ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

IN VITRO BIOACCESSIBILITY OF ANTHOCYANINS IN BLACK CHOKEBERRY (*ARONIA MELANOCARPA*) ADDED YOGURTS

M.Sc. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

SİYAH KUŞ KİRAZI (*ARONIA MELANOCARPA*) İLAVE EDİLMİŞ YOĞURTLARDA ANTOSİYANİNLERİN *IN VITRO* BİYOYARARLILIĞININ İNCELENMESİ

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4 MAYIS 2015

Gizem ÇATALKAYA, a **M.Sc.** student of ITU **Graduate School of Science Engineering And Technology** student ID **506121535,** successfully defended the **thesis** entitled "***IN VITRO* BIOACCESSIBILITY OF ANTHOCYANINS IN BLACK CHOKEBERRY (*ARONIA MELANOCARPA*) ADDED YOGURTS**", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Date of Submission : **4 May 2015**
Date of Defense : **3 June 2015**

To my family,

FOREWORD

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Gizem ÇATALKAYA
Food Engineer

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LIST OF ABBREVIATIONS

AF	**:**After fermentation
BF	**:**Before fermentation
BSA	**:**Bovine serum albumin
°Bx	**:**Brix
Cya-3-ara	**:**Cyanidin-3-arabinoside
Cya-3-gal	**:**Cyanidin-3-galactoside
Cya-3-glu	**:**Cyanidin-3-glucoside
Cya-3-xyl	**:**Cyanidin-3-xyloside
DM	**:**Dry matter
DPPH	**:**1,1-diphenyl-2-picrylhydrazil
GAE	**:**Gallic acid equivalent
GI digestion	**:**Gastrointestinal digestion
HAS	**:**Human serum albumin
Nc	**:**Neocuproine
SDS	**:**Sodium dodecyl sulfate
SDS PAGE	**:**Sodium dodecyl sulfate polyacrylamide gel electrophoresis
TEAC	**:**Trolox equivalent
TNBS	**:**Trinitrobenzenesulfonic acid hydrate

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IN VITRO BIOACCESSIBILITY OF ANTHOCYANINS IN BLACK CHOKEBERRY (*ARONIA MELANOCARPA*) ADDED YOGURTS

SUMMARY

Milk and other dairy products are consumed all over the world. Among these, yogurt is one of the most popular and unique dairy products, which is being enjoyed for its refreshing taste and beneficial properties. It is easily digestible and has high nutritive and therapeutic properties. The importance of yogurt as part of a balanced and healthy diet is admitted by regulatory authorities and scientific institutions in most countries. An increasing number of epidemiologic and clinical proof suggests that yogurt consumption may act beneficially on weight regulation and metabolic risk factors.

Aronia melanocarpa, known as black chokeberry are one of the richest plant sources of phenolic compounds especially anthocyanins, which have beneficial effects on health like prevention of cancer and cardiovascular diseases. It was demonstrated that, aronia berries exhibited different biological effects both in vitro and in vivo such as antioxidant, gastroprotective, hepatoprotective, and antiproliferative activities.

Therefore, fruit added yogurts have been gaining more attention by consumers and manufacturers because of their additional health benefits beyond normal nutritional value. However, despite the presence of large amounts of polyphenols present in ingested food, only a very small portion can be actually absorbed. Which may be a result of processing factors or interactions between food components.

In this study, *in vitro* simulated gastrointestinal digestion of anthocyanins from black chokeberry (*Aronia melanocarpa*) added to homemade yogurts was investigated in terms of bioaccessibility, antioxidant activity and the possible fate of anthocyanins.

Two types of black chokeberry yogurts were prepared by adding pulp (1%) and minced fresh berries (10%), aiming to add similar amounts of anthocyanins. The pulp and berries were added to plain yogurt, which were prepared based on full fat cow's milk, after fermentation, or directly to the milk before fermentation. All batches were prepared in duplicate. Methanolic extracts of samples were analyzed for their contents of total monomeric anthocyanins, and total phenolics as well as their antioxidant capacity after 1 day and 8 days of storage. In addition to that, major individual anthocyanin content of juice, pulp, minced berries and chokeberry added undigested yogurts was analyzed by high performance liquid chromatography (HPLC) coupled with photodiode array detector (PDA).

The dry matter content of pulp was 25.5 ± 0.4% while it was 17.1 ± 0.7% for fresh berries. Also, the dry matter content of all yogurt batches ranged between 14.8 and 15.3%.

The highest total phenolic content and antioxidant capacity measured by DPPH assay were observed in fresh berries, which were found to be 3242±37.4 mg GAE/100 g DM and 22.2±0.49 mM TEAC/100g DM, respectively. In contrast, the anthocyanin content and antioxidant capacity measured by CUPRAC assay of the black chokeberry pulp was higher than the other samples, which were determined as 7564±139 mg cya-3-glu/100 g DM and 42.2±4.23 mM TEAC/100 g DM respectively. However, although the anthocyanin content of pulp was higher than the berries, no real difference was

observed between antioxidant activity of pulp and berries. On the other hand, all measured values were greatly lower in chokeberry juice than in berries and pulp.

By the HPLC analysis, four individual anthocyanins were observed in the extracts of chokeberry juice, berries and pulp as well as chokeberry added yogurt samples. These anthocyanins can be ordered from abundant to rare one as follow: cya-3-gal, cya-3-ara, cya-3-glu, cya-3-xyl. Cya-3-galactoside was the main anthocyanin in chokeberry juice, berries and pulp. It composed 70%, 68% and 66% of total chokeberry anthocyanins, respectively.

No differences in any of the measured parameters was observed for the plain yogurts, indicating that batches were reproducible. In all batches, the highest total phenolics, total anthocyanins and antioxidant capacity were observed in berry added yogurts. On the other hand, the higher the anthocyanin content, the higher the total phenolic content and antioxidant capacity. In undigested yogurts, no effect of storage days on the anthocyanin content was observed ($p>0.05$). For both yogurts with added berries or pulp, a lower amount of anthocyanins was measured when the berries and pulp ($p<0.001$) were added before compared to added after fermentation. On the other hand, even when the fruits were added to the yogurt after fermentation, only 70% of the anthocyanins from the berries were measured, and even not 20% of the pulp anthocyanins.

A significant effect on the total phenolic content of undigested yogurts was observed depending on the type of fruits added ($p < 0.001$) in the following order: no<pulp<berries. Independent if the type of fruit was added before or after fermentation, no significant difference between the storage days was observed on the total phenolic content ($p>0.05$). The 2-way interaction of type of fruit x type of fermentation was highly significant on the antioxidant capacity of undigested yogurts ($p<0.001$), as well as the main effects, type of fermentation and fruit ($p<0.001$). No significant effect of the 2-way interaction terms (type of fruit x storage days; type of fermentation x storage days) was observed ($p>0.05$ for each) on the antioxidant activity of undigested yogurts, as well as no influence of the storage days ($p>0.05$).

During the buccal phase of digestion no or slight amount of phenolic compounds were observed in plain yogurts and pulp yogurts. On the other hand, the measured total phenolic compounds in salivary berry samples were much higher (approximately 83-84% of the total phenolic content of undigested yogurts) compared to plain and pulp added yogurts. After gastric digestion and intestinal digestion much higher total phenolic content was observed both in plain yogurts and pulp or berry added yogurts, even values that are higher than what was measured in the undigested samples. A higher antioxidant capacity observed in fruit added yogurts compared to plain and pulp added yogurts.

The bioaccessibility of anthocyanins was greatly decreased after the intestinal digestion. Higher amounts of anthocyanins can be recovered in berry added yogurts compared to pulp added yogurts. The highest recoveries were 3.5% and 32.1% (for IN and OUT sample respectively) for pulp added yogurt as well as 6.3% and 34.3% (for IN and OUT sample respectively) for berries added yogurt. Our results show that, anthocyanins are more stable at acidic conditions and highly sensitive to pancreatic digestion.

On the antioxidant activity, samples showed higher antioxidant capacity when they were analysed with CUPRAC method in comparison to DPPH method. In all berry added yogurt samples a higher DPPH radical scavenging activity and cupric ion reducing ability were observed compared to plain and pulp added yogurts and this higher antioxidant capacity remains stable during the digestion process. However the antioxidant activity substantially decreased in the dialysed fractions.

The degree of hydrolysis increased when the samples were subjected to pancreatic digestion. No differences in the degree of hydrolysis were observed between the yogurt varieties in each digestion step except oral phase, reflecting that the digestion of proteins was not affected by the anthocyanins.

According to SDS-PAGE analysis, Bovine serum albumin and caseins found to be the prevailing proteins in yogurt samples. A distinct band over the 66.2 kDa molecular weight area was observed in undigested yogurt samples which might be the BSA (bovine serum albumin). Also, outstanding bands were observed around 31 kDa molecular weight range which are likely to be the caseins. After the addition of saliva, the protein bands remained the same with undigested yogurts. At the end of the gastric phase, a clear degradation of the large molecular weight proteins to low molecular weight proteins was observed and undefined bands appeared in the low molecular weight region (<6.5 kDa) of the gels. Similar trends in gel patterns between BID-OUT samples and completely digested samples (PG-OUT) were observed. When the pancreatic solution was introduced, a further degradation of small molecular weight proteins resulting leaner bands in the low molecular weight range was observed and some new bands in the higher molecular weight area, which were indicated as digestive juice proteins by the other studies. The gel patterns of proteins showed that proteins were not affected by salivary digestion and started to degrade continuously until the digestion finishes.

Further analysis of the different digestive fractions are needed to elucidate the fate of the anthocyanins i.e. interaction with major yogurt components, or transformation in new metabolites during digestion.

SİYAH ARONİA MEYVESİ (*ARONIA MELANOCARPA*) İLAVE EDİLMİŞ YOĞURTLARDA ANTOSİYANİNLERİN *IN VITRO* BİYOERİŞİLEBİLİRLİĞİNİN İNCELENMESİ

ÖZET

Süt ve diğer süt ürünleri tüm dünyada yaygın olarak kullanılmaktadır. Yoğurt ise süt ürünleri arasında tazeleyici tadı ve yararlı etkileri nedeniyle tercih edilen en popüler ürünlerden biridir. Yoğurt, kolay sindirilebilirdir ve yüksek beslenme değeri ve terapötik özelliklere sahiptir. Yoğurdun bireylerin dengeli ve sağlıklı beslenmesinde önemli bir role sahip olduğu birçok düzenleyici kurul ve bilimsel enstitü tarafından kabul edilmiştir. Günden güne artan sayıdaki epidemiyolojik ve klinik kanıtlar yoğurt tüketiminin kilo düzenlemesi ve metabolik risk faktörleri üzerine yararlı şekilde etki edeceğini belirtmektedir.

Siyah aronia meyvesi (*Aronia melanocarpa*), fenolik bileşenler açısından, özellikle de kanser ve kardiyovasküler hastalıklar üzerine yararlı etkisi olduğu bilinen antosiyaninler açısından, zengin bitkisel kaynaklardan birisidir. Aronia meyvesinin hem in vivo hem de invitro çalışmalarda antioksidan, mide koruyucu, karaciğeri koruyucu ve hücre büyümesini engelleyici olması gibi farklı biyolojik aktiviteler göstermiştir.

Buna bağlı olarak, meyveli yoğurtlar hem üreticiler hem de tüketiciler tarafından meyveli yoğurtların sağlığa karşı faydalarının normal yoğurtlara göre artmasından dolayı daha fazla ilgi görmeye başlamıştır. Fakat, her ne kadar tüketilen gıdada yüksek miktarda polifenoller bulunsa da, sindirim sonunda üretim prosesine ait faktörler ya da gıda matrisi içerisinde oluşan etkileşimler sonucu sadece küçük bir kısmı absorbe olabilmektedir.

Bu çalışmada, siyah Aronia meyvesi ilave edilmiş ev yapımı yoğurtlarda bulunan antosiyaninlerin in vitro simüle edilmiş gastrointestinal sindirimi biyoerişilebilirlik, antioksidan aktivite ve antosiyaninlerin muhtemel gidişatı açısından incelenmiştir.

Benzer miktarlarda antosiyanin ilave edilmesi amaçlanarak, pulp (%1) ve parçalanmış bütün meyvelerin (%10) sade yoğurda eklenmesiyle iki çeşit yoğurt hazırlanmıştır. Tam yağlı inek sütü temelli yoğurtlar, pulp ve parçalanmış meyvelerin fermentasyon sonrası sade yoğurda veya fermentasyon öncesi direkt olarak süte eklenmesi ile üretilmiştir. Tüm örnekler çift parallelli hazırlanmıştır.

Örneklerin metanolik ekstraktları toplam monomerik antosiyanin içeriği, toplam fenolik bileşik içeriği ve antioksidan aktiviteleri açısından 1 gün ve 8 gün muhafaza edildikten sonra analiz edilmiştir. Bunlara ek olarak, aronia meyve suyu, pulp ve parçalanmış meyve ve aronia eklenmiş yogurtların başlıca antosiyanin profil içerikleri fotodiyot dizisi dedektöre (PDA) bağlı yüksek performans sıvı komatografisi (HPLC) ile analiz edilmiştir.

Siyah aronia pulpunun kuru madde miktarı %25,5 ± 0,4 olarak, parçalanmış meyvenin ise %17,1 ± 0,7 olarak bulunmuştur. Ayrıca analiz edilmek üzere hazırlanan tüm yoğurt gruplarının kuru madde mitarının %14,8 ile 15,3 arasında olduğu tespit edilmiştir.

En yüksek toplam fenolik bileşik (3242±37,4 mg GAE/100 g kuru madde) ve DPPH metoduyla belirlenen antioksidan kapasitesinin (22,2±0,49 mM TEAC/100g kuru madde) parçalanmış meyvelerde en yüksek olduğu gözlenmiştir. Buna karşın, siyah aronia pulpunun toplam antosiyanin içeriğinin (7564±139 mg cya-3-glu/100 g kuru madde) ve CUPRAC metoduyla ölçülen antioksidan aktivitesinin (42.2±4.23 mM TEAC/100 g kuru madde) meyve suyu ve parçalanmış meyvelere oranla daha yüksek olduğu gözlenmiştir. Fakat, pulpun antosiyanin içeriğinin diğer örneklerden fazla olmasına rağmen, pulp ve parçalanmış meyvelerin antioksidan aktiviteleri arasında bir fark gözlenmemiştir. Diğer taraftan, ölçülen tüm değerlerin parçalanmış meyve ve pulpa kıyasla siyah aronia meyve suyunda oldukça düşük olduğu tespit edilmiştir.

