ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

EFFECT OF FERMENTATION ON PHENOLIC CONTENT, ANTHOCYANIN STABILITY, ANTIOXIDANT CAPACITY AND *IN VITRO* BIOACCESSIBILITY DURING SHALGAM (ŞALGAM) BEVERAGE PRODUCTION

M.Sc. THESIS

Betül TOKTAŞ

Department of Food Engineering

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Thesis Advisor: Prof. Dr. Beraat ÖZÇELİK

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ŞALGAM SUYU FERMENTASYONUNUN FENOLİK MADDE VE ANTOSİYANİN İÇERİĞİ, ANTİOKSİDAN KAPASİTESİ VE İN VİTRO BİYOYARARLILIK ÜZERİNE ETKİSİ.

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Betül Toktaş, a M.Sc. student of ITU Graduate School of Science Engineering and Technology student ID 506141503 successfully defended the thesis/dissertation entitled "EFFECT OF FERMENTATION ON PHENOLIC CONTENT, ANTHOCYANIN STABILITY, ANTIOXIDANT CAPACITY AND *IN VITRO* BIOACCESSIBILITY DURING SHALGAM (ŞALGAM) BEVERAGE PRODUCTION", 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 lovely family and all humanity…

FOREWORD

When I compare myself with the five years previous version of me I would never think that i can finish my BSc. It was like I had trapped in time line. I really can not say it has changed however, it is pleasure to be able to write foreword of my own MSc thesis. My trial with shalgam beverage started with an extremely minor project when I was 2nd class of BSc and after that although I have never taste shalgam at all untill this year, practically my whole life purpose become this sour but delicious beverage a little bit more each passing day. Eventually it not also become my BSc thesis subject but also MSc.

Primarily, I would like to express my appreciation for my precious advisor Prof. Dr. Beraat Özçelik for her supporting and guiding me all the time. She helped me patiently to complete my master thesis in this long, instructive process.

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I would also like to thank my friends for their encouragement and humour especially who has unsparing in assist to complete my thesis in this process.

At the end, thanks to you. If you are reading this line after the others, you at least read one page of my thesis; hope you can learn something. Thank you

May, 2016

Betül TOKTAŞ
(Food Engineer)

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ABBREVIATIONS

A	**:** Absorbance
AC	**:** Anthocyanin Content
CUPRAC	**:** Cupric Reducing Antioxidant Capacity
DF	**:** Dilution Factor
DPPH	**:** Diphenyl-1-pictylhydrazyl
TAC	**:** Total Antioxidant Capacity
TFC	**:** Total Flavonoid Content
TPC	**:** Total Phenolic Content
DPPH	**:** 2,2-diphenyl-1-picrylhydrazyl
GAE	**:** Gallic Acid Equivalent
HPLC	**:** High Performance Liquid Chromotography
MW	**:** Moleculer weight
pH	**:** Power of Hydrogen
Trolox	**:** 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid

SYMBOLS

ε **:** Molar absorptive coefficient

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EFFECT OF FERMENTATION ON PHENOLIC CONTENT, ANTHOCYANIN STABILITY, ANTIOXIDANT CAPACITY AND *IN VITRO* BIOACCESSIBILITY DURING SHALGAM (ŞALGAM) BEVERAGE PRODUCTION

SUMMARY

Shalgam beverage due to existing benefits to humanity health could be in the category of functional beverages. In order to put forth the potential bioavailabiltiy of shalgam which is characterized as functional fermented beverage by public, current study is conducted.

In order to obtain sample during fermentation three different shalgam beverage were pickled by the instructions of Doganay Gıda in Adana, direct method. The raw materials for producing shalgam beverage in laboratory was also provided from Doğanay Gıda. Shalgams beverages, which is used in the scope of verification of laboratory made product, was taken from 3 different local markets in Istanbul. Phenolics of black carrot was extracted for experiments. pH analysis, dry matter analysis, total flavonoid analysis, total phenolic analysis (Folin-Ciocalteu method), anthocyanin analysis (pH differential method) and antioxidant analysis (DPPH and CUPRAC) were performed to the samples collected on 1st, 12th, and 24th days of the fermentation, three different commercial shalgam beverages, black carrot and bulgur extracts. DPPH - Diphenyl-1-pictylhydrazyl- method shows radical-scavenging capacity is directly related with antioxidant capacity, CUPRAC -Cupric Reducing Antioxidant Capacity- method is performed for determining antioxidants in terms of trolox equivalent. Folin-Ciocalteu method determines total phenolic content of samples. Bioaccessibility tests were conducted to the twelve-diffeent shalgam beverage samples. Furthermore, initial samples, mouth samples and intestine samples were analyzed in High Performance Liquid Chramatograph (HPLC) to discover the types of phenolics and anthocyanins in shalgam beverage samples.

The pH of the shalgam beverage sample continuously decreased during lactic acid fermentation. Spectrophotometric experiments show that the total flavonoid content, total phenolic content, anthocyanin compound and antioxidant capacity of black carrot are significantly higher compared to shalgam beverages before bioaccessibility tests. In general total flavonoid content, total phenolic content, anthocyanin compound and total antioxidant capacity of shalgam sample collected on the 1st days of fermentation and bulgur sample were observed to be at the lowest levels. As the fermentation progress total flavonoid content, total phenolic content, anthocyanin compound and total antioxidant capacity raised quickly at first twelve day and at end of the fermentation those compounds continues to increase with decreasing tendency ratio compared with first twelve days of fermentation.

In vitro bioaccessibility tests demonstrate that containing high amounts of health benefit compound does not imply that those benefit substance can be utilized by our system. The bioaccessibility of total flavonoid content, total phenolic content, anthocyanin compound and total antioxidant capacity in mouth, stomach and intestine were observed to be lower compared to the initial samples. As the samples pass through from mouth to intestine, the percentage of bioaccessibility was decreased. For instance at the end of the mouth digestion recovery percent of black carrot, shalgam sample collected on 1st days of fermentation, 12th days of fermentation, 24th days of fermentation and commcercial shalgam samples changes between 43.9-56.0 % and

statistically identical for flavonoid content. As the sample pass stomach and reach to intestine, the recovery percent of samples was decline, which changes between 6.7 to 13.9 %. Similar results were obtained in terms of phenolic content, anthocyanin compound and total antioxidant capacity recovery percent.

16 different phenolics were detected from initial shalgam beverage samples namely; 3-4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, caffeinne, catechin, chlorogenic acid, elagic acid, epicatechin, ethyl-3-4-dihydroxybenzoat, ferulic acid, fumaric acid, gallic acid, p-coumaric acid, quercetin, sinapic acid, syringic acid and vanilin. After performing bioaccessibility tests, no phenolic was detected from shalgam sample collected on 1st days of fermentation. On the other hand 5 different phenolics namely 3-4-dihydroxybenzoic acid, p-coumaric acid, chlorogenic acid, 4-dihydroxybenzoic acid and epicatechin were detected from shalgam sample collected on 12th, 24th days of fermentation and commercial shalgam samples. As anthocyanin, only cyanidin was detected except for mouth samples and intestine samples of 1st days of fermentation.

It is revealed that fermentation process has positive effect on beneficial substance such as flavonoid, phenolic, anthocyanin and antioxidant. Besides, although the total flavonoid content, total phenolic content, anthocyanin compound and total antioxidant capacity of black carrot has the highest level among all samples; following the end of digestion it has proven with current study that the bioaccessible total flavonoid content, total phenolic content, anthocyanin compound and total antioxidant capacity of shalgam sample collected on 24th days of fermentation and commcercial shalgam beverage sample had elevated or identical value with black carrot extract samples. To sum up, it is possible to say that fermentation process has positive effect on bioaccessible total flavonoid content, total phenolic content, anthocyanin compound and total antioxidant capacity.

ŞALGAM SUYU FERMENTASYONUNUN FENOLİK MADDE VE ANTOSİYANİN İÇERİĞİ, ANTİOKSİDAN KAPASİTESİ VE İN VİTRO BİYOYARARLILIK ÜZERİNE ETKİSİ

ÖZET

Şalgam suyunun insan sağlığına olan pozitif etkilerinin halk tarafından biliniyor olması sebebiyle fonksiyonel içecekler kategorisinde olarak değerlendirilen içecekler arasındadır. Son dönemlerde artan tüketim trendi ile birlikte yalnızca Adana ve çevresinde değil İstanbul ve İzmir gibi büyük şehirlerde de sıkça tüketilen önemli bir fermente içecek haline gelmiştir. Şalgam suyunun fonksiyonel özellikleri solunum yolunu temizlemesi, sindirim sistemini düzenlemesi, kalp ve damar hastalıklarını önlemesi, bağışıklık sistemini pozitif yönde etkilemesi olarak sıralanabilir. Şalgam suyunun sağlık üzerinde olumlu etkisi olan fonksiyonel özellikleri ürünün ana hammaddesi olan kara havucun flavonoid, fenolik madde, antosiyanin gibi yararlı bileşenleri yüksek oranda içermesi ve bu nedenle antioksidan kapasitesinin fazla olmasından kaynaklanır.

Kara havuç zengin antosiyanin içeriği sebebiyle doğal renklendirici olarak kullanılan sebzelerden bir tanesidir. Şalgam suyuda rengini antosiyanin madde içeriği zengin olan kara havuçtan alır. Şalgam suyu fermentasyonu sırasında başlıca laktik asit fermentasyonu gerçekleşir ve ürün kendine özel olan tat ve kokusu fermentasyon sırasında üretilen laktik asit öncelikli olmak üzere etaol gibi çeşitli organik bileşenlerden kaynaklanır. Ayrıca laktik asit bakterileri antioksidan özelliğe sahip olduğu bilinen mikroorganizmalar olduğundan son ürünün antioksidan kapasitesine olumlu yönde etki eder. Şalgam suyu içerisinde ayrıca potasyum, fosfor, kalsiyum, demir ve diğer minerallerde bulunmaktadır. Yapılan bir çalışmada ürünün üretimi sırasında şalgam bitkisi kullanılmasının toprak kokusu oluşturduğu tüketiciler tarafından belirlenmiştir.

Bu çalışmada şalgam suyu içerisindeki flavonoid madde, fenolik madde, antosiyanin bileşiği ve toplam antioksidan kapasitesinin fermentasyon süresince değişimi incelenmiş ve içeceğin ana hammaddesi olan kara havuç ve bulgur ekstraktları ve doğrulama amaçlı rafta bir ay bekletilen marketten alınan şalgam suları ile kıyaslanmıştır karşılaştırılmıştır. Kullanılan şalgam suyu örnekleri İstanbul'da üç farklı yerel marketten alınmıştır. Laboratuvarda hazırlanan şalgam suyu için hammaddeler Adana'da bulunan Doğanay Gıda'dan tedarik edilmiştir ve üretimi için Doğanay Gıda'nın verdiği reçete kullanılarak numuneler üç farklı paralel olacak şekilde hazılanmıştır. Şalgam suyu üretimi için doğrudan üretim ve geleneksel üretim olmak üzere iki farklı metod mevcuttur. Bu çalışmada laboratuvarda yapılan şalgam suyu direk metot (doğrudan metot) kullanılarak kara havuç, bulgur, tuz ve suyun yalnızca laktik asit fermentasyona bırakılması ile üretilmiştir. Bulgur laktik asit fermentasyonu sırasında laktik asit bakterileri için uygun besin ortamını oluşturken, eklenen tuz laktik asit bakterilerinin gelişimini sınırlar.

Fermentasyon süresince gerçekleşen değişiklikleri ölçebilmek amacıyla fermentasyonun 1., 12. ve 24. günlerinden numune alınmıştır. Şalgam suyu üretiminde

ana hammadde olarak kullanılan kara havucun, fermentasyon sırasında alınan 1., 12., 24. gün numunelerinin ve doğrulama amaçlı marketten alınan şalgam suyu numunelerinin başlangıçtaki flavonoid madde miktarı, fenolik madde miktarı, antosiyanin madde miktarı ve toplam antikosidan kapasitesi belirlenmiştir. Toplam fenolik madde miktarı Folin-Ciocalteu reaktifi kullanılarak, antosiyanin içeriği pH differential metodu yardımıyla belirlendi. Antioksidan kapasitesi DPPH - Diphenyl-1-pictylhydrazyl- metodu yardımıyla radikal yakalama kapasitesi ölçülerek ve CUPRAC –Cupric Reducing Antioxidant Capacity- metodu ile fenollerin bakır iyonlarını indirgemesi ölçülerek belirlenmiştir. Biyoyararlılık deneyini gerçekleştirebilmek için laboratuar ortamında ağız, mide ve bağırsak ortamı hazırlanarak yapay sindirimin gerçekleşmesi sağlanmıştır. Biyoyararlılık testi fermentasyonun 1., 12. ve 24. günü alınan şalgam suyu numuneleri ve ticari şalgam numunelerine uygulanmıştır. Yapılan bioyararlılık testlerinden elde edilen ağız, mide ve bağırsak sindirimi sonrası alınan numunelere spektrofotometrik analizler; toplam flavonoid madde içeriği, toplam fenolik madde içeriği, antosiyanin bileşen içeriği ve toplam antioksidan kapasitesi testi; uygulanmıştır. Fermentasyonun 1., 12. ve 24. günü alınan numuneler ve ticari şalgam numuneleri içerisinde bulunan fenolik ve antosiyanin madde içeriklerinin tanımlanabilmesi açısından yüksek basınçlı sıvı kromatografisi (HPLC) cihazında analiz edilmiştir.

