ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

THE EFFECT OF FOOD PROCESSING ON ANTIOXIDANT CAPACITY OF PURPLE CARROT

M.Sc. THESIS

Sezen SÜZME

Department of Food Engineering

Food Engineering Programme

Thesis Advisor: Prof. Dr. Dilek BOYACIOĞLU

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

GIDA PROSESİNİN KARA HAVUCUN ANTİOKSİDAN KAPASİTESİ ÜZERİNE ETKİLERİ

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Sezen Süzme, a **M.Sc.** student of ITU **Graduate School of.Science Engineering and Technology** student ID **506101511**, successfully defended the **thesis/dissertation** entitled "**THE EFFECT OF FOOD PROCESSING ON ANTIOXIDANT CAPACITY OF PURPLE CARROT**", which he/she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my family,

FOREWORD

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ABBREVIATIONS

AOAC	: Association Official of Analytical Chemists
CUPRAC	: Copper Reducing Antioxidant Capacity
DPPH	: 1,1-Diphenyl-2- picrylhydrazyl
GAE	: Gallic acid equivalents
HPLC	: High Performance Liquid Chromatography
LC-MS	: Liquid Chromatography-Mass Spectrometry
TEAC	: Trolox Equivalent Antioxidant Capacity

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THE EFFECT OF FOOD PROCESSING ON ANTIOXIDANT CAPACITY OF PURPLE CARROT

SUMMARY

Black carrot concentrate has gained increasing interest in recent years as a natural colorant due to expected health benefits related to the substantial content of bioactive compounds, especially anthocyanins. Since black carrots are mostly consumed as concentrate, it is desirable to investigate how these components are affected during processing treatments. Furthermore, there have been very few studies to evaluate the absorption of acylated anthocyanins *in vitro* and limited research have been performed dealing with the absorption/metabolism of these components *in vitro*.

In general, black carrot concentrate production includes 13 steps of raw material, milling, citric acid treatment, heating and cooling, mashing, pressing, extraction-I, extraction-II, total juice, pasteurization, depectinization, ultrafiltration, and concentrating. In this study, every step of processing was investigated in detail to elucidate the changes in antioxidative compounds by analyzing the moisture content by gravimetric method, total phenolic and total flavonoid contents, total antioxidant capacity and polymeric color by spectrophotometric methods and phenolic acid and anthocyanin contents by HPLC.

The objective of this study was to investigate the effects of processing of black carrot to concentrate such on phenolics, flavonoids and anthocyanins at molecular level. This study performed at industrial-scale processing provided information about the effect of processing on healthy compounds of black carrots.

The results showed that in black carrot samples, moisture content was found to be 87.3 ± 0.5 %. The total moisture loss was about 44% by processing raw material into the concentrate. On the other hand, the moisture changes from the milling step to concentrate step were observed to be between 91.2%-98.9%. In black carrot concentrate, due to evaporation process, the moisture content decreased significantly as expected. Values for all analyses were given on fresh weight basis due to very high moisture content values of samples collected from each step (87%-99% except concentrate 44%). pH of processing samples ranged from 3.8 to 4.1.

Prior to the spectrophotometric analysis, the best extraction solvent out of acetone:water:acetic acid at ratios of 70:29.5:0.5 and 70:28:2 and 7% acetic acid in 80% methanol was investigated by analyzing the amount of total phenolics, total flavonoids and antioxidant capacities with DPPH and CUPRAC methods. According to the results, total phenolic content of black carrot in acetone:water:acetic acid (70:29.5:0.5) extract were 9 % and 25 % higher than that of acetone:water:acetic acid (70:28:2, v/v/v) and acetic acid methanol extract. Similarly, the results of DPPH and CUPRAC methods showed that acetone:water:acetic acid (70:29.5:0.5) extract yielded with 17%-15% and 29%-18% higher values of antioxidant capacity than acetone:water:acetic acid (70:28:2, v/v/v) and acetic acid in methanol extractions,

respectively. However, total flavonoid contents of black carrot in %7 acetic acid in %80 methanol extract were 12% and 30% higher than those of acetone:water:acetic acid (70:29.5:0.5) and acetone:water:acetic acid (70:28:2, v/v/v) extracts. As a result, acetone:water:acetic acid (70:29.5:0.5) was found to be the most effective solvent system.

The antioxidant capacity methods of DPPH and CUPRAC yielded significant higher values by the 2nd extraction method (first removal of hydrophilic fraction, then lipophilic fraction) in hydrophilic fractions ($p \leq 0.05$). The antioxidant capacity values were about 81%-88% lower in hydrophilic fractions obtained by the 1st extraction method first (removal of lipophilic fraction, then hydrophilic fraction) as analyzed by both methods and solvent systems. On the other hand, there was no significant difference in antioxidant capacity values of lipophilic fractions by two different extraction methods ($p \leq 0.05$). As a result, due to the loss in hydrophilic antioxidant capacity, 2nd extraction method was preferred in further analysis of bioactive compounds and lipophilic antioxidant capacity was neglected.

In black carrot samples, total amount of phenolics was found to be 319.5 ± 58.1 mg GAE/100 g fresh weight (25.3 mg GAE/g dry weight). The phenolic contents of samples collected from each step were found to be statistically different ($p \leq 0.05$) ranging between 48.1±8.9 and 433.3±29.8 mg GAE/ 100 gr fresh weight. Processing black carrot into concentrate resulted in an overall increase of 35% in phenolic content on a fresh weight basis. If the results were calculated on a dry weight basis, the concentration of polyphenols in concentrate was 69% lower than that of the black carrot sample. The steps with the highest influence on the total phenolic content were milling, juice extraction-I (EXT-I) and extraction-II (EXT-II) steps. The high amount of recovery of phenolics occurred as expected in juice collecting step (TOJ), total phenolic content was reached to 104.6±24.3 mg GAE / 100 gr fresh weight. Phenolic losses during black carrot concentrate processing are mainly due to milling, physical removal of the peels in the presscake and concentrating into black carrot juice (dry weight basis), whereas citric acid treatment, heat treatment, juice depectinization, pasteurization, clarification steps result in minor changes.

Total flavonoid content of the fruits was found to be 1048.4 ± 226.0 mg quercetin/100 g fresh weight. The flavonoid contents of samples collected from each step were found to be statistically different ($p \leq 0.05$) ranging between 93.7±8.9 and 1268.0±136.5 mg quercetin/ 100 gr fresh weight. Total flavonoid content showed similar pattern at each step observed in total phenolic contents, although percent changes were different for those compounds during processing. The total flavonoid content increased from 100% in black carrots to 117% in its concentrate. However, if the results were calculated on a dry weight basis, the concentration of flavonoids in concentrate was 73% lower than in the black carrots. Milling (MIL) led to a strong decrease of total flavonoids (by 65%) compare to raw material as observed similarly for total phenolic content. Two extraction steps (EXT-I and EXT-II) resulted in extensive losses of flavonoids. Juice extraction carried out in two steps was the processing step showing the highest loss in flavonoids by 87% - 91%. It was important to note that changes founded in total flavonoid contents were higher than total phenolic content. The differences in changes may be attributed to difference of localization, stability conditions and processing effects on flavonoids.

Total monomeric anthocyanin content of black carrots was found 53.7 ± 15.2 mg cyanidin-3-glucoside / 100 g fresh weight. The monomeric anthocyanin contents of

samples collected from each step were found to be statistically different ($p \leq 0.05$) ranging between 10.2± 1.1 and 151.8± 27.6 mg cyanidin-3-glucoside/ 100 gr fresh weight. The concentration of monomeric anthocyanins in concentrate was 28% higher than in the black carrots as fresh weight basis. If the results were calculated on a dry weight basis, processing of black carrots resulted in total monomeric anthocyanin losses of 36% compared to levels found in raw material. Milling of black carrots (MIL) led to loss 66% of monomeric anthocyanins due to cell wall or membrane distruption. Total monomeric anthocyanin content showed a different pattern than phenolics and flavonoid contents during extraction. However, only extraction-II step (EXT-II) showed extensive decrease by 80% of total monomeric anthocyanins. The high amount of recovery of monomeric anthocyanins occurred as expected in juice collecting step (TOJ), total monomeric anthocyanin content was reached to 26.6±3 mg cyanidin-3-glucoside/ 100 gr fresh weight.

The percent polymeric color values of black carrot was 6.2±4.0. Significant ($p \leq 0.05$) changes in the percent polymeric color occurred in all processing samples. Percent polymeric color during processing changed between 3.6±2.1and 16.2±3.7. Polymeric color in milling step (MIL) was 2.5 fold higher than raw material. On the other hand, after heating and cooling step (HAT) 2.1 fold reduction occurred in polymeric color whereas extraction- II step (EXT-II) resulted in 1.8 fold higher polymeric color value compared to raw material. The losses in total monomeric anthocyanins may be attributed high values of polymeric color in some steps. The decline in anthocyanin content in samples with increasing polymeric color (percent) might be related to the polymerization reaction of anthocyanins.

The mean values of total antioxidant capacity of black carrot samples analyzed by DPPH and CUPRAC methods were 492.6± 84.9 and 1568.7342.3 μmol TEAC/100 g fresh weight, respectively. The total antioxidant capacity of samples collected from each step were found to be statistically different ($p \leq 0.05$). Processing black carrot in concentrate resulted in overall 30% (DPPH) and 22% (CUPRAC) increase in fresh weight basis whereas in dry weight 67% (DPPH) and 71% (CUPRAC) decreases were observed. In the second step of process, milling led to 54% (DPPH) and 57% (CUPRAC) reduction in antioxidant capacity. With reference to the raw material, DPPH and CUPRAC showed the largest decreases of 82-83% and 89-91% by extraction-I and extraction-II steps.

The results clearly indicate that phenolic contents of samples collected from each step were found to be statistically different ($p \leq 0.05$). Chlorogenic acid (5-O-caffeoylquinic acid), neocholorogenic acid (trans-5-O-caffeoylquinic acid) and caffeic acid were detected in black carrot samples. Chlorogenic acid which is a hydroxycinnamate, was most abundant phenolic acid with the amount of 266.8±52.6 mg/100 g fresh weight. Processing to concentrate led to 4.2, 10.0 and 22.0 fold increases in the amounts of chlorogenic acid, neochlorogenic acid and caffeic acids, respectively, as compared to raw material in fresh weight basis. If results were calculated on dry weight basis, the concentrate sample has 2.2 fold higher neochlorogenic acid and 4.9 fold higher caffeic acid as compared to black carrot, whereas chlorogenic acid showed a 1.1 fold decrease as a result of processing. Following milling step, chlorogenic acid and neochlorogenic acid decreased by 74.9% and 52.2%, in contrast to other phenolic acids, caffeic acid which showed a 70% increase. Extraction steps caused a decrease of chlorogenic and neochlorogenic acids in the range of 80-94%, whereas a moderate level of loss occurred in caffeic acid amount (45-75%). The results indicate that heating and cooling, pasteurization

and concentration steps showed apparent increases in phenolic acid contents of black carrot samples with heating treatments.

Five anthocyanins were detected and identified by HPLC order as cyanidin- 3-glucoside (kuromanin chloride), cyanidin derivative no. 1, cyanidin derivative no. 2, pelargonidin-3-glucoside and pelargonidin-3,5-diglucoside. Processing black carrot into concentrate led to increase in all individual anthocyanins in fresh weight. Black carrot concentrate has 10 (cyanidin-3-glucoside), 6.8 (pelargonidin-3-glucoside), 4.9 (pelargonidin,3-5-diglucoside), 3.8 (cyanidin derivative no. 1) and 5.3 (cyanidin derivative no. 2) fold higher anthocyanin amounts as compared to black carrot. If results were calculated on dry weight basis, concentrate has 2.3 (cyanidin-3-glucoside), 1.5 (pelargonidin-3-glucoside), 1.1 (pelargonidin,3-5-diglucoside) and 1.2 (cyanidin derivative 2) fold higher anthocyanin amounts as compared to black carrot whereas cyanidin derivative 1 showed a 1.2 fold decrease which shows a different tendency from other anthocyanin compounds as a result of processing. Extraction steps were the processing step involving the biggest loss of anthocyanins. Extractions led to extensive losses ranged between 75-91%. The results indicate that heating and cooling, pasteurization and concentration steps showed apparent increases in anthocyanin contents of black carrot samples with heating treatments.

The statistically significant correlations were found for all methods ($p \leq 0.05$). Highest correlation coefficient value was observed between two antioxidant methods, DPPH and CUPRAC (r = 0.984). On the other hand, total monomeric anthocyanin values showed lowest correlations either with the amounts of total phenolics (r = 0.827) or total flavonoids (r = 0.799). It was interesting to observe lower correlation of monomeric anthocyanin values with flavonoid content, though anthocyanins were classified as flavonoids. In consistent with this data, the amount of flavonoids calculated by summing up the individual flavonoids as analyzed by HPLC were extensively higher than anthocyanin content obtained by the pH differential method.

GIDA PROSESİNİN KARA HAVUCUN ANTİOKSİDAN KAPASİTESİ ÜZERİNE ETKİLERİ

ÖZET

Kara havuç içerdiği sağlık açısından faydalı bileşilekler özellikle antosiyaninlerce zengin olması nedeniyle günümüzde gittikçe kullanımı yaygınlaşan doğal bir renklendirici haline gelmiştir. Kara havuç genellikle konsantreye işlenmiş halde kullanıldığından sağlık açısından olumlu etki yaratan bu bileşenlerin proses sırasında değişiminin incelenmesi önemlidir. Bunun yanı sıra, kara havucun içerdiği açil antosiyaninlerin *in vitro* absorpsiyon ve metabolizasyon mekanizmaları ile kısıtlı çalışma ve araştırma bulunmaktadır.

Genel olarak kara havuç üretimi; ham madde, parçalama, sitrik asit ile muamele, eşanjör çıkışı, mayşeleme, presleme, ekstraksiyon-I, ekstraksiyon-II, ham su, pastörizasyon, depektinizasyon, ultrafiltrasyon ve konsantre olmak üzere 13 basamaktan oluşmaktadır. Bu çalışmada üretim sürecinin her bir aşaması, gravimetrik yöntemle nem içeriği, spektrofotometrik yöntemler ile toplam fenolik, flavonoid, toplam antioksidan kapasitesi, toplam monomeric antosiyanin, polimerik renk, HPLC ile fenolik asit ve antosiyanin profilleri incelenmiştir.

Bu çalışmada, kara havucun konsantreye işlenmesi sırasında fenoliklerin, flavonoidlerin ve antosiyaninlerin değişiminin moleküler düzeyde incelenmesi amaçlanmıştır. Proses etkisinin araştırılması için endüstriyel örneklerle çalışılmıştır.

Çalışmadan elde edilen sonuçlar kara havuç örneklerinin nem miktarının % 87.3 ± 0.5 olduğunu göstermiştir. Kara havucun konsantreye işlenmesi esnasında %44'lük bir nem kaybına neden olduğu bulunmuştur. Bunun yanı sıra, parçalama basamağından konsantreye kadar nem miktarı %91-%99 aralığında değişmiştir. Kara havuç konsantresinin nem miktarı evaporasyon basamağında beklenildiği gibi önemli bir düşüş göstermiştir. Tüm analiz dataları yaş baz üzerinden verilmiştir, bunun nedeni proses örneklerinin nem miktarlarının çok yüksek olmasıdır (Konsantre (%44) hariç %87-%99). Örneklerin pH değerleri 3.8 to 4.1 aralığında değişmiştir.

Spektrofotometrik analizler öncesinde, kara havuç örnekleri 70:29.5:0.5 and 70:28:2 yüzdelerinde aseton:su:asetik asit ve %7 asetik asitli %80'lik metanol ile ekstrakte edilip; toplam fenolik, total flavonoid ve toplam antioksidan kapasiteleri DPPH ve CUPRAC metotları ile analizlenip, bu metotlar için en iyi ekstraksiyon çözgeni belirlenmiştir. Sonuçlar ışığında, toplam fenolik madde miktarı 70:29.5:0.5 aseton:su:asetik asit çözgeni ile ekstrakte edilen örneklerde sırasıyla 70:28:2 yüzdelerinde aseton:su:asetik asit ve %7 asetik asitli %80'lik metanol çözgenlerine göre %9 ve %25 oranlarında daha yüksek sonuçlar vermiştir. Benzer şekilde , DPPH ve CUPRAC antioksidan metotları aseton:su:asetik asit (70:29:0.5) ile muamele edilmiş ekstraktlar sırasıyla 70:28:2 yüzdelerinde aseton:su:asetik asit ve %7 asetik asitli %80'lik metanol çözgenlerine nazaran %17-%15 and %29 -%18 aralığında değişen yüksek sonuçlar vermiştir. Diğer taraftan, kara havuçların flavonoid

içerikleri %7 asetik asitli %80'lik metanol ile muamele edilmiş ekstraktlar, 70:29.5:0.5 and 70:28:2 yüzdelerinde aseton:su:asetik asit le işlem görmüşlere oranla % 12 and %30 düzeyinde yüksek sonuçla vermiştir. Sonuç olarak, 70:29.5:0.5 aseton:su:asetik asit çözgeni en iyi ekstraksyion çözgeni olarak belirlenmiştir.

Ekstraksiyon metotları kıyaslandığında hidrofilik fraksiyonun lipofilik fraksiyondan önce ekstrakte edildiği örneklerde hidrofilik fraksiyonun toplam antioksidan kapasite miktarları DPPH ve CUPRAC metotlarıyla %81-%88 oranında daha yüksek sonuç vermiştir. Bunun yanı sıra, lipofilik fraksiyonu iki ekstraksiyon metotunda da istatistiksel olarak önemli farklılıklar vermemiştir ($p \leq 0.05$). Hidrofilik fraksiyonun ilk olarak alındığı ekstraksiyon yöntemi uygun yöntem olarak seçilip, lipofilik fraksiyonun antioksidan kapasitesi çok düşük olduğundan katkısı ihmal edilmiştir.

Kara havuç örneklerinin toplam fenolik madde miktarları 319.5 ± 58.1 mg GAE/100 g yaş baz (25.3 mg GAE/g kuru baz) olarak bulunmuştur. Toplam fenolik madde miktarı prosesin her basamağında istatistiksel olarak önemli farklılıklar göstermiştir ($p \leq 0.05$). Numuneler arası toplam fenolik madde miktarı 48.1±8.9 ve 433.3±29.8 mg GAE/ 100 gr yaş baz aralığında değişmiştir. Kara havuçların konsantreye işlenmesi sırasında toplam fenolik madde miktarı yaş bazda %35'lik oranında bir artış göstermiştir. Sonuçlar kuru bazda hesaplandığında prosesin %69'luk bir azalışa neden olduğu bulunmuştur. En büyük kayıplar parçalama ve ekstraksiyon basamaklarında meydana gelmiştir. Preslenen tüm suyun tanklarda birleştirilmesi ile toplam fenolik madde miktarı artıp, 104.6±24.3 mg GAE / 100 gr yaş baz miktarına ulaşmıştır. Toplam fenolik madde miktarı posanın atılması, parçalama ve kuru bazda konsantre edilme basamaklarında en büyük düşüşleri diğer basamaklar ufak değişimlere neden olmuştur.

Kara havuçların toplam flavonoid içerikleri 1048.4 ± 226.0 mg küersetin/100 g yaş baz olarak bulunmuştur. Toplam flavonoid miktarı prosesin her basamağında istatistiksel olarak önemli farklılıklar göstermiştir ($p \leq 0.05$). Numuneler arası toplam flavonoid miktarı 93.7±8.9 -1268.0±136.5 mg küersetin/ 100 gr yaş baz olarak değişmiştir. Toplam flavonoid miktarı proses boyunca fenolik madde miktarı ile aynı değişimleri göstermiş fakat yüzde kayıplar toplam flavonoid içeriğinde daha çok olmuştur. Yaş bazda proses sonunda toplam flavonoid miktarı %17 artış göstermiştir. Fakat sonuçlar kuru bazda hesaplandığında, proses %73'lük bir düşüşe neden olmuştur. En büyük kayıplar parçalama ve ekstraksiyon basamaklarında meydana gelmiştir. Flavonoid kayıplarının fenolik madde kaybından yüksek olmasının nedeni, flavonoidlerin hücredeki bulunduğu yer ile ilişkili olabileceği bilinmektedir.

Kara havuçların toplam monomerik antosiyanin içerikleri 53.7 ± 15.2 mg siyanidin-3-glukozit/ 100 g yaş baz olarak belirlenmiştir. Toplam monomerik antosiyanin miktarı prosesin her basamağında istatistiksel olarak önemli farklılıklar göstermiştir ($p \leq 0.05$). Numuneler arası toplam monomerik antosiyanin miktarı 10.2± 1.1-151.8± 27.6 mg siyanidin-3-glukozit/ 100 gr yaş baz aralığında değişmiştir. Proses sonunda toplam monomeric antosiyanin miktarı yaş bazda %28'lik bir artışa neden olurken, kuru bazda %36'lık bir kayba neden olmuştur. Parçalama toplam monomerik antosiyanin miktarını önemli ölçüde düşürmüştür ($p \leq 0.05$). Toplam monomeric antosiyanin miktarı fenolik ve flavonoidlerden farklı bir değişim grafiği çizmiştir. En büyük kayıp yalnızca ektraksiyon-II basamağında %80 oranında gerçekleşmiştir. Preslenen suların tanklarda toplanmasıyla toplam monomeric antosiyanin miktarı 26.6±3 mg siyanidin-3-glukozit/ 100 gr yaş baza ulaşmıştır.

Kara havuçların polimerik renk değerleri %6.2±4.0 bulunmuştur. Toplam monomerik antosiyanin miktarı prosesin her basamağında istatistiksel olarak önemli farklılıklar göstermiştir ($p \leq 0.05$). Polimerik renk miktarı proses boyunca %3.6±2.1ve %16.2±3.7 aralığında değişmiştir. Parçalama basamağında hammaddeye göre polimerik renk 2.5 kat artmıştır. Diğer yandan, eşanjör basamağı 2.1 düşüş gösterirken ekstraksiyon-II basamağı 1.8 kat artış göstermiştir. Toplam monomerik antosiyanin miktarındaki düşüşler polimerik renk miktarının artışıyla ilişkilendirilmiştir. Bu artışların antosiyaninlerin polimerleşme reaksiyonlardan kaynaklandığı önerilmektedir.

DPPH ve CUPRAC metotları ile kara havuçların toplam antioksidan kapasiteleri sırasıyla 492.6± 84.9 and 1568.7342.3 μmol TEAC/100 g yaş baz olarak bulunmuştur. Toplam antioksidan kapasite miktarları prosesin her basamağında istatistiksel olarak önemli farklılıklar göstermiştir ($p \leq 0.05$). Proses sonucunda toplam antioksidan kapasite miktarları yaş bazda %30 (DPPH) ve %22 (CUPRAC) oranlarında artmıştır. Kuru bazda ise %67 (DPPH) ve %71 (CUPRAC) oranlarında düşüş gözlenmiştir. İkinci basamak olan parçalama basamağı %54 (DPPH) ve % 57 (CUPRAC) oranlarında düşüşe neden olmuştur. Antioksidan kapasitede en büyük kayıplar DPPH ve CUPRAC metotları ile sırasıyla olarak ekstraksiyon-I ve ekstraksiyon-II basamaklarında gözlenmiştir.

HPLC analizleri doğrultusunda her basamakta fenolik asit profilleri istatistiksel olarak önemli farklılılar göstermiştir ($p \leq 0.05$). Klorojenik asit, neoklorojenik asit ve kafeik asit bileşikleri kara havuç numunelerinde tespit edilmiştir. Klorojenik asit kara havuç numunelerinde en fazla bulunan fenolik asit olarak belirlenmiş ve miktarı 266.8±52.6 mg/100 g yaş baz olarak bulunmuştur. Konsantreye işleme sırasında sırasıyla 4.2 (klorojenik asit), 10.0 (neoklorojenik asit) and 22.0 (kafeik asit) kat azalma meydana gelmiştir. Sonuçlar kuru bazda verildiğinde neoklorojenik asit 2.2 ve kafeik asit 4.9 kat artış gösterirken, klorojenik asit 2.2 kat azalma göstermiştir. Parçalama basamağında klorojenik asit ve neoklorojenik asit sırasıyla %75 ve %52, kafeik asit %70 oranında artış göstermiştir. Buna ek olarak ekstraksiyon basamakları klorojenik ve neoklorojenik asit miktarları %80-94 azalmaya neden olurken, kafeik asit miktarında diğer fenolik asit miktarlarına göre daha az miktar azalmaya neden olmuştur (%45-75). Ayrıca sonuçlar sıcaklık uygulamalarının olduğu eşanjör, pastörizasyon ve evaporasyon basamaklarında artış olduğunu göstermiştir.

Kara havuçta 5 farklı antosiyanin birleşiği HPLC analizi ile tespit edilmiştir.Bunlar sırasıyla siyanidin- 3-glukozit, siyanidin türevi no.1, siyanidin türevi no.2, pelargonidin-3-glukozit ve pelargonidin-3,5-diglukozittir. Konsantreye işleme prosesi yaş bazda tüm antosiyaninlerin artışına neden olmuştur. Kara havuç konsantresi kara havuçla kıyaslandığında 10 (siyanidin-3-glukozit), 6.8 (pelargonidin-3- glukozit), 4.9 (pelargonidin, 3-5-diglukozit), 3.8 (siyanidin türevi no.1) and 5.3 (siyanidin türevi no.2) katlarında artış gözlenmiştir. Sonuçlar kuru bazda hesaplandığında ise 2.3 (siyanidin-3-glukozit), 1.5 (pelargonidin-3- glukozit), 1.1 (pelargonidin,3-5-diglukozit) ve 1.2 (siyanidin türevi no.2) artış gözlenirken, bunların yanı sıra siyanidin türevi no.1 diğerlerinden farklı olarak 1.2 kat azalmıştır. Ekstraksiyon basamakları en büyük antosiyanin kayıplarının olduğu basamaklardır ve kayıplar bu basamaklarda %75-%91'e ulaşmıştır. Ayrıca sonuçlar fenolik asitlerde olduğu gibi sıcaklık uygulamalarının olduğu eşanjör, pastörizasyon ve evaporasyon basamaklarında antosiyaninlerde artış olduğunu göstermiştir.