Aronia meyve suyu, pulpu ve parçalanmış meyveleri ile aronia eklenmiş *in vitro* simüle sindirim işlemi uygulanmamış taze yoğurt ekstraktlarında HPLC analizi ile belirlenen 4 farklı antosiyanin olduğu gözlenmiş ve bunların en yüksek miktarlıdan en düşük miktarlıya siyanidin-3-galaktozid, siyanidin-3-glukozid, siyanidin-3-arabinosid ve siyanidin-3-ksilosid olduğu görülmüştür. Meyve suyu, parçalanmış meyve ve pulp ektraklarının üçünde de siyanidin-galaktozidin baskın antosiyanin olduğu gözlemlenmiş ve sırasıyla örneklerdeki toplam antosiyanin miktarının %70, %60 ve %66 sını oluşturduğu gözlemlenmiştir.

Sade yoğurtlar için, ölçülen hiçbir parametrede farklılık görülmemiş olup, tüm yoğurt setlerinin tekrarlanabilir olduğu gözlenmiştir. Tüm yoğurt setlerinde, en yüksek toplam fenol ile toplam antosiyanin içeriği ve antioksidan aktivitesi parçalanmış meyve eklenen örneklerde görülmüştür. Bunun yanısıra, örneklerdeki antosiyanin içeriği artışına bağlı olarak, toplam fenolik bileşen içeriği ve antioksidan aktivitesi özelliklerinde de artış olduğu görülmüştür. Sindirim işlemi uygulanmayan taze yoğurtlarda muhafaza süresinin antosiyanin içeriği üzerinde bir etkisinin bulunmadığı gözlenmiştir ($p>0,05$). Pulp ve parçalanmış meyve ilaveli yoğurtların her ikisinde de antosiyanin içeriğinin fermentasyon öncesi eklenmiş yoğurtlarda, fermentasyon sonrası eklenenlere kıyasla daha düşük olduğu gözlenmiştir ($p<0,001$). Buna ek olarak, pulp ya da parçalanmış meyveler fermentasyondan sonra eklense bile, antosiyaninlerin asıl aronia pulp ve meyvelerine oranla parçalanmış meyve ilaveli yoğurtlardan sadece %70'i, pulp ilaveli yoğurtlardansa sadece yaklaşık %20'si ekstrakte edilebilmiştir.

Eklenen meyve çeşidinin sindirim uygulanmamış taze yoğurtların toplan fenolik bileşen içeriği üzerinde sade<pulp<parçalanmış meyve sıralamasında önemli bir etkiye sahip olduğu gözlenmiştir. Aronia meyvelerinin fermentasyondan önce ya da sonra eklenmesinden bağımsız olarak, muhafaza süresinin birinci ve sekizinci günü arasında toplam fenolik bileşen açısından önemli bir fark olmadığı gözlenmiştir ($p>0,05$).

2 yollu eklenen meyve çeşidi x fermentasyon çeşidi etkileşiminin ve ana etkiler olan meyve çeşidi ile fermentasyon çeşidinin sindirim uygulanmayan yoğurtların antioksidan kapasitesi üzerinde önemli bir etkiye sahip olduğu görülmüştür ($p<0,001$). İki yollu etkileşim terimleri olan meyve çeşidi x muhafaza süresi ($p>0,05$); fermentasyon çeşidi x muhafaza süresi ($p>0,05$)'nin ve tek başına muhafaza süresinin ($p>0,05$) antioksidan aktivite üzerinde önemli bir etkisinin olmadığı tespit edilmiştir.

Ağız sindirimi boyunca sade ve pulp eklenmiş yoğurtlarda hiç ya da çok az fenolik bileşen içeriği olduğu gözlenmiştir. Diğer bir taraftan, parçalanmış meyve eklenen yoğurtlarda ağız sindirimi sonunda sade veya pulp eklenmiş yoğurtlara göre çok daha fazla (yaklaşık olarak sindirim uygulanmamış yoğurtların toplam fenolik içeriğinin %83-84'ü kadar) toplam fenolik bileşen içeriği olduğu görülmüştür. Mide ve bağırsak sindiriminden sonra, her üç sade, pulp ilaveli ve parçalanmış meyve ilaveli yoğurt örneğinin de toplam fenol içeriğinin oldukça arttığı, hatta elde edilen değerlerin sindirim uygulanmamaış olan taze yoğurt örneklerine ait toplam fenolik bileşen

içeriklerinden daha yüksek olduğu bulunmuşur. Ayrıca, sindirim boyunca parçalanmış meyve eklenen yoğurtlar sade ve pulp eklenmiş yoğurtlara oranla daha yüksek bir antioksidan aktivite göstermişlerdir.

Antosiyaninlerin biyoerişilebilirliği bağırsak sindiriminden sonra yüksek derecede düşmüştür. *In vitro* simüle sindirim sonunda elde edilen geri kazanımlar parçalanmış meyve eklenen yoğurtlarda, sade ve pulp eklenenlere göre daha fazladır. Elde edilen en yüksek geri kazanımlar pulp eklenmiş yoğurtlarda IN örnekleri için %3,5, OUT örnekleri için %32,1 olup parçalanmış meyve eklenen yoğurtlarda ise IN örnekleri için %6,3 ve OUT örnekleri için %34,3 olduğu gözlenmiştir. Elde edilen sonuçlar, antosiyaninlerin asidik koşullarda daha stabil olduğunu göstemiştir.

Antioksidan aktivite bakımından CUPRAC ve DPPH metotları karşılaştırıldığında, CUPRAC metoduyla analizlenen örneklerin DPPH ile analizlenenlere göre daha yüksek antioksidan aktivite gösterdiği gözlenmiştir. Parçalanmış meyve eklenen yoğurtların sade ve pulp eklenenlere kıyasla daha yüksek bir DPPH radikal yakalama aktivitesi ve bakır iyonu indirgeme kabiliyeti gösterdiği ve durumun sindirim işlemi boyunca stabil kaldığı gözlenmiştir. Buna karşın, sindirim işlemi sonunda tüm örneklerin diyaliz fraksiyonlarında antioksidan aktivitenin yüksek derecede düştüğü görülmüştür.

Sindirim işleminin ağız aşaması harıç diğer basamaklarında örneklerdeki protein hidrolizasyon dereceleri arasında önemli bir fark olmadığı ve bu durumun yoğurt proteinlerinin sindiriminin ilave edilen antosiyaninler tarafından kısıtlanmadığı gözlenmiştir. Hidroliz derecesi analizinin sonucuna göre, örnekler pankreatik sindirime tabi tutlduğunda proteinlerin hidrolizasyon derecesinin arttığı gözlenmiştir.

SDS-PAGE analizi sonucuna göre, sığır serum albümini (BSA) ve kazeinler yoğurttaki baskın proteinler olarak bulunmuştur. 66,2 kDa moleküler ağırlığı alanında sığır serum albümini olduğu düşünülen belirgin bir bant oluştuğu gözlenmiştir. Ayrıca, 31 kDa moleküler ağırlığı aralığında kazeinler olduğu tespit edilen göze çarpıcı bantlar oluşmuştur. Ağız sıvısı eklenmiş örneklerde oluşan protein bantlarının sindirim uygulanmamış yoğurtlardaki protein bantları ile aynı görünümde oldukları tespit edilmiştir. Mide sindiriminin sonunda ise, büyük molekül ağırlıklı proteinlerin küçük molekül ağırlıklı proteinler degrade olduğu açık bir şekilde gözlenmiş ve jellerin 6,5 kDa'dan küçük moleküler ağırlıktaki alanında belirlenemeyen yeni bantlar oluştuğu bulunmuştur. Bağırsak sindirimi öncesi (BID-OUT) ve sonrası (PG-OUT) örneklerinin benzer jel desenlerine sahip olduğu gözlenmiştir. Sindirim işelminin bağırsak sindirimi aşamasında pankreatik çözelti eklendiğinde mide fazında oluşan küçük moleküler ağırlıklı proteinler ileri degradasyona uğramış ve küçük moleküler ağırlıklı bölgede daha ince ve açık renkli bantlar oluşmuştur. Ayrıca bağırsak fazında yüksek moleküler ağırlıklı bölgede daha önce mide fazında gözlenmeyen ve diğer çalışmalar tarından sindirim sıvısı proteinleri olarak tanımlanan yeni bantlar belirmiştir. Sonuç olarak, elde edilen jel desenleri proteinlerin sindirimlerinin ağız sindirimden etkilenmediğini ve ağız aşamasından sonra sindirim tamamlanana kadar sürekli bir şekilde degrade olmaya başladıklarını göstermiştir.

Yapılan çalışmalar, antosiyaninlerin muhtemel akıbetini (örneğin major yoğurt bileşenleri ile etkileşimi veya sindirim sırasında yeni bileşenlere dönüşmesi) aydınlatmak için sindirim aşamalarından alınan fraksiyonlara destekleyici ilave analizlerin yapılması gerektiğini göstermiştir.

1 INTRODUCTION

Yogurt is a well-known fermented dairy product obtained by the lactic acid fermentation of milk by the action of yogurt starter bacteria, *Streptococcus salivarius* subsp. *thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Cossu, Juliano, Pisu, & Alamanni, 2009). It is an accessible, easy-to-digest, and tasty food that provides important nutrients and thus forms part of a balanced nutrient-rich diet during development and growth. Recent studies have shown that yogurt consumption may have a beneficial role on body weight regulation and cardiovascular health as well as bone health (Marette & Picard-Deland, 2014) (Rizzoli, 2014). Additionally, it supplies minor components that have been shown to play a role in decreasing the risk of particular diseases, especially gastrointestinal disorders such as infantile diarrhea, and increasing the host resistance to bacterial infection, gastro-enteritis, and constipation (Cano, Agüero, & Perdigon, 2002).

Yogurt with added antioxidants from natural sources appears to be a convenient food form to satisfy the consumer's interest in original yogurt nutrients, beneficial effects of starter cultures, and health benefits of added antioxidants. For this reason, several attempts to produce yogurts fortified with natural antioxidant-rich extracts have been undertaken (Chouchouli, et al., 2013).

Aronia melanocarpa berries (black chokeberries) are one of the richest plant sources of phenolic compounds, especially anthocyanins. Different useful effects on health have been reported for black chokeberries and their extracts, such as prevention and treatment of cardiovascular diseases and colon cancer, antidiabetes and antimutagenic effects. This may principally be due to the antioxidant activity exhibited by phenolic compounds, and particularly of the anthocyanins, in these berries and their extracts (D'Alessandro, et al., 2013).

When evaluating the potential functionality of a compound, its bioavailability in food is more important than the quantity of that compound. Research concerning the bioaccessibility of phenolic compounds and other antioxidants from solid matrices is significant, since only the compounds released from the food matrix and/or absorbed in the small intestine are potentially bioavailable and able to show their beneficial effects (Sengul, Surek, & Nilufer-Erdil, 2014). Despite the presence of large amounts

of polyphenols present in ingested food, only a very small portion can be actually absorbed. For instance, polyphenols may be associated with food matrix and thus become unavailable for absorption (Correa-Betanzo, et al., 2014). Milk proteins such as bovine serum albumin (BSA), β-lactoglobulin and γ-globulin are known for their binding capacity with dietary polyphenols (Xiao, et al., 2011), which could restrict the amount of phenolic compounds available for absorption.

Within this context, the objectives of this study were;

(i) to evaluate the fermentation effect on the antioxidant properties and anthocyanin content of the yogurt mixtures which black chokeberry berries and pulp added before and after the fermentation process,

(ii) to understand the possible interactions between the anthocyanins found in black chokeberry berry as well as pulp and milk proteins by using in vitro gastrointestinal digestion model.

(iii) to recover the anthocyanins remained in pulp which is a valuable by-product of juice processing.

2 LITERATURE REVIEW

2.1 Yogurt

Milk and other dairy products are consumed all over the world. Among these, yogurt is one of the most popular and unique dairy products, which is being enjoyed for its refreshing taste and beneficial properties. It is easily digestible and has high nutritive and therapeutic properties (Serafeimidou, Zlatanos, Laskaridis, & Sagredos, 2012) (Singh, Singh Kapoor, & Singh, 2011). Figure 2.1 shows the yearly global yogurt consumption per capita for 15 countries according to Euromonitor International's 2013 data.

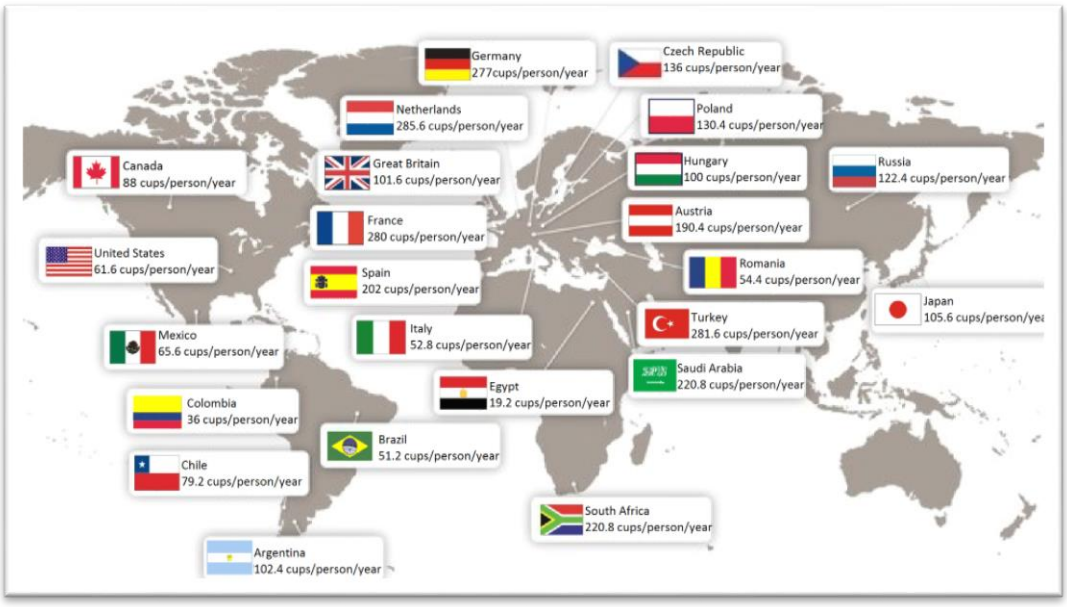

Figure 2.1: Global yogurt consumption per capita and per year (1 cup=125g) (Danone, 2014)

The first direct description of yogurt is found in a dictionary called Divanu Lugati-t Turk, compiled by Kasgarli Mahmut in 1072–1073 in the Middle East. The consumption of yogurt spread rapidly throughout the geographic and cultural region known as the Levant, which surrounded the westernmost protrusion of Asia, involving most of the Republic of Turkey (Donovan & Shamir, 2014).

