ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

POLYPHENOL CONTENT AND *IN VITRO* BIOAVAILABILITY OF BLACK CARROT (*DAUCUS CAROTA*) POMACE AND PEEL

M.Sc. THESIS

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Department of Food Engineering

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JUNE 2015

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

KARA HAVUÇ (*DAUCUS CAROTA*) POSA VE KABUĞUNUN POLİFENOL KAPASİTESİNİN VE *İN VİTRO* BİYOYARARLILIĞININ İNCELENMESİ

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Fatma Damla BİLEN, a **M.Sc** student of ITU **Graduate School Of Science Engineering And Technology** student ID **506131508**, successfully defended the **thesis** entitled "**POLYPHENOL CONTENT AND *IN VITRO* BIOAVAILABILITY OF BLACK CARROT (*DAUCUS CAROTA*) POMACE AND PEEL**", which 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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May 2015

Fatma Damla BİLEN
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ABBREVIATIONS

ABTS	**:** 2,2- azinobis 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt
ACY	**:** Anthocyanin
ANOVA	**:** Analysis of variance
AOAC	**:** Association Official of Analytical Chemists
BA	**:** Bioaccessible
BC	**:** Black Carrot
C3G	**:** Cyanidin-3-glucoside
CUPRAC	**:** Copper Reducing Antioxidant Capacity
DAD	**:** Diode array detection
DF	**:** Dilution factor
DPPH	**:** 1,1-Diphenyl-2- picrylhydrazyl
DW	**:** Dry weight
FD	**:** Freeze dried
FRAP	**:** Ferric Reducing Antioxidant Capacity
GA	**:** Gallic acid
GAE	**:** Gallic Acid Equivalent
GI	**:** Gastrointestinal
LOD	**:** Limit of Detection
LOQ	**:** Limit of Quantification
NBA	**:** Non-bioaccessible
ND	**:** Not detected
PA	**:** Phenolic Acid
PE	**:** Peel
PO	**:** Pomace
HPLC	**:** High Performance Liquid Chromatography
SCFA	**:** Short Chain Fatty Acid
SPE	**:** Solid Phase Extraction
TAC	**:** Total antioxidant capacity
TE	**:** Trolox Equivalent
TMA	**:** Total monomeric anthcyanin content
TPC	**:** Total phenolic content
UPLC-ESI-MS	**:** Liquid Chromatography-Electrospray Ionization Mass Spectrometry
VC	**:** Vacuum belt-dried

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POLYPHENOL CONTENT AND *IN VITRO* BIOAVAILABILITY OF BLACK CARROT (*DAUCUS CAROTA*) POMACE AND PEEL

SUMMARY

Carrots belong to the *Apiaceae* family; which is one of the most consumed vegetable in the world. Although orange colored carrots are common, black carrots have recently gained interest especially as a natural food colorant due to their high heat, light and pH stability. Black carrots (*Daucus carota*) originate from Turkey and the Middle and Far East, where they have been cultivated for at least 3000 years. They have an attractive bluish-purple color with high levels of anthocyanins. Black carrot is usually consumed after processing into various products, such as concentrate, jams and juices. As a result of processing, large amounts of by-products including peel and pomace are generated. These by-products are usually disposed, both in environmental and economic terms. Several literature studies proved that by-products of different fruit and vegetables contain high levels of total phenolics, flavonoids, anthocyanins and antioxidant capacity. However, there is no study on black carrot by-products; peel and pomace.

In this study the aim is to investigate characterization and *in vitro* bioaccessibility of polyphenols of black carrot and its by-products. This study is performed on industrial waste materials that will also provide information about the effect of processing. For the experimental setup of the *in vitro* gastrointestinal digestion, pepsin, pancreatic enzymes and intestinal bacteria were used. Total phenolic, total anthocyanin and total antioxidant capacities and polymeric color were determined by a spectrophotometer. For the identification and quantification of phenolic metabolites high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography-electrospray ionization mass spectrometry (UPLC-ESI-MS) were used. Statistical differences between samples were evaluated by one-way ANOVA ($p<0.05$).

Five major anthocyanin pigments; two non-acylated (cyanidin-3-xylosylglucosyl galactoside and cyanidin-3-xylosylgalactoside) and three acylated with sinapic acid (cyanidin-3-sinapoylxylosylglucosylgalactoside), ferulic acid (cyanidin-3-feruloyl xylosylglucosylgalactoside) and *p*-coumaric acid (cyanidin-3-*p*-coumaroylxylosyl glucosylgalactoside) were investigated. In the group of phenolic acids; neochlorogenic acid, cryptochlorogenic acid, chlorogenic acid caffeic acid, ferulic acid were determined. After *in vitro* digestion, a decrease in the content of neochlorogenic, cryptochlorogenic and chlorogenic acid were observed, whereas an increase in the content of caffeic and ferulic acid was found.

Significantly lower amounts of total anthocyanins were present in the stomach (26%-49%), small intestine (20%-38%) and colon (18%-47%). Similarly, total phenolics in the stomach (29%-57%), small intestine (48%-73%) and colon (45%-64%) were also lower than undigested total phenolic content. During colonic digestion, acylated anthocyanins were converted into their parent anthocyanins as a result of microbial

activity. The proportional production of major SCFAs after 24 hours were observed for pomace and peel samples.

Among all four antioxidant capacity methods performed in this study (ABTS, DPPH, FRAP and CUPRAC), DPPH, CUPRAC and FRAP assays showed similar trends of decrease or increase. CUPRAC resulted in the highest antioxidant capacity for undigested black carrot (17426.1 mg TE/100 g dw); whereas it was measured 7198.7 mg TE/100 g dw with DPPH, 9257.2 mg TE/100 g dw with FRAP and 2489 mg TE/100 g dw with ABTS. In total, the CUPRAC assay gave the highest and DPPH assay the lowest values for each sample.

In overall, the current study highlighted black carrot and its by-products as valuable health promoting sources.

SİYAH HAVUÇ (*DAUCUS CAROTA*) POSA VE KABUĞUNUN POLİFENOL KAPASİTESİNİN VE *İN VİTRO* BİYOYARARLILIĞININ İNCELENMESİ

ÖZET

Apiaceace familyasında havuç yüzyıllar boyunca dünyada en çok tüketilen sebzelerden birsi olmuştur. Her ne kadar turuncu renkli havuçlar yaygın olsa da, siyah havuç özellikle yüksek sıcaklık, ısı ve pH stabilitesi yüzünden doğal renklendirici olarak kullanılmaya ve değer kazanmaya başlamıştır. Siyah havuç (*Daucus carota*) Türkiye, Orta ve Uzak Doğu'da en az 3000 yıldır üretilmektedir. Antosiyaninler açısından oldukça zengin mavimsi-mor renge sahiptirler. Siyah havuç genellikle reçel, konsantre, içecek gibi ürünlerde kullanılmaktadır ve üretim sonunda yüksek miktarlarda atık ürün ortaya çıkmaktadır. Ve bu atıklar hem ekonomik kayıp hem de çevre kirliliği gibi sıkıntılara neden olmaktadır. Daha önce başka sebze ve meyvelerin atıkları ile ilgili pek çok çalışma yapılmıştır ve bu atıkların toplam fenolik, flavonoid, antosiyanin içerikleri ile antioksidan kapasitilerinin yüksek olduğu kanıtlanmıştır.

Bu çalışmada amaç siyah havuç ve atıklarının polifenollerinin karakterizasyonu ve bunların biyoyararlılıklarının incelenmesini amaçlamaktadır. Çalışmada endüstiyel atıklar kullanılmış ve ürünler üzerinde proses etkileri de gözlemlenmiştir. Deneysel planda *in vitro* sindirim için pepsin, pankreatik enzimler ve kolonik bakteriler kullanılmıştır. Çalışmada kullanılan *in vitro* sindirim üç basamaklı bir prosedüre sahiptir ve mide, ince bağırsak, kalın bağırsak basamaklarından oluşmaktadır. Fabrikadan alınan örnekler sıvı nitrojen altında öğütülüp, donrulurak kurutularak deneylerde kullanılmak üzere hazırlanmışlardır. Başlangıç örnekleri ise %0.1 (v/v) formik asit içeren %75'lik methanol ve %0.1 (v/v) formik asit içeren %75'lik ethanol ile hazırlanmıştır.

Ürünün toplam fenolik, toplam antosiyanin, toplam antioksidan kapasiteleri ve polimerik renk analizleri spektrofotometre ile ölçülmüştür. Fenolik kapasite analizleri Folin-Ciocalteu metodu; toplam monomeric antosiyanin kapasite pH differential metodu; toplam antioksidan kapasite ise dört farklı metot (ABTS, CUPRAC, DPPH ve FRAP) kullanılarak yapılmıştır. Her spektrofotometrik yöntem için örnekler üç parallel hazırlanmış ve sonuçların ortalaması alınmıştır. Fenolik metabolitler yüksek performanslı sıvı kromatografisi (HPLC) ve mass spectrometry (UPLC-ESI-MS) kullanılarak tespit edilmiştir. Bunların dışında kısa zincirli yağ asitleri gaz kromatografisi (GC) kullanılarak tepit edilmiştir. Örnekler arası istatistiksel farklılıklar tek yollu ANOVA (p<0.05) ile tespit edilmiştir.

Çalışma sonunda LS-MS analizlerinden elde edilen sonuca göre siyah havuç ve atıklarında toplam beş ana antosiyanin; iki tanesi non-acylated (cyanidin-3-xylosylglucosylgalactoside ve cyanidin-3-xylosylgalactoside) üçü ise sinapik asit (cyanidin-3-sinapoylxylosylglucosylgalactoside), ferulik asit (cyanidin-3-feruloyl xylosylglucosylgalactoside) ve *p*-kumarik asit (cyanidin-3-*p*-coumaroylxylosyl glucosylgalactoside) türevleri olarak bulunmuştur. Her antosiyaninin karakterizasyonu UV-görülür karakterleri, MS ve fragmantasyonları dolayısıyla pozitif modda gerçekleştirilmiştir. Antosiyanin profilleri daha önce yapılmış olan

literatürdeki çalışmalarla paralellik göstermektedir. Sindirimden önceki başlangıç örneklerinde posa en yüksek antosiyanin içeriğine sahip ürün olarak tespit edilmiştir. Cyanidin-3-xylosyl-feruloyl-glucosyl-galactoside başlangıç örnekleri için en baskın (40-69%) antosiyanin olarak gözlemlenmiştir. Toplam antosiyaninlerin 61-88%'ini acylated antosiyaninler oluşturmaktadır. Bu da acylated antosiyaninlerin non-acylated antosiyaninlere göre pH ve sıcaklık değişimlerine daha toleranslı olduklarını gösterir. Buna göre, siyah havuç atıkları acylated antosiyanin kaynağı olarak gıda uygulamalarında rahatça kullanılabilir. Non-acylated antosiyaninler için posa en yüksek sonuçlara sahipken (81.0 mg C3G/100 kuru madde ve 507.8 C3G/100 kuru madde); siyah havuç sinapik, ferulik ve *p*-kuromik türevleri açısından diğer örneklerden daha zengin içeriğe sahip olduğu görülmüştür (282.2 mg C3G/100 kuru madde, 844.9 C3G/100 kuru madde ve 97.5 C3G/100 kuru madde). *In vitro* mide sindirimi sonunda non-acylated antosiyaninler için %37-%89 civarında; diğerleri için ise %39-%89 civarında düşüş tespit edilmiştir. Tüm *in vitro* sindirim sonunda ise cyanidin-3-xylosylglucosylgalactoside dışında diğer antosiyaninlerde düşüş görülmüştür. Sonuçlar istatiksel olarak birbirlerine paralellik göstermektedir ($p<0.05$).

Başlıca fenolik asitler ise neoklorojenik asit (3-*O*-caffeoylquinic acid), kriptoklorojenik asit (4-*O*-caffeoylquinic acid), klorojenik asit (5-*O*-caffeoylquinic acid), kafeik asit ve ferulik asit olarak gözlenlenmiştir. Literatürdeki diğer sonuçlarla paralel olmak üzere klorojenik asit tüm örneklerde en baskın (%71-84) bileşen olarak tespit edilmiştir. Caffeoylquinic asit türevleri ise toplam fenolik asitlerin %83-89'unu meydana getirmektedir. Sindirim sonucu özellikle neoklorojenik ve kafeik asit artışları dikkat çekicidir. *In vitro* sindirim sonunda neoklorojenik asit, kriptoklorojenik asit ve klorojenik asit içeriklerinde azalma (%0.03-%95) gözlenirken, ferulik ve kafeik asitte artma tespit edilmiştir. Özellikle kabuk örneklerindeki artış dikkat çekicidir (kafeik asit için %620; ferulik asit için %240).

Prosesin ürünler üzerindeki etkisinin incelenmesi için polimerik renk analizi MeOH ve EtOH kullanılarak gerçekleştirilmiş ve sonucunda kabuk ürünlerinde diğer ürünlere kıyasla daha yüksek sonuçlar elde edilmiştir. Tüm ürünler için MeOH ekstraksiyonu, EtOH ekstraksiyonuna göre daha yüksek sonuçlar alınmasına neden olmuştur.

Başlangıç örneklerinde toplam fenolik madde ve toplam antioksidan kapasite siyah havuç atıklarına göre sırasıyla %10-28 ve %12-31 daha düşük olsa da sonuçlar arasında istatistiksel olarak bir fark yoktur ($p<0.05$). Bunun dışında başlangıç örnekleri için toplam monomerik antosiyanin içerikleri karşılaştırıldığında posa örneklerinin diğerlerine göre %3 daha yüksek olduğu söylenilebilir. Toplam fenolik madde içeriği siyah havuç, kabuk ve posa için sırasıyla 5743.0 mg GAE/100 g kuru madde, 5170.1 mg GAE/100 g kuru madde ve 4151.3 mg GAE/ 100 g kuru madde olarak bulunmuştur. Kabuğun ve posanın, siyah havuç örneklerinden daha düşük fenolik madde içeriğine sahip olduğu görülmüştür. Mide sindirimi sonrasında örneklerde sırasıyla %43, %57 ve %71 düşüş; ince bağırsak sindirimi sonunda %52, %27 ve %28'lik düşüş, kalın bağırsak sindirimi sonucunda ise %36, %39 ve %47'lik düşüşler tespit edilmiştir. İnce bağırsak ve kalın bağırsak sindirimleri sonunda istatiksel olarak benzer sonuçlar elde edilmiştir ($p<0.05$). İnce bağırsak örneklerininde mide örneklerine kıyasla gözlemlenen artış literatürle paralellik göstermiş, bunun nedeni ise ek ekstraksiyon süresi (4 saat), ince bağırsak sindirim enzimlerinin (lipaz, pankreatin) örnekler üzerindeki etkisi olarak açıklanabilir.

Toplam monomerik antosiyanin madde içeriği posa için en yüksek sonuç (1703.4 mg C3G/100 g kuru madde) gözlemlenirken en düşük sonuç kabuk (1221.1 mg C3G/100 g kuru madde) için tespit edilmiştir. Mide sindirimi sonrasında örneklerde sırasıyla %51, %73 ve %74 düşüş; ince bağırsak sindirimi sonunda %80, %62 ve %68'lik düşüş, kalın bağırsak sindirimi sonucunda ise %81, %53 ve %57'lik düşüşler tespit edilmiştir. İnce bağırsak ve kalın bağırsak sindirimleri sonunda istatiksel olarak benzer sonuçlar elde edilmiştir (p<0.05). Toplam monomerik antosiyanin içeriğinde toplam fenolik madde içeriğine göre daah yüksek düşüşler gözlemlenmiştir.

Bu çalışmada uygulanan dört toplam antioksidan kapasite metodundan (ABTS, DPPH, FRAP ve CUPRAC), CUPRAC metodu sindirilmemiş siyah havuç için en yüksek antioksidan kapasite değerini verirken (17426.1 mg TE/100 g kuru madde); Aynı ürün için DPPH analizi sonunda sonuç, 7198.7 mg TE/100 g kuru madde; FRAP için 95257.2 mg TE/100 g kuru madde ve ABTS için 2489 mg TE/100 g kuru madde olarak ölçülmüştür. *In vitro* mide sindirim sonunda siyah havuç için CUPRAC, DPPH ve FRAP analizleri sonucunda %31-50 düzeylerinde düşüş gözlemlenirken; ABTS sonuçlarında %263'lük bir artış elde edilmiştir. Tüm sindirim tamamlandığında ise başlangıç örneğine oranla CUPRAC, DPPH ve FRAP analizleri sonucunda %56-62 düzeyinde düşüş; ABTS sonunda %106'lık artış tespit edilmiştir. Siyah havuç posası için yapılan antioksidan kapasite analizlerinde ise bu dört metot aynı artış ve azalış trendlerini gösterirken; kabuk için siyah havuca benzer şekilde DPPH, CUPRAC ve FRAP metotları birbiriyle paralellik göstermektedir. Sindirimden önce siyah havuç, posa ve kabuk ürünleri için antioksidan kapasiteleri ABTS hariç; sırasıyla siyah havuç, kabuk ve posa olarak tespit edilmiştir. Posa ürünlerinde dört metotta ve kabuk ürünlerinde DPPH, FRAP ve CUPRAC metotlarında ince bağırsak ve kalın bağırsak sindirimleri sonunda elde edilen değişimler istatiksel olarak birbirlerine benzerlik göstermektedirler (p<0.05). Sonuçlardaki farklılıklar her bir metotun birbirinden farklı pH değerlerinde daha etkin olması, polifenollerin kimyasal yapıları ve sindirim sırasındaki bunların diğer bileşenlerle olan etkileşimleri olarak gösterilebilir. Bu yüzden her çalışma için en az iki metotla antioksidan kapasite araştırması yapılması önerilmektedir.

Kısa zincirli yağ asit (SCFA) analizleri sonucunda ise blankler 24 saat ve 48 saat sonucunda birbirlerine benzer sonuçlar göstermiştir. Bu da ekstra bir SCFA üretimi olmadığını kanıtlamaktadır. 24 saat sonucunda tespit edilen ana SCFA'ler asetik, propionic ve bütirik asittir. Özellikle posa örnekleri için asetik asit içeriğinde büyük bir artış görülmüştür. Bunun dışında bütirik asitte 48 saat sonunda; 24 saate kıyasla iki kat artış tespit edilmiştir. Genel olarak asetik asitte siyah havuç örnekleri için (sırasıyla 24 saat ve 48 saat) %73-%73, posa örnekleri için %78-%75 ve kabuk örnekleri için %73-%68 artış görülmüştür. Posada meydana gelen toplam SCFA oluşumu, siyah havuç ve kabukta meydana gelenden yüksektir (15-24%) (p<0.05). SCFA sağlıklı bir kalın bağırsak demek olduğundan, siyah havuç posasının sağlıklı bir kalın bağırsak için güzel bir kaynak olduğu varsayılabilir.

In vitro gastrointestinal sindirim metodları daha önce üzüm, mango ve nar örnekleri için kullanılmış olsa da bu çalışma siyah havuç kabuk ve posası üzerine yapılmış ilk çalışmadır. Üretim prosesleri sırasında açığa çıkan siyah havuç atıkları endüstri için ciddi bir problem yaratmaktır. Oysa ki siyah havuç atıkları özellikle antosiyanince zengin polifenol kaynağı olarak değerlendirilebilir. Bu çalışma siyah havuç ve atıklarının polifenol içerikleri, antioksidan kapasiteleri ve bunların biyoyararlılıkları üzerine gerçekleştirilmiştir. TPC, TMAC ve TAC *in vitro* sindirim sonucu belirgin bir şekilde (23-82%) azalmıştır. Posa örneklerinin *in vitro* sindirim basamaklarının

hepsinde açığa çıkan antosiyanin miktarı siyah havuçtan fazladır; bu da siyah havuç posasının iyi bir bioaccessible antosiyanin kaynağı olduğunu göstermektedir.

Yukarıda da özetlendiği üzere, bu çalışmanın amacı toplam fenoliklerin, toplam monomerik antosiyaninlerin ve siyah havuç, kabuk ve posasının içerdiği ana fenolik asitlerin ve de antosiyaninlerin sindirim stabilitesini incelemek ve mide, ince bağırsak ve kalın bağırsak sindirimi sırasında antioksidan aktivitedeki değişimi gözlemlemek üzerine yapılmıştır. Bunlara ek olarak mikrobiyal metobolik aktivitenin bir sonucu olan kısa zincirli yağ asitlerinin oluşumu (SCFAs) da incelenmiştir.

1. INTRODUCTION

Fruits and vegetables can be consumed as processed products such as juices, jams or canned as well as unprocessed raw material. They have gained interest due to their significant content of bioactive compounds, vitamins and minerals, which can result in positive health effects. It is proven by scientific data that consumption of fruit and vegetables can be preventivee against cardiovascular diseases or cancer (Capanoglu *et al.*, 2012).

Many processed fruit and vegetables are peeled off, taken apart from their seeds, stalks or some other parts. However; according to the literature, there are significant decreases on anthocyanin content of fruits and vegetables as a consequence of those processing steps (Peschel *et al.*, 2006; Capanoglu *et al.*, 2008).

Black carrots (*Daucus carota*) originate from Turkey and the Middle and Far East, where they have been cultivated for at least 3000 years. They have a 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 (Kamiloglu *et al*., 2015). Black carrots accumulate five major anthocyanin pigments, two of them are nonacylated, and three are derivatives of cyanidin acylated with sinapic acid, ferulic acid and p-coumaric acid (Elham *et al.*, 2006; Netzel *et al.*, 2007). Black carrot is often consumed after processing into various products and as a result of processing, large amounts of by-products including peel and pomace are generated. These by-products are usually disposed, both in environmental and economic terms (Montilla *et al.*, 2011; Tatoglu, 2014).

In recent years, bioaccessibility of phenolic constituents in a variety of food materials became a popular topic. Although daily consumption of anthocyanins is pretty high, there are no sufficient amounts of research papers on bioavailability of phenolics. Moreover, there are several parameters including the food matrix, pH, temperature, presence of enzymes, host and other factors that have a significant effect on potential bioavailability of anthocyanins. *In vitro* digestion method can be well correlated with conclusions from *in vivo* models which can be useful for determination of

bioavailability (Bouayed *et al.*, 2011; Liang *et al.*, 2012; Erdil, 2013). Considering the above, the aim of the current study to investigate the stability of polyphenols in black carrot, peel and pomace during *in vitro* gastrointestinal digestion.

This research thesis is presented as literature, materials and methods, results and discussion and conclusion parts. In the literature chapter, black carrots, polyphenols of black carrots as well as their bioavailability were reviewed. Fruit and vegetable by-products literature review was given also in the literature part. Materials and methods section included the detailed protocols followed for the analysis. Results and discussion part included spectrophotometric measurements, HPLC analysis and simulation of *in vitro* bioavailability of black carrots.

2. LITERATURE REVIEW

2.1. General Characteristics of Black Carrots

Carrot or *Daucus carota*, is part of the *Apiaceae* (also known as *Unbelliferae)* family. This two years old vegetable can be divided into two groups; anthocyanin group (*Daucus carota subsp. sativus var. atrorubans*) and carotene group (*Daucus carota ssp. sativus*) (Rodriguez-Sevilla *et al.*, 1999; Utus, 2008). Anthocyanin group is grown in eastern countries like Turkey, India, Egypt and Afghanistan; whereas, carotene group is a worldwide product (Pistrick, 2001; Kammerer *et al.*, 2004).

Black carrots originate from Turkey and Middle and Far East, where they have been cultivated for at least 3000 years (Kamiloglu *et al.*, 2015). Orange carrots first produced by Dutch horticulturists in the 16^{th} century with crossing domesticated and wild species (Navazio *et al.*, 2010; Wivel *et al.*, 2012). Although orange is a predominate color for carrots, recently black carrots have attracted interest due to their bluish-color with high levels of anthocyanins (1750 mg/kg). Apart from their colorant properties, anthocyanins may serve an important role in promoting health by reducing the risk of atherosclerosis and cancer, preventing inflammation, and acting as antioxidants. Besides anthocyanins as the major polyphenols, black carrots also contain significant amounts of phenolic acids, including hydroxycinnamates and caffeic acid. (Utus, 2008; Khandare *et al.*, 2011; Kamiloglu *et al.*, 2015). Black carrot is shown in Figure 2.1.

Edible fleshy root of carrot can be grown in a year, however, for flowers and seed production two years is required (Ozen, 2008). Plants, are not harvested for the first year, have small white flowers and seeds in the second year (Navazio *et al.*, 2010; Tatoglu, 2014). Carrot is one of the most economically important crops in worldwide. Especially black carrot seeds have significantly high economic value. Accordingly, seed growers produce hybrid black carrot seeds (İyicinar, 2007). Temperatures between 15-21^{o}C, low light and high humidity are optimum growth conditions for black carrots. Growth conditions of black carrots have direct effect on

their colors. Vegetables that are harvested in spring have more bright color compared to the ones are harvested in fall and winter season (Tatoglu, 2014).

Figure 2. 1 : Appearance of *Daucus carota* (Black Carrot).

Black carrots are rich sources of fiber (2.48 g/100 g), sugars like glucose (1.10-5.60 g/100 g), fructose (0.14-4.36 g/100 g) and saccharose (1.20-3.31 g/100 g), minerals such as calcium (33 mg/100 g), magnesium (17 mg/100 g), potassium (256 mg/100 g), phosphorus (29 mg/100 g), iron (0.26 mg/100 g) and zinc (0.15 mg/100g), polyphenols and they are low in fat (0.14 g/100 g). Besides, they contain 142.3-159.6 g/kg dry matter and 7.0-1.38 g/kg protein (Tatoglu, 2014; Anon, nd).

As many fruits and vegetables, black carrots are seasonal and perishable, and difficult to preserve as a raw material. As a result of processing, large amounts of by-products including peel and pomace generated. Black carrots can be consumed as fresh, preserved and canned. Besides, they can serve as a natural food colorant due to their high heat, light, and pH stability. Hence, black carrot extracts are used to give colour for beverage (fruit juices and nectars, non-alcoholic and fermented beverages), conserves, jellies, confectionary and ice cream production (Montilla *et al.*, 2011).

Orange carrots, the most consumed vegetables after potatoes in worldwide; however, they ranked 7th in United States (U.S.) after tomatoes, head lettuce, onions, snap beans, sweet corn and bell peppers (Brunke and Boriss, 2006; Algarra *et al.*, 2014). Consumption of fresh and processed carrots in U.S. is shown in Figure 2.2.

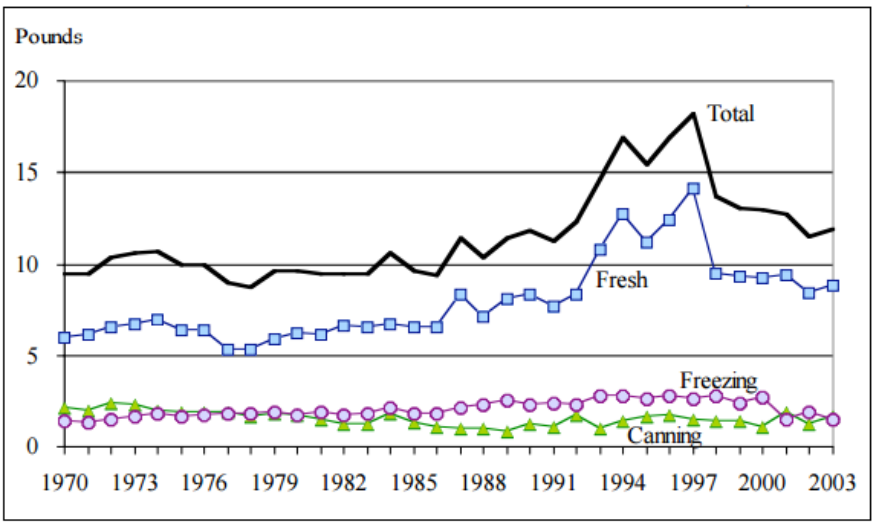

Figure 2. 2 : Consumption of fresh and processed carrots in U.S., per capita (Brunke, 2006).

2.2. Black Carrot Polyphenols

Recent clinical studies show that free radicals in bodies have a significant role on cardiovascular diseases, cancer and diseases of ageing (Mathew and Abraham, 2006). Free radicals are quite reactive atoms or molecules that have one or more unpaired electron. Free radicals gain or give electrons due to their unpaired electrons. Because of these features, proteins, carbohydrates, fats and plasma membranes can be damaged. Antioxidants prevent formation and reaction of free radicals and shatter molecules that were damaged. Even at very low concentrations, antioxidants prevent oxidation (Yildiz, 2007).

Consumers are increasingly aware of diet related health problems, hence looking for health and safe promoting natural ingredients. Due to high antioxidant activity and polyphenols of vegetable and fruits, plant-based diet can be used in order to reduce development of cardiovascular diseases or cancer (Halvorsen *et al.*, 2002). Indeed, it was proved that there is an inverse correlation between the consumption of vegetables and fruits and cancer risk by epidemiological studies (Vinson, 1999). A chart, illustrating the increase in mortality from cancer in people consuming the least amount of fruits and vegetables compared to those consuming the most, is showed in Figure 2.3. The significant association between the consumption of fruits and vegetables, and positive health effects is exerted by the antioxidant activity of

polyphenols. Hence, to determine the nutritional quality of fig fruit, it is important to analyze all the main compounds showing antioxidant activity.

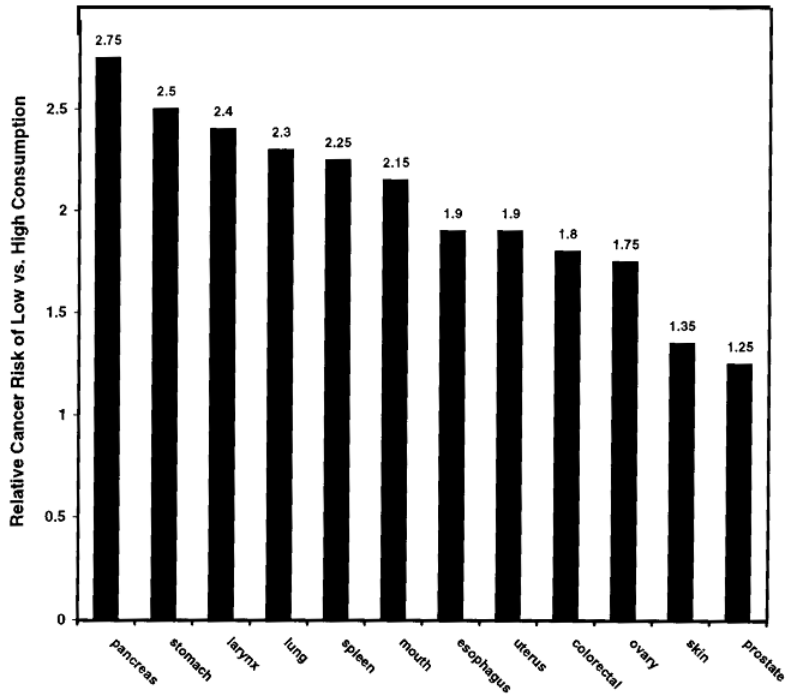

Figure 2. 3 : The protective effect of fruits and vegetables in reducing the risk of cancers (Vinson, 1999).

