journal of Agricultural and Food Chemistry* 59: 7700- 7707.

Wang, T., He, F. and Chen, G. (2014). Improving bioaccessibility and bioavailability of phenolic compounds in cereal grains through processing technologies: A concise review. *Journal of Functional Foods* 7: 101-111.

Xu, B. and Chang, S.K.C. (2007). A Comparative Study on Phenolic Profiles and Antioxidant Activities of Legumes as Affected by Extraction Solvents. *Journal of Food Science* 72 (2): 159- 166.

Xu, B. and Chang, S.K.C. (2008). Effect of soaking, boiling, and steaming on total phenolic content and antioxidant activities of cool season food legumes. *Food Chemistry* 110: 1- 13.

Xu, B. and Chang, S.K.C. (2009). Phytochemical Profiles and Health-Promoting Effects of Cool-Season Food Legumes As Influenced by Thermal Processing. *Agricultural and Food Chemistry* 57: 10718-10731.

Yıldız, N. and Yalçın, E. (2013). Chemical, Nutritional and Technological Properties of Buckwheat. *GIDA* 38(6): 383-390.

Zhang, M., Chen, H., Li, J. and Liang, Y. (2010). Antioxidant properties of tartary buckwheat extracts as affected by different thermal processing methods. *Food Science and Technology* 43: 181-185.

Zhao, Y., Du, S., Wang, H. and Cai (2014). In vitro antioxidant activity of extracts from common legumes. *Food Chemistry* 152: 462–466.

Zhou, Y., Li, D., Wang, L., Li, Y., Yang, B., Bhandari, B., Chen, X.D. and Mao, Z. (2009). Effect of water content on thermal behaviors of common buckwheat flour and starch. *Journal of Food Engineering* 93: 242–248.

Zhou, K., Slavin, M., Lutterodt, H., Whent, M., Eskin, N.A.M. and Yu, L. (2013). Cereals and Legumes. In: Biochemistry of Foods. Elsevier Inc, pp: 3-48.

Zielinska, D., Szawara-Nowak, D. and Zielinski, H. (2007). Comparison of Spectrophotometric and Electrochemical Methods for the Evaluation of the Antioxidant Capacity of Buckwheat Products after Hydrothermal Treatment. *Journal of Agricultural Food Chemistry* 55 (15): 6124-6131.

Zia-Ul-Haq, M., Iqbal, S., Ahmad, S., Bhanger, M.I., Wiczkowski, W., Amarowicz, R. (2008). Antioxidant Potential of Desi Chickpea Varieties Commonly Consumed in Pakistan, Journal of Food Lipids, 15(3): 326–342.

APPENDICES

APPENDIX A: Calibration Curves

APPENDIX B: Anova Tables

APPENDIX A

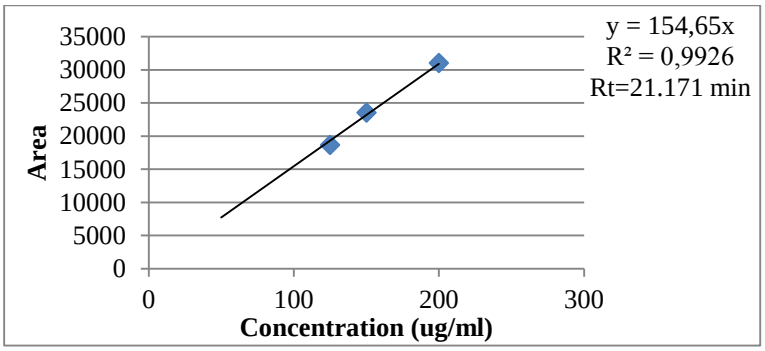

Figure A.1: Standard calibration curve of delphinidin chloride for HPLC.

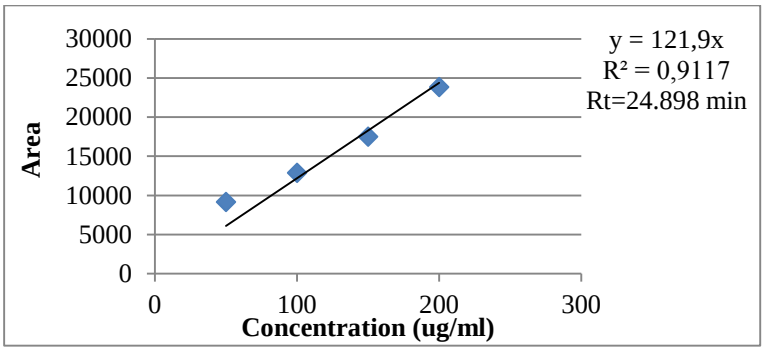

Figure A.2: Standard calibration curve of cyanidin chloride for HPLC.

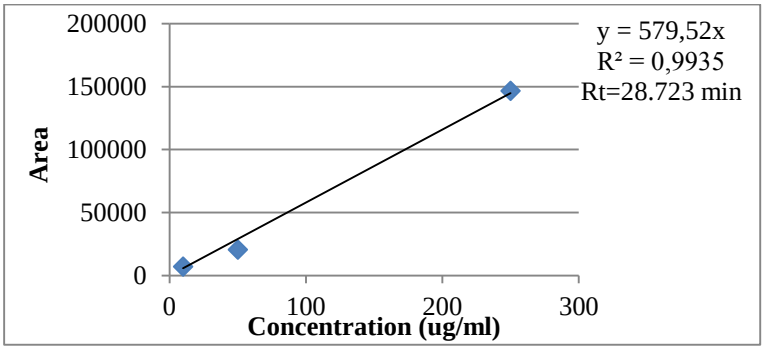

Figure A.3: Standard calibration curve of pelargonidin chloride for HPLC.