Şalgam suyu fermentasyonu süresince pH değişimi ölçülmüş ve ortamın pH değerinin beklenildiği gibi fermentasyon ilerledikçe azaldığı gözlemlenmiştir. Biyoyararlılık öncesi başlangıç numunelerine yapılan analizler sonucunda kara havuç fenolik madde, antosiyanin içerik ve antioksidan kapasite açısından şalgam suyu numunelerinden istatiksel olarak önemli ölçüde fazla miktarda ingrediyen içerdiği belirlenmiştir. Fermentasyonun ilk gününde flavonoid madde, fenolik madde ve antosiyanin içeriği ve antioksidan kapasitesi minimum seviyedeyken fermentasyon ilerledikçe bu miktarlarda artış gözlemlenmiştir. Fermentasyonun 1. ve 12. günleri arasında toplam flavonoid fenolik ve antosiyanin madde içeriği ve antioksidan kapasitesi hızla artarken fermentasyonun 12. ve 24. günlerinde alınan numunelere yapılan deneyler sonucunda ilgili içeriklerin artış oranının fermentasyon süresinin ilk yarısına kıyasla daha az olduğu gözlemlenmiştir. Piyasadan alınan şalgam suyu numuneleri analiz edildiğinde ürünün flavonoid, fenolik, antosiyanin madde içeriği ve antioksidan kapasitesinin fermantasyonun 24. gününde alınan numuneyle eşit veya daha az olduğu gözlemlenmiştir.

Yapılan *in vitro* biyoyararlılık deneyleri ile herhangi bir ürünün sağlık üzerine pozitif etkisi olan yararlı bileşenleri fazla miktarda içermesinin bu faydalı maddelerin tamamının insan vücudu tarafından kullanılamayabileceğini göstermiştir. Ağız, mide ve bağırsak ortamında ürün sindirimi sağlandıktan sonra alınan numunelere yapılan deneyler, başlangıç numunelerine yapılan deney sonuçları ile kıyaslandığında toplam flavonoid madde içeriği, toplam fenolik madde içeriği, antosiyanin madde içeriği ve toplam antioksidan kapasitesinin azaldığı gözlemlenmiştir. İncelenen örnekler mide ortamından bağırsak ortamına geçerken biyoyararlılık yüzdesinin mide ortamındaki değerler ile kıyaslandığında daha da azaldığı gözlemlenmiştir. Numunelerin ağız sindirimini takiben kara havuç, fermentasyonun 1., 12. ve 24. günü alınan şalgam suyu numuneleri ve marketten tedarik edilen ticari şalgam suyu numunesinin başlangıç değerlerine göre geri dönüşüm yüzdesi flavonoid madde içeriği için % 43,9 ve 56 arasında değişiklik gözlemlenmektedir. Numuneler mide sindirimi sonrasında bağırsak ortamına geçtikten sonra geri kazanım yüzdesi % 6.7 ve 13.9 değerlerine düştüğü gözlemlenmiştir. Toplam fenolik madde, antosiyanin madde içeriği ve toplam

antioksidan kapasitesi için geri kazanım yüzdesi açısından benzer sonuçlar elde edilmiştir.

Üç paralel olacak şekilde 4 farklı şalgam suyu numunesinin HPLC cihazında analiz edilmesi sonucunda başlangıç numuneleri içerisinde tanımlı 16 farklı fenolik madde tespit edilmiştir. Bu fenolik maddelerin isimleri; 3-4-dihidroksi benzok asit, 4-hidroksi benzoik asit, kafein, kateşin, klorojenik asit, elajik asit, epikateşin, etil-3-4-dihidroksi benzoat, ferulik asit, fumarik asit, gallik asit, p-kumarik asit, kuersetin, sinapik asit, siringik asit and vanilin. Yapılan biyoyararlılık testleri sonrası fermentasyonun birinci günü alınan şalgam suyu numunesi incelendiğinde fenolik madde tespit edilememiştir. Fakat fermentasyonun on ikinci günü, yimi dördüncü günü alınan şalgam suyu numuneleri ve marketten alınan şalgam suyu numunelerinden beş farklı fenolik madde tanımlanmıştır. Biyoyararlılık testi sonrası alınan numunelerden tanımlanan fenolik maddeler 3-4-dihidroksi benzoik asit, p-kumarik acit, klorojenik asit, 4-dihidroksi benzoik asit and epikateşin olarak sıralanabilir. Numunelerin HPLC'ye verimesi sonucu antosiyanin olarak fermentasyonun on ikinci günü, yimi dördüncü günü alınan şalgam suyu numuneleri ve marketten alınan şalgam suyu numunelerinden yalnızca siyanidin tanımlanmıştır. Biyoyararlılık testi sonrası fermentasyonun birinci günü ağız ve bağırsak sindirimi sonrası alınan numune içerisinde antosiyanin madde içeriği tespit edilememiştşir.

Bu çalışma sonucunda başlangıçta kara havuç için toplam flavonoid madde, antosiyanin madde ve toplam fenolik madde içeriği ve antioksidan kapasitesi ciddi anlamda yüksek seviyelerde olsa da, ürünün sindirimi sonrasında insan vücudunun kullanabildiği biyoyararlanılabilir fenolik madde, antosiyanin ve antioksidan kapasitesi miktarının laboratuarda üretilen 24. gün numunesi ve fermentasyona uğramamış kara havuç için aynı veya 24. gün numunesinin daha yüksek seviyelerde olduğunu ortaya çıkarmıştır. Sonuç olarak kontrollü laktik asit fermentasyonunun flavonoid madde, fenolik madde, antosiyanin madde içeriği ve toplam antioksidan kapasitesini arttırıcı etkisi olduğu söylenebilir. To our knowledge, this is the first study that has focused on the changes in total phenolic contents, total flavonoid content, anthocyanin content, antioxidant capacity and *in vitro* bioaccessibility during spontaneous shalgam fermentation.

1. INTRODUCTION

Traditional foods and beverages have been an important part of our lives all over the world. Their production is one of the oldest manufacturing and preservation methods of human consumption, dating back to ancient times (Caplice and Fitzgerald, 1999).

Shalgam is a traditional beverage highly popular not also in southern Turkey, particularly in the cities of Adana, Mersin, and Hatay but also in other parts of country, especially in Istanbul, Ankara, and Izmir metropolises (Erten et al, 2008). According to Turkish Standart Enstitue (TSE) shalgam is a traditional lactic acid fermented beverage in which bulgur, drinkable water, black carrot, salt and if desired chilli peppers are mixed and exposed to fermentation (TSE 11149, 2003). In shalgam production, black carrot is used as major raw material. It gives aroma, taste and color to the shalgam beverage (Tanguler, 2010). Black carrots are considered to be one of the most important sources of natural food colorant. Pigmentation (red colour) that derives from anthocyanins is the main factor for using black carrot in the production of shalgam beverage (Erten et al, 2008). Shalgam beverage also known as having therapeutic properties, such as developing the immune system and preventing digestion disorders (Canbas and Fenercioglu, 1984).

In literature, the terms of bioavailability and biaccessibility are known as different. FDA stated that bioavailability means the rate and extent to which the active ingredient is absorbed from a product and becomes available (1996). Amount of ingested food product, which is reachable for absorption in the intestine after digestion, is decribes as bioaccessibility (Hedrén et al, 2002). It is possible to measure bioavailability and bioaccessibility by *in vivo* and *in vitro* experiments. *In vivo* assays carry out by living beings to obtain a result after consumption of a nutrient (Yeum and Russell, 2002). *In vitro* digestion techniques have been developed as an alternative approach to *in vivo* assay and if two methods are compared *in vitro* digestion methods considered as simple, cheap and reproducible tools to assess the digestive stability of different food constituents (Rodríguez-Roque et al, 2013). *In vitro* digestion is measured by made of digestion stimulation in a laboratory such as gastrointestinal models which is known

as stimulation of mouth, stomach and intestinal condition in laboratory (Parada and Aguilera, 2007).

Various studies have been conducted on phenolic content including flavonoid, anthocyanins, and antioxidant capacity in black carrots and shalgam (Kammerer et al, 2004; Turker et al, 2007; Montilla et al, 2011; Baser et al., 2012). Phenolic composition of black carrots may be altered during shalgam fermentation due to instable structure of anthocyanins (Kirca et al, 2007). On the other hand, due to relatively poor bioavailability of anthocyanins, in vitro bioaccessibility should be evaluated. Although, there are a few studies on the effect of *in vitro* digestion on anthocyanins and polyphenols of fruits and vegetables such as black mulberry (Tomas et al, 2015), tomato (Huélamo et al., 2015), chokeberry (Bermudez-Soto et al, 2007), fruit beverages (grape, orange, apricot) (Cilla, et al, 2011), there is not exist any available research to evaluated the effect of *in vitro* gastrointestinal digestion on shalgam beverage during fermentation. To our knowledge, this is the first study that has focused on the changes in total phenolic contents, total flavonoid content, anthocyanin content, antioxidant capacity and *in vitro* bioaccessibility during spontaneous shalgam fermentation.

1.1 Purpose of Thesis

The aim of this study was to investigate the effect of black carrot fermentation on *in vitro* bioaccessibility in terms of total phenolic content, total flavonoid content, anthocyanin content, total antioxidant capacity and detection of phenolics and anthocyanins by HPLC analysis during shalgam beverage production.

1.1.1 Secondary Purpose of Thesis

Secondary purpose of thesis is to explore that how bioaccessibility in terms of total phenolic content, total flavonoid content, anthocyanin content and total antioxidant capacity changes at the end of the fermentation process and major raw material, black carrot and bulgur.

1.2 Literature Review

1.2.1 Production of shalgam beverage

There are two main production methods for shalgam: traditional method and direct method that is differ depending on manufacturer (Tanguler and Erten, 2012). Traditional method contains two different fermentation process. First fermentation is known as dough fermentation in which bulgur flour, salt, dough and water mixed and exposed to fermentation for 3-5 days at ambient temperature. First fermentation made lactic acid compound at elevated level. During second fermentation, carrot fermentation, after the addition of black carrot and adequate water anthocyanin compound from black carrot pass to liquid. Carrot fermentation performed at ambient temperature for 3-10 days. In this research, direct production method is used. Direct method does not include dough fermentation (Tanguler, 2010).

1.2.1.1 Raw materials

The raw materials used for shalgam production are black carrot, salt, bulgur flour and drinkable water.

Black carrot

Black carrot, *Daucus carota spp. sativus var. atrorubens Alef* is traditionally grown in Turkey, Egypt, Pakistan, and India considered being one of the most important sources of natural food colorants. Roots of black carrots are used as the main raw material for the formation of lactic acid as the predominant product during sugar fermentation and for the red color of shalgam. Pigmentation (red colour) that derives from anthocyanin is the main factor for using black carrot in the production of shalgam (Erten et al, 2008). Anthocyanins are extracted from black carrot into shalgam beverage during fermentation (Kammerer et al, 2004).

The major anthocyanin compounds of black carrots are acylated and unacylated cyanidin derivatives (Tanguler, 2010). The anthocyanins mostly present in black carrot could be specified as cyaniding-3-xylosyl-galactose and cyaniding-3-xylosyl-glucosyl-galactoside, which is further monoacylated with ferulic, sinapic and p-coumaric acids (Turker et al, 2007). Besides anthocyanins, p-coumaric acid, caffeic

acid, ferulic acid, 3-hydroxybenzoic acid derivatives and 1quercetin glycoside, chlorogenic acid, mono- and dihydroxycinnamoylquinic acid are the other phenolics reported in black carrot (Kammerer et al, 2004).

Black carrot is added between 10-20% levels at the beginning of fermentation to achieve adequate acidity and colour at the end. If black carrot is added at required amounts, the sugar level is sufficiently high to support fermentative process (Erten et al, 2008).

Bulgur

Bulgur flour is a minor ingredients used apprixomately 3% for the shalgam production. It also provides nutrients to microorganisms during fermentation process (Tanguler, 2010).

Salt

Salt is an essential material in production of shalgam, which has several functions during fermentation. It not only inhibits the spoilage but also assists the growth of lactic acid bacteria. Besides salt enhances shalgam beverage its salty taste (Tanguler, & Erten, 2012).