Korelasyon analizleri sonucunda metotlar arası korelasyon istatistiksel olarak önemli bulunmuştur ($p \leq 0.05$). En yüksek korelasyon antioksidan metotları arasında bulunmuştur ($r = 0.984$). Bunun yanı sıra, toplam monomerik antosiyanin metodu diğer tüm metotlarla düşük korelasyon göstermiştir ($r = 0.799$). Toplam flavonoid içeriği ve toplam monomerik antosiyanin miktarının düşük korelasyonu dikkat çekici bir noktadır, çünkü antosiyaninler flavonoid sınıfı bileşikleridir. Bu bilgiyle eş olarak HPLC ile yapılan antosiyanin profil analizi sonuçları ile toplam monomerik antosiyanin metoduyla alınan sonuçlar tutarsız olup, HPLC sonuçları toplam monomerik antosiyanin metodunda bulunan monomerik antosiyanin miktarından daha yüksektir.

1. INTRODUCTION

Consumption of fruits and vegetables which are rich in antioxidant compounds may improve health outcomes related to a number of diseases, such as cardiovascular malfunction, common cancers, diabetes, obesity, cataracts as shown in many epidemiological studies. Polyphenolics have been pointed out, by using several animal model and human subject studies, to be bioavailable and play a protective role in the molecular mechanisms of chronic diseases triggered by oxidative stress and free radical damage (Arts and Hollman, 2005; Manach *et al*., 2005; Williamson and Manach, 2005; Heber, 2008).

Black carrots play an important role in human nutrition as they constitute a rich source of health-promoting ingredients. Black carrots (*Daucus carota* ssp. *sativus* var. *Atrorubens* Alef.) originate from Turkey and the Middle and Far East, where they have been cultivated for at least 3000 years. Black carrots have an attractive bluish-purple color with high levels of anthocyanins and can serve as a natural food colorant due to their high heat, light, and pH stability (Cevallos-Casals and Cisneros-Zevallos., 2004; Rein and Heinonen, 2004; Bakowska- Barczak, 2005; Sadilova *et al*., 2006; Kammerer *et al*., 2004). Nowadays, black carrots are gaining increasing popularity as a source of natural food colorants. Due to legal restrictions and the consumers' demand for natural food, synthetic additives and especially pigments are increasingly replaced by colorings originating from plants, such as anthocyanins. Black carrot juice can be a good choice for coloring fruit juices and nectars, soft drinks, conserves, jellies, and confectionery (Khandere *et al*., 2010).

Since black carrot pigments exhibit extraordinary quality parameters and health benefit effects, the phenolic and anthocyanin profile in black carrots has been studied in detail (Kammerer *et al*., 2004; Khandere *et al*., 2010; Montilla *et al*., 2011). Black carrot contains significant amounts of phenolic acids, including hydroxycinnamates (chlorogenic acid and neochlorogenic acid), caffeic acid (Kammerer *et al*., 2004). Furthermore, the main anthocyanins that have been related to such health effects

consist of cyanidin-based acylated and non-acylated pigments (Kammerer *et al*., 2004, Montilla *et al*., 2011).

Because of the fact that black carrot is not consumed in fresh form but after processing to different kinds of products, the effects of processing on health-enhancing phytochemicals should be investigated. Food processing, including various physical and biological factors such as temperature increase and enzymatic activity, is believed to have a significant influence on the destruction or change of bioactive compounds. On the other hand, there is limited information available on the changes of phenolics, especially anthocyanins, after processing. Thus, the phytochemistry of anthocyanins and other phenolics in processed black carrot concentrate is still unclear.

Recently, the bioavailability issue of phenolic components has gained increasing interest. Although there are many studies investigating the in vitro antioxidant potential of bioactive compounds (Uyan *et al*., 2004; Alasavar *et al*., 2005; Sun *et al*., 2009) and numerous studies have focused on anthocyanin bioavailability (Kurilich *et al*., 2005; Charron *et al*., 2009), further investigation is necessary to reveal whether key compounds are sufficiently bioavailable to facilitate the expected health benefits. There have been very few attempts to evaluate the absorption and metabolism of acylated anthocyanins and limited researchhas been performed dealing with the absorption/metabolism of these components in the human body.

The purpose of this study was to investigate the effects of processing of black carrot to concentrate on its phenolics, flavonoids and anthocyanins by focusing at industrial-scale processing.

This research thesis is presented as literature, materials and methods, results and discussion and conclusion parts. In the literature chapter, black carrot and black carrot concentrate, healthy compounds of black carrot; mainly carotenoids, anthocyanins and phenolic acids, were reviewed. The changes during industrial food processing on black carrot antioxidants were also presented in the literature. Materials and methods section included the detailed protocols followed for the analysis. Results and discussion part was including; the effect of each processing step on antioxidative compounds in black carrot and black carrot concentrate. In the

conclusion part, the implication of findings and recommendations on the changes of healthy compounds were presented.

2. LITERATURE

2.1 Black Carrot and Black Carrot Concentrate

Black carrot or *Daucus carota* ssp. *sativus* var. *atrorubens* Alef. is part of the *Apiaceae family Daucus carota* species which have different root colors such as white, yellow, red, purple and black (Erten and Tanguler, 2012). It has been grown and consumed in the East for at least 3,000 years. Historical records indicate that it was cultivated in parts of Europe in the 12th century and in Holland by the 14th century. It was commonly consumed until about 1750 when Dutch breeders developed the orange variety. The black carrot is still consumed in some parts of the world, such as Spain, Pakistan, Afghanistan, Turkey, and Egypt (Kammerer, 2003; Schwarz *et al.*, 2004).

Black carrot root contains approximately 88% water, 1% protein, 7% carbohydrate, 0.2% fat, and 3% fiber (Gajewski *et al., 2007*). The carbohydrate fraction is almost exclusively simple sugars, predominantly sucrose, glucose and fructose (Alasavar *et al.*,2001). Black carrot is a significant source of vitamin A (Mills *et al.*, 2008) and phytonutrients, including carotenoids, anthocyanins, and other phenolic compounds (Sun *et al.*,2009). Similar to black carrot, purple carrot contains higher amounts of antioxidant vitamins, carotenoids and phenolics than other colored carrot varieties including orange, yellow and white (Alasalvar *et al.*, 2005). Due to appreciable levels of a variety of different compounds, carrot is considered a functional food with significant health-promoting properties (Arscott and Tanumihardjo, 2010).

Black carrot is an economically important horticultural crop that has gained popularity in recent decades due to increases in the awareness of its nutritional value. Black carrot is mostly consumed as a processed product in the form of sauce, juice, and concentrate. Furthermore, in Turkey, black carrot is used as a major raw material in shalgam production which is a traditional lactic acid fermentation beverage (Erten *et al.*, 2008). There is a demand for food colorants from natural sources that can serve as alternatives to the use of synthetic dyes due to the consumer concerns over

the use of synthetic additives. Health benefits of anthocyanin extracts intensified the interest in anthocyanin-rich foods. Today extracts of black carrots are commonly used in juices, candies, confectionery, ice cream, soft drinks, or other fermented beverages as a healthier alternative to synthetic colorants (Khandere *et al.,* 2010). Cevallos-Casals and Cisneros-Zevallos (2004) showed that colorants rich in acylated anthocyanins, such as sweet potato and purple carrot, are more resistant to the variations in pH than colorants rich in unacylated anthocyanins such as red grapes. Black carrot is an excellent source of acylated anthocyanins. Rein and Heinonen, (2004) have shown that black carrot extract immediately increased the color intensity of strawberry juice and raspberry juice by 166% and 58%, respectively. Also, it has been reported that the initial intensifying of the strawberry juice color and the stability of color during storage increased by the addition of black carrot extracts. Kammerer *et al*. (2007) studied on improving the visual appearance of canned strawberries by adding black carrot and elderberry concentrates as natural colorants. The values of the fruits colored with black carrot anthocyanins were expected to be superior to those fruits, where elderberry concentrate was added, due to the lack of acylated pigments. The black carrot concentrate superior to the elderberry extract in terms of color retention of canned strawberries. Pigment stability was only significantly improved when black carrot concentrate was added, whereas the fruits colored with the elderberry concentrate showed almost the same relative pigment loss as the control. According to Kırca *et al.* (2003), anthocyanins from black carrots can impart the desired red color to blood orange juice due to their higher stability at processing temperatures. Also, anthocyanins from black carrot juice have good stability during both heating and storage in colored fruit juices/nectars including apple, grape, orange, grapefruit, tangerine, and lemon juices and apricot, peach and pineapple nectars (Kırca *et al.*, 2006).

Generally, the industrial juice-making process involves several steps as outlined in Figure 2.1. Briefly, process starts with sound fruit, freshly harvested from the field or taken from refrigerated or frozen storage. Thorough washing is usually necessary to remove dirt and foreign objects and may be followed by a sanitation step to decrease the load of contaminants. The juicing process continues with the crushing step to break down the cell tissue. Soluble pectin found in fresh juice is a result of physical breakup of the cells and the activity of pectolytic enzymes that are primarily located

in the cell wall of the fruit. This soluble pectin is the cause of difficulty in extraction due to increased juice viscosity , resulting in reduced extraction effectiveness. Then, the fruit mash is heated to 45 to 50ºC followed by the addition of pectolytic enzyme preparations. Reaction time can take up to 1 to 2 h. Special tubular heat exchangers have to be used for this production step. Heating the mash can improve extraction of the cell wall material and color. Depectinization is designed to reduce the viscosity and slipperiness of the pulp and thus permit the effective use of decanters and presses with proper press aids as needed. Most commercial pectolytic enzymes contain certain anthocyaninase side activity, which degrade anthocyanins into a colorless form called anthocyanidin. To ensure juice color stability, the level of anthocyaninase side activity must be minimal. A good pectolytic enzyme preparation for processing therefore needs to possess the following characteristics: heat tolerance and low pH tolerance, with little or no anthocyaninase activity. Traditionally, the term pectinases includes the three enzymes pectin methyl esterase, pectin lyase, and polygalacturonase. Clarification is a process by which the semi-stable emulsion of colloidal plant carbohydrates that support the insoluble cloud material of a freshly pressed juice is "broken". Hence the viscosity is dropped and the opacity of the cloudy juice is changed to an open splotchy look. This can be accomplished in one of two general ways: enzymatically and nonenzymatically. Ultrafiltration is a membrane filtration process that separates particles based on molecular weight. The process uses a cross-flow method of operation. Fruit juice is concentrated by controlled evaporation of water, the major constituent of the juice. Fruit juice concentration can offer significant advantages to the processor; by concentrating the juice, the bulk is reduced, thereby reducing storage volume requirement and transportation costs. Storage of the cold concentrate is less likely to exhibit yeast growth because of the high sugar concentration and also if frozen concentrate is the primary end product (Höhn *et al.*, 2005; McLellan and Padilla-Zakour , 2005).

2.2 Healthy Compounds of Black Carrot

Fruits and vegetables provide a complex mixture of nutrients and non-nutrients, such as phytochemicals, that work together to protect against disease. Diets rich in fruits and vegetables have been associated with reduced risk of degenerative diseases including some cancers and cardiovascular diseases.

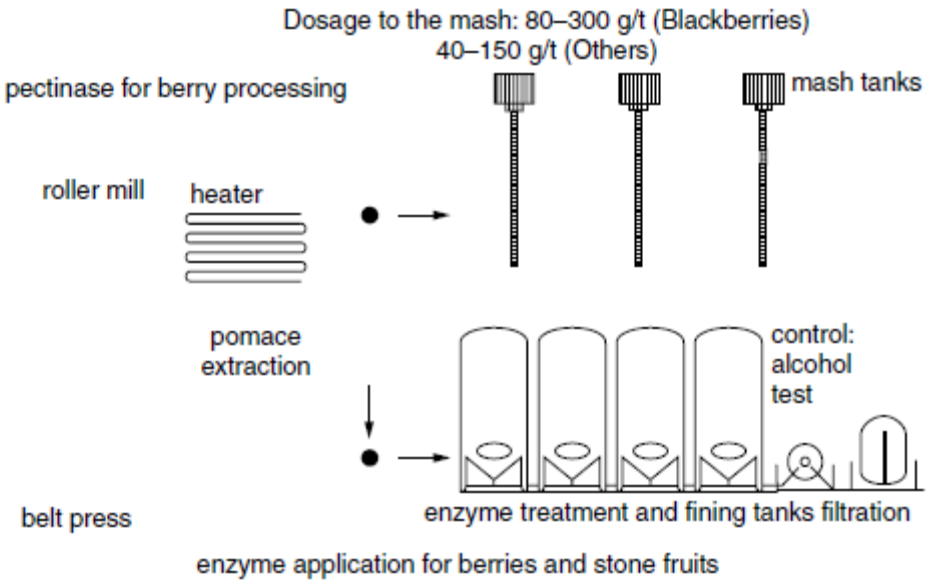

Figure 2.1 : Process line- berries and stone fruits (Höhn *et al.*, 2005).

Fruits and vegetables are a rich source of phytochemicals such as carotenoids, flavonoids, and other phenolic compounds. Different colors of fruits and vegetables are due to pigmented disease-fighting phytochemicals, which is one reason many dietary guidelines recommend choosing a variety of fruits and vegetables (Heber, 2207; Paredes-López *et al.*, 2010).

It is well-known that the positive effect on health associated with the consumption of fresh fruits and vegetables is exerted by the pool of antioxidants, with noticeable synergistic effects. Therefore, to assess the nutritional quality of black carrot, it is important to study all of the main compounds having antioxidant activity. The protective effects of black carrots and its products have been attributed to their high contents of phenolics such as chlorogenic acids, anthocyanins, vitamin A, and carotenoids. In the following sections, carotenoids phenolic acids, and anthocyanins,will be evaluated.

2.2.1 Carotenoids

Carotenoids are compounds comprised of eight isoprenoid units whose order is inverted at the molecule center. All carotenoids can be considered as lycopene ($C_{40}H_{56}$) derivatives by reactions involving: (1) hydrogenation, (2) dehydrogenation, (3) cyclization, (4) oxygen insertion, (5) double bond migration, (6) methyl migration, (7) chain elongation, (8) chain shortening. Carotenoids are classified by their chemical structure as: (a) carotenes that are constituted by carbon and

hydrogen; (b) oxycarotenoids or xanthophylls that have carbon, hydrogen, and, additionally, oxygen (Delgado-Vargas *et al.,* 2000; Krinsky and Johnson, 2005; Voutilainen *et al.,* 2006). Also, carotenoids have been classified as primary or secondary. Primary carotenoids group those compounds required by plants in photosynthesis (β-carotene, violaxanthin, and neoxanthin), whereas secondary carotenoids are localized in fruits and flowers (α-carotene, β-cryptoxanthin, zeaxanthin, antheraxanthin, capsanthin, capsorubin) (Mortensen, 2006; Rodriguez-Amaya, 2010).

Carotenoids are a group of phytochemicals that comprise a family of over 700 compounds in nature and are responsible for the pigmentation in many fruits and vegetables. Those that predominate and are often quantified in human serum include lutein, zeaxanthin, β-cryptoxanthin, lycopene, and α- and β-carotene (Yeum and Russel, 2002). Carotenoids are accessory light-harvesting pigments to chlorophyll in the chloroplasts of photosynthetic tissues and in the chromoplasts of nonphotosynthetic tissues such as fruits, flowers, and the roots of carrots (Hager and Howard, 2006; Mortensen, 2006). Other functions of carotenoids in plants include photoprotection by quenching the excess energy of excited chlorophyll or singlet oxygen (Arscott and Tanumihardjo, 2010). Most carotenoids act as macular pigments (lutein and zeaxanthin) and they have antioxidant and biochemical properties other than pro-vitamin A activity (Krinsky *et al.,* 2005). The carotenoids in carrot roots may likely serve none of the previously mentioned purposes and may be the result of mutation. Carrot root carotenoids occur as pure pigment crystals in chromoplasts. Each crystal is surrounded by a membrane to form a carotene body (Arscott and Tanumihardjo, 2010).

The primary source of dietary carotenoids include carrots (*Daucus carota* L.), tomatoes (*Lycopersicon esculentum* Mill.), sweet potatoes (*Ipomoea batatas*) and spinach (*Spinacia oleracea* L.) (Voutilainen *et al.,* 2006). Carrot roots are rich in carotenoids. Four carotenes (α- carotene , β- carotene, lutein and lycopene) can be routinely quantified in various colors of carrots. The predominant carotenoids are the provitamin A carotenes, that is, α- and β-carotene in carrots (Arscott and Tanumihardjo, 2010).

Carotenoids are abundant in plant-based foods and have been implicated as the beneficial substances in these diets in the prevention of disease. The proposed

mechanisms include provitamin A activity, antioxidant free radical scavenger activity that offers protection against LDL oxidation (Krinsky *et al.,* 2005), increased cell-to-cell communication via gap junctions (Delgado-Vargas *et al.,* 2000; Krinsky *et al.,* 2005), and immunomodulatory effects (Rodriguez-Amaya, 2010).

Carotenoids are a complex group of chemicals, and studies of the health effects of carotenoids are very heterogeneous, it is difficult to undertake a meta-analysis or even a detailed systematic review about the health effects of carotenoids. The epidemiologic literature relating carotenoids to CVD and cancer is conflicting and has been reviewed extensively (Krinsky *et al.,* 2005; Voutilainen *et al.,* 2006). The results of a large-scale prospective study reported that both α- and β-carotene intake, and carrot consumption, but not tocopherols,vitamin C, or other carotenoids were inversely related to CVD mortality in elderly men (Buijsse *et al.,* 2008). There exists evidence of the effectiveness of β-carotene in the treatment of certain kinds of cancer, for example, smoking related cervical intraepithelial neoplasia and cervical and stomach cancer. Although a higher plasma-carotene concentration has been associated with a reduced risk of heart disease in several crosssectional and prospective studies, 4 large randomized trials did not reveal any reduction in cardiovascular events with carotene use, and, in fact, there may even be an increase in heart disease and total mortality in male smokers (Voutilainen *et al.*, 2006).

Total carrot root carotenoid content can vary significantly between cultivars as shown in Table 2.1 (Alasavar *et al.*, 2001; Grassmann *et al.*,2007) and is the major source of variation in reported carrot carotenoid concentrations. Additionally, the growing season, soil, maturity, and genetic factors also influence carotenoid content of carrots (Arscott and Tanumihardjo, 2010).

Current cultivars of purple varieties include solid purple carrots, often referred to as "black carrots," and carrots with purple phloem and white, yellow, or orange xylem (core). Purple carrots with a white core contain very low levels of carotene (4 to 6 ppm), whereas purple–orange carrots (38 to 130 ppm) can contain as much or more total carotene as typical orange carrots (Grassmann *et al.,* 2007). The continued research in carrot cultivar development has produced a novel purple–orange–red cultivar that contains approximately 40 ppm carotenes (Mills *et al.*, 2008). The high amount of various carotenoids in purple carrots suggests that they have pro-vitamin A activity and may be a good source of lutein.

Table 2.1 : Carotenoid content of raw carrots of different colors[1].

	Total (ppm)			
	α-Carotene	**β-Carotene**	**Lutein**	**Lycopene**
Orange				
Surles *et al.*, 2004	22	128	2.6	nd
Sun *et al.*, 2009	27	69	0.4	0.6
Alasavar *et al.*, 2001	40	69	nm	nm
Nicolle *et al.*, 2004	13 to 31	32 to 66	0.6 to 1.8	nm
Rodriguez-Amaya *et al.*, 2008	10 to 22	18 to 38	nm	nm
Grassmann *et al.*, 2007	57 to 70	45 to 52	4 to 5	nd
Dark Orange				
Surles *et al.*, 2004	31	185	4.4	17
Sun *et al.*, 2009	45	113	0.7	0.9
Nicolle *et al.*, 2004	75.8	172	1	nm
Yellow				
Surles *et al.*, 2004	0.5	1.8	5.1	nd
Sun *et al.*, 2009	0.2	3.6	2.4	0.04
Alasavar *et al.*, 2001	nr	nr	nm	nm
Nicolle *et al.*, 2004	nd	3.3	1.4 to 2.3	nm
Grassmann *et al.*, 2007	nr	nr	5 to 10	nd
Red				
Surles *et al.*, 2004	1.1	3.4	3.2	61
Sun *et al.*, 2009	0.2	22	0.2	50
Purple-white				
Grassmann *et al.*, 2007	2 to 3	2 to 3	9 to 10	nd
Purple-orange				
Surles *et al.*, 2004	4	123	11	nd
Sun *et al.*, 2009	10	28	1.1	0.2
Alasavar *et al.*, 2001	87	161	nm	nm
Grassmann *et al.*, 2007	62 to 100	65	8 to 10	nd
Purple-yellow				
Sun *et al.*, 2009	2	15	3	0.4
Nicolle *et al.*, 2004	nd	3.1 to 3.8	1.8 to 2.2	nm
White				
Surles *et al.*, 2004	nd	0.06	0.09	nd
Sun *et al.*, 2009	0.05	0.34	1.7	0.04

[1] nd: not detected, nm: not measured, nr: not reported.

Alasalvar *et al*. (2005) reported these carrots to contain 2.2 and 2.3 times more α and β carotene than the typical orange carrots they studied, whereas Surles *et al*. (2004) found this to be true for only α-carotene.

2.2.2 Anthocyanins

Anthocyanins from the Greek anthos, a flower, and kyanos, dark blue,are flavonoids (flavan like), and consequently based on a C15 skeleton with a chromane ring bearing a second aromatic ring B in position 2 (C_6-C_3-C_6) and with one or more sugar molecules bonded at different hydroxylated positions of the basic structure. Anthocyanins are substituted glycosides of salts of phenyl-2- benzopyrilium (anthocyanidins) (Mortensen, 2006; Zafra-Stone *et al.,* 2007). The basic C_6-C_3-C_6 anthocyanin structure is the source of an infinity of colors produced by its chemical combination with glycosides and/or acyl groups and by its interaction with other molecules and/or media conditions. The six anthocyanidins are the most abundant and they are water soluable pigments. The six common anthocyanidin backbones are cyanidin, malvidin, delphinidin peonidin, petunidin, and pelargonidin. These backbones can vary in the number and position of hydroxyl groups, methoxyl groups, and type, position, and number of attached sugar molecules which may also be acylated by various aromatic or aliphatic acids (Wrolstad *et al.,* 2005). Anthocyanins are responsible for many of the attractive colors, from scarlet to blue, of flowers, fruits, leaves, and storage organs (Patras *et al.,* 2010). In aqueous phase, anthocyanins exist as a mixture of four molecular species and their relative color depends upon pH. At pH 1–3, the flavylium cation is red colored, at pH 5, the colorless carbinol pseudobase is generated, and at pH 7–8 the blue purple quinoidal base is formed (Castaneda-Ovando *et al.,* 2009).

Some fruits are a source of one anthocyanin: cyanidin in apple, cherry, fig, and peach; delphinidin in eggplant and pomegranate; some fruits have two main anthocyanins such as cherry sweet and cranberry (cyanidin and peonidin), while others have several anthocyanins (grape) (Castaneda-Ovando *et al.,* 2009).

Anthocyanins occur in nature as glycosides of anthocyanidins and may have aliphatic or aromatic acids attached to the glycosidic residues. Acylation with aromatic acids including *p*-coumaric, caffeic, ferulic, sinapic, gallic, or *p*-hydroxybenzoic acids has an important stabilizing effect on anthocyanins. These acyl substituents are commonly bound to the C_3 sugar, esterified to the 6-OH or less frequently to the 4-OH group of the sugars. Research involving the development of anthocyanin containing food colorants has led to the discovery of anthocyanin molecules with complex patterns of glycosylation and acylation that exhibit remarkable stability to

pH changes, heat treatment and light exposure (Castaneda-Ovando *et al.,* 2009). Cevallos-Casals and Cisneros-Zevallos (2004) showed that colorants rich in acylated anthocyanins, such as sweet potato and purple carrot, were more resistant to the solution pH than colorants rich in unacylated anthocyanins such as red grape.

Recent trend in food production to replace synthetic pigments for natural alternatives is steadily increasing (Stintzing *et al.,* 2004). They are often used in industry as natural colorants in foods and beverages. Unfavorably, anthocyanins are affected with a high lability toward exogenic factors such as oxygen, light, and especially pH and temperature. Therefore, to secure optimal color and nutritional quality, minimization of anthocyanin degradation during processing is a real need (Bakowska-Barczak, 2005; Wrolstad, 2005).

Dietary anthocyanins may play a role in health promotion and protection from cardiovascular disease (Zafra-Stone *et al.,* 2007; Wang and Stoner, 2008; (Arscott and Tanumihardjo, 2010), diabetes (Rojo *et al.,* 2012) and exhibit anticarcinogenic activity against multiple cancer cell types in vitro and tumor types *in vivo* such as colon (Zhao *et al.,* 2004; Jing *et al.,* 2008), breast (Fernandes *et al.,* 2010), skin and lung cancer (Wang *et al.,* 2008) by acting as dietary antioxidants, reducing inflammation and lipid oxidation, causing induction of anti-inflammatory and vasoprotective effects, phase II enzymes, and apoptosis (Zafra-Stone1 *et al.,* 2007; Wang *et al.,* 2008).