The uniqueness of yogurt is attributed to the symbiotic fermentation involved in its production process. According to the FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), yogurt is a coagulated milk product obtained by lactic acid fermentation through the activity of *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus*. Fermentation of lactose by these bacteria produces mainly lactic acid, which reacts with the milk proteins to give yogurt its typical texture and its characteristic sensorial properties (Serafeimidou, Zlatanos, Kritikos, & Tourianis, 2013). To meet the National Yogurt Association's criteria for "live and active culture yogurt," the finished yogurt product must contain live lactic acid bacteria in amounts 10^8 organisms/g at the time of manufacture, and the cultures must remain active at the end of the stated shelf life, as verified with the use of a specific activity test (Adolfsson, Meydani, & Russell, 2004).

2.1.1 Starter cultures of yogurt

The starter culture is a must component in the production of high quality yogurt delivering consistent quality attributes desired by consumers. Yogurt is made with live and active cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, and the US Food and Drug Administration (FDA) requires that these two specific bacteria be present as living organisms in a product for it to be called yogurt.

The flavor of plain yogurt is achieved through a protocooperation between rods and cocci, which is influenced by factors such as incubation temperature and acid concentration. Protocooperation, previously described as biochemical mutualism, involves the exchange of metabolites and/or stimulatory factors as seen in Figure 2.2 and Figure 2.3. Since *Streptococcus thermophilus* have less nutritional requirements and hence grow preferentially in milk, during the first exponential growth of *Streptococcus thermophilus*, no growth of *Lactobacilus bulgaricus* is observed. In the second phase, as the pH of milk begins to drop, growth of *Streptococcus thermophilus* (less acid tolerant) slows down and it provides several growth factors such as formate, pyruvate, folate, CO_2, and some long-chain fatty acids that stimulate *Lactobacilus bulgaricus* (more acid tolerant) to grow exponentially. On the other hand, *Lactobacilus bulgaricus* releases cell wall proteases and cytoplasm peptidases that hydrolyze caseins into peptides, following broken down to amino acids. These amino acids served by *Lactobacillus bulgaricus* support the second exponential growth phase and stimulate the growth of *Streptococcus thermophilus.* The growth of *Lactobacillus*

bulgaricus continues in the third growth phase. In brief, *Lactobacillus bulgaricus* produces amino acids and peptides required by *Streptococcus thermophilus* as growth factors, while folate is produced by *Streptococcus thermophilus* to support the growth of *Lactobacillus bulgaricus*. Volatile compounds include small amounts of acetic acid, diacetyl, and acetaldehyde produced by *Lactobacillus bulgaricus*, which contributes much to the unique flavor of yogurt (Surono & Hosono, 2002) (Hill & Kethireddipalli, 2013).

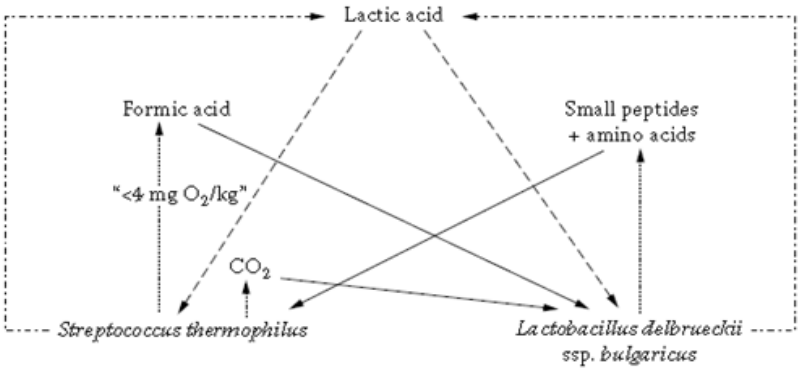

Figure 2.2: Outline of the stimulation and the inhibition of the growth of yogurt bacteria in milk. (-.-.-.-): formation of lactic acid; (……): formation of growth factors; (——): stimulation; (- - - -): inhibition (Walstra, Wouters, & Geurts, 2006)

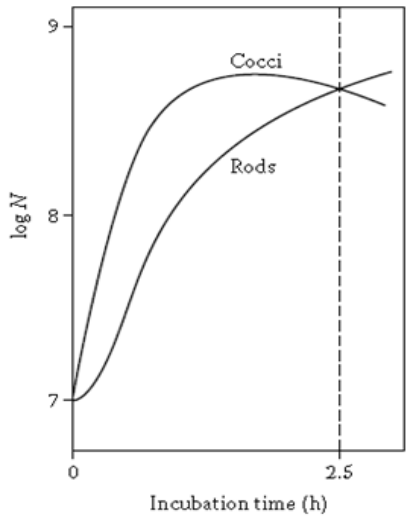

Figure 2.3: Growth of cocci and rods in yogurt (starter) cultured at 45°C in intensely heated milk. Inoculum percentage equals 2.5 N= count in ml^{-1}. Approximate results (Walstra, Wouters, & Geurts, 2006)

The optimum growth temperature is 45 °C for *Lactobacillus bulgaricus and* 37 °C *for Streptococcus thermophilus*. The value of 42 °C is selected for the commercial production of yogurt. Although the production temperature is higher than the optimum activity temperature of *Streptococcus thermophilus,* it is adequately thermophilic in

nature to grow along with *Lactobacillus bulgaricus* during the commercial production of yogurt (Robinson, 2002).

2.1.2 Types of yogurt

Yogurts differ according to several factors, such as their chemical composition, method of production, flavor used and the nature of post-incubation processing. Bovine milk is most commonly used to make yogurt, but milk from water buffalo, goats, ewes, mares, camels, and yaks is also used in various parts of the world (Donovan & Shamir, 2014). Based on the fat content, there are three main types of yogurt: full-fat yogurt, reduced-fat yogurt, and low-fat yogurt. On the basis of the method of production and the physical structure of the coagulum, yogurts are classified as set, stirred or drinking yogurt. Set yogurt is the product formed when the fermentation of milk is carried out in a retail container, and the yogurt produced is characterized by a firm, gel-like structure. In contrast, stirred yogurt results when the coagulum is produced from milk, and the gel structure is broken before cooling and packaging. Drinkable yogurt can be considered as stirred yogurt of low viscosity (Shah, 2003) (Hill & Kethireddipalli, 2013). In addition to those, some new varieties have been added over years including frozen-, concentrated-, dried, and pasteurized yogurt. Based upon the flavorings, yogurts are divided into three categories; plain or natural yogurt, fruit yogurt and flavored yogurt. Natural yogurt is the traditional product, which has a typical sharp 'nutty' flavor. Fruit yogurts are made by addition of fruits, usually in the form of fruit preserves, puree or jam. Flavored yogurts are prepared from natural yogurt by adding sugar and/or other sweetening agents, flavorings and colorings (Shah, 2003). A summation of yogurt types is given in Table 2.1 (Chandan & O'Rell, 2006).

Table 2.1: Types of commercial yogurts and their definitions

Type of Yogurt	**Definition**
Plain	Unflavored yogurt may be cultured in cups or cultured in a vat and dispend into cups. Sugar is not added to the formulation.
Fruit flavored	This type of yogurt is cultured in a vat or bulk and then flavored with a fruit preparation. Styles consist of blended/stirred and fruit-on-the-bottom.

Table 2.1 (continuing): Types of commercial yogurts and their definitions

Blended/stirred	Fermented base is blended with fruit preparation to disperse the fruit throughout and packaged. On cooling, the product thickens and viscous custard-like texture is formed. This style is further subdivided into Swiss- (containing stabilizers and viscous texture) and French- (containing no stabilizers and less viscous texture) style blended yogurt.
Light	Nonfat yogurt in which no sugar added and high intensity of sweeteners are used, resulting in significant reduction in calories.
Lo carb	Nonfat yogurt in which high intensity sweeteners are used instead of sugar. Fruit preparations are replaced with fruit flavors. Lactose content of nonfat milk is reduced by membrane processing. Milk protein concentrate and whey protein isolate are used to decrease the lactose content further.
Custard	It is designed for children. It has a very viscous body like custard. Only fruit puree/juice is used for fruit flavoring. Usually, fermented in the cup.
Sundae/ fruit-on-the-bottom	The fruit is layered in the bottom of the cup, followed by a top layer of unfermented or fermented yogurt. Before consumption it requires blending to mix the fruit preparation.
Natural	Contains only natural ingredients. Generally, it does not contain stabilizers, artificial colors or flavors.
Organic	Contains only ingredients certified as organic.
Yogurt drink/smoothie	Drinkable yogurt is fluid enough to drink. May be sweet and fruit flavored. Smoothies are drinking yogurt, often fortified with minerals and vitamins, prebiotics and probiotics. Some may be designed as a meal replacement.
Whips/mousse	Contains up to 50% (by volume) of inert gas/air to create a fluffy/light texture.
Yogurt with topping	Sweetened fermented base is packaged separately in a cup and sealed. Topping consisting of cereals, nuts, or fruits and is packaged in a smaller cup and sealed. Then the consumer mixes the toppings before the consumption.

Table 2.1 (continuing): Types of commercial yogurts and their definitions

Concentrated/ Greek/strained	It is relatively high in milk fat and milk solids-non-fat. It has a creamy texture and mild flavor as a result of whey removal by centrifugal/membrane separation or by stirring through cloth.
Frozen	The fermented yogurt is blended with low fat/nonfat ice cream to obtain pH of 6.0. The yogurt mix is then extruded through a soft serve machine at 50% overrun and decorated with nuts and other foods to get soft serve frozen yogurt.

2.1.3 Yogurt production

The process for producing yogurt can be summarized in the following sequence of steps: standardization of milk solids, heat treatment, cooling to 40 – 45 °C, inoculation with the specific microorganisms, and incubation at 40–45 °C until pH 4.6–4.7. The following steps are cooling, handling, and packaging. Milk is the basic ingredient of the preparation. Its composition can be modified to meet economic, practical, and consumer acceptance. Especially the solids content has a significant effect on the firmness of the yogurt (De Oliveira, 2014). The main steps of yogurt production are detailed in Table 2.2 (FAO Corporate Document Repository, 2014) and the basic process of making yogurt is summarized in Figure 2.4 (De Oliveira, 2014).

Table 2.2: Main steps of yogurt production and required equipment

Ingredients	***Process***	***Equipment***
Milk	Preheat to 70°C for 15-20 minutes Other alternative temperature/time combinations: 90-95 °C for 2-5 min (De Oliveira, 2014), (Early, 2012), 85 °C for 30 min (Shah, 2003), (Selvamuthukumaran & Farhath, 2014)	Heat source
		Thermometer
		Boiling pan
	Cool to 40-45°C (De Oliveira, 2014)	Thermometer
	Addition of starter culture (2 %)	Measuring and weighing equipment
	Pour into bottles/pots	Funnel or Liquid filler
		Sealing machine
		or Capping machine
	Incubate at 43-45°C	Commercial incubator
		Thermometer
	Store at 4°C	Refrigerated storage

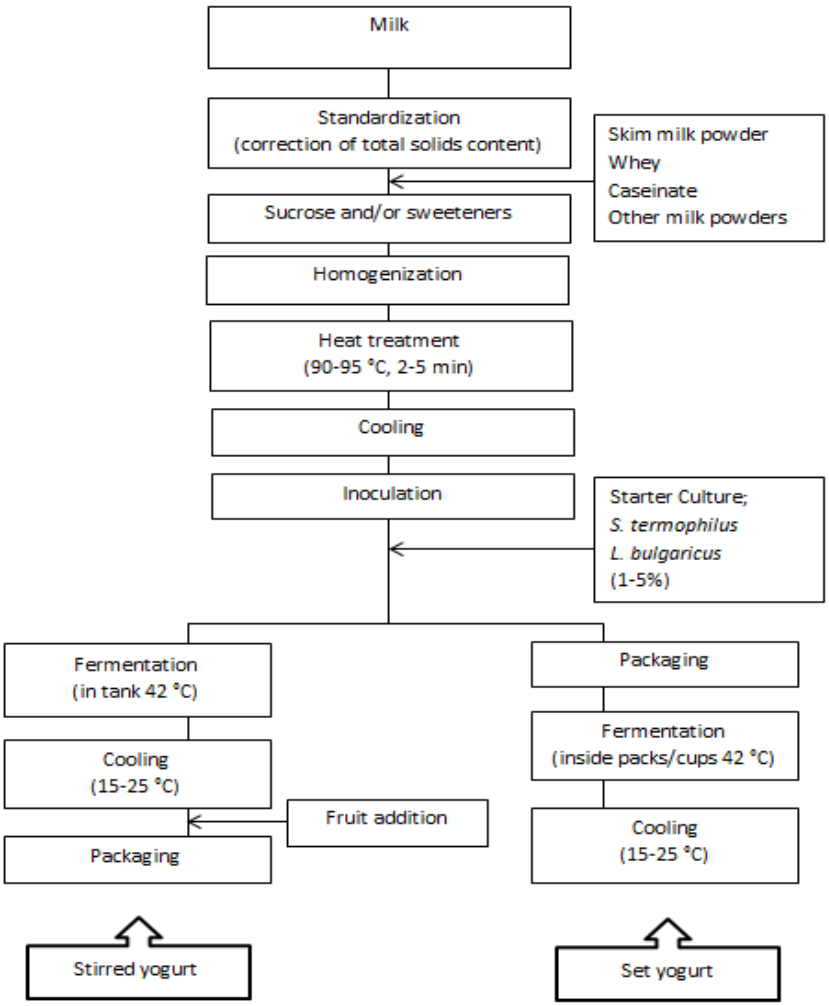

Figure 2.4: Basic process of yogurt production (De Oliveira, 2014)

2.1.4 Health benefits of yogurt

Yogurt is an attainable, easy-to-digest, and tasty food that provides important nutrients to children and adolescents and in this way forms part of a balanced nutrient-rich diet during development and growth. The importance of yogurt as part of a balanced and healthy diet is admitted by regulatory authorities and scientific institutions in most countries. An increasing number of epidemiologic and clinical proof suggests that yogurt consumption may act beneficially on weight regulation and metabolic risk factors (Marette & Picard-Deland, 2014).