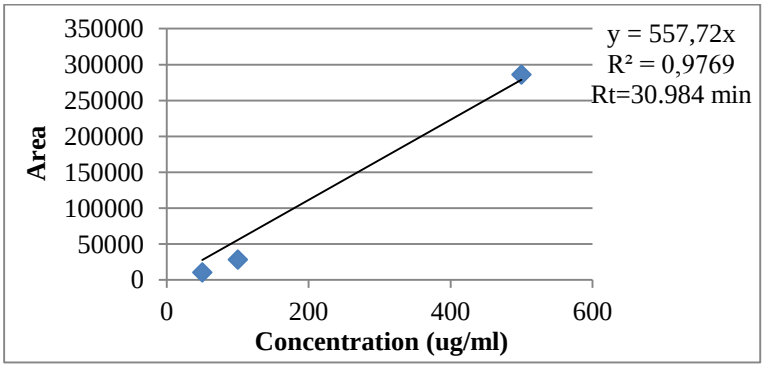

Figure A.4: Standard calibration curve of malvidin chloride for HPLC.

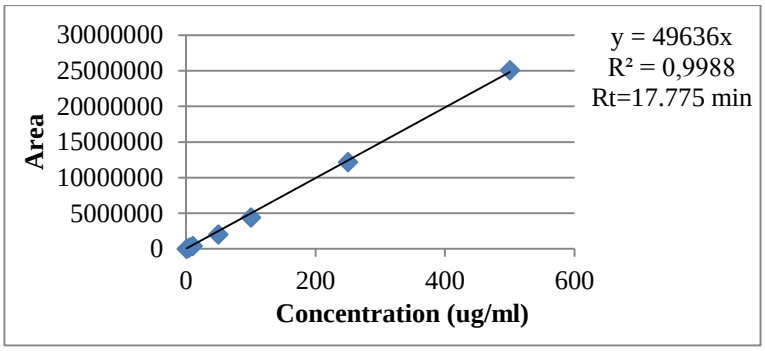

Figure A.5: Standard calibration curve of cyanidin3-O-glucoside for HPLC.

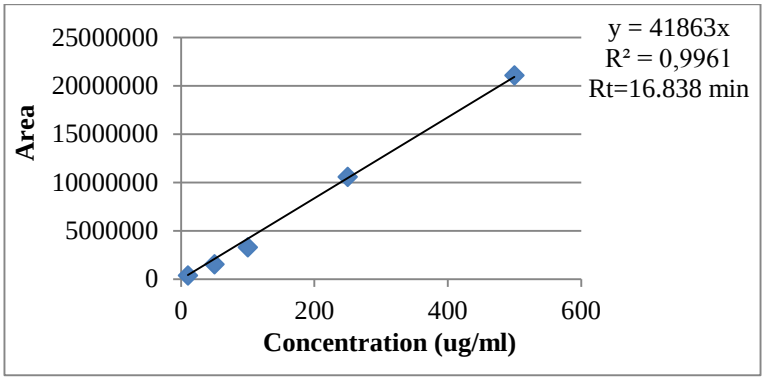

Figure A.6: Standard calibration curve of cyanidin 3-O-galactoside for HPLC.

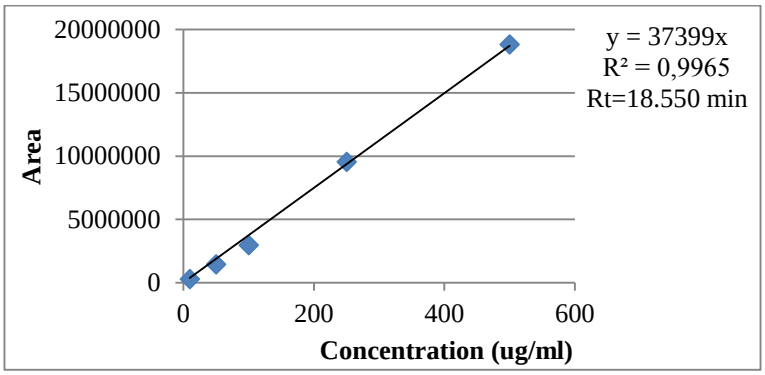

Figure A.7: Standard calibration curve of cyanidin 3-O-rutinoside for HPLC.

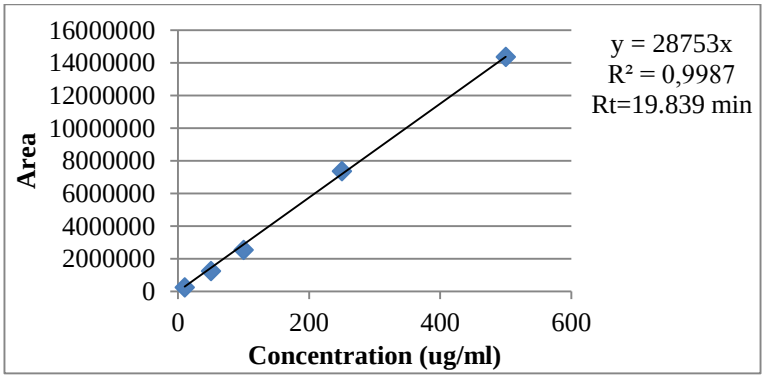

Figure A.8: Standard calibration curve of pelargonidin 3-O-glucoside for HPLC.