Chemical structure may play an important role in the inhibitory activity. In one study, commercially prepared grape (*Vitis vinifera*), bilberry (*Vaccinium myrtillus* L.), and chokeberry (*Aronia meloncarpa* E.) anthocyanin-rich extracts were investigated for their potential chemopreventive activity against colon cancer. The varying compositions and degrees of growth inhibition suggest that the anthocyanin chemical structure may play an important role in the growth inhibitory activity of commercially available anthocyanin-rich extracts. Chokeberry, containing monoglycosylated cyanidin derivatives, inhibited colon cancer cell growth to a greater extent than grape and bilberry, when inhibition was compared at similar concentrations of monomeric anthocyanin (Zhao *et al.,* 2004). Furthermore, Jing *et al*. (2008) compare the chemoprotective properties of anthocyanin-rich extracts with variable anthocyanin profiles to understand the relationship between anthocyanin chemical structure and chemoprotective activity, measured as inhibition of colon

cancer cell proliferation. Anthocyanin-rich extracts with different anthocyanin profiles from purple corn, chokeberry, bilberry, purple carrot, grape, radish, and elderberry were tested for growth inhibition using a human colorectal adenocarcinoma cell line. All anthocyanin-rich extracts suppressed cell growth to various degrees as follows: purple corn > chokeberry and bilberry > purple carrot and grape > radish and elderberry. The study suggested that anthocyanin chemical structure affected chemoprotection, with nonacylated monoglycosylated anthocyanins having greater inhibitory effect on cell proliferation, whereas anthocyanins with pelargonidin, triglycoside, and/or acylation with cinnamic acid exerted the least effect (Jing *et al.,* 2008). On the other hand, the antiproliferation activity of a black carrot anthocyanin-rich extract on human cancer cells and metabolism of its characteristic anthocyanins (acylated and nonacylated forms) in humans were investigated, and it was concluded that anthocyanin-rich extract from black carrot concentrate, containing acylated and nonacylated anthocyanin derivatives, inhibited significantly the growth of colorectal adenocarcinoma and promyelocytic leukaemia cells *in vitro* (Netzel *et al.,* 2007).

The primary anthocyanins found in purple carrots (sometimes referred to as black carrots), are derivatives of cyanidin, but pelargonidin and peonidin glycosides have also been identified (Kammerer *et al.,* 2003 and Kammerer *et al.,* 2004b). Black carrots accumulate five major anthocyanin pigments, among them are two nonacylated, cyanidin 3-xylosylglucosylgalactoside and cyanidin 3-xylosylgalactoside and three derivatives of cyanidin acylated with sinapic acid (cyanidin 3-sinapoylxylosylglucosylgalactoside, ferulic acid (cyanidin 3-feruloylxylosylglucosylgalactoside and *p*-coumaric acid (cyanidin 3-p-coumaroylxylosylglucosylgalactoside (Kammerer *et al.,* 2004b; Montilla *et al.,* 2011). Reports of total anthocyanin content range from 0 mg/100 g fresh weight in orange carrots to 350 mg/ 100 g fresh weight in dark purple carrots (Kammerer *et al.,* 2004b; Sun *et al.,* 2009). Total anthocyanin concentration in the roots of purple carrots can vary widely between cultivars and even within a cultivar based on the degree of root coloring (Kammerer *et al.,* 2004b). Montilla *et al*. (2011) aimed to identify the pigment composition of black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) cultivars Antonina, Beta Sweet, Deep Purple, and Purple Haze.The amount of phenolic compounds in roots of Deep Purple (97.9 mg GAE/100 g fw)

was slightly higher than in Antonina (75.3 mg GAE/100 g fw), and considerably higher in comparison with the Beta Sweet (28.5 mg GAE/100 g fw) and Purple Haze (17.9 mg GAE/100 g fw) cultivars. The cultivars Antonina, Deep Purple, and Beta Sweet contained higher levels of anthocyanins than the cultivar Purple Haze, which was already visually indicated by the degree of root coloring (Montilla *et al.,* 2011). The anthocyanin contents for black carrot were lower than those reported for black currant and blueberries (476-365 mg/100 g fw), but they were similar to other foods such as plum (19-124 mg/100 g fw), strawberries (21-40 mg/100 g fw), red onion (49 mg/100 g fw), and red grapes (27 mg/100 g fw) (Wu *et al.,* 2006).

Some pyranoanthocyanins (i.e., anthocyanins that contain an additional pyran ring between C-4 and the hydroxyl group attached to C-5) that occur a direct reaction between anthocyanins and free hydroxycinnamic acids (Rentzscha *et al.,* 2007). Six novel pyranoanthocyanins were identified by in black carrot (*Daucus carota* ssp. *sativus* var. *atrorubens* Alef.) juice. The two major compounds, namely, the vinylcatechol adducts of cyanidin 3-O-(6-O-feruloyl-β-D-glucopyranosyl)-(1-6)-[β-D-xylopyranosyl-(1-2)]-β-D galactopyranoside and cyanidin 3-O-[β-D-xylopyranosyl-(1-2)]-β-D-galactopyranoside, respectively, were isolated by a combination of high-speed countercurrent chromatography with semipreparative HPLC. The four remaining pigments were characterized as the vinylphenol and vinylguaiacol adducts of cyanidin 3-O-[β-D-xylopyranosyl-(1-2)]-β-D-galactopyranoside, the vinylguaiacol adduct of cyanidin 3-O-(6-O-feruloyl-β -D-glucopyranosyl)-(1-6)-[β-D-xylopyranosyl- (1-2)]-β-D-galactopyranoside, and the vinylcatechol adduct of cyanidin 3-O-(6-O-sinapoyl-β -Dglucopyranosyl)-(1-6)-[β-D-xylopyranosyl-(1-2)]-β-D-galactopyranoside (Schwarz *et al.,* 2004).

2.2.3 Phenolic acids

Phenolic acids consist of two subgroups, the hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids include gallic, *p*-hydroxybenzoic, protocatechic, vanillic and syringic acids, which in common have the C6–C1 structure. Hydroxycinnamic acids, on the other hand, are aromatic compounds with a three-carbon side chain (C_6–C_3), with caffeic, ferulic, *p*-coumaric and sinapic acids being the most common are monophenolic antioxidants that help to stabilize most vegetable oils and occur widely in nature. Chlorogenic acid is formed by the

esterification of hydroxycinnamic acids, such as caffeic, ferulic, and *p*-coumaric, with quinic acid. Hydroxycinnamic acids are one of the major classes of phenolic compounds. They are present in a large variety of fruits and vegetables (Manach *et al.*, 2004). The major representative of hydroxycinnamic acids in food is caffeic acid. It occurs largely conjugated with quinic acid, as in chlorogenic acid (5-O-caffeoylquinic acid). Caffeic and ferulic acids are the most common phenolic acids in berries, and they are rarely found free; in general, are esterified with other molecules as carbohydrates and organic acids. They are mainly present in the bound form, linked to cell-wall structural components, such as cellulose, lignin, and proteins through ester bonds. Ferulic acids occur primarily in the seeds and leaves of plants, mainly covalently conjugated to mono- and disaccharides, plant-cell-wall polysaccharides, glycoproteins, polyamines, lignin, and insoluble carbohydrate biopolymers (Luie, 2004).

Carrots contain phenolic compounds with a single aromatic ring known as phenolic acids. The main phenolic compounds found in carrots are chlorogenic acids, which are hydroxycinnamic acid derivatives formed by the esterification of cinnamic acids, such as caffeic, ferulic, and *p*-coumaric acids, with quinic acid. These compounds contribute to the organoleptic properties of fresh and processed carrots. Phenolic acids in carrots are present throughout the root, but highly concentrated in the periderm tissue (Zang and Hamauzu, 2004). In a study, the phenolic contents detected in different tissues decreased in the following order: peel > phloem > xylem (Kang *et al.*, 2008).

Carrots were classified as a “low phenolic content” vegetable (<100 mg catechin equivalents/ 100 g fresh weight having just 12.9 mg, but were joined in that grouping by tomato (30.8 mg), green bean (36.8 mg), red onion (73.9 mg), and spinach (82.1 mg). In comparison, collard greens (348.3 mg) and red cabbage (213.1 mg) were in the “high phenolic content” group (Arscott and Tanumihardjo, 2010). Carrots had 37.6% as bound phenolics, second only to potato with the highest bound-phenolic content. Bound phenolics are found mostly in ester form and are associated with cell-wall components (Kang *et al.*, 2008). They lend cross-linking ability to confer structural stability in the cell-wall matrix. Carrot cell walls have been shown to contain significant quantities of esterified *p*-hydroxybenzoic acid, which is presumed to be esterified to cell wall polymers (Padayache *et al.*, 2012).

Alasavar *et al*. (2001) identified 11 different phenolic acids in orange, purple, yellow, and white carrots. Chlorogenic acid predominated in every color carrot, its concentration being 54.1, 8.5, 4.5, and 4.4 mg/100 g in purple, orange, white, and yellow carrots, respectively. Total concentration of all the identified phenolic acids was greatest in the purple carrots and followed the same color order as chlorogenic acid concentration. Moreover, it was founded that total phenolics content of purple carrots was initially 2.9-fold higher than that of orange carrots (Alasavar *et al.,* 2005). Furthermore, Kammerer *et al*. (2004a) investigated most of the compounds detected were identified as composed of *p*-coumaric, caffeic and ferulic acids. Additionally, three hydroxybenzoic acid derivatives and one quercetin glycoside were detected. 5-O-Caffeoylquinic acid (chlorogenic acid) represented the predominant compound amounting to 657 mg/kg in the roots and 5815 mg/kg in the concentrate. The specific fragmentation patterns of mono- and dihydroxycinnamoylquinic acids allowed the distinction of several stereoisomers. According to Sun *et al.,* (2009) purple-yellow carrot contained the highest amount of chlorogenic acid, followed by purple-orange carrot, and it was much higher than that in other carrot colors. Chlorogenic acid in purple-yellow carrot was 2.5, 13.9, 29.8, 16.3, 61.5, and 19.2 times that in purple-orange, red, dark orange, typical orange, yellow, and white carrots, respectively. The rank of caffeic acid content in carrots was the same as for chlorogenic acid. Caffeic acid content was much less than chlorogenic acid in all carrots; for example, chlorogenic acid content was 52 times that of caffeic acid content in purple-yellow carrots, and the ratio ranged from 33 to 258 across all colors. The predominant phenolic acids in carrots are chlorogenic acid. Chlorogenic acid represented 52.4, 57.1, 51.4, and 72.5% of the total phenolic compounds in orange, yellow, white, and purple carrots, respectively. On the other hand, Grassmann *et al.,* (2007) found a similar ranking by color of total phenolics and included red carrots, which had a slightly higher concentration than yellow, orange, and white.

Phenolic acids may play a role and protection from diabetes, cardiovascular disease (Olthof *et al.,* 2001), and obesity (Chao *et al.,* 2010). Karthikesan *et al*. (2010) reveals that chlorogenic acid restore the activities of regulatory enzymes involved in lipids and lipoprotein metabolism. This effect should be the consequence of the enhancement of insulin secretion as well as the antioxidant activity of chlorogenic

acid (Karthikesan *et al.,* 2010). Furthermore, chlorogenic acid seemed to be more potent for body weight reduction and regulation of lipid metabolism than caffeic acid in high-fat diet-induced-obese mice (Chao *et al.,* 2010). Moreover, *in vivo* studies confirmed that chlorogenic acid improved glucose tolerance and mineral pool distribution in obese Zucker (fa/fa) rats (Rodriguez de Sotillo *et al.,* 2006). In a study, it was reported that chlorogenic acid can modify lipids and glucose metabolism, which may improve insulin sensitivity. Chlorogenic acid may be used in the prevention and treatment of chronic metabolic diseases, such as metabolic syndrome and type 2 diabetes (Li *et al.,* 2009). Bonita *et al*. (2007) reported chlorogenic acid was effective in preventing oxidative damage to human epithelial cells. Of interest for heart health is the study with rat cardiomyocytes. Chlorogenic acid and other hydroxycinnamic acids were proved non-cytotoxic, and they both stabilized membranes and improved the energetic status of cardiomyocytes (Bonita *et al.,* 2007). Moreover, Abdel-Wahab *et al*. (2003) proved the beneficial effect of the natural *p*-coumaric acid in protecting animals against oxidative damage. This protecting potential of *p*-coumaric acid could be due to its free radicals scavenging capability.

2.2.4 Antioxidant activity of black carrot bioactive compounds

Phenolic acids and flavonoids, especially anthocyanins as a flavonoid, made a greater contribution to the total antioxidant capacity in foods. They are the major compounds in hydrophilic extracts. Some papers have also reported a linear correlation between the values of the antioxidant capacity with total phenolic content and total anthocyanin content.

Carotenoids were the major antioxidants in hydrophobic extracts. Papers reporting the antioxidant activity of foods in relation to these compounds are much fewer and the results lack coherence. Although some papers reported that carotenoids contributed to the antioxidant activity of foods (Grassmann *et al.,* 2007; Zanfini *et al.*, 2010), in others no correlation was found between the carotenoid content and the antioxidant capacity (Choi *et al.*, 2007; Lavelli *et al.,* 2009).

Gajewski *et al*. (2007) found purple carrots, rich in anthocyanins, to have greater *in vitro* antioxidant capacity than orange and yellow carrots as measured from methanolic extract, which may more efficiently extract hydrophilic (phenolics),

rather than hydrophobic (carotenoids) compounds. Furthermore, in a study antioxidant activities of fresh orange and purple carrots, measured by ORAC fluorescein assay were 76.7±1.6 and 217± 2.9 μmol TE/g, respectively . Purple carrot contained, initially, a 2.8-fold higher ORAC value than its orange counterpart. Antioxidant activity of orange carrots (Alasavar *et al.*, 2005). Sun *et al*. (2009) also included both the DPPH and ABTS methods showed that purple-yellow carrot had the highest antioxidant capacity, followed by purple-orange carrot, whereas the antioxidant capacities of the other carrots did not significantly differ. Hydrophilic extracts had much higher antioxidant capacity than hydrophobic extracts for all carrots. The antioxidant capacity of the hydrophilic extract of purple-yellow carrot was >90 times higher than that of the hydrophobic extract as determined by the ABTS and DPPH methods. For the hydrophobic extract as determined by the ABTS method, the antioxidant capacities of orange, dark orange, and red carrots did not significantly differ and were higher than the antioxidant capacities of purple-yellow and purple-orange carrots, whereas yellow and white carrots had the lowest antioxidant capacity. The results of the DPPH method agreed with the ABTS method except that purple-yellow carrot did not significantly differ in antioxidant capacity with orange, dark orange, and red carrots (Sun *et al.*, 2009).

2.3 Effect of Industrial Processing of Black Carrot Bioactive Compunds

Lorem Black carrots are a rich source of stable anthocyanins. Türkyılmaz et *al.* (2012) evaluated the effects of clarification and pasteurization on anthocyanins of black carrot juice. Monomeric anthocyanins, anthocyanins profile and percent polymeric color were evaluated during processing of black carrot juice. While depectinization and bentonite treatments resulted in 7% and 20% increases in monomeric anthocyanin content of black carrot juice, respectively, gelatine–kieselsol treatment and pasteurization resulted in 10% and 3–16% reduction. The depectinization caused a 7% increase in anthocyanin content due to release of anthocyanins from the cell. Contrary to depectinization and bentonite treatment, clarification with gelatin–kieselsol resulted in 10% reduction in anthocyanin contents. After pasteurization, there were 3–16% reductions the anthocyanin contents of black carrot juice samples. There was lower anthocyanin losses in unclarified (7%) and depectinized juices (3%) compared to juices clarified with bentonite (16%)

and gelatin–kieselsol (13%) after pasteurization. Percent polymeric color decreased after clarification, but substantially increased in samples subjected to heat. Significant increases in the percent polymeric color occurred in depectinized black carrot juice (21%) compared to unclarified black carrot juice (18%). In contrast to depectinization, the percent polymeric color values significantly decreased in black carrot juice samples after the clarification with bentonite and gelatin– kieselsol. Contrary to this decrease during clarification, the percent polymeric color increased 6% after the pasteurization of clarified black carrot juice. According to screening of anthocyanins individually, pectinization resulted in formation of two more anthocyanins (cyanidin-3-galactoside-xyloside and cyanidin-3-galactoside-xyloside-glucoside-sinapic acid). However, after pasteurization treatment those anthocyanins were not detected due to heat treatment. In general, there was a steady increase in individual anthocyanins during depectinization and bentonite treatment, but decrease after gelatin–kieselsol clarification. Similar changes in the proportion of individual anthocyanins were also reported after gelatin–kieselsol clarification. Clarification and pasteurization also caused changes in the proportion of acylated compounds. The highest proportion of acylated anthocyanins were found in unclarified black carrot juice as 90%. However, the proportion of acylated anthocyanins decreased to 83% and 84% after depectinization and clarification with bentonite, gelatin and kiselsol treatment, respectively. The lowest proportion of acylated anthocyanins (79.5%) was found in unclarified samples after pasteurization. The clarification with bentonite and gelatin–kieselsol resulted insignificant changes in the proportions of acylated anthocyanins (Türkyılmaz *et al.*, 2012).

The effect of pre-press maceration treatment with different doses of cell wall degrading enzyme pectinase on antioxidant composition of black carrot juice was investigated by Khandere et *al* (2011). Enzyme-assisted processing significantly improved the antioxidant composition of black carrot juice. There was an overall increase of 33% in juice yield, 27% in total phenolics and 46% in total flavonoids. The total anthocyanin content in black carrot juice was almost doubled. There was 30% increase in total antioxidant activity of black carrot juice extracted with enzyme over straight pressed juice. With increasing pectinase dosage as predicted, there was a significant increase in polymeric color (Khandere *et al.*, 2011).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

The following chemicals were purchased for extract preparation and determination of total phenolic, flavonoid, anthocyanin, antioxidant contents and polymeric color; gallic acid (≥98%), quercetin (≥98%), acetone (≥99.8%), ethanol (≥99.8%), hexane (≥95%), Folin-Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium disulfite ($K_2S_2O_5$), and neocupraine (Nc) from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); methanol (≥99.9%), formic acid (≥98%), hydrochloric acid (37%), sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), sodium hydroxide (NaOH), sodium acetate trihydrate ($CH_3COONa.3H_2O$), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), copper (II) chloride ($CuCl_2$) and ammonium acetate (NH_4Ac) from Merck KGaA (Darmstadt, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and aluminum chloride ($AlCl_3$) from Fluka Chemie (Buchs, Switzerland); potassium chloride (KCl) from Riedel-de Haen Laborchemikalien GmbH (Hanover, Germany) The following standards and reagents were used for the quantification of phenolic compounds: gallic acid (≥99%) and cyanidin 3-*O*-glucoside (≥96%), pelargonin chloride (pelargonidin-3,5-di-O-glucoside chloride, ≥90%) from Extrasynthese (Genay, France); caffeic acid (≥98%), chlorogenic acid (5-caffeoylquinic acid, ≥98%) from Fluka; neochlorogenic acid (≥98%), kuromanin chloride callistephin chloride (≥97%), trifluoroacetic acid (TFA, 99%) and acetonitrile (%99.8) from Sigma-Aldrich. For simulation of *in vitro* gastrointestinal system, pepsin, pancreatin, bile salt, dialysis bags (Membra-Cel MD34) from Sigma-Aldrich and sodium bicarbonate from BDH Chemicals Ltd. (Poole, UK) were purchased. Water used for all analysis was distilled and purified with the water purification system (TKA GenPure).

3.1.2 Plant material-black carrots

Black carrots (*Daucus carota* L. ssp. *Sativu*s var. *atrorubens* Alef.) were collected two times in December 2010 and 2011 from the Ereğli district of the city of Konya and processed into its concentrate in a fruit juice company in Karaman, Turkey. During processing of the same batch starting from raw material, intermediate processing materials yielded from the different steps, and the final black carrot concentrate products were collected. In total, 52 samples from 4 different processing events using independent black carrot batches (biological replicates) were collected. All samples were ground to a fine powder using a precooled grinder (IKA Model A10) and were stored at -80 ºC until analysis.

The scheme of industrial-scale black carrot concentrate production is shown in Figure 3.1. The first sample ("RAW- Raw Material") was taken from black carrot bulk as they arrived at the factory. The second sample ("MIL-Milling ") was taken from the crushing and milling equipment unit where black carrots had been washed and milled. In this unit black carrots were crushed with knives in a few seconds and collected in the tanks. The third sample ("CAT-Citric acid treatment) was taken after the material had been treated with %50 (w/v) citric acid solution to provide optimum pH for pectinase activitythe pH of material was adjusted from 6.0 to 3.8. The fourth sample was taken after material was heated to 85-90 °C for 1 minute and allowed to cool to 50-55°C in the heat-exchanger unit, which was followed by the mashing of black carrots in a mash tank. Mashing was performed by adding with Fructozym® Color (highly concentrated pectolytic enzyme preparation) and incubating the mash (fifth sample "MAS- Mashing"). Following enzymatic treatment, the mash was pressed in a bladder press and sixth sample was taken after pressing (sample "PRS-Pressing). After the juice and presscake were isolated, presscake extracted two times more (samples "EXT-I and EXT-II- Extraction-I, II"). Ninth sample ("TOJ- Total juice) was the juice that were combined from pressing (PRS) and extraction (EXT-I and EXT-II) steps. The combined juice phase was pasteurized at 90-95 °C for 90 seconds and allowed to cool down to 50-55°C for providing depectinization activity under optimum conditions (sample "PAS- Pasteurization & Cooling). After pasteurization, depectinization of juice was performed by adding Pectinex® Ultra Color and incubating juice for 1 hour (sample "PEC-Depectinization"). A negative alcohol precipitation test was used as an indication of complete depectinization.

Following depectinization, the juice was clarified by ultrafiltration (sample "ULF- Ultrafiltration"). Finally, the juice was evaporated until a Brixº value of 65 was reached (sample "CON- Concentrate").

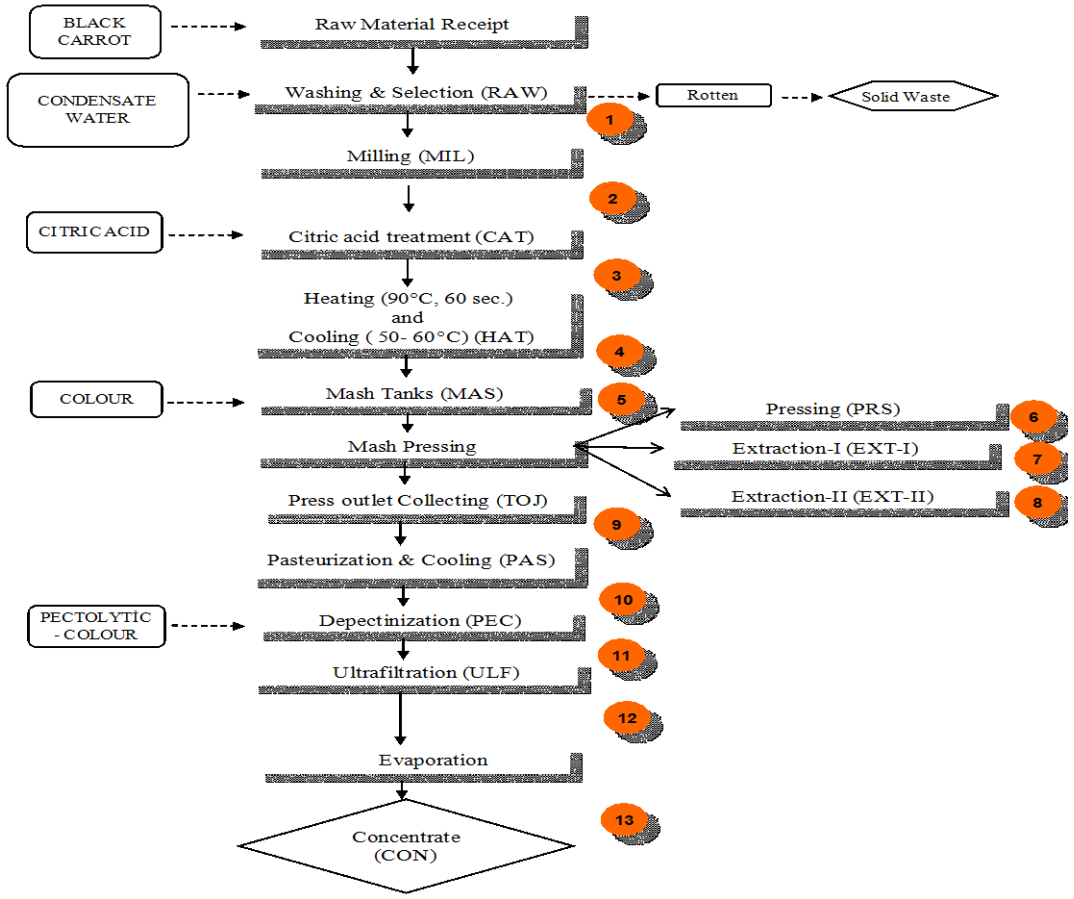

Figure 3.1 : Production scheme of black carrot processing with sampling points.