Sodium benzoate

Sodium benzoate is added as preservative after fermentation process with recipe from Doganay Gıda.

1.2.1.2 Fermentation process

Fermentation is defined as the extraction of energy from carbohydrates and other organic substrates without using O_2 as an electron acceptor. Li (2005) states that during fermentation process, complex foods including proteinaceous material, carbohydrate and carbohydrate-like material, fatty substances undergo different irreversible change according to nature of food, types of microorganisms and the environmental condition as three major factor pH value, anaerobic conditions and temperatures influencing their growth and metabolic pattern. The addition of salt controls the growth of microorganisms during fermentation (Li et al, 2006).

After fermentation, number of volatile organic compounds such as terpenes for carrot and fennel and alcohol content observed to increase and solid content and aldehydes

decrease (Beshkova et al, 2003). According to Tanguler and Erten, lactic acid, which aid to preserve and enhances taste and aroma is the principle end product of shalgam fermentation and also acetic acid formed during fermentation is the basis volatile acidity (2012). Tanguler (2010) stated that, forty-four volatile compounds such as carbonyl compound, volatile acids, higher alcohol, esters, and volatile phenols have been identified in shalgam following fermentation. Moreover, biogenic amines, which are depend on the concentration of the amino acid substrates and the activity of the amino acid decarboxylase enzymes, are formed (Erten et al, 2008). Bacterial decarboxylation of amino acids at elevated concentration may cause a health hazard (Ozdestan and Uren, 2010).

The lactic acid bacteria's are known to have antioxidant activity, which also has been reported in several studies. The highest activity was obtained by heterofermentative Lactobacillus species (Annuk, 2003). The antioxidant activity observed to increase during the fermentation with the development of radical-scavenging activity (Virtanen et al, 2006).

1.2.2 Characteristic of shalgam beverage

1.2.2.1 Chemical composition

Canbas and Deryaoglu analyzed commercial shalgam samples from four well-known producers in Adana (1993). According to this research, the pH of shalgam beverage ranges from 3.33 to 3.67. One of the most important component of shalgam is lactic acid, which preserves the beverage and enhances taste and aroma. The concentration of anthocyanin ranges from 88.3 mg/L to 134.6 g/L with the average of 114.1. Besides shalgam beverage contains potassium, phosphorus, calcium, iron and some other minerals (Erten et al, 2008). It also include high levels of amino acids, water-soluble vitamins (B_1, B_2 and C), natural antioxidants and phenolics (Ersan and Turan, 2012). In a study which investigate the quality and composition of shalgam using red beet instead of black carrot has shown that sensory properties of shalgam which produce by black carrot is more acceptable for consumers while soil odor is detected with production of red beet (Tanguler, 2010).

Phenolic compounds

Phenolic compounds being secondary metabolites are polyphenolic antioxidant, which can be found in natural structure of fruits and vegetables. The phenolic compounds are divided into two groups as phenolic acids and flavonoids. Hydroxyl benzoic and hydroxyl cinnamic acids are two groups of phenolic acids, which are shown in Figure 1.1. Phenolic compounds being the source of bitterness and sourness in foods contribute to the taste and aroma (Nizamoglu and Nas, 2010). Additionally, Nizamoglu and Nas stated that phenolic compounds, natural antioxidants, have the ability of preventing many disease such as cancer, heart disease, lung disease by stopping or inhibiting the free radical reactions (2010).

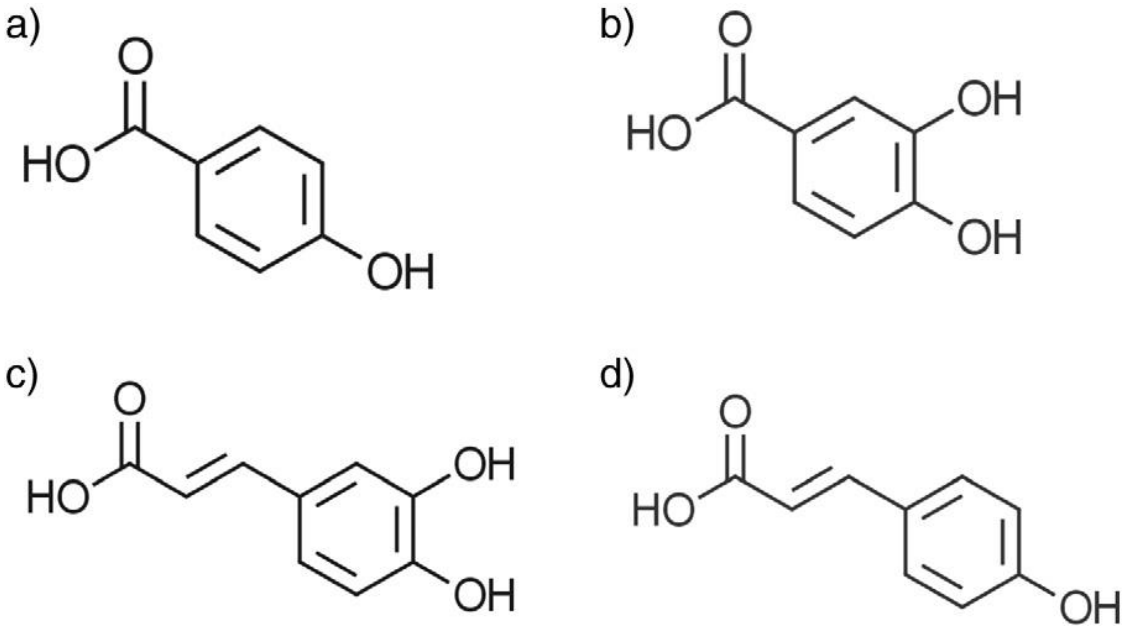


Figure 1.1: Phenolic acids a**.** p-hydroxybenzoic acid **b**. 3,4-dihydroxybenzoic acid **c.** caffeic acid **d**. p-coumaric acid.

Flavonoid compounds

Flavonoids are divided into five groups as anthocyanidin, flavone and flavonone, flavononlar, catechin and eucoanthocyanidin and proanthocyanidin. Flavonoids, which are in the large class of phytochemicals, are widely distibuted in the plant kingdom especially in the epidermis of leaves, skin of fruits (Caballero, 2009). Antioxidant, anti-thrombotic, anti-inflammatory, anti-proliferative, antibacterial and lipid metabolism regulator properties of flavonoid types are beneficial for human health (Manthey and Busling, 1998). Figure 1.2 shows structures of the major

subclasses of flavonoids; Isoflavone, Flavone, Flavonol, Flavanone and Flavan-3-ols (Caballero, 2009).

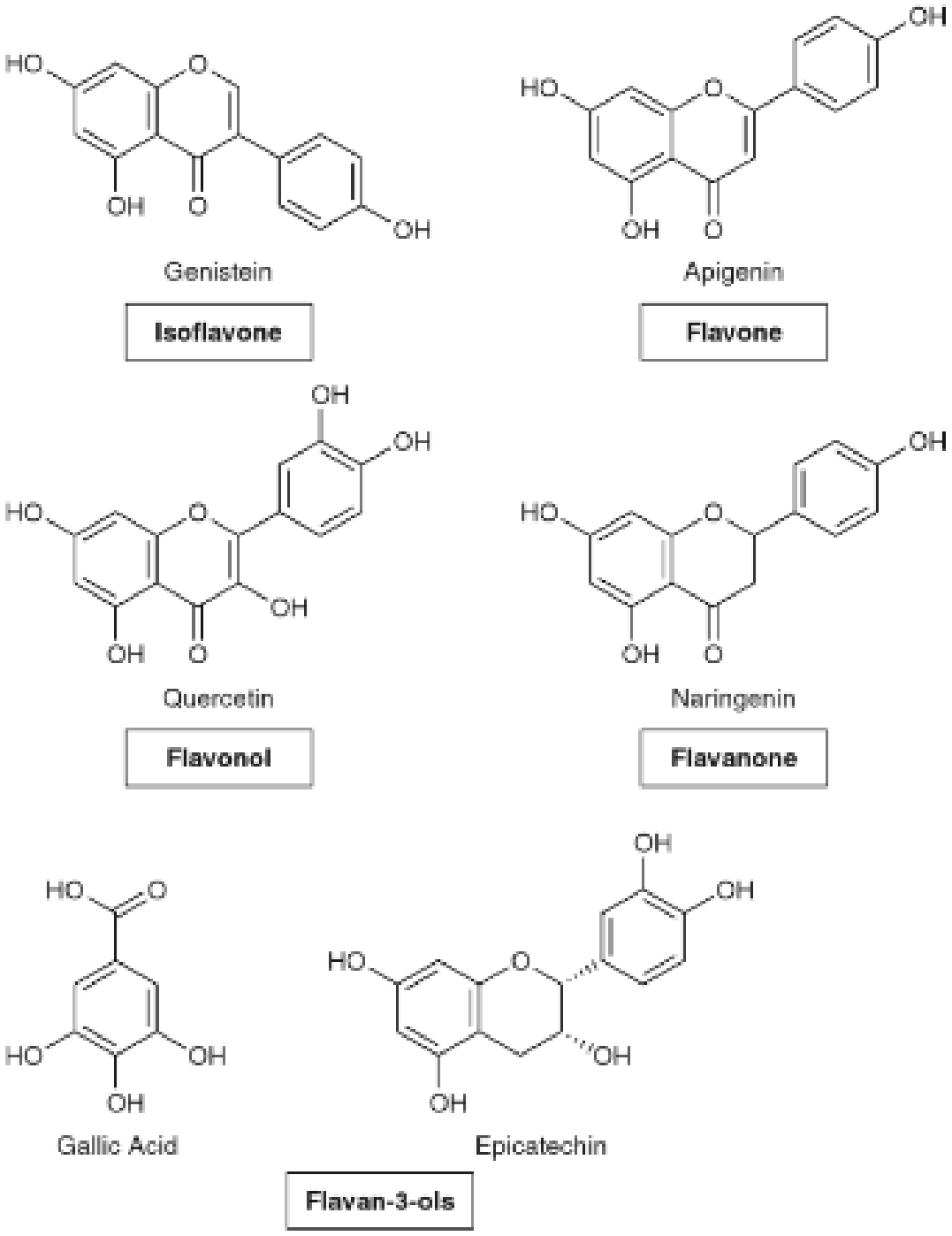


Figure 1.2: Structures of the major subclasses of flavonoids adopted from Caballero (2009).

Anthocyanin compounds

Anthocynidins, which does not occur free in nature, make glycosides with sugar and are known as anthocyanins. These compunds are most abundant flavonoids.

Anthocyanins act as natural colorant and responsible for the pink, red, blue, purple color of vegetable, fruits, fruit juices and wines (Nizamoglu and Nas, 2010). They are phenolic compounds, which constitute the largest group of water soluble pigments (Algarra et al, 2014). Anthocyanins occur in nature as glycosides of anthocyanidins and may have aliphatic or aromatic acid attached to the glycosidic residues (Turker et al, 2004). Algarra et al, (2014) states that there has been 539 anthocyanins isolated reported so far, nevertheless commonly known anthocyanins based on six anthocyanidins; cyaniding, delphinidin, malvidin, pelargonidin, peonidin and petunidin. The color of black carrot is originates from its high anthocyanin content (Tanguler, 2010). The anthocyanins present in black carrots, derivatives of cyanidins, are cyanidin-3-xylosyl-galactoside and cyanidin-xylosyl-glucosyl-galactoside, and also further monoacylated with ferulic, snapic and p-coumaric acids (Turker et al, 2004). Figure 1.3 demonstrates the most abundant anthocyanins existing in black carrot. The consumption of anthocyanins lowers the risk for diabetes, arthritis, cardiovascular disease and cancer due to their anti-inflammatory and antioxidant activities (Miguel, 2011).