Although it generally has a similar micronutrient composition as milk, yogurt is highly concentrated with proteins, vitamins and minerals, such as vitamin B_2 and B_{12}, calcium, magnesium, potassium, phosphorus, zinc, and others (Wang, Livingston, Fox, Meigs, & Jacques, 2013). It supplies minor components that have been shown to play a role in decreasing the risk of certain diseases, particularly gastrointestinal

disorders such as infantile diarrhea, and increasing the host resistance to bacterial infection, gastro-enteritis, and constipation (Cano, Agüero, & Perdigon, 2002).

It has been shown that yogurt consumption has a protective effect on *Helicobacter pylori* seropositivity and helps to extirpate the infection among those already infected. In other words, eating yogurt may protect against acquiring *Helicobacter pylori* infection (Ornelas, Galvan-Potrillo, & López-Carrillo, 2007).

There is a substantial body of evidence to indicate that fermented dairy products such as yogurt are well tolerated by sufferers of lactose intolerance. It has been suggested that this is because of the bacterial enzyme, β-galactosidase produced by the culture in 'live' yogurt. This enzyme, which is able to digest lactose to glucose and galactose, is intracellular and hence is thought to survive gastric digestion (Eskin, 1990) (Buttriss, 2005) (Savaiano, 2014).

The health and strength of our bones rely on a balanced diet and a steady stream of nutrients, most importantly, calcium and vitamin D. Dairy products may represent the best dietary sources of calcium because of the high content, high absorptive rate, and relatively low cost (Caroli, Poli, Ricotta, Banfi, & Cocchi, 2011) (Sunyecz, 2008). The beneficial effect of consumption of yogurt on bone health was reviewed by Rizzoli (2014).

Epidemiologic studies and clinical trials dealing with the interactions of yogurt nutrients and bacteria within the food matrix are warranted to evaluate the effect of yogurt on the modulation of the gut microbiota and the prevention of obesity and cardiometabolic diseases (Marette & Picard-Deland, 2014). Several studies indicate that consumption of yogurt has antihypertensive and hypocholesterolemic effects in spontaneously hypertensive rats (Ramchandran & Shah, 2011), enhancing immunity in the respiratory tract (Racedo, Villena, Salva, & Alvarez, 2009), reducing the risk of type 2 diabetes (Tong, Dong, Wu, Li, & Qin, 2011).

The role of proteins as physiologically active components in the diet is being increasingly accepted. In recent years it has been recognized that dietary proteins provide a rich source of biologically active peptides (Korhonen & Pihlanto, 2006). The formation of bioactive peptides from proteins and oligopeptides in dairy products can be produced by digestion. Various bioactive peptides were detected in vivo in the human and animal intestine after ingestion of milk, yogurt or bovine casein (Alhaj & Kanekanian, 2014). Chabance et al. (1998) reported that caseinophosphopeptides (CPPs) were found in the stomach and duodenum of adults after ingestion of milk or yogurt.

2.2 Black Chokeberry (Aronia melanocarpa)

Aronia melanocarpa, known as black chokeberry, originates from the eastern parts of North America. It was transferred to Europe around 1900 and in the 1960s the plant was established as a cultivar in the former Soviet Union. Nowadays black chokeberries are widely distributed mainly in the east-south and central parts of Europe and cultivated as an industrial crop (Denev, Kratchanov, Ciz, Lojek, & Kratchanova, 2012). It is widely used in the food industry, both on its own, or blended with other fruits such as in juice and soft drinks, wine production, food coloring and natural health products (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007).

2.2.1 Chemical composition of black chokeberry

It is well known that factors as variety, light intensity, soil moisture, time of harvest and growth rate result in differences in the chemical composition of plants (Eheart & Massey Jr., 1962). A summary of chemical composition of black chokeberry and its products, adapted from Kulling and Rawel (2008), is shown in Table 2.3.

The dry matter content of black chokeberries reported by Ochmian et al. (2012) ranged from 15.3-19.5% in their fresh form. However, in another report the maximum dry matter content of black chokeberry reaches levels up to 29% (Kulling & Rawel, 2008).

It was demonstrated that fresh black chokeberry and its pomace are a rich source of dietary fiber among the several dietary fiber rich fruits. In addition, the fiber powders obtained from the berries still contain notewothy amounts of anthocyanins, as indicated by their dark violet color. Black chokeberry pomace preparations have been suggested to be a good source of dietary fiber containing high amounts of cellulose, hemicellulose and lignin (Wawer, Wolniak, & Paradowska, 2006) (Nawirska & Uklanska, 2008).

Chokeberry fruit is characterized by a relatively low acidity in comparison with other berry fruits. The low content of free acids found in chokeberry pomace is linked to the transfer of free acids to the juice together with other soluble substances like sugars and potassium salts. Thus low soluble calcium and magnesium salts of native organic acids and acidic products of enzymatic pectin hydrolysis can remain in pomace most of all, resulting the low titratable acidity which can only be determined with free carboxylic groups. Additionally, galacturonic acid is claimed as the dominant organic acid among the determined ones in black chokeberry (Sójka, Kołodziejczyk, & Milala, 2013). In contrast, the main organic acids in chokeberry identified by Ochmian et al.

(2012) and Šnebergrová et al. (2014) were L-malic acid-citric acid and malic acid-quinic acid, respectively.

Table 2.3: Chemical composition of black chokeberry fruits and juices

Constituent		Sample	Concentration	Reference
Basic Composition	Soluble solids, °Bx	Fresh pressed juice	19.5	(Ara, 2002)
		Berries	15.2-22.9	(Šnebergrová, et al., 2014)
	Dry matter, %	Pasteurized juice	15.5	(Kulling & Rawel, 2008)
		Berries	15.6; 20; 16.7-28.8	(Kulling & Rawel, 2008)
		Pomace	93.6-94.8	(Sójka, Kołodziejczyk, & Milala, 2013)
	pH	Fresh pressed juice	3.6	(Ara, 2002)
		Pasteurized juice	3.3	(Kulling & Rawel, 2008)
		Berries	3.3-3.7	(Kulling & Rawel, 2008)
	Dietary Fibers	Berries, g/kg FW	56; 3.4-5.8	(Kulling & Rawel, 2008)
		Pomace, g/100g DM	63.5-77.9	(Sójka, Kołodziejczyk, & Milala, 2013)
	Protein	Berries, g/100g FW	0.7	(Kulling & Rawel, 2008)
		Pomace, g/100g DM	4.9-24.1	(Sójka, Kołodziejczyk, & Milala, 2013)
	Fat	Berries, g/100g FW	0.14	(Kulling & Rawel, 2008)
		Pomace, g/100g DM	2.9-13.9	(Sójka, Kołodziejczyk, & Milala, 2013)
	Ash	Fresh pressed juice, g/L	6.4; 4.6	(Kulling & Rawel, 2008)
		Pasteurized juice, g/L	3.6; 4.1	(Kulling & Rawel, 2008)
		Berries, g/kg FW	4.4; 5.8; 4.2-11.8	(Kulling & Rawel, 2008); (Šnebergrová, et al., 2014)
		Pomace, g/100g DM	1.4-3.9	(Sójka, Kołodziejczyk, & Milala, 2013)
Saccharides	Glucose	Fresh pressed juice, g/L	41	(Ara, 2002)
		Pasteurized juice, g/L	40	(Kulling & Rawel, 2008)
		Pomace, g/100g DM	0.39-0.80	(Sójka, Kołodziejczyk, & Milala, 2013)

Table 2.3 (continuing): Chemical composition of black chokeberry fruits and juices

Saccharides	Fructose	Fresh pressed juice, g/L	38	(Ara, 2002)
		Pasteurized juice, g/L	37	(Kulling & Rawel, 2008)
		Pomace, g/100g DM	0.48-0.58	(Sójka, Kołodziejczyk, & Milala, 2013)
	Sorbitol	Fresh pressed juice, g/L	80	(Ara, 2002)
		Pasteurized juice, g/L	55.6	(Kulling & Rawel, 2008)
		Pomace, g/100g DM	1.06-2.32	(Sójka, Kołodziejczyk, & Milala, 2013)
	Saccharose	Pomace, g/100g DM	0.03-0.043	(Sójka, Kołodziejczyk, & Milala, 2013)
Organic Acids	Malic acid	Fresh pressed juice, g/L	9	(Ara, 2002)
		Pasteurized juice, g/L	11	(Kulling & Rawel, 2008)
		Berries g/kg FW	13.1	(Kulling & Rawel, 2008)
		Pomace, g/kg DM	1.51-3.01	(Sójka, Kołodziejczyk, & Milala, 2013)
	Citric acid	Fresh pressed juice, g/L	0.5	(Ara, 2002)
		Pasteurized juice, g/L	0.247	(Kulling & Rawel, 2008)
		Berries g/kg FW	2.1	(Kulling & Rawel, 2008)
		Pomace, g/kg DM	0.489-0.942	(Sójka, Kołodziejczyk, & Milala, 2013)
	Isocitric acid	Fresh pressed juice, g/L	0.065	(Ara, 2002)
	Shikimic acid	Fresh pressed juice, g/L	0.08	(Ara, 2002)
	Succinic acid	Fresh pressed juice, g/L	1.5	(Ara, 2002)
		Pasteurized juice, g/L	160	(Kulling & Rawel, 2008)
		Berries, g/kg FW	0.8 (3 months stored)	(Kulling & Rawel, 2008)
	Galacturonic acid	Pomace, g/kg DM	5.35-15.6	(Sójka, Kołodziejczyk, & Milala, 2013)
	Quinic acid	Berries, g/kg FW	4.1-6.8	(Šnebergrová, et al., 2014)

Table 2.3 (continuing): Chemical composition of black chokeberry fruits and juices

Vitamins	Vit C	Fresh pressed juice, mg/L	200	(Ara, 2002)
		Berries, mg/kg FW	137; 13-270	(Kulling & Rawel, 2008)
	Folic acid	Pasteurized juice, mg/L	0.035	(Kulling & Rawel, 2008)
		Berries, mg/kg FW	0.2	(Kulling & Rawel, 2008)
	Vit B1	Fresh pressed juice, mg/L	0.5	(Ara, 2002)
		Berries, mg/kg FW	0.18	(Kulling & Rawel, 2008)
	Vit B2	Fresh pressed juice, mg/L	0.6	(Ara, 2002)
		Berries, mg/kg FW	0.2	(Kulling & Rawel, 2008)
	Vit B6	Fresh pressed juice, mg/L	0.55	(Ara, 2002)
		Berries, mg/kg FW	0.28	(Kulling & Rawel, 2008)
	Niacin	Fresh pressed juice, mg/L	3.4	(Ara, 2002)
		Berries, mg/kg FW	3	(Kulling & Rawel, 2008)
	Panthotenic acid	Fresh pressed juice, mg/L	2.2	(Ara, 2002)
		Berries, mg/kg FW	2.79	(Kulling & Rawel, 2008)
	Tocopherols	Berries, mg/kg FW	17.1	(Kulling & Rawel, 2008)
	Vit K	Berries, mg/kg FW	0.242	(Kulling & Rawel, 2008)
Minerals	Na	Fresh pressed juice, mg/L	5	(Ara, 2002)
		Pasteurized juice,mg/L	5.7	(Kulling & Rawel, 2008)
		Berries, mg/kg FW	26	(Kulling & Rawel, 2008)
		Pomace, mg/kg DM	52.5-89	(Sójka, Kołodziejczyk, & Milala, 2013)
	K	Fresh pressed juice, mg/L	2850	(Ara, 2002)
		Pasteurized juice,mg/L	1969	(Kulling & Rawel, 2008)
		Berries, mg/kg FW	2180; 1356.3-3659.7	(Kulling & Rawel, 2008); (Šnebergrová, et al., 2014)
		Pomace, mg/kg DM	1814.3-3075.9	(Sójka, Kołodziejczyk, & Milala, 2013)
	Ca	Fresh pressed juice, mg/L	150	(Ara, 2002)
		Pasteurized juice,mg/L	185	(Kulling & Rawel, 2008)

Table 2.3 (continuing): Chemical composition of black chokeberry fruits and juices

Minerals	Ca	Berries, mg/kg FW	322	(Kulling & Rawel, 2008)
		Pomace, mg/kg DM	2186.8-4080.4	(Sójka, Kołodziejczyk, & Milala, 2013)
	Mg	Fresh pressed juice, mg/L	140	(Ara, 2002)
		Pasteurized juice,mg/L	160	(Kulling & Rawel, 2008)
		Berries, mg/kg FW	162	(Kulling & Rawel, 2008)
		Pomace, mg/kg DM	370.8-2501	(Sójka, Kołodziejczyk, & Milala, 2013)
	Fe	Fresh pressed juice, mg/L	4	(Ara, 2002)
		Pasteurized juice,mg/L	0.4	(Kulling & Rawel, 2008)
		Berries, mg/kg FW	9.3	(Kulling & Rawel, 2008)
		Pomace, mg/kg DM	68.9-86.2	(Sójka, Kołodziejczyk, & Milala, 2013)
	Zn	Fresh pressed juice, mg/L	1.3	(Ara, 2002)
		Pasteurized juice,mg/L	0.6	(Kulling & Rawel, 2008)
		Berries, mg/kg FW	1.47	(Kulling & Rawel, 2008)
		Pomace, mg/kg DM	5.6-36.9	(Sójka, Kołodziejczyk, & Milala, 2013)
	I	Pasteurized juice,µg/L	<5	(Kulling & Rawel, 2008)
	P	Berries, mg/kg FW	257-417.5	(Šnebergrová, et al., 2014)
	Cu	Pomace, mg/kg DM	5-12.4	(Sójka, Kołodziejczyk, & Milala, 2013)
Phytochemicals	Carotenoids	Pasteurized juice,µg/L	70	(Kulling & Rawel, 2008)
		Berries, mg/kg FW	46	(Kulling & Rawel, 2008)
	Total phenolics	Pasteurized juice,µg/L	6.3-6.95	(Kulling & Rawel, 2008)
		Berries, mg/100 g FW	2010; 2556; 6902	(Kulling & Rawel, 2008)
		Berries, mg/100 g DM	3760; 7465; 7849	(Kulling & Rawel, 2008)
	Amygdalin	Fresh pressed juice, mg/kg	57.5	(Ara, 2002)
		Berries, mg/kg FW	201	(Kulling & Rawel, 2008)
	Total anthocyanins	Berries mg/ kg FW	2055-6231	(Šnebergrová, et al., 2014)

According to Sojka et al. (2013) the content of saccharides in chokeberry pomace was in a range of 2.7–3.5%. Sorbitol (sugar alcohol) was a dominant component, correspond to above 60% of saccharides for seedless fractions and about 40% for seed fractions. Seed fractions of pomaces possessed a considerably higher content of saccharose and glucose. The sugar content estimated by Ochmian et al. (2012) was 6.2-10.8 g in 100 grams of fruits for total sugars, 8.83-12.48 g in 100 grams of fruits for reduced sugars.