3.2 Methods

3.2.1 Moisture analysis

Moisture contents of the samples were analyzed according to "Association Official of Analytical Chemists" (AOAC, 1990) method 934.01. Approximately, 2 g of each sample was placed on a pre-weighed aluminum pans and weighed. The pans were kept in vacuum oven (Gallenkamp, Germany) at 90ºC until an equilibrium moisture content was reached (5 h). After drying, the pans were removed from the oven, allowed to cool in a desiccator and weighed again. All samples were analyzed in triplicate and average values were reported.

3.2.2 Determination of optimum solvent for the analysis of phenolic, flavonoid contents and antioxidant capacity

The most effective extraction solvent system and extraction method for the analysis of total phenolics, and antioxidant activity were investigated. Three different solvent systems including acetone:water:acetic acid (70:29.5:0.5, v/v/v) (Sun *et al*., 2009), %7 acetic acid in %80 methanol (Teow *et al*., 2007) and acetone:water:acetic acid (70:28:2, v/v/v) (Jimenez-Alvarez *et al*., 2008) were applied. 2±0.01 g of each sample was extracted with 5 ml of 3 different solvent system in a cooled ultrasonic bath (Azakli, Turkey) for 15 min. The treated samples were centrifuged (Hettich Zentrifugen Universal 32R) for 10 min at 4000 rpm and the supernatant was collected. Another 5 ml of solvent system was added to the pellet and the extraction procedure was repeated. This procedure was again repeated by addition of another 5 ml of solvent system to the remaining pallet. All supernatants were combined and adjusted to a final volume of 20 ml. For analysis of hydrophilic and lipophilic antioxidant activity, 2 different extraction methods were tested and compared for their efficiency. In the first method, lipophilic fraction was extracted with hexane twice (10*2 ml) and hexane was evaporated until dryness under nitrogen in a water bath at 50ºC. Then hydrophilic fraction was extracted by the method as explained above (Sun *et al*., 2009). In the second method, the hydrophilic fraction was first extracted followed by the lipophilic fraction extraction with hexane (10*2 ml). Prepared extracts were stored at -20ºC until analysis. The supernatants were analyzed for their total phenolic content and antioxidant capacities using CUPRAC and DPPH methods. Based on those results acetone:water:acetic acid (70:29.5:0,5, v/v/v), solvent mixture was selected for the extraction of hydrophilic phenolic compounds due to higher extraction yields. As lipophilic antioxidant capacity was observed to be low lipophilic fraction was not analysed for optimum solvent testing.

The procedure applied for the preparation of hydrophilic extracts for total phenolics, antioxidant capacity and total flavonoid content were as follows: 2±0.01 g of each sample was extracted with 5 ml of acetone:water:acetic acid (70:29.5:0,5, v/v/v) in a cooled ultrasonic bath (Azakli, Turkey) for 15 min. The treated samples were centrifuged (Hettich Zentrifugen Universal 32R) for 10 min at 4000 rpm and the supernatant was collected. Another 5 ml of acetone:water:acetic acid (70:29.5:0,5, v/v/v) was added to the pellet and the extraction procedure was repeated. This

procedure was again repeated by addition of another 5 ml of acetone:water:acetic acid (70:29.5:0,5, v/v/v) to the remaining pellet. All supernatants were combined and adjusted to a final volume of 20 ml. Prepared extracts were stored at -20 ^{o}C until analysis (Capanoglu *et al*., 2008). For total monomeric anthocyanin content, polymeric color analysis 75% aqueous-methanol containing 0.1% (v/v) formic acid solvent system was used for anthocyanin stability based on available information in the literature (Awika *et al*., 2004). The calibration curves are given in Appendix, Figure A.1-A.13.

3.2.3 Analysis of phenolics

The total phenolic content of extracts was determined using Folin-Ciocalteu reagent according to the method modified from Velioglu *et al*. (1998) using gallic acid as a standard. One hundred μL of extract was added to 0.75 mL of freshly prepared Folin-Ciocalteu reagent (1:10, v/v with distilled water). The mixture was allowed to stand for 5 min and then 0.75 mL of 6% sodium carbonate solution was added to the mixture. After 90 min of incubation at room temperature, absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Shimadzu UV-1700, Japan). The total phenolics concentration of extracts was expressed on fresh weight basis as milligrams of gallic acid equivalence (GAE) per 100 g of fresh weight of sample. Samples of each extraction were analyzed in triplicate. The calibration curve is shown in Appendix, Figure A.1.

3.2.4 Analysis of total flavonoids

The total flavonoid content was measured colorimetrically as described by Kim *et al*. (2003) at 510 nm . At time zero, 1 mL of sample was mixed with 0.3 mL of 5% $NaNO_2$ solution. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. At 6 min, 2 mL of 1 M NaOH was added to the mixture. Immediately, 2.4 mL of distilled water was added and mixed well. The total flavonoid content of extracts was determined by a quercetin standard curve and expressed as milligrams of quercetin equivalence (QUE) per 100 g of fresh weight of sample. Triplicate samples were analyzed for each extract. The calibration curve is shown in Appendix, Figure A.10.

3.2.5 Total antioxidant capacity assays

Two different *in vitro* tests were applied to estimate differences in total antioxidant levels. The calibration curves obtained by each method are shown in the Appendix, Figures A.4 and A.7.

The DPPH assay was performed as described by Karuman and Karunakaran (2006). 100 µL of each sample extract was mixed with 2 mL of 0.1 mM DPPH in methanol. After 30 min of incubation at room temperature, the absorbance of the mixture was measured at 517 nm against methanol.

The CUPRAC (Copper Reducing Antioxidant Capacity) method was performed according to the method previously described by Apak *et al*., (2004 and 2006) and Bektaşoğlu (2006). The method involved the preparation of 10^{-2} M Copper (II) chloride solution, ammonium acetate (NH_4Ac) buffer (pH=7), and $7.5x10^{-3}$ M Neocuproin (Nc) solutions. To a test tube 1 mL each of Cu (II), Nc, and NH_4Ac buffer solutions were added. Sample extract or standard solution (100 mL) and H_2O (1 mL) were added to the initial mixture so as to make the final volume of 4.1 mL. The tubes were stoppered, and after 1 h, the absorbance at 450 nm was recorded against a reagent blank. The results were expressed in terms of µmol TEAC/100 g fresh weight.

3.2.6 Total monomeric anthocyanin content

The monomeric anthocyanin content was determined according to the pH differential method (Lee *et al*., 2005). Absorbance was measured at 520 and 700 nm in buffers at pH 1.0 and pH 4.5. The total monomeric anthocyanin content expressed as cyanidin-3-glucoside equivalents, as follows **(3.1)**:

$$\text{Total monomeric anthocyanin} = (A \times MW \times DF \times 103)/(\varepsilon \times l) \qquad \textbf{(3.1)}$$

Where; $A = (A_{520nm} - A_{700nm})_{pH\ 1.0} - (A_{520nm} - A_{700nm})_{pH\ 4.5}$, MW=molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF=dilution factor, 103=factor for conversion from g to mg, ε=molar extinction coefficient of cyanidin-3-glucoside (26900 L/(mol.cm)), and l=pathlength (cm). The total monomeric anthocyanin content of extracts was expressed as milligrams of cyanidin-3-glycoside (cyd-3-glu) per 100 g of fresh weight of sample.

3.2.7 Polymeric color

Percent polymeric color was determined using the method described by Giusti and Wrolstad (2001). Sample extracts were diluted with water to have an absorbance reading between 0.5 and 1.0 at 512 nm when evaluated by an using a UV-Vis spectrophotometer (Shimadzu UV-1700, Japan). For analysis, 0.2 mL of 0.90 M potassium metabisulfite was added to 2.8 mL diluted sample (bisulfite bleached sample) and 0.2 mL of deionide water was added to 2.8 mL diluted sample (nonbleached, control sample). After equilibrating for 15 min, but not more than 1 h, samples were evaluated at 700, 512, and 420 nm wavelengths. Color density was calculated using the control sample according to the following formula **(3.2)** (Giusti and Wrolstad, 2001):

$$\text{Color density} = [(A_{420nm} - A_{700nm}) + (A_{512nm} - A_{700nm})] \times \text{dilution factor} \quad \textbf{(3.2)}$$

Polymeric color was determined using the bisulfite-bleached sample using the following formula **(3.3)** (Giusti and Wrolstad, 2001):

$$\text{Polymeric color} = [(A_{420nm} - A_{700nm}) + (A_{512nm} - A_{700nm})] \times \text{dilution factor} \quad \textbf{(3.3)}$$

Percent polymeric color was calculated using the formula **(3.4)** (Giusti and Wrolstad, 2001):

$$\%\ \text{Polymeric color} = (\text{polymeric color}/\text{color density}) \times 100 \quad \textbf{(3.4)}$$

3.2.8 HPLC analysis of phenolic acids and anthocyanin composition

Major phenolic compounds were determined following the method of Capanoglu *et al.* (2008). Extracts were filtered through a 0.45-µm membrane filter and 1 mL was injected into a Waters 2695 HPLC system with PDA (Waters 2996) detector. Supelco SUPERCOSIL® LC-18 column (25×4.6 mm, 5µm) was used as the stationary phase. The mobile phase consisted of solvent **A**, Milli-Q water with 0.1% (v/v) TFA and solvent **B**, 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 initial conditions. The flow rate was 1 ml/min. Detection was 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. All analyses were performed in triplicate.

3.2.9 Statistical analysis

Data were collected from three independent extractions for each fraction and reported as mean ± standard deviation. For multiple comparisons, data was subjected to statistical analysis using SPSS software (version 16.0 for Windows, SPSS Inc.) for the analysis of variance (ANOVA). Duncan's new multiple range test was used to analyze differences between treatments ($p \leq 0.05$). Statistical analysis tables are given in the Appendix Table C.1.

In addition, Pearson's correlation analysis between antioxidant capacity, total phenolic content, total flavonoid content and total monomeric anthocyanin content of black carrot processing samples was also performed. Statistical analysis tables are given in the Appendix Table C.2.

Besides, t-test analyses were performed using the same statistical package to elucidate if any significant difference existed between extraction methods and results were given in Appendix C, Table C.3.

4. RESULTS AND DISCUSSION

4.1 Effect of Black Carrot Concentrate Processing on Antioxidative Compounds

In this study samples representing a number of different well-defined processing steps were taken from a juice producer. In order to decrease batch-to-batch variations, each process step was independently sampled four times using different batches processed during two successive years. Each sample was analyzed for the contents of moisture, pH, total phenolics, total flavonoids, antioxidant capacity, polymeric color, and phenolic acid and anthocyanin profiles.

4.1.1 Moisture contents and pH

Moisture contents of samples taken from each step during processing are shown in Table 4.1.

Table 4.1 : Moisture contents of processing steps[1].

Processing Steps	Moisture content, %
RAW	87.3 ± 0.5
MIL	92.3 ± 0.2
CAT	91.2 ± 0.8
HAC	94.0 ± 1.0
MAS	95.5 ± 0.2
PRS	96.4 ± 0.3
EXT-I	97.9 ± 0.2
EXT-II	98.9 ± 0.1
TOJ	96.9 ± 0.2
PAS	95.8 ± 0.4
PEC	95.7 ± 0.2
UFL	95.8 ± 0.6
CON	43.7 ± 0.9

[1]Data represent mean values and ± standard deviation of 4 independent processing events.

In black carrot samples, moisture content was found to be 87.3 ± 0.5 %. Uyan *et al.* (2004) and Ersus *et al*. (2006) reported moisture contents of 81.2% and 86.9%, respectively. It is possible to have different moisture contents because of different

climate, growing conditions and harvesting time. The total moisture loss was found to be about 44% by processing raw material into the concentrate. On the other hand, the moisture changes from the milling step to concentrate step were observed to be between 91.2%-98.9%. In black carrot concentrate, due to evaporation process, the moisture content decreased significantly as expected. Values for all analyses were given on fresh weight basis due to very high moisture content values of samples collected from each step. pH of processing samples ranged from 3.8 to 4.1.

4.1.2 Solvent choice of spectrophotometric methods

The results of three different solvent systems (acetone:water:acetic acid at ratios of 70:29.5:0.5 and 70:28:2 and 7% acetic acid in 80% methanol) are given in Table 4.2.

According to the results, total phenolic content of black carrot in acetone:water:acetic acid (70:29.5:0,5) extract were 9 % and 25 % higher than that of acetone:water:acetic acid (70:28:2, v/v/v) and acetic acid methanol extract. Similarly, the results of DPPH and CUPRAC methods showed that acetone:water:acetic acid (70:29.5:0,5) extract yielded with 17% -15% and 29% -18% higher values of antioxidant capacity than acetone:water:acetic acid (70:28:2, v/v/v) and acetic acid in methanol extractions, respectively. However, total flavonoid contents of black carrot in %7 acetic acid in %80 methanol extract were 12% and 30% higher than those of acetone:water:acetic acid (70:29.5:0,5) and acetone:water:acetic acid (70:28:2, v/v/v) extracts, respectively. As a result, acetone:water:acetic acid (70:29.5:0,5) was found to be the most effective solvent system for black carrot phenolics similar to the findings of Sun *et al*. (2009). In consistent with this study, Tabart *et al*. (2007) revealed that for extraction of the total phenolics from black currant leaves, aqueous acetone was found to be more effective than methanol and water.

The most widely used solvents for extracting phenolic substances are methanol, acetone and their water solutions. Acetone and methanol seem to have distinct specificities in the extraction of polyphenolic substances. This fact is in accordance with polarity of the solvent used for the extraction and solubility of phenolic compounds. Aqueous methanol, due to its polarity, is more effective at extracting polyphenols linked to polar fibrous matrices. Acetone/water mixtures are more useful for extracting polyphenols from protein matrices, since such solvent mixtures appear to degrade the polyphenol–protein complexes (Tabart *et al*., 2007).

Table 4.2 : Comparison of three solvent systems for determination of total phenolics, flavonoids and antioxidant capacities in black carrot samples[1].

	Extraction Solvent	Black Carrot-Raw Material
Total Phenolics (mg GAE/100 g fresh weight)	acetone:water:acetic acid (70:29.5:0.5, v/v/v)	190.2±1.4
	acetone:water:acetic acid (70:28:2, v/v/v)	172.3±4.9
	7% acetic acid in 80% methanol	141.1±1.5
DPPH method (mg TEAC/100 g fresh weight)	acetone:water:acetic acid (70:29.5:0.5, v/v/v)	167.3±2.0
	acetone:water:acetic acid (70:28:2, v/v/v)	137.6±0.4
	7% acetic acid in 80% methanol	141.1±1.5
CUPRAC method (mg TEAC/100 g fresh weight)	acetone:water:acetic acid (70:29.5:0.5, v/v/v)	649.4±8.7
	acetone:water:acetic acid (70:28:2, v/v/v)	459.3±6.9
	7% acetic acid in 80% methanol	526.4±8.8
Total Flavonoids (mg Quercetin/ 100 g fresh weight)	acetone:water:acetic acid (70:29.5:0.5, v/v/v)	360.6±15.8
	acetone:water:acetic acid (70:28:2, v/v/v)	285.6±4.2
	7% acetic acid in 80% methanol	411.7±0.0

[1]Data represent mean values and ± standard deviation of 3 replicates. All contents are expressed per 100 g fresh weight.

Since extraction yield is also affected by the method of extraction (Tabart *et al*., 2007), two extraction methods were evaluated either first hydrophilic fraction or lipophilic fraction was removed.

The antioxidant capacity methods of DPPH and CUPRAC yielded significant higher values by the 2nd extraction method in hydrophilic fractions ($p \leq 0.05$) as presented Table 4.3. The antioxidant capacity values were about 81%-88% lower in hydrophilic fractions obtained by the 1st extraction method as analyzed by both methods and solvent systems. On the other hand, there was no significant difference in antioxidant capacity values of lipophilic fractions by two different extraction methods ($p \leq 0.05$) (Table 4.3).

Table 4.3 : Comparison of two extraction methods to determine antioxidant capacities in black carrot samples[1].

Methods	Fraction	Solvent System	Extraction 1	Extraction 2
DPPH method (mg TEAC/100 g fresh weight)	Hydrophilic fraction	acetone:water:acetic acid (70:29:0,5, v/v/v)	22.3±2.6	167.3±2.0*
		acetone:water:acetic acid (70:28:2, v/v/v)	16.1±0.8	137.6±0.4*
		7% acetic acid in 80% methanol	24.8±2.4	141.1±1.5*
	Lipophilic fraction	acetone:water:acetic acid (70:29:0,5, v/v/v)	3.9±0.1	3.3±0.1
		acetone:water:acetic acid (70:28:2, v/v/v)	3.8±0.1	3.8±0.1
		7% acetic acid in 80% methanol	4.2±0.2	4.3±0.2
CUPRAC method (mg TEAC/100 g fresh weight)	Hydrophilic fraction	acetone:water:acetic acid (70:29:0,5, v/v/v)	95.4±13.3	649.4±8..7*
		acetone:water:acetic acid (70:28:2, v/v/v)	60.7±2.4	459.3±6.9*
		7% acetic acid in 80% methanol	45.9±5.6	526.4±8.8*
	Lipophilic fraction	acetone:water:acetic acid (70:29:0,5, v/v/v)	15.6±0.1	17.7±0.4
		acetone:water:acetic acid (70:28:2, v/v/v)	20.3±0.6	22.1±0.2
		7% acetic acid in 80% methanol	16.8±0.2	17.6±0.5

[1]Data represent mean values and ± standard deviation of 3 replicates. All contents are expressed per 100 g fresh weight. (*) in the rows represent statistically significant differences ($p \leq 0.05$)

In addition, lipophilic antioxidant capacity was found to be much lower compared to that of hydrophilic fractions, which is in full alignment with findings of Sun *et al.* (2009). As a result, due to the loss in hydrophilic antioxidant capacity, 2nd extraction method was preferred in further analysis of bioactive compounds.

4.1.3 Total phenolics

Total phenolic contents of the samples are presented in Table 4.4 and Figure 4.1. In black carrot samples, total amount of phenolics was found to be 319.5 ± 58.1 mg

GAE/100 g fresh weight (25.3 mg GAE/g dry weight). These findings were higher than results provided by Alasavar *et al*. (2001), Mills *et al*. (2008), and Sun *et al*. (2009) reporting amounts of 74.6±3.32 mg GAE/100 g fresh weight, 12.0±0.3 mg GAE/g dry weight and 15.0±1.1 mg GAE/ g dry weight, respectively.

The phenolic contents of samples collected from each step were found to be statistically different ($p \leq 0.05$) ranging between 48.1±8.9 and 433.3±29.8 mg GAE/ 100 gr fresh weight (Table 4.4).

Table 4.4 : The content of total phenolics of black carrot processing samples[1].

Processing Steps	Moisture content, %	Total phenolics (mg GAE/100 g fresh weight)
RAW	87.3 ± 0.5	319.9 ± 58.1 b
MIL	92.3 ± 0.2	163.8 ± 37.4 c
CAT	91.2 ± 0.8	160.9±30.3 c
HAC	94.0 ± 1.0	143.5±24.1 cd
MAS	95.5 ± 0.2	148.9±26.0 cd
PRS	96.4 ± 0.3	148.5±13.4 cd
EXT-I	97.9 ± 0.2	66.7±6.6 ef
EXT-II	98.9 ± 0.1	48.1±8.9 f
TOJ	96.9 ± 0.2	104.6±24.3 de
PAS	95.8 ± 0.4	147.1±23.3 cd
PEC	95.7 ± 0.2	148.8±17.5 cd
UFL	95.8 ± 0.6	156.7±17.6 c
CON	43.7 ± 0.9	433.3±29.8 a

[1]Data represent mean values and ± standard deviation of 4 independent processing events. All contents are expressed per 100 g fresh weight. Different letters in the columns represent statistically significant differences ($p \leq 0.05$)

Processing black carrot into concentrate resulted in an overall increase of 35% in phenolic content on a fresh weight basis (Figure 4.1). If the results were calculated on a dry weight basis, the concentration of polyphenols in concentrate was 69% lower than that of the black carrot sample. Consistent with these results, Hartman *et al*. (2008) and Holtung *et al*. (2011) reported that 70% and 57% losses of polyphenols were obtained by processing strawberries in to puree (laboratory-scale) and berries to juice (industrial scale), respectively.

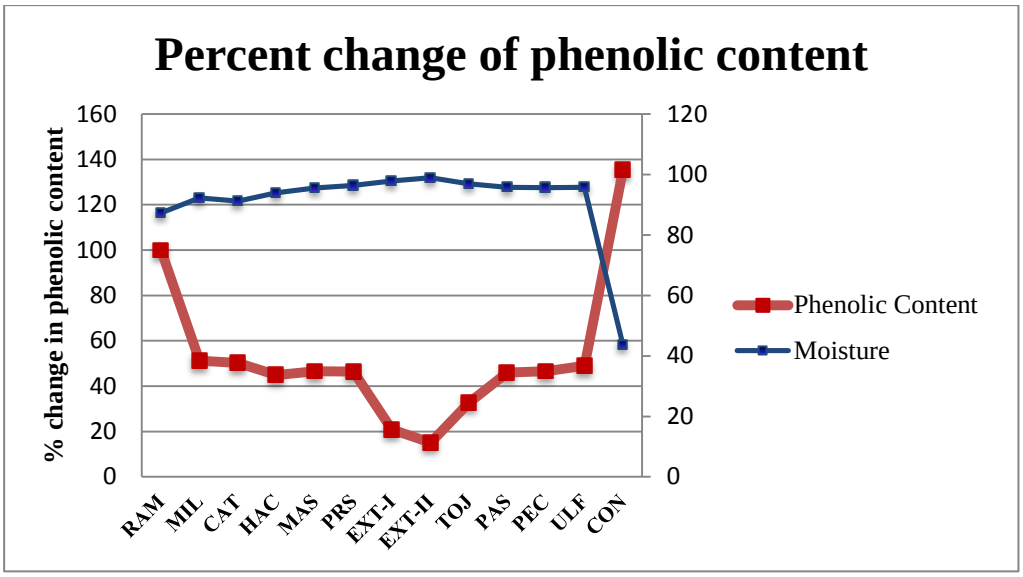

Figure 4.1 : Percent change in phenolic contents of samples collected from each processing step with normalized values (The percent values were calculated by accepting the initial content of each component in the vegetable as 100 unit).

The effect of each processing step on total phenolics was significantly different from each other ($p \leq 0.05$) (Table 4.4). The first step where the black carrots were crushed in a mill (MIL) led to a strong decline of total phenolics to 49% in comparison to raw material (Figure 4.1). There are contradictory findings in the literature depending on the source of fruit or vegetable. For instance, Reyes *et al.* (2003) proposed that wounding of purple potatoes resulted in an increase of total phenolics by 60% due to abiotic stress. In addition, it has been proposed that the milling step may facilitate the release of phenolic compounds associated with cell wall breakage (Renard *et al.*, 2011). On the other hand, our findings were in align with the results of Fang *et al.* (2006) who reported high losses in phenolic amount of billberries after crushing step during processing at industrial scale. Pronounced losses of polyphenolics during milling is believed to be related to native polyphenol oxidase enzyme in fruits and vegetables (Renard *et al.*, 2011). Polyphenol oxidases contributing to the oxidation and degradation reactions of polyphenols play a major role in polyphenol degradation. Therefore, initial blanching treatment applied during processing is important to inactivate those enzymes (Skrede *et al.*, 2000). Based on several studies compared the effect of steam blanching of fruits on polyphenol contents revealed that blanching treatment improves the retention of polyphenols in juices both industrial and laboratory scale (Fang *et al.*, 2006; White *et al.*, 2011). Other potential mechanism responsible for the observed decreases in phenolic amounts could be

associated to the possible polymerization reactions of anthocyanins with other phenolic compounds, such as flavan-3-ols or polyflavan-3-ols (Reed *et al*., 2005). The increase in polymeric color % values when raw black carrots were milled also supported a possible polymerization reaction occurring during processing (Section 4.1.4).

In the third step, where black carrots exposed to 50% citric acid solution treatment (CAT), total phenolic contents of samples were found to be 160.9±30.3 mg GAE/100 gr fresh weight. In the following steps of heating up to 90ºC then cooling down to 50-60ºC in heat-exchanger (HAT) resulted in about 55% reduction in total phenolics amount compared to the raw material (Figure 4.1).

After mashing step (MAS), total phenolic content was 148.9±26.0 mg GAE/ 100 gr fresh weight (Table 4.4). Mashing led to 53.5% decrease compare to raw material. Previous studies have shown that enzymatic mash treatment seems to be favorable for the release of phenolics as it leads to greater recovery of compounds. Hartman *et al*. (2008) reported through enzymatic treatment of the strawberry mash, a yield of about 72% of the possible phenolic recovery was reached, while the yield without enzymatic treatment was only about 55%. On the other hand, it was reported that the largest loss of phenolics was caused by mashing of strawberries at laboratory scale (Klopotek *et al*., 2005). The maceration reaction parameters are critical due to recovery of phenolics. The optimal maceration was determined at laboratory scale by Lando *et al*. (2004), using an enzyme dosage of 0.18% by wet weight fruits and vegetables with a reaction at 60 ºC for 30 min on finely crushed mash. Furthermore, warming up the mash also added to the higher yield of total polyphenols through enhancement of the solubility of the poor water soluble polyphenols (Hartman *et al*., 2008). Moreover, it has been shown that increased enzyme dosage and maceration time together with increased maceration temperature, in general, increased the amount of total phenols (Landbo *et al*., 2004), whereas higher doses of enzyme pectinase used may result in lower yield of important phytochemicals such as anthocyanins and flavonoids. The negative influence of increased enzyme doses may be due to enzyme catalyzed degradation of the phenolics caused by either polyphenol oxidase activity or glucosidase side activities in the enzyme (Khandere *et al*., 2010). Another critical point is enzyme mixtures due to release of polyphenols correlated to struction of cell wall and the degree of plant cell wall breakdown (Landbo *et al*.,

2004). Landbo *et al*. (2004) and Bucher *et al*. (2005) determined Pectinex® BE, which a mixture of pectin esterase, pectin lyase and polygalacturonase that is a cloned *Aspergillus niger* preparation, consistently tended to be slightly better than the others in giving high anthoycanins yields, high total phenols yields for black currant and billberry, respectively, at laboratory scale. It was interesting to note that the enzyme-assisted processing of black carrot juice was found to be more efficient in increasing the total anthocyanin content (99%) in comparison to total phenolics (27%) (Khandere *et al*., 2010).This suggests that anthocyanin glucosides are more readily released from the cell wall matrix as compared to phenolics. This might be due to the differences in the precise location of phenols and anthocyanin and their type of bonding, possible physical entrapment in the lignin and plant cell wall network which are largely unknown at present (Khandere *et al*., 2010). Enzymatic preparations for specific to black carrots cell wall structure composed of branched pectin, cellulose, and lignin (Padayachee *et al*., 2012) have to be investigated in further studies.