Besides the presence of known antioxidants such as vitamins C and E, black carrots have attracted the attention of the scientific community due to their phenolic compounds (secondary metobilites of plants) content.

Derivatives of benzoic acid, hydroxybenzoic acids (gallic, *p*-hydroxybenzoic, protocatechuic, vanillic and syringic acids) and derivatives of cinnamic acid, hydroxycinnamic acids (caffeic, ferulic, *p*-coumaric and sinapic acids) are the two main groups of phenolic acids (Figure 2.4). Hydroxybenzoic acids have C_6-C_1 structure; whereas, hydroxycinnamic acids have C_6-C_3 structure (Manach *et al.*, 2004; Balasundram *et al*., 2006).

Hydroxycinnamic acids occur frequently in foods as simple esters with quinic acid or glucose. Probably, the most abundant soluble bound hydroxycinnamic acid is chlorogenic acid (5-*O*-caffeoylquinic acid), which is combined from caffeic and quinic acids (Lafay *et al*., 2006). Unlike hydroxycinnamic acids, hydroxybenzoic acids are mainly present in foods in the form of glucosides (Mattila *et al*., 2006).

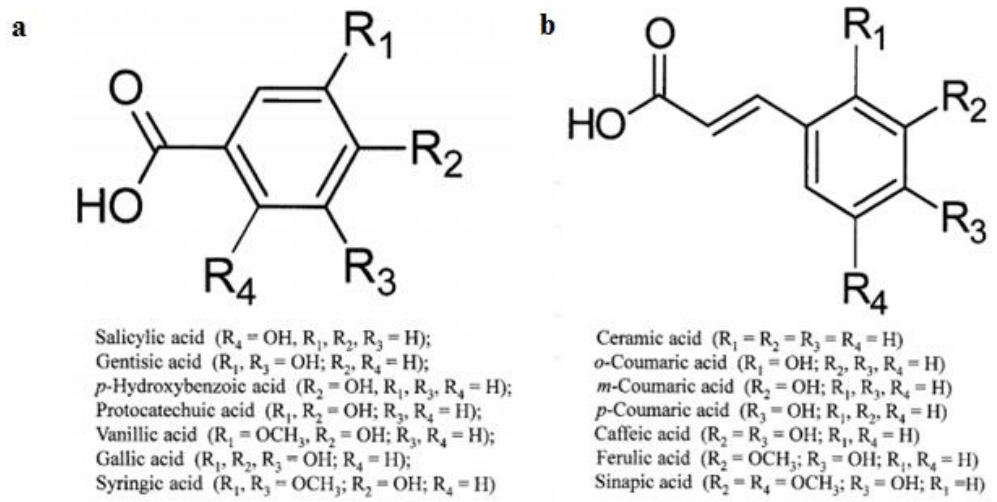

Figure 2. 4 : Examples of hydroxybenzoic (a) and hydroxycinnamic (b) acids (Reis Giada, 2013).

Flavonoids are low molecular weight polyphenolic compounds that have diphenyl propane with fifteen carbon atoms structure ($C_6C_3C_6$). They are the most abundant phenolics present in plants (Kahraman, 2002). General formula of flavonoids is shown in Figure 2.5.

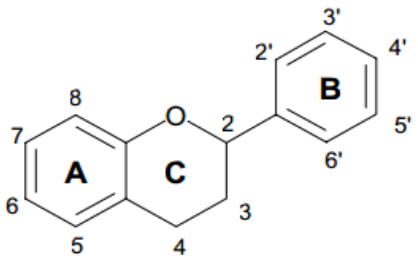

Figure 2. 5 : General Structure of Flavonoids (Kahraman, 2002).

Flavonoids can spirit off free radicals by participating redox reactions. This occurs due to their aromatic ring structured hydroxyl groups. Aromatic rings and multiple unsaturated bindings provide a durable structure to flavonoids. Formation of reactive oxygen species can be blocked out with metal chelating capability of flavonoids. As a result of those three important properties, flavonoids are strong antioxidants (Dincer, 2007).

Flavonoids can be divided into six main subgroups of flavones (apigenin, luteolin), flavonols (quercetin, myricetin), flavanones (naringenin, hesperitin), isoflavones

(genistein, glycitein), flavanols (catectin, epicatechin), and anthocyanidins (cyanidin, delphinidin, peonidin, malvidin) (Figure 2.6).

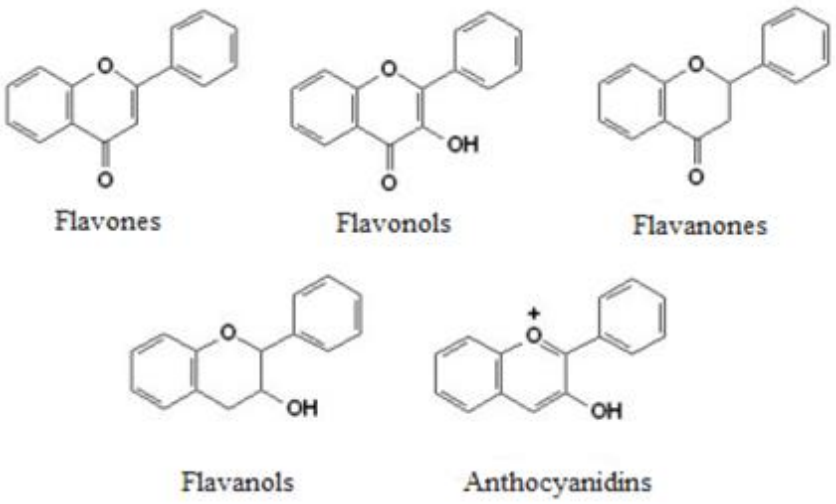

Figure 2. 6 : Chemical structures of flavonoids (Yildiz, 2007).

Celery, olive and parsley contain high amounts of flavone; whereas, flavanones are mainly found in orange and grapefruit. Tea, onion, apple and cabbage are rich in quercetin, flavonols. Flavanols are present in large amounts in green tea, vine and peach; though anthocyanins are found in red and blue flowers or fruits (Yildiz, 2007). Anthocyanin pigments protect leaves of plants from pathogens and harmful UV beams. Also, they can participate in enzyme inhibitors of antioxidant (Avci, 2006; Karatas *et al.*, 2013).

Several authors have determined the profile of phenolic compounds of black carrots (Ersus-Uyan *et al.*, 2004; Kammerer *et al*., 2004; Alasavar *et al*., 2005; Kırca *et al.*, 2006; Gizir *et al.*, 2008; Charron *et al.*, 2009; Algarra *et al.*, 2014; Koley *et al.*, 2014; Suzme *et al.*, 2014; Kamiloglu *et al.*, 2015). In these studies, phenolic acids and anthocyanins were identified.

Black carrots mainly consist of cyanidin glycosides that are often acylated with hydroxycinnamic (such as caffeic, ferulic and *p*-coumaric acids) and hydroxybenzoic acids as well as minor anthcocyanins as peonidin and pelargonidin glycosides (Kammerer *et al.*, 2004).

Black carrots contain five major anthocyanin pigments, among them are two nonacylated, (cyanidin-3-xylosylglucosylgalactoside and cyanidin-3-xylosyl

galactoside) and three of anthocyanins were acylated with sinapic acid (cyanidin-3-sinapoylxylosylglucosylgalactoside), ferulic acid (cyanidin-3-feruloylxylosyl glucosylgalactoside) and *p*-coumaric acid (cyanidin-3-*p*-coumaroylxylosyl glucosylgalactoside) (Elham *et al.*, 2006; Netzel *et al.*, 2007; Ozkan, 2009).

2.3. Fruit and Vegetable By-products Rich in Anthocyanins

By-products of plant food processing represent a major disposal problem for the industry concerned, however they are also promising sources of bioactive compounds. In fact, several by-products especially the ones from wine industry are shown to be rich sources of polyphenols. Recently, there is a rapidly growing interest on plant by-products, their chemical values and potential positive effects on human health. In addition, by products of plants is a considerable disposal problem for food processing industry (Kosseca, 2011). By-products of fruit contains peels, seeds, flesh, pulp and stems that are produced by different industrial steps and usually wasted or discarded (Ajila *et al.*, 2007).

There are several successful trials on production of aroma compounds, enzymes, ethanol, organic acids, polysaccharides, pigments, antibiotics, biopolymers, films; dietary fibers, coloring agents, antioxidants, phenolic compounds from by-products of fruits or vegetables (Kosseca, 2011).

By-products that are rich by means of phenolic compounds, powerful natural antioxidants, are generated during food and agricultural production. For instance, in the European Union, approximately 450,000 tons of onion waste is generated by the year. Onions have been studied for their high flavonoid and sulfer containing compound capacities; moreover antioxidant properties in different in vitro models. Due to those promising results, phenolic content and antioxidant stability of onion by-products were investigated (Kosseca, 2011).

Annually more than 9 million tons of grape by-products are produced by wine making process. As a consequence of that large by-product capacity; grapes are the most studied plant. Grape by-products have been investigated as a good source of phenolic compounds.

A number of studies on bioactive compounds of different plant by-products were summarized in Appendix, Table A.1. Overall, it has been reported that the by-products of fruit and vegetables contain high levels of total phenolics, flavonoids, anthocyanins and antioxidant capacity. Usage of those waste materials as food enrichment constituents could have economic benefits to producers, environmental impact; more importantly, health supplement for consumers.

Taking into account the potential use of compound present in by-products of different plants that were listed below and no previous study evaluated the antioxidant activity and polyphenol composition of black carrot by-products; this study focused on characterization of polyphenols from black carrot by-products; peel and pomace.

2.4. Bioavailability of Black Carrot Polyphenols

The positive health effects of polyphenols depend on the consumption amount and most importantly on their bioavailability (Manach *et al.*, 2004). Bioavailability is a fraction of a nutrient which is ingested and available to the body for utilization in normal physiological functions or for storage (Castenmiller *et al.*, 1999). Bioavailability of polyphenols changes greatly from one to another, and for some compounds it depends on dietary source (Manach *et al.*, 2004). The bioavailability of a dietary compound depends on its digestive stability, quantity or fraction that is released from the food matrix in the gastrointestinal (GI) tract that is defined as bioaccessibility (Tagliazucchi *et al.*, 2010).

For the assessment of bioavailability and bioaccessibility, there are four main methods; *in vitro* (simulated GI digestion; Caco-2 cell cultures), *ex vivo* (gastrointestinal organs in laboratory conditions), *in situ* (intestinal perfusion in animals) and *in vivo* (animal and human studies) methods (Carbonell *et al.*, 2014). Due to stability of *in vitro* models under GI conditions and well correlation with *in vivo* models; *in vitro* studies can be used as a trustworthy determination method for bioavailability of polyphenols (Bouyed *et al.*, 2011).

In vitro digestion is being extensively used since they are relatively inexpensive, technically simple, rapid (screening of numerous samples) and do not have the same ethical restrictions as *in vivo* methods (Liang *et al.*, 2012; Carbonell *et al.*, 2014).

Studies considering *in vitro* digestion of some polyphenols for different foods such as almond skins (Mandalari *et al.*, 2010), 23 commercial vegetable juices (Wooton-Beard *et al.*, 2011), apple varieties (Bouayed *et al.*, 2012), blueberries (Correa-Betanzo *et al.*, 2014), edible artichoke heads (Garbetta *et al.*, 2014) and cinnamon beverages (Helal *et al.*, 2014) were performed.

As far as it is known, in the literature, there is a study on evaluating the *in vitro* GI digestion of black carrot jams and marmalades polyphenols (Kamiloglu *et al.*, 2015). *In vitro* gastrointestinal digestion methods have already been used to study the release of polyphenols from by-products of grape, mango and pomegranate. Nevertheless, this is the first study that has focused on the changes in polyphenols from black carrot peel and pomace during *in vitro* gastrointestinal digestion.

3. MATERIAL AND METHODS

3.1. Materials

3.1.1. Chemicals

For simulation of *in vitro* gastrointestinal (GI) system chemicals that were used are shown in Table 3.1.

Table 3.1 : Chemicals for *in-vitro* GI simulation.

Chemical	**Company**	**CAS Number**
Bile (extract porcine)	Sigma_Aldrich	8008-63-7
Bovine Serum Albumin	Sigma_Aldrich	9048-46-8
Calcium Chloride Dihydrate ($CaCl_2.2H_2O$)	Sigma_Aldrich	10035-04-8
Sodium Dihydrogen Phosphate ($Na_2H_2PO_4$)	Sigma_Aldrich	7558-80-7
Lipase (from porcine pancreas)	Sigma_Aldrich	9001-62-1
Magnesium Chloride 6 aq ($MgCl_2.6H_2O$)	Chem_Lab NV	7791-18-6
Mucin (from porcine stomach)	Sigma_Aldrich	84082-64-4
Pancreatin (from porcine pancreas)	Sigma_Aldrich	8049-47-6
Pepsin (from porcine gastric mucosa)	Sigma_Aldrich	9001-75-6
Potassium Chloride (KCl)	Sigma_Aldrich	7447-40-7
Potassium Dihydrogen Phosphate (KH_2PO_4)	Chem_Lab NV	7778-77-0
Sodium Bicarbonate ($NaHCO_3$)	Chem_Lab NV	144-55-8
Urea	Chem_Lab NV	57-13-6

Chemicals that were used for extract preparation, short chain fatty acid (SCFAs) and determination of total phenolic (TP), total anthocyanin (TA) and antioxidant contents are shown in Table 3.2.

Table 3.2 : Chemicals for extraction, SCFA, TP, TA and antioxidant content.

Chemical	**Company**	**CAS Number**
ABTS salt	Sigma_Aldrich	30931-67-0
Acetic Acid (glacial/Analytical grade)	Fischer Scientific	64-19-7
Ammonium Acetate ($C_2H_7NO_2$)	Acros	631-61-8
Citric Acid (99.5-101%) ($C_6H_8O_7.1H_2O$)	Chem_Lab NV	5949-29-1
Copper Dichloride ($CuCl_2$)	UCB	7447-39-4
Diethylether	Chem_Lab NV	60-29-7

Table 3.3 : (Continued) Chemicals for extraction, SCFA, TP, TA and antioxidant content.

Chemical	**Company**	**CAS Number**
Ethanol, absolute (C_2H_6O)	Fisher Scientific	64-17-5
DPPH (2,2-Diphenyl-1-pikryl-hydrazl)	Sigma_Aldrich	1898-66-4
Folin- Ciocalteu's reagent	Fluka	
Formic Acid 99-100%	Chem_Lab NV	64-18-6
Gallic Acid	Acros Organics	149-91-7
Hydrochloric Acid (HCl)	Chem_Lab NV	7647-01-0
Iron Chloride ($FeCI_3.6H_2O$)	Janssen Chemical	1.0025-77-1
Methanol (Analytical reagent grade& HPLC grade)	Fischer Scientific UK	67-56-1
Monopotassium Phosphate (KH_2PO_4)	Chem_Lab NV	7778-77-0
Neocupraine	Sigma_Aldrich	484-11-7
Potassium Hydroxide (KOH)	Chem_Lab NV	1310-58-3
Potassium Metabisulfite ($K_2S_2O_5$)	Merck	16731-55-8
Potassium Persulfate ($K_2S_2O_8$)	Acros Organics	7727-21-1
Sodium Acetate Trihydrate ($CH_3COONA.3H_2O$)	Chem_Lab NV	6131-90-4
Sodium Carbonate (Na_2CO_3)	Merck	497-19-8
TPTZ (2,4,6- Triphridyl-s-triazine)	Sigma_Aldrich	3682-35-7
Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)	Sigma_Aldrich	53188-07-1

Standards and reagents were used for the quantification of phenolic compounds and anthocyanins were shown in Table 3.3:

Table 3.3 : Chemicals for quantification of phenolic compounds and anthocyanins.

Chemical	**Company**	**CAS Number**
Caffeic acid	Sigma_Aldrich	331-39-5
Chlorogenic acid	Sigma_Aldrich	327-97-9
Cryptochlorogenic acid	Sigma_Aldrich	905-99-7
Cyanidin-3-*O*-glucoside	Sigma_Aldrich	7084-24-4
Ferulic acid	Fluka	1135-24-6
Neochlorogenic acid	Sigma_Aldrich	906-33-2

Water used for all analysis was distilled and purified with the water purification system (Millipore, Direct Q 3 UV).

3.1.2. Plant Material

Black carrots, pomace and peel were obtained from the juice concentrate production company Erkonsantre, Konya/Turkey. Pomace and raw material were taken in 3

different batches, peel was taken from raw material. Pomace was gathered during black carrot juice concentrate production. The flow chart of concentrate production is shown in Figure 3.1. Black carrot, peel and pomace samples were ground under liquid nitrogen to a fine powder. Then they were freeze-dried for 24 hours and stored -80^{o}C before further analysis.

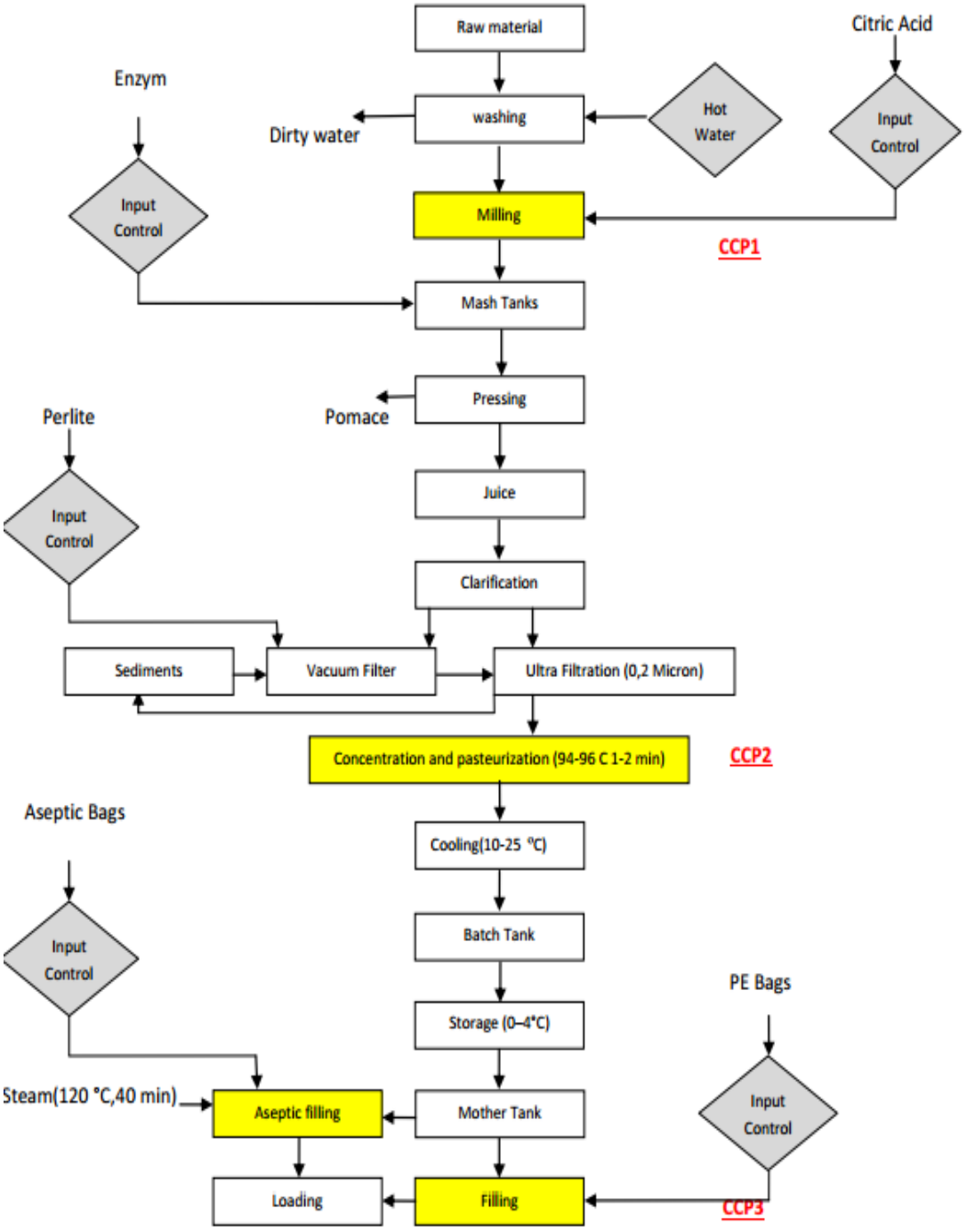

Figure 3. 1 : Flow chart of Black Carrot Juice Concentrate.

3.2. Methods

3.2.1. Simulated in vitro gastrointestinal (GI) digestion

The simulated GI digestion system contained 3 main stages; gastric, small intestine and colon (sampling time: 24 and 48 hours) stage. To follow the release of phenolics and anthocyanins, analysis was performed for samples that were collected from each stage. Antioxidant activity was also determined. Main stages of *in vitro* digestion of samples from raw material are shown in Figure 3.2.

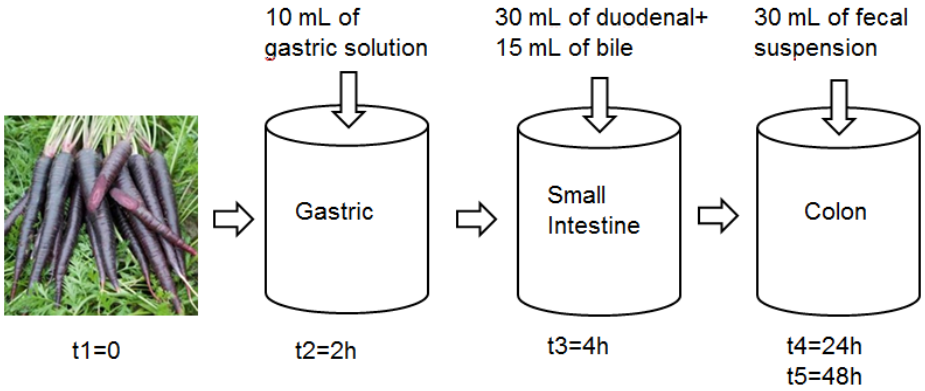

Figure 3. 2 : Stages of GI digestion simulation.

3.2.1.1. Gastric Phase

62 mL of distilled water was added to 3 grams of freeze-dried black carrot, peel and pomace powders that were added to penicillin bottles (65 mL of distilled water for the blank). After mixing them, 15 mL of sample was immediately taken as time point zero. After 10 mL of gastric juice addition samples were incubated in a shaker for 2 hours at 37^{o}C (pH 3). Gastric solution contains; 16.5 g/L sodium chloride (NaCl), 2.1 g/L sodium dihydrogen phosphate (NaH_2PO_4), 4.92 g/L potassium chloride (KCl), 2.4 g/L calcium chloride dehydrate ($CaCl_2.2H_2O$), 0.54 g/L urea, 2.1 g/L mucin, 6 g/L bovine serum albumin and 6 g/L pepsin. After 2 hours, 15 mL of sample was collected. 2 mL of sample was kept in an eppendorf tube, while the rest (13 mL) was centrifugated at 3000 g for 10 minutes at 4^{o}C. Supernatants and pellets were kept separately and stored at -20^{0}C until further analysis.

3.2.1.2. Small Intestine Phase

For the small intestine stage, 15 mL sample was collected from the gastric phase, 30 mL of duodenal and 15 mL of bile medium were added in penicillin bottles. Samples were incubated at 37^{o}C for 4 hours (pH 7). Duodenal medium contained 7.01 g/L

NaCl, 0.56 g/L KCl, 0.2 g/L $CaCl_2.2H_2O$, 5.61 g/L sodium bicarbonate ($NaHCO_3$), 0.08 g/L potassium dihydrogen phosphate (KH_2PO_4), 0.5 g/L magnesium chloride 6 aqueous ($MgCl_2.6H_2O$), 0.1 g/L urea, 1 g/L bovine serum albumin, 3 g/L pancreatin and 0.5 g/L lipase, whereas; bile medium contained 5.26 g/L NaCl, 0.38 g/L KCl, 0.22 g/L $CaCl_2.2H_2O$, 5.79 g/L $NaHCO_3$, 0.25 g/L urea, 1.8 g/L bovine serum albumin and 6 g/L of bile. After 4 hours, 15 mL of sample was collected. Same procedure as gastric phase (2 mL of sample was kept in an eppendorf tube, while the rest (13 mL) was centrifugated at 3000 g for 10 minutes at 4^oC) was applied. Supernatants and pellets were collected and they were stored at -20^0C separately.

3.2.1.3. Colon Phase

30 mL of fecal suspension (SHIME- Simulator of the Human Intestinal Microbial Ecosystem) was added to penicillin bottles and samples were incubated for 24 hours at 37^oC (pH 6.5). Samples were kept close to the heat, metal caps were used and squeezed tightly in order to avoid contamination during and after addition of fecal suspension. After 24 hours 15 mL of samples were collected (2 mL for eppendorf tube). Same procedure as gastric and small intestine phase was applied for collected 13 mL samples and they were stored at -20^0C.

Samples in the penicillin bottles were incubated for 24 hours more at 37^oC. After 24 hours, 15 mL of samples were collected. Same procedure as for all previous stages was applied for collected 13 mL samples and they were stored at -20^0C.

The bacterial wastes were autoclaved before disposal.

3.2.2. Short-Chain Fatty Acid (SCFAs)

500 µL of black carrot, peel and pomace extracts from colon fermentation (sampling time: 24 and 48 hours) were mixed with 500 µL of H_2SO_4 solution (1/1 dilution). After addition of 400 µL of internal standard (2-methyl hexanoic acid), 0.4 g NaCI and 2 mL of diethylether; samples were vortexed for 3 minutes. Samples were centrifuged at 3000 g for 3 minutes (Sigma Laboratory Centrifuges 4K15, Santorius AG). After centrifugation supernatents were collected into vials and analyzed using a GC-2014 gas chromatograph (Shimadzu, ‘s-Hertogenbosch, the Netherlands), equipped with a capillary fatty acid-free EC-1000 Econo-Cap column (dimensions: 25 mm*0.53 mm, film thickness 1.2 mM; Alltech, Laarne, Belgium), a flame ionization detector and a split injector.

3.2.3. Extraction of Phenolics from Plant Material

Prior to spectrophotometric analysis, a detailed literature research concerning the best extraction solvent was carried out and as a result 75% aqueous-ethanol (pH: 3.5, adjusted using 1M citric acid) and 75% aqueous-methanol containing 0.1% (v/v) formic acid were selected as the best solvents for extraction.

Methanolic extractions for each fraction were carried out as described previously by Capanoglu *et al*. (2008). 0.1 g of freeze-dried powder from each sample was extracted with 5 ml of 75% aqueous-methanol containing 0.1% (v/v) formic acid in a cooled ultrasonic bath (Elma S60H Elmasonic, Elma-Hans Scmidboviev GmbHQ Co.KG) for 15 min. The treated samples were centrifuged for 10 min at 3000 g, at 4^{o}C and the supernatants were collected. Another 5 ml of 75% aqueous-methanol containing 0.1% (v/v) formic acid was added to the pellet and this extraction procedure was repeated for one more time. All supernatants were combined and diluted to their final volume of approximately 10 ml. Prepared extracts were stored at -20^{0}C until analysis.

For ethanolic extractions, 0.2 g of freeze-dried powder was collected in 50 ml tubes and mixed with 20 mL of 75% aqueous-ethanol. After 3 hours incubation in a shaking water bath at 50^{o}C, samples were cooled by ultrasonication for 15 minutes. The treated samples were centrifuged for 10 min at 5000 g, at 4^{o}C and the supernatants were collected. Prepared extracts were stored at -20^{0}C until analysis.

Since aqueous methanol extracted significantly more polyphenols than aqueous ethanol, 75% aqueous methanol containing 0.1% formic acid was used as the extraction solvent for all other subsequent analyses.

3.2.4. Alkali Hydrolysis of Pellets for Non-Extractable Polyphenol Extraction

0.1 g of pellet from each sample was mixed with 2 mL of 2N NaOH and incubated at 40^{o}C for 30 minutes in a sonicated bath. By addition of 2N of HCI (~1800-2000 mL), samples were neutralized. After addition of MeOH (0.1% acetic acid), samples were vortexed for 2 minutes. The treated samples were centrifuged for 10 min at 10000 g, at 4^{o}C and the supernatants were collected. Another 4 ml of methanol containing 0.1% (v/v) acetic acid was added to the pellet and this extraction procedure was repeated for one more time. All supernatants were combined and

adjusted to a final volume of 20 ml by using methanol. Prepared extracts were stored at -20^{0}C until analysis.

3.2.5. Determination of Total Monomeric Anthocyanin Content (TMA)

The TA content of samples was investigated according to the pH differential method (AOAC, 2006). After dilution of samples with KCl and $CH_3COO.Na$, absorbance was measured against blank at 520 and 700 nm in buffers at pH 1.0 and pH 4.5. The TMA was expressed as cyanidin-3-glucoside equivalents, as follows:

$$\text{TA (cyanidin-3-glucoside equivalents, mg/L)} = (A \times MW \times DF \times 10^3)/(\varepsilon \times l) \quad \textbf{(3.1)}$$

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, 10^3 factor for conversion from g to mg, ε molar extinction coefficient of cyanidin-3-glucoside (26900 L/(mol.cm)), and *l* pathlength (cm). The results were expressed as mg cyanidin-3-glucoside (C3G)/100g dry weight. Analysis for samples of each extraction was performed in triplicate. The calibration curves are shown in Appendix, Figure B.