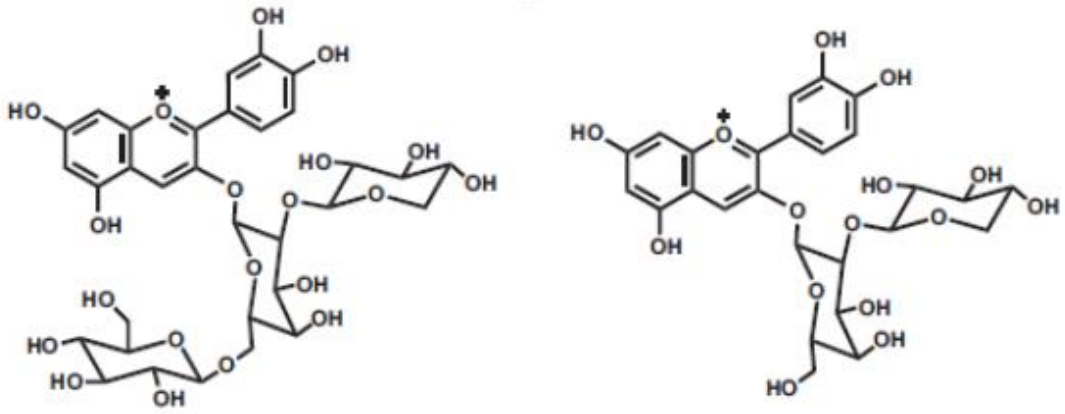


Figure 1.3: Anthocyanin which is mostly found in black carrot. Cyanidin 3-xylosyl glucosyl galactoside and cyanidin 3-xylosil galactoside respectvely adapted from (Algarra et al, 2014)

Antioxidant capacity

Antioxidant can be divided into two major groups; primary or chain breaking; moreover they can also be classified into five type; primary antioxidant, oxygen scavengers, secondary antioxidants, enzymatic antioxidant, and chelating agents (Kolakowska and Bartosz, 2011). The antioxidant capacity is positively affected by the presence of phenolic compound content (Algarra et al, 2014). Black carrot has

powerful antioxidant activity due to its total phenolic and anthocyanin content (Tanguler, 2010). Free radicals, which can lead to cancer, heart disease, lung disease and cataract, are done oxidative damage to lipids, proteins and nucleic acids and occur when atoms, molecules and ion lose their electrons. Antioxidant compounds such as vitamin C and E stop or inhibit the reaction that free radicals start due to unpaired electrons and prevent the damage by connecting the oxygen and metal. Free radicals and other reactive oxygen species are derived from metabolic process of human body or from external sources such as exposure to X-rays, ozone, cigarette, smoking, air pollutants and industrial chemicals (Lobo et al, 2010). Food products which has high antioxidant capacity can inhibit free radical induced diseases such as cancer, cardiovascular disease, carcinogenesis, aging, and Alzheimer (Nizamoglu and Nas, 2010; Lobo et al, 2010).

The total antioxidant capacity of the extracts was determined using DPPH and CUPRAC assays since antioxidant activity occurs by different mechanisms which means employing a method depending on one mechanism may not reflect the true antioxidant capacity (Karadag et al., 2009).

DPPH, which is dark colored and stable free radical molecules, is soluble in methanol and ethanol (Wollinger et al., 2016). Brand - Williams et al. stated that the interaction of a potential antioxidant with DPPH depends on its structural conformation. At a wavelength around 515 nm due to presence of an unpaired electron, DPPH has strong absorption (1995). Observation of color change from violet to yellow occur leading to determination of antioxidant activity by reducing a number of DPPH electron molecules corresponding to the number of available hydroxyl groups (Wollinger et al., 2016).

The absorbance of the orange–yellow colored chelate formed as a result of the redox reaction of cupric neocuproine reagent with antioxidants is measured at 450 nm in CUPRAC assay (Apak et al, 2004).

1.2.2.2 Functional properties of shalgam beverage

Potassium plays a role in the regulation of osmotic pressure, blood pressure, acid-base balance. Minerals, calcium and phosphorus plays a fundamental role in the rapid skeletal development of young. Iron has various vital functions; carrying oxygen from the lungs to tissues as a component of hemoglobin in red blood cells and electron

transport within cells, and it participates in important enzyme system in various tissues in the body. Moreover, it decrease the incidence of anemia. Copper and zinc are essential minerals for all life forms and required cofactors for enzymatic reactions (Ersan and Turan, 2012).

1.2.3 Bioavailability and bioaccessibility

According to FDA bioavailability is the rate and extent to which the active ingredient is absorbed from a product and becomes available (1996); summarizing; amount of nutrient in blood plasma (Blum and Hummon, 2000). On the other hand, bioaccessiblity is described as the amount of ingested food product, which is reachable for absorption in the intestine after digestion (Hedrén et al, 2002). Bioavailability and bioaccessibility of food products can be measured by both in *vivo* and *in vitro* assay. *In vivo* assays carry out by analysing living beings after consumption of a nutrient (Yeum and Russell, 2002). *In vitro* digestion techniques have been developed as an alternative approach to *in vivo* assay. *In vitro* digestion is known as stimulation of mouth, stomach and intestinal condition in laboratory conditions (Parada and Aguilera, 2007). If comparision is done between *in vivo* and *in vitro* approach; *in vitro* techniques are simpler, cheaper and more reproducible tools to assess the digestive stability of different food constituents (Rodríguez-Roque et al, 2013). It is possible to state that there are some deviation between the results of *in vitro* and *in vivo* assessment; but in general there are some in vitro methods, which is valid; in consistent with *in vivo* human studies. Furthermore altohugh *in vivo* assay measures bioavailability directly with lesser value; there are some limitation for *in vivo* studies such as individual difference, physiological state, dose and existence of other food components influence the results (Faulks and Southon, 2005), ethical restriction about existence of human and animal in the experiments (Van Het Hof et al, 2000; Van Het Hof et al, 2000).

Despite the fact that the bioavailability of phenolic content of nutrient are presumed to have bioavailable properties, bioavailability feature of this beneficial components may vary depending on release from the food matrix, stability against several biochemical factors, absorption capacities (Celep et al, 2015; Manach et al, 2004), possible interactions with other food components, presence of suppressors or cofactors (Parada and Aguilera, 2007). Besides there are other studies which has proved that

concentration of anthocyanin profile may be affected by technological process such as fermentation (Wickzowski et al, 2016; Wiczkowski et al, 2015).

In order to evaluate the potential role of anthocyanins in the human body, changes occurring in the gastrointestinal tract should be taken into account. For simulating the gastrointestinal conditions, in vitro digestion and dialysis methods are being commonly used due to being rapid, safe, and do not have the same ethical restrictions as in vivo methods (Liang et al., 2012). These *in vitro* methods have already been tested in various fruits rich in anthocyanins including raspberry (McDougall et al, 2005a), chokeberry (Bermudez-Soto et al, 2007), apples (Bouayed et al, 2012), mulberry (Liang et al., 2012), fig (Kamiloglu and Capanoglu, 2013) sour cherry (Toydemir et al, 2013), broccoli (Vallejo et al, 2004), red cabbage (McDougall, Fyffe, Dobson, & Stewart, 2007) and pepper (Hervert- Hernandez et al, 2010).

To our knowledge, no study to date evaluated the effect of in vitro gastrointestinal digestion during fermentation of shalgam beverage.

1.2.4 Hypothesis

Lactic acid fermentation of black carrot has positive effects on total phenolic content, anthocyanin content, total antioxidant capacity and *in vitro* bioaccessibility of shalgam beverage.

End product of fermentation process; sample collected on 24^{th} day of fermentation, has equal or higher bioaccessible total phenolic content, anthocyanin content and total antioxidant capacity than major raw material, black carrot.

2. MATERIALS AND METHODS

2.1 Raw Materials

Black carrots (Daucus carota L. ssp. sativus var. atrorubens Alef.) and bulgur were supplied from Doganay Inc. in Adana, Turkey. Sampling was performed shalgam samples collected on the 1st, 12th, and 24th days of fermentation. Commercial shalgam beverages were supplied from three different local markets. Experiment were conducted to the samples of 1st, 12th, and 24th days of fermentation which are also kept in refrigerator at -80°C in order to perform bioavailability tests and main raw material; black carrot. All of the studies have done three replicates to obtain more acceptable and reliable results.

2.2 Manufacturing Process

In current study, direct production method is used for shalgam production according to instructions of Doganay Inc, Adana, Turkey. In direct production method (Figure 2.1), black carrots are sorted out and cut into pieces (approximately 5 cm length and 1.5 cm width). Bulgur is placed into a thin cloth and tied with rope. The chopped black carrots (17 %), salt (1.5 %), bulgur (1.5 %) and adequate water (80 %) are transferred to the container to employ lactic acid fermentation. Lactic acid fermentation gives shalgam its typical taste due to producing mainly lactic acid and ethanol. (Erten, Tanguler, & Canbas, 2008). Fermentation takes place at ambient temperatures (22°C) for 24 days. Shalgam beverage was produced twice in three different containers.

2.3 Chemicals and Reagents

DPPH (2,2-Diphenyl-1-pictylhydrazyl), Neocupraine, Trolox, α-amilaz (300-1500 U/ml), pepsin (≥2500 U/ml), pancreatin (≥100 U/ml) and bile salts were purchased from Sigma-Aldrich Chemie GmbH & Co. KG (Steinheim, Germany). Potassium chloride, mono-potassium phosphate, sodium bicarbonate, sodium chloride, magnesium chloride hexahydrate, ammonnium carbonate, hydrochloric acid, calcium chloride dihydrate, sodium hydroxide Folin-Ciocalteu reagent was purchased from

MERCK (Germany). Dialysis bags (Membra-Cel MD34 - 14 x 100 CLR) used for in vitro gastrointestinal digestio n were obtained from Serva Electrophoresis GmbH (Heidelberg, Germany). All chemicals and reagents used for the analyses were of analytical grade.

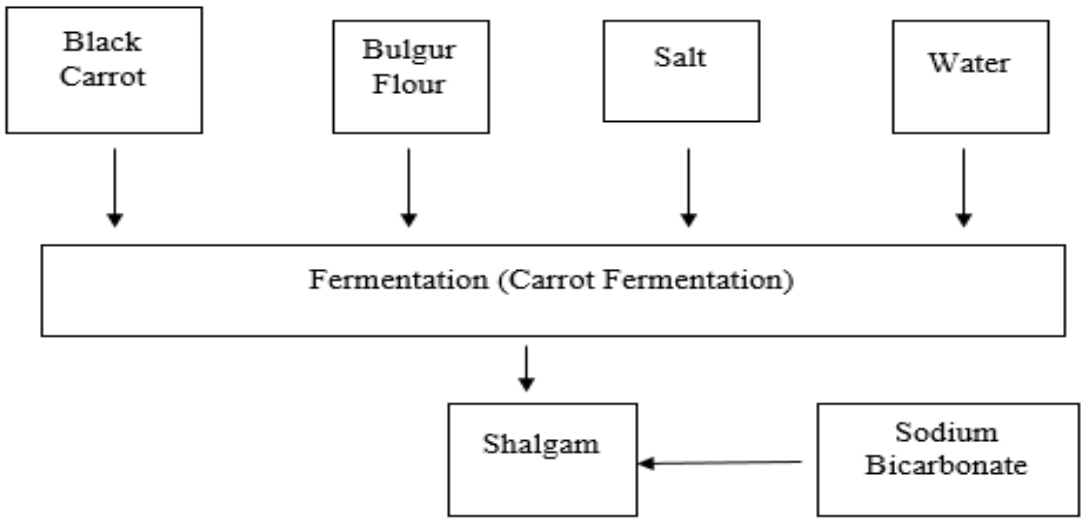


Figure 2.1: The production of Shalgam by direct method

2.4 Extraction of Phenolic Compounds

75 % MeOH (10 ml) was used to extract phenolics of milled black carrot samples (2 g) in falcon tubes. After incubation in an iced ultrasonic bath (USC900TH, VWR ultrasonic cleaner, Radnor, PA, USA), tubes are centrifuged (Universal 32R, Hettich Zentrifugen, Tuttlingen, Germany) at 4°C, 4500 g for 10 minutes. Extraction was repeated twice and top phases were pooled. Prepared extracts were stored at -20°C.

2.5 Determination of Brix, Dry Matter Content and pH

Dry matter content and pH analysis were determined according to AOAC method (1984). All samples were analyzed in triplicate in order to compare results more reliable.

2.6 Simulation of *In Vitro* Gastrointestinal Digestion

Bioaccessibility of shalgam beverage was investigted by *in vitro* gastrointestinal digestion model which is stimulated according to Dinnella et al methodology (Dinnella

et al, 2007). In order to constitute mouth environment, for solid 5 gr and for liquid 5 ml sample were dissolved in 3.5 ml saliva solution, 0.5 ml α-amilaz solution, 25µL $CaCl_2$ solution and 975µl distilled water. After shaking and incubating for 2 minutes at 37°C, 2 ml sample was taken (Mouth sample: MS). For stumulating gastric conditions, remaining solution was dissolved in 6 ml of gastric fluid, 1.26 ml pepsin solution, 4µL $CaCl_2$, 0.16 mL HCl nd 0,556µl distilled water. The solution acidified with 6 M HCl to obtain pH 3 under constant stirring. The prepared solution was placed to shaking water bath and incubated at 37°C. After incubation, 2 ml of sample has taken from stomach environment (Stomach sample: SS). 7.7 ml of intestinal fluid, 3.5 ml of pancreatic solutiın, 1.75 ml of bile solution, 28 µl of $CaCl_2$ solution, 0.105 ml of 1 M NaOH was added to remaining solution and final volume was arrange to 10 ml with distilled water in order to obtain intestinal environment. After 2 hours of incubation in shaking water bath at 37°C, 2 ml of the solution was collected to analyze post gastric condition (Intestine sample: IS).