Following first press step (PRS), total phenolic content was found 148.5±13.4 mg GAE/ 100 gr fresh weight as shown in Table 4.4. The steps with the highest influence on the total phenolic content were juice extraction-I (EXT-I) and extraction-II (EXT-II) steps. As shown in Figure 4.1, juice extractions in these two steps showed the largest losses (79%-85%). These results are in align with those of Gil-Izquierdo *et al*. (2002), Lee *et al*. (2004), Hartmann *et al*. (2008), Holtung *et al*. (2011), Renard *et al*. (2011), White *et al*. (2011), and Rajasekar *et al*., (2012), who reported significant losses in polyphenolics due to removal of polyphenol rich seeds and skins at both laboratory and industrial scale. In a study it was reported that, the press-residue consisted of peels, seeds, branches, and some remaining flesh of the berries had a higher concentration of dry matter and the amount of total phenolics in press-residue was approximately 3 fold higher than in the berries, and approximately 4 fold higher than in the raw juice (Holtung *et al*., 2011). Retention of polyphenols played a major role in pressing step depending on heating and pressing conditions (press type, waiting conditions, exposure to air), the use of pressing aids such as hulls, and skin thickness, and presence of seeds which contribute to the phenolic content (Gil-Izquierdo *et al*.,2002; Lee *et al*., 2004, Hartmann *et al*., 2008, Holtung *et al*., 2011, Renard *et al*., 2011, White *et al*., 2011). Renard *et al*. (2011) reported an

increase in polyphenol concentration with pressing temperature and a decrease with mash oxidation during pressing step in juice processing (Renard *et al*., 2011). According to Holtung *et al*. (2011) the total phenolics concentrations increased with longer extraction time. Although increased extraction time was the only factor which significantly increased the levels of total phenolics, an interaction between temperature and extraction time had a significant effect on the level of total phenolics. The highest concentrations of total phenolics was obtained with 90ºC extraction temperature and 15 min extraction time (Holtung *et al*., 2011).

The high amount of recovery of phenolics occurred as expected in juice collecting step (TOJ), total phenolic content was reached to 104.6±24.3 mg GAE / 100 gr fresh weight as shown in Table 4.4.

Pasteurization of total juice (PAS) at 95ºC for 90 seconds led total phenolic content to reach 147.1±23.3 mg GAE/ 100 gr fresh weight (Table 4.4.). There are contradictory findings in the literature depending on the source of fruit or vegetable. For instance, Fang *et al*. (2006) proposed that the contents of hydroxybenzoic acids in pasteurization were 50% higher than the non-pasteurized bilberry juices. On the other Klopotek *et al*. (2005), Brownmiller *et al*. (2008) and Hartman *et al*. (2008) reported losses between 10%-27% in phenolic contents of strawberry and blueberry products after pasteurization step during processing at laboratory scale.

In depectinization (PEC) and ultrafiltration (ULF) steps total phenolic contents were found 148.8±17.5 and 156.7±17.6 mg GAE/ 100 gr fresh weight, respectively. Depectinization step was applied prior to clarification due to an increase in juice clarity yield. In previous studies clarification resulted in phenolic losses between 15-42% at laboratory scale (Klopotek et al., 2005; Pinelo et al.,2006).

The final stage of processing, concentrating black carrot juice to 66 ºBrix (CON) led to increase of total phenolic content from 319.5 ± 58.1 mg GAE/100 g fresh weight to 433.3±29.8 mg GAE/ 100 gr fresh weight (Tablo 4.4.). Concentrating led to 35% increase in fresh weight basis however; if results were calculated on dry weight basis 69% decrease was obtained in black carrot concentrate. According to Gil-Izquierdo *et al*. (2002) concentration processes did not affect the initial hydroxycinnamic content of orange juice at industrial scale. It is clear that phenolic losses during black carrot concentrate processing are mainly due to milling, physical removal of the

peels in the presscake and concentrating into black carrot juice (dry weight basis), whereas citric acid treatment, heat treatment, juice depectinization, pasteurization, clarification steps resulted in minor changes.

4.1.4 Total flavonoids, total monomeric anthocyanins and polymeric color

Changes in total flavonoid content, total monomeric anthocyanin content and polymeric color were evaluated during different steps in black carrot concentrate processing. Total flavonoid content of the fruits was found to be 1048.4 ± 226.0 mg quercetin/100 g fresh weight (Table 4.5). There is only one study reporting the total flavonoid content of black carrot juice by Khandere *et al.* (2010) as 118±12 mg catechin /100 ml. The flavonoid contents of samples collected from each step were found to be statistically different ($p \leq 0.05$) ranging between 93.7±8.9 and 1268.0±136.5 mg quercetin/ 100 gr fresh weight (Table 4.5).

Table 4.5 : The contents of total flavonoids, total monomeric anthocyanin content and percent polymeric color of black carrot processing samples[1].

Processing Steps	Moisture content, %	Total flavonoids[2]	Total monomeric anthocyanin[3]	Polymeric color, %
RAW	87.3 ± 0.5	1048.4±226.0 b	53.7±15.2 b	6.2±4.0 cd
MIL	92.3 ± 0.2	364.0±102.6 c	18.2±6.4 cd	15.3±10.2 ab
CAT	91.2 ± 0.8	347.0±113.0 c	21.5±2.3 cd	16.2±3.7a
HAC	94.0 ± 1.0	298.4±53.4 c	27.3±6.1 c	7.8±4.3 cd
MAS	95.5 ± 0.2	292.7±24.9 c	28.3±10.8 c	9.9±2.8 bcd
PRS	96.4 ± 0.3	262.1±72.8 cd	26.4±3.6 c	4.4±2.4 d
EXT-I	97.9 ± 0.2	138.5±18.5 de	20.8±2.1cd	5.3±2.3 cd
EXT-II	98.9 ± 0.1	93.7±13.8 e	10.2±1.1 d	11.4±1.6 abc
TOJ	96.9 ± 0.2	315.7±97.7 c	26.6±3.9 c	4.2±2.1 d
PAS	95.8 ± 0.4	294.2±77.1 c	27.4±3.1 c	4.9±2.0 d
PEC	95.7 ± 0.2	298.7±63.9 c	28.2±3.1 c	5.8±2.0 cd
UFL	95.8 ± 0.6	304.0±52.5 c	33.8±7.4 c	3.6±2.1 d
CON	43.7 ± 0.9	1268.0±136.5 a	151.8±27.6 a	4.2±1.0 d

[1]Data represent mean values and ± standard deviation of 4 independent processing events. All contents are expressed per 100 g fresh weight. Different letters in the columns represent statistically significant differences ($p \leq 0.05$)
[2]Total flavonoid content is expressed as mg quercetin / 100 gr fresh weight.
[3]Total anthocyanin content is expressed as mg cyanidin-3-glucoside / 100 gr fresh weight.

Total flavonoid content showed similar pattern at each step observed in total phenolic contents, although percent changes were different for those compounds during processing. The total flavonoid content increased from 100% in black carrots to 117% in its concentrate as shown in Figure 4.2. However, if the results were

calculated on a dry weight basis, the concentration of flavonoids in concentrate was 73% lower than in the black carrots.

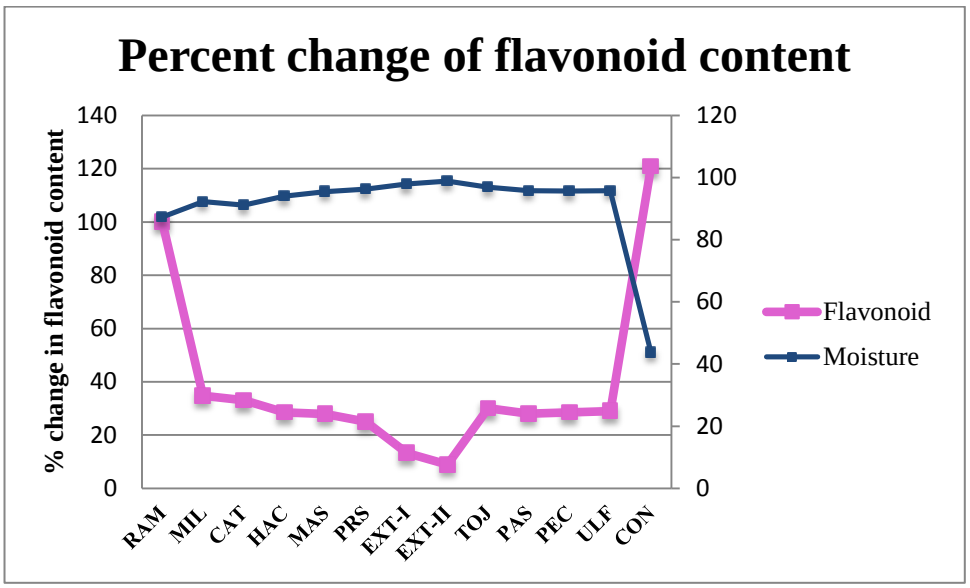

Figure 4.2 : Percent change in flavonoid contents of samples collected from each processing step with normalized values (The percent values were calculated by accepting the initial content of each component in the vegetable as 100 unit).

In second step, milling (MIL) led to a strong decrease of total flavonoids (by 65%) compare to raw material as observed similarly for total phenolic content (Section 4.1.3). During citric acid treatment (CAT), heating and cooling (HAT), mashing (MAS) and first pressing (PRS), flavonoid content changed between 262.1±72.8-347.0±113.0 mg quercetin/ 100 gr fresh weight (Table 4.5). Following first press, two extraction steps (EXT-I and EXT-II) resulted in extensive losses of flavonoids. Juice extraction carried out in two steps was the processing step showing the highest loss in flavonoids by 87% - 91% (Figure 4.2). Such high decreases indicate that some part of the flavonoids is lost as the pomace is removed in those steps. Similar results were reported by White *et al*. (2011) as additional losses could be attributed to the binding of the flavonols to cell wall material, which may have caused them to be unextractable.

Following the extraction steps, the observed flavonoid recovery in juice collecting step (TOJ) was obtained as the total flavonoid content reached 315.7±97.7 mg quercetin /100 gr fresh weight in total juice (TOJ) shown in Table 4.5. In further steps; pasteurization (PAS), depectinization (PEC) and ultrafiltration (ULF) resulted in flavonoid losses of about 72% compared to raw material. In last step, flavonoid

content of black carrot concentrate (CON) was determined to be 1268.0±136.5 mg quercetin /100 gr fresh weight as presented Table 4.5.

It was important to note that changes founded in total flavonoid contents were higher than total phenolic content. The differences in changes may be attributed to difference of localization, stability conditions and processing effects on flavonoids (Khandere *et al*., 2010).

Total monomeric anthocyanin content of black carrots was found 53.7 ± 15.2 mg cyanidin-3-glucoside / 100 g fresh weight as represented in Table 4.5. Previous studies reported 17.9 ± 8.6 μmol/g dry weight (Sun *et al*., 2009), 17.7±1.9 mg / 100 g fresh weight (Montilla *et al*., 2011), and 125.2 ± 17.2 mg /100 g fresh weight (Ersus *et al*., 2007) of total monomeric anthocyanin contents of black carrot. Based upon results of different studies, it appears that total monomeric anthocyanin contents of black carrots show differences due to degree of root coloring, cultivar and growing conditions (Kammerer *et al*., 2004b; Montilla *et al*., 2011).

The monomeric anthocyanin contents of samples collected from each step were found to be statistically different ($p \leq 0.05$) ranging between 10.2±1.1 and 151.8±27.6 mg cyanide-3-glucoside/ 100 gr fresh weight (Table 4.5).

The concentration of monomeric anthocyanins in concentrate was 28% higher than in the black carrots as fresh weight basis as presented in Figure 4.3. If the results were calculated on a dry weight basis, processing of black carrots resulted in total monomeric anthocyanin losses of 36% compared to levels found in raw material. Consistent with these results, Klopotek *et al*. (2005) and Hager *et al*. (2008) reported 33% and 65% overall losses of anthocyanins processed in juice at laboratory scale. In the second step, milling of black carrots (MIL) led to loss 66% of monomeric anthocyanins due to cell wall or membrane distruption. This distruption may release both peroxidase (POD) and polyphenoloxidase from cells (Ranst *et al*., 2009). These enzymes have been shown to cause anthocyanin degradation in the presence of cofactors such as chlorogenic acid (Hager *et al*., 2008). Other reason for observed decreases could be associated to the polymerization of anthocyanins with phenolic acids as mentioned in Section 4.1.3. Moreover, the reduction in concentration of anthocyanins might be associated to the particle size of the crushed vegetable as the

finest crushed mash was reported to have the highest amounts of anthocyanins (Landbo *et al*., 2004).

Monomeric anthocyanin content of samples, following citric acid treatment (CAT) and heating in exchanger (HAT) were found 21.5±2.3 and 27.3±6.1 mg cyanidin-3-glucoside as shown in Table 4.5. In further step, mashing of black carrots (MAS) resulted in 47% reduction of total monomeric anthocyanins mostly due to the reasons that were stated in Section 4.1.3 (Figure 4.2).

Monomeric anthocyanin content was found 26.4±3.6 and 20.8±2.1 mg cyanidin-3-glucoside after initial pressing of black carrot mash (PRS) and extraction-I (EXT-I) step, respectively. Total monomeric anthocyanin content showed a different pattern than phenolics and flavonoid contents during extraction. However, only extraction-II step (EXT-II) showed extensive decreases by 80% of total monomeric anthocyanins. Consistent with these results, several studies reported 18% (Skrede *et al*., 2000), 42% (blanched berries), 55% (nonblanched berries) (Lee *et al*., 2002), 15% (Brownmiller *et al*., 2008), and 13% (Hartmann *et al*., 2008) losses of anthocyanin content as a result of pressing step. Therefore, it appears that blanching may also facilitate softening of the skin, allowing for a greater extraction of anthocyanins (Howard *et al*., 2012). For instance, White *et al*. (2011) revealed that blanching resulted in 24% loss of total anthocyanins. Those differences reported in various studies may be attributed to the differences in anthocyanin structure of different fruits and vegetables.

The high amount of recovery of monomeric anthocyanins occurred as expected in juice collecting step (TOJ), total monomeric anthocyanin content was reached to 26.6±3 mg cyanidin-3-glucoside/ 100 gr fresh weight as shown in Table 4.5.

In pasteurization step (PAS), monomeric anthocyanin content was 27.4±3.1 mg cyanidin-3-glucoside/100 gr fresh weight as presented in Table 4.5. Pasteurization led to slight effect on black carrot anthocyanins due to high stability of acylated anthocyanin structure of black carrots at 90 ºC (Sadilova *et al*.,2006). Similar results were obtained by Türkyılmaz *et al*. (2012) pasteurization led to 3-16% reductions the anthocyanin contents of black carrot juices or did not affect the stability of black carrot anthocyanins (Türker *et al*., 2004). Furthermore, Brownmiller *et al*. (2008)

reported that the short pasteurization time used for blueberry juices, typically 60−90s at 90 °C, generally results in minor losses (<10%) of anthocyanins since short time and low temperature improves anthocyanin retention (Keshinski *et al*., 2010). On the other hand, Lee *et al*. (2002) reported a substantial loss of anthocyanins (76%) in pasteurized blueberry juices, and Klopotek *et al*. (2005) revealed that the pasteurization led to a decrease of 27% in juice and 39% in nectar. An explanation for the degradation of anthocyanins during the pasteurization of samples might be associated to the activation of enzymes which degrade anthocyanins, such as polyphenol oxidase (Turfan *et al*., 2011).

As presented in Figure 4.3, slight changes of total monomeric anthocyanins were observed after depectinization step (PEC) (28.2±3.1 mg cyaniding-3-glucoside/ 100 gr fresh weight) following pasteurization step (27.4±3.1 mg cyanidin-3-glucoside/100 gr fresh weight) similar to the results of White *et al*. (2011). Contrary to these findings, *Türkyılmaz et al*. (2012) reported a 7% increase in monomeric anthocyanin content of black carrot juice by depectinization treatment. Similarly, Buchert *et al*. (2005) compared the effects of different enzyme preparations on anthocyanin composition of bilberry juice and illustrated the importance of screening enzyme preparations used in juice processing for optimizing the dosage to minimize anthocyanin degradation.

Clarification (ULF) resulted in 37% losses of monomeric anthocyanins (Figure 4.3), whereas *Türkyılmaz et al*. (2012) who reported 10% reduction in black carrot anthocyanin contents. Previous studies showed anthocyanin losses of 8% (Lee *et al*., 2002) and 25% in blueberries (Brownmiller *et al*., 2008), and 1.3% in black raspberries (Hager *et al*., 2008). In another study with pomegranates, clarification caused 4% loss of anthocyanins in juice from sacs and 19% loss in juice from whole fruit (Turfan *et al*., 2011). A reason for these losses could be binding of anthocyanins to cell wall polysaccharides/proteins (Howard *et al*., 2012).

Total monomeric anthocyanin content in black carrot concentrate (CON) was 151.8±27.6 mg cyanidin-3-glucoside/100 gr fresh weight. Kırca *et al*. (2007) proposed degradation of black carrot monomeric anthocyanins increased with increasing solid content during heating. In consistent with Kırca *et al*. (2007), concentrating blueberry juice to 65−73.5 °Brix has been shown to have only a minor effect (<10% loss) on anthocyanins.

Polymeric color is a measure of the extent of anthocyanin polymerization and browning. Percent polymeric color is a ratio between polymerized color and color density is used to determine the percentage of the color that is contributed by polymerized anthocyanins. This assay is based on the principle that monomeric anthocyanins react with sodium bisulfate. Monomeric anthocyanins will combine with bisulfate at C-4 position. Polymeric anthocyanins will not undergo this reaction, as the position is not available, being covalently linked to another phenolic compound (Wrolstad *et al*., 2005; Howard *et al*. 2012).

The percent polymeric color values of black carrot was 6.2±4.0 as presented in Table 4.5. Significant ($p \leq 0.05$) changes in the percent polymeric color occurred in all processing samples. Percent polymeric color during processing changed between 3.6±2.1and 16.2±3.7 as presented in Table 4.5.

Polymeric color in milling step (MIL) was 2.5 fold higher than raw material. This may be due to the release of polyphenols from the cell by the action of polyphenol oxidase enzymes. Rapid oxidation of anthocyanins into brown pigment was reported in the presence of hydrogen peroxide and peroxidases (Fang *et al*., 2006; Hager *et al*., 2008). Another potential mechanism for the observed increase could be related to the polymerization and condensation reactions of anthocyanins with other phenolic compounds, including flavan-3- ols or polyflavan-3-ols (Reed *et al*., 2005). In a study, phenolic acids such as ferulic and syringic acids have also been shown to complex with anthocyanins in strawberry and raspberry juices (Rein *et al*., 2005). However, following the milling step, samples treated with citric acid (CAT) also had 16.2% polymeric value as shown in Table 4.5.

On the other hand, after heating and cooling step (HAT) 2.1 fold reduction occurred in polymeric color whereas total monomeric anthocyanin content showed an increase. In contrast, Turfan *et al*. (2011) revealed that heat applied during juice processing caused the degradation of monomeric anthocyanins, polymerization of anthocyanins, and the formation of brown color in fruit products. Due to high stability of acylated black carrot anthocyanins (Sadilova *et al*., 2006) heat treatment might not cause any degradation but may release monomeric anthocyanins from cell wall by breaking cell wall.

In further step, mash treatment (MAS) led to 27% increase in polymeric color (Figure 4.3). This finding was in align with Khandere *et al*. (2010) who studied the effect of pectinase enzyme on polymeric color of black carrot. On the other hand, first pressing step (PRS) caused a 55% reduction in polymeric color. Following first pressing step, percent polymeric color of extraction-I sample (EXT-I) was observed 5.3±2.3% whereas extraction- II step (EXT-II) resulted in 1.8 fold higher polymeric color value compared to raw material. Juice collecting step (TOJ) decreased polymeric color up to 4.2±2.1%. Pasteurization (PAS) and pectinization (PEC) steps showed slight effects on polymeric color, 4.9±2.0 and 5.8±2.0, respectively, as presented in Table 4.5 and Figure 4.3. Clarification (ULF) caused a 37% increase similar to the findings of Türkyılmaz *et al*. (2012). In final stage of process (CON), polymeric color value of black carrot concentrate was found 4.2±1.0% as shown in Table 4.5.

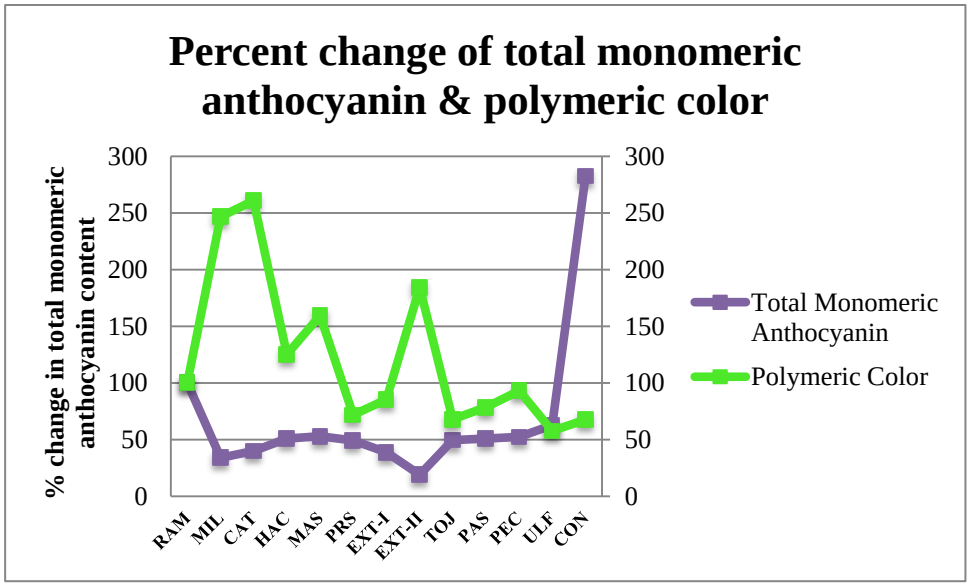

Figure 4.3 : Percent change of total monomeric anthocyanin content and percent polymeric color of samples collected from each processing step with normalized values (The percent values were calculated by accepting the initial content of each component in the vegetable as 100 unit).

Based upon the losses in total monomeric anthocyanin may be attributed high values of polymeric color in these steps as given in Figure 4.3. The decline in anthocyanin content in samples with increasing polymeric color (percent) was related to the polymerization reaction of anthocyanins. Increased polymerization is usually attributed to the condensation reactions that might lead to anthocyanin discoloration. Certain sugars, amino acids, polyphenols, or other nucleophiles, when condensed in

the presence of flavylium salts, yield colorless products, which are reactive and undergo further changes (Khandere *et al*., 2011).

4.1.5 Total antioxidant capacity

The antioxidant capacity of samples using 2 different methods including DPPH, and CUPRAC are represented in Table 4.6.

The mean values of total antioxidant capacity of black carrot samples analyzed by DPPH and CUPRAC methods were 492.6± 84.9 and 1568.7 ± 342.3 μmol TEAC/100 g fresh weight, respectively. A previous study using the ABTS and DPPH method reported antioxidant capacity of purple carrots 285.0±23.5 and 131.1±7.6 μmol TEAC/g dry weight, respectively (Sun *et al*., 2009). A reason of extensive higher values in this study could be associated to some factors such as cultivar, growing conditions, harvesting time of raw material. As presented in Table 4.6 the DPPH method gave about 69% lower total antioxidant capacity values compared to the CUPRAC method. Data from all two applied methods showed similar changes of the antioxidant capacity in terms of percent change during processing as shown in Figure 4.4.

The total antioxidant capacity of samples collected from each step were found to be statistically different ($p \leq 0.05$) (Table 4.6).

Processing black carrot in concentrate resulted in overall 30% (DPPH) and 22% (CUPRAC) increase in fresh weight basis whereas 67% (DPPH) and 71% (CUPRAC) decreases based on dry weight were observed as presented in Figure 4.4. Similar to our study result, Klopotek *et al*. (2005) reported that the antioxidant capacity of strawberry puree determined as a FRAP value decreased by 34% during the process. Moreover, Hager et al. (2008) revealed 55% losses in $ORAC_{FL}$ values in processing of blackberry juices in laboratory scale.