3.2.6. Determination of Total Phenolic Content (TPC)

TPC of digestion samples and extracts was determined according to the Folin-Ciocalteu method (Velioglu *et al.*, 1998). After addition of 0.75 mL of Folin-Ciocalteu reagent (1:10 v/v with distilled water) to 100 µL of extract, mixture was incubated for 5 minutes at room temperature. Then 0.75 mL of Na_2CO_3 solution was added. After 90 min of incubation at room temperature, absorbance was read at 725 nm against blank by UV-Vis spectrophotometer (Vanian, Cary 50 Bio UV-Visible Spectrophotometer). Gallic acid was used as a standard for calibration curve. Results were expressed as miligrams per 100 g dry weight of gallic acid equivalents (mg GAE/100 g dw). Samples of each extraction were analyzed in triplicate. The calibration curves are shown in Appendix, Figure B.

3.2.7. Determination of Total Antioxidant Capacity (TAC)

ABTS, DPPH, FRAP and CUPRAC assays were performed in order to estimate TAC. In all assays Trolox® was used as a standard and results were given as Trolox equivalence per 100 grams of dry weight of original sample (mg TE/100 g dw). Samples were analyzed in triplicate for each assay.

The ABTS (2,2- azinobis 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) assay was performed according to Miller and Rice-Evans (1997). ABTS and potassium persulfate solutions were mixed and kept at room temperature in the dark for overnight. ABTS stock solution was diluted in dipotassium phosphate and monopotassium phosphate mixture (buffer: pH 8.0) to an absorbance of 0.90 (±0.05) at 734 nm to prepare the ABTS-working solution. After 1 minute from ABTS working solution addition to 100 µL samples, absorbance was measured at 734 nm.

The method which is described in the literature (Kumaran and Karunafaran, 2006) was followed for the DPPH (1,1-diphenyl-2- picrylhydrazyl) assay). After 2 mL of DPPH in methanol (0.0098 g powder in 250 mL of methanol) addition to 100 µL of samples, samples were incubated for 30 minutes at room temperature. The absorbance of mixture was measured at 517 nm against blank.

The FRAP (Ferric Reducing Antioxidant Power) assay developed by Benzie and Strain (1996) was used in this study. To perform the assay, a 900 µL aliquot of freshly prepared FRAP reagent (a mixture of acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM ferric chloride in proportions of 10:1:1 (v/v/v), respectively) was combined with 100 µL of extract. The absorbance of the reaction mixture was measured at 593 nm after 4 min.

The CUPRAC (Copper Reducing Antioxidant Capacity) assay was carried out according to the procedure of Apak *et al*. (2004). 100 µL of extract was mixed with 1 mL of 10 mM $CuCl_2$, 7.5 mM neocuproine and 1 M NH_4Ac (pH:7). Immediately, 1 mL of distilled water was added to the mixture so as to make the final volume 4.1 mL. After 60 min of incubation at room temperature, absorbance was read at 450 nm against a reagent blank. The calibration curves are shown in Appendix, Figure B.

3.2.8. Determination of Percent Polymeric Color (%)

Polymeric color is a measure of the extent of anthocyanin polymerisation and browning. Percentage polymeric color, the ratio between polymerised color and color density, is used to determine the percentage of the color that is contributed by polymerised anthocyanins (Wrolstad *et al.*, 2005; Holzwarth *et al.*, 2012). Polymetric color analysis of samples was performed according to the method which was developed by Guisti and Wrolstad (2001). Samples were diluted with water to have an absorbance reading in between 0.5-1.0 at 512 nm (1/20). 0.2 mL 0.90M potassium

metabisulfite was added to 2.8 mL diluted (bleached) samples. 0.2 mL MQ was added to 2.8 mL diluted (non-bleached) sample. After 15 minutes, absorbance of samples was read at 400, 520 and 712 nm against blank. %Polymeric color was calculated as follows:

$$\text{Color Density} = [(A_{420}-A_{700})+(A_{512}-A_{700})]*DF \text{ (non-bleached)} \quad \textbf{(3.2)}$$

$$\text{Polymeric Color} = [(A_{420}-A_{700})+(A_{512}-A_{700})]*DF \text{ (bleached)} \quad \textbf{(3.3)}$$

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

3.2.9. MS Analysis of Major Individual Phenolic Acids and Anthocyanins

Before LC-MS analysis, samples from the *in vitro* gastrointestinal digestion were purified using a solid-phase extraction (SPE) method. Initially, 500 mg/4 mL C18 SPE cartridges (Grace Davison Discovery Sciences, Deerfield, IL, USA) were conditioned by rinsing with 6 mL formic acid/methanol (1:100, v/v) followed by 4 mL formic acid/MQ water (1:100, v/v). 1.5 mL aliquots of samples were acidified with 30 μL formic acid and centrifuged (Labnet Spectrafuge 16M, Labnet International Inc., Woodbridge, NJ, USA) at 16000 *g* for 10 min. Afterwards, the supernatants were loaded to activated cartridges which were subsequently washed with 5 mL formic acid/MQ water (1:100, v/v). Samples were eluted with formic acid/methanol (1:100, v/v) and then dried using nitrogen. Prior to LC-MS analysis, samples were redissolved in DMSO/MQ water (1:10, v/v) and filtered through 0.45-μm membrane filters.

The identification of polyphenol metabolites was achieved with ultra-performance liquid chromatography-electrospray ionization mass spectrometry (UPLC-ESI-MS) (Waters Synapt HDMS, column: Actuity BEH C18). The mobile phase was solvent A, water 0.1% formic acid and solvent B, methanol with 0.1% formic acid. A linear gradient was used as follows: at 0 min, 95% solvent A and 5% solvent B; at 10 min, 85% solvent A and 15% solvent B; at 15 min, 85% solvent A and 15% solvent B; at 23 min, 5% solvent A and 95% solvent B; at 28 min, 5% solvent A and 95% solvent B; at 30 min, 95% solvent A and 5% solvent B and at 32 min 95% solvent A and 5% solvent B. The flow rate was 0.25 mL/ min. Mass range was 100-1500 Da (ESI+), capillary voltage was 2.75V, sampling cone was 40V, extraction cone was 4V, for

collision energy, low energy, trap was 6V, transfer was 4V; for collision energy, high energy, trap-ramp was 15-30V, transfer was 4V. Cooling temperature was 40^{o}C; whereas sampler temperature was 10^{o}C. Masslynx 4.1 was used as a software system.

3.2.10. HPLC Analysis of Major Individual Phenolic Acids and Anthocyanins

The quantification of polyphenol metabolites were carried out using high liquid chromatography-diode array detection (HPLC-DAD) (Capanoglu *et al.*, 2008). Extracts were cleaned by solid phase extraction (SPE) method.1.5 mL of sample mixed with 30 µL formic acid and centrifuged at 14000 rpm for 10 minutes (Spektrofuge 10M, Labnet Internatial). After SPE (C18 500 mg 14 mL Part 205250/ 512284 catalog: 205250, Grace Davison Discovery Science) conditioned with 6 mL of 1% formic acid in MeOH and 4 mL 1% formic acid in water; sample was loaded. Coloums washed with 5 mL of 1% formic acid in water. 1.5 mL of 1% formic acid in MeOH was eluted, collected and analyzed by HPLC system (Thermo Scientific, Ultimate 3000) (Figure 3.3), and a diode array detector (DAD). Grace C18 250x4.60 mm column (Grace) was used. The mobile phase was 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 to initial conditions. The flow rate was 1 mL/ min. Measurement was done at 280, 312, 360 and 520 nm. All analyses were performed in triplicates and the results were expressed as mg per 100 g dw of sample. . The content of anthocyanin glycosides were tentatively quantified using cyanidin-3-*O*-glucoside, whereas phenolic acids were quantified using their authentic standards. HPLC chromatograms are shown in Appendix, Figure C.

3.2.11. Statistical Analysis

Data were collected in triplicate and reported as mean ± standard deviation. Statistical analysis was applied using SPSS software (version 20.0, SPSS, Chicago, IL, USA). Mean values were compared by analysis of variance (ANOVA) followed by Tukey's post hoc test ($p < 0.05$). Statistical analysis tables are given in the Appendix Table D.

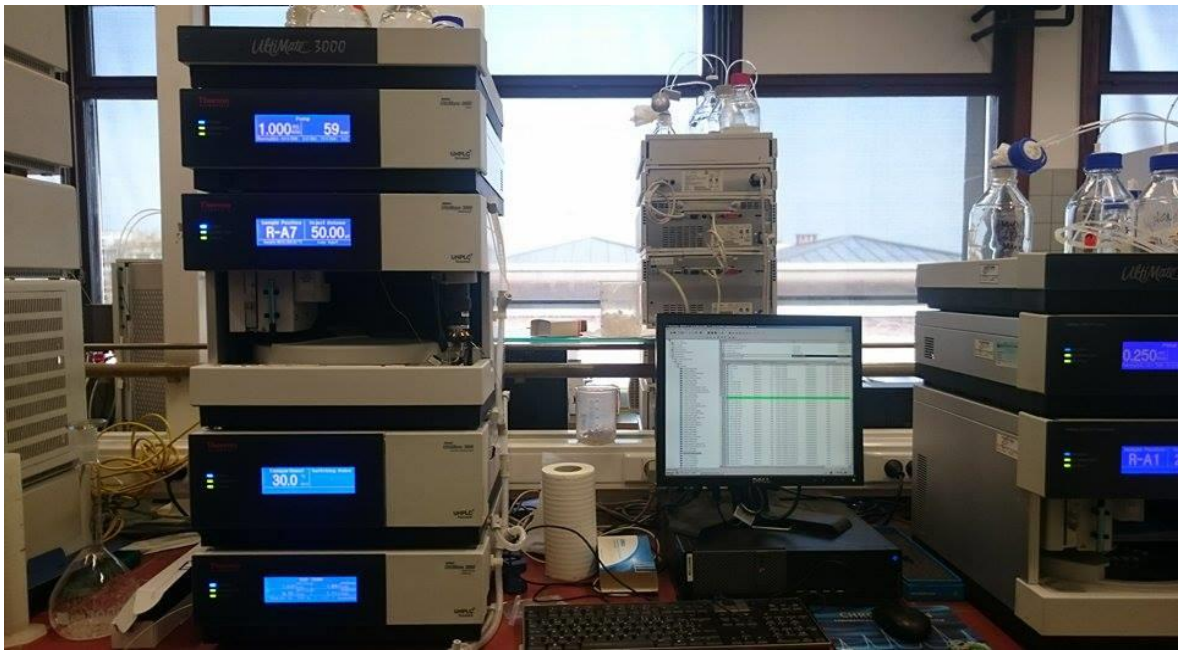

Figure 3. 3 : Thermo Scientific, Ultimate 3000 HPLC.

4. RESULTS

4.1. Short Chain Fatty Acids

SCFA content of all samples are shown in Figure 4.1. Results for blanks after 24 and 48 hours were the same; which can be a proof for no extra SCFA production. Major SCFAs (acetic, propionic and butyric acid) produced after 24 hours were similar for pomace and peel samples. Acetate yield increased for all samples, especially pomace after 48 h. Besides, After 48 h butyric acid concentrations increased twice as much whereas changes in acetic and propionic acid were not significant. In general acetic acid was the main SCFA for black carrot (73% after 24h; 73% after 48h), for peel (73% after 24h; 68% after 48h), for pomace (78% after 24h; 75% after 48h) followed by propionic and butyric acids. Propionic acid production after 24 hours for black carrot and its by-products were between 18% and 23%; whereas they were 18%-24% at 48h. At both time points, total amount of SCFAs produced from pomace samples was significantly higher (15-24%) than black carrot and peel ($p < 0.05$).

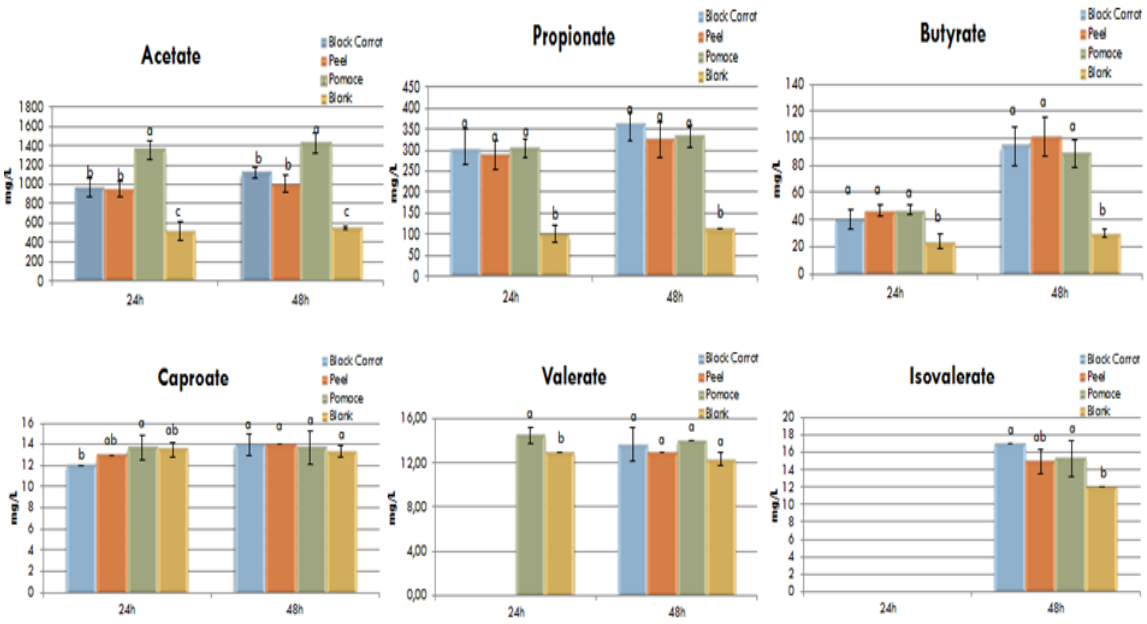

Figure 4. 1 : SCFA productions in colon after 24 h and 48 h

4.2. Total Monomeric Anthocyanin Content (TMA)

The influence of GI digestion on TMA of black carrots and by-products are shown in Table 4.1. TMA ranged from 1703.4 mg of C3G/100 g dw for pomace to 1221.1 mg of C3G/100 g dw for peel. Among undigested samples, pomace showed the highest anthocyanin content. After gastric digestion there were significant decreases (51%-74%) in TMA, for all samples.

For black carrot samples, TMA content of the bioacessible fraction was the highest for undigested samples, followed by stomach samples. Decrease in the colon after 48 h was 82% (the highest decrease); whereas gastric conditions gave the lowest (51%) decrease.

Bioaccessible fraction of peel had the highest decrease (73%) after gastric digestion and the lowest (53%) after colon fermentation for 48 h; which was similar to pomace samples (74% and 43%, respectively).

For non-bioaccessible fractions of black carrot and by-products there were decreases (75%-88%) from gastric phase to small intestine; whereas increases (131-156%) were observed from small intestine to colon.

Table 4.1 : Changes in the total monomeric anthocyanin (TMAC) contents of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
Black Carrot	BA	1653.8 ± 183.1^{a}	804.0 ± 62.8^{b}	337.5 ± 54.9^{c}	451.1 ± 38.7^{c}	304.6 ± 50.9^{c}
	NBA	nd	24.8 ± 5.4^{a}	6.2 ± 1.5^{c}	15.9 ± 3.8^{b}	8.5 ± 2.3^{bc}
Peel	BA	1221.1 ± 236.2^{a}	324.3 ± 36.0^{b}	470.0 ± 64.0^{b}	367.9 ± 32.3^{b}	577.9 ± 13.9^{b}
	NBA	nd	38.7 ± 6.2^{a}	6.8 ± 1.5^{bc}	15.7 ± 2.6^{b}	10.8 ± 2.8^{b}
Pomace	BA	1703.4 ± 164.1^{a}	446.1 ± 52.3^{c}	545.6 ± 54.3^{bc}	449.7 ± 58.9^{c}	730.0 ± 69.8^{b}
	NBA	nd	198.5 ± 110.1^{a}	23.9 ± 5.3^{b}	59.0 ± 11.3^{b}	33.8 ± 3.9^{b}

*The data presented in this table consist of average values ± standard deviation of three independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$). BA: Bioaccessible; NBA: Non-bioaccessible; nd: not detected

4.3. Total Phenolic Content (TPC)

TPC of all fractions are expressed on dry weight (DW) basis in Table 4.2. Results shows that undigested black carrot samples were the richest by means of TP (5753.0 mg GAE/100 g dw) compared to undigested peel (5170.1 mg GAE/100 g dw) and pomace (4151.3 mg GAE/100 g dw) samples. TPC of by-products after gastric phase showed significant (57-71%) loss in comparison to the undigested (initial) values; whereas the decrease for black carrot was 43%.

During GI digestion of black carrot, for bioaccessible polyphenols, decrease was in the range of 36-54%; whereas for non-bioaccessible polyphenols it was 63-89%. The highest decrease was recorded during colon digestion after 24 h for both samples.

For non-bioaccessible phenolics of by-products the highest (0.54-91%) decrease was observed in colon phase after 24 h; whereas for bioaccessible fraction the highest decrease was during gastric phase (57-71%). In addition, the lowest decreases (27-28%) for by-products are observed during small intestine digestion.

Table 4.2 : Changes in the total phenolic (TPC) contents of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
		TPC (mg GAE/100 g dw)				
Black Carrot	BA	5743.0 ± 910.8^{a}	3296.0 ± 751.5^{ab}	2768.5 ± 711.8^{b}	2653.0 ± 697.2^{b}	3669.3 ± 964.4^{ab}
	NBA	1123.1 ± 35.1^{a}	411.7 ± 78.3^{b}	204.6 ± 37.1^{c}	120.9 ± 46.5^{c}	163.1 ± 41.8^{c}
Peel	BA	5170.1 ± 620.4^{a}	2234.4 ± 351.2^{b}	3761.2 ± 796.3^{ab}	3218.6 ± 345.7^{b}	3136.7 ± 214.0^{b}
	NBA	1699.9 ± 88.9^{a}	489.5 ± 74.5^{b}	235.5 ± 32.0^{c}	147.3 ± 54.0^{c}	174.2 ± 43.1^{c}
Pomace	BA	4151.3 ± 224.8^{a}	1202.9 ± 142.6^{d}	2987.2 ± 223.0^{b}	1883.3 ± 293.0^{cd}	2215.8 ± 577.9^{bc}
	NBA	464.0 ± 71.7^{a}	662.8 ± 121.4^{a}	215.7 ± 25.0^{b}	215.1 ± 53.9^{b}	229.1 ± 30.5^{b}

*The data presented in this table consist of average values ± standard deviation of three independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$). BA: Bioaccessible; NBA: Non-bioaccessible; nd: not detected.

4.4. Total Antioxidant Capacity (TAC)

Table 4.3 shows the effect of *in vitro* GI digestion on antioxidant capacity of black carrots, peel and pomace determined using for different methods ABTS, CUPRAC, DPPH and FRAP assays. Similarly to the outcomes observed in case of bioaccessible TA and TP, values obtained after gastric digestion were significantly lower (7%–64%) compared to undigested values ($p < 0.05$), except for black carrot samples that were measured by ABTS assay (263% increase was observed). In agreement with the above results, TAC for black carrot was the highest, ranging from 7198.7 -17426.1 mg of TEAC/100 g dw for DPPH, FRAP and CUPRAC assays. However; the lowest TAC for black carrot was measured as 2489.0 mg of TEAC/100 g dw for ABTS assay. On the other hand, TAC of pomace was found to be slightly higher (3%) than black carrots.

Especially for pomace samples; for all four assays, trend of decreases and increases were quite similar to each other. After gastric digestion there is a decrease between 42% and 69%. However; there was a correlation between DPPH, FRAP and CUPRAC results for black carrot and peel samples, whereas ABTS results were quite different from those three assays.

Table 4.3 : Changes in the total antioxidant capacity of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
		DPPH (mg TE/100 g dw)				
Black Carrot	BA	7198.7 ± 1027.3^{a}	3583.3 ± 469.3^{b}	3095.7 ± 1136.6^{b}	2686.4 ± 605.8^{b}	2443.6 ± 630.1^{b}
	NBA	787.7 ± 238.3^{a}	247.0 ± 54.7^{b}	191.3 ± 37.8^{b}	166.5 ± 35.7^{b}	196.6 ± 12.1^{b}
Peel	BA	4943.9 ± 1316.3^{ab}	2702.5 ± 260.1^{b}	4995.6 ± 1188.6^{a}	3750.8 ± 450.8^{ab}	3058.9 ± 338.7^{ab}
	NBA	1206.5 ± 61.6^{a}	281.1 ± 17.7^{b}	205.2 ± 20.9^{bc}	148.8 ± 18.8^{c}	159.8 ± 24.2^{c}
Pomace	BA	5247.4 ± 770.4^{a}	1868.8 ± 151.5^{c}	4064.5 ± 300.0^{ab}	2187.9 ± 518.6^{c}	2534.5 ± 903.8^{bc}
	NBA	387.4 ± 89.7^{ab}	482.8 ± 195.8^{a}	180.5 ± 20.0^{b}	246.8 ± 56.1^{ab}	232.9 ± 22.5^{ab}

(Continued)

Table 4.3 : (Continued) Changes in the total antioxidant capacity of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
			FRAP (mg TE/100 g dw)			
Black Carrot	BA	9257.2 ± 1537.0^{a}	4605.6 ± 1231.4^{b}	3548.1 ± 1170.4^{b}	3183.8 ± 1094.8^{b}	3489.3 ± 629.1^{b}
	NBA	1389.7 ± 272.5^{a}	387.2 ± 47.4^{b}	178.5 ± 50.1^{b}	145.1 ± 29.7^{b}	111.7 ± 36.3^{b}
Peel	BA	7641.7 ± 913.0^{a}	2719.6 ± 272.9^{c}	4761.2 ± 1055.1^{b}	3468.2 ± 533.8^{bc}	3818.4 ± 389.3^{bc}
	NBA	1752.5 ± 140.0^{a}	492.8 ± 49.1^{b}	224.4 ± 35.7^{c}	160.5 ± 27.0^{c}	125.7 ± 35.1^{c}
Pomace	BA	6937.5 ± 587.7^{a}	2167.1 ± 250.5^{c}	4372.3 ± 695.8^{b}	2893.9 ± 617.3^{bc}	3197.5 ± 803.0^{bc}
	NBA	539.5 ± 70.6^{ab}	877.6 ± 240.0^{a}	206.0 ± 21.1^{b}	326.5 ± 45.9^{b}	222.2 ± 27.1^{b}
			ABTS (mg TE/100 g dw)			
Black Carrot	BA	2489.0 ± 478.0^{b}	9031.2 ± 1601.6^{a}	4812.6 ± 1132.2^{b}	5061.4 ± 1361.6^{b}	5120.0 ± 871.8^{b}
	NBA	2393.6 ± 469.9^{a}	512.2 ± 87.4^{b}	294.9 ± 24.5^{b}	225.2 ± 43.5^{b}	259.0 ± 30.0^{b}
Peel	BA	4799.2 ± 424.7^{bc}	4461.7 ± 548.7^{c}	5783.5 ± 607.8^{ab}	6205.5 ± 464.5^{a}	5781.2 ± 283.0^{ab}
	NBA	3729.2 ± 194.6^{a}	666.0 ± 86.2^{b}	391.4 ± 62.1^{c}	242.3 ± 40.1^{c}	291.3 ± 37.0^{c}
Pomace	BA	4316.7 ± 453.1^{a}	2521.1 ± 182.0^{b}	5242.1 ± 340.0^{a}	4105.6 ± 638.7^{ab}	4703.4 ± 981.5^{a}
	NBA	982.5 ± 109.0^{ab}	1310.0 ± 233.9^{a}	526.8 ± 35.0^{b}	656.8 ± 55.6^{b}	537.6 ± 26.9^{b}
			CUPRAC (mg TE/100 g dw)			
Black Carrot	BA	17426.1 ± 2935.5^{a}	11992.6 ± 1474.7^{ab}	7817.9 ± 2291.5^{b}	7195.6 ± 1828.3^{b}	7647.3 ± 1301.4^{b}
	NBA	4040.1 ± 740.1^{a}	1313.9 ± 207.9^{b}	846.4 ± 207.5^{b}	728.4 ± 176.6^{b}	429.0 ± 39.7^{b}
Peel	BA	15324.0 ± 1802.4^{a}	7311.3 ± 944.9^{b}	11343.5 ± 2655.6^{ab}	9250.8 ± 961.8^{b}	8583.1 ± 337.0^{b}
	NBA	6470.9 ± 258.1^{a}	1561.5 ± 281.0^{b}	981.9 ± 214.7^{c}	821.4 ± 240.5^{cd}	477.1 ± 72.5^{d}
Pomace	BA	12960.7 ± 852.6^{a}	4447.4 ± 785.1^{c}	8919.1 ± 959.5^{b}	5542.2 ± 1012.1^{bc}	6924.4 ± 2066.4^{bc}
	NBA	1953.2 ± 311.2^{a}	2247.2 ± 331.3^{a}	741.2 ± 127.9^{b}	841.6 ± 82.3^{b}	695.5 ± 98.1^{b}

*The data presented in this table consist of average values ± standard deviation of three independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$). BA: Bioaccessible; NBA: Non-bioaccessible; nd: not detected.

4.5. Percent Polymeric Color (%)

% Polymetric colors for black carrot samples were measured as 13.37±2.66% (extracted with EtOH) and 13.70±1.44% (extracted with MeOH); whereas they were 11.48±0.13% (extracted with EtOH) and 3.53±0.31% (extracted with MeOH) for pomace samples. The highest results were observed for peel samples 19.99±2.59% (extracted with EtOH) and 22.86±3.01% (extracted with MeOH).

4.6. Major Individual Phenolic Acids and Anthocyanins

Anthocyanin content of black carrot, peel and pomace were determined using both spectrophotometric pH differential and HPLC methods. LC-MS analysis of black carrots and by-products led to the identification of five major anthocyanins. The major anthocyanins detected were cyanidin-based, two of them were non-acylated (cyanidin-3-xylosyl-glucosyl-galactoside and cyanidin-3-xylosyl-galactoside), and three of them were acylated with sinapic acid (cyanidin-3-xylosyl-sinapoyl-glucosyl-galactoside), ferulic acid (cyanidin-3-xylosyl-feruloyl-glucosyl-galactoside) and coumaric acid (cyanidin-3-xylosyl-coumaroyl-glucosylgalactoside). Acylated anthocyanins of black carrots constitute 85% of total anthocyanins; whereas 89% for peel and 42% for pomace. Predominant anthocyanin for black carrot and peel was cyanidin-3xylosyl-feruloyl-glucosyl-galactoside; whereas, cyanidin-3-xylosyl-galactoside was the major one for pomace samples. In Figure 4.2 and Figure 4.3 HPLC chromatograms for black carrot, peel and pomace anthocyanins before and after *in vitro* digestion are showed.

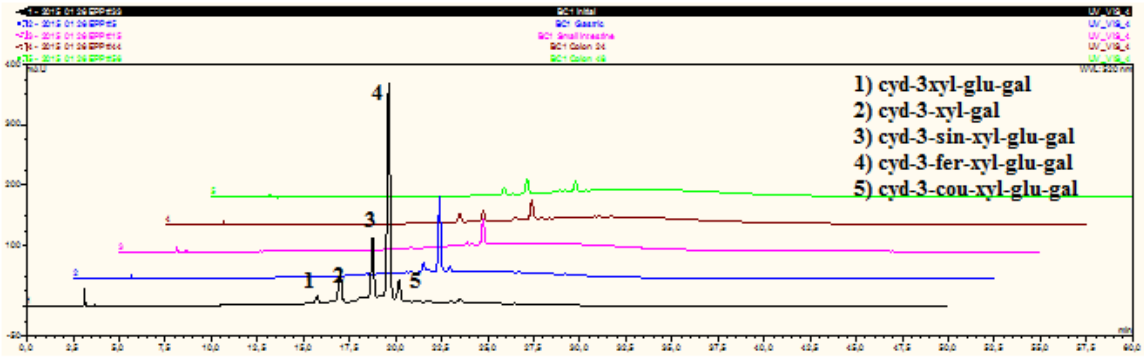

Figure 4. 2 : Anthocyanins of black carrot after *in vitro* digestion.

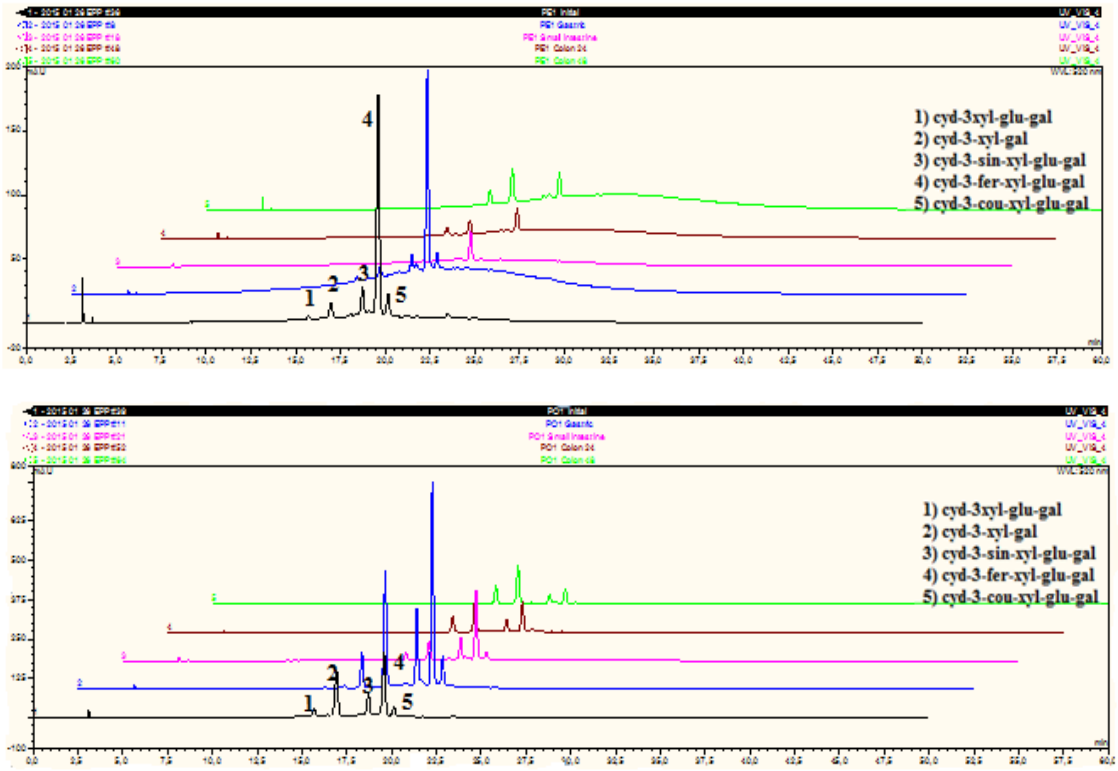

Figure 4. 3 : Anthocyanins of peel and pomace after *in vitro* digestion.