The lipid composition of chokeberry was investigated by Zlatanov (1999). The seeds contain 19.3 g/kg glyceride oil. The content of phospholipids, mainly phosphatidylcholine, phosphatidylinositol and phosphatidylethanolamine, was 2.8g/kg. The total amounts of sterols were 1.2g/kg. The main component was β-sitosterol, followed by campesterol and Δ^5-avenasterol. In the tocopherol fraction α-tocopherol (55.5 mg/kg) predominated in chokeberry oil. With another approach, the lipid composition from waste of black chokeberry extraction with freon134a (1,1,1,2-tetrafluorethane) and with freon 134a+acetone was analysed by Merdzhanov et al. (2013). The main components found in the triacylglycerol fractions were linoleic (47.8-57.2%), oleic (26.4-28.4%) and palmitic (11.0-15.5%) acids. β-tocopherol (59.3 – 61.4%) predominated in the tocopherol fractions, and β-sitosterol (74.3%) in the sterol fraction.

Basic composition of black chokeberry pomace fractions with seed and without seed in different sizes was analysed by Sojka et al. (2013). According to their data, the highest protein content was in the seed fraction of the fruits (24.1%). However, the seedless parts of the fruit fractions possessed remarkably lower content of protein (6%). In this sense, the recovery of seeds as valuable raw material in the processing of chokeberry pomace is an important matter.

Ash content refers to the inorganic residue such as minerals remaining after either burning or complete oxidation of organic matter in foods (Harbers, 1998). The mineral content (ash values) of black chokeberries was reported as 4.4-5.8 g/kg (Kulling & Rawel, 2008) and 2-3.8% in dry matter (Wawer, Wolniak, & Paradowska, 2006). In another study, ash content of different fractions of dried chokeberry pomace varied between 1.4-3.9% in dry matter (Sójka, Kołodziejczyk, & Milala, 2013).

Anthocyanins are responsible for the red, purple, and blue hues present in fruits, vegetables, and grains. There are 6 common anthocyanidins (pelargonidin, cyanidin, peonidin, delphinidin, petunidin, and malvidin), whose structures can vary by glycosidic substitution at the 3 and 5 positions (Lee, Durst, & Wrolstad, 2005). Aronia

berries are among the richest plant sources of anthocyanins (class of flavonoids): cyanidin-3-O-galactoside, cyanidin-3-O-arabinoside, cyanidin-3-O-xyloside and cyanidin-3-O-glucoside which are responsible for dark red, blue, and purple color of berries. About 25% of the total polyphenols in chokeberry fruits are anthocyanins. The high content of these phenolics seems to correlate to the antioxidant activity reported for these berries (Galván D'Alessandro, Dimitrov, Vauchel, & Nikov, 2013) (Ramić, et al., 2015). Aside from anthocyanins, chokeberry fruit is a rich source of proanthocyanidins with a high degree of polymerisation. Chokeberry fruits also contain flavonols, including quercetin glycosides as well as hydroxycinnamic acids, i.e. chlorogenic acid and neochlorogenic acid (Sójka, Kołodziejczyk, & Milala, 2013).

A recent study on antioxidant potential of chokeberry products indicates that the highest total phenolic and total anthocyanin contents were in chokeberry pomace, whereas the highest total flavonoid content and antioxidant activity values were in dried fruits. Four major anthocyanins, including cyanidin-3-galactoside, cyanidin-3-arabinoside, cyanidin-3-glucoside and cyanidin-3-xyloside, were detected in chokeberry fruit (Kapci, et al., 2013).

According to Mladin et al. (2011) chokeberries contain important amounts of anthocyanins (509 mg/100g fruit mean value). In addition, chokeberry juice had a strong and stable color during 1 week under room storage. Within this context, it can be combined with apple or sour cherry juice. High tannin content gives to the fruits an astringent flavor, and by the discovery of their antioxidant capacity richness in these compounds represents an added value of such kind of fruits. In point of this aspect, chokeberry which accumulated the largest amounts of tannins, are by far the most valuable for the antioxidant property.

Najda and Labuda (2013) showed that black chokeberry fruits had the highest total phenolics and anthocyanin content and the lowest flavonoid content among eight different orchard shrub species.

2.2.2 Positive effects on health

In recent years, black chokeberries have gained popularity due to their high content of polyphenols with antioxidant activity. Several reports indicated that extracts from *Aronia* berries exhibited different biological effects both *in vitro* and *in vivo* (antioxidant, gastroprotective, hepatoprotective, and antiproliferative activities), not only through antioxidant pathways, but also via impacting signal transduction/intracellular signaling cascades, impacting apoptosis, etc. (Ciocoiu, Badescu, Miron, & Badescu M., 2013).

Results of Bijak et al. (2011) demonstrated that chokeberry polyphenols has an anticoagulant effect in blood. This findings show that chokeberry extracts can be used in the future as directly edible natural thrombin inhibitors having a safe origin to prevent thrombosis, which may be alternative to vitamin K antagonists.

Pancreatic α-amylase and lipase are the key enzymes in the digestive system, catalyzing the hydrolysis of complex food ingredients to simple and easily digestible molecules. The inhibition of these enzymes could help to reduce energy value of food, by reducing its availability and extension of the digestion process, thereby reducing the body weight and causing far-reaching health benefits. Worsztynowicz et al. (2014) have shown that both anthocyanins and phenolic acids in chokeberry are compounds which inhibit the ability of the reaction catalyzed by α -amylase and lipase.

Bräunlich et al. (2013) showed that different polyphenolic compounds of black chokeberry can have beneficial effects in reducing blood glucose levels due to inhibition of α-glucosidase and may have a potential to suppress oxidative stress.

It has been shown that flavonoids can prevent the oxidation of the LDL fraction and delay the development of experimental atherosclerosis. A study by Naruszewicz et al. (2007) shows that flavonoids from chokeberry fruits has an ability to reduce oxidative stress and to decrease in cardiovascular risk markers in patients with the history of myocardial infraction treated with statins. Likewise, Skoczyñska et al. (2007) showed that drinking of *Aronia melanocarpa* fruit juice may have a beneficial effect on reduction of cardiovascular risk by decreasing total cholesterol and LDL cholesterol level and increasing HDL cholesterol level.

It is known that *Aronia melanocarpa* has an antimicrobial effect on some microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, and type A influenza virus. In a recent study, it was shown that chokeberry extracts has a non-toxic inhibitory effect on biofilm formation of *E. coli* and *Bacillus cereus* (Bräunlich, et al., 2013).

In an animal study it has shown that, black chokeberry red pigments have an inhibitory effect on gastric damage induced by ethanol in rats (Matsumoto, Hara, Chiji, & Kasai, 2004).

2.3 Bioavailability of Flavonoids and Phenolic Compounds

2.3.1 Definition of bioavailability, bioaccessibility and bioactivity

On oral consumption, the uptake of micronutrients and phytochemicals into the body is not complete, as a certain percentage is not absorbed. To measure the amount that is actually absorbed, distributed to the tissue, metabolized and eventually excreted, the term bioavailability was introduced. Bioavailability describes the concentration of a given compound or its metabolite at the target organ. The Food and Drug Administration defines bioavailability as 'the rate and the extent to which the therapeutic moiety is absorbed and becomes available to the site of drug action' (Holst & Williamson, 2008).

Bioaccessibility has been defined as the fraction of a compound which is released from the food matrix in the gastrointestinal lumen and therefore becomes available for intestinal absorption. Chewing in the mouth initiates the process and several digestive fluids containing different enzymes continue to break down the food matrix in the stomach and throughout the rest of the gastrointestinal lumen (Figure 2.5). Bioaccessibility is influenced by the composition of the digested food matrix, the synergisms and antagonisms of the different food components, but also by physicochemical properties, such as pH, temperature and texture of the matrix (Rein, et al., 2013).

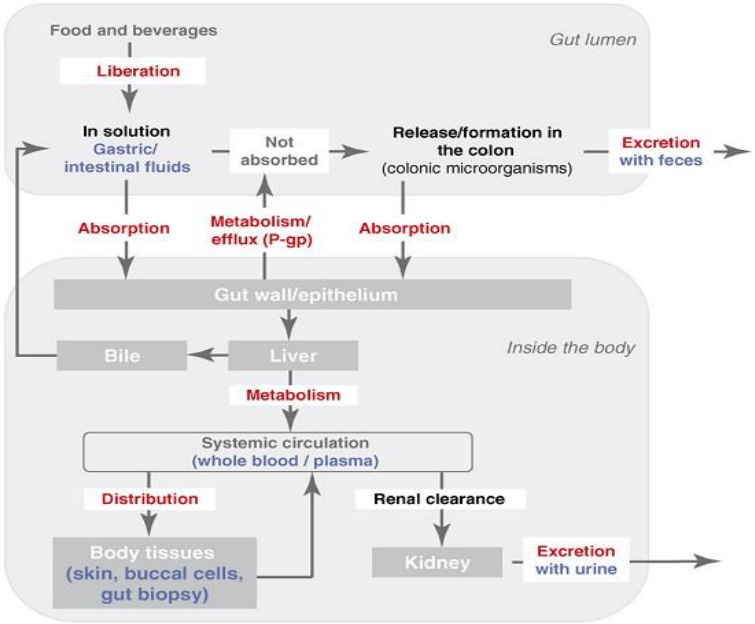

Figure 2.5: Basic incidents describing the fate of nutrients (Holst & Williamson, 2008)

Bioactivity is the specific effect upon exposure to a substance. It includes tissue uptake and the consequent physiological response (such as antioxidant, anti-inflammatory). It can be evaluated *in vivo*, *ex vivo*, and *in vitro* (Figure 2.6 and Figure 2.7).

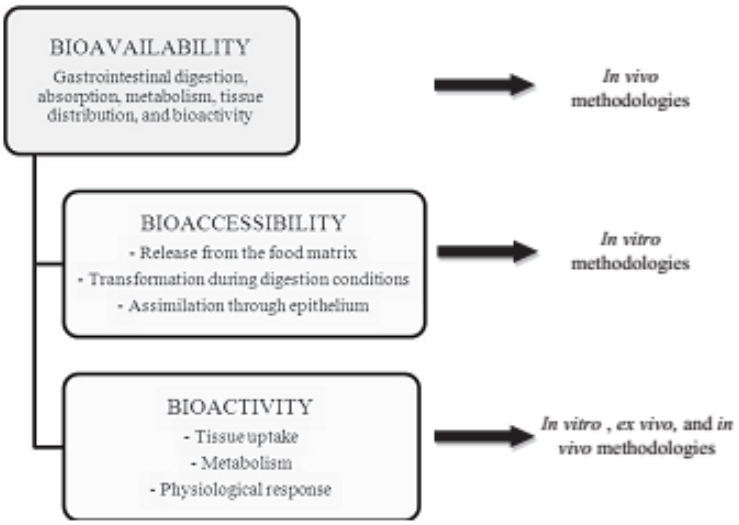

Figure 2.6: Description of bioavailability, bioaccessibility, bioactivity and their potential assessment methodologies (Carbonell-Capella, Buniowska, Barba, Esteve, & Frigola, 2014)

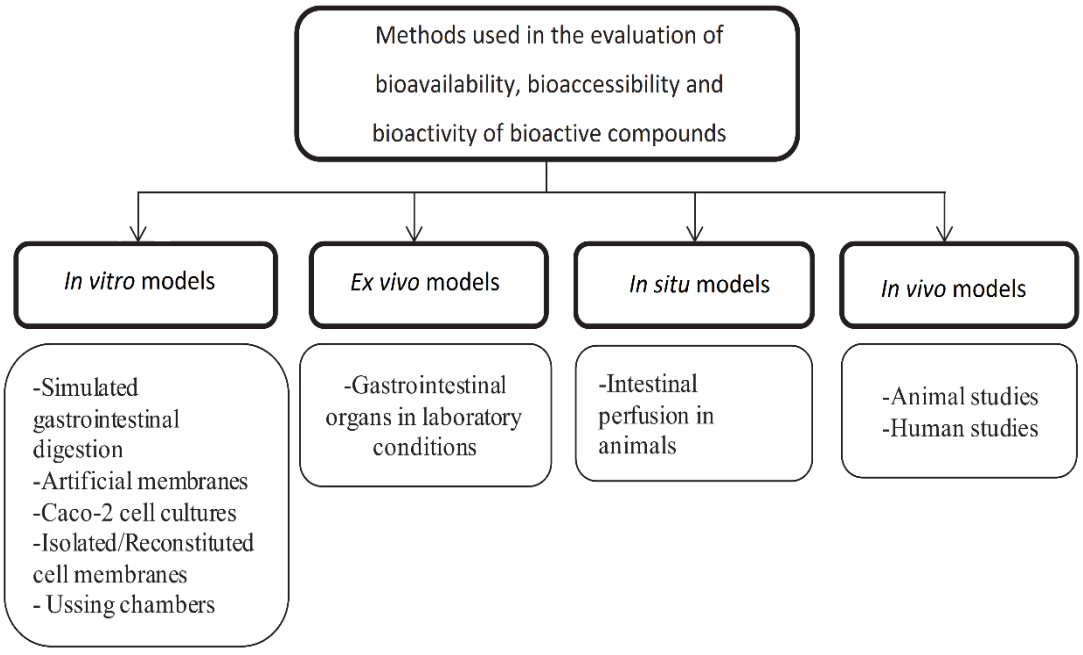

Figure 2.7: Methodologies used in the evaluation of bioavailability, bioaccessibility and bioactivity of bioactive compounds (Carbonell-Capella, Buniowska, Barba, Esteve, & Frigola, 2014).