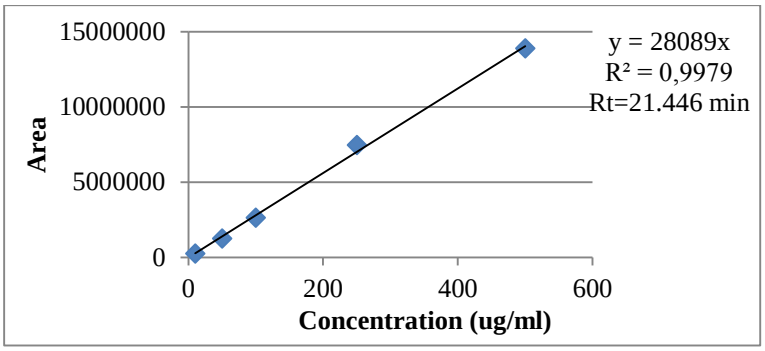

Figure A.9: Standard calibration curve of malvidin 3-O-galactoside for HPLC.

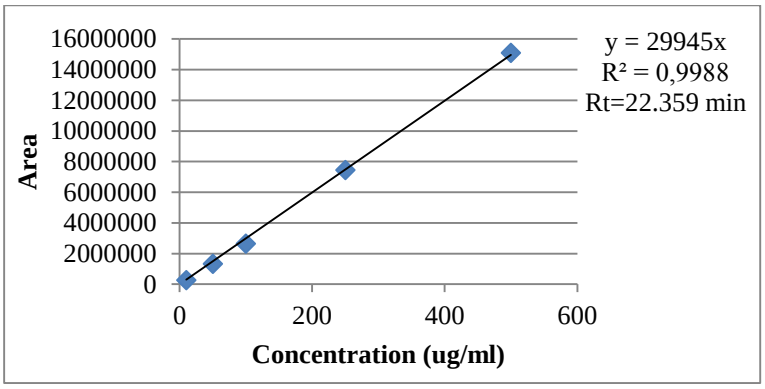

Figure A.10: Standard calibration curve of malvidin 3-O-glucoside for HPLC.

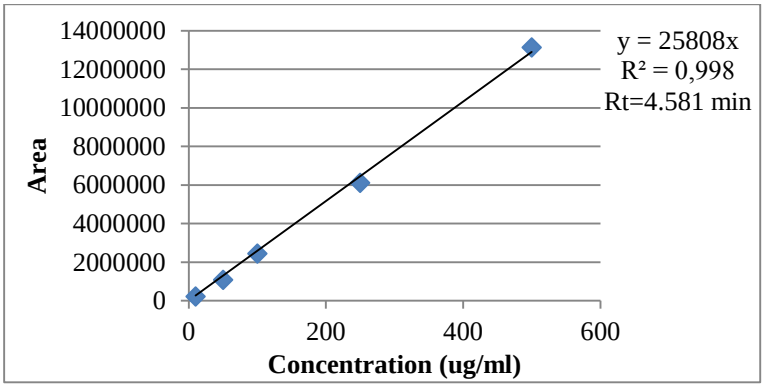

Figure A.11: Standard calibration curve of gallic acid for HPLC.

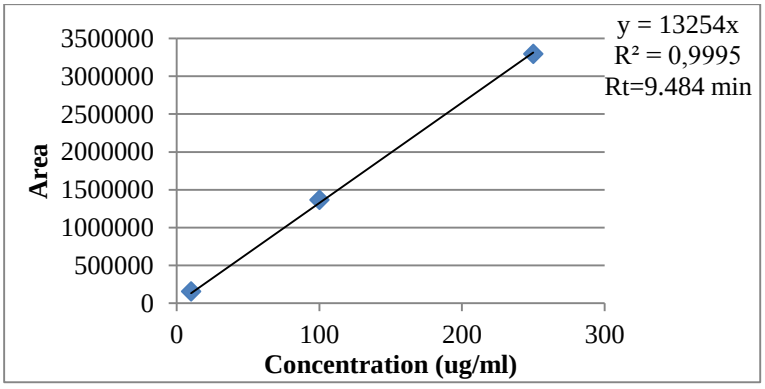

Figure A.12: Standard calibration curve of P-hydroxybenzoic acid for HPLC.

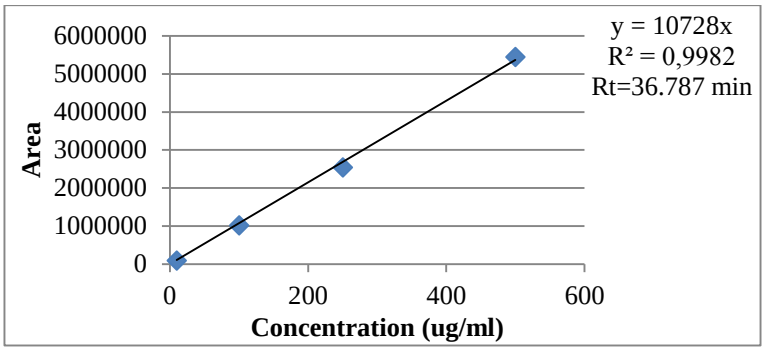

Figure A.13: Standard calibration curve of genistein for HPLC.