Among the undigested samples, pomace contained the highest amount of total anthocyanins, which was consistent with the results obtained spectrophotometrically. Gastric digestion caused a decrease by 64%-89% for black carrots and 51%-67% for pomace samples. Except for cyanidin-3-xylosyl-glucosyl-galactoside (31% increase), there is a decrease between 39% and 69% for peel samples after gastric digestion. No anthocyanins were detected for the non-bioaccessible fraction of black carrot and peel samples; however, acylated anthocyanins were observed for non-bioaccessible parts of pomace. A detailed chart is given below in Table 4.4.

Table 4.4 : Changes in the anthocyanins of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
	cyanidin-3-xylosyl-glucosyl-galactoside (mg C3G/100 g dw)					
Black Carrot	BA	44.1 ± 7.4^{a}	11.0 ± 3.1^{b}	12.4 ± 5.5^{b}	33.1 ± 11.5^{a}	33.2 ± 8.2^{a}
	NBA	nd	nd	nd	nd	nd
Peel	BA	10.5 ± 4.8^{b}	13.8 ± 3.2^{b}	10.9 ± 2.0^{b}	31.7 ± 13.0^{a}	32.0 ± 2.2^{a}
	NBA	nd	nd	nd	nd	nd

Table 4.4 : (Continued) Changes in the anthocyanins of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
Pomace	BA	81.0 ± 8.7^{a}	50.8 ± 6.7^{b}	24.6 ± 4.4^{c}	82.4 ± 3.9^{a}	96.2 ± 12.9^{a}
	NBA	nd	4.9 ± 3.4^{a}	nd	1.2 ± 0.6^{ab}	1.2 ± 0.9^{ab}
cyanidin-3-xylosyl-galactoside (mg C3G/100 g dw)						
Black Carrot	BA	176.9 ± 54.9^{a}	19.2 ± 2.5^{b}	19.2 ± 8.3^{b}	41.2 ± 13.8^{b}	44.9 ± 17.2^{b}
	NBA	nd	nd	nd	nd	nd
Peel	BA	75.8 ± 30.7^{a}	23.2 ± 6.8^{b}	11.1 ± 1.1^{b}	44.8 ± 20.5^{ab}	52.6 ± 14.8^{ab}
	NBA	nd	nd	nd	nd	nd
Pomace	BA	507.8 ± 38.2^{a}	165.1 ± 31.4^{b}	64.3 ± 10.7^{c}	179.8 ± 4.2^{b}	220.4 ± 41.6^{b}
	NBA	nd	49.3 ± 26.4^{a}	nd	7.4 ± 1.6^{b}	7.5 ± 1.7^{b}
cyanidin-3-sinapoyl-xylosyl-galactoside (mg C3G/100 g dw)						
Black Carrot	BA	282.2 ± 92.1^{a}	31.7 ± 7.7^{b}	22.9 ± 10.4^{b}	24.3 ± 12.0^{b}	27.1 ± 9.7^{b}
	NBA	nd	nd	nd	nd	nd
Peel	BA	96.9 ± 22.3^{a}	29.5 ± 9.1^{b}	12.2 ± 1.9^{b}	26.6 ± 8.6^{b}	26.6 ± 5.3^{b}
	NBA	nd	nd	nd	nd	nd
Pomace	BA	250.6 ± 34.7^{a}	110.8 ± 10.9^{b}	75.5 ± 15.1^{b}	82.9 ± 13.2^{b}	66.1 ± 10.6^{b}
	NBA	nd	17.8 ± 10.4^{a}	0.1 ± 0.1^{b}	3.3 ± 1.2^{b}	0.3 ± 0.1^{b}
cyanidin-3-feruloyl-xylosyl-galactoside (mg C3G/100 g dw)						
Black Carrot	BA	844.9 ± 181.1^{a}	110.8 ± 35.6^{b}	50.4 ± 20.1^{b}	62.8 ± 28.5^{b}	46.1 ± 19.9^{b}
	NBA	nd	3.9 ± 1.5^{a}	nd	nd	Nd
Peel	BA	519.5 ± 62.7^{a}	98.2 ± 29.3^{b}	41.2 ± 4.6^{b}	70.5 ± 30.3^{b}	49.7 ± 13.4^{b}
	NBA	nd	8.1 ± 2.2^{a}	nd	nd	nd
Pomace	BA	610.6 ± 43.0^{a}	265.2 ± 37.5^{b}	198.5 ± 16.6^{bc}	143.3 ± 0.6^{cd}	86.5 ± 11.1^{d}
	NBA	nd	48.7 ± 28.6^{a}	4.0 ± 0.7^{b}	8.1 ± 2.0^{b}	1.6 ± 0.0^{b}

Table 4.4 : (Continued) Changes in the anthocyanins of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
cyanidin-3-coumaroyl-xylosyl-galactoside (mg C3G/100 g dw)						
Black Carrot	BA	97.5 ± 3.5^{a}	35.0 ± 15.9^{b}	19.6 ± 6.8^{b}	17.9 ± 5.7^{b}	31.0 ± 14.0^{b}
	NBA	nd	nd	nd	nd	nd
Peel	BA	46.5 ± 9.1^{a}	28.4 ± 11.6^{ab}	13.8 ± 3.4^{b}	33.3 ± 18.2^{ab}	31.1 ± 11.6^{ab}
	NBA	nd	nd	nd	nd	nd
Pomace	BA	77.4 ± 5.1^{a}	38.3 ± 6.4^{b}	25.2 ± 6.3^{c}	17.5 ± 1.1^{c}	24.3 ± 2.8^{c}
	NBA	nd	5.1 ± 3.7^{a}	nd	nd	nd

*The data presented in this table consist of average values ± standard deviation of three independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$). BA: Bioaccessible; NBA: Non-bioaccessible; nd: not detected.

Five major phenolic acids, namely neochlorogenic acid (3-*O*-caffeoylquinic acid), cryptochlorogenic acid (4-*O*-caffeoylquinic acid), chlorogenic acid (5-*O*-caffeoylquinic acid), caffeic acid and ferulic acid were detected in black carrot and by-products using LC-MS in negative mode. Table 4.5 shows the impact of *in vitro* gastrointestinal digestion on major phenolic acids present in black carrot and by-products. Chlorogenic acid was identified as the most abundant compound for all samples accounting for 71-84% of total phenolic acids, whereas caffeoylquinic acids represented 83-89% of the phenolic acids. Chlorogenic acid amount of pomace was 14% higher than black carrot. Due to gastric digestion; neochlorogenic, cryptochlorogenic and chlorogenic acid contents of black carrot (54%, 78%, 89%), peel (51%, 78%, 75%) and pomace (57%, 14%, 42%), also caffeic and ferulic acid of pomace (54%, 40%) were decreased while caffeic and ferulic acid amounts of black carrot (305%, 8%) and peel (340%, 38%) were increased. At the end of *in vitro* fermentation in the colon, 27% further decrease in the content of chlorogenic acid was observed for pomace, along with the formation of caffeic acid. Neochlorogenic acid and cryptochlorogenic acid were not detected in non-bioaccesible part of black carrot and by-products. However, chlorogenic, caffeic and ferulic acid were observed in some stages of digestion for pomace non-bioaccesible fraction of pomace samples.

Table 4.5 : Changes in the phenolic acids of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
		Neochlorogenic acid (mg/100 g dw)				
Black Carrot	BA	9.5 ± 3.5^{a}	4.4 ± 1.0^{b}	1.6 ± 1.1^{b}	3.7 ± 0.5^{b}	5.1 ± 0.4^{ab}
	NBA	nd	nd	nd	nd	nd
Peel	BA	6.8 ± 0.9^{a}	3.3 ± 1.2^{bc}	0.9 ± 0.4^{c}	3.3 ± 0.9^{bc}	5.3 ± 0.8^{ab}
	NBA	nd	nd	nd	nd	nd
Pomace	BA	16.2 ± 0.9^{a}	7.0 ± 1.3^{b}	4.3 ± 0.4^{cd}	2.9 ± 0.7^{d}	5.7 ± 0.4^{bc}
	NBA	nd	nd	nd	nd	nd
		Cryptochlorogenic acid (mg/100 g dw)				
Black Carrot	BA	33.8 ± 18.3^{a}	7.6 ± 2.6^{b}	4.1 ± 0.4^{b}	8.6 ± 1.1^{b}	9.6 ± 0.7^{b}
	NBA	nd	nd	nd	nd	nd
Peel	BA	23.7 ± 5.7^{a}	9.2 ± 2.8^{b}	6.1 ± 0.9^{b}	9.3 ± 0.3^{b}	10.7 ± 0.4^{b}
	NBA	nd	nd	nd	nd	nd
Pomace	BA	16.7 ± 2.1^{a}	14.3 ± 1.9^{ab}	11.0 ± 0.5^{b}	16.8 ± 0.9^{a}	16.2 ± 1.2^{a}
	NBA	nd	nd	nd	nd	nd
		Chlorogenic acid (mg/100 g dw)				
Black Carrot	BA	335.5 ± 141.6^{a}	37.0 ± 11.0^{b}	26.0 ± 12.0^{b}	39.6 ± 4.7^{b}	25.8 ± 0.7^{b}
	NBA	nd	nd	nd	nd	nd
Peel	BA	170.2 ± 60.0^{a}	43.0 ± 16.3^{b}	34.5 ± 4.1^{b}	35.3 ± 12.3^{b}	27.0 ± 4.0^{b}
	NBA	nd	nd	nd	nd	nd
Pomace	BA	475.7 ± 43.4^{a}	277.1 ± 27.3^{b}	156.9 ± 6.3^{c}	51.4 ± 8.2^{d}	26.1 ± 7.1^{d}
	NBA	nd	22.7 ± 16.1^{a}	2.5 ± 0.3^{b}	4.7 ± 1.3^{ab}	2.8 ± 0.2^{b}
		Caffeic acid (mg/100 g dw)				
Black Carrot	BA	2.2 ± 0.5^{c}	8.9 ± 2.2^{ab}	5.9 ± 0.5^{bc}	12.8 ± 1.2^{a}	11.8 ± 2.4^{a}
	NBA	nd	nd	nd	nd	nd
Peel	BA	2.0 ± 0.2^{b}	8.8 ± 2.9^{ab}	5.4 ± 0.8^{b}	16.1 ± 5.1^{a}	14.4 ± 2.5^{a}
	NBA	nd	nd	nd	nd	nd
Pomace	BA	19.1 ± 2.2^{bc}	8.8 ± 0.8^{c}	4.3 ± 0.9^{c}	28.6 ± 4.0^{b}	46.1 ± 12.3^{a}
	NBA	nd	3.6 ± 1.3^{a}	1.8 ± 0.0^{b}	nd	nd

Table 4.5 : (Continued) Changes in the phenolic acids of black carrot and by-products during *in vitro* gastrointestinal digestion*.

Sample	Fraction	Undigested	Stomach	Small Intestine	Colon t=24h	Colon t=48h
		Ferulic acid (mg/100 g dw)				
Black Carrot	BA	63.3 ± 13.7^{b}	68.5 ± 15.3^{b}	98.4 ± 7.4^{ab}	93.7 ± 23.1^{ab}	108.0 ± 1.0^{a}
	NBA	nd	nd	nd	nd	nd
Peel	BA	38.2 ± 7.6^{b}	52.8 ± 11.3^{b}	79.0 ± 15.3^{ab}	128.7 ± 49.8^{a}	129.7 ± 21.6^{a}
	NBA	nd	nd	nd	nd	nd
Pomace	BA	41.5 ± 7.3^{b}	24.8 ± 1.1^{b}	34.0 ± 4.2^{b}	32.2 ± 3.9^{b}	72.1 ± 16.5^{a}
	NBA	nd	nd	nd	1.2 ± 0.3^{a}	1.2 ± 0.2^{a}

* The data presented in this table consist of average values ± standard deviation of three independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$). BA: Bioaccessible; NBA: Non-bioaccessible; nd: not detected.

Limit of detection (LOD), limit of quantification (LOQ), slope, intercept and R^2 results are given in Table 4.6.

Table 4.6 : LOQ, LOD, Slope, Intercept and R^2 results for digestion samples.

		Un-digested	**Gastric**	**Small Intestine**	**Colon 24h**	**Colon 48h**
Neo chlorogenic Acid	LOD (ppm)	0.02	0.12	0.15	0.06	0.08
	LOQ (ppm)	0.08	0.40	0.51	0.19	0.26
	Slope	0.34	0.37	0.38	0.40	0.43
	Intercept	-0.14	0.17	0.17	0.15	-0.09
	R^2	0.994	0.994	0.997	0.997	0.999
Crypto chlorogenic Acid	LOD (ppm)	0.06	0.18	0.12	0.13	0.15
	LOQ (ppm)	0.19	0.59	0.40	0.42	0.52
	Slope	0.30	0.31	0.33	0.34	0.34
	Intercept	0.29	0.05	0.02	0.06	-0.08
	R^2	0.992	0.997	0.999	0.999	0.998
Chlorogenic Acid	LOD (ppm)	0.09	0.17	0.10	0.18	0.10
	LOQ (ppm)	0.29	0.57	0.32	0.60	0.34
	Slope	0.39	0.37	0.44	0.43	0.42
	Intercept	-0.01	-0.01	-0.13	-0.03	-0.06
	R^2	0.998	0.999	0.997	0.999	0.999
Caffeic Acid	LOD (ppm)	0.02	0.03	0.09	0.04	0.07
	LOQ (ppm)	0.05	0.10	0.30	0.12	0.23
	Slope	0.78	0.71	0.72	0.73	0.73
	Intercept	-0.12	-0.05	0.00	-0.40	-0.37
	R^2	0.999	0.998	0.998	0.994	0.998

Table 4.6 : (Continued) LOQ, LOD, Slope, Intercept and R^2 results for digestion samples.

		Un-digested	Gastric	Small Intestine	Colon 24h	Colon 48h
Ferulic Acid	LOD (ppm)	0.03	0.06	0.17	0.26	0.06
	LOQ (ppm)	0.10	0.22	0.55	0.86	0.19
	Slope	0.79	0.72	0.77	0.75	0.74
	Intercept	-0.04	-0.05	-0.64	-0.51	-0.30
	R^2	0.999	0.998	0.996	0.996	0.996
Cyanidin-3-glucoside	LOD (ppm)	0.06	0.11	0.12	0.07	0.13
	LOQ (ppm)	0.22	0.38	0.41	0.25	0.45
	Slope	0.67	0.70	0.75	0.76	0.85
	Intercept	0.09	0.15	-0.03	-0.69	-2.00
	R^2	0.998	0.997	0.999	0.998	0.991

4.7. MS Analysis of Major Individual Phenolic Acids and Anthocyanins

Mass spectrometric data and identification of the polyphenols are given in Table 4.7.

Table 4.7 : Mass spectrometric data and identification of the polyphenols detected in the black carrot and by-products by LC-MS.

Retention time (min)	Identity	UPLC/DAD UV spectrum λ (nm)	Mode	Mass (m/z)	MS^2 main fragment(m/z)
8.97	Neochlorogenic acid	330	-	353	191
11.85	Cryptochlorogenic acid	330	-	353	191
12.4	Chlorogenic acid	330	-	353	191
15.42	Caffeic acid	330	-	179	135
17.57	cyanidin-3-xylosyl-glucosyl-galactoside	500	+	743	287
18.34	cyanidin-3-xylosyl-galactoside	500	+	581	287
18.98	cyanidin-3-sinapoyl-xylosyl-galactoside	500	+	949	287
19.61	cyanidin-3-feruloyl-xylosyl-galactoside	500	+	919	287
19.67	cyanidin-3-coumaroyl-xylosyl-galactoside	500	+	889	287
21.76	Ferulic acid	330	-	193	178

5. DISCUSSION

Short-chain fatty acids (SCFAs) are the major end products that are formed from anaerobic bacterial fermentation of indigestible carbohydrates in the colon. Digestion of peptides and proteins also increases SCFAs production. Mainly acetic, propionic and butytic acids are produced during colonic fermentation. Most procedures involve pretreatment of the fecal samples followed by gas chromatography (GC) (Cummings, 1995; Tangerman and Nagengast, 1996; Zhao *et al.*, 2005; Garcia-Villalba *et al.*, 2012).

Acetic acid was the most abundant SCFA for all samples after 24 hours (73%-78%) which is similar for literature studies on millet grains (50%); rye, wheat and oat bran (58%-60%). Acetate followed by propionate for samples (18%-23%), for millet grains (37%-41%) and butyrate for black carrot samples (3%-4%), for millet grains (9%-13%) (Shahidi and Chandrasekara 2012). Pomace had more acetate content (78%) compared to black carrot (73%) and peel (73%) due to its higher fermentable sugar concentration. Propionate also increased after 48 hours for black carrot and by-products (9%-16%) which is beneficial for health. Increased production of propionate may inhibit cholesterol synthesis (Hijova *et al.*, 2009). Butyrate has preventative effects against colon cancer and adenoma development as well as it stimulates immunogenicity of cancer cells (Hijova *et al.*, 2009). Butyrate production was increased as well as acetate, because bacteria isolated from human intestine are capable of utilizing acetate for butyrate production in the colon (Duncan *et al.*, 2002). In total acetic, propionic and butyric acids represented 97%-99% of SCFAs for black carrots which was also found by Sanchez- Patan *et al.* (2015) for more than 90% in cranberries. Valerate is formed from the condensation of propionate and acetate, whereas; caproate is formed from 3 acetate units or from butyrate by *Clostridium* and *Megaspaera* species (Gibson and Roberfroid, 1999).

After gastric digestion, significantly lower TPC (43%-71%) of black carrot and by-products were determined ($p< 0.05$). Our results were in accordance with other

studies performed by Tavares *et al.*, 2012, who observed a TPC reduction for blackberries (7%); Tagliazucchi *et al.* (2010), who determined around 30%-45% decreases for grape TPC of grapes and Bouayed *et al.* (2011), who reported 35% reduction for TPC of apples. Other studies performed on chokeberry, pomegranate, red cabbage, eight different fruit beverages, araticum and papaya extract, also observed reduction in TPC after gastric digestion (Perez-Vicente *et al.*, 2002; Bermudez-Soto *et al.*, 2007; McDougall *et al.*, 2007; Cilla et al., 2011; Pavan *et al.*, 2014). In addition to those studies Liang *et al.* (2012); found out that there was a decrease for TPC of mulberries around 39.4%. The lost in TPC during gastric conditions was lower than in TMA (51%-74%) capacity. Due to the modifications of anthocyanins to different phenolic compounds, TMA lost was higher (Sengul *et al.*, 2014).

After intestinal digestion, there was a decrease between 28-52% for TPC of black carrot and by-products; while it was around 62%-80% for TMA content. Those differences might be explained by additional time of extraction, effect of intestinal digestive enzymes on the food matrix, facilitating the release of phenolics bound to the matrix, the chemical environment of the gastro-intestinal tract, pH changes and interactions of phenolics with other dietary constituents released during digestion. Phenolic compounds could also bind to proteins in aqueous media through hydrogen bonding, covalent bonding or hydrophobic interactions thus making them unavailable for absorption (Tagliazucchi *et al.*, 2010; Bouayed *et al.*, 2011; Stanisavljevic´ *et al.*, 2015). Tagliazucchi *et al.*, 2010, observed 80% of decrease of TMA for grape samples which is similar to our results. In addition, the reason for the high loss of anthocyanins may be related to that they are metabolized to some non-colored forms, oxidized, or degraded into other chemicals, which may not be detected under the present conditions (Perez-Vicente *et al.*, 2002). The results of this study are compatible with other studies in which a low bioavailability of anthocyanins is described (Perez-Vicente *et al.*, 2002; McDougall *et al.*, 2005; Bouayed *et al.*, 2011). Lastly it was proven that acylated anthocyanins were more stable than nonacylated forms (McDougall *et al.*, 2005; Ryan and Prescott, 2010). The anthocyanin profile of black carrots that was identified with HPLC was consistent with those reported previously by Kammerer *et al.* (2003), Montilla *et al.* (2011), Algarra *et al.* (2014) and Suzme *et al.* (2014). In vitro simulated

gastrointestinal digestion system was followed by a colon fermentation to analyze the stability of anthocyanins and other polyphenols of black carrot and by-producs. For almost each sample there was a decrease in terms of TPC, TMA and TAC which are correlated with the results of the blueberry study (Correa-Betanzo *et al.*, 2014). For blueberry study; after gastric digestion anthocyanin levels dropped nearly 50%, after intestinal digestion it dropped nearly 60%-75% and 77%-78% during colonic fermentation compared to the initial undigested samples. For black carrot and by-products anthocyanin levels were reduced by 51%-73% for gastric, 62%-80% for intestinal and 57%-82% for colonic digestion. After in vitro digestion, a decrease in the content of chlorogenic acid was observed, whereas; an increase in the content of caffeic acid which is totally in accordance with blueberry study. Caffeic acid has been reported as the major product resulting from the hydrolysis of chlorogenic acid by colonic microbiota (Gonthier *et al.*, 2003; Correa-Betanzo *et al.*, 2014).

Black carrots are good sources of natural antioxidants. Antioxidant capacity of black carrots was determined by other researchers like Algarra *et al.* (2014) and Kamiloglu *et al.* (2015). Compared to our observations from DPPH and FRAP analysis, other results were significantly low, which might be explained by cultivar differences between samples, growth conditions or developmental stage of black carrots. DPPH, FRAP and CUPRAC results showed that there was a significant decrease for TAC (31%-69%) of black carrot and by-products; which are comparable with apple (Bouayed *et al.*, 2011), blackberry (Tavares *et al.*, 2012) and tomato juice (Wootton-Beard et al., 2011) studies.

6. CONCLUSION AND RECOMMENDATIONS

As far as we know, the current study is the first research dealing with bioavailability of *in vitro* gastrointestinal (GI) and colonic digestion of black carrot by-products. In this study; total phenolic, total monomeric anthocyanin and antioxidant capacities of *in vitro* digested black carrot, peel and pomace samples were discussed. To obtain more accurate results, HPLC and LC-MS analysis of individual phenolic compounds and anthocyanins were performed in addition to spectrophotometric analysis.

The results showed that black carrot and its by-products contain five major anthocyanin pigments, among them two are non-acylated (cyanidin-3-xylosyl glucosylgalactoside and cyanidin-3-xylosylgalactoside) and three of anthocyanins are acylated with sinapic acid (cyanidin-3-sinapoylxylosyl glucosylgalactoside), ferulic acid (cyanidin-3-feruloylxylosylglucosylgalactoside) and *p*-coumaric acid (cyanidin-3-*p*-coumaroyl xylosylglucosylgalactoside). HPLC analysis of individual phenolic compounds allowed determination of the detailed alteration of each compound as a black carrot, pomace and peel. For all three samples, the predominant phenolic compound was chlorogenic acid, followed by ferulic and cryptochlorogenic acid. Neochlorogenic, cryptochlorogenic and chlorogenic acid contents were decreased during in vitro digestion; whereas, ferulic and caffeic acid levels were increased. The highest loss (86%-90%) after digestion was observed in cyanidin-3-feruloylxylosyl glucosylgalactoside for all samples.

One of the major findings of this study was the high TP and TMA capacities and TAC of by-products. TP capacity of pomace is 28%; peel is 10% lower than black carrot; whereas TMA capacity of peel is 26% lower, pomace is 3% higher than black carrot. Percent recovery of total phenolics after simulated *in vitro* GI digestion were not significantly different from each other (36%-47%); whereas, recovery of total antioxidants was significantly different (53%-82%).

Therefore, in addition to *in vitro* studies, clinical studies investigating the bioavailability of those compounds would provide valuable data for elucidating the effect of food by-products on human health.

In conclusion, this study focused on polyphenol content, antioxidant activity and bioavailabilty of black carrot and by-products. Overall, it was found that black carrot, peel and pomace are good sources of polyphenols with high bioaccessibility levels. Although the results obtained with the model of simulated *in vitro* GI digestion cannot directly predict the human *in vivo* conditions, still this model is helpful for investigating the bioavailability of polyphenols.

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APPENDICES

APPENDIX A: Literature review on polyphenols contents of by-products

APPENDIX B: Calibration Curves

APPENDIX C: HPLC and SCFA Chromatograms

APPENDIX D: Anova Tables

Table A.1 : Literature review on polyphenol contents of by-products*.

Material	Process	By-product	Results	Reference
Apple	Manual peeling	-Peel -Flesh	Peel had significantly higher amounts of simple phenols (75%) and flavonoids (66%) compared to flesh. Flesh did not contain any ACY. The shaded peel contained more simple phenols (36.4%), less ACY (52%) and less flavonoids (12.5%) than sun exposed peel.	Ju et al., 1996
Apple	Manual peeling	-Peel	Peel had higher content of ACY (70.7%), TPC (54.0%) and flavonols (70.4%) compared to whole fruit.	Lata, 2007
Apple	Apple cider and juice production	-Freeze (FD) and vacuum belt (VD) dried (at 80-95-110^{o}C) pomace	VD pomace at 80^{o}C had 1.5-65.7% higher levels of TPC compared to VD pomace at 95^{o}C and 110^{o}C and FD fresh apple and pomace. VD80 pomace had 12.2% higher amount of ACYs than VD95, 39.2% higher than VD110, 81.1% higher than FD fresh apple and 9.46% higher than FD apple pomace.	Yan and Kerr, 2012
Apple	Manual peeling	-Peel	Sun-exposed peel was richer in phenolic acids (16.9%), flavonols (74.6%) and ACYs (97.9%); whereas shaded peels had 1.49% higher concentrations of flavanols.	Feng et al., 2013
Black Chokeberry	Juice production	-Pomace	ACYs were the second largest group that was present in pomace among the total polyphenols (7.7-9.3%). Cyanidin-3-galactoside (60.4%) and cyanidin-3-arabinoside (31.6%) were the main ACYs. Chokeberry pomace had relatively high amounts of ACYs compared to cranberry (88.4%), red grape (89.7%) and blueberry (85.5% higher) pomace.	Sojka et al., 2013
Blueberry	Juice production	-Pomace (stems, skins and seeds)	Extrusion had an adverse effect on total ACYs (33-42% reduction). Temperature, screw speed, or their interaction had no significant effect on total ACY content.	Khanal et al., 2009

Table A.1 : (Continued) Literature review on polyphenol contents of by-products*.

Caneberry (blackberryand raspberry)	Manual pressing	-Press residues (in laboratory conditions)	Cyanidin-3-glucoside was the most prevalent ACY component (87%) in press residues. Fresh blackberries were richer in ACYs compared to fresh raspberries (69.3%). Similarly, press residues of blackberries were also richer in ACYs (58.6%).	Saponjac et al., 2013
Ceylon Gooseberry	Manual pressing	-Pulp	Pulp had 92.5% and 92.9% lower amounts of TPC and ACY compared to fresh sample. Ceylon gooseberry has a potential to be used as a source of ACY with industrial purposes, since it can be extracted with pure water.	Bochi et al., 2014
Cranberry	Juice production	-Pomace (skins and seeds)	Cyanidin 3-arabinoside was the most prominent ACY (59.1%) in cranberry pomace. ACY concentration in pomace was decreased around 71.5% compared to fresh cranberries.	White et al., 2010
Grape	Wine production	-Pomace	Nerello Cappuccio grape pomace had the highest concentration of TPC (43.8%) and TMA (45.3%) compared to other four varieties (Nero d'Avola, Nerello Mascalese, Frappato and Cabernet Sauvignon); whereas Nero d'Avola had the highest antioxidant activity (30.5%).	Ruberto et al., 2007
Grape	Manual peeling	-Skin	Malvidin-derivatives were the most common (25.6-30.7%) ACYs in majority of samples. Malvidin 3-*O*-glucoside was found as main ACY in table and wine grapes of Vitis vinifera (37.6%). Wine grapes had higher content of ACYs compared to table grapes (75%).	Liang et al., 2008
Grape	Manual peeling	-Skin -Seeds	TPC of seeds were 46.9% higher than skins. Among the grape skins, Shiohitashibudou cultivar had the highest TPC (18.7%) and ACYs (26%); whereas Muscat of Alexandria seeds had the highest content of TPC (39.6%).	Poudel et al., 2008
Grape	Manual peeling/ pressing	-Peel -Seed -Pulp	Purple-skinned grapes had 97% higher amounts of ACYs than bronze-skinned grapes. Skins of purple grapes had 62.1% higher ACY than whole fruit. ACYs were unevenly distributed in different parts of muscadine grapes; they were accumulated in grape skin, while the levels in pulp and seed were almost negligible.	Huang et al., 2009

Table A.1 : (Continued) Literature review on polyphenol contents of by-products*.