2.3.2 Factors affecting the bioavailability

Many factors affect the bioavailability of a compound; these may be divided into exogenous factors such as bioaccessibility, the complexity of the food matrix, the chemical form of the compound of interest, structure and amount of co-ingested compounds, as well as endogenous factors including mucosal mass, intestinal transit time, rate of gastric emptying, metabolism and extent of conjugation, and protein-binding in blood and tissues (Table 2.4) (Holst & Williamson, 2008) (Rein, et al., 2013). Since the lipophilic and hydrophilic compounds have a different solubility, absorption of these compounds occurs by different mechanisms (Richelle, Sabatier, Steiling, & Williamson, 2006). Bioactivity (in some sources referred as bioefficacy) may be improved through enhanced bioavailability. Therefore, several technologies have been developed to improve the bioavailability of xenobiotics, including structural modifications, nanotechnology and colloidal systems (Rein, et al., 2013).

Table 2.4: Main factors affecting the bioavailability of dietary polyphenols in humans (D'Archivio, Filesi, Vari, Scazzocchio, & Masella, 2010)

External factors	Environmental factors (*i.e.*, sun exposure, degree of ripeness); food availability
Food processing related factors	Thermal treatments; homogenization; liophylization; cooking and methods of culinary preparation; storage
Food related factors	Food matrix; presence of positive or negative effectors of absorption (*i.e.*, fat, fiber)
Interaction with other compounds	Bonds with proteins (*i.e.*, albumin) or with polyphenols with similar mechanism of absorption
Polyphenols related factors	Chemical structure; concentration in food; amount introduced
Host related factors	Intestinal factors (*i.e.*, enzyme activity; intestinal transit time; colonic microflora). Systemic factors (*i.e.*, gender and age; disorders and/or pathologies; genetics; physiological condition)

2.3.3 Bioavailability of anthocyanins

Even though a compound has strong antioxidative or other biological activities in vitro, it will have almost no biological activity in vivo if only a small amount or none of the compound gets to the target tissues. The most abundant polyphenols in our diet are not necessarily those that have the best bioavailability profile. Consequently, it is not only important to know how much of a nutrient is present in specific food or dietary supplement, but it is even more important to know how much of it is bioavailable (D'Archivio, Filesi, Vari, Scazzocchio, & Masella, 2010).

Anthocyanins are broadly distributed and many plants, including berries, contain several structurally diverse anthocyanins. They seem to be poorly absorbed in the

small intestine, so significant amounts probably pass into the large intestine where bacterial degradation occurs. There are reports that cyanidin-based anthocyanins undergo cleavage of the sugar section followed by ring fission of the released cyanidin, which produces 3,4-dihydroxybenzoic acid. Detecting and quantifying the trace levels of complex anthocyanin profiles in plasma and urine after absorption, excretion, and potential phase I and phase II metabolism appears to be very difficult (Denev, Kratchanov, Ciz, Lojek, & Kratchanova, 2012).

Also, a study on stability of polyphenols in black chokeberry subjected to an in vitro gastric and pancreatic digestion showed that gastric digestion had no considerable effect on any of the main phenolic compounds present in chokeberry (anthocyanins, flavan-3-ols, flavonols and caffeic acid derivatives). However, these compounds were significantly altered during the pancreatic digestion and this effect was more remarkable for anthocyanins as approximately 43% was lost during the 2h treatment with pancreatin, while flavonols and flavan-3-ols decreased by 26% and 19%, respectively. Neochlorogenic acid decreased by 28% whereas chlorogenic acid was increased by 24% (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007). Likewise, Liang et al. (2012) demonstrated that, bioaccessibility of mulberry anthocyanins was greatly decreased after the intestinal digestion. On the other hand, the radical scavenging activity showed that the digest has good antioxidant capacity due to the phenolic compounds generated from anthocyanin degradation under intestinal digestion.

In an *in vivo* study it has been shown that, after consumption of natural chokeberry juice, the native compounds and their glucuronidated and methylated metabolites are present in human plasma and urine during 24 h. Moreover, the study showed that 70% of anthocyanins were excreted with urine as metabolites with the dominance of peonidin monoglucuronide (Wiczkowski, Romaszko, & Piskula, 2010).

Kay et al. (2004) investigated the metabolic conversion of chokeberry-derived cyanidin glycosides in human subjects. Volunteers consumed approximately 20 g chokeberry extract containing 1.3 g cyanidin 3-glycosides after a 2 weeks anthocyanin wash-out diet and their blood samples were taken before consumption and 0.5, 1 and 2 hours after consumption. Cyanidin-3-galactoside accounted for 55.4% and 66% of the detected anthocyanins in urine and serum samples respectively. The metabolites were identified as glucuronide conjugates, as well as methylated and oxidized derivatives of cyanidin-3-galactoside and cyanidinglucuronide. The consumption of four cyanidin glycosides (cyanidin 3-galactoside, cyanidin 3-arabinoside, cyaniding 3-xyloside, and cyanidin 3-glucoside) resulted in the appearance of at least 10 individual

anthocyanin metabolites in human urine and serum. As the authors suggested, conjugation probably affects the biological activity of anthocyanins and these metabolic products are likely in part responsible for the reported health benefits associated with the consumption of anthocyanins.

Kamonpatana et al. (2014) worked on the effect of anthocyanin structure on the bioavailability at the buccal mucosa. According to their findings, in chokeberry juice, loss of cyanidin-3-xyloside exceeded that of other anthocyanins, whereas cyanidin-3-glucoside preferentially accumulated in epithelium cells. These results suggest that anthocyanin structure affects the stability and the buccal cell uptake and therefore the potential efficacy of anthocyanin-rich products for the promotion of oral health.

2.3.4 Protein-anthocyanin interactions

Recent studies indicate that the bioavailability of anthocyanins is extremely low. One of the possible reasons could be their binding to proteins. Therefore, the binding affinity of cyanidin-3-glucoside (Cya-3-glu) to human serum albumin (HSA) and its influence on *α*-amylase activity was investigated by the quenching of protein tryptophan fluorescence by Wiese et al. (2009). They observed that, the strongest affinity of cyanidin-3-glucoside for HSA being at pH 7 underlines its potential in transport and distribution of the phenolic compounds in organisms. An influence on salivary amylase activity is possible when drinking berry juices with high anthocyanins content. Within this context they assumed that, taking chokeberry (*Aronia melanocarpa*) juice into consideration, then 100 ml would provide *ca*. 200 mg anthocyanins and saliva would contain 1–2 mg protein/ml. Therefore, the ratios of these two components would be such that an influence on activity can be expected.

Similarly, the interaction between cyanidin-3-glucoside and bovine serum albumin (BSA) was investigated in another study. According to their findings, cyanidin-3-glucoside can be bound within the hydrophobic cavity in site II′ of BSA. Since, serum albumin is the most abundant carrier protein in plasma with a high affinity for a wide range of drugs and metabolites, it plays key physiological roles in the transportation, distribution and metabolism of many endogenous and exogenous ligands. (Shi, Wang, Zhu, & Chen, 2014).

3 MATERIALS AND METHODS

3.1 Black Chokeberry *(Aronia melanocarpa)* and Yogurt Material

Fresh black chokeberry berries and black chokeberry pulp were obtained from a local producer from Mol, Belgium at their harvest season. All samples were ground using a laboratory scale grinder, and stored at -20 °C until analysis. A picture of black chokeberry is given in Appendix A, Figure 1.A.

UHT full-fat cow's milk was supplied from a local supermarket. Yogurt starter culture (*Streptococcus thermophilus* and *Lactobacillus delbrueckii spp. bulgaricus)* used in yogurt manufacturing was supplied from BMS Wine Depot SA, Kuurne, Belgium.

3.2 Preparation of Berries and Pulp Added Yogurt Samples

UHT full-fat cow's milk was used for yogurt production. Firstly, a stock yogurt culture was prepared one night before the day of yogurt production. One liter of milk was inoculated with a lyophilized starter culture (1 packet) and left at 45°C in a (non-shaking) water bath overnight.

Two types of black chokeberry yogurts, further indicated as fruit yoghurts, were prepared. In the first method, berries or pulp were added to plain yogurt after fermentation of the milk and in the second method, berries or pulp were added directly to the milk before fermentation. Here, the aim is to see if the fermentation has an effect on the anthocyanin content of the fruit added yogurts.

To produce a plain set yogurt, 4% of sugar (w/v) was dissolved in one liter of milk. After that, the temperature of the milk was brought to 45 °C and inoculated with three spoons of stock yogurt culture. The mixture was fermented in a 45°C water bath until the final pH was 4.5 (about 4.5 hours) (Tseng & Zhao, 2013). After the milk was coagulated, the amount of pulp and berries added into yogurt was calculated to achieve approximately the same anthocyanin content. Therefore, 1% of pulp (w/w) and 10% of minced berries (w/w) were added to yogurt. Blank yogurt samples were also prepared without adding pulp or berries, further indicated as plain yogurt.

Similarly, 4% of sugar (w/v) was dissolved in one liter of milk and the temperature of the milk was brought to 45 °C. Afterwards, the mixture was inoculated with three spoons of stock yogurt culture and 1% of pulp (w/v) and 10% berries (w/v) were added immediately to the milk. The fruit added milk mixtures were then incubated in a water bath at 45 °C until the pH dropped to approximately 4.5 (about 4.5 hours). Blank yogurt samples were also prepared without adding pulp or berries. Flow diagrams of yogurt production are presented in Figure 3.1 and Figure 3.2 and pictures of the yogurt samples are given in Appendix A, Figure A.2 and Figure A.3.

All yogurt samples were divided into two batches and stored in at 4 °C until the analyses. The first batch was analyzed at the first day of storage and the second batch was analyzed at the eighth day of storage. All fermentations were done in duplicate.

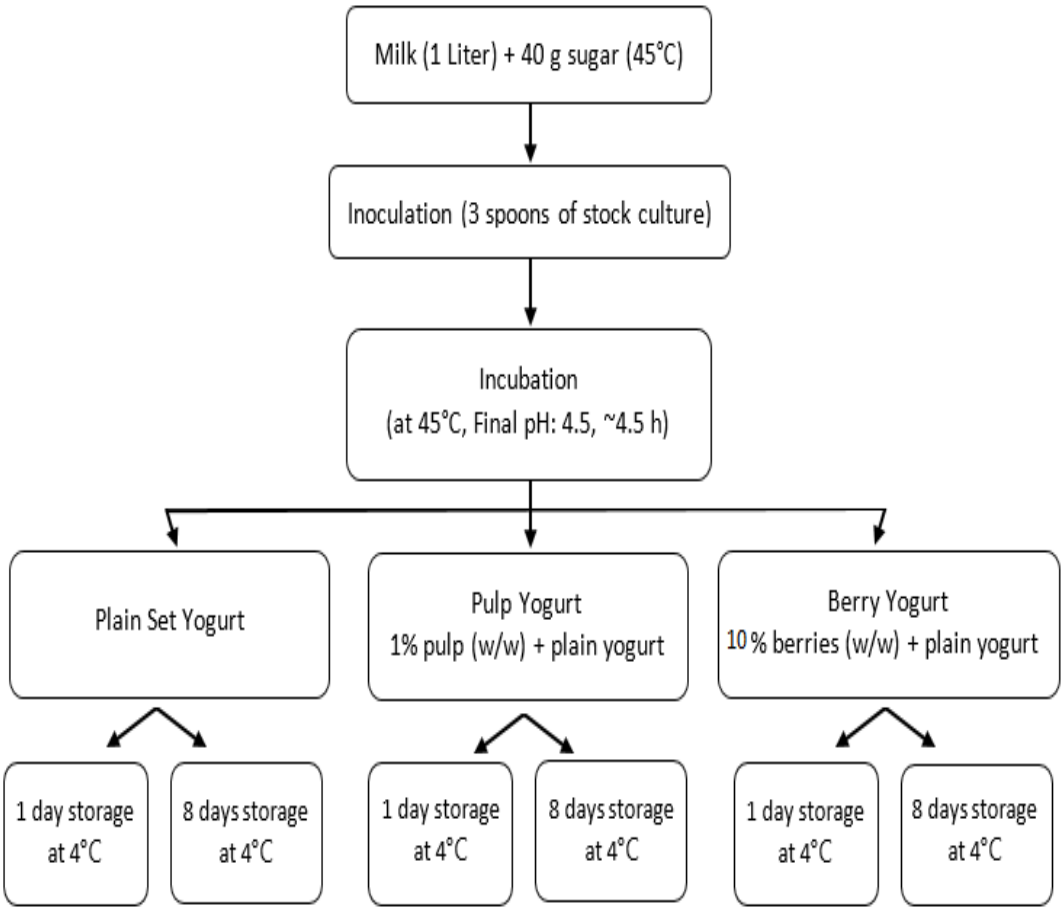

Figure 3.1: Flow diagram of yogurt production in which pulp/berries were added after fermentation