In the second step of process, milling led to 54% (DPPH) and 57% (CUPRAC) reduction in antioxidant capacity. Other steps resulted in antioxidant capacity losses from 54% to 66% and from 57% to 70% in DPPH and CUPRAC assays, respectively; except for the extraction steps as shown in Figure 4.4. The steps with the highest influence on the antioxidant capacity were extraction-I and II steps by about 82-91% reductions, which is similar to the findings of Klopotek *et al*. (2005).

Table 4.6 : Total antioxidant capacity of black carrot processing samples[1].

Processing Steps	Total Antioxidant Capacity	
	DPPH (µmol TEAC/100 g)	CUPRAC(µmol TEAC/100 g)
RAW	492.6 ± 84.9b	1568.7 ± 342.3b
MIL	225.1 ± 85.2c	650.0 ± 186.0c
CAT	196.3 ± 84.3c	625.8 ± 201.0c
HAC	166.3 ± 69.1cd	536.2 ± 87.6c
MAS	183.6 ± 47.9c	554.5 ± 68.3c
PRS	169.4 ± 17.3cd	522.6 ± 101.7c
EXT-I	91.1 ± 13.3de	261.5 ± 21.1d
EXT-II	53.3 ± 20.6e	137.5 ± 29.9d
TOJ	165.5 ± 38.3cd	469.9 ± 96.2c
PAS	174.0 ± 40.2cd	495.2 ± 63.3c
PEC	204.9 ± 42.8c	557.6 ± 67.5c
UFL	200.7 ± 32.2c	565.2 ± 107.9c
CON	713.0 ± 46.8a	2029.8 ± 90.7a

[1]Data represent mean values and ± standard deviation of 4 independent processing events. All contents are expressed per 100 g fresh weight. Different letters in the columns represent statistically significant differences ($p \leq 0.05$)

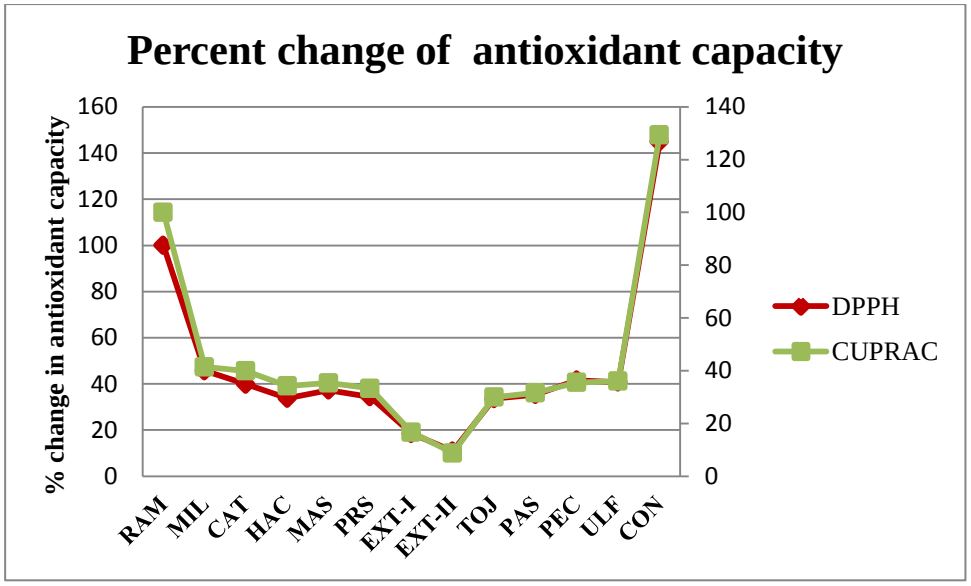

Figure 4.4 : Percent change of antioxidant capacity of samples collected from each processing step with normalized values (The percent values were calculated by accepting the initial content of each component in the fruit as 100 unit).

4.1.6 Phenolic acid profile

The phenolic acid contents of processing samples are shown in Table 4.8. The results clearly indicate that phenolic contents of samples collected from each step were found to be statistically different ($p \leq 0.05$) as presented in Table 4.7. Chlorogenic acid (5-O-caffeoylquinic acid), neocholorogenic acid (trans-5-O-caffeoylquinic acid)

and caffeic acid were detected in black carrot samples as shown in Figure 4.5. Chlorogenic acid which is a hydroxycinnamate, was most abundant phenolic acid with the amount of 266.8±52.6 mg/100 g fresh weight (Table 4.7). According to the studies carried out by Alasalvar *et al*. (2001), Kammerer *et al.* (2004b), and Sun *et al*. (2009) the amount of chlorogenic acid was reported to be much lower as 54±3 mg/100g fresh weight, 657 ± 2 mg/kg fresh weight and 18790±38 µg/g dry weight, respectively.

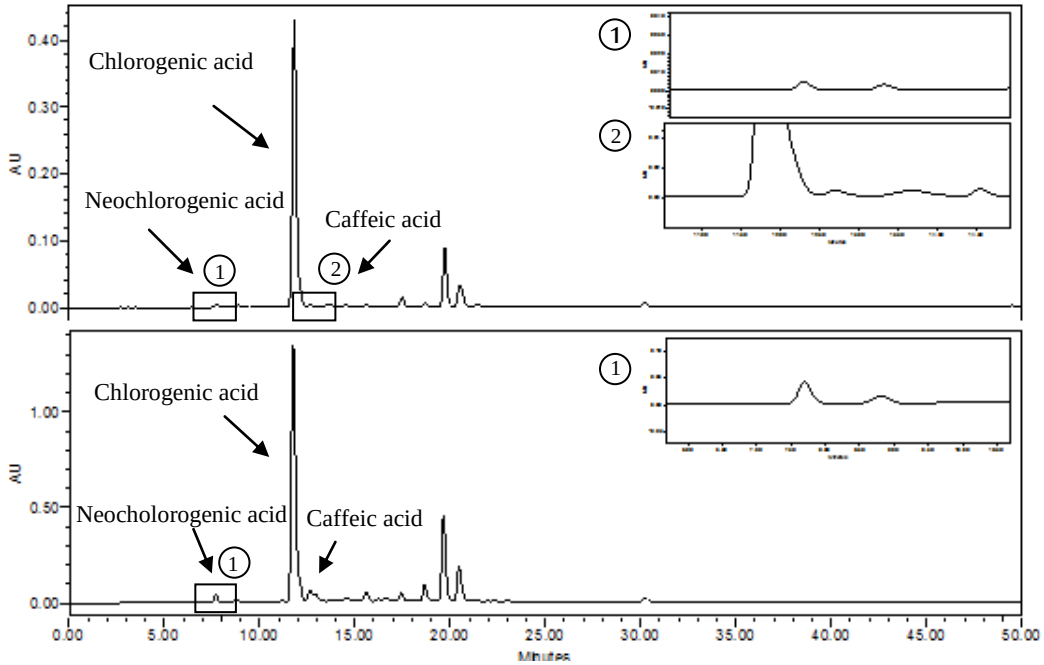

Figure 4.5 : HPLC chromatograms of raw material extract (upper panel) and concentrate (lower panel) - recorded at 312 nm.

The amount of other phenolic acids, caffeic acid and neochlorogenic acid were much lower than that of chlorogenic acid as 0.6±0.1 and 1.5±0.3 mg/100 g fresh weight, respectively (Table 4.7). In a study, caffeic acid content of black carrot was found 358.5±38 µg/g dry weight (Sun *et al*., 2009). However, no data is available on the amount of neochlorogenic acid in black carrots.

The results clearly indicate that phenolic contents of samples collected from each step were found to be statistically different ($p \leq 0.05$) as shown in Table 4.7.

Processing to concentrate led to chlorogenic acid, neochlorogenic acid and caffeic acid 4.2, 10.0 and 22.0 fold increase respectively, as compared to raw material in fresh weight basis. According to Kammerer *et al*. (2004b), amount of chlorogenic acid in concentrate was 8.9 fold higher than black carrots. If results were calculated

on dry weight basis, concentrate has 2.2 (neochlorogenc acid) and 4.9 (caffeic acid) fold higher phenolic acid amounts as compared to black carrot, whereas chlorogenic acid showed an 1.1 fold decrease presenting a different behaviour from other phenolic acids as a result of processing.

Table 4.7 : The contents of phenolic acids of black carrot processing samples[1].

Processing Steps	Chlorogenic acid	Neocholorogenic acid	Caffeic acid
RAW	266.8±52.6b	1.5±0.3b	0.6±0.1bcd
MIL	67.1±30.5cd	0.7±0.4bc	1.0±0.3b
CAT	51.3±3.0cde	0.5±0.4c	0.7±0.4bcd
HAC	63.8±18.8cd	0.5±0.4bc	0.8±0.5bc
MAS	63.0±4.7cd	0.7±0.4bc	0.8±0.2bc
PRS	64.1±9.0cd	0.7±0.1bc	0.7±0.1bc
EXT-I	34.9±9.0de	0.3±0.1c	0.3±0.1cd
EXT-II	15.7±2.9e	0.1±0.1c	0.2±0.0d
TOJ	53.1±7.2cde	0.6±0.2bc	0.6±0.1bcd
PAS	80.1±6.5c	0.8±0.4bc	0.8±0.3bc
PEC	76.0±3.4cd	0.8±0.3bc	0.9±0.3b
UFL	75.6±16.4cd	1.0±0.2bc	0.9±0.2b
CON	1110.3±64.5a	15.0±2.0a	13.4±1.0a

[1]Data represent mean values and ± standard deviation of 4 independent processing events. All contents are expressed per 100 g fresh weight. Different letters in the columns represent statistically significant differences ($p \leq 0.05$)

4.1.7 Anthocyanin profile

The anthocyanin contents of processing samples are shown in Table 4.8. Five anthocyanins were detected and identified by HPLC as cyanidin- 3-glucoside (kuromanin chloride), cyanidin derivative no. 1, cyanidin derivative no. 2, pelargonidin-3-glucoside, and pelargonidin-3, 5-diglucoside. The areas of the peaks for cyanidin derivatives were quantified based on cyanidin-3-glucoside which has a similar spectrum index. Quantifications of other anthocyanins were carried out using calibration curves of the each external reference standard. The cyanidin based acylated and nonacylated anthocyanins of black carrot have been reported by Kammerer *et al*. (2004), Sun *et al*. (2009), and Montilla *et al*. (2011). Cyanidin derivative no.1 and no.2 compounds (Figure 4.6) may be one of the unknown acylated cyanidin derivatives that have been determined by Kammerer *et al*. (2004), Sun *et al*. (2009), and Montilla *et al*. (2011). Moreover, peonidin, pelargonidin glycosides and cyanidin based pyranoanthocyanins were detected in other studies (Kammerer *et al*., 2003; Schwarz *et al*., 2004). Amongst the five anthocyanins

identified, cyanidin derivative no. 2 was the major anthocyanin in black carrots, with 76.0±1.5 mg/100 g fresh weight followed by cyanidin derivative no. 1 (65.4±12.8 mg/100 g fresh weight), pelargonidin-3,5-diglucoside (37.3±1.5 mg/100 g fresh weight), pelargonidin-3-glucoside (24.1±2.4 mg/100 g fresh weight), and cyanidin-3-glucoside (4.4±1.8 mg/100 g fresh weight) as shown in Table 4.8. According to previous studies, cyd-3-gal-xyl-glc-fer was found the major anthocyanin (Kammerer *et al*., 2004; Schwarz *et al*., 2004). Similarly, Sadilova *et al*. (2006) also reported cyd-3-gal-xyl-glc-fer as the major anthocyanin in black carrot concentrate. Cyanidin derivative no. 2 could be cyd-3-gal-xyl-glc-fer anthocyanin in this study. On the other hand, contrary to those findings, Sadilova *et al*. (2006) found that the second major anthocyanin was different from other studies. The available information shows that composition and proportion of anthocyanins may differ depending on the differences in cultivar and harvest time.

Since the acylated anthocyanins show greater stability during processing, anthocyanin contents of samples collected from each step were found to be statistically different ($p \leq 0.05$) as shown in Table 4.8.

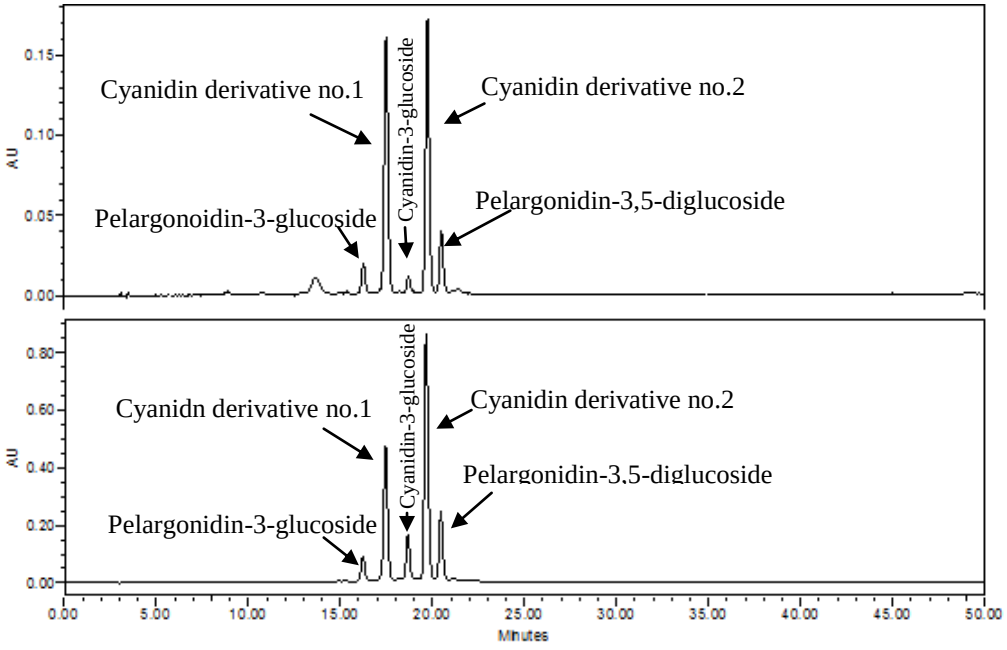

Figure 4.6 : HPLC chromatograms of raw material extract (upper panel) and concentrate (lower panel) - recorded at 520 nm.

Processing black carrot into concentrate led to the increases in all individual anthocyanins in fresh weight. It was found that black carrot concentrate had 10 (cyanidin-3-glucoside), 6.8 (pelargonidin-3-glucoside), 4.9 (pelargonidin,3-5-

diglucoside), 3.8 (cyanidin derivative no.1) and 5.3 (cyanidin derivative no.2) fold higher anthocyanin amounts as compared to that of black carrot. On the other hand, if the results were calculated based on dry weight, concentrate had 2.3 (cyanidin-3-glucoside), 1.5 (pelargonidin-3-glucoside), 1.1 (pelargonidin,3-5-diglucoside), and 1.2 (cyanidin derivative no. 2) fold higher anthocyanin amounts as compared to black carrot, whereas cyanidin derivative no. 1 showed an 1.2 fold decrease as a result of processing.

In the second step of process, milling resulted in reductions in anthocyanin content ranging between 33-74% due to cell wall or membrane distruption as pointed in section 4.1.3. In the further step where citric acid was added, a decrease of 63-69% in individual anthocyanins was observed, however it reached to 83% in cyanidin derivate no. 1.

Except for the pelargonidin-3,5-diglucoside, other anthocyanins showed an increase after heating and cooling step ranged between 29-45%, whereas the amount of pelargonidin-3,5-diglucoside declined 30% (compared to citric acid treatment (CAT) sample) most probably due to its unstability during heat treatment.

Furthermore, mash treatment led to 31.6% and 64.6% increases of cyanidin-3-glucoside and pelargonidin-3, 5-diglucoside compared to heating and cooling (HAT) sample, respectively. This may be as a result of the release of cyanidin-3-glucoside and pelargonidin-3,5-diglucoside from the cells after mashing (Turfan *et al*., 2011) where commercial pectinase enzyme preparation was also added.

Extraction steps were observed to induce biggest losses of anthocyanins by about 75-91%. After collecting pressed juice in tanks, 16-22% of the anthocyanins were recovered in total juice (TOJ) fraction following extraction step, however a higher recovery ratio about 37 % was observed for cyanidin-3-glucoside compound. Furthermore, pasteurization treatment increased individual anthocyanins in the range of 44-48% compared to the total juice (TOJ) sample. The possible explanation for this phenomenon would be the release of anthocyanins from the plant cell by the effect of the heat applied during pasteurization (Türkyılmaz *et al*., 2012). Following pasteurization, no change was observed in anthocyanin compounds at depectinization and clarification steps. On the other hand, at the final step of concentrating of

clarified juices increase between 12-15% (compared to ultrafiltration (ULF) sample) was obtained in individual anthocyanin components.

The results indicate that applying heat in heating and cooling (HAT), pasteurization (PAS) and concentrating (CON) steps showed apparent increases in anthocyanin contents of black carrot process samples. Those increases might be associated to the inactivation of endogenous oxidative enzymes which cause oxidative losses of these compounds in black carrots. In addition, heating application may lead to the ease of extraction by sample tissue breakdown, increased cell permeation and may provide thermally induced release of bound anthocyanin components (Skrede *et al*., 2000; Dewanto et al., 2002; Lee *et al*., 2002; and Fang *et al*., 2006).

4.1.8 Correlation between total phenolics, total flavonoids, total monomeric anthocyanins and total antioxidant capacities

The correlations between the total phenolic content, total flavonoid content, total monomeric anthocyanin content and total antioxidant capacities of black carrot processing samples were investigated. The Pearson's correlation coefficients are represented in Table 4.9.

The statistically significant correlations were found for all methods ($p \leq 0.05$). Highest correlation coefficient value was observed between two antioxidant methods, DPPH and CUPRAC, as presented in Table 4.9 ($r = 0.984$). These high level of correlation coeffients indicate that all antioxidant assay methods were well correlated to each other. Different phenolics contents might have different degrees of contributions for overall antioxidant activities of processing black carrot samples.

Furthermore, significant ($p \leq 0.05$) correlations existed between total phenolics and total antioxidant capacity methods, CUPRAC ($r = 0.981$) and DPPH ($r = 0.978$) similar to the high correlation flavonoid contents with those methods ($r = 0.968$ for DPPH method and $r = 0.979$ for CUPRAC method) (Table 4.9).

On the other hand, total monomeric anthocyanin values showed lowest correlations either with the amounts of total phenolics ($r = 0.827$) or total flavonoids ($r = 0.799$) (Table 4.9). It was interesting to observe lower correlation of monomeric anthocyanin values with flavonoid content, though anthocyanins were classified as flavonoids. In consistent with this data, the amount of flavonoids calculated by summing up the individual flavonoids as analysed by HPLC were extensively higher

Table 4.8 : The anthocyanin profile of black carrot processing samples[1].

Processing steps	Kuromanin chloride (cyanidin -3-glucoside)	Callistephin chloride (Pelargonidin-3-glucoside)	Pelargonin chloride	Cyanidin derivative 1	Cyanidin derivative 2
RAM	4.4±1.8b	24.1±2.4b	37.3±1.5b	65.4±12.8b	76.0±1.5b
MIL	2.9±1.5bc	8.6±4.2cd	18.3±10.5c	14.5±7.1cd	33.6±18.5c
CAT	1.6±0.9bc	7.9±3.7cd	11.5±5.7def	10.9±3.7cd	23.8±12.0cd
HAC	1.9±0.4bc	10.2±2.8cd	8.1±0.8ef	16.0±7.2cd	32.8±14.8cd
MAS	2.6±1.8bc	11.0±2.8c	13.3±4.0cd	18.5±3.1cd	32.2±13.5c
PRS	2.4±1.1bc	9.8±1.0c	13.3±1.4cd	18.3±3.6cd	26.2±3.6cd
EXT-I	1.1±0.4c	4.7±0.3de	6.1±0.8ef	11.1±4.4cd	13.2±2.1de
EXT-II	0.5±0.1c	2.7±0.4e	3.0±0.5f	5.4±1.7d	7.0±1.7e
TOJ	2.1±0.9bc	8.0±0.8cd	10.3±0.7def	16.2±3.4cd	21.2±4.1de
PAS	3.1±1.7bc	11.6±2.9c	15.2±4.2cd	23.4±1.6c	30.4±9.6c
PEC	2.9±1.3bc	11.4±1.7c	13.9±2.1cd	22.8±2.2c	29.6±6.7c
ULF	3.0±0.7bc	12.0±1.9c	14.6±2.1cd	21.7±4.9c	30.7±5.2c
CON	44.7±5.0a	165.1±5.3a	180.6±16.3a	248.0±23.1a	399.1±4.7a

[1]Data represent mean values and ± standard deviation of 4 independent processing events. All contents are expressed per 100 g fresh weight. Different letters in the columns represent statistically significant differences ($p \leq 0.05$)

Table 4.9 : Correlation coefficients (*r* values) between total phenolics, total flavonoids, totalmonomeric anthocyanins and total antioxidant capacities.

	Total Phenolics	**Total Flavonoids**	**Total Monomeric Anthocyanins**	**Antioxidant Capacity, DPPH**	**Antioxidant Capacity, CUPRAC**
Total Phenolics		0.967*	0.827*	0.978*	0.981*
Total Flavonoids	0.967*		0.799*	0.968*	0.979*
Total Monomeric Anthocyanins	0.827*	0.799*		0.839*	0.828*
Antioxidant Capacity, DPPH	0.978*	0.968*	0.839*		0.984*
Antioxidant Capacity, CUPRAC	0.981*	0.979*	0.828*	0.984*	

* Represent statistically significant correlation ($p \leq 0.05$).

than anthocyanin content obtained by the pH differential method (Section 4.1.7). A similar trend has also been reported by Lee and Finn (2007) who reported lower amounts of total anthocyanins obtained through the pH differential method than those by the HPLC quantification method in elderberries since elderberries have acylated anthocyanin structure. Results clearly demonstrated that the pH differential method might be incapable of determining total monomeric anthocyanin content due to the presence of acylated anthocyanins in black carrots (Montilla *et al*., 2011).

5. CONCLUSIONS AND RECOMMENDATIONS

Black carrot concentrate has gained increasing interest in recent years as a natural colorant in addition to expected health benefits due to its substantial content of bioactive compounds, especially anthocyanins. Since black carrots are mostly consumed as concentrate, it is desirable to investigate the fate of those components during processing treatments. In the available literature, although there are several reports focusing on the impact of processing on phytochemicals of various fruits and vegetables, there is still limited information about the changes of black carrot phenolics at industrial scale. Furthermore, there have been very few studies to evaluate the absorption of acylated anthocyanins *in vitro* and limited research have been performed revealing the absorption/metabolism of these components *in vitro*.

The objective of this study was to investigate the effects of processing of black carrot to its concentrate on contents of phenolics, flavonoids, and anthocyanins in samples collected from industrial-scale.

The results of the choice of extraction solvent and method indicated that an efficient solvent system needs to be investigated to extract phenolic compounds besides anthocyanins. Removal of lipophilic fraction before hydrophilic fraction causes extensive losses of hydrophilic antioxidant capacity. Therefore, lipophilic fraction of black carrots can be neglected due to its low antioxidant activity.

Comparisons of processing samples based on either wet-weight or dry-weight were confusing since addition of water in black carrot concentrate processing cause inconsistency in calculating exact values of phenolic compounds in samples. In some steps such as extraction steps, dry matter content decrases extensively to about 1%. Therefore, calculation of the amount of phenolic compounds based on dry matter content gives enourmously high amounts as it multiples the concentration by about 100 times and it deviates the real values extensively. Accordingly, the calculation of the phenolics only in raw material and concentrate samples, which both have higher dry matter contents based on dry weight basis gives more accurate results.

Processing of black carrots into its concentrate increases the amounts of total phenolics, total antioxidant capacity, total flavonoids and total monomeric anthocyanins on fresh weight basis. According to the HPLC results, each phenolic compound showed different behaviour. HPLC analysis is necessary to screen individual changes in compounds. In other processing steps besides concentrating, the steps with the highest influence on the phenolic compounds are milling of raw material and juice extraction steps in fresh weight basis when compared to raw material.

The results of correlation analysis clearly indicate that there is a strong correlation between the total phenolic content, total flavonoid content and antioxidant capacity values according to DPPH and CUPRAC methods. Polymeric color and total monomeric anthocyanin assays are sufficient to understand polymerization behavior of anthocyanins. Total monomeric anthocyanin method (pH differential) is insufficient to reflect the changes in monomeric anthocyanins during processing, and, therefore, it is not recommended for monitoring changes in monomeric anthocyanins during black carrot processing as a single method. Due to the lack of specificity of this method for anthocyanins, the presence of acylated anthocyanins in black carrots might reduce the accuracy of the assay. Furthermore, total phenolic and total monomeric anthocyanin assays yield less accurate results in accordance with individual analysis of phenolic acids and and monomeric anthocyanins as performed by HPLC. HPLC is sufficient to reflect the changes in phenolic acids and monomeric anthocyanins during processing. Since structure of black carrot phenolic compunds, especially acylated anthocyanins, are complex matrices, LC-MS analyses are necessary for a more detail understanding in changes of phenolic compounds.

Due to the lack of knowledge about total flavonoid content and total antioxidant capacity during black carrot concentrate production, this study provides new information on quantitative changes occur in flavonoid content and total antioxidant capacity at industrial scale. The results of this study explaining the qualitative and quantitative changes of antioxidant compounds may then be exploited to determine industrial strategies for avoiding undesirable modifications while maximizing the content of these desirable and health-promoting components.