Grape	Manual pressing	-Pomace (stems, skins and seeds) -Seeds	Extrusion process reduced total ACYs in pomace by 18-53%. Both temperature and screw speed, but not their interaction, affected total ACY content. The total ACY content decreased when temperature increases.	Khanal et al., 2009
Grape	Juice Production	-Seed and pulp (for white and red grapes)	Seeds of red grapes had higher amounts of TPC (36.5%) and ACY (57.6%) compared to red grape pulp. Phenolic content of white grape seeds were 31.1% richer than white grape pulp; whereas pulp had 50% higher amounts of ACYs.	Spanghero et al., 2009
Grape	Wine production	-Skins of white (two cultivars) and red (three cultivars) grapes	ACYs were not detected in the white grape skins. For the red skinned grapes; Merlot had higher concentrations of ACYs (30.6-78.1%) compared to the other two cultivars (Cabernet Sauvignon, Pinot Soir). TPC of white grape cultivars were significantly lower (43%) compared to red ones. Antioxidant capacity of red grapes was higher than white ones (36.3%).	Deng et al., 2011
Grape	Wine production	-Pomace (of red grapes)	Cabernet Sauvignon grape pomace was found to have higher content of TPC (15.3-56.3%), antioxidant activity (26.8-62.8%) and reducing power (16.4-52.8%) compared to other varieties (Merlot, Bordeaux and Isabel). Bordeaux had the highest content of total ACYs (37.4-64.8% higher than other varieties).	Rockenbach et al., 2011
Grape	Wine making	-Pomace (skin and seed)	Seeds of grapes were found to be richer than skins by means of TPC (87.5%) and antioxidant activity (76.5%). Skins of Cabernet Sauvignon had higher ACYs (10.9-67.7%) compared to the other 6 species. In seed extract ACYs were not detected.	Rockenbach et al., 2011
Grape	Manual peeling	-Skin	Antioxidant capacity of Noble grape skin was enhanced by 18-38% after exogenous abscisic acid treatment. TPC and ACY content of treated samples were 30% and 39-51% higher than control samples, respectively. Cyanidin-3-5 diglucoside had the highest increase (63%).	Sandhu et al., 2011

Table A.1 : (Continued) Literature review on polyphenol contents of by-products*.

Grape	-Juice making -Wine making	-Pomace -Fresh skin	The skin of Cabernet Sauvignon had 65.3% higher ACY content compared to Kyoho skin. However, Kyoho pomace had 32.2% higher ACYs than C. Sauvignon pomace. Wine and juice processing decreased the ACY content of C. Sauvignon and Khoyo pomace by 95.4% and 80.5%, respectively. Juice-making pomace is a better source of ACYs than winemaking pomace.	Li et al., 2013
Grape	Wine making	-Skin (from grape marc) -Lees	Lees had 61.93% higher amounts of flavonols compared to fresh grape; whereas skin had 46.51% more than fresh ones.	Barcia et al., 2014
Grape	Wine making	-Pomace	The Cabernet Sauvignon pomace had the highest TPC (26.2-40.9%) and ACY content (24.7-40.4%) compared to other two grape varieties (Merlot and Tanat).	Iora et al., 2014
Grape	Wine making	-Pomace	Spray-dried pomace at 130-170^{o}C had higher TPC (43.5-85.8%), ACY (47.51-81.3%), and antioxidant activity (40.3-89.6%) than freeze dried pomace. ACYs of samples dried at 130^{o}C with 20% carrier were more stable (20.4% loss) compared to the other drying techniques (23.4-48.0% loss).	Souza et al., 2014
-Grape -Blueberry	Manual pressing	-Pomace (stems, skins and seeds)	For unheated samples, blueberry pomace had 28.9% higher levels of ACYs compared to grape pomace. Total ACY contents of both pomaces were decreased when heating temperature is increased from 70 to 125^{o}C (32-87% and 64-90% for grape and blueberry pomace, respectively). Heating at 40^{o}C up to 3 days was not harmful to ACYs.	Khanal et al., 2010
Mango	Manual peeling	-Peel -Seed	Non dried (ND) peel had 63.3% higher amount of phenolics compared to ND seed; whereas freeze dried (FD) peel had 22.1% higher than FD seed, static air dried (OS) peel had higher 8.4% than OS seed and forced air dried (OF) peel had 44% higher compared to OF seed. According to DPPH results, ND peel had 59.5% higher antioxidant activity than ND seed; whereas FD peel was 2.8% higher than FD seed, OS peel 50%	Dorta et al., 2012

Table A.1 : (Continued) Literature review on polyphenol contents of by-products*.

			greater than OS seed and OF peel 74% higher than OF seed. According to ABTS results, ND seed had 50% lower antioxidant activity than ND skin, 72% lower results for OS seed compared to OS skin and 37.4% lower results for OF seed than OF skin; however, FD seed had 7.81% higher than FD skin. FD skin had the highest (28.8-47.4%) ACY concentration.	
Orange	-Fresh juice production -Citrus based drinks	-Peels -Leaves	Peels of Bigarade cultivar had the highest (25.6%) concentration of TPC which was followed by Thomson (20.7%). Leaves of Bigarade had the highest TPC (30.4%). Bigarade had strong antioxidant activity (DPPH scavenger, slows (77%) the rate of oxidation of linoleic acid and inhibition of β-carotene bleaching). Leaves of oranges had 15.4% higher TPC compared to peels of oranges. Peels of Sanguinelli had the highest (43%) levels of ACY followed by Double fine (36.9%) and Portugaise (20%), the rest had no ACY.	Lagna-Benanmrouche and Madani, 2013
Raspberry	Juice production	-Pomace	Total phenolic content of Willamette cultivar was 62.4% higher than Meeker, whereas; Meeker had 64.84% higher amounts of ACYs. Both pomaces exhibited good DPPH radical-scavenging activity.	Cetojevic-Simin et al., 2014
Sour cherry	Cherry juice production	-Pomace	Compared to results from literature; antioxidant activity of the sour cherry pomace had 3 times lower than black currant pomace.	Kolodziejczyk et al., 2013
Tropical fruits from Brazil	Fruit processing plants	-Pulp -By-products (pulp's leftovers, seed and peel)	In total, ACY content and TPC of pulps were higher (68% and 27.2%, respectively) than their by-products. Surinam cherry by-products had the highest levels of ACYs (58.5%); whereas, acerola pulp had the highest TPC (%34.5).	Silva et al., 2014

*ACY: Anthocyanin, DPPH: 1,1-Diphenyl-2- picrylhydrazyl, TMA: Total monomeric anthocyanin content, TPC: Total phenolic content

APPENDIX B: Calibration Curves

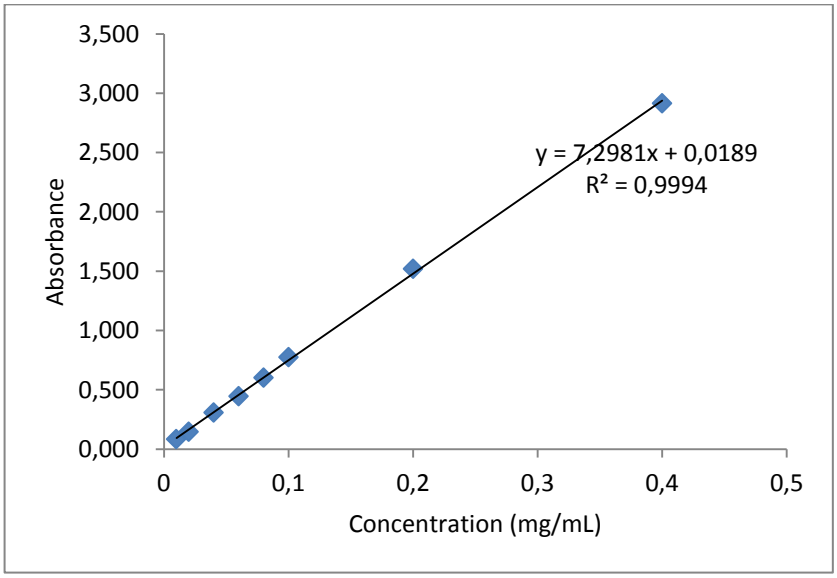

Figure B. 1 : Calibration curve for total phenolics in methanol (for bioaccessible).

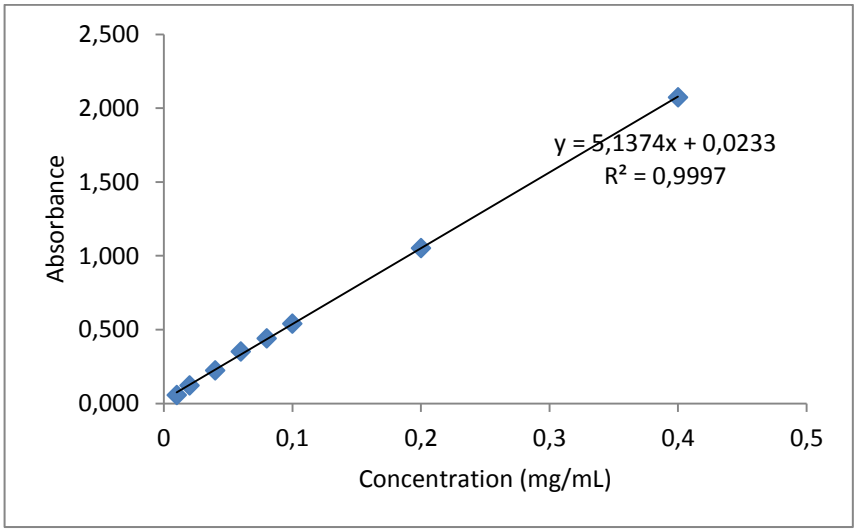

Figure B. 2 : Calibration curve for total phenolics in water (for bioaccessible).

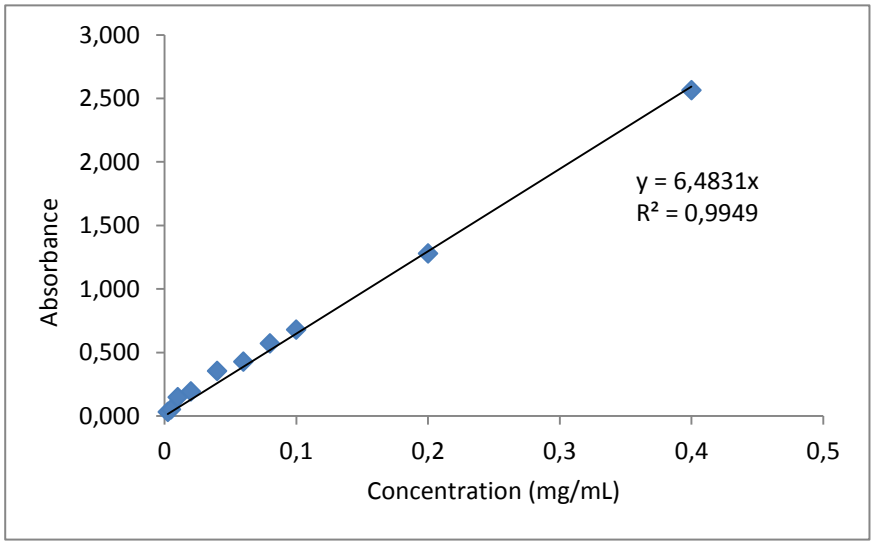

Figure B. 3 : Calibration curve for total phenolics in methanol (non-bioaccessible).

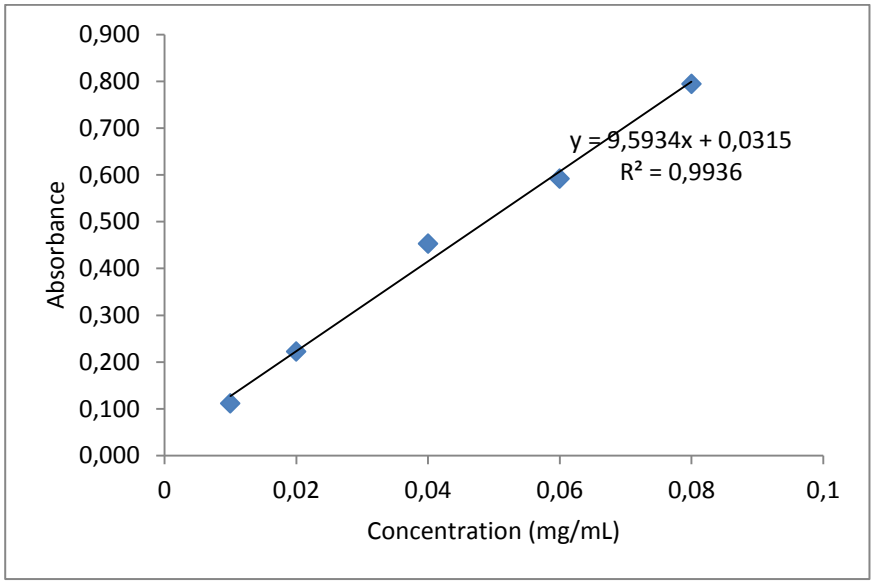

Figure B. 4 : Calibration curve for ABTS assay in methanol (for bioaccessible).

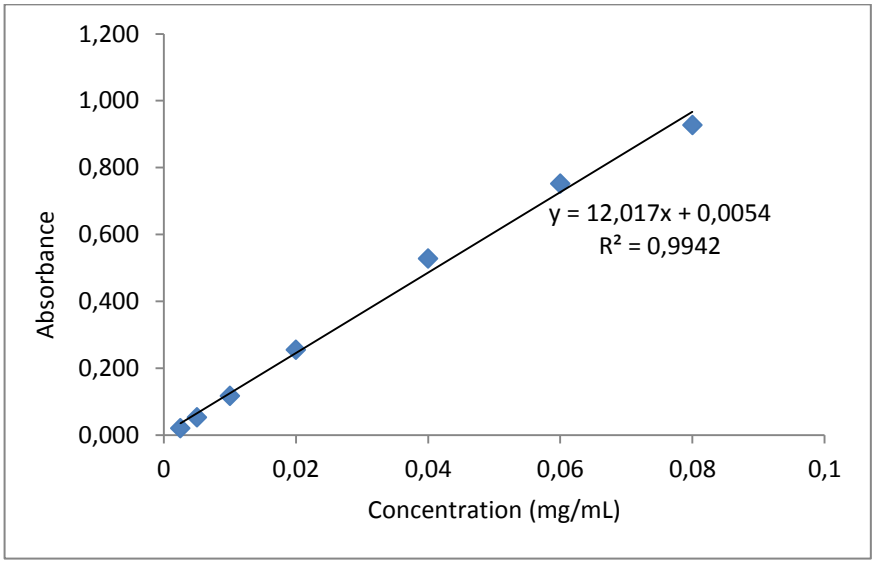

Figure B. 5 : Calibration curve for ABTS assay in methanol (for nonbioaccessible).

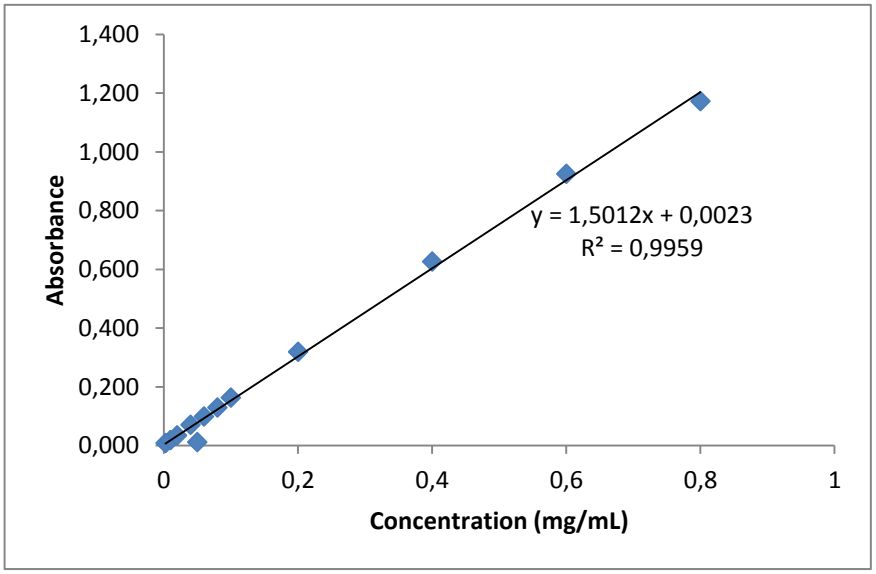

Figure B. 6 : Calibration curve for CUPRAC assay in methanol (for bioaccessible).

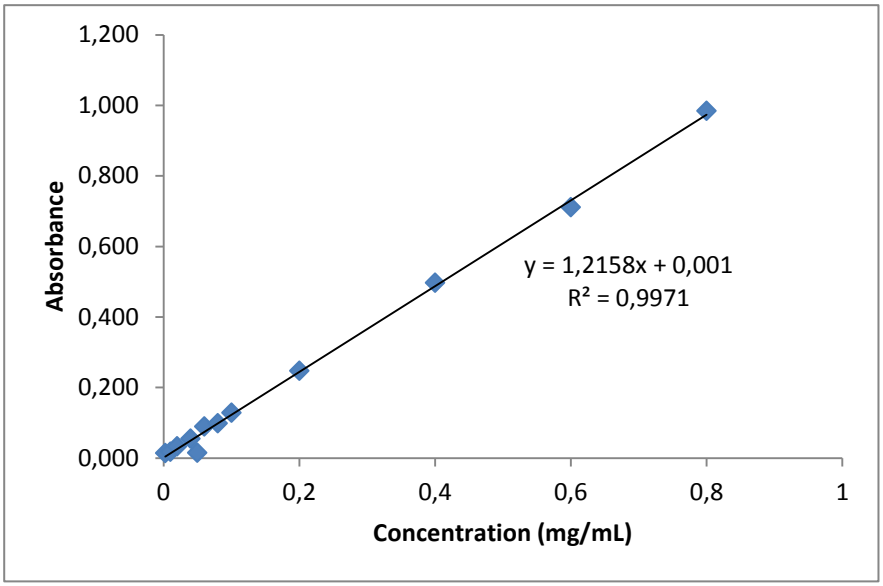

Figure B. 7 : Calibration curve for CUPRAC assay in methanol (for nonbioaccessible).

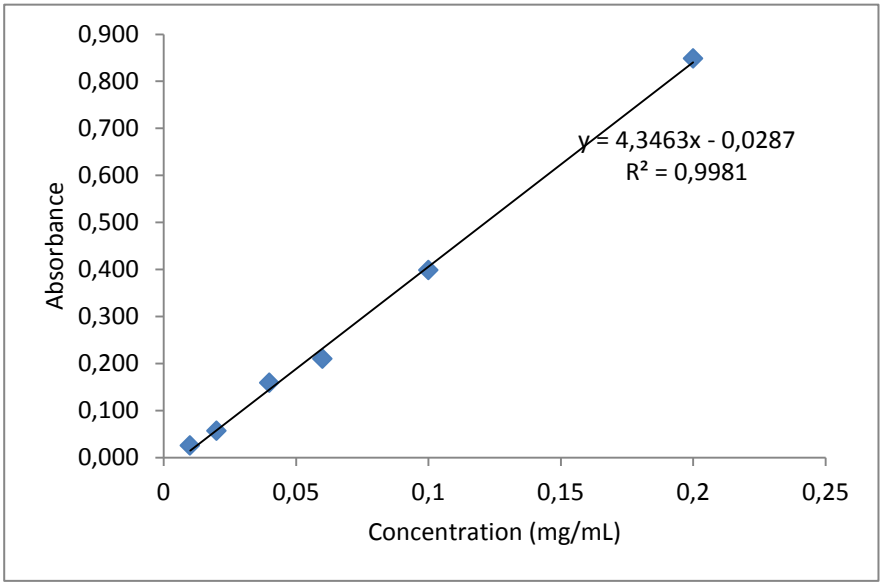

Figure B. 8 : Calibration curve for DPPH assay in methanol (for bioaccessible).

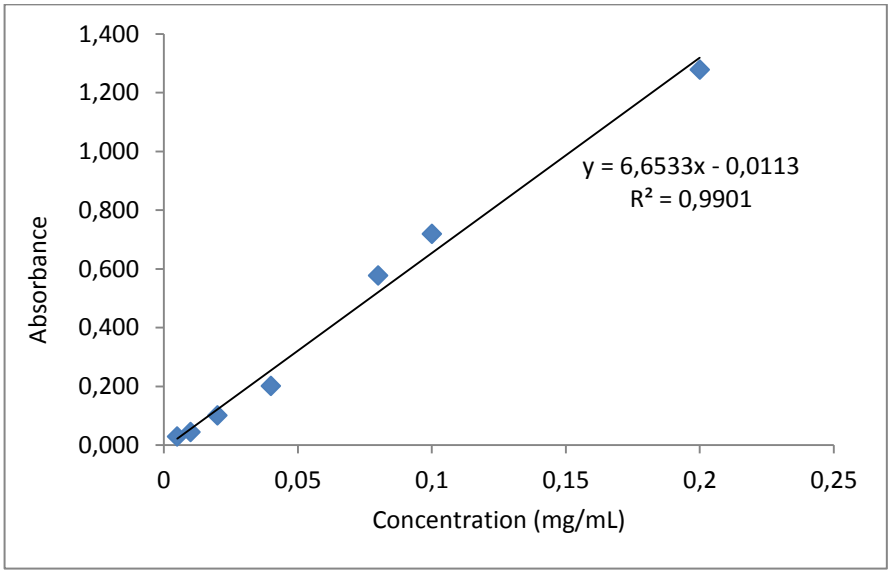

Figure B. 9 : Calibration curve for DPPH assay in methanol (for nonbioaccessible).

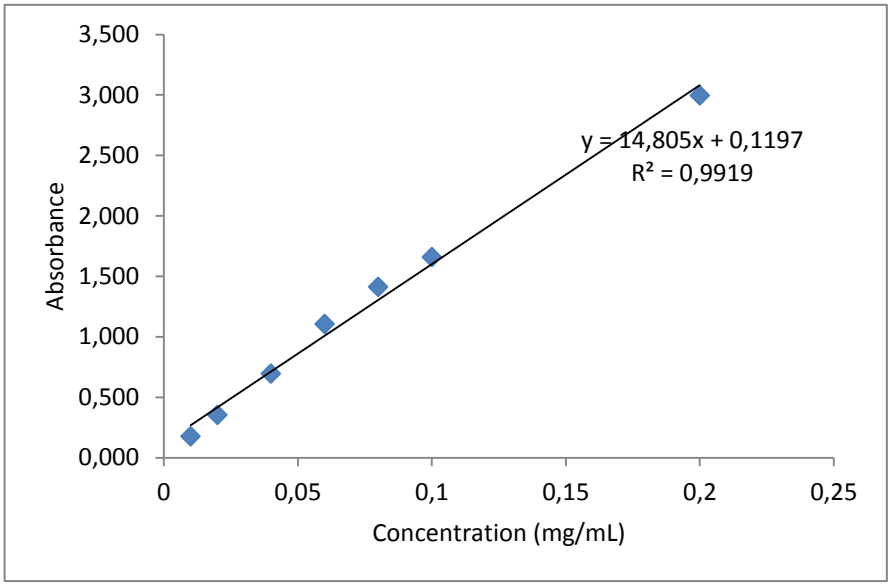

Figure B. 10 : Calibration curve for FRAP assay in methanol (for bioaccessible).

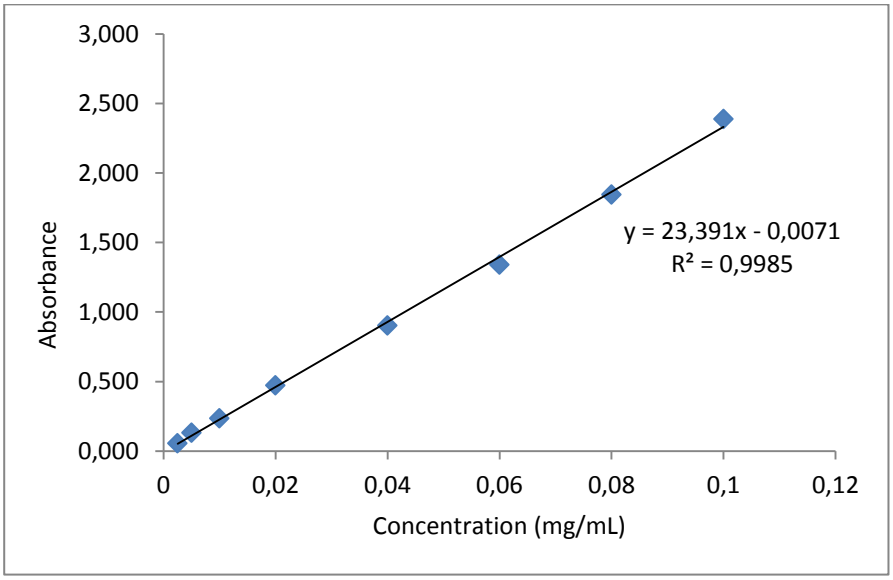

Figure B. 11: Calibration curve for FRAP assay in methanol (for nonbioaccessible).

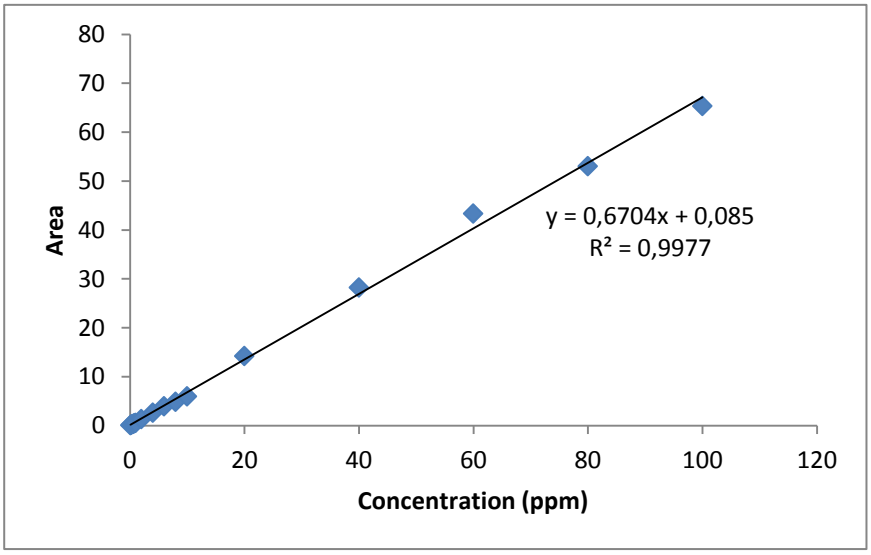

Figure B. 12 : Cyanidin-3-glucoside calibration curve- Undigested.

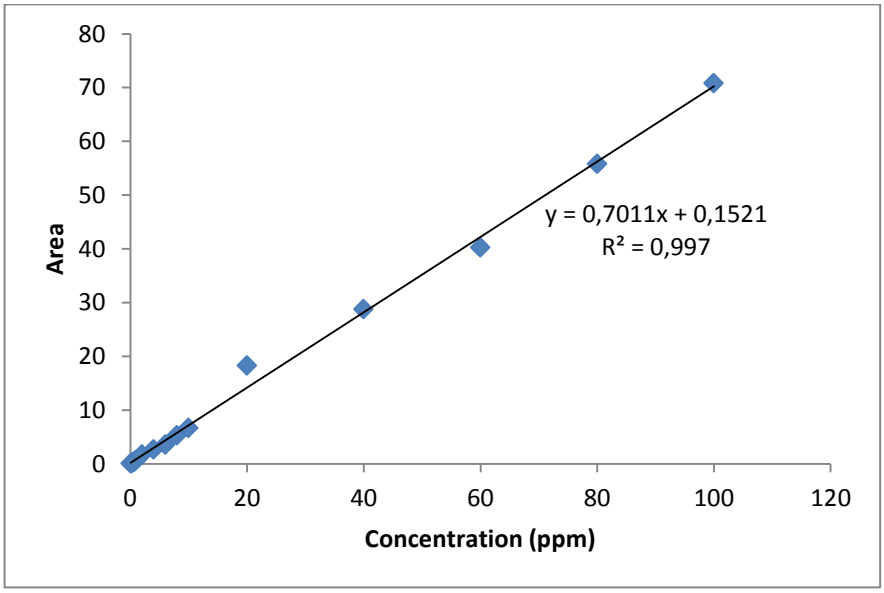

Figure B. 13 : Cyanidin-3-glucoside calibration curve- Gastric.

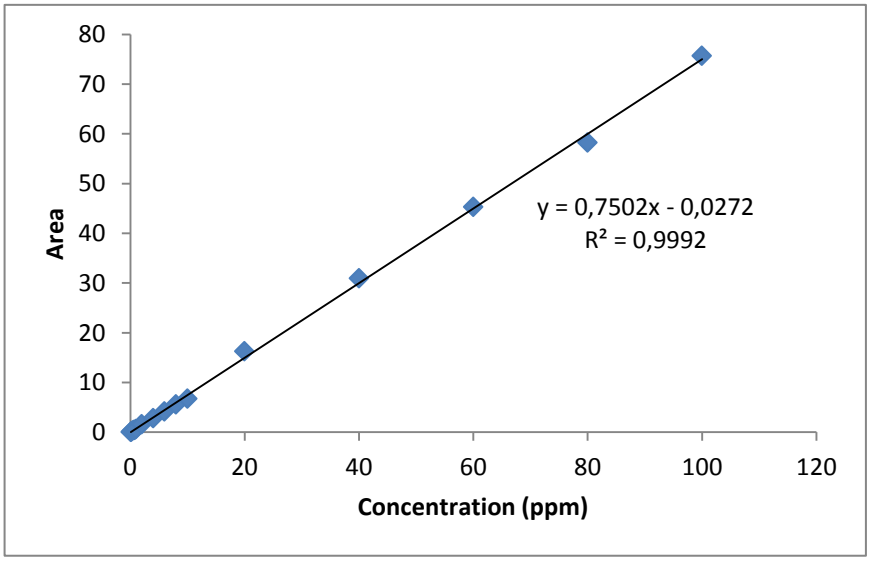

Figure B. 14 : Cyanidin-3-glucoside calibration curve- Small Intestine.

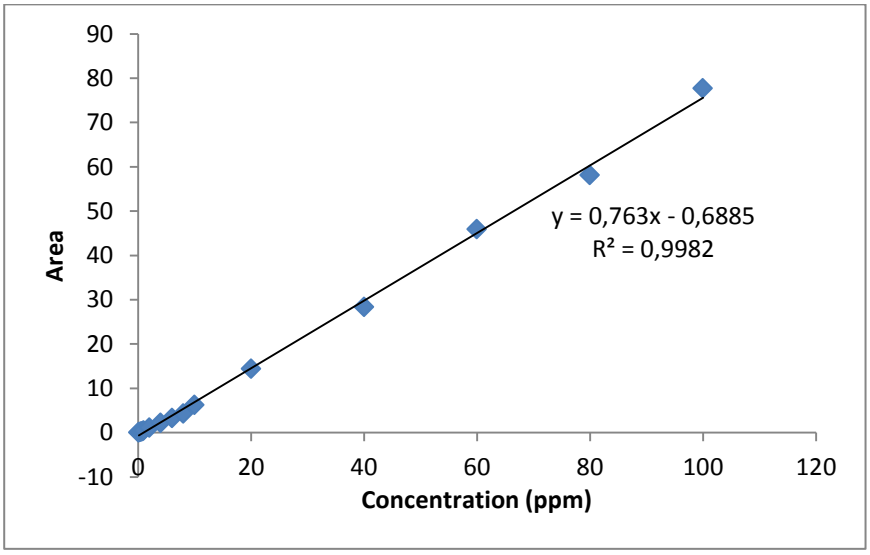

Figure B. 15 : Cyanidin-3-glucoside calibration curve- Colon 24 hour.