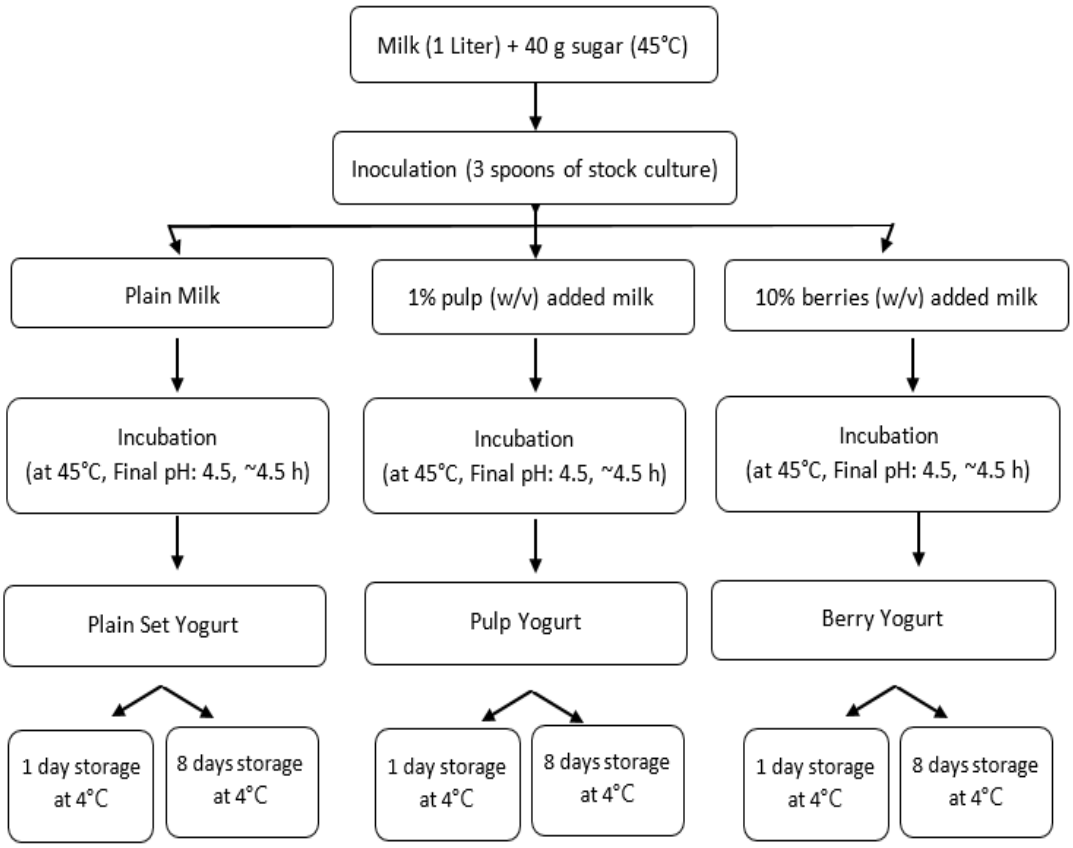

Figure 3.2: Flow diagram of yogurt production in which pulp/berries were added before fermentation

3.3 In-vitro Gastrointestinal Digestion

Saliva, gastric juice, duodenal juice and bile juice were prepared according to Table 3.3-1, and based on Rinaldi, Gauthier, Britten, & Turgeon (2014), Van Hecke et al. (2014) and Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips (2005). The organic and inorganic solutions were completed to 500 ml with distilled water. After mixing of the organic and inorganic solutions, some further constituents were added and dissolved. The pH of the juices were checked and adjusted to appropriate intervals which were indicted in Table 3.1. For the determination of the free soluble polyphenols in yogurt samples mixed with berries and pulp, which might be potentially available for uptake under GI digestion conditions, *in vitro* digestion was carried out based on Rinaldi, Gauthier, Britten, & Turgeon (2014), Van Hecke et al. (2014) and Versantvoort, Oomen, Van de Kamp, Rompelberg, & Sips (2005). The release of phytochemicals from the yogurt matrices was analyzed at different stages of digestion.

Table 3.1: Constituents and concentrations of the synthetic juices of *in vitro* digestion

	Saliva	Gastric Juice	Duodenal Juice	Bile Juice
Inorganic Solution	10 ml KCl 89.6 g/L	15.7 ml NaCl 175.3 g/L	40 ml NaCl 175.3 g/L	30 ml NaCl 175.3 g/L
	10 ml KSCN 20 g/L	3.0 ml NaH_2PO_4 88.8 g/L	40 ml $NaHCO_3$ 84.7 g/L	68.3 ml $NaHCO_3$ 84.7 g/L
	10 ml NaH_2PO_4 88.8 g/L	9.2 ml KCl 89.6 g/L	10 ml KH_2PO_4 8 g/L	4.2 ml KCl 89.6 ml
	10 ml $NaSO_4$ 57 g/L	18 ml $CaCl_2 \cdot 2H_2O$ 22.2 g/L	6.3 ml KCl 89.6 g/L	150 µl HCl 37% g/g
	1.7 ml NaCl 175.3 g/L	10 ml NH_4Cl 30.6 g/L	10 ml $MgCl_2$ 5 g/L	
	20 ml $NaHCO_3$ 84.7 g/L	6.5 ml HCl 37% g/g	180 µl HCl 37% g/g	
Organic Solution	8 ml urea	10 ml glucose 65 g/L	4 ml urea 25 g/L	10 ml urea
		10 ml glucoronic acid 2 g/L		
		3.4 ml urea 25 g/L		
		10 ml glucose amine hydrochloride 33 g/L		
Organic+ Inorganic Solution	290 mg α-amylase	1 g BSA	9 ml $CaCl_2 \cdot 2H_2O$ 22.2 g/L	10 ml $CaCl_2 \cdot 2H_2O$ 22.2 g/L
	15 mg uric acid	2.5 g pepsin	1g BSA	1.8 g BSA
	25 mg mucin	3 g mucin	9 g pancreatin	30 g bile
			1.5 g lipase	
pH	6.8±0.2	1.30±0.02	8.1±0.2	8.2±0.2

An illustration of the different sampling steps is shown in Table 3.2. Briefly, the experiment was started by the addition of 9 g of sample in a 100 ml schott flask maintained at 37 °C in a water bath. Then 6 ml of saliva were added. The mixtures were agitated by gentle movements in the shaking water bath for 2 min. For the gastric digestion phase, 6 ml of gastric fluid was added to the flask and the mixture was agitated. After 30 min, 6 ml of gastric fluid was added one more time and the mixture was agitated during an additional 30 min. To simulate intestinal digestion, segments of dialysis bags (Sigma Aldrich, Molecular weight cut-off 12400 Da) were cut to a specific length (15.5 cm), rinsed with tap water and then one end of each strip was sealed with clips. Bags were filled with bubble free 5.5 ml NaCl (0.9%) and 5.5 mL $NaHCO_3$ (0.5 M), sealed with clips, and completely immersed into the gastric digesta immediately after gastric digestion. The samples were then incubated in the shaking water bath for 45 min at 37°C. Subsequently, 12 ml of duodenal juice and 6 ml of bile juice were added to the digesta, which was further incubated in shaking water bath for an additional 2 h at 37°C. Aliquots of each phase were diluted to same volume (36 ml) by adding distilled water and stored at -20 °C until further analysis. The dialysis bags were taken out and outer side of the bags were rinsed with water, carefully dried using a paper cloth. The content of the bags were then transferred to a 15 ml screw cap plastic tube, weighed and diluted to a final volume of 14 ml with 0.9% NaCl and stored at -20 °C until further analysis.

Table 3.2: Sampling steps of *in vitro* gastrointestinal digestion

Addition Time of Digestion Fluids \ Sampling Time	Saliva (S) sample - 0h 2min	After Gastric Digestion (AGD) Sample - 1h 2min	Before Intestinal Digestion (BID-IN and BID-OUT) Samples - 1h 47min	Post Gastrointestinal Digestion (PG-IN and PG-OUT) Samples - 3h 47min
Saliva 0h 0min				
Gastric Juice 0h 2min	-			
Duodenal Juice + Bile Juice 1h 47min	-	-		
Duodenal Juice + Bile Juice 1h 47min	-	-	-	

3.4 Chemical Analysis

Various analyses were done to the undigested and digested yogurt samples. An overview of the chemical analyses is shown in Table 3.3.

Table 3.3: A summary of overall analyses[1]

Sample \ Analysis			Digestion	Dry Matter Content	Total Phenolic Content	DPPH	CUPRAC	Total Anthocyanin Content	HPLC	Degree of Hydrolysis	SDS Page
Juice					x	x	x	x	x		
Pulp				x	x	x	x	x	x		
Berries				x	x	x	x	x	x		
Berries and pulp added after fermentation (1st and 2nd batch)	1st day	PY	x	x	x	x	x	x	x		
		Y+P	x	x	x	x	x	x	x		
		Y+B	x	x	x	x	x	x	x		
	8th day	PY	x	x	x	x	x	x	x		
		Y+P	x	x	x	x	x	x	x		
		Y+B	x	x	x	x	x	x	x		
Berries and pulp added before fermentation (1st and 2nd batch)	1st day	PY	x	x	x	x	x	x	x	x	x
		Y+P	x	x	x	x	x	x	x	x	x
		Y+B	x	x	x	x	x	x	x	x	x
	8th day	PY	x	x	x	x	x	x	x		
		Y+P	x	x	x	x	x	x	x		
		Y+B	x	x	x	x	x	x	x		

[1]PY: Plain yogurt, Y+P: Pulp added yogurt, Y+B: Berries added yogurt

3.4.1 List of chemicals

For dry matter content assay;

95 % ethanol

For extraction;

Pure methanol

For total phenolic content assay;

0.2 N Folin-Ciocalteau reactive

20% Na_2CO_3 solution

Gallic acid standard

For DPPH assay;

0.1 mM DPPH in methanol

Trolox standard

For CUPRAC assay;

Copper (II) chloride solution (10^{-2} M)

Amonnium acetate (NH_4Ac) buffer (pH 7)

Neocuproine (Nc) Solution ($7.5x10^{-3}$ M)

Trolox standard

For total monomeric anthocyanins assay;

pH 1.0 buffer (0.025 M KCl)

pH 4.5 buffer (0.4 M $CH_3CO_2Na \cdot 3H_2O$)

For degree of hydrolysation assay;

0.21 M Sodium phosphate buffer (pH 8.0)

5% TNBS stock solution

1% SDS

Leucine standard

For SDS-PAGE assay;

Demineralized water

Biosafe Coomassie blue G250

Non-reducing sample buffer: XT (4x, Biorad)

Reducing sample buffer: 990 µl XT (4x, Biorad) + 10 µl XT red (20x) [Should be prepared before use, cannot be stored.]

Molecular weight markers comprised the following mix of proteins: aprotinin (6500 Da), lysozyme (14 400 Da), trypsin inhibitor (21 500 Da), carbonic anhydrase (31 000 Da), ovalbumin (45 000 Da), bovine serum albumin (66 200 Da), phosphorylase b (97 400 Da), β-galactosidase (116 250 Da) and myosin (200 000 Da).

3.4.2 List of instruments and analysis materials

Oven set at 105 °C

Analytical balance

Desiccator

Ultra-turrax

Centrifuge

Filter paper (WWR 413, 5-13 µm)

Spectrophotometer

pH meter

Electrophoresis tank

Precast gel cassette

Power supply

Scanner

HPLC

3.4.3 Dry matter content

The dry matter content of samples was determined by ISO 1442-1973 method. Therefore, 15 grams of sea sand was added to aluminum foil recipients and recipients were placed in a preheated oven at 105 °C for one hour, after which they were cooled down in a desiccator for at least 30 minutes and weighed (=M_0). Then, 5 grams of berries, pulp or undigested yogurt sample was added to the aluminum recipients and weighed again (=M_1). Samples were then mixed with 5 ml of 95% ethanol and placed in the oven for 3,5 hours after which they were cooled down in a desiccator for about 45 minutes to one hour and weighed (=M_2). Dry matter content was calculated as shown in equation 3.1:

$$\%DM = \frac{(M_2 - M_0)}{(M_1 - M_0)} x100 \quad \textbf{(3.1)}$$

Where;

% DM = gram dry matter per 100 g sample

M_0= mass of the preheated sea sand (g)

M_1= mass of the sea sand and sample before drying (g)

M_2= mass of the sea sand and sample after drying (g)

All samples were performed in double.

3.4.4 Extraction of the phenolic compounds from the samples

To 5 gram of minced berries, pulp, yogurt or 5 ml of digesta (except BID-IN and PG-IN samples) 15 ml methanol was added in a plastic tube with screw cap and homogenized by using an ultra-turrax for 30 seconds at 4000 rpm. Homogenized samples were placed in ice for 15 minutes. Then the mixture was centrifuged for 10 minutes at 4000 rpm at 4 °C and filtered through a filter paper. Similarly, the pellet was re-extracted with 10 ml methanol:water (80:20 v/v). Homogenization was performed for 20 seconds at 4000 rpm and centrifugation was made as the former step. The extracts were combined and dilutions were made with methanol:water (90:10 v/v) (Olsen, Aaby, & Borge, 2009).

3.4.5 Total phenolic content

The Folin-Ciocalteau reagent is a solution of complex polymeric ions formed from phosphomolybdic and phosphotungstic heteropoly acids. It oxidizes phenolates, reducing the heteropoly acids to a blue Mo-W complex. The phenolates are only present in alkaline solution but the reagent and products are alkali unstable. Hence a moderate alkalinity and a high reagent concentration are used in the procedure.

Total phenolic content was determined by the Folin-Ciocalteau procedure using gallic acid as standard (Singleton, Orthofer, & Lamuela-Raventos, 1999).

Briefly, 1 ml of different concentrations of gallic acid (ranging between 0 and 50 mg/L) or 1 ml of sample (methanolic extracts of berries, pulp, undigested yogurts and digesta as well as BID-IN and PG-IN samples) were diluted with 1 ml of deionized water. Then, 0.5 ml of 0.2 N Folin-Ciocalteau reagent was added, and the contents were vortexed. After 6 min incubation, 1.5 ml of Na_2CO_3 (20%) solution and 1 ml of deionized water were added, and, after vortexing, the mixture was incubated for 2 h at 22 °C in the dark. The absorbance was measure at 760 nm at the end of the incubation period. The concentration of total phenolic compounds was calculated as mg of gallic acid equivalents (GAE)/ 100 g of dry weight samples, by using a standard calibration curve. The calibration curve is shown in Appendix B, Figure B.1.

3.4.6 DPPH (1,1-diphenyl-2-picrylhydrazyl) method

Total antioxidant capacity was estimated by DPPH (1,1-diphenyl-2-picrylhydrazil) radical scavenging method (Kumaran & Karunakaran, 2006). The method is based on the reduction of DPPH free-radical into 1,1-diphenyl-2-picryl hydrazine in the presence of a hydrogen-donating antioxidant. The ability to scavenge the stable DPPH radical is measured by a decrease in absorbance.

To evaluate the DPPH radical scavenging activity, a 0.1 mM solution of DPPH in methanol was prepared. Then, 0.1 ml of standard Trolox with concentrations of 0 - 100 mg/L or 0.1 ml sample (methanolic extracts of berries, pulp, undigested yogurts and digesta as well as BID-IN and PG-IN samples) was mixed with 2 ml of 0.1 mM DPPH (in methanol) and vortexed. After 30 min of incubation in dark at 22°C, absorbance at 517 nm was measured against blank which contained methanol instead of sample extract. The results were expressed as mM Trolox equivalent (TEAC)/100g of dry weight, based on the obtained standard curve. The calibration curve is shown in Appendix B, Figure B.2.