2.7 Spectrophotometric Assays

2.7.1 Determination of total flavonoid content

The total flavonoid content was determined according to Dewanto et al (2002). 100 µl extract was mixed with 500 µl distilled water and 30 µl 5% aqueous-$NaNO_2$ solution. 60 µl 10% $AlCl_3.6H_2O$ solution was added after 6 minutes. Than 5 minutes, 200 µl 1 M NaOH was mixed. Final volume of mixture was filled with distilled water to 1000 µl. The absorbance was read at 510 nm against distilled water immediately and rutin was used for the standard. The results were expressed as mg rutin equivalent/g for solid and mg rutin equivalent/ml for liquid samples.

2.7.2 Determination of total phenolic content

Total phenolic content of samples was determined using Folin-Ciocalteu method according to Javanmardi et al (2003). 200 µL of each sample was mixed with 1.5 mL 10 fold diluted Folin-Ciocalteu's reagent and 1.2 mL Na_2CO_3 (7.5 g/100g). The mixture was allowed to stand for 90 minutes at room temperature before the absorbance was measured at 765 nm. Results were expressed as gallic acid equivalents (GAE) per 100 g. All samples were analyzed in triplicate to obtain reliable results.

2.7.3 Determination of anthocyanin content

pH Differential method (Lee et al, 2005) was employed to evaluate anthocyanin content. The samples were diluted by potassium chloride buffer at pH 1.0 (25 mmol/L) and sodium acetate buffer at pH 4.5 (0.4 mol/L). Absorbance was measured at 512 and 700 nm. Results were expressed as cyanidin-3-glucoside (Cy-3-glc) and calculated according to the equation 4.1.

$$\mathrm{AC}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) = \frac{\mathrm{A.MW.DF.10^3}}{\varepsilon.1} \qquad (4.1)$$

A absorbance ($A_{pH1.0}$-$A_{pH4.5}$), MW molecular weight (449.2 g /mol Cy-3-gly),

NW is taken 445.2 to the based on cyanidin molecular weight.

DF is the dilution factor, ε molar absorptive coefficient (ε = 26.900).

2.7.4 Determination of antioxidant capacity by DPPH assay

Antioxidant capacities of the samples were determined by DPPH assay described by Tezcan et al. (2008). 100 µL sample and 2 ml of 0.1 mol/L DPPH in methanol were mixed. Absorbance at 517 nm was determined after incubation for 30 minutes in dark environment at room temperature. Results were expressed as micromole trolox equivalent (TE) per 100 milliliter or 100 gram sample.

2.7.5 Determination of antioxidant capacity by CUPRAC assay

CUPRAC Cupric Reducing Antioxidant Capacity analysis was performed based on the method developed by Apak et al. (2004). The method comprised mixing of the 100 µL sample with a 1 mL of 10 mmol/L copper (II) chloride solution, 1 mL of 7.5 mmol/L neocupraine and 1 mL of 1 mol/L ammonium acetate buffer (pH 7). 1 mL of distilled water was added to the mixture to make the final volume 4.1 mL. After 30 min of incubation at room temperature, absorbance was measured at 450 nm. All analysis were performed triplicate and the results are expressed by micromole trolox equivalent per milliliter or gram sample.

2.8 Identification of Phenolic Acids by UPLC Analysis

A Shimadzu LC -10A apparatus (Kyoto,Japan) equipped with a SPD-M10A photodiode array dedector (PDA) was used for analytical UHPLC separations.Reversed-phase chromatography was performed with 250 x 4.6 mm

Kromasil 100 C-18 column packed with 5µm particles (teknokroma,Barcelona,Spain), fitted with a security guard C18 ODS (4 x3.0 mm i.d).Gradient were formed with He-degassed solvent.Solvent A was H2O containing %0.1 formic acid, and solvent B was MeCN by applying different elution conditions. Separation was accomplished starting with 5% A for 2 min at pressure of 115 bar,followed by a linear gradient was performed for 10 min from 5% B to 95 % A and subsequent linear gradient from 20 to 95% A in 5 min.The flow rate was 0.5 mL.min-1, and the operating temperature was 40 0C. The injection volume was 10µL.The chromatogram was recorded at 286 nm; measures phenolic acids.

2.9 Determination of Anthocyanin Profile by HPLC-PDA Analysis

Phenolic acids were determined following the method (Capanoglu et al, 2008). Samples were passed through 0.45 µm membrane filters and injected into high-performance liquid chromatography (HPLC) system. The separation in Waters 2695 HPLC (Waters Co., Milford, MA, USA) took place in photodiode array (PDA) detector (Waters 2996) with a Supelcosil LC-18 25 cm 4.60 mm, 5 µm column (Sigma–Aldrich). The following solvents with a flow rate of 1 mL/min and 10 µL injection volume were used for spectral measurement at 312 nm: as mobile phase solvent A, Milli-Q water with 0.1% (v/v) TFA and solvent B, acetonitrile with 0.1% (v/v) TFA. The 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 returning to initial conditions. Quantification was done using external standards. All analyses were performed in triplicates and the results were expressed as mg per 100 g dw for solid sample and ml per 100 l dw for liquid samples.

2.10 Statistical Analysis

All measurements were repeated three times using triplicate samples and reported as mean ± standard deviation. IBM SPSS Statistics software (version 21.0, SPSS, Chicago, IL, USA) was employed for statistical analysis. Mean values were compared by one-way analysis of variance (ANOVA). Differences were analyzed by Tukey’s

Test comparisons and p value of < 0.05 was chosen to determine significant differences.

3. RESULTS AND DISCUSSIONS

3.1 Changes In pH During Fermentation

The pH of the shalgam beverage sample was 4.9±0.5 at the beginning, decreased to 3.43±0.32 and 3.38±0.4 shalgam samples collected on the 12th and 24th days of fermentation, respectively (Figure 3.1). pH of commercial shalgam juice is 3.49±0.24 which is in accordance with the pH results of shalgam beverage examined in this study. As the fermentation progress, the more pH of sample was decreased, the more the acidity of beverage was increased. Similarly, Turker et al (2004) reported the pH of commercial shalgam juice as 3.5, and it changed between 3.54 and 3.76 during storage.

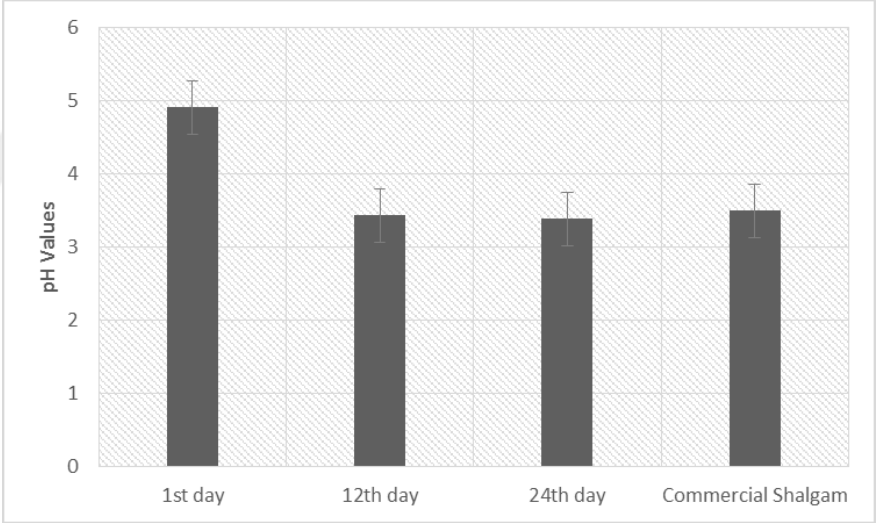


Figure 3.1: pH values of shalgam during fermentation and commercial shalgam ($p < 0.05$)

3.2 Effect of Lactic Acid Fermentation on Total Flavonoid Content

The TFC of black carrot was found as 643.2±22 mg rutin/100 g sample based on dry weight in current research. Chatatikun and Chiabchalard measured TFC of black carrot extract between 3.7 and 17.7 mg QE/g dw (2013). In another research on effect of preteratment on black carrot flavonoid the TFC of samples were found between 217 and 421 mg QE/g samples (Garba and Kaur, 2014). During fermentation the TFC content of shalgam were observed to rise. While sample collected on 12th and 24th days

of fermentation were statistically identical; the TFC value of commercial shalgam and blach carrot extraction sample were statistically equal and higher (Table 3.1).

Table 3.1: Changes in total flavonoids, phenolics, anthocyanins, and antioxidant capacity of shalgam beverage, black carrot and bulgur samples

Samples	Total Flavonoid Content (mg rutin/100 ml)	Total Phenolic Content (mg GA/100 ml)	Anthocyanin Content (mg/l)	DPPH (μmol TE/100 mg dw)	CUPRAC (μmol TE/mg dw)
1st day	15.8 ± 1^{a}	12.3 ± 1^{a}	306.0 ± 7^{a}	3.5 ± 1^{a}	58.6 ± 2^{b}
12st day	197.0 ± 12^{b}	62.7 ± 3^{b}	2834.5 ± 148^{b}	57.7 ± 7^{b}	178.7 ± 10^{c}
24st day	250.5 ± 8^{b}	81.5 ± 3^{c}	3250.6 ± 49^{c}	69.7 ± 5^{b}	214.0 ± 8^{d}
Commercial Shalgam	252.0 ± 44^{c}	74.5 ± 7^{bc}	2845.6 ± 141^{b}	60.9 ± 7^{b}	162.0 ± 14^{c}
Black Carrot	643.2 ± 22^{c}	112.7 ± 11^{d}	5291.3 ± 254^{d}	104.1 ± 5^{c}	546.2 ± 135^{e}
Bulgur	0.3 ± 0.1^{a}	0.2 ± 0.1^{a}	1.9 ± 0.3^{a}	0.3 ± 0.1^{a}	0.8 ± 0.1^{a}

3.3 Effect of Lactic Acid Fermentation on Total Phenolic Content

The results of TPC of raw material (black carrot), shalgam samples collected during fermentation, and commercial shalgam beverage are shown in Table 3.1 according to current study, TPC of black carrot was found to be higher (720.3±32 mg GA/100 g fw) than most of the research data in literature which is ranged between 125±17.22 mg/100 g (Ersus and Yurdagel, 2007) to 350.5±12.9 mg/100g (Kaur and Kapoor, 2002).

At the beginning of the fermentation process, TPC was 12.3±1 mg GA/100 ml dw, gradually increased to 62.7±3 mg GA/100 ml dw and reached to 81.5±3 mg GA/100 ml dw at the end of the fermentation. TPC of shalgam collected on the 24th day of fermentation was statistically equal to commercial shalgam beverage sample (74.5±7 mg GA/ml dw). Bulgur and shalgam sample collected on first days of fermentation shows the lowest but statistically identical value (Table 3.1).

3.4 Effect of Lactic Acid Fermentation on Anthocyanin Content

The color of the shalgam comes from the anthocyanin pigments of black carrots. The AC of samples obtained by extraction of black carrot found in current study (731.0±4.6 mg/kg fw) was similar with the literature (716.8±15.2 mg/kg fw) which is reported by

Kammerer et al (2004). Black carrot had the highest amount of anthocyanins (5291.3±254 mg/kg dw) if comparision is done between shalgam beverage samples.

The AC of shalgam samples collected during fermentation process increased rapidly in early fermentation including the first twelve days. This increment proceeded from 306.0±7 mg/L dw to 2834.5±148 mg/L dw during the first twelve days of the fermentation, the increase of AC content was observed more slowly compared to increment of AC content at the end of the fermentation (3250.6±49 mg/L dw). It is probably due to the reduction of lactic acid bacteria count each passing day between samples collected on the 12^{th} and 24^{th} day of fermentation. (Tanguler and Erten, 2012). The AC of commercial shalgam and shalgam sample collected on 12th days were observed to be statistically identical (Table 3.1).

Consequently, lactic acid fermentation considerably increased the total phenolics and anthocyanins in shalgam beverage ($p < 0.05$).

Figure 3.2 shows that as the total phenolic content increases, anthocyanin content also rises. It is noticeable that there is high correlation between TPC and AC ($R^2 = 0.91$).