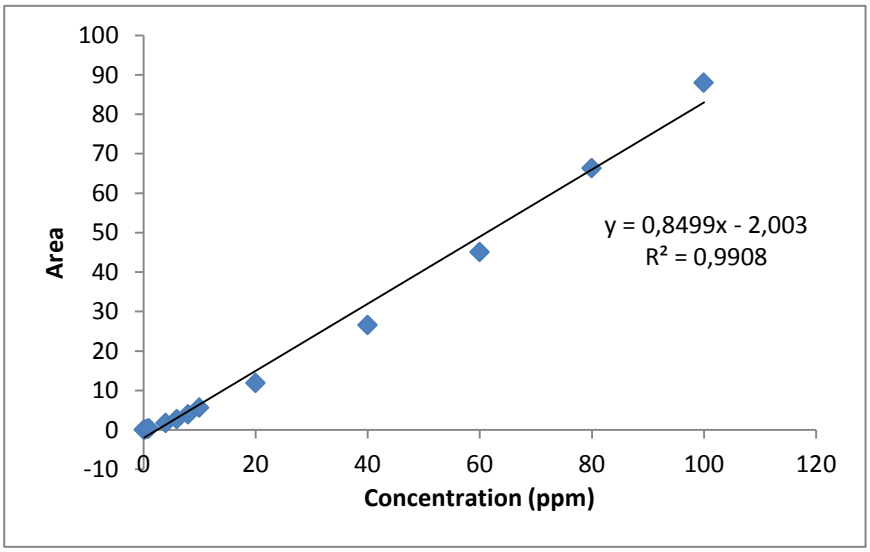

Figure B. 16 : Cyanidin-3-glucoside calibration curve- Colon 48 hour.

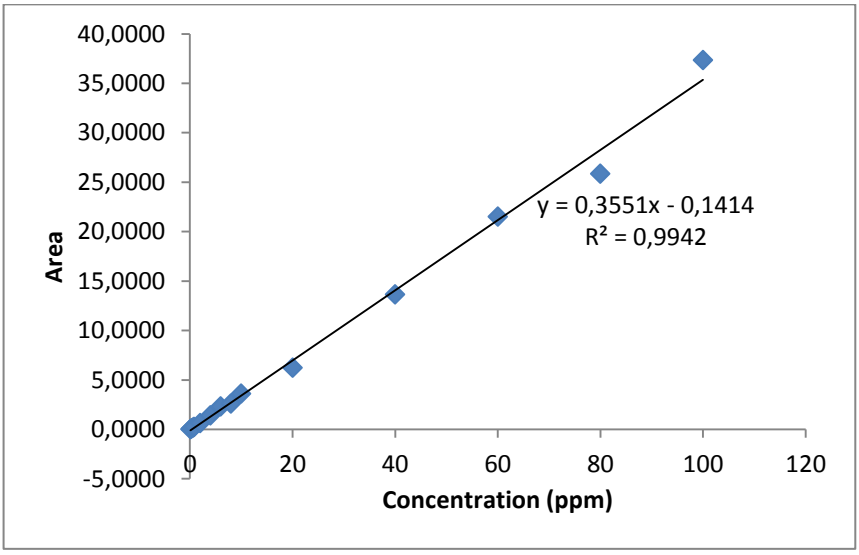

Figure B. 17 : Neochlorogenic acid calibration curve- Undigested.

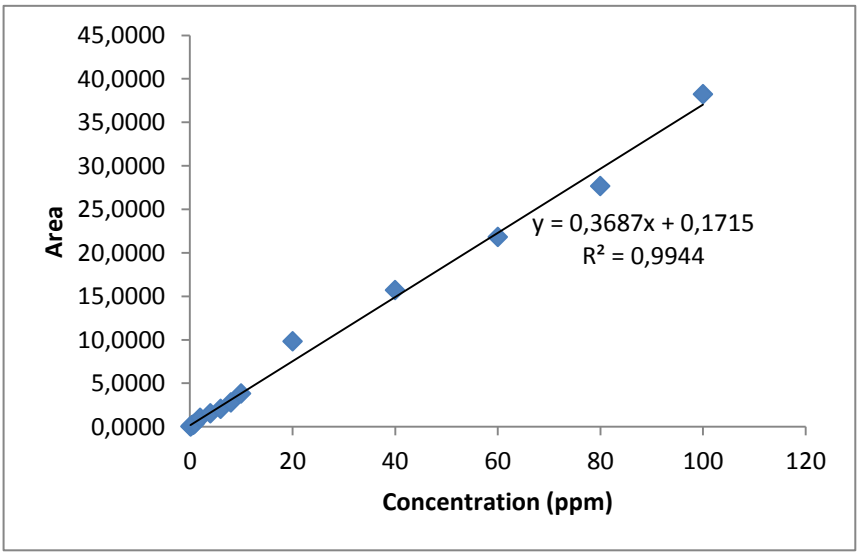

Figure B. 18 : Neochlorogenic acid calibration curve- Gastric.

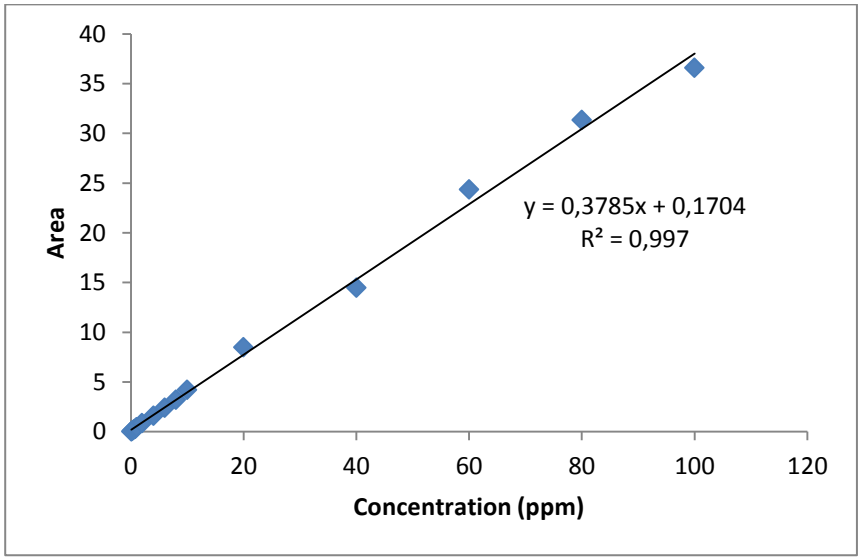

Figure B. 19 : Neochlorogenic acid calibration curve- Small Intestine.

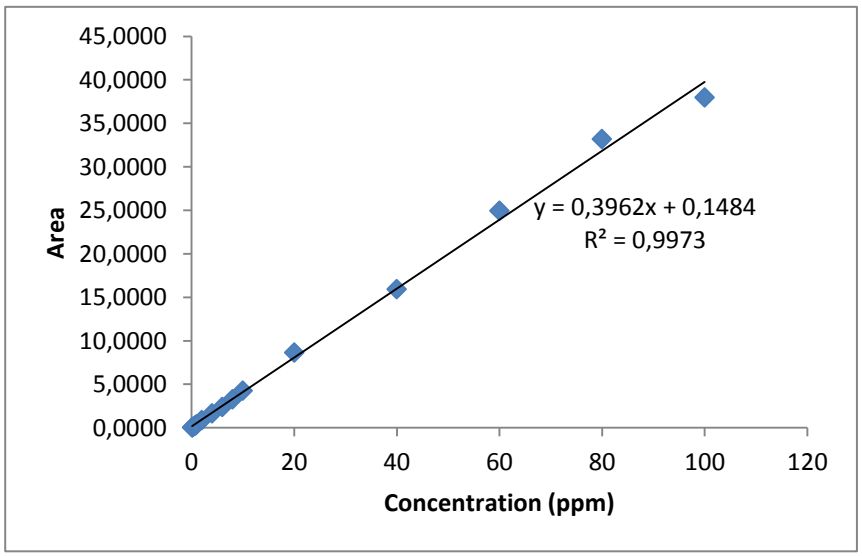

Figure B. 20 : Neochlorogenic acid calibration curve- Colon 24 hour.

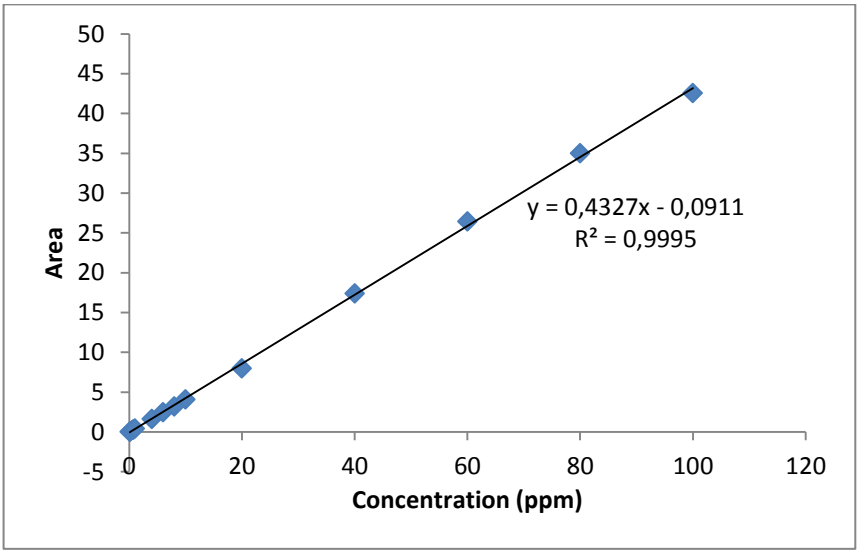

Figure B. 21 : Neochlorogenic acid calibration curve- Colon 48 hour.

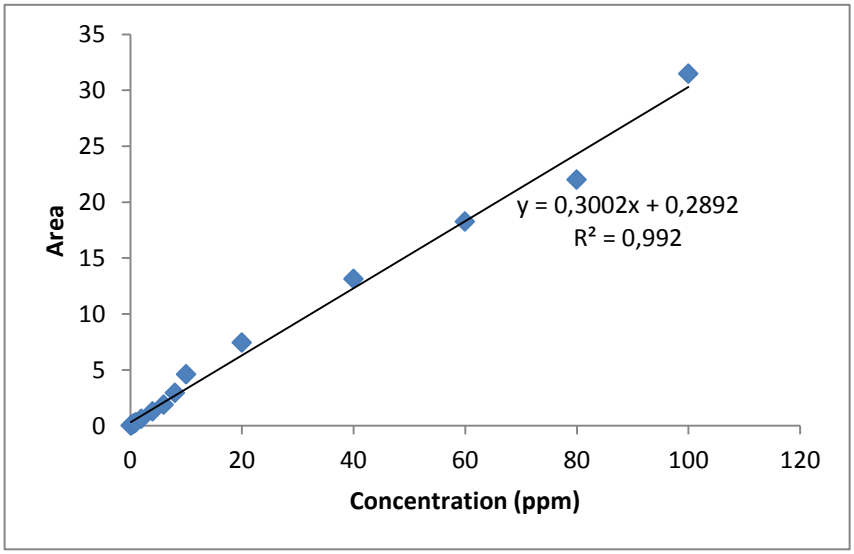

Figure B. 22 : Cryptochlorogenic acid calibration curve- Undigested.

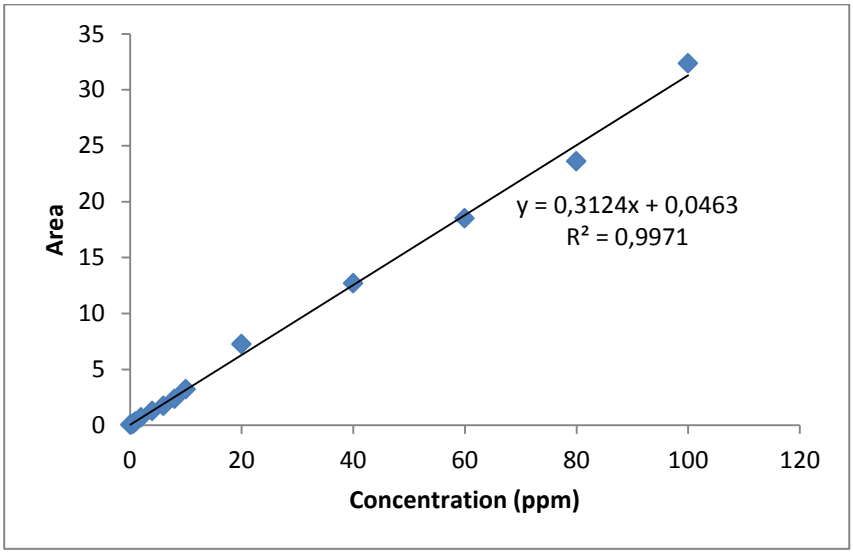

Figure B. 23 : Cryptochlorogenic acid calibration curve- Gastric.

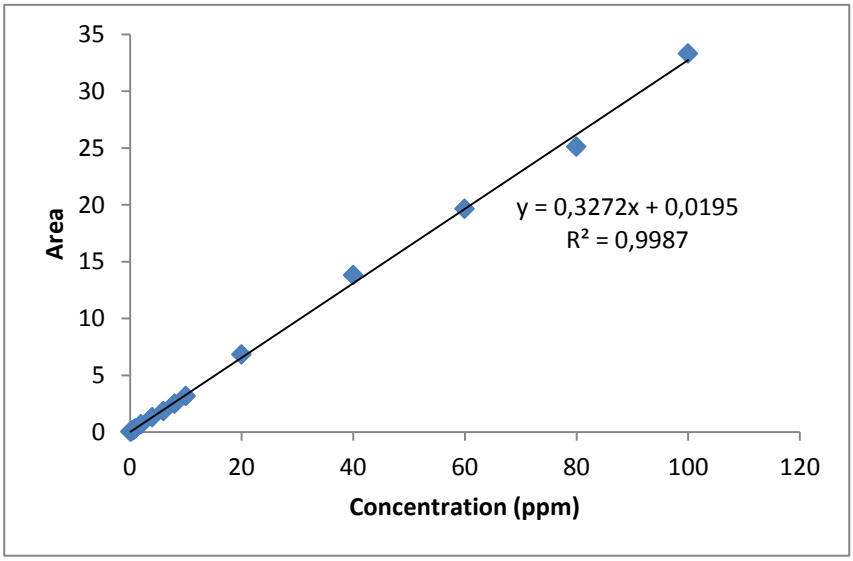

Figure B. 24 : Cryptochlorogenic acid calibration curve- Small Intestine.

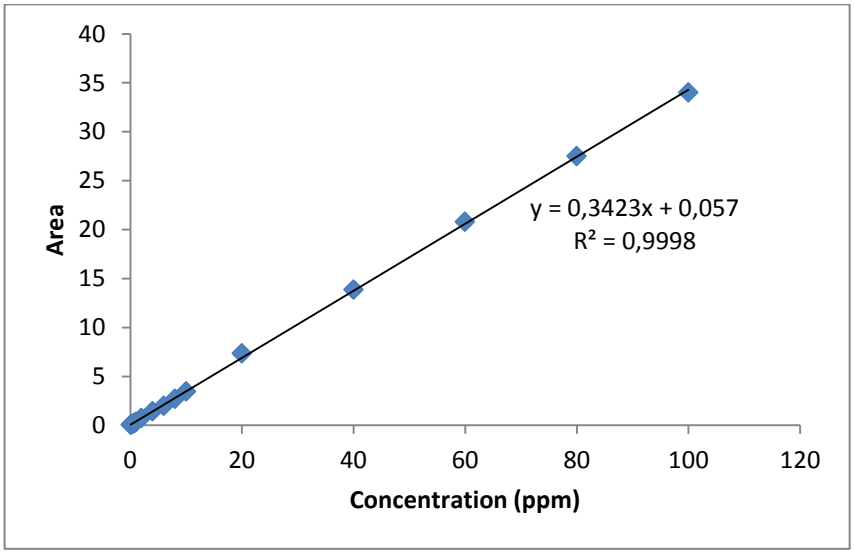

Figure B. 25 : Cryptochlorogenic acid calibration curve- Colon 24 hour.

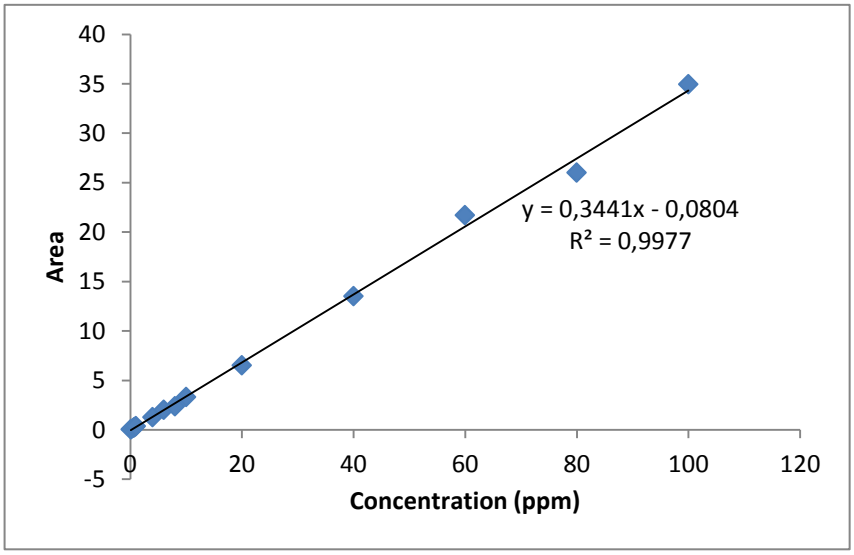

Figure B. 26 : Cryptochlorogenic acid calibration curve- Colon 48 hour.

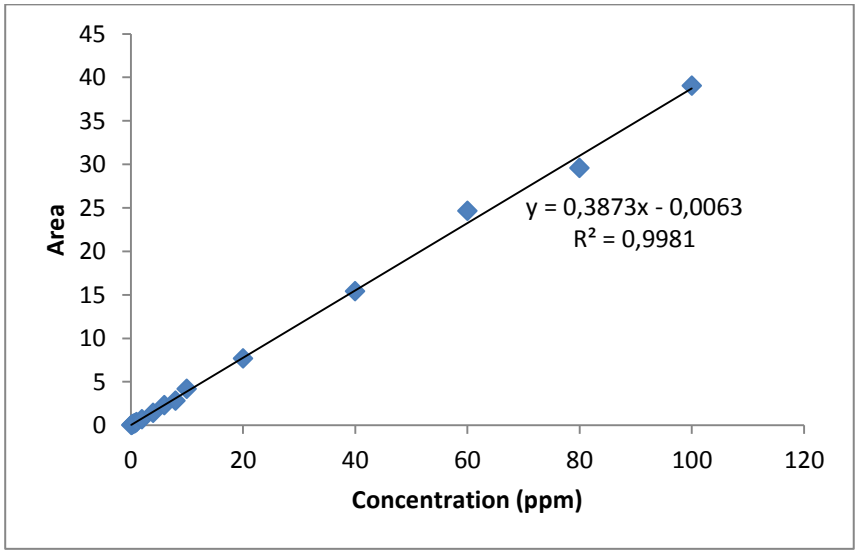

Figure B. 27 : Chlorogenic acid calibration curve- Undigested.

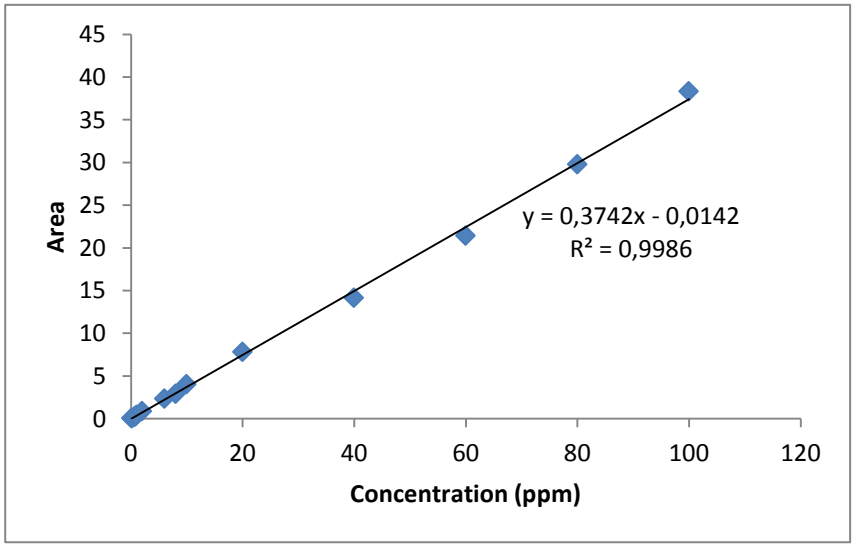

Figure B. 28 : Chlorogenic acid calibration curve- Gastric.

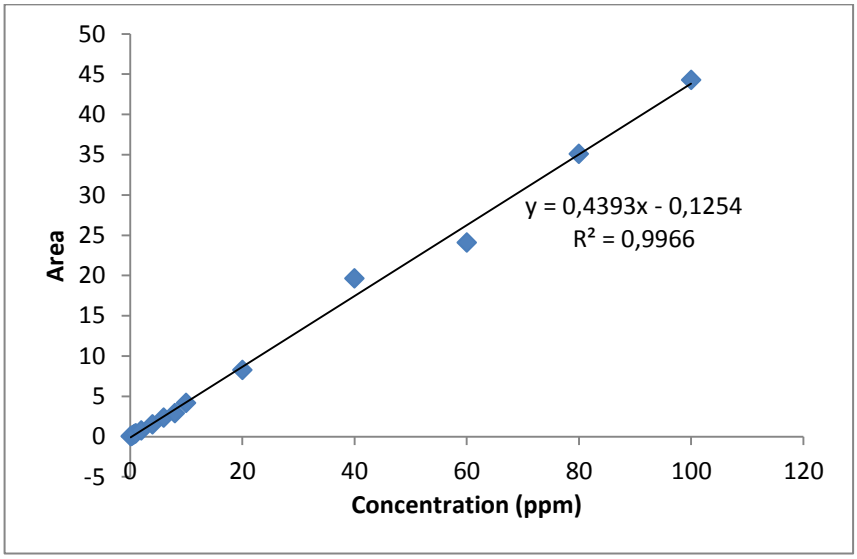

Figure B. 29 : Chlorogenic acid calibration curve- Small Intestine.

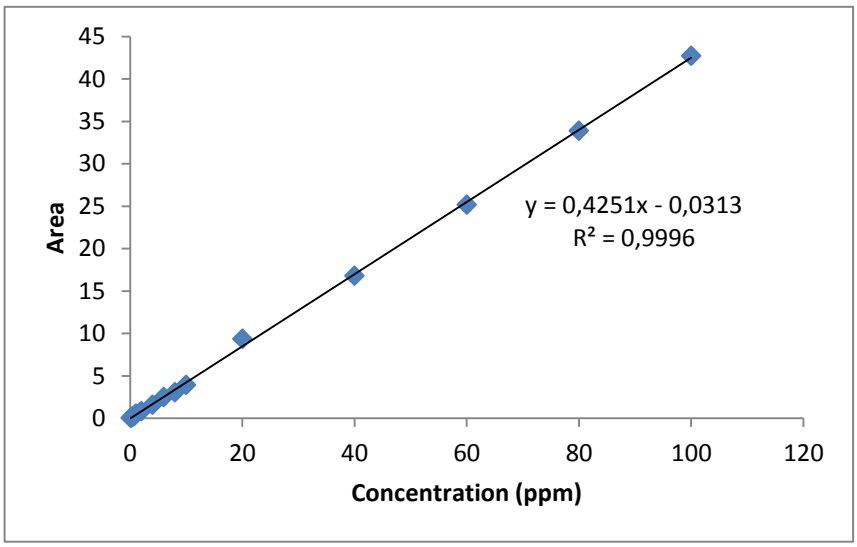

Figure B. 30 : Chlorogenic acid calibration curve- Colon 24 hour.

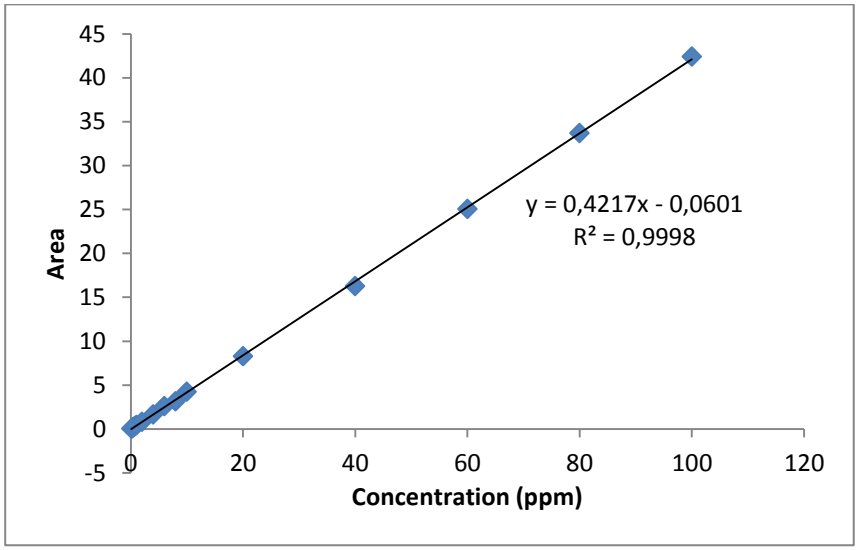

Figure B. 31 : Chlorogenic acid calibration curve- Colon 48 hour.

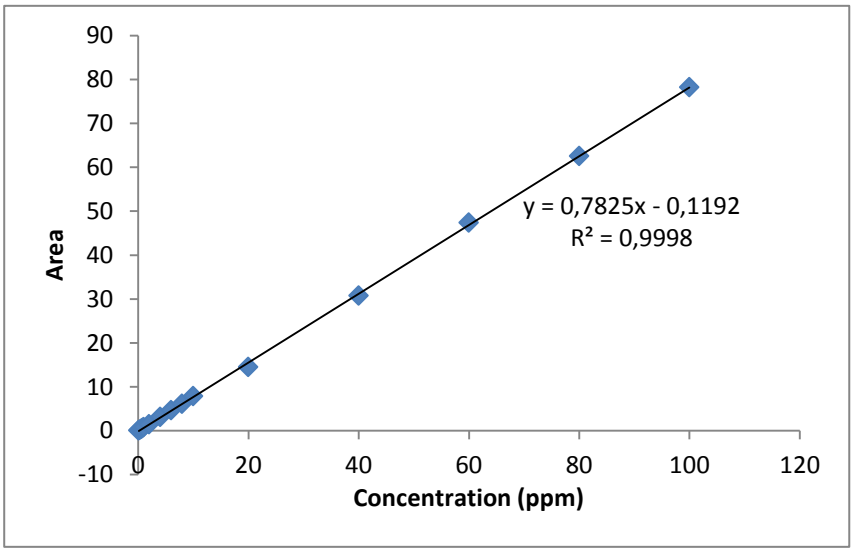

Figure B. 32 : Caffeic acid calibration curve- Undigested.

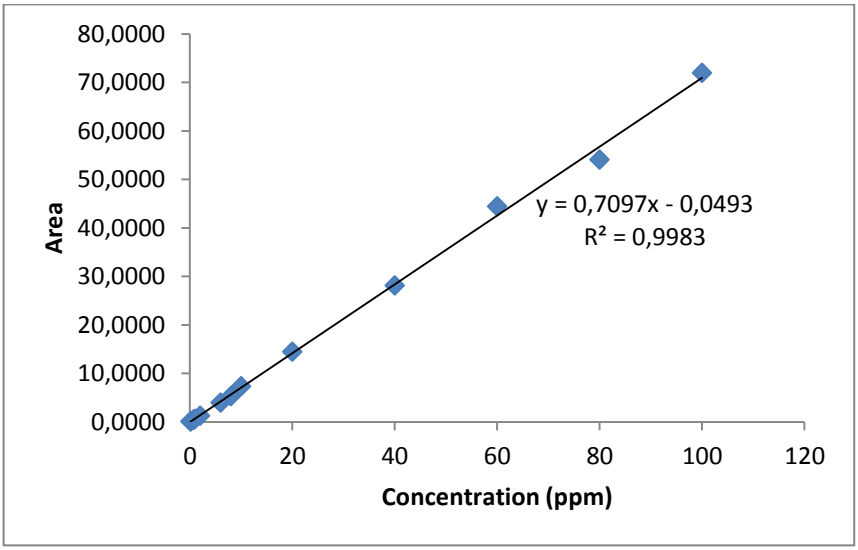

Figure B. 33 : Caffeic acid calibration curve- Gastric.