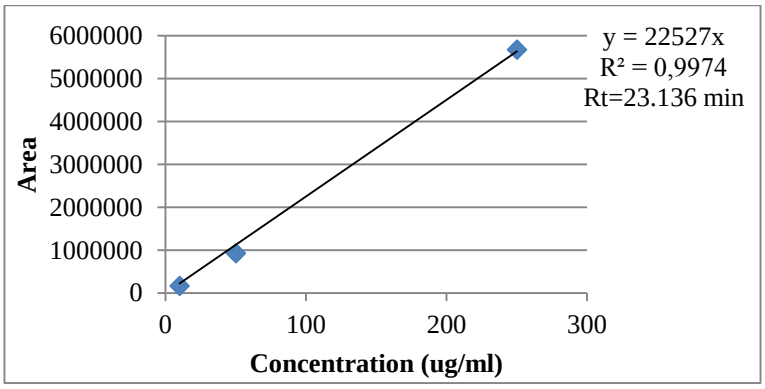

Figure A.14: Standard calibration curve of genistin for HPLC.

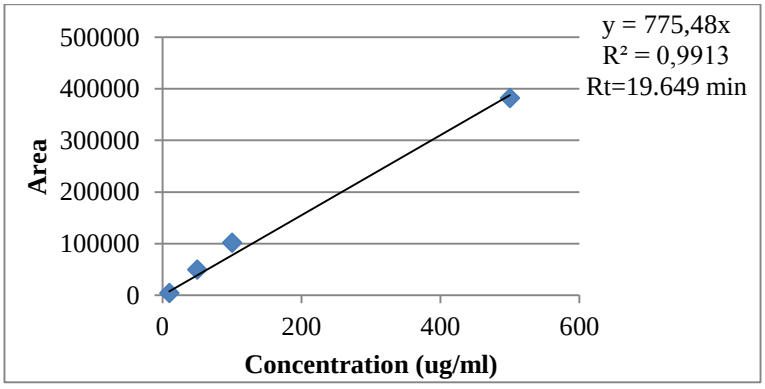

Figure A.15: Standard calibration curve of glycitin for HPLC.

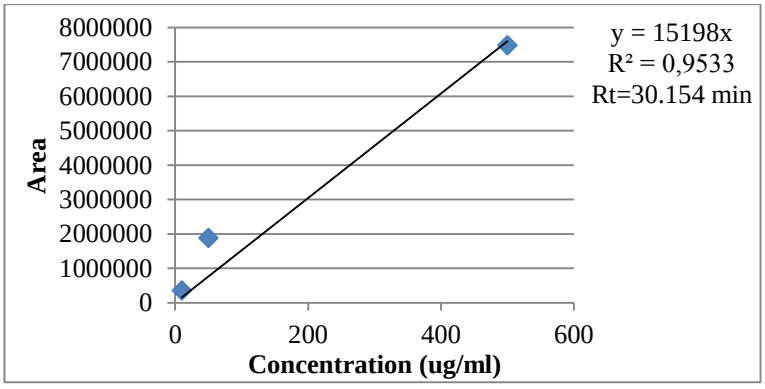

Figure A.16: Standard calibration curve of daidzein for HPLC.

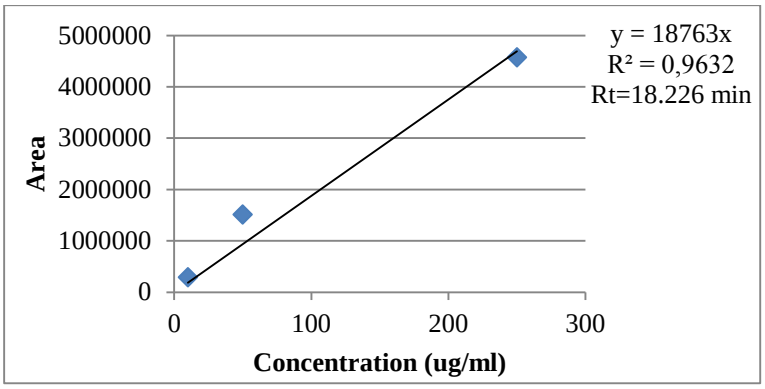

Figure A.17: Standard calibration curve of daidzin for HPLC.

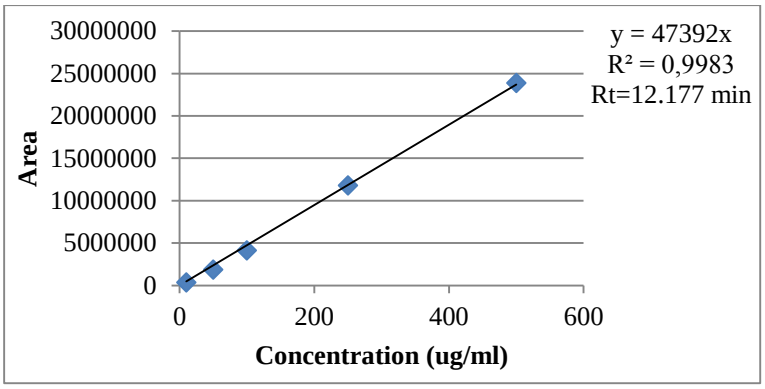

Figure A.18: Standard calibration curve of cafeic acid for HPLC.