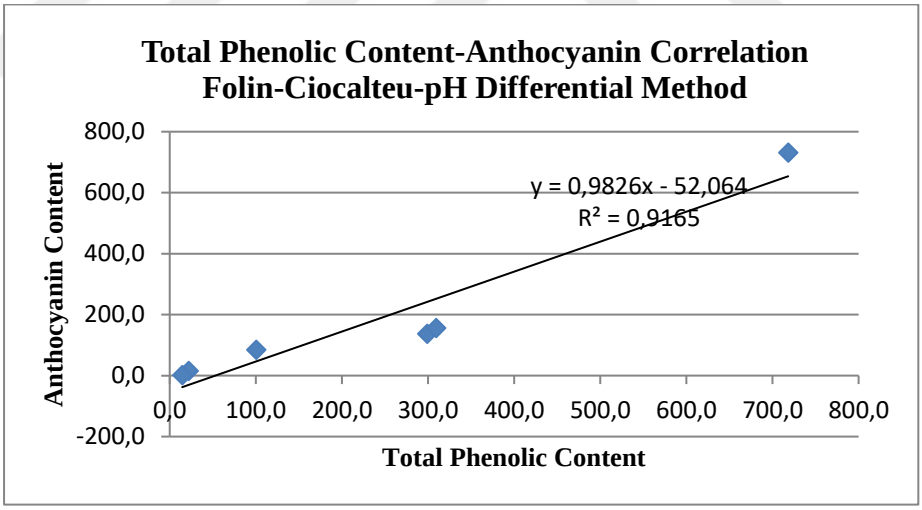


Figure 3.2: The correlation between total phenolic content and anthocyanin content ($R^2 = 0,9165$)

3.5 Effect of Lactic Acid Fermentation on Total Antioxidant Capacity

The total antioxidant capacity (TAC) of raw material (black carrot, bulgur), shalgam samples collected during fermentation, and commercial shalgam beverage samples are also represented in Table 3.1.

In terms of DPPH assay, black carrot exhibited the highest TAC (104.1±5 μmol TE/100g dw, 58.6±2 μmol TE/g dw for DPPH and CUPRAC assays, respectively) among all samples. As reported by previous study (Algarra et al, 2014), the TAC of black carrot extracts ranged between 17.6 and 240 μM TE/100 g fw, which is compatible with our results (28.1±2.54 μmol TE/100 g fw) in terms of DPPH assay. The variation of TAC may be due to differences in black carrot maturity and growing conditions (Kader et al, 1977).

TAC of shalgam increased rapidly from 3.5±1 μmol TE/100g dw and 58.6±2 μmol TE/ml dw to 57.7±7 μmol TE/100g dw and 178.7±10 μmol TE/ml dw sample collected on the 12th day of fermentation for DPPH and CUPRAC assays, respectively. This increment was observed to be decreased in the last twelve days of the fermentation compared to first twelve days as previous assay. In terms of DPPH assay there was not statistical difference between sample collected on 12th day and 24th day of fermentation however in terms of CUPRAC assay increment was observed. TAC of shalgam collected on the 24th day of fermentation and commercial shalgam product was determined to be 5.1±0.35 μmol TE/g fw and 3.2±0.7 μmol TE/100 g fw, respectively. Our findings are in agreement with Baser et al. whose results are in the range of 3.53 and 5.93 μmol TE/g fw (2012).

In summary, lactic acid fermentation caused a significant increase in TAC of shalgam beverage ($p < 0.05$). As a result of experiments the total phenolic, anthocyanin and antioxidant contents were increased while the pH decreased during fermentation, however the ingredient of those that in commercial shalgam were less than laboratory made shalgam which is correlated with pH increment.

3.6 Effect of *In Vitro* Bioaccessibility on Total Flavonoid Content

Table 3.2 shows the effect of *in vitro* gastrointestinal digestion on TFC, TPC, TAC and AC. After mouth digestion, significantly lower TFC for all samples (38.8-56.0 %). Only 32.3 % of TFC initially presence in shalgam sample collected on 24th days of fermentation. The stomach condition represented 16.5-33.2% of the initial TFC. The non-dialyzed flavonoid content accounted as 6.7-13.9% of the initial TFC. In previous studies, bioaccessible total flavonoid was changed between 15.9 and 20.1 % for soymilk, blended fruit juice and mixture of blended fruit juice and soymilk beverage (Rodríguez-Roque et al, 2013).

Table 3.2: TFC changes of *in vitro* bioaccessibility of shalgam beverage, black carrot and bulgur samples based on % recovery ($p < 0.05$)

Sample and Analysis	Mouth	Stomach	Intestine
1st day	$43,9 \pm 4^{ab}$	$21,7 \pm 5^{ab}$	$6,9 \pm 3^{b}$
12th day	$53,4 \pm 5^{b}$	$32,1 \pm 6^{b}$	$6,7 \pm 0^{b}$
24th day	$56,0 \pm 2^{b}$	$32,3 \pm 5^{b}$	$8,9 \pm 6^{b}$
Commercial Shalgam	$44,9 \pm 13^{ab}$	$33,2 \pm 3^{b}$	$8,5 \pm 2^{b}$
Black carrot	$50,4 \pm 3^{ab}$	$23,0 \pm 3^{ab}$	$13,9 \pm 6^{c}$
Bulgur	$38,8 \pm 7^{a}$	$16,5 \pm 5^{a}$	$12,1 \pm 3^{a}$

3.7 Effect of *In Vitro* Bioaccessibility on Total Phenolic Content

Effect of gastrointestinal digestion on the percent recovery of bioaccessible total phenolics is shown in Table 3.3. Gastric digestion significantly reduced TPC in terms of all samples ($p < 0.05$). After gastric digestion, bioaccessible phenolics of black carrot (48.4±4.0%), shalgam samples collected on the 24th day, and on 12th day of fermentation (45.4±3.7% and 44.6±4.0%, respectively) did not change significantly ($p> 0.05$). In previous studies, percent recovery of bioaccessiable phenolics after gastric digestion was reported to be 107.8±2.1% for red cabbage (McDougall et al, 2007) and 29% for pomegranate (Pérez-Vicente et al, 2002).

It is demonstrated frankly that when the sample was passed through the stomach, the dialyzed fraction of phenolics which correspond to IN were significantly reduced. The percent of recovery of the bioaccessible phenolics of black carrot (14.6±1.3%), shalgam beverage collected on the 1st day of fermentation (14.2±4.1%), on the 12th day of fermentation (15.9±1.9%), on the 24th day of fermentation (16.0±1.8%), and commercial shalgam (14.7±2.5%) in IN fraction were similar ($p>0.05$). Our findings are comparable with the results noted by Kamiloglu et al. (2015) who determined the percent recovery of TPC of black carrot jam and marmalade in dialyzed fraction in the range of 4.9-17.5%. Additionally, dialyzed fraction for percent recovery of TPC was determined as 7.2±0.3% in wine (McDougall et al, 2005b) and 44.6 - 62.7% in apple (Bouayed et al, 2012).

Non-dialyzed phenolic fraction (OUT) was substantially decreased ($p < 0.05$) compared to initial results. In current study, the percent recovery of phenolics in OUT

fractions were detected to be 39.0±2.3% in black carrot, 29.0±5.0% in shalgam samples collected on the 1st day of fermentation, 32.5±3.2% on the 12th day of fermentation, 43.0±2.1 % on the 24th day of fermentation, and 21.5±3.5 % in commercial shalgam. The results were consistent with the data reported by Kamiloglu et al (0.1-58.1%) previously for black carrot jam and marmalade (2015). Meanwhile, the percent recovery of phenolics in non-dialyzed fraction was reported as 24.9±0.9 % in red cabbage (McDougall et al, 2007), and 54.71±2.31 % in mulberry (Liang et al., 2012).

Table 3.3: TPC changes of *in vitro* bioaccessibility of shalgam beverage, black carrot and bulgur samples based on % recovery ($p < 0.05$)

Sample and Analysis	Mouth	Stomach	Intestine
1st day	41.6 ± 8^{ab}	21.3 ± 5^{b}	10.8 ± 3^{a}
12th day	46.5 ± 9^{ab}	29.4 ± 3^{c}	14.9 ± 2^{a}
24th day	59.5 ± 8^{b}	35.5 ± 4^{c}	17.1 ± 3^{ab}
Commercial Shalgam	58.3 ± 10^{b}	30.1 ± 4^{c}	18.5 ± 3^{b}
Black carrot	58.4 ± 6^{b}	29.7 ± 4^{c}	15.7 ± 2^{ab}
Bulgur	33.0 ± 4^{a}	18.0 ± 10^{a}	15.7 ± 9^{ab}

3.8 Effect of *In Vitro* Bioaccessibility on Anthocyanin Content

Table 3.4 represents impact of gastrointestinal digestion on the percent recovery of bioaccessible anthocyanins. Gastric digestion did not have a significant effect on bioaccessible anthocyanins ($p > 0.05$) of black carrot (36.5 ± 1.6%), samples collected on the 1st day of fermentation (34.6 ± 3.7%), on the 12th day of fermentation (36.5 ± 4.8%), and commercial shalgam (34.9 ± 1.7%) observed to be similar while it has substantial effect on shalgam collected on the 24th day of fermentation (46.6 ± 3.0%) ($p< 0.05$). In previous studies, percent recovery of anthocyanins in gastric digestion was determined as 10% in pomegranate juice (Pérez-Vicente et al, 2002) and 7% in chokeberry juice (Bermudez-Soto et al, 2007).

After intestinal digestion, bioaccessible anthocyanins was substantially decreased as it is in case in bioaccessible of phenolics. Low bioaccessible anthocyanins were also shown for purple tomato, red cabbage (Li et al, 2014; McDougall et al, 2007). Bioaccessible anthocyanins were different for each sample in dialyzed fraction ($p <$

0.05). Percent recovery of anthocyanins of shalgam collected from 24th day of fermentation (6.8±0.4) was higher than black carrot (6.2±0.6) ($p < 0.05$) which is comparable to the results reported by Toydemir et al. (2013) indicated that the recovery of total anthocyanins obtained from IN sample of nectar was higher than fresh fruit sample. Besides, McDougall, Fyffe, Dobson, and Stewart (2005b) indicated that recovery of anthocyanins in red wine after in vitro digestion for dialyzed fraction was 7.2±0.3 %, which is also compatible with our findings. In other studies, the recovery of anthocyanin content for dialyzed fraction was found as 5% in raspberry and 0.34± 0.3 % in mulberry (McDougall et al, 2005a; Liang et al., 2012).

Table 3.4: AC changes of *in vitro* bioaccessibility of shalgam beverage, black carrot and bulgur samples based on % recovery ($p < 0.05$)

Sample and Analysis	Mouth	Stomach	Intestine
1st day	48.5 ± 1^{b}	30.5 ± 2^{b}	13.5 ± 1^{b}
12th day	49.9 ± 5^{bc}	30.1 ± 3^{b}	19.2 ± 2^{bc}
24th day	58.1 ± 2^{d}	45.2 ± 3^{c}	26.6 ± 2^{c}
Commercial Shalgam	57.6 ± 6^{d}	43.5 ± 5^{c}	24.2 ± 5^{bc}
Black carrot	55.9 ± 2^{cd}	40.1 ± 15^{bc}	24.5 ± 8^{c}
Bulgur	39.6 ± 1^{a}	14.8 ± 2^{a}	0.3 ± 0.2^{a}

Regarding non-dialyzed phenolic fraction, similar bioaccessiable anthocyanins was observed between shalgam collected on the 12th day of fermentation and commercial shalgam samples ($p>0.05$), and between samples collected on the 24th day of fermentation and black carrot ($p > 0.05$). In point of view of recovery percent, it was observed that there was no significant difference among commercial shalgam (35.6±2.9%), black carrot (%35.2±2.1%), shalgam collected on the 12th day (34.1±1.9%) and on the 1st day of fermentation (32.0±2.4%) ($p>0.05$), but the bioaccessibility of shalgam beverage collected on the 24th day of fermentation (47.5±2.3%) was higher than all samples ($p< 0.05$). In other studies, it was reported that 70% anthocyanins of raspberries and 37% anthocyanins of wine were recovered in OUT samples (McDougall et al, 2005a; McDougall et al, 2005b).

The bioaccessibility of TPC and AC in gastric digestion, dialyzed and non-dialyzed fraction was observed to be lower compared to the initial samples. This situation is caused by degradation of anthocyanins and phenolics due to reaction with solubilized

proteins and polymerization of phenolics, which result in losing activity and solubility of phenolics (Gibis and Weiss, 2012).

3.9 Effect of *In Vitro* Bioaccessibility on Total Antioxidant Capacity

The influence of gastrointestinal digestion on antioxidant capacity of black carrots, and shalgam samples were determined using two different assays, namely DPPH and CUPRAC. According to DPPH and CUPRAC assays, increment in percent recovery of TAC was observed as fermentation proceeded, similarly with previous experiments. In MS, there was a sharp reduction in TAC when compared with initial samples (Table 3.5).