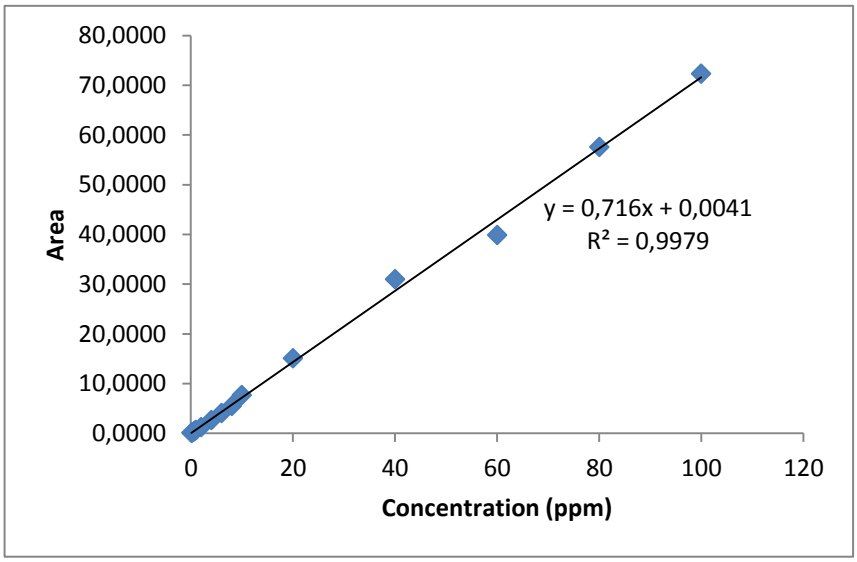

Figure B. 34 : Caffeic acid calibration curve- Small Intestine.

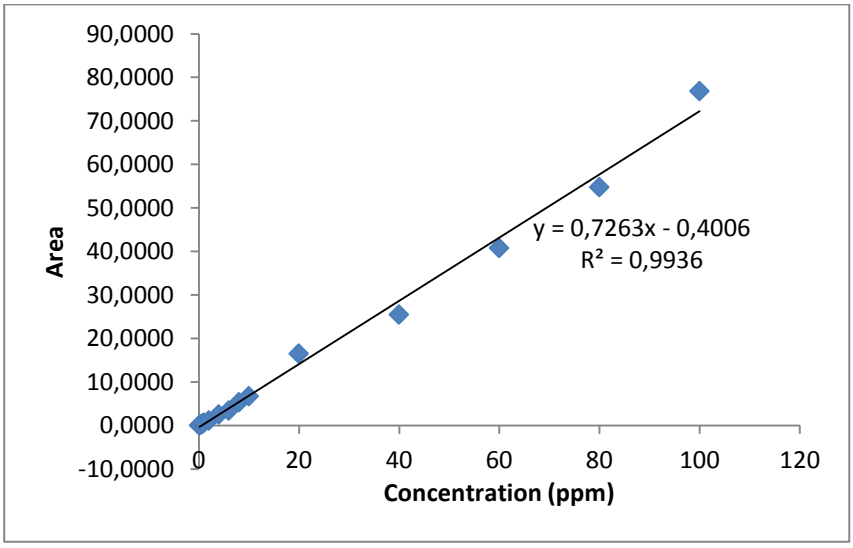

Figure B. 35 : Caffeic acid calibration curve- Colon 24 hour.

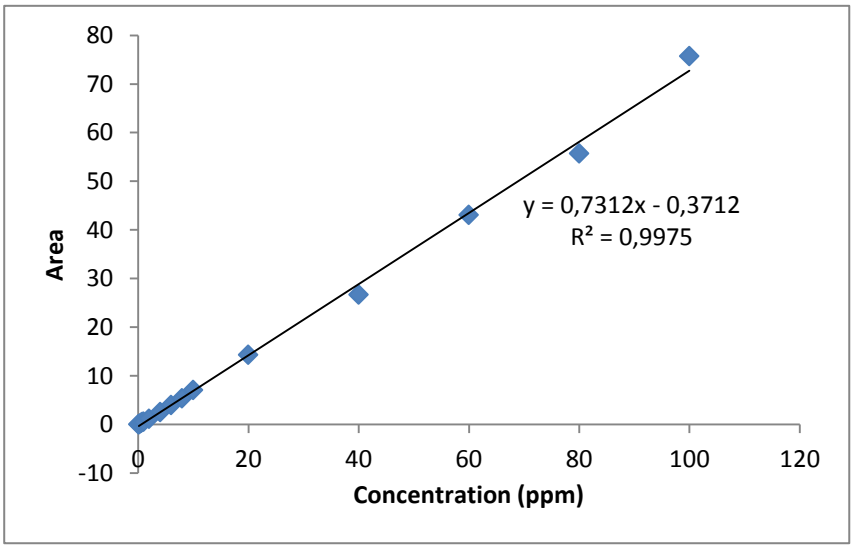

Figure B. 36 : Caffeic acid calibration curve- Colon 48 hour.

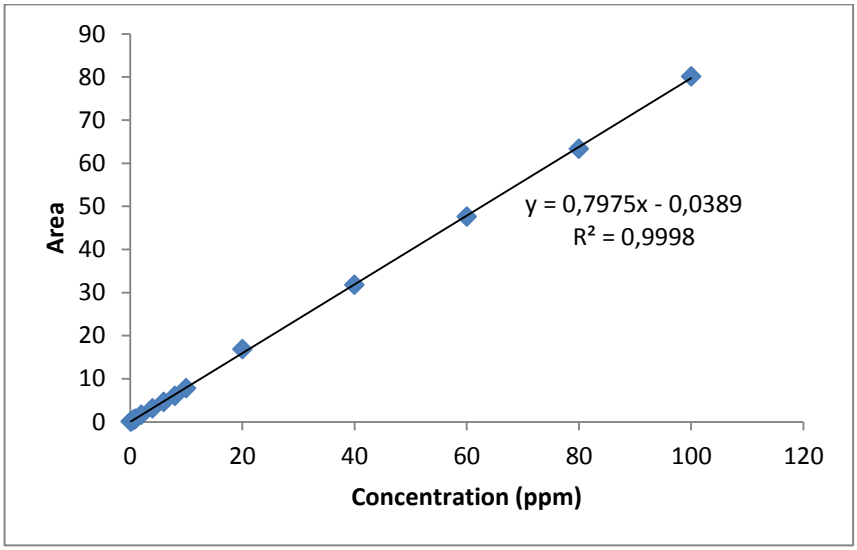

Figure B. 37 : Ferulic acid calibration curve- Undigested.

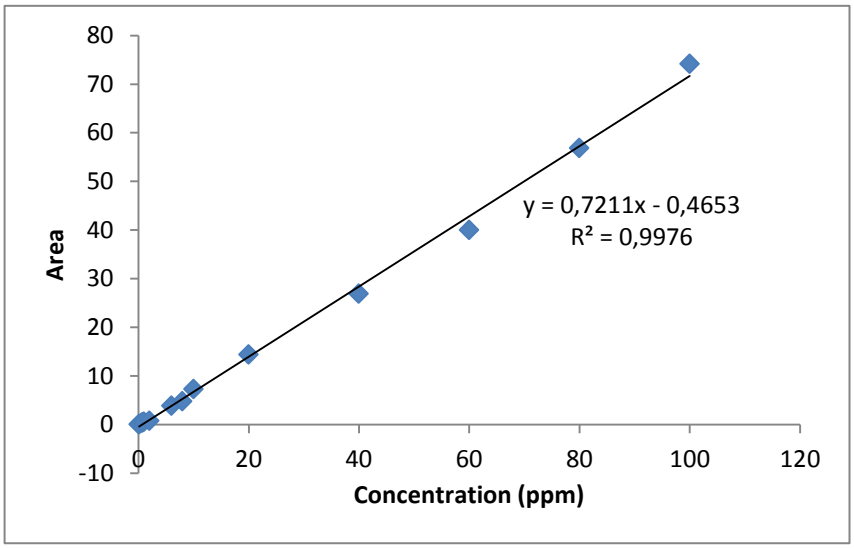

Figure B. 38 : Ferulic acid calibration curve- Gastric.

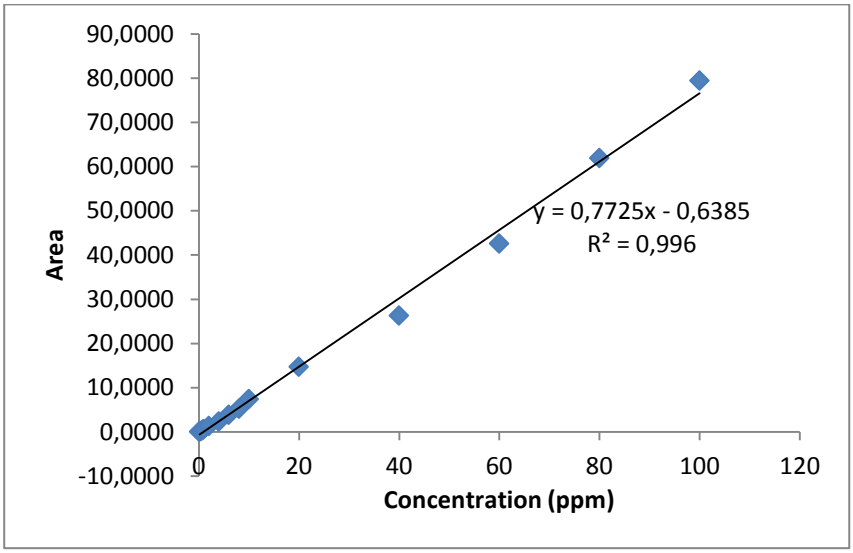

Figure B. 39 : Ferulic acid calibration curve- Small Intestine.

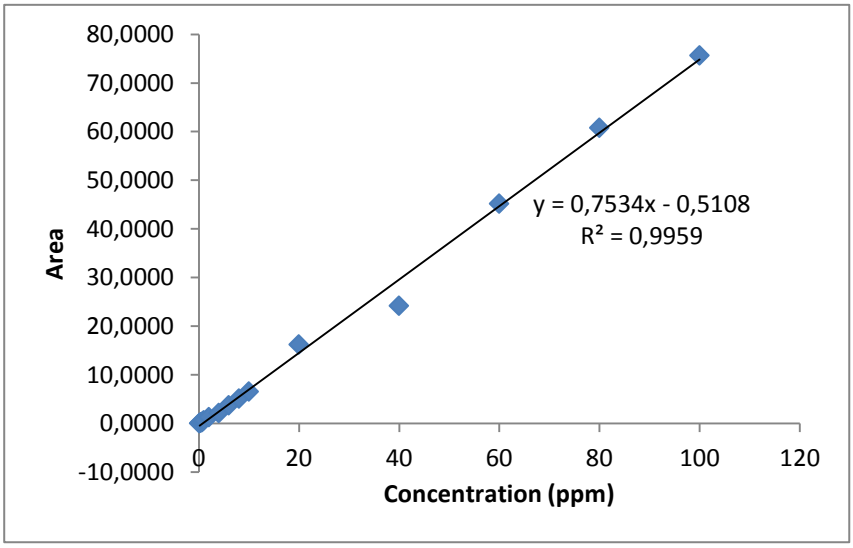

Figure B. 40 : Ferulic acid calibration curve- Colon 24 hour.

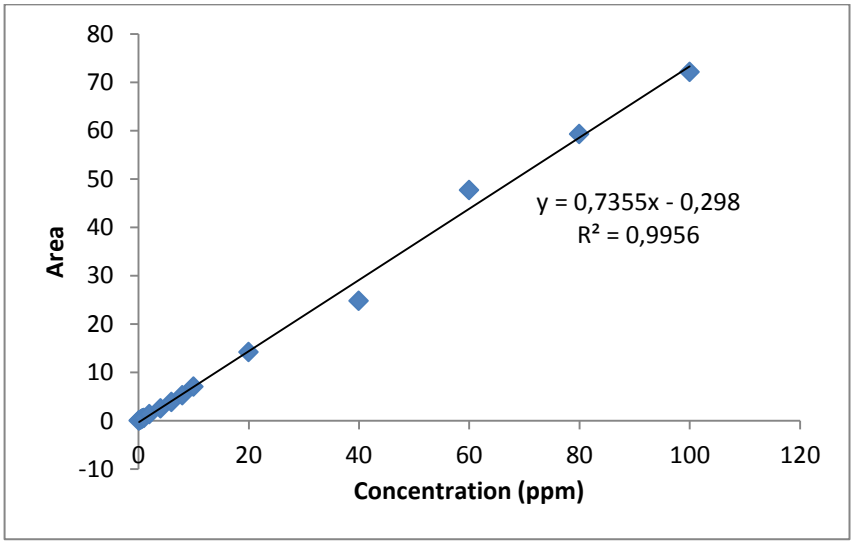

Figure B. 41 : Ferulic acid calibration curve- Colon 48 hour.

APPENDIX C. HPLC and SCFA Chromatograms

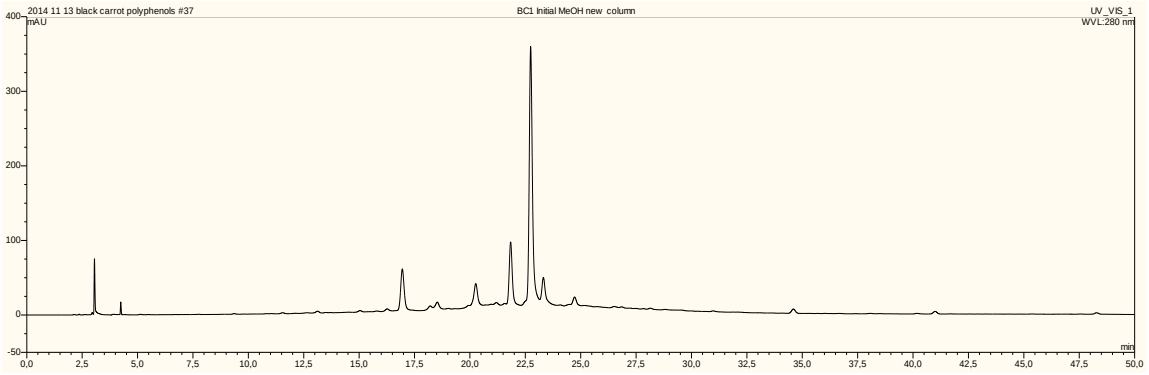

Figure C. 1 : HPLC chromatogram (recorded at 280 nm) of Black carrot (plant extract).

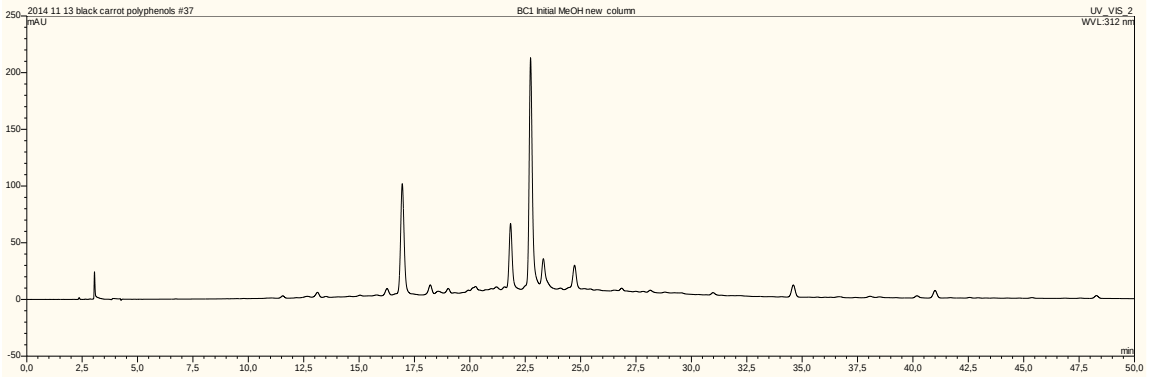

Figure C. 2 : HPLC chromatogram (recorded at 312 nm) of Black carrot (plant extract).

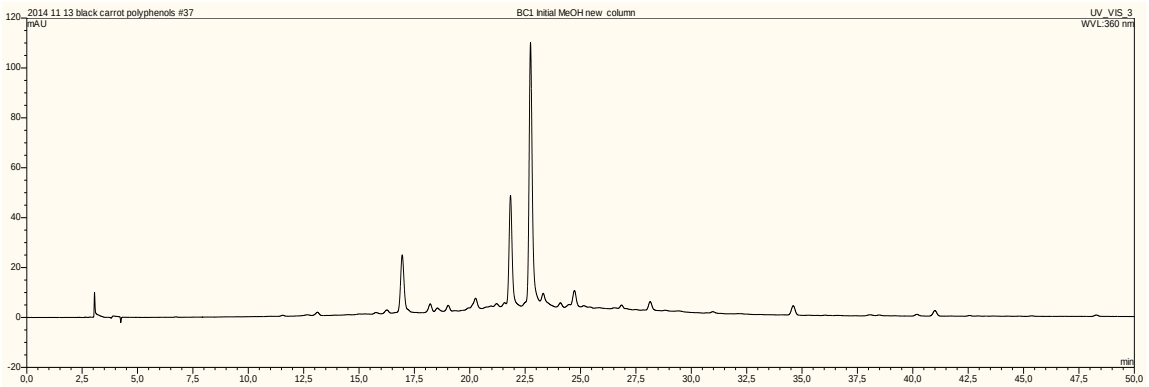

Figure C. 3 : HPLC chromatogram (recorded at 360 nm) of Black carrot (plant extract).

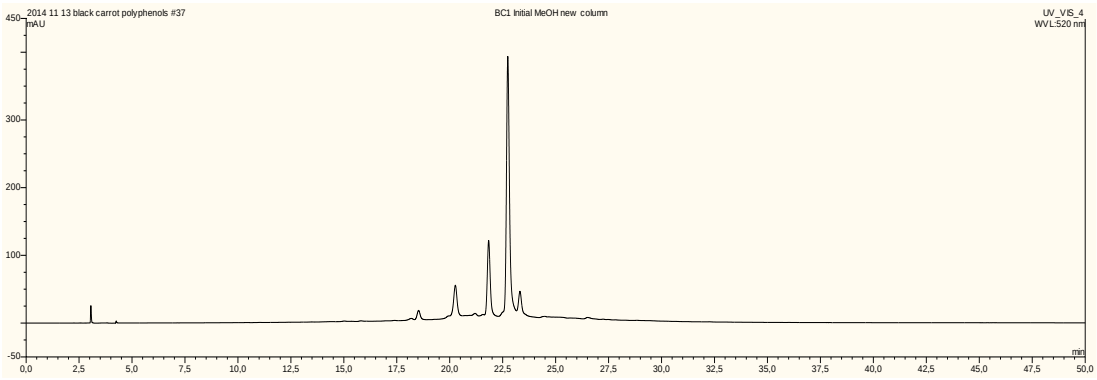

Figure C. 4 : HPLC chromatogram (recorded at 520 nm) of Black carrot (plant extract).

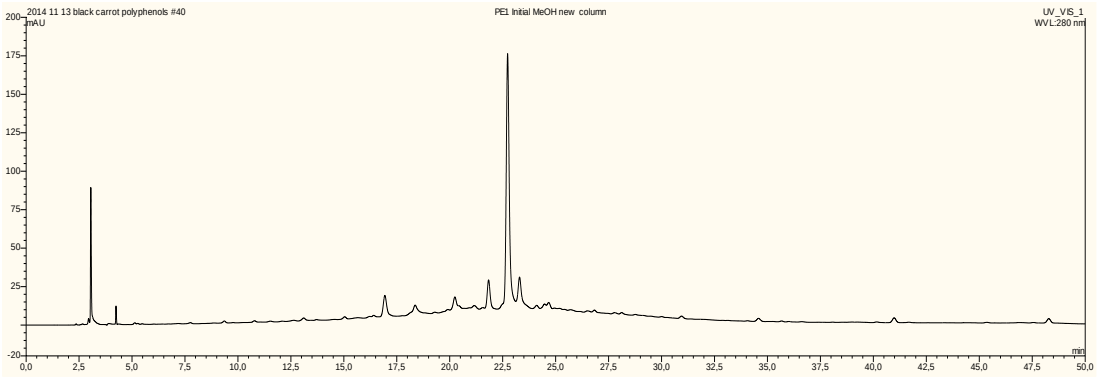

Figure C. 5 : HPLC chromatogram (recorded at 280 nm) of peel (plant extract).

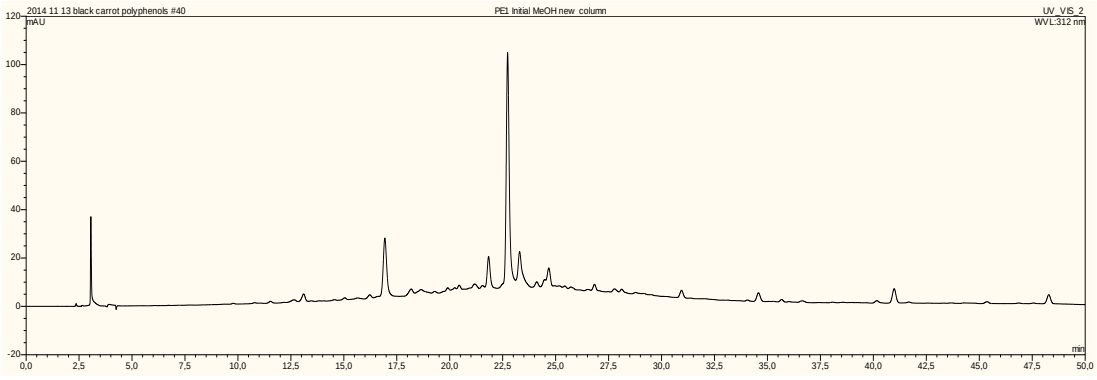

Figure C. 6 : HPLC chromatogram (recorded at 312 nm) of peel (plant extract).

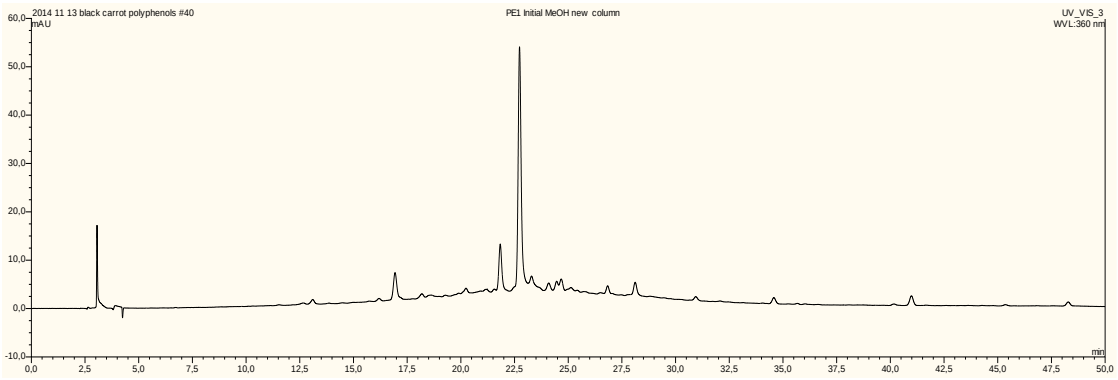

Figure C. 7 : HPLC chromatogram (recorded at 360 nm) of peel (plant extract).

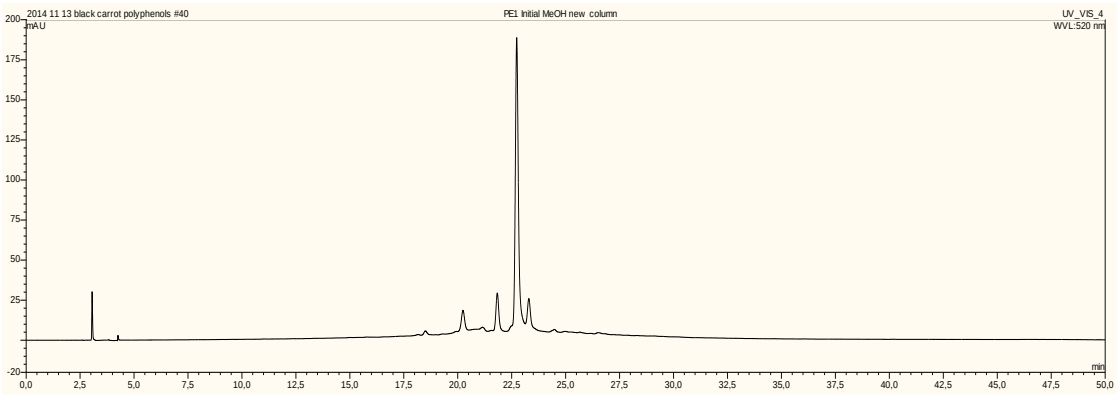

Figure C. 8 : HPLC chromatogram (recorded at 520 nm) of peel (plant extract).

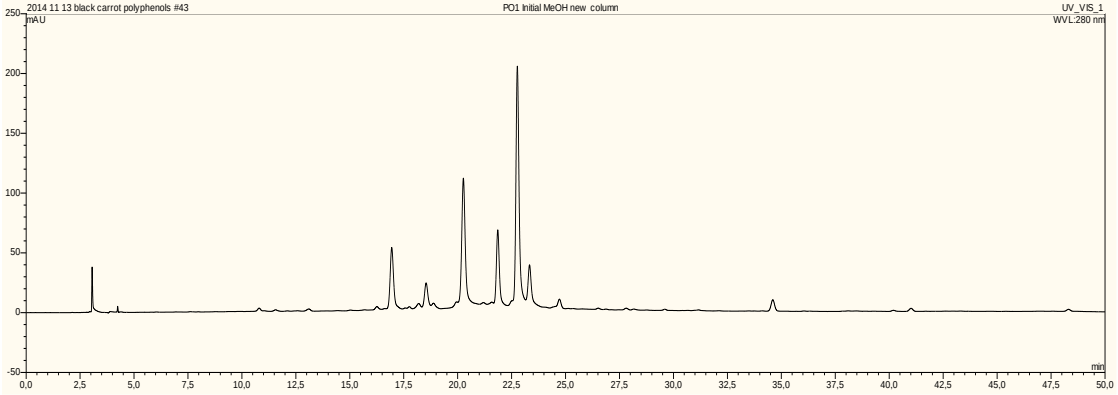

Figure C. 9 : HPLC chromatogram (recorded at 280 nm) of pomace (plant extract).

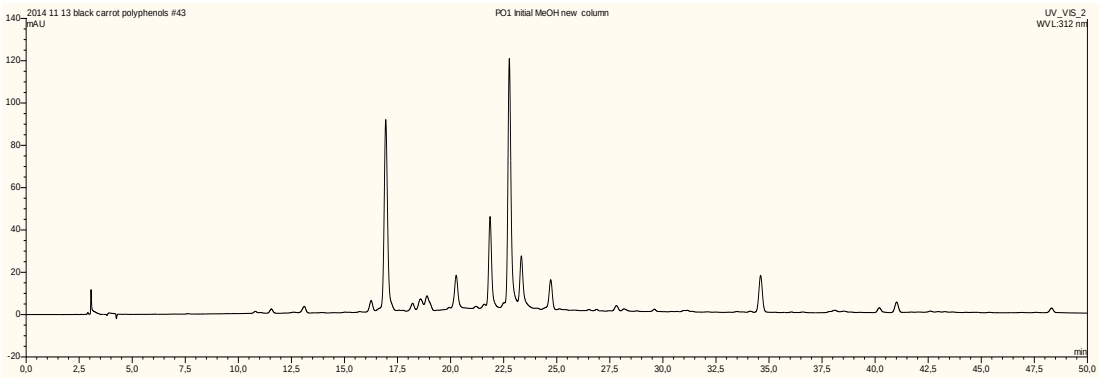

Figure C. 10 : HPLC chromatogram (recorded at 312 nm) of pomace (plant extract).

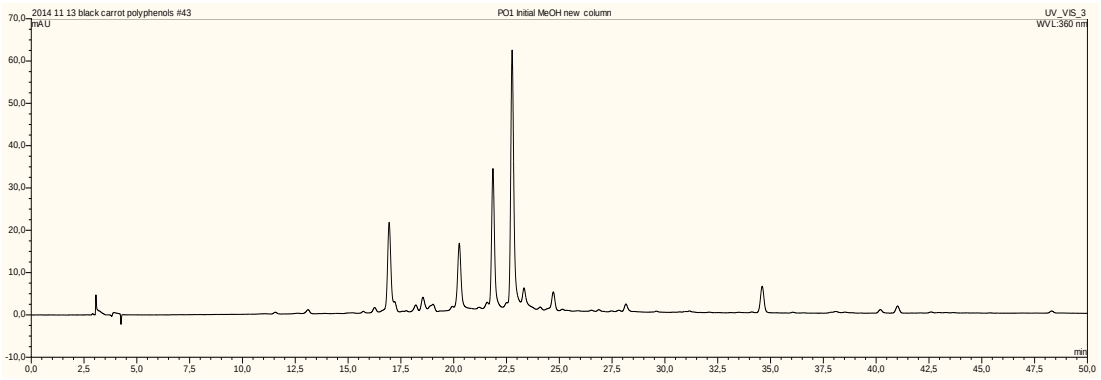

Figure C. 11 : HPLC chromatogram (recorded at 360 nm) of pomace (plant extract).

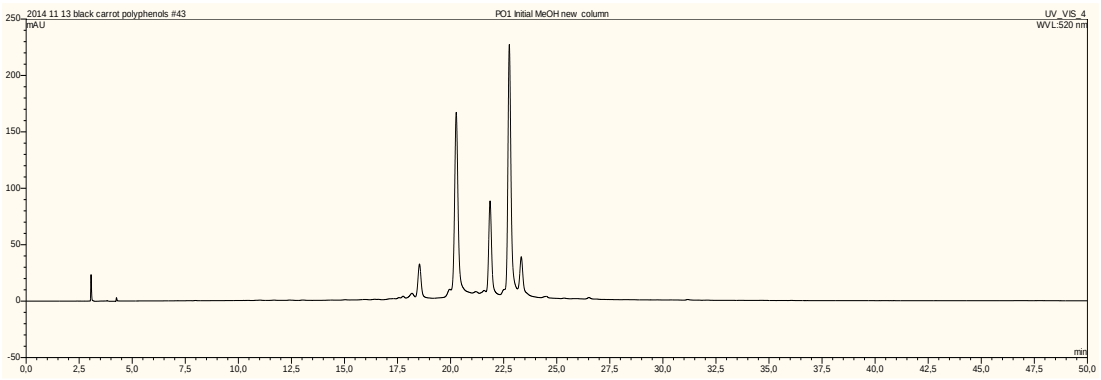

Figure C. 12 : HPLC chromatogram (recorded at 520 nm) of pomace (plant extract).