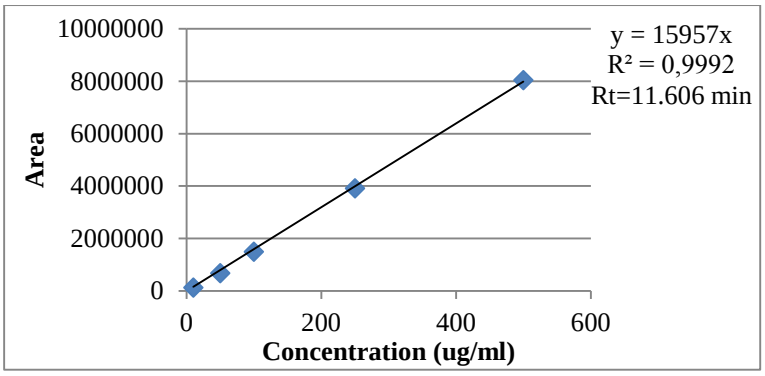

Figure A.19: Standard calibration curve of vanilic acid for HPLC.

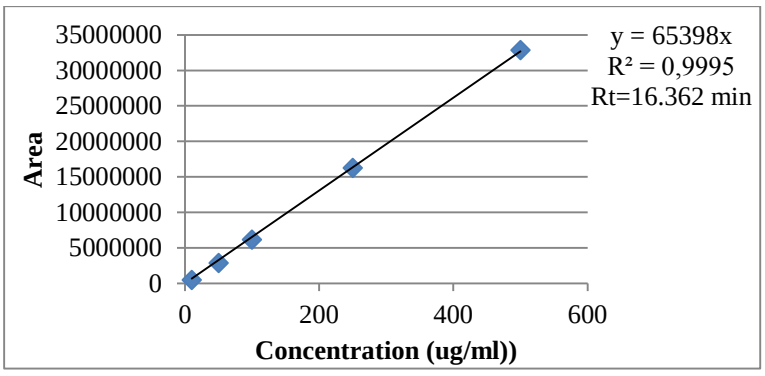

Figure A.20: Standard calibration curve of P-coumaric acid for HPLC.

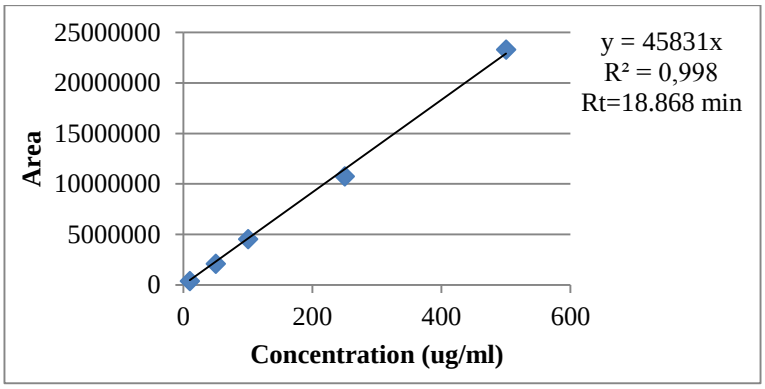

Figure A.21: Standard calibration curve of ferulic acid for HPLC.

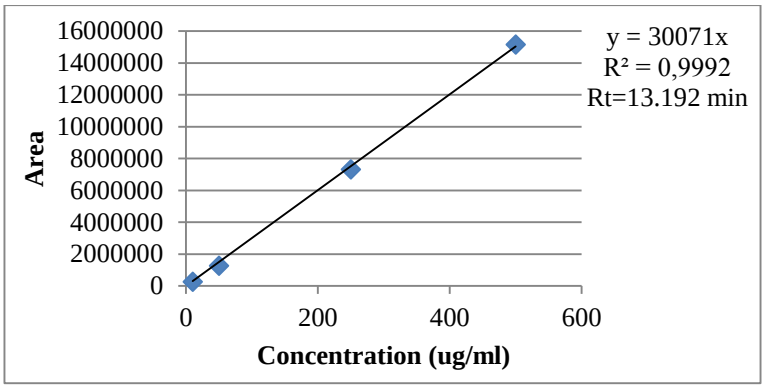

Figure A.22: Standard calibration curve of syringic acid for HPLC.

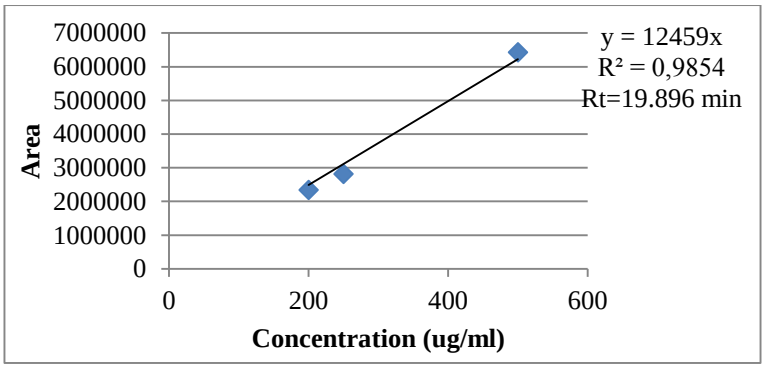

Figure A.23: Standard calibration curve of sinapic acid for HPLC.