In further studies, to prevent phenolic and anthocyanin losses, inserting an initial blanching operation prior to milling step and also using enzymes that are specific to

black carrot cell wall structure at mashing step can be investigated at industrial scale. Furthermore, LC-MS analyses can be performed to screen changes of phenolic compounds in detail. In addition *in vitro* studies with Caco-2 cells and future clinical studies investigating the bioavailability of phenolic compounds would provide valuable data for elucidating the effect of black carrot processing on human health.

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APPENDICES

APPENDIX A: Calibration Curves

APPENDIX B: HPLC Chromotograms

APPENDIX C: Statistical Tables

APPENDIX A

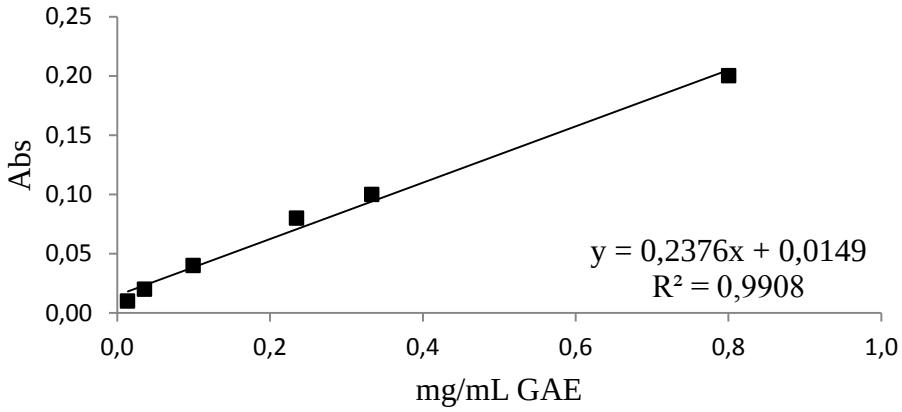

Figure A.1 : Calibration curve for total phenolics in acetone:water:acetic acid (70:29.5:0.5, v/v/v).

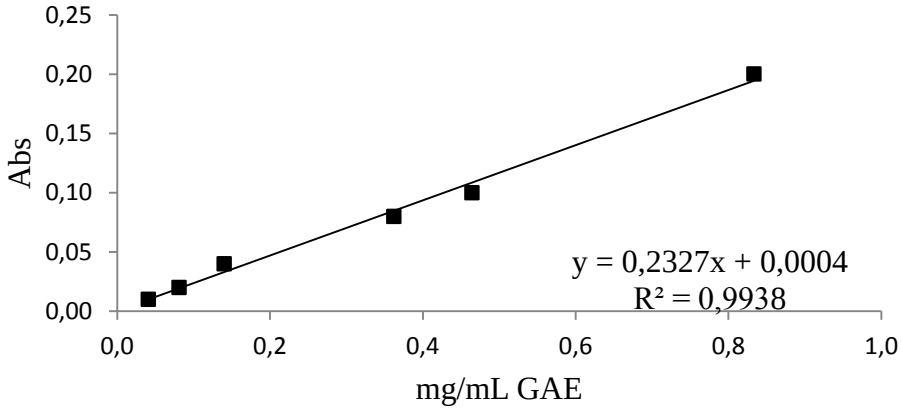

Figure A.2 : Calibration curve for total phenolics in acetone:water:acetic acid (70:28:2, v/v/v).

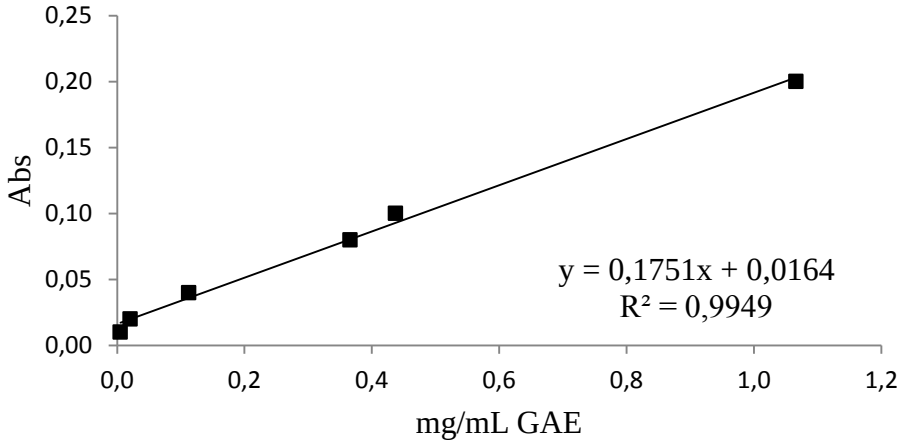

Figure A.3 : Calibration curve for total phenolics in 80% aqueous-methanol containing 7% (v/v) acetic acid.

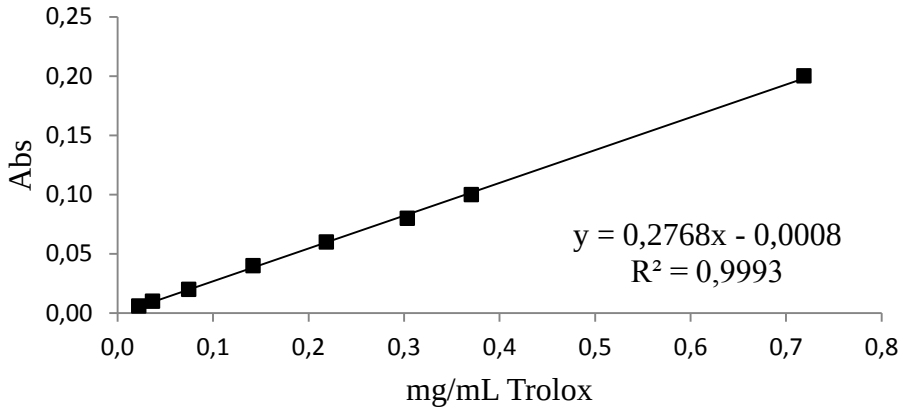

Figure A.4 : Calibration curve for DPPH assay in acetone:water:acetic acid (70:29.5:0.5, v/v/v).

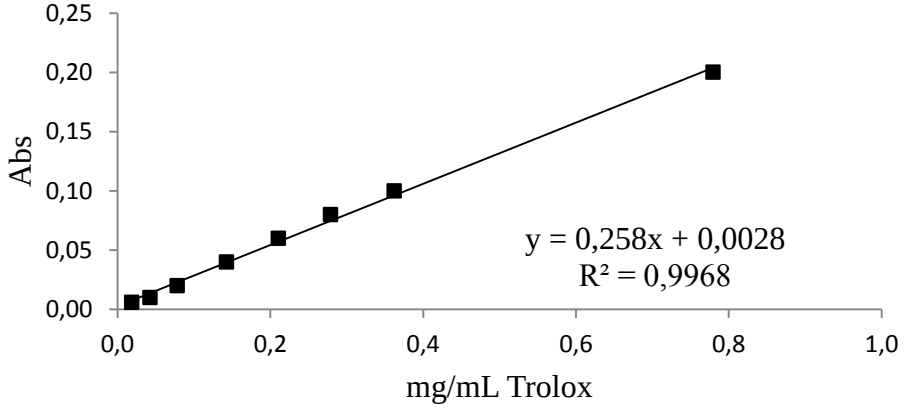

Figure A.5 : Calibration curve for DPPH assay in acetone:water:acetic acid (70:28:2, v/v/v).

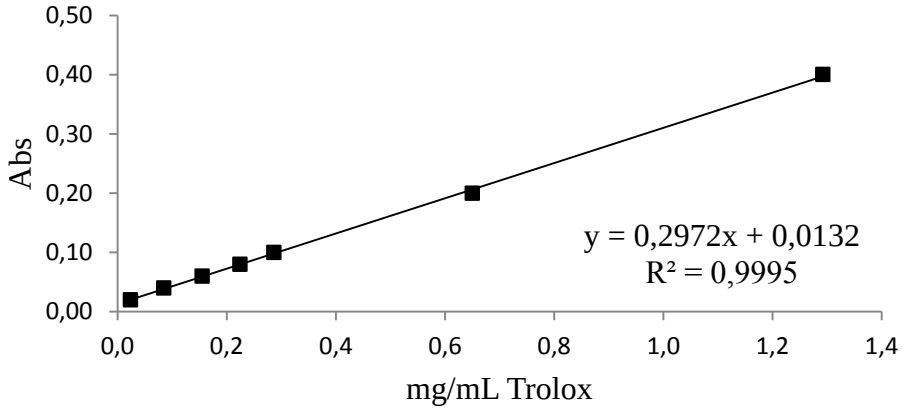

Figure A.6 : Calibration curve for DPPH assay in 80% aqueous-methanol containing 7% (v/v) acetic acid.

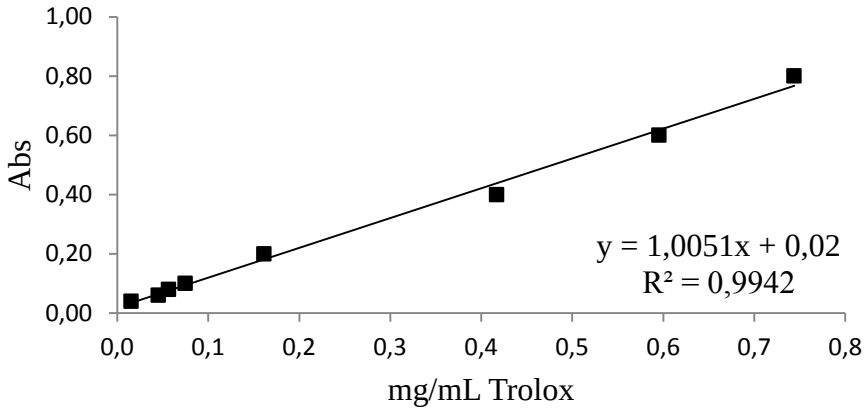

Figure A.7 : Calibration curve for CUPRAC assay in acetone:water:acetic acid (70:29.5:0.5, v/v/v).

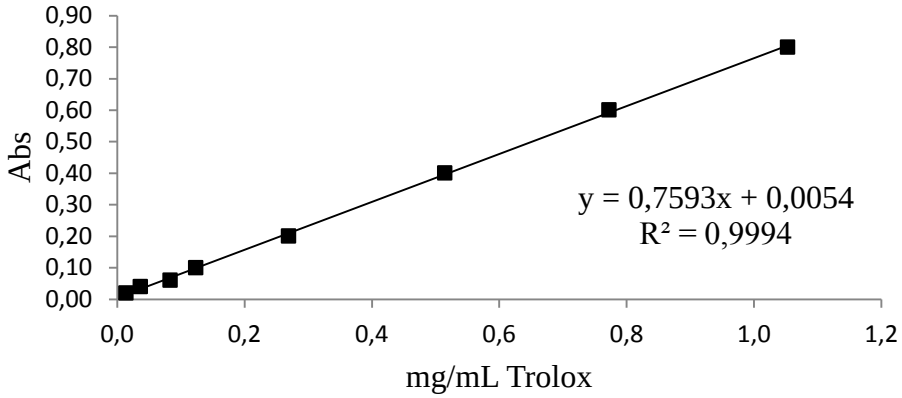

Figure A.8 : Calibration curve for CUPRAC assay in acetone:water:acetic acid (70:28:2, v/v/v).

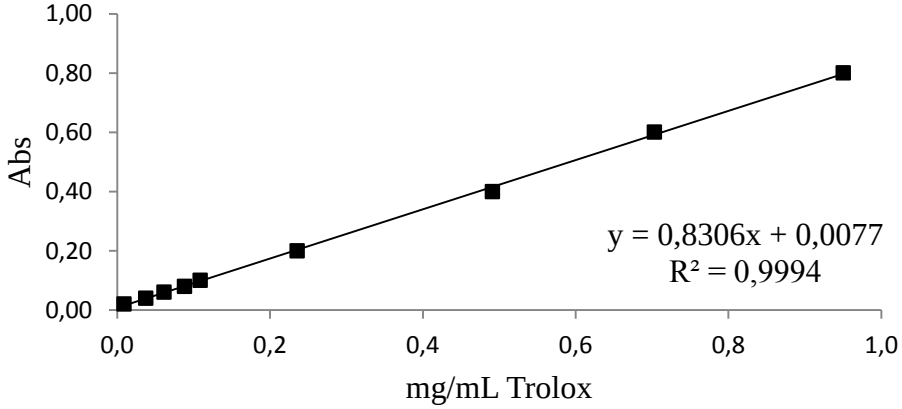

Figure A.9 : Calibration curve for CUPRAC assay in 80% aqueous-methanol containing 7% (v/v) acetic acid.

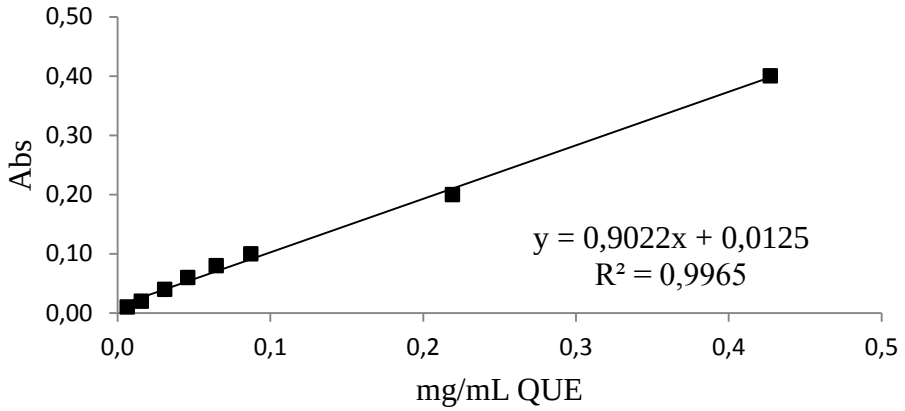

Figure A.10 : Calibration curve for total flavonoids in acetone:water:acetic acid (70:29.5:0.5, v/v/v).

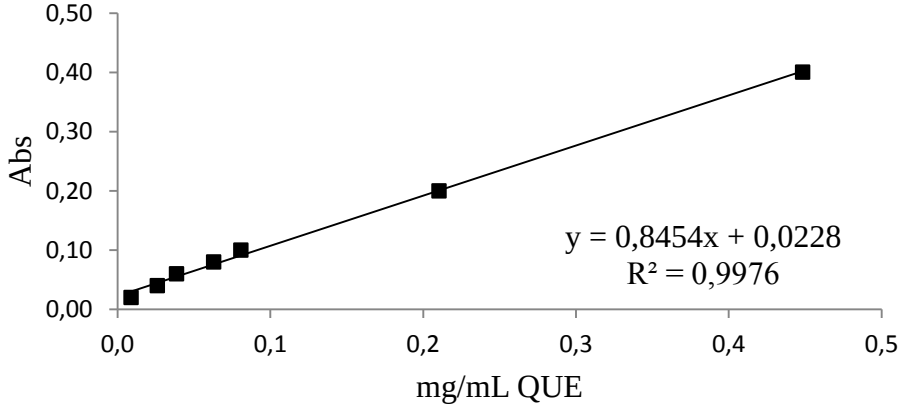

Figure A.11 : Calibration curve for total flavonoids in acetone:water:acetic acid (70:28:2, v/v/v).

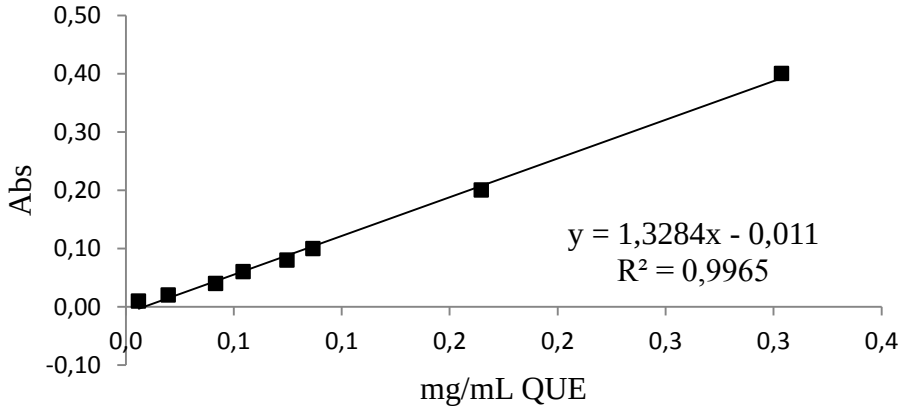

Figure A.12 : Calibration curve for total flavonoids in 80% aqueous-methanol containing 7% (v/v) acetic acid.

APPENDIX B

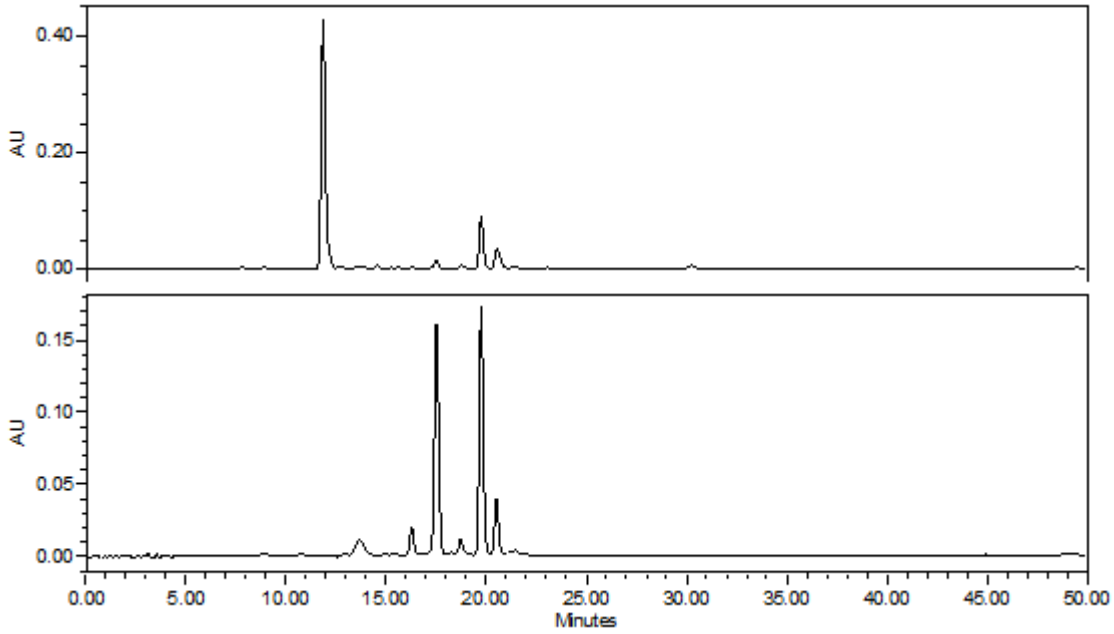

Figure B.1 : HPLC chromatograms of raw material extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

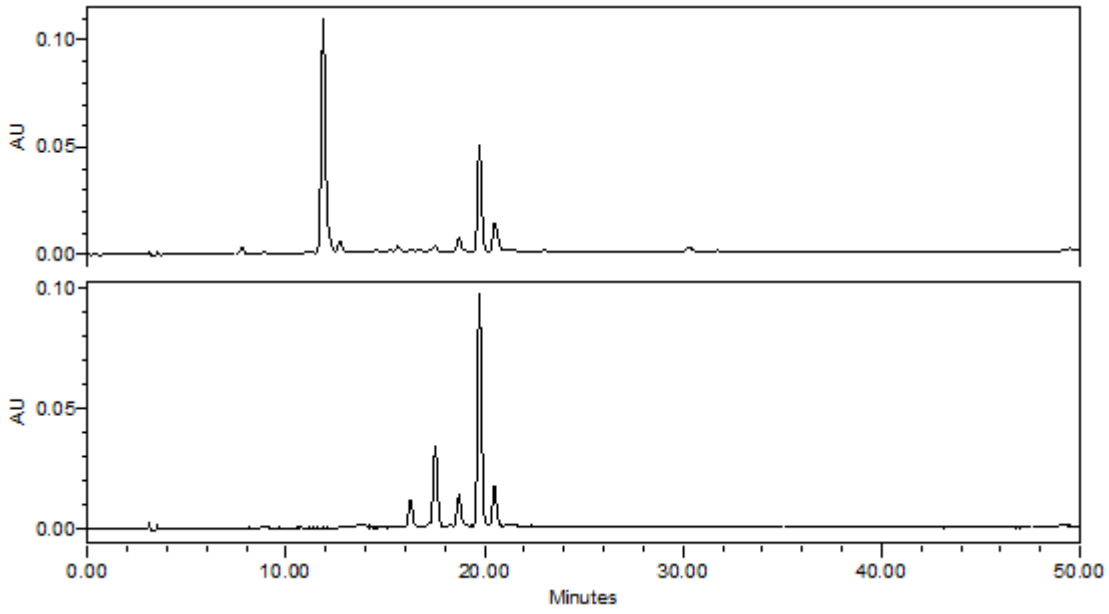

Figure B.2 : HPLC chromatograms of milling step (MIL) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

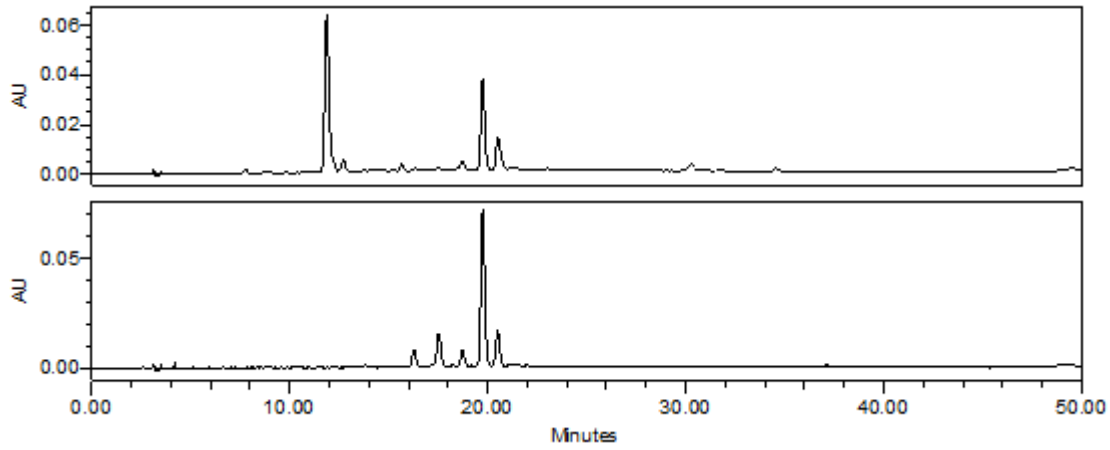

Figure B.3 : HPLC chromatograms of citric acid treatment step (CAT) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

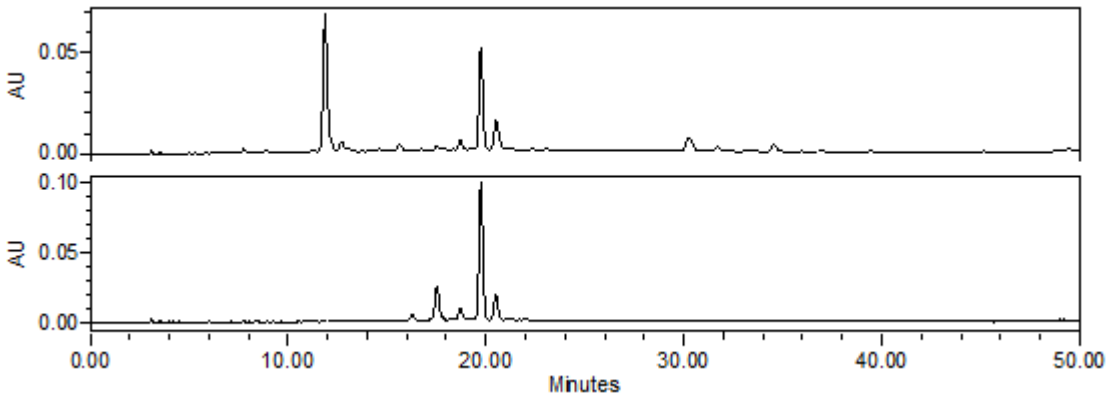

Figure B.4 : HPLC chromatograms of heating and cooling step (HAT) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

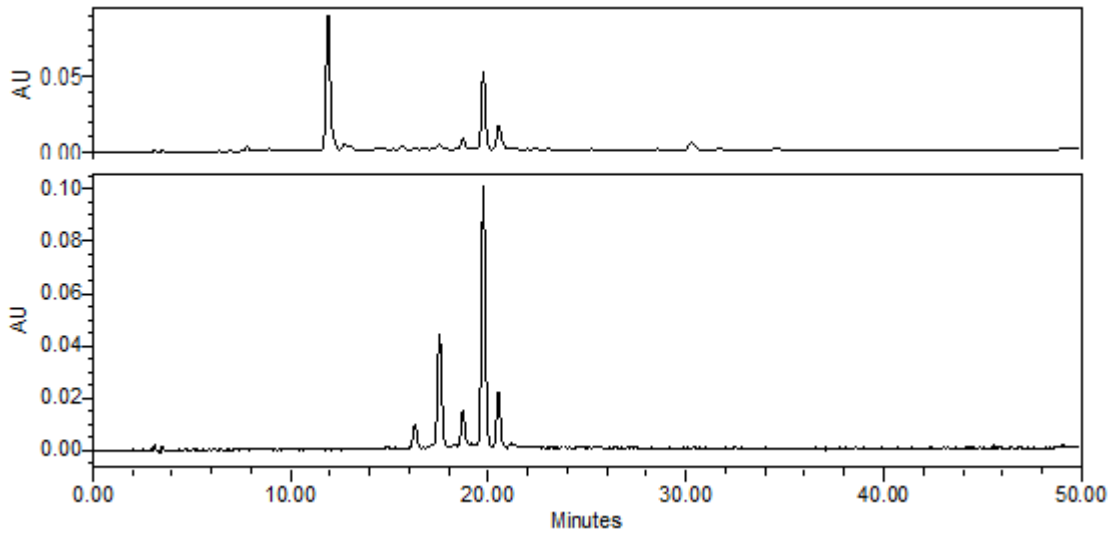

Figure B.5 : HPLC chromatograms of mashing step (MAS) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

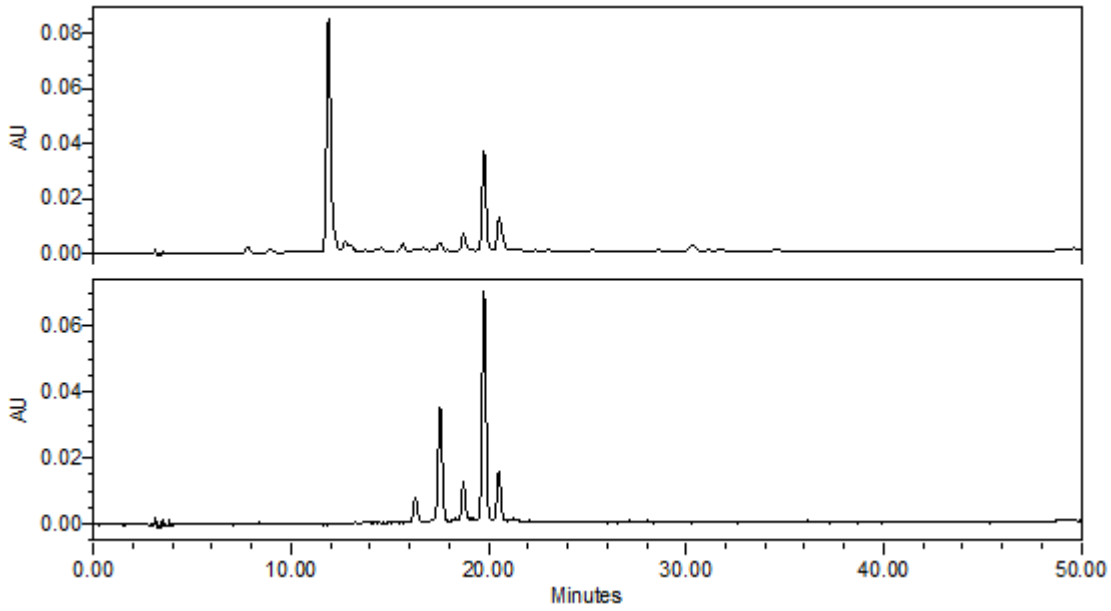

Figure B.6 : HPLC chromatograms of pressing step (PRS) extracts (upper panel- recorded at 312 nm and lower panel- recorded at 520 nm).