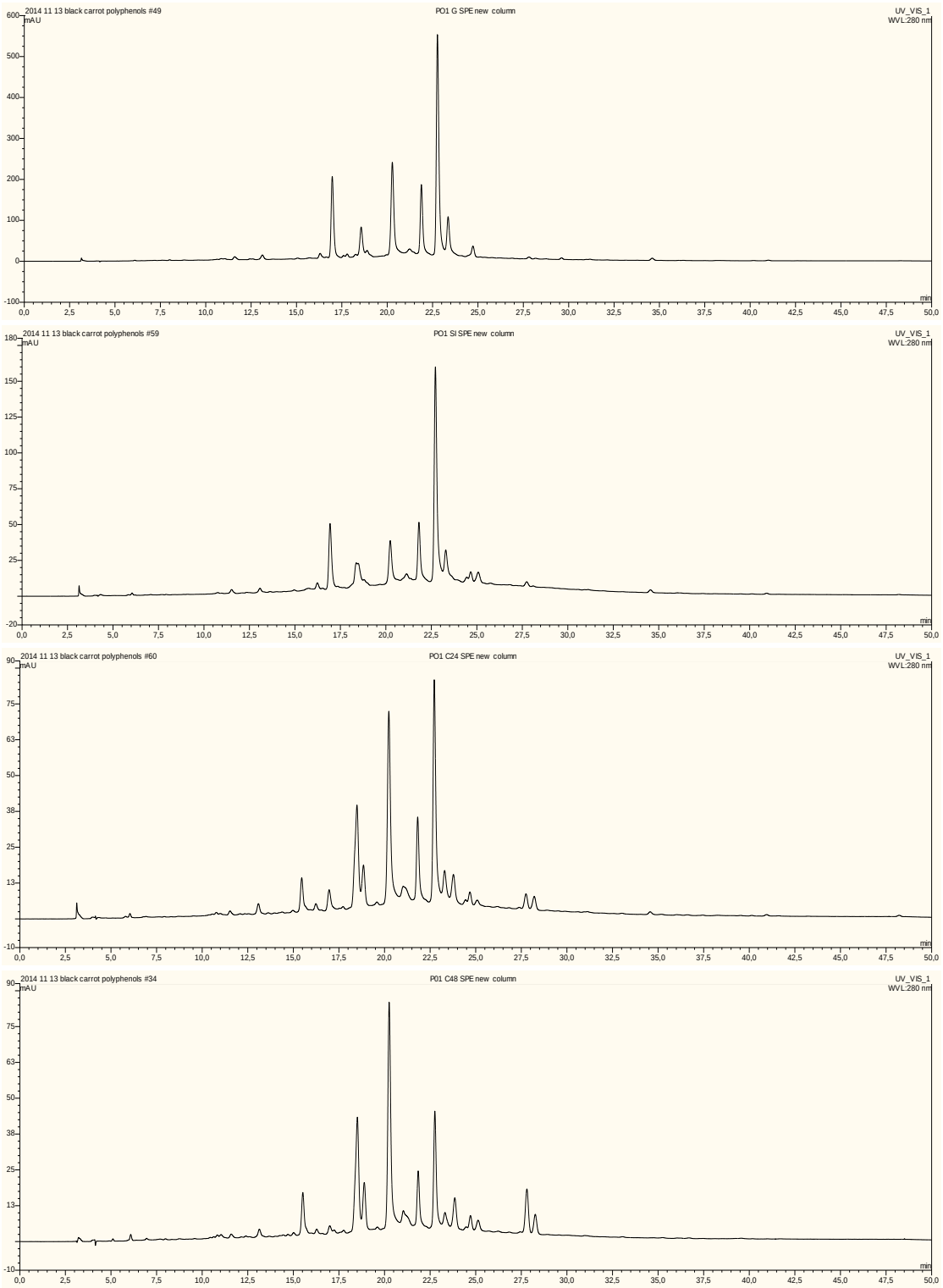

Figure C. 13 : HPLC chromatograms (recorded at 280 nm) of *in vitro* gastrointestinal digestion results for Pomace.

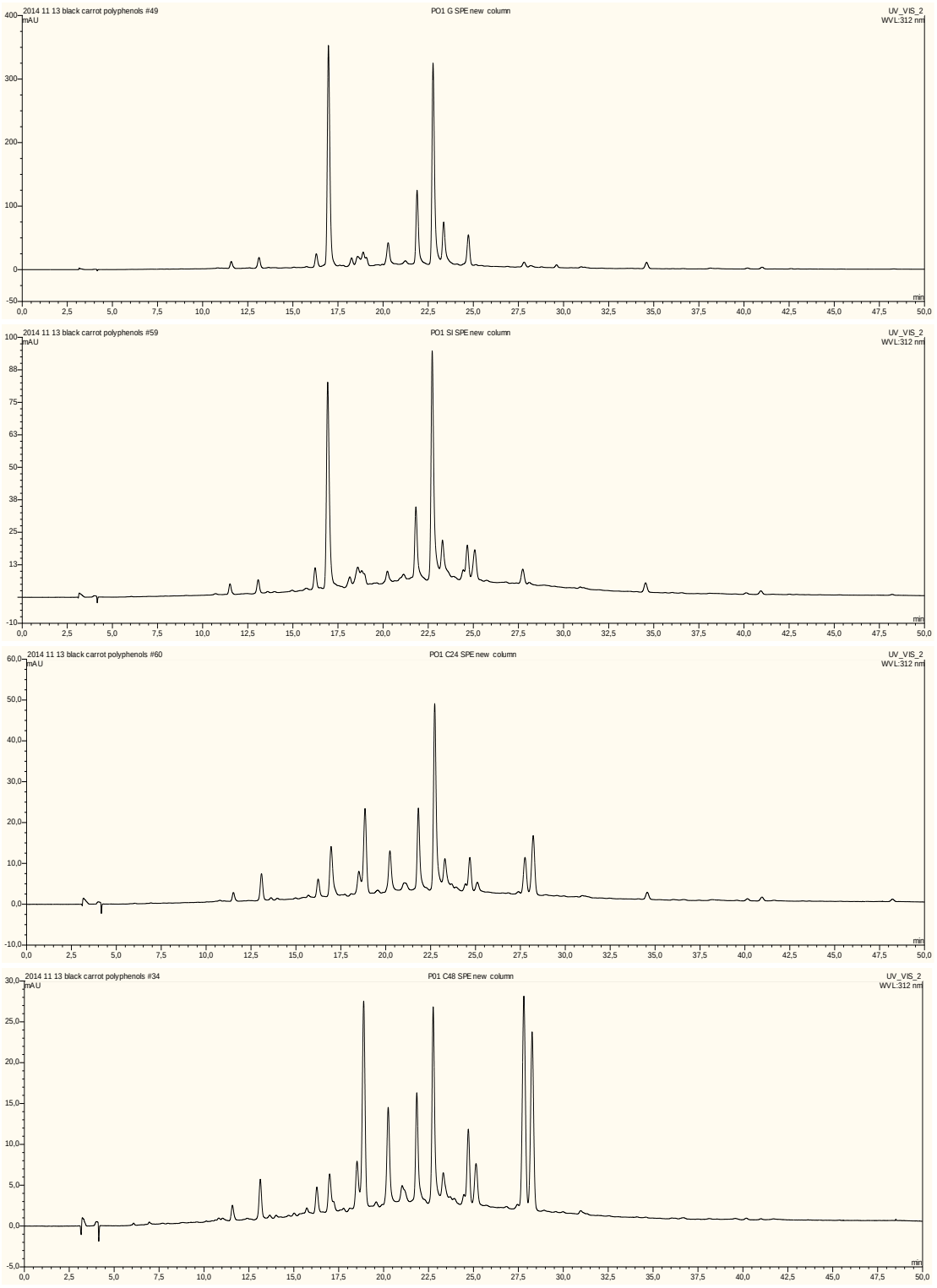

Figure C. 14 : HPLC chromatograms (recorded at 312 nm) of *in vitro* gastrointestinal digestion results for Pomace.

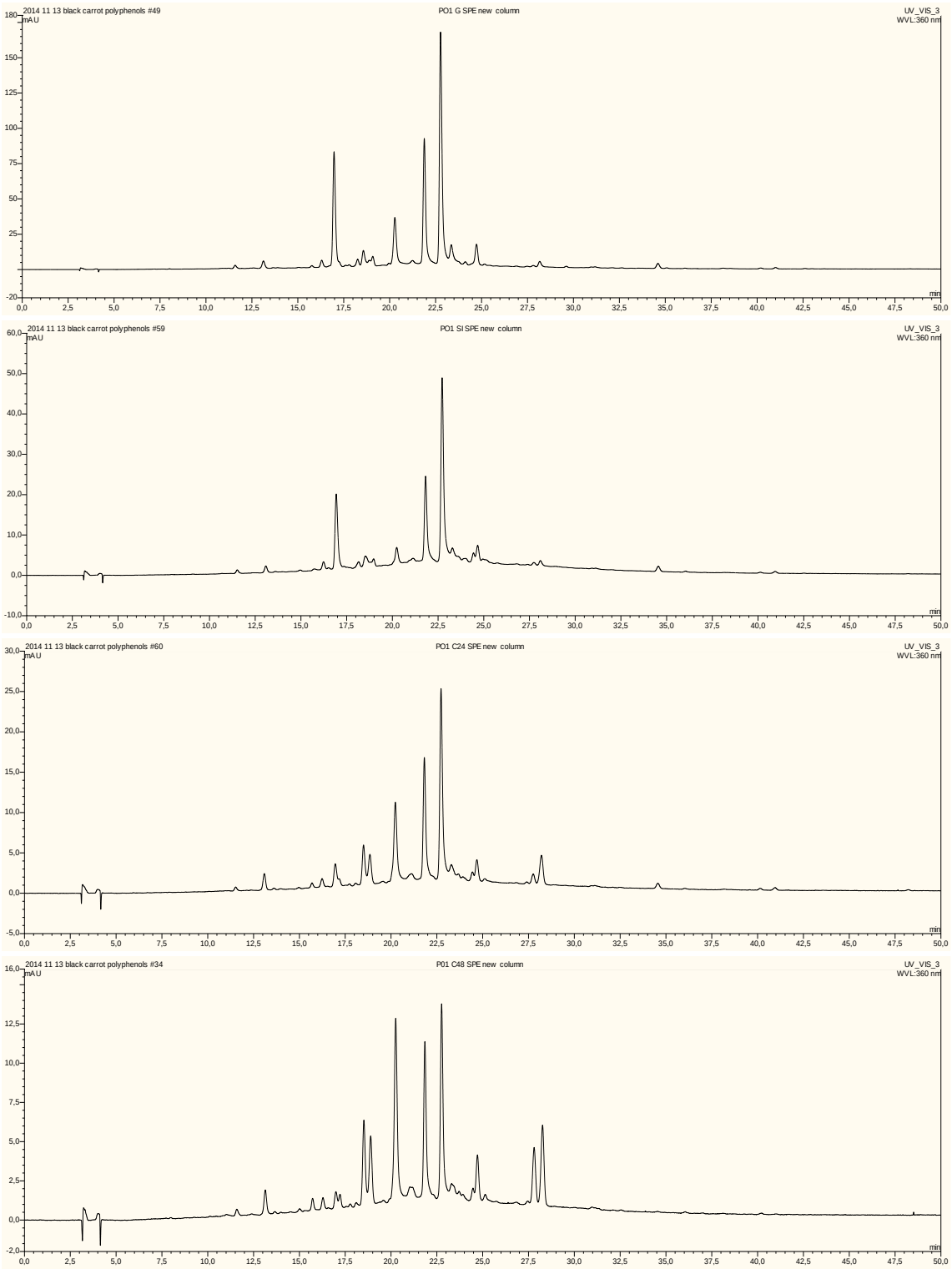

Figure C. 15 : HPLC chromatograms (recorded at 3600 nm) of *in vitro* gastrointestinal digestion results for Pomace.

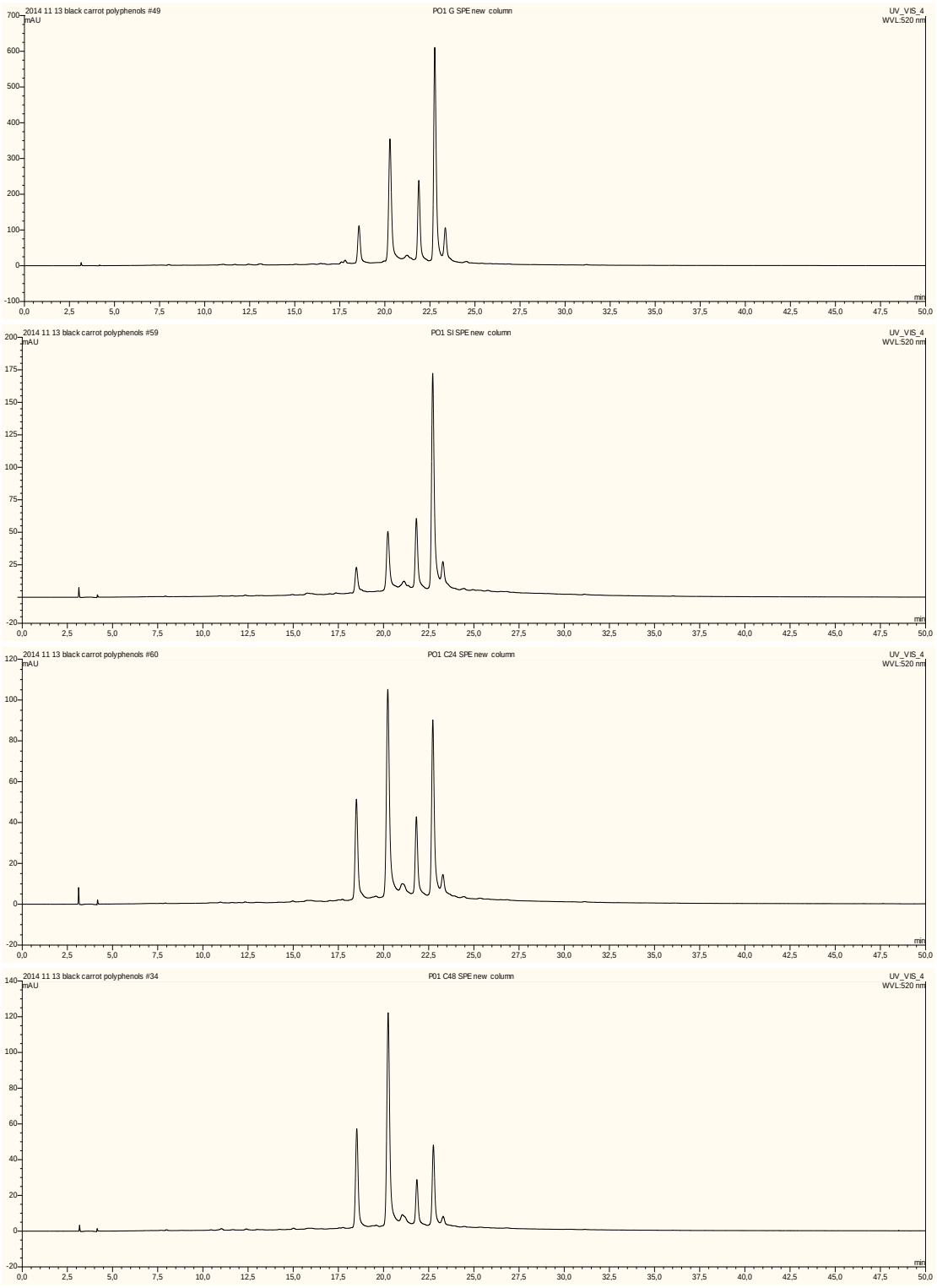

Figure C. 16 : HPLC chromatograms (recorded at 520 nm) of *in vitro* gastrointestinal digestion results for Pomace.

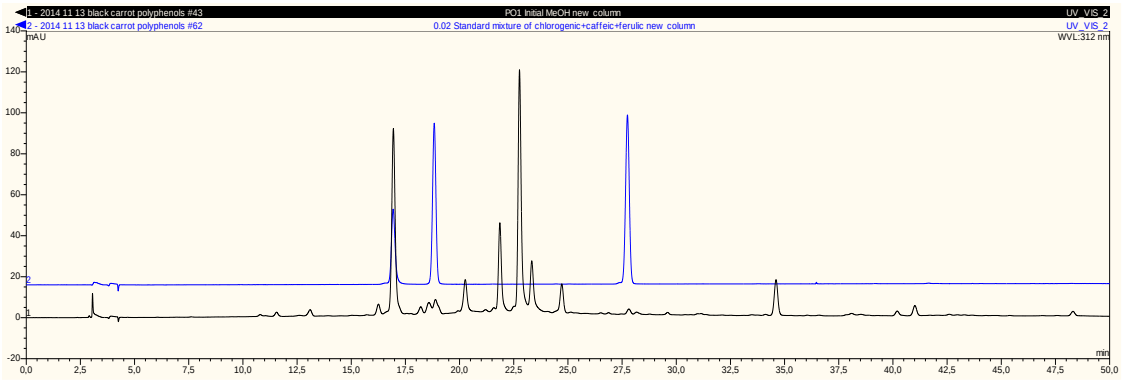

Figure C. 17 : Overlay with standarts.

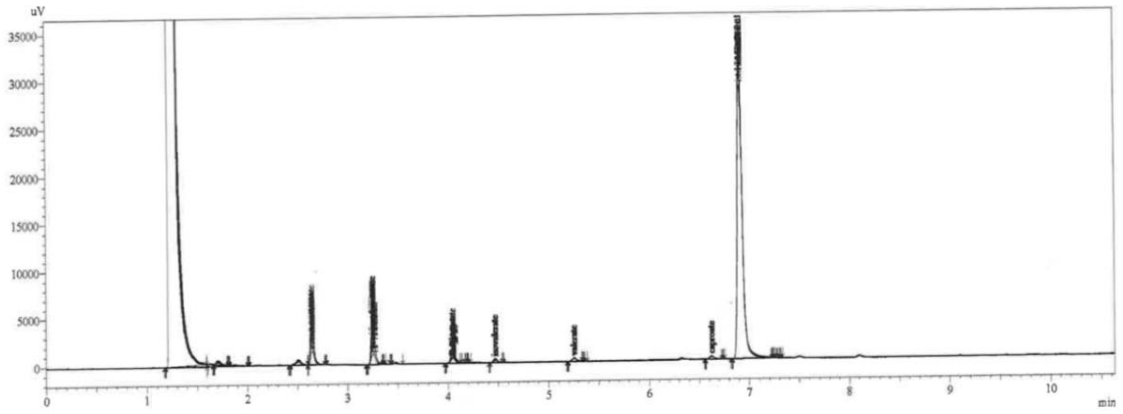

Figure C. 18 : SCFA chromatograms.

APPENDIX D. Anova Tables

Table D.1 : Statistical analysis results of black carrot samples.

		Sum of Squares	df	Mean Square	F	Sig.
BC.TP	Between Groups	2022457.943	4	505614.486	261.832	.000
	Within Groups	19310.633	10	1931.063		
	Total	2041768.576	14			
PE.TP	Between Groups	5090287.663	4	1272571.916	476.701	.000
	Within Groups	26695.413	10	2669.541		
	Total	5116983.076	14			
PO.TP	Between Groups	451152.580	4	112788.145	17.183	.000
	Within Groups	65639.793	10	6563.979		
	Total	516792.373	14			
BC.DPPH	Between Groups	1011909.431	4	252977.358	16.446	.000
	Within Groups	153821.607	10	15382.161		
	Total	1165731.037	14			
PE.DPPH	Between Groups	2757759.871	4	689439.968	652.000	.000
	Within Groups	10574.233	10	1057.423		
	Total	2768334.104	14			
PO.DPPH	Between Groups	305356.711	4	76339.178	6.172	.009
	Within Groups	123678.727	10	12367.873		
	Total	429035.437	14			
BC.FRAP	Between Groups	3109102.260	4	777275.565	42.615	.000
	Within Groups	182394.373	10	18239.437		
	Total	3291496.633	14			

Table D.1 : (Continued) Statistical analysis results of black carrot samples.

PE.FRAP	Between Groups	5152148.413	4	1288037.103	242.326	.000
	Within Groups	53153.007	10	5315.301		
	Total	5205301.420	14			
PO.FRAP	Between Groups	714048.091	4	178512.023	8.676	.003
	Within Groups	205758.673	10	20575.867		
	Total	919806.764	14			
BC.ABTS	Between Groups	10442100.391	4	2610525.098	43.644	.000
	Within Groups	598143.993	10	59814.399		
	Total	11040244.384	14			
PE.ABTS	Between Groups	26958617.700	4	6739654.425	1022.962	.000
	Within Groups	65883.733	10	6588.373		
	Total	27024501.433	14			
PO.ABTS	Between Groups	1371968.069	4	342992.017	11.728	.001
	Within Groups	292464.187	10	29246.419		
	Total	1664432.256	14			
BC.CUPRAC	Between Groups	25526074.800	4	6381518.700	40.547	.000
	Within Groups	1573864.133	10	157386.413		
	Total	27099938.933	14			
PE.CUPRAC	Between Groups	74020258.617	4	18505064.654	882.082	.000
	Within Groups	209788.487	10	20978.849		
	Total	74230047.104	14			
PO.CUPRAC	Between Groups	6356064.604	4	1589016.151	23.008	.000
	Within Groups	690645.893	10	69064.589		
	Total	7046710.497	14			

Table D. 2 : Statistical analysis results of black carrot samples (HPLC, phenolic acids, supernatent).

		Sum of Squares	df	Mean Square	F	Sig.
BC.Neo	Between Groups	100.625	4	25.156	8.309	.003
	Within Groups	30.276	10	3.028		
	Total	130.901	14			
PE.Neo	Between Groups	60.780	4	15.195	19.637	.000
	Within Groups	7.738	10	.774		
	Total	68.518	14			
PO.Neo	Between Groups	331.175	4	82.794	126.055	.000
	Within Groups	6.568	10	.657		
	Total	337.743	14			
BC.Crypto	Between Groups	1715.483	4	428.871	6.274	.009
	Within Groups	683.563	10	68.356		
	Total	2399.046	14			
PE.Crypto	Between Groups	567.416	4	141.854	16.827	.000
	Within Groups	84.302	10	8.430		
	Total	651.717	14			
PO.Crypto	Between Groups	71.904	4	17.976	8.601	.003
	Within Groups	20.900	10	2.090		
	Total	92.804	14			
BC.Chlorogenic	Between Groups	221402.662	4	55350.665	13.614	.000
	Within Groups	40656.744	10	4065.674		
	Total	262059.406	14			

Table D. 2 : (Continued) Statistical analysis results of black carrot samples (HPLC, phenolic acids, supernatent).

PE.Chlorogenic	Between Groups	44264.833	4	11066.208	13.651	.000
	Within Groups	8106.471	10	810.647		
	Total	52371.304	14			
PO.Chlorogenic	Between Groups	408314.352	4	102078.588	183.391	.000
	Within Groups	5566.180	10	556.618		
	Total	413880.531	14			
BC.Caffeic	Between Groups	228.041	4	57.010	23.300	.000
	Within Groups	24.468	10	2.447		
	Total	252.508	14			
PE.Caffeic	Between Groups	421.942	4	105.485	12.700	.001
	Within Groups	83.058	10	8.306		
	Total	505.000	14			
PO.Caffeic	Between Groups	3350.893	4	837.723	24.200	.000
	Within Groups	346.172	10	34.617		
	Total	3697.065	14			
BC.Ferulic	Between Groups	4556.812	4	1139.203	5.653	.012
	Within Groups	2015.275	10	201.527		
	Total	6572.087	14			
PE.Ferulic	Between Groups	21481.894	4	5370.474	7.982	.004
	Within Groups	6728.346	10	672.835		
	Total	28210.240	14			
PO.Ferulic	Between Groups	4063.972	4	1015.993	14.106	.000
	Within Groups	720.252	10	72.025		
	Total	4784.225	14			

Table D. 3 : Statistical analysis results of black carrot samples (HPLC, phenolic acids, pellet).

		Sum of Squares	df	Mean Square	F	Sig.
BC.Neo	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PE.Neo	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PO.Neo	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
BC.Crypto	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PE.Crypto	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PO.Crypto	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
BC.Chlorogenic	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			

Table D. 3 : (Continued) Statistical analysis results of black carrot samples (HPLC, phenolic acids, pellet).

PE.Chlorogenic	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PO.Chlorogenic	Between Groups	1007.240	4	251.810	4.832	.020
	Within Groups	521.154	10	52.115		
	Total	1528.394	14			
BC.Caffeic	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PE.Caffeic	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PO.Caffeic	Between Groups	31.242	4	7.810	22.424	.000
	Within Groups	3.483	10	.348		
	Total	34.725	14			
BC.Ferulic	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PE.Ferulic	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			

Table D. 3 : (Continued) Statistical analysis results of black carrot samples (HPLC, phenolic acids, pellet).

PO.Ferulic	Between Groups	4.958	4	1.239	55.267	.000
	Within Groups	.224	10	.022		
	Total	5.182	14			

Table D. 4 : Statistical analysis results of black carrot samples (HPLC, anthocyanins, supernatent).

		Sum of Squares	df	Mean Square	F	Sig.
BC.ACY1	Between Groups	2507.498	4	626.875	10.609	.001
	Within Groups	590.874	10	59.087		
	Total	3098.373	14			
PE.ACY1	Between Groups	1474.485	4	368.621	8.741	.003
	Within Groups	421.692	10	42.169		
	Total	1896.176	14			
PO.ACY1	Between Groups	10045.905	4	2511.476	39.117	.000
	Within Groups	642.036	10	64.204		
	Total	10687.941	14			
BC.ACY2	Between Groups	52732.890	4	13183.222	18.450	.000
	Within Groups	7145.378	10	714.538		
	Total	59878.268	14			

Table D. 4 : (Continued) Statistical analysis results of black carrot samples (HPLC, anthocyanins, supernatent).

PE.ACY2	Between Groups	7701.006	4	1925.252	5.912	.010
	Within Groups	3256.612	10	325.661		
	Total	10957.618	14			
PO.ACY2	Between Groups	334166.851	4	83541.713	96.906	.000
	Within Groups	8620.894	10	862.089		
	Total	342787.744	14			
BC.ACY3	Between Groups	157019.844				
	Within Groups	17787.765	10	1778.777		
	Total	174807.609	14			
PE.ACY3	Between Groups	13399.543	4	3349.886	24.504	.000
	Within Groups	1367.054	10	136.705		
	Total	14766.598	14			
PO.ACY3	Between Groups	70083.828	4	17520.957	47.783	.000
	Within Groups	3666.758	10	366.676		
	Total	73750.586	14			
BC.ACY4	Between Groups	1458174.058	4	364543.514	51.114	.000
	Within Groups	71320.133	10	7132.013		
	Total	1529494.191	14			
PE.ACY4	Between Groups	501698.629	4	125424.657	106.185	.000
	Within Groups	11811.935	10	1181.193		
	Total	513510.564	14			

Table D. 4 : (Continued) Statistical analysis results of black carrot samples (HPLC, anthocyanins, supernatent).

PO.ACY4	Between Groups	511311.135	4	127827.784	175.356	.000
	Within Groups	7289.610	10	728.961		
	Total	518600.745	14			
BC.ACY5	Between Groups	12933.123	4	3233.281	29.999	.000
	Within Groups	1077.783	10	107.778		
	Total	14010.905	14			
PE.ACY5	Between Groups	1640.029	4	410.007	2.951	.075
	Within Groups	1389.554	10 14	138.955		
	Total	3029.583				
PO.ACY5	Between Groups	6946.079	4	1736.520	74.467	.000
	Within Groups	233.192	10	23.319		
	Total	7179.271	14			

Table D. 5 : Statistical analysis results of black carrot samples (HPLC, anthocyanins, pellet).

		Sum of Squares	df	Mean Square	F	Sig.
BC.ACY1	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PE.ACY1	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			

Table D. 5 : (Continued) Statistical analysis results of black carrot samples (HPLC, anthocyanins, pellet).

PO.ACY1	Between Groups	49.494	4	12.374	4.774	.021
	Within Groups	25.916	10	2.592		
	Total	75.411	14			
BC.ACY2	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PE.ACY2	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PO.ACY2	Between Groups	5154.140	4	1288.535	9.187	.002
	Within Groups	1402.593	10	140.259		
	Total	6556.733	14			
BC.ACY3	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PE.ACY3	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PO.ACY3	Between Groups	708.933	4	177.233	8.079	.004
	Within Groups	219.387	10	21.939		
	Total	928.320	14			

Table D. 5 : (Continued) Statistical analysis results of black carrot samples (HPLC, anthocyanins, pellet).

BC.ACY4	Between Groups	36.754	4	9.189	20.075	.000
	Within Groups	4.577	10	.458		
	Total	41.331	14			
PE.ACY4	Between Groups	156.946	4	39.237	40.350	.000
	Within Groups	9.724	10	.972		
	Total	166.670	14			
PO.ACY4	Between Groups	5023.459	4	1255.865	7.645	.004
	Within Groups	1642.712	10	164.271		
	Total	6666.170	14			
BC.ACY5	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PE.ACY5	Between Groups	.000	4	.000	.	.
	Within Groups	.000	10	.000		
	Total	.000	14			
PO.ACY5	Between Groups	62.587	4	15.647	5.622	.012
	Within Groups	27.834	10	2.783		
	Total	90.421	14			

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PUBLICATIONS/PRESENTATIONS ON THE THESIS

- Kamiloglu, S., Capanoglu, E., **Bilen, F. D.**, Gootaert, C., Van Camp, J. 2015: Characterization of polyphenols and antioxidant potential of black carrot (Daucus carota) by-products: Peel and Pomace: *20th National Symposium for Applied Biological Sciences (NSABS'15),* 30th January 2015, Louvain-la-Neuve, Belgium (Poster presentation).

- Kamiloglu, S., Capanoglu, E., **Bilen, F. D.**, Gootaert, C., Gonzales, G. B., Van de Wiele, T., Van Camp, J. 2015: *In vitro* gastrointestinal digestion of polyphenols from plant processing by-products of black carrot (*Daucus carota*) *4th International Conference of Food Digestion (INFOGEST '15),* 17-19 March 2015, Naples, Italy (Poster presentation).

- Kamiloglu, S., Capanoglu, E., **Bilen, F. D.**, Gonzales, G. B., Gootaert, C., Van de Wiele, T., Van Camp, J. 2015: *In vitro* colonic fermentation of black carrot (*Daucus carota*) and its by-products: *Belgium Nutrition Society 5th Annual Meeting (BSN '15),* 03 April 2015, Brussels, Belgium (Poster Presentation).