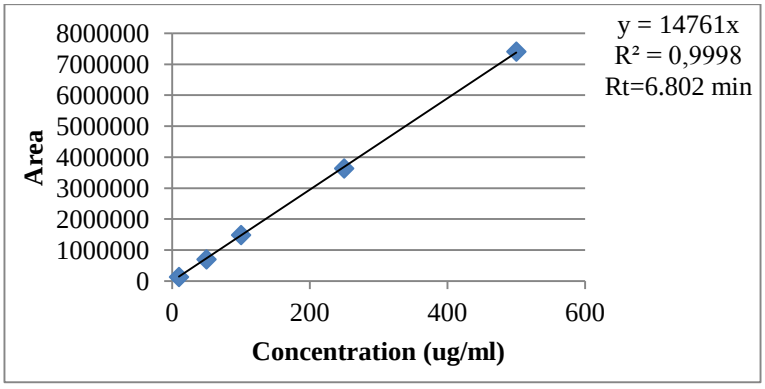

Figure A.24: Standard calibration curve of protocatechuic acid for HPLC.

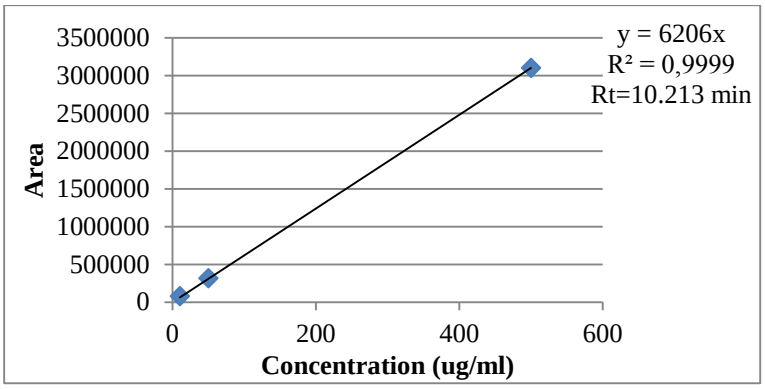

Figure A.25: Standard calibration curve of catechin hydrate for HPLC.

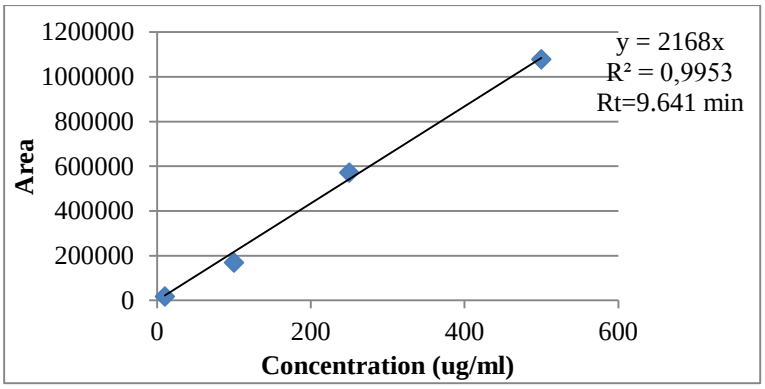

Figure A.26: Standard calibration curve of epigallocatechin for HPLC.

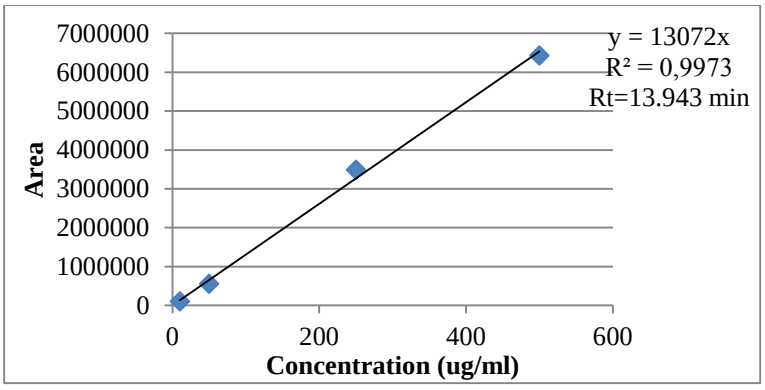

Figure A.27: Standard calibration curve of epigallocatechin gallate for HPLC.

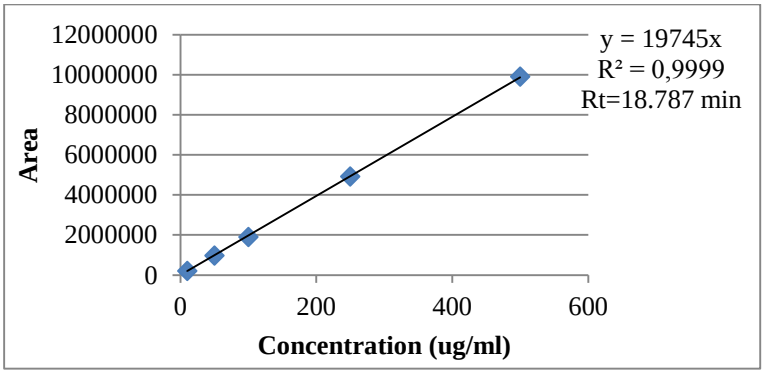

Figure A.28: Standard calibration curve of epicatechin gallate for HPLC.