Table 3.5: TAC changes of *in vitro* bioaccessibility of shalgam beverage, black carrot and bulgur samples based on % recovery ($p < 0.05$) in terms of DPPH and CUPRAC

Sample and Analysis	Mouth	Stomach	Intestine
DPPH			
1st day	50.3 ± 6^{ab}	26.5 ± 3^{c}	6.2 ± 2^{ab}
12th day	50.7 ± 1^{ab}	26.0 ± 2^{c}	9.9 ± 1^{bc}
24th day	55.2 ± 12^{b}	22.2 ± 7^{bc}	5.4 ± 1^{ab}
Commercial Shalgam	44.7 ± 4^{ab}	19.7 ± 4^{ab}	7.4 ± 3^{bc}
Black carrot	48.7 ± 3^{b}	25.9 ± 5^{c}	12.3 ± 3^{c}
Bulgur	39.0 ± 7^{a}	12.1 ± 3^{a}	3.0 ± 1^{a}
CUPRAC			
1st day	44.7 ± 6^{ab}	18.2 ± 4^{ab}	7.6 ± 1^{ab}
12th day	43.7 ± 6^{ab}	21.6 ± 2^{abc}	12.6 ± 1^{bc}
24th day	56.4 ± 9^{c}	32.7 ± 7^{cd}	18.5 ± 3^{d}
Commercial Shalgam	56.6 ± 3^{c}	30.1 ± 4^{bcd}	18.4 ± 3^{d}
Black Carrot	48.3 ± 3^{b}	36.0 ± 6^{d}	18.0 ± 1^{d}
Bulgur	37.3 ± 5^{a}	11.2 ± 1^{a}	1.2 ± 2^{a}

Similar total antioxidant capacity in terms of percent recovery were observed shalgam collected on the 1st day of fermentation (50.3 ± 6^{ab} %), on the 12th day of fermentation (50.7 ± 1^{ab} %), on the 24th day of fermentation (55.2 ± 12^{b} %), commercial shalgam (44.7 ± 4^{ab} %) and black carrot extraction (48.7 ± 3^{b} %) ($p > 0.05$) in terms of DPPH assay.Furthermore, commercial shalgam sample (56.6 ± 3^{c} %) and shalgam collected

on the 24th day of fermentation (56.4 ± 9^{c} %) exhibited higher TAC in terms of CUPRAC assay compared to sample collected on the 1st day of fermentation (44.7 ± 6^{ab} %), on the 12th day of fermentation (43.7 ± 6^{ab} %) and bulgur sample (37.3± 5^{a} %) (p<0.05).

As the samples pass through from mouth into stomach, the percent bioaccessibility was decreased. Results indicated that there was no significant difference observed between shalgam collected on the 1st day of fermentation (26.5 ± 3^{c} %), on the 12th day of fermentation (26.0 ± 2^{c} %), on the 24th day of fermentation (22.2 ± 7^{bc} %) and black carrot samples (25.9 ± 5^{c} %). Bulgur samples shows the lowest TAC values in terms of DPPH experiment. CUPRAC analysis results indicated that black carrot (36.0 ± 6^{d} %), commercial shalgam (30.1 ± 4^{bcd} %), sample collected on the 24th day of fermentation (32.7 ± 7^{cd} %) shows highest and similar value.

In intestine condition; the highest TAC value among all samples was belong to black carrot (12.3 ± 3^{c} %) in terms of DPPH assay and shalgam being 24th day of fermentation (18.5 ± 3^{d} %), commercial shalgam (18.4 ± 3^{d} %) and black carrot (18.0 ± 1^{d} %) for CUPRAC assay. TAC values for IS was ranged from 3.0 ± 1^{a} % to 12.3 ± 3^{c} % for DPPH and from 1.2 ± 2^{a} % to 18.5 ± 3^{d} % for CUPRAC experiments.

3.10 Effect of Fermentation on Bioaccessible Phenolics

According to result in current study, it is possible to say that fermentation process affected positively bioaccessible TFC, TPC, AC and TAC. Regarding MS, SS and IS it is obvious that recovery percent of bioaccessible TFC, TPC, AC and TAC was decreased as the sample pass. Besides a sharp reduction in TFC, TPC, AC and TAC was observed compared with initial samples.

It is possible to occurence of interaction due to release from the food matrix, absorption capacities, ability of a compound to cross membranes, possible interactions with other food components and ingestion of pigments, presence of suppressors or cofactors, technological process such as fermentation, mixing, cooking etc, the effect of pH, biliary acids, microbiota, the lack of sensitivity of the analytical method.

3.11 HPLC Analysis

In current study 16 different phenolics was detected namely 3-4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, caffeinne, catechin, chlorogenic acid, elagic acid,

epicatechin, ethyl-3-4-dihydroxybenzoat, ferulic acid, fumaric acid, gallic acid, p-coumaric acid, quercetin, sinapic acid, syringic acid and vanilin from shalgam samples. In another rsearch on black carrot jams and marmalades chlorogenic acid, crytochlorogenic acid, neochlorogenic acid and caffeic acid was detected (Kamiloglu et al, 2015).

The concentration of 3-4-dihydroxybenzoic acid, chlorogenic acid quercetin, and sinapic acid were observed to increase as the fermentation progress, however there were not significant difference between shalgam sample collected on 24th days of fermentation and commercial shalgam sample in terms of 3-4-dihydroxybenzoic acid and sinapic acid. Furthermore the concentration of 4-hydroxybenzoic acid, caffeinne, catechin, ethyl-3-4-dihydroxybenzoat, fumaric acid, gallic acid, p-coumaric acid, syringic acid and vanilin were statistically identical among shalgam sample collected on 1st, 12th, and 24th days of fermentation and commercial shalgam samples (Table 3.6). In general, it is possible to explain that sample collected on 24th days of fermentation contains higher phenolic content compared to other samples.

Table 3.6: Phenolic content of shalgam beverage samples

	Retention time	Concentration	Area
3-4-dihydroxybenzoic Acid			
1st day	16.0±0.0^{a}	854.9±193.9^{a}	8.8±1.7^{a}
12th day	15.9±0.0^{a}	1386.9±152.3^{b}	12.9±0.19^{b}
24th day	16.0±0.0^{a}	1794.6±66.8^{c}	16.1±1.5^{c}
Commercial Shalgam	16.0±0.0^{a}	1869.6±89.1^{c}	16.5±0.8^{c}
4-hydroxybenzoic Acid			
1st day	7.8±0.1^{a}	4.7±0.5^{a}	8.7±0.8^{a}
12th day	7.7±0.1^{a}	4.8±3.0^{a}	7.8±1.4^{a}
24th day	7.9±0.0^{a}	4.4±3.2^{a}	6.9±4.5^{a}
Commercial Shalgam	7.8±0.2^{a}	5.4±0.8^{a}	8.7±1.3^{a}
Caffeinne			
1st day	16.9±0.0^{a}	1872.0±124.7^{a}	1.7±0.1^{a}
12th day	16.9±0.0^{a}	24761±473.9^{a}	2.1±0.6^{a}
24th day	16.9±0.0^{a}	2338.8±297.6^{a}	1.9±0.2^{a}
Commercial Shalgam	16.9±0.0^{a}	2087.1±74.3^{a}	1.7±0.1^{a}

Table 3.6 (continued): Phenolic content of shalgam beverage samples

	Retention time	Concentration	Area
Catechin			
1st day	8.5±0.1[a]	9.3±1.7[a]	4.5±0.8[a]
12th day	8.6±0.1[a]	9.9±2.3[a]	4.3±0.7[a]
24th day	8.4±0.1[a]	7.4±4.2[a]	3.1±1.5[a]
Commercial Shalgam	8.5±0.2[a]	10.3±1.1[a]	4.3±0.5[a]
Chlorogenic Acid			
1st day	15.6±0.0[a]	176.0±1.7[bc]	1.8±0.3[b]
12th day	15.5±0.1[a]	128.3±41.3[ab]	0.9±0.3[a]
24th day	15.5±0.0[a]	155.6±41.9[bc]	1.3±0.3[ab]
Commercial Shalgam	15.6±0.1[a]	200.6±11.9[c]	1.7±0.1[b]
Ellagic Acid			
1st day	11.1±0.1[a]	443.2±61.4[b]	1.3±0.2[a]
12th day	11.2±0.1[a]	621.7±57.6[a]	1.7±1.1[a]
24th day	11.1±0.1[a]	647.2±165.8[a]	1.7±0.5[a]
Commercial Shalgam	11.2±0.1[a]	434.2±10.2[b]	1.1±0.0[a]
Epicatechin			
1st day	6.7±0.0[a]	118.8±3.0[ab]	2.4±0.2[ab]
12th day	6.7±0.0[a]	154.2±20.6[c]	2.8±0.2[c]
24th day	6.7±0.1[a]	105.2±27.7[a]	1.8±0.3[a]
Commercial Shalgam	6.7±0.1[a]	113.7±13.6[ab]	2.0±0.0[ab]
Ethyl-3-4-dihydroxybenzoat			
1st day	8.9±0.1[a]	1.6±0.1[a]	3.3±0.2[a]
12th day	8.9±0.1[a]	2.1±0.4[a]	3.9±0.4[a]
24th day	8.8±0.1[a]	2.0±0.5[a]	3.5±0.6[a]
Commercial Shalgam	8.9±0.2[a]	1.9±0.2[a]	3.2±0.4[a]
Ferulic Acid			
1st day	7.0±0.1[a]	91.9±5.1[a]	1.4±0.1[a]
12th day	7.1±0.2[a]	138.4±10.8[a]	1.9±0.3[a]
24th day	7.0±0.0[a]	137.0±51.3[a]	1.8±0.6[a]
Commercial Shalgam	7.0±0.1[a]	90.1±13.0[a]	1.2±0.2[a]
Fumaric Acid			
1st day	13.2±0.0[a]	756.3±335.0[a]	1.8±0.7[a]
12th day	13.2±0.0[a]	838.5±43.6[a]	1.8±0.8[a]
24th day	13.2±0.0[a]	1265.3±101.2[a]	2.6±0.0[a]
Commercial Shalgam	13.2±0.0[a]	1275.6±66.6[a]	2.6±0.1[a]

Table 3.6 (continued): Phenolic content of shalgam beverage samples

	Retention time	Concentration	Area
Gallic Acid			
1st day	11.5±0.1[a]	375.8±47.3[a]	1.3±0.2[a]
12th day	11.6±0.0[a]	431.4±66.6[a]	1.3±0.3[a]
24th day	11.6±0.1[a]	422.5±31.7[a]	1.2±0.2[a]
Commercial Shalgam	11.6±0.0[a]	395.8±27.2[a]	1.1±0.1[a]
p-Coumaric Acid			
1st day	12.2±0.0[a]	538.5±28.5[a]	1.8±0.2[a]
12th day	12.3±0.0[a]	576.7±22.7[a]	1.7±0.1[a]
24th day	12.3±0.0[a]	830.6±105.4[a]	2.4±0.5[a]
Commercial Shalgam	12.3±0.0[a]	513.0±60.0[a]	1.5±0.2[a]
Quercetin			
1st day	15.8±0.0[a]	993.7±34.5[a]	39.7±0.9[a]
12th day	15.8±0.0[a]	1096.5±4.1[b]	39.6±4.1[b]
24th day	15.8±0.0[a]	1071.2±4.6[b]	37.1±2.4[b]
Commercial Shalgam	15.8±0.0[a]	1062.5±6.0[b]	36.2±0.2[b]
Sinapic Acid			
1st day	14.7±5.9[a]	1141.7±289.8[ab]	2.2±0.5[ab]
12th day	14.7±0.0[a]	740.7±95.4[a]	1.3±0.3[a]
24th day	14.7±0.0[a]	1437.8±134.9[b]	2.4±0.4[b]
Commercial Shalgam	14.7±0.0[a]	1308.3±46.7[b]	2.2±0.1[b]
Syringic Acid			
1st day	5.9±0.0[a]	348.8±27.1[a]	15.8±.7[a]
12th day	5.8±0.0[a]	355.2±15.8[a]	13.1±0.8[a]
24th day	5.8±0.0[a]	332.7±2.7[a]	11.8±0.9[a]
Commercial Shalgam	5.8±0.0[a]	364.0±27.7[a]	12.7±1.0[a]
Vanilin			
1st day	9.3±0.1[a]	4.0±5.1[a]	3.4±0.4[a]
12th day	9.5±0.3[a]	0.9±0.3[a]	2.9±0.8[a]
24th day	9.4±0.1[a]	1.4±0.3[a]	4.3±1.1[a]
Commercial Shalgam	9.6±0.1[a]	1.1±0.2[a]	3.4±0.3[a]
Fumaric Acid			
1st day	13.2±0.0[a]	756.3±335.0[a]	1.8±0.7[a]
12th day	13.2±0.0[a]	838.5±43.6[a]	1.8±0.8[a]
24th day	13.2±0.0[a]	1265.3±101.2[a]	2.6±0.0[a]
Commercial Shalgam	13.2±0.0[a]	1275.6±66.6[a]	2.6±0.1[a]

Table 3.7 shows the retention time, concentration and area of phenolics detected from shalgam beverage colletcted on 1st, 12th and 24th days of fermentation and commercial shalgam samples after performing bioaccessibility tests. Five different phenolics was detected from bioaccessiblity samples (MS and IS) however no phenolic detected from the sample 1st days of fermentation.