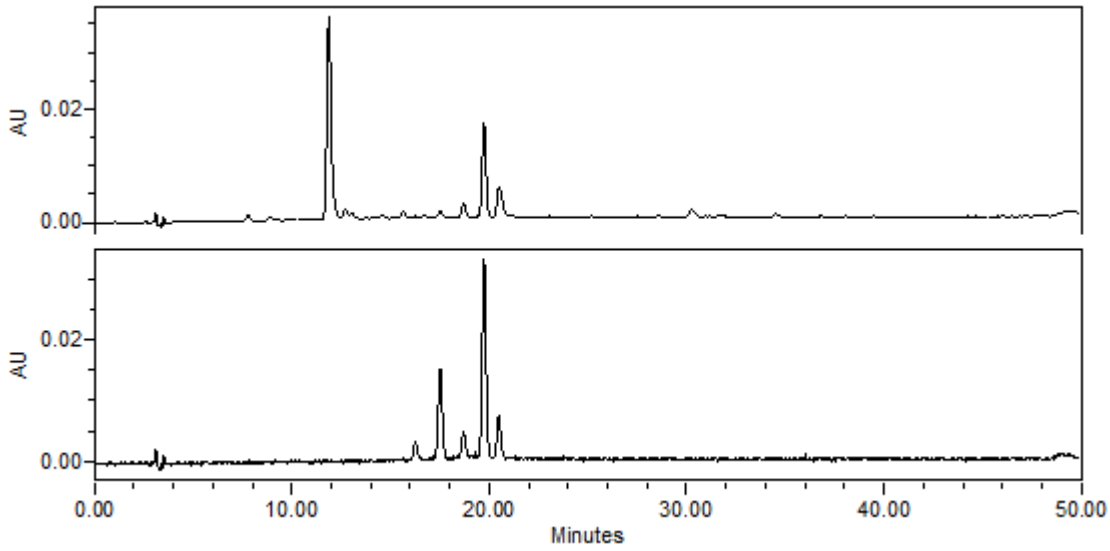

Figure B.7 : HPLC chromatograms of extraction-I (EXT-I) extracts (upper panel- recorded at 312 nm and lower panel- recorded at 520 nm).

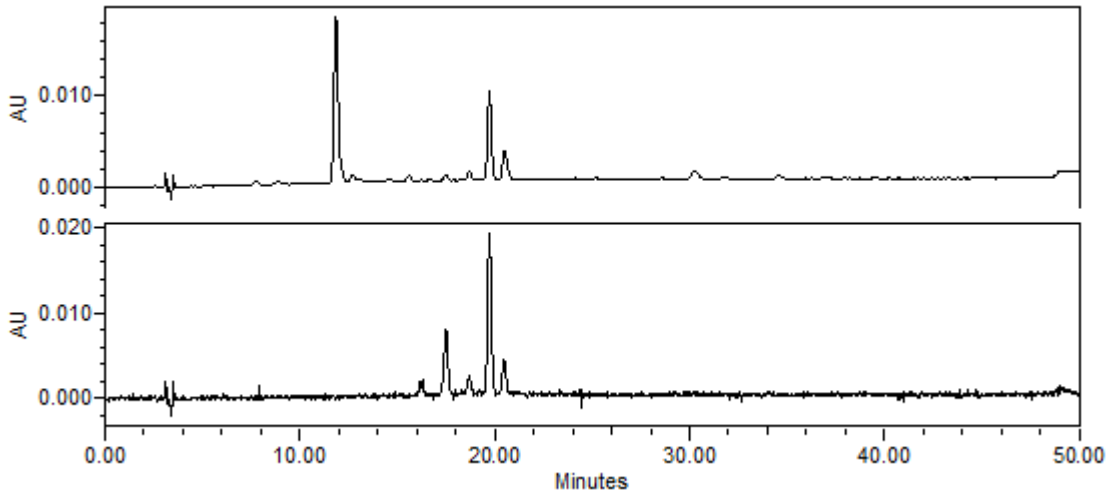

Figure B.8 : HPLC chromatograms of extraction-II (EXT-II) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

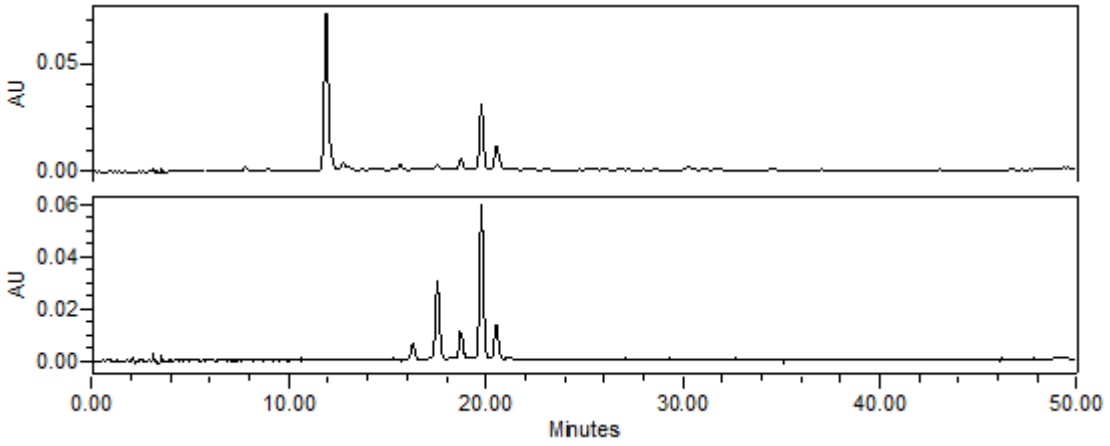

Figure B.9 : HPLC chromatograms of total juice (TOJ) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

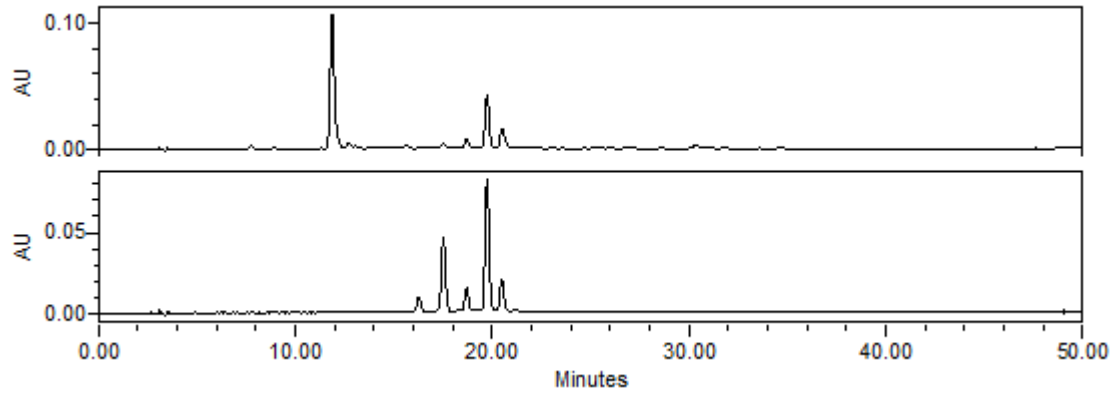

Figure B.10 : HPLC chromatograms of pasteurization step (PAS) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

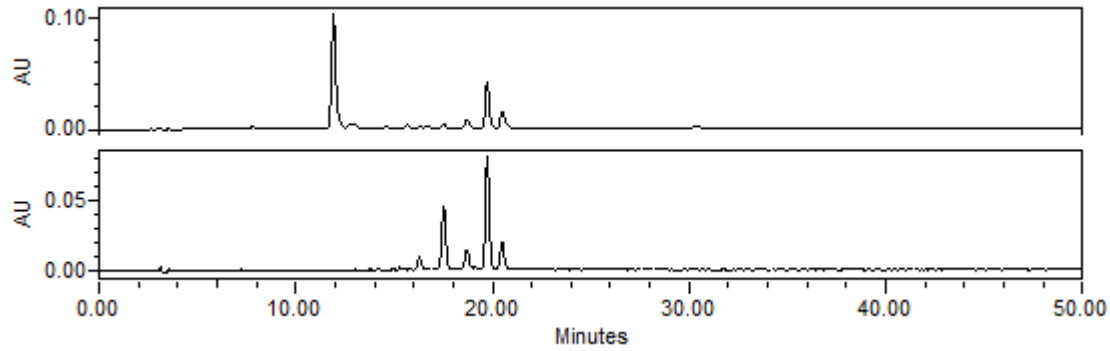

Figure B.11 : HPLC chromatograms of depectinization step (PEC) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

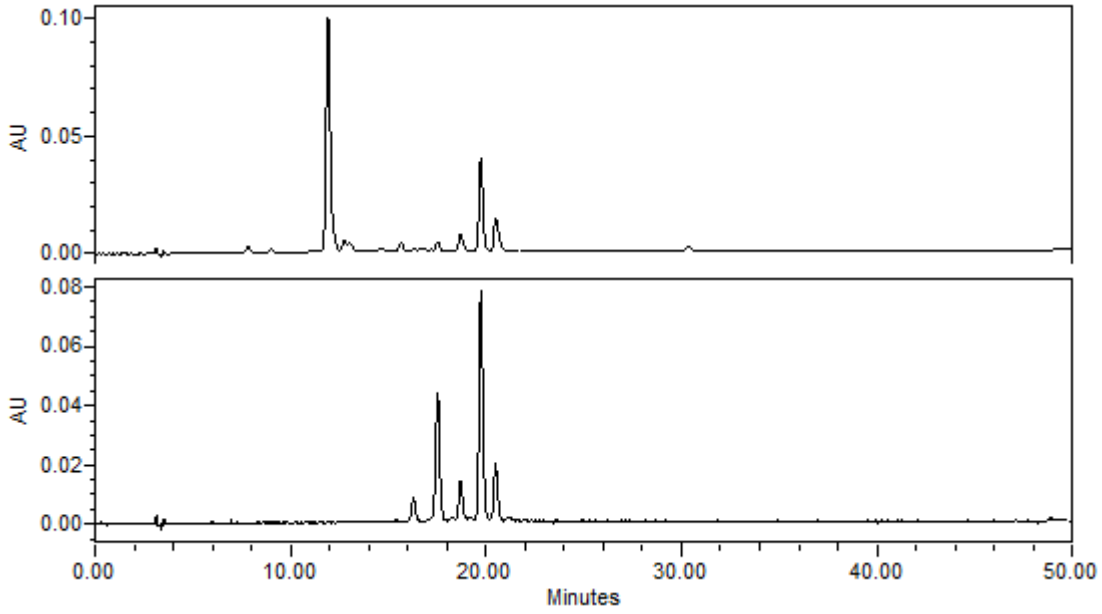

Figure B.12 : HPLC chromatograms of ultrafiltration step (ULF) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

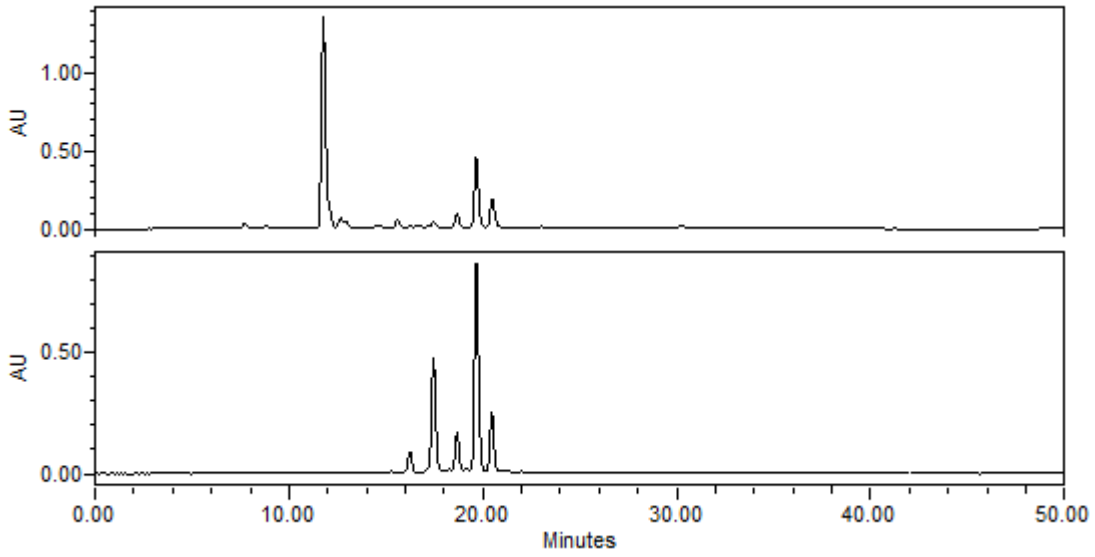

Figure B.13 : HPLC chromatograms of concentration step (CON) extracts (upper panel-recorded at 312 nm and lower panel- recorded at 520 nm).

APPENDIX C

Table C.1 : Statistical analysis results of black carrot processing samples.

		Sum of Squares	df	Mean Square	F	Sig.
Total Phenolics	Between Groups	497787.461	12	41482.288	54.546	0.000
	Within Groups	29659.570	39	760.502		
	Total	527447.031	51			
CUPRAC	Between Groups	1.301E7	12	1083751.235	55.482	0.000
	Within Groups	761807.225	39	19533.519		
	Total	1.377E7	51			
DPPH	Between Groups	1489404.367	12	124117.031	42.839	0.000
	Within Groups	112995.355	39	2897.317		
	Total	1602399.722	51			
Total Flavonoid Content	Between Groups	5670133.604	12	472511.134	49.292	0.000
	Within Groups	373851.785	39	9585.943		
	Total	6043985.389	51			
Total Monomeric Anthocyanin Content	Between Groups	62423.343	12	5201.945	51.976	0.000
	Within Groups	3903.295	39	100.084		
	Total	66326.638	51			
Polymeric Color	Between Groups	878.016	12	73.168	4.979	0.000
	Within Groups	573.107	39	14.695		
	Total	1451.123	51			
Chlorgenic Acid	Between Groups	4123833.339	12	343652.778	508.331	0.000
	Within Groups	26365.637	39	676.042		
	Total	4150198.975	51			
Neocholorogenic Acis	Between Groups	762.923	12	63.577	166.593	0.000
	Within Groups	14.884	39	.382		
	Total	777.806	51			
Caffeic Acid	Between Groups	599.507	12	49.959	385.192	0.000
	Within Groups	5.058	39	.130		
	Total	604.566	51			
Cyanidin-3-glucoside	Between Groups	6676.988	12	556.416	168.133	0.000
	Within Groups	129.066	39	3.309		
	Total	6806.054	51			
Pelargonidin-3-glucoside	Between Groups	90129.726	12	7510.810	803.133	0.000
	Within Groups	364.723	39	9.352		
	Total	90494.449	51			
Pelargonin chloride	Between Groups	106305.236	12	8858.770	243.532	0.000
	Within Groups	1418.670	39	36.376		
	Total	107723.906	51			

Table C.1: Statistical analysis results of black carrot processing samples (continuing).

		Sum of Squares	df	Mean Square	F	Sig.
Cyanidin derivative 1	Between Groups	202202.645	12	16850.220	231.558	0.000
	Within Groups	2837.982	39	72.769		
	Total	205040.627	51			
Cyanidin derivative 2	Between Groups	518119.280	12	43176.607	428.509	0.000
	Within Groups	3929.640	39	100.760		
	Total	522048.920	51			

Table C.2 : Correlation results of black carrot processing samples.

		Total Phenolic	CUPRAC
Total Phenolic	Pearson Correlation	1	0.981**
	Sig. (2-tailed)		0.000
	N	52	52
CUPRAC	Pearson Correlation	0.981**	1
	Sig. (2-tailed)	0.000	
	N	52	52
		Total Phenolic	DPPH
Total Phenolic	Pearson Correlation	1	0.978**
	Sig. (1-tailed)		0.000
	N	52	52
DPPH	Pearson Correlation	0.978**	1
	Sig. (1-tailed)	0.000	
	N	52	52
		CUPRAC	DPPH
CUPRAC	Pearson Correlation	1	0.984**
	Sig. (2-tailed)		0.000
	N	52	52
DPPH	Pearson Correlation	0.984**	1
	Sig. (2-tailed)	0.000	
	N	52	52

Table C.3 : Correlation results of black carrot processing samples (continuing).

		Total Phenolic	Total Flavonoid Content
Total Phenolic	Pearson Correlation	1	0.967**
	Sig. (1-tailed)		0.000
	N	52	52
Total Flavonoid Content	Pearson Correlation	0.967**	1
	Sig. (1-tailed)	0.000	
	N	52	52
		Total Phenolic	Total Monomeric Anthocyanin Content
Total Phenolic	Pearson Correlation	1	0.827**
	Sig. (1-tailed)		0.000
	N	52	52
Total Monomeric Anthocyanin Content	Pearson Correlation	0.827**	1
	Sig. (1-tailed)	0.000	
	N	52	52
		Total Flavonoid Content	Total Monomeric Anthocyanin Content
Total Flavonoid Content	Pearson Correlation	1	0.799**
	Sig. (2-tailed)		0.000
	N	52	52
Total Monomeric Anthocyanin Content	Pearson Correlation	0.799**	1
	Sig. (2-tailed)	0.000	
	N	52	52
		Total Flavonoid Content	DPPH
Total Flavonoid Content	Pearson Correlation	1	0.968**
	Sig. (2-tailed)		0.000
	N	52	52
DPPH	Pearson Correlation	0.968**	1
	Sig. (2-tailed)	0.000	

Table C.3: Correlation results of black carrot processing samples (continuing).

	N	52	52
		Total Monomeric Anthocyanin Content	DPPH
Total Monomeric Anthocyanin Content	Pearson Correlation	1	0.839**
	Sig. (1-tailed)		0.000
	N	52	52
DPPH	Pearson Correlation	0.839**	1
	Sig. (1-tailed)	0.000	
	N	52	52
		Total Monomeric Anthocyanin Content	CUPRAC
Total Monomeric Anthocyanin Content	Pearson Correlation	1	0.828**
	Sig. (1-tailed)		0.000
	N	52	52
Total Antioxidant Capacity (Cuprac)	Pearson Correlation	0.828**	1
	Sig. (1-tailed)	0.000	
	N	52	52
		Total Monomeric Anthocyanin Content	DPPH
Total Monomeric Anthocyanin Content	Pearson Correlation	1	0.839**
	Sig. (1-tailed)		0.000
	N	52	52
DPPH	Pearson Correlation	0.839**	1
	Sig. (1-tailed)	0.000	
	N	52	52

**Correlation is significant at the 0.05 level.

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Professional Experience and Rewards:

Project Assistant of AnThocyanin and polyphenol bioactives for Health Enhancement through Nutritional Advancement (ATHENA) - Funded under 7th FWP (Seventh Framework Programme European Commission) (August 2010-June 2012).

List of Publications:

- Nilüfer Erdil D,. Sunay Elif A., Boyacıoğlu D., Demir N., Süzme S. "*Crystallization Behavior of Turkish Honeys by DSC*" 2009, IFT Annual Meeting&Expo 2009, Anaheim, CALIFORNIA, USA (Poster presentation).
- Nilüfer Erdil D,. Sunay Elif A., Boyacıoğlu D., Demir N., Süzme S. "*Crystallization Behavior of Turkish Honeys by DSC*" 2009, APImondia, Sepetember 2009, Montpellier, France (Poster presentation).
- Toydemir Otkun, G., Suzme, S., Sunay, A. E., Boyacioglu, D. 2010. "The Changes in Phenolic Content and Antioxidant Activity of Tea Infusions with Honey Addition". 2010 EFFoST Annual Meeting, Food & Health, November 2010, Dublin, Ireland (Poster presentation).
- Ozer, F., Islek, M., Toydemir, G., Suzme, S., Nilufer Erdil, D., Boyacioglu, D. "Evaluating The Effect of Almond Inclusion on Antioxidant and Phenolic Contents of Grape-Seed Added Breads". IFT 2011 Annual Meeting & Food Expo, June 2011, New Orleans, Louisiana, USA (Poster presentation).
- Suzme, S., Toydemir, G., Capanoglu, E., Boyacioglu, D. "Investigating The Antioxidant Potential of Red Fruit Juice Concentrates". Novel Approaches In Food

Industry (NAFI 2011) – International Food Congress, May, 2011, Izmir, Turkey (Poster presentation).

- Kurtlar, T., Boyacioglu, D., Capanoglu, E., Suzme, S. (2011). Descriptive Sensory Analysis of Dark Chocolates with Different Almond Content. "NAFI International Food Congress", 26-29 May, 2011, Izmir, Turkey (Poster presentation).
- Sunay A.E., Akdogan G , Boyacioglu D.,Suzme' S. "A valuable tool for new product development: consumer acceptance of creamed honey" 9th Pangborn Sensory Science Symposium, September 2011, Toronto, Canada (Poster presentation).
- Sunay A.E., Akdogan G , Boyacioglu D.,Suzme' S. "Mixing time affects consumer acceptance of creamed honey" 9th Pangborn Sensory Science Symposium, September 2011, Toronto, Canada (Poster presentation).
- Boyacıoğlu, D., Toydemir, G., Süzme, S., Çapanoğlu, E. 2011. Çaya Bal Katılmasının Fenolik Madde Miktarı ve Antioksidan Aktivite Üzerine Etkisi. Gıda Teknolojisi, 2:86-90 (National Journal).

PUBLICATIONS/PRESENTATIONS ON THE THESIS

- Çapanoğlu Güven, E., Nilüfer Erdil, D., Kapçı, B., Sürek, E., **Süzme, S**., Boyacıoğlu, D. 2012. Meyve suyuna işleme sırasında antioksidan özelliklerde meydana gelen değişimler. Gıda Teknolojisi, 2:100-104
- **Süzme, S**., Boyacıoğlu, D., Çapanoğlu, E. 2012. Investigating The Effect Of Processing On Phenolic And Anthocyanin Profile Of Black Carrot. IFT 2012 Annual Meeting & Food Expo, June 2012, Las Vegas, Nevada, USA (Poster presentation).
- **Süzme, S**., Boyacıoğlu, D., Çapanoğlu, E. 2012. Processing Effect on Phenolic and Anthocyanin Profile Of Black Carrots And Black Carrot Concentrate. Advanced NonthermalProcessing in Food Technology: Effects on Quality and Shelf life of Food and Beverages (ANPFT2012), 07-10 May, 2012, Kusadasi, TURKEY (Poster presentation).