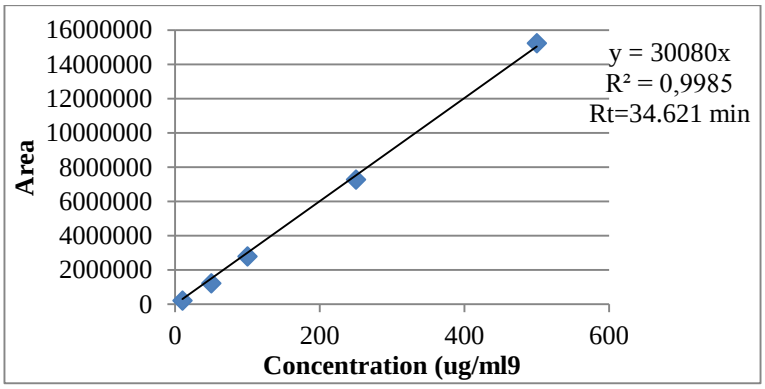

Figure A.29: Standard calibration curve of naringenin for HPLC.

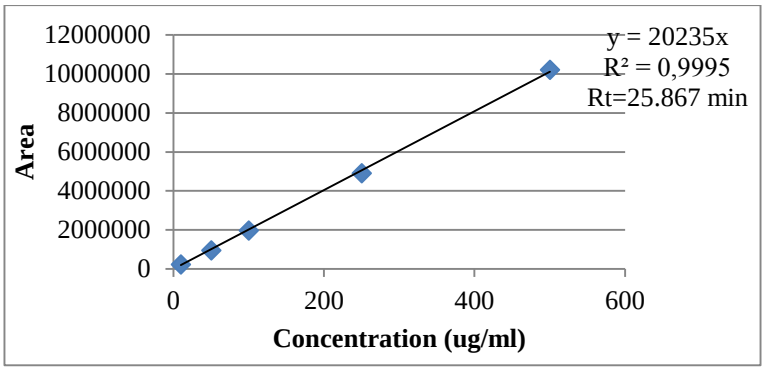

Figure A.30: Standard calibration curve of myricetin for HPLC.

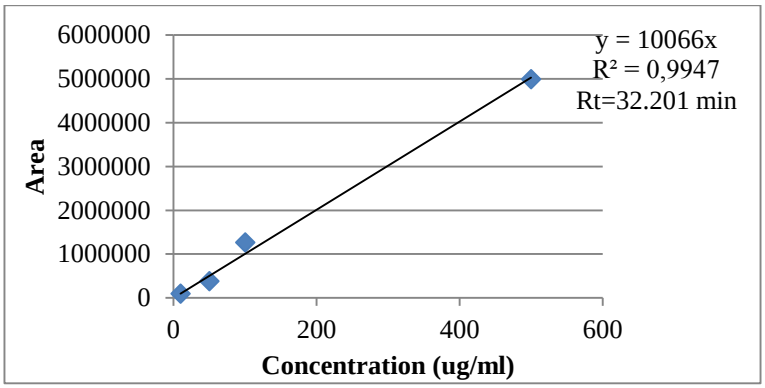

Figure A.31: Standard calibration curve of quercetin for HPLC.

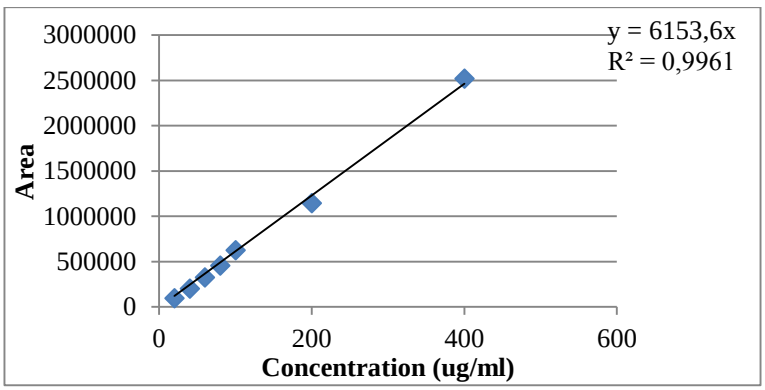

Figure A.32: Standard calibration curve of epicatechin for HPLC.

Table A.1: Wavelength and retention time of phenolic compounds by HPLC-PDA.