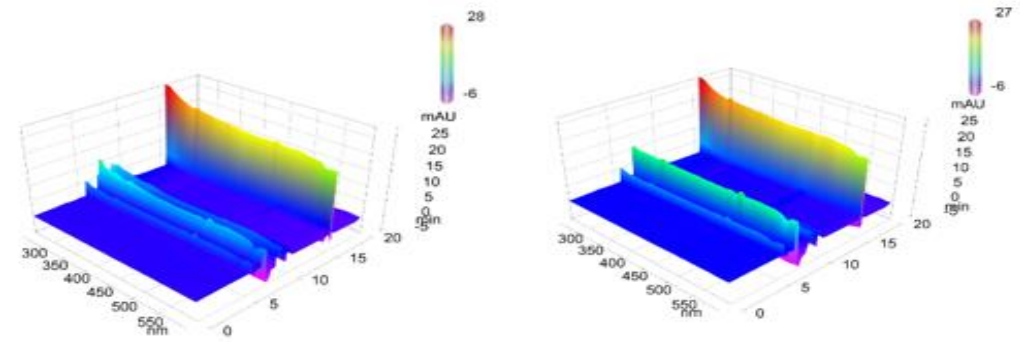
Table 3.7: Phenolic content of bioaccessiblity samples of shalgam beverage

	Retention time	Concentration	Area
3-4 Hydroxy benzoic acid			
Mouth Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	11.3±0.3[a]	0.00006±2E-4[b]	2741.0±1144.1[b]
24th day of fermentation	11.1±0.1[a]	0.00164±2.E-03[a]	101420.3±2424.2[a]
Commercial Shalgam	11.2±0.05[a]	0.00004±2E-4[b]	1697.0±755.0[b]
Intestine Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	11.1±0.07[a]	0.00002±0.00[a]	428.02±67.3[a]
24th day of fermentation	11.1±0.00[a]	0.00004±0.00[ab]	1534.3±740.4[ab]
Commercial Shalgam	11.2±0.05[a]	0.00007±0.00[b]	1823.6±252.5[b]
p-coumaric acid			
Mouth Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	18.6±0.2[a]	0.00004±2E-05[a]	4416.0±937.6[a]
24th day of fermentation	18.7±0.1[a]	0.00006±3E-06[a]	6081.3±352.4[a]
Commercial Shalgam	18.8±0.1[a]	0.00009±0.5E-05[a]	5641.03±3073.8[a]
Intestine Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	18.5±0.00[a]	0.00005±5.2E-06[a]	3247.0±734.9[a]
24th day of fermentation	18.4±0.3[a]	0.00012±0.00001[a]	6234.0±1803.9[a]
Commercial Shalgam	18.5±0.04[a]	0.00012±0.0001[a]	6956.5±970.1[a]
Chlorogenic acid			
Mouth Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	12.1±0.1[a]	0.00024±0.0001[a]	7277.7±2566.2[a]
24th day of fermentation	12.2±0.3[a]	0.00015±2.2E-05[a]	3020.2±443.4[a]
Commercial Shalgam	12.3±0.2[a]	0.00009±5E-05[a]	2703.0±1451.7[a]

Table 3.7 (continued): Phenolic content of bioaccessiblity samples of shalgam beverage

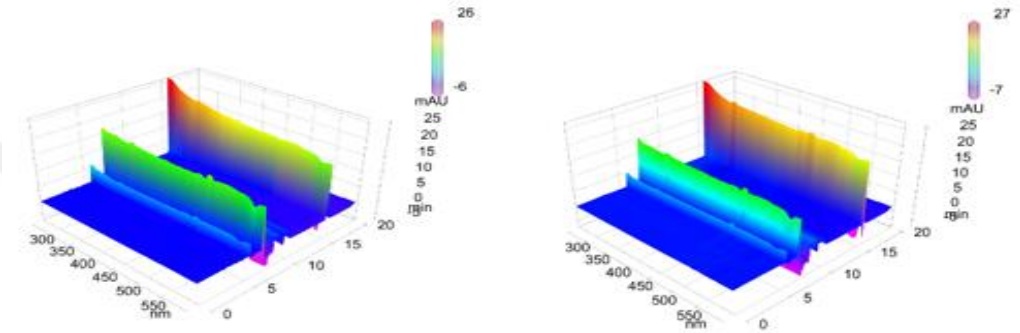
	Retention time	Concentration	Area
Intestine Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	12.1±0.00[a]	0.00005±0.00[a]	1408.0±522.0[a]
24th day of fermentation	12.1±0.00[a]	0.00009±0.00[a]	2840.7±780.8[a]
Commercial Shalgam	Not detected	Not detected	Not detected
4 Hydroxy benzoic acid			
Mouth Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	11.6±0.1[a]	0.00004±0.3E-05[a]	1889.7±527.3[a]
24th day of fermentation	11.6±0.1[a]	0.00012±2.3E-05[ab]	18957.4±3253.6[ab]
Commercial Shalgam	11.6±0.06[a]	0.0003±8.9E-05[b]	137696±4492.4[b]
Intestine Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	11.7±0.04[a]	0.00002±0.000001[a]	866.3±86.78[a]
24th day of fermentation	11.7±0.02[a]	0.00021±0.00001[ab]	10375.7±849.4[ab]
Commercial Shalgam	11.6±0.07[a]	0.00012±0.0001[ab]	5977.5±813[ab]
Epicatechin			
Mouth Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	15.8±0.03[a]	0.00002±0.00001[a]	1426.0±762.3[a]
24th day of fermentation	15.7±0.01[a]	0.00014±0.0001[b]	4522.5±342.6[b]
Commercial Shalgam	15.8±0.01[a]	0.0008±0.00005[b]	5518.3±2242.9[b]
Intestine Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	15.8±0.0[a]	0.00001±0.00[a]	821.7±115.8[a]
24th day of fermentation	15.9±0.0[a]	0.00004±0.00[a]	2801.7±774.7[a]
Commercial Shalgam	Not detected	Not detected	Not detected

Figure 3.3 shows the 3D analysis report and Figure 3.4 shows 2D analysis report for the sample collected on 1st, 12th, 24th days of fermentation and commercial shalgam sample.



a. Shalgam beverage collected on 1st days of fermentation

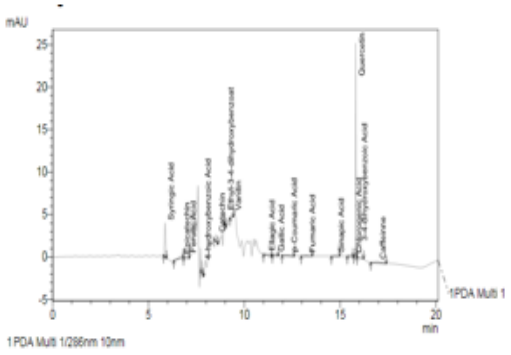
b. Shalgam beverage collected on 12th days of fermentation



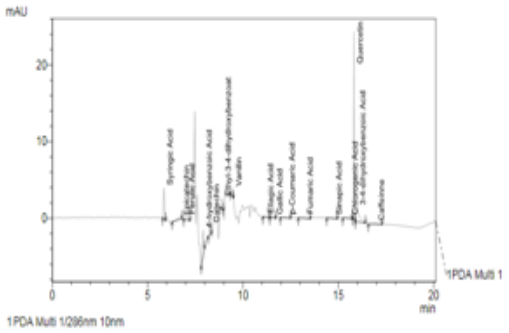
c. Shalgam beverage collected on 24th days of fermentation

d. Commercial shalgam sample

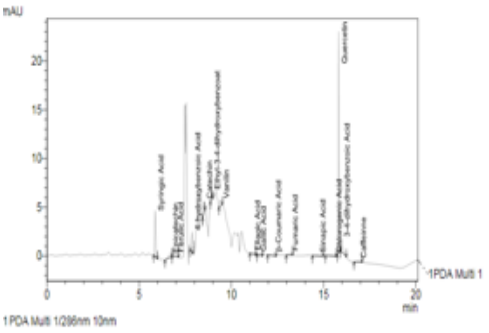
Figure 3.3: 3-D Analysis Report of samples



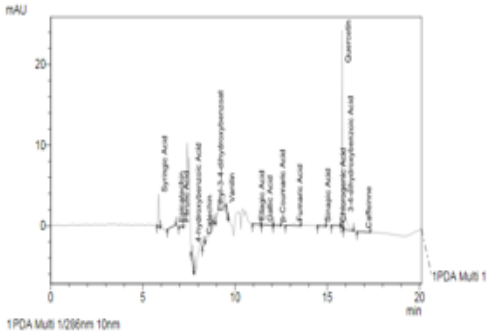
a. Shalgam beverage collected on 1st days of fermentation



b. Shalgam beverage collected on 12th days of fermentation



c. Shalgam beverage collected on 24th days of fermentation



d. Commercial shalgam sample

Figure 3.4: 2-D Analysis report of sample

As anthocyanin only cyanidin content was detected (Table 3.8) which is in compatible with literature. On the other hand, after bioaceessibility tests cyanidin was not detected from the sample collected on 1st days of fermentation.

Table 3.8: Anthocyanin content of shalgam beverage samples

Cyanidin Content	Retention Time	Concentration	Area
Initial Samples			
1st day of fermentation	19.7±0.00[a]	0.00001±0.00[a]	509±0.00[a]
12th day of fermentation	19.7±0.00[a]	0.007±0.01[b]	73577.7±0.00[b]
24th day of fermentation	19.7±0.00[a]	0.016±0.01[c]	105428.3±0.00[c]
Commercial Shalgam	19.7±0.00[a]	0.008±0.01[bc]	78511.3±0.00[bc]
Mouth Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	19.6±0.06[a]	0.003±0.001[a]	26123.3±6194.3[a]
24th day of fermentation	19.7±0.04[a]	0.011±0.002[b]	101315±3525.2[b]
Commercial Shalgam	19.6±0.03[a]	0.003±0.001[a]	31873.7±7770.1[a]
Intestine Samples			
1st day of fermentation	Not detected	Not detected	Not detected
12th day of fermentation	19.6±0.00[a]	0.0013±0.03[a]	14785.7±3205.9[a]
24th day of fermentation	19.7±0.04[a]	0,006±0.001[c]	64248.6±3151.2[c]
Commercial Shalgam	19.7±0.07[a]	0.005±0.001[b]	44555.5±7306.3[b]

4. CONCLUSION

Fruits and vegetables are source of phenolic and anthocyanin compounds, which has important health benefit on people such as improving immune system and preventing digestion disorders and cancer due to having antioxidant activity and use as natural colorants in food product. Black carrot is the main raw material of the shalgam beverage which pigmentation comes from black carrot also has phenolic and anthocyanin compounds excessive amounts. During shalgam fermentation process, depending on lacticacid bacteria activity the more the acidity of beverage is increase, the more flavonoids, phenolics, anthocyanins and antioxidant capacity also increase which improves the benefit of product comes from black carrot. In current study, the amount of flavonoid content value is raised as the fermentation progress, this increment supported in terms of phenolic content, anthcyanin content and antioxidant capacity. Initial samples demonstrated that black carrot has the highest value of flavonoid, phenolic, anthocyanin content and antioxidant capacity; during fermentation benefitial compound observed to increase; but when compared to commercial shalgam beverage stayed on shelves for one month compounds were statistically equal or decreased.

After performing biaccessibility tests, it has proven with current study by the results that the bioaccessible flavonoid, phenolic, anthocyanin compound and antioxidant capacity of end product of shalgam beverage is elevated or identical with black carrot values. The bioaccessiblity value of total flavonoid content, total phenolic content, anthocyanin compound and total antioxidant capacity in mouth, stomach and intestine were observed to be lower compared to the initial samples. As the samples pass through from mouth to intestine, the recovery percentage was decreased. For instance at the end of the mouth digestion recovery percent of black carrot, shalgam sample collected on 1^{st} days of fermentation, 12^{th} days of fermentation, 24^{th} days of fermentation and commcercial shalgam samples changes between 43.9-56.0 % and statistically identical for flavonoid content. As the sample pass stomach and reach to intestine, the recovery percent of samples was decline changing between 6.7 to 13.9

%. Similar results were obtained in terms of phenolic content, anthocyanin compound and total antioxidant capacity recovery percent.

16 different phenolics were detected from initial shalgam beverage samples. However, after performing bioaccessibility tests no phenolic was detected from shalgam sample collected on 1st days of fermentation. On the other hand, 5 different phenolics were detected from shalgam sample collected on 12th, 24th days of fermentation and commercial shalgam samples. As anthocyanin, only cyanidin was detected except for mouth samples and intestine samples of 1st days of fermentation.

In vitro bioavailability tests demonstrate that containing high amounts of health benefit compound does not imply that those benefit substance can be utilized by our system. It is possible to say that fermentation process has positive effect on bioaccessiblity of phenolic and anthocyanin content and total antioxidant capacity. Further studies are needed to explore in order to understand how beneficial compound in food matrix effect human health.

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