Compound	Retention time	Factor	Max. Wavelength
Catechin	11.8	5000000	280
Catechin hydrate	11.8	9000000	280
Chlorogenic acid	11.9	20000000	312
4-O-caffeoyl-quinic acid	12.1	30000000	312
Gentisic acid	12.6	745071	312
Syringic acid	12.7	20000000	280
Caffeic acid	12.8	60000000	312
Epicatechin	15.1	6000000	280
p-coumaric acid	17.0	80000000	312
Cynarin	17.6	30000000	312
Ferulic acid	18.8	40000000	312
2-hydroxycinnamic acid	22.7	50000000	280
Rutin	23.2	6000000	360
Quercetin-3-galactoside	23.5	20000000	360
Quercetin-3-B-D-glucoside	24.0	20000000	360
Kaempferol-3-o-rutinoside	24.8	30000000	360
Hesperidin	26.0	10000000	280
procatechuic acid	26.4	10000000	280
Luteolin	35.5	50000000	360
Quercetin dihydrate	36.0	30000000	360
Naringenin	36.6	30000000	280
Gallic Acid	4.7	20000000	280
Kaempferol	40.7	50000000	360
Pinocembrin	48.0	30000000	280
Chrysin	48.8	30000000	280
Galangin	49.4	20000000	360
Neocholorogenic acid	7.7	30000000	312

APPENDIX B

Table B.1: ANOVA table of each analysis for raw products.

		Sum of Squares	df	Mean Square	F	Sig.
Folin	Between Groups	44498.956	11	4045.360	59.449	.000
	Within Groups	1633.149	24	68.048		
	Total	46132.106	35			
Flavanoid	Between Groups	1793686.825	11	163062.439	61.338	.000
	Within Groups	63802.454	24	2658.436		
	Total	1857489.279	35			
ABTS	Between Groups	39923.513	11	3629.410	490.903	.000
	Within Groups	177.440	24	7.393		
	Total	40100.953	35			
CUPRAC	Between Groups	6283862.493	11	571260.227	546.723	.000
	Within Groups	25077.154	24	1044.881		
	Total	6308939.647	35			
DPPH	Between Groups	12812.245	11	1164.750	249.203	.000
	Within Groups	112.173	24	4.674		
	Total	12924.418	35			

Table B.2: ANOVA table of each analysis for steam-cooked products.

		Sum of Squares	df	Mean Square	F	Sig.
Folin	Between Groups	471639.666	8	58954.958	362.160	.000
	Within Groups	16115.908	99	162.787		
	Total	487755.574	107			
Flavanoid	Between Groups	1945344.280	8	243168.035	85.398	.000
	Within Groups	281899.775	99	2847.472		
	Total	2227244.055	107			
ABTS	Between Groups	430023.502	8	53752,938	501.692	.000
	Within Groups	10607.193	99	107.143		
	Total	440630.695	107			
CUPRAC	Between Groups	19787963.705	8	2473495,463	282.348	.000
	Within Groups	867285.383	99	8760.458		
	Total	20655249.088	107			
DPPH	Between Groups	51323.486	8	6415.436	118.405	.000
	Within Groups	5364.039	99	54.182		
	Total	56687.524	107			

Table B.3: ANOVA table of each analysis for raw and steam cooked products.

		Sum of Squares	df	Mean Square	F	Sig.
Folin	Between Groups	681829.915	17	40107.642	268.860	.000
	Within Groups	21481.422	144	149.177		
	Total	703311.337	161			
Flavanoid	Between Groups	15876678.706	17	933922.277	212.633	.000
	Within Groups	632473.152	144	4392.175		
	Total	16509151.858	161			
ABTS	Between Groups	603592.410	17	35505.436	435.015	.000
	Within Groups	11753.121	144	81.619		
	Total	615345.531	161			
CUPRAC	Between Groups	34735365.725	17	2043256.807	291.365	.000
	Within Groups	1009829.835	144	7012.707		
	Total	35745195.560	161			
DPPH	Between Groups	99485.836	17	5852.108	148.356	.000
	Within Groups	5680.280	144	39.446		
	Total	105166.116	161			

Table B.4: ANOVA table of each analysis for bioavailability (PG, IN and OUT) of raw and steam cooked products.

		Sum of Squares	df	Mean Square	F	Sig.
Folin	Between Groups	1244725.590	17	73219.152	44.820	.000
	Within Groups	235242.714	144	1633.630		
	Total	1479968.304	161			
Flavanoid	Between Groups	1009370.456	17	59374.733	15.525	.000
	Within Groups	550710.486	144	3824.378		
	Total	1560080.942	161			
ABTS	Between Groups	14464.484	17	850.852	429.519	.000
	Within Groups	285.256	144	1.981		
	Total	14749.739	161			
CUPRAC	Between Groups	9467574.001	17	556916.118	101.280	.000
	Within Groups	791823.926	144	5498.777		
	Total	10259397.927	161			
DPPH	Between Groups	5487.910	17	322.818	103.924	.000
	Within Groups	447.308	144	3.106		
	Total	5935.217	161			

Table B.5: ANOVA table of bioavailability recovery of raw and steam cooked products.

		Sum of Squares	df	Mean Square	F	Sig.
Folin	Between Groups	537340.763	17	31608.280	43.627	.000
	Within Groups	104330.693	144	724.519		
	Total	641671.457	161			
Flavanoid	Between Groups	36509.094	17	2147.594	26.200	.000
	Within Groups	11803.726	144	81.970		
	Total	48312.820	161			
ABTS	Between Groups	7642.774	17	449.575	735.694	.000
	Within Groups	87.997	144	.611		
	Total	7730.771	161			
CUPRAC	Between Groups	70043.279	17	4120.193	78.274	.000
	Within Groups	7579.912	144	52.638		
	Total	77623.191	161			
DPPH	Between Groups	17079.309	17	1004.665	129.175	.000
	Within Groups	1119.969	144	7.778		
	Total	18199.278	161			

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