3.4.7 Cupric ion reducing antioxidant capability (CUPRAC)

In this method, the copper (II) chloride (or cupric) ion reducing ability of polyphenols is measured. The method comprises mixing of the antioxidant solution (directly or after acid hydrolysis) with a copper (II) chloride solution, a neocuproine alcoholic solution, and an ammonium acetate aqueous buffer at pH 7 and subsequent measurement of the developed absorbance at 450 nm after 30 min (Apak, Güçlü, Özyürek, & Karademir, 2004).

Briefly, 100 µl of sample extract or standard, 1 ml of 10^{-2} M Cu (II), 1 ml of Nc solution, 1 ml of NH_4Ac buffer and 1 ml of distilled water were respectively pipetted to a test tube and vortexed. Absorbance was measured at 450 nm against reagent blank after 30 min incubation. The calibration curve is shown in Appendix B, Figure B.3.

3.4.8 Total monomeric anthocyanin content

The total monomeric anthocyanin content was determined using pH differential method (Lee, Durst, & Wrolstad, 2005). Monomeric anthocyanin pigments reversibly change color with a change in pH; the colored oxonium form exists at pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance of the pigments at λ_{max} is proportional to the pigment concentration. Degraded anthocyanins in the polymeric form are resistant to color change regardless of pH and are not included in the measurements because they absorb at pH 4.5 as well as pH 1.0.

Shortly, methanolic extracts of berries, pulp, undigested yogurts and digesta as well as BID-IN and PG-IN samples were diluted 1:10 with 0.025 M KCl-solution (pH=1) and with 0.4 M acetate buffer (pH=4.5). Each sample was vortexed and left in the dark for 15 min at 22°C. After the incubation, samples were centrifuged at 3000 rpm and filtered through a cartridge filter to remove turbidity. The absorbance of the samples

at λ_{max} (536 nm for black chokeberry sample) and 700nm were measured. The reason for measuring the absorbance at 700 nm was to correct for haze. The anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, was calculated as shown in equation 3.2:

$$\text{Total Monomeric Anthocyanin Concentration } \left(\text{cyanidin} - 3 - \text{glucoside equivalents, mg / L}\right) = \frac{AxMWxDFx10^3}{\varepsilon xl} \quad \textbf{(3.2)}$$

Where;

$A = (A_{536nm}-A_{700nm})_{pH1}-(A_{536nm}-A_{700nm})_{pH4.5}$

MW (molecular weight)= 449.2 g/mol for cyanidin-3-glucoside

D= Dilution factor

l= path length in cm

ε= 26900 molar extinction coefficient in $Lxmol^{-1}xcm^{-1}$ for cyanidin-3-glucoside

10^3= Factor for conversion from g to mg.

3.4.9 HPLC analysis

For the identification and quantification of specific black chokeberry anthocyanins by HPLC, extracts of juice, pulp and fruits as well as undigested yogurts were analysed using a W600 Waters HPLC system coupled to a Waters 996 PDA detector. Compounds were separated using a Supelcosil LC-18 column (250 × 4.6 mm, 5 µ) heated to 40 °C and applying a gradient from 95% to 25% MQ water and a 5–75% acetonitrile gradient, both in 0.1% trifluoroacetic acid (1 mL/min flow rate) across a period of 50 min (Toydemir, et al., 2013). Anthocyanins were detected at 520 nm (Chandra, Rana, & Li, 2001). Peak identification was done by comparing absorbance spectra and retention times of eluting peaks with available standards or with data taken from the literature (Chandra, Rana, & Li, 2001). All analyses were performed in duplicate and the obtained data were expressed as mg cya-3-glu/100 g dry weight.

3.4.10 Degree of hydrolysis

The degree of hydrolysis represents the percentage of peptide bonds hydrolyzed during hydrolysis of protein. To evaluate the amount of amino groups before and after protein hydrolysis, a spectrophotometric reaction technique with TNBS (Trinitrobenzenesulfonic acid hydrate) is used. TNBS reacts with amino groups in their unprotonated state, producing a yellow product which gives absorbance at 340 nm.

A 0.21 M Sodium phosphate as buffer was prepared and pH was set at 8.0. TNBS solution and SDS were prepared with the concentrations of 0.05% and 1%, respectively. 20x, 10x, 4x, 2x times diluted ±3mM leucine in 1% SDS was used to prepare a standard curve. Samples were prepared by dilution in 1% SDS to a concentration of 0.5 mg/ml in duplicate and 150 µl of leucine standard or undigested yogurt or S, AGD, BID and OUT samples was pipetted in a screw cap glass tube, followed by 450 µl of the sodium phosphate buffer and 450 µl 0.05% TNBS. Then, the tubes were sealed with caps and they were incubated for 60 minutes in a 50°C stove. After the incubation, 900 µl 0.1 N HCl was added to tubes to stop the reaction. All tubes were centrifuged at 2000 rpm at 4°C for 5 minutes and the absorbance of the supernatants was measured at 340 nm. The degree of hydrolysis was calculated using hydrolysis equivalents (h), the number of peptide bonds cleaved during hydrolysis, expressed as meq/g protein (Adler-Nissen, 1979).

3.4.11 SDS-PAGE analysis

SDS-PAGE analysis was performed based on BioRad (2015). Protein samples are prepared using heat and SDS to denature the proteins. SDS minimizes charge variability among proteins, giving them the same charge to mass ratio and forcing them into rod-like shapes. This effectively eliminates the effects of protein conformation and native charge density on the electrophoretic migration distance.
Under reducing conditions the denaturing sample buffer eliminate protein secondary structure by reducing disulfide bonds.

Sample Preparation:

In a 2 ml Eppendorf tube, 30 µl pH<5 sample + 70 µl non-reducing sample buffer + 100 µl demineralized water or 50 µl pH>5 sample + 50 µl non-reducing sample buffer + 100 µl demineralized water were pipetted. Then, the samples were shaked gently at ambient temperature for 60 minutes and after 60 minute duration they were left to a boiling water bath (95-100°C) for maximum 5 minutes. All samples were immediately cooled at the end of the heating process by putting the Eppendorf tubes in the freezer.

Gel loading and electrophoresis:

The precast gel cassette was removed from the BioRad package, rinsed with deionized water, the tape in the bottom of the cassette was peeled off and the comb was pulled out of the cassette with care. Then the gels were placed into the slots of the Criterion Cell.

The upper and lower buffer chamber were filled with 1X running buffer. 20 µl sample was loaded into the wells and electrophoresis was performed for 1 hour in the following conditions: 160V, 300A, 300W. After 1 hour duration the gels were gently removed from the cassettes.

Staining and de-staining:

The gels were first washed with demineralized water and they were put in fresh demineralized water twice for 10 minutes by gently shaking. Then the gels were stained during maximum 60 minutes in staining solution (Biosafe Coomassie blue G250) with gentle mixing. Afterwards, the staining solution was removed and demineralized water was added. The demineralized water refreshed after 10-30-15 minutes washing and finally the gels were left for de-staining overnight on a shaker.

Gel conservation and scanning:

Stained gels were rapidly scanned to prevent drying of the gels after covering with a cellophane membrane.

3.5 Statistical Analysis

Data were collected from two independent extracts of berries and pulp as well as one extract of each digested and undigested sample from two independent yogurt batches and reported as mean ± SD. For multiple comparisons data were subjected to statistical analysis using SPSS for the analysis of variance (ANOVA). Tukey's test was used to analyze differences between samples ($p<0.05$). 2-way interaction terms (type of fruit added x storage days and type of fermentation x storage days) and all 3-way interaction terms of the samples were non-significant and were thus removed from the model. Statistical analysis results are given in Appendix F.

4 RESULTS AND DISCUSSION

4.1 Characterization of Black Chokeberry Berries, Pulp and Juice

The dry matter content of pulp was 25.5 ± 0.4% while it was 17.1 ± 0.7% for fresh berries. The total phenolic and total monomeric anthocyanin contents as well as DPPH radical scavenging activities of berries, pulp and juice are presented in Table 4.1.

Table 4.1: Total phenolic, total monomeric anthocyanin and antioxidant properties of berries, pulp and juice[1]

Sample	Total Phenolics (mg GAE[2])	DPPH (mM TEAC[2])	CUPRAC (mM TEAC[2])	Total anthocyanins (mg cya-3-glu[2])
Juice (100 ml)	122±5.85	0.79±0.01	1.78±0.37	38.4±1.18
Berries (100g DM)	3242±37.4	22.2±0.49	37.8±2.42	1364±3.46
Pulp (100g DM)	1927±235	18.7±3.64	42.2±4.23	7564±139

[1]Data represent average values ± standard deviation of duplicates. [2]GAE: Gallic acid equivalent, TEAC: Trolox equivalent antioxidant capacity, cya-3-glu: Cyanidin-3-glucoside; n = 2

According to the obtained data, the highest total phenolic content and antioxidant capacity, as measured by the DDPH and CUPRAC methods, were observed in fresh berries. In contrast, the anthocyanin content of the chokeberry pulp was higher than for the other samples. However, although the anthocyanin content of pulp was higher than the berries, no real difference was observed between radical scavenging activity of pulp and berries. The loss of antioxidant activity of phenolic compounds in fruits during processing could be the reason of the lower antioxidant capacity in pulp. On the other hand, all measured values were greatly lower in chokeberry juice than in berries and pulp. In this study, the findings associated with berries, pulp and chokeberry juices were comparable with the existing literature (Kapci, et al., 2013) (Najda & Łabuda, 2013) (Mayer-Miebach, Adamiuk, & Behsnilian, 2012) (Wangensteena, et al., 2014) (Benvenuti, Pellati, Melegari, & Bertelli, 2004) (Ochmian, Grajkowski, & Smolik, 2012) (Rop, et al., 2010) (Zheng & Wang, 2003). However, we observed some differences which might be a result of several factors including variety/cultivar, growing conditions, climatic conditions, and ripening stage (Šnebergrová, et al., 2014).

By the HPLC analysis, four anthocyanins were detected in chokeberry juice: Cya-3-galactoside, Cya-3-glucoside, Cya-3-arabinoside and Cya-3-xyloside (chromatograms were given in Appendix C, Figure C1). As shown in Table 4.2, Cya-3-galactoside was the main anthocyanin in chokeberry juice, berries and pulp. It composed 70%, 68% and 66% of total chokeberry anthocyanins, respectively. A similar anthocyanin profile was reported for chokeberry juice (Wiczkowski, Romaszko, & Piskula, 2010) and chokeberry berries (Gasiorowski, et al., 1997). On the other hand, Kapci et al. (2013) reported slightly higher values of chokeberry anthocyanins in pomace than ours, except for cya-3-arabinoside.

Table 4.2: Anthocyanin profile of chokeberry juice, berries and pulp[1]

Sample	Cya-3-galactoside	Cya-3-glucoside	Cya-3-arabinoside	Cya-3-xyloside	TOTAL
Juice (mg/100 ml)	22.5±0.56	0.73±0.04	7.94±0.06	0.85±0.07	32.0±0.65
Berries (mg/100g DM)	1028±19.8	28.3±1.79	405±4.84	48.8±3.21	1510±23.2
Pulp (mg/100g DM)	1058±14.0	36.2±1.08	456±5.81	49.3±1.33	1600±7.92

[1]Data represent average values ± standard deviation of duplicates.

4.2 Dry Mater Content of Undigested Yogurts

The dry matter content of the undigested yogurts is shown in Table 4.3. The dry matter content of all batches ranged between 14.8 and 15.3%. All of the measured dry matter contents were compatible with the dry matter content of commercial yogurts (13-17%) as reported by Patel (2011).

Table 4.3: Dry matter content of undigested yogurt samples[1,2]

Sample Name	Mean Value ± Standard Deviation	Sample Name	Mean Value ± Standard Deviation
AF1-1 plain	15.2 ±0.01	**BF1-1 plain**	15.2 ±0.08
AF1-1 pulp	15.2 ±0.16	**BF1-1 pulp**	14.9 ±0.12
AF1-1 berry	15.1 ±0.00	**BF1-1 berry**	15.2 ±0.05
AF1-8 plain	15.2 ±0.05	**BF1-8 plain**	15.1 ±0.15
AF1-8 pulp	15.3 ±0.13	**BF1-8 pulp**	14.9 ±0.11
AF1-8 berry	14.9 ±0.13	**BF1-8 berry**	15.2 ±0.15
AF2-1 plain	15.2 ±0.17	**BF2-1 plain**	15.2 ±0.11
AF2-1 pulp	14.9 ±0.12	**BF2-1 pulp**	15.2 ±0.02
AF2-1 berry	15.2 ±0.03	**BF2-1 berry**	15.0 ±0.14
AF2-8 plain	15.1 ±0.12	**BF2-8 plain**	15.2 ±0.09
AF2-8 pulp	15.2 ±0.01	**BF2-8 pulp**	14.8 ±0.29
AF2-8 berry	15.0 ±0.04	**BF2-8 berry**	15.0 ±0.13

[1]Data represent average values ± standard deviation of duplicates. [2]Different letters in the column represent statistically significant differences between the samples within each yogurt set ($p < 0.05$).

4.3 Total Phenolics, Total Monomeric Anthocyanins and Antioxidant Capacity of Undigested Yogurt Samples

On the plain yogurt, no differences in any of the parameters were observed, confirming that the four different batches were prepared and stored in a reproducible way (Table 4.4, Table 4.5, Table 4.7, and Table 4.8). In all batches, the highest total phenolics, total anthocyanins and antioxidant capacity were observed in berry added yogurts. On the other hand, the higher the anthocyanin content, the higher the total phenolic content and antioxidant capacity.

The total phenolic content of undigested yogurt samples is shown in Table 4.4 and Figure 4.1. All data were given in terms of mg GAE/100 g on the dry matter basis. For the total phenolic content, the 2-way interaction terms (type of fruit added x type of fermentation, and type of fruit added x storage days) were not significant ($p > 0.05$) and were therefore removed from the model.

Table 4.4: Total phenolic content of undigested yogurts (mg GAE/100g DM)[1,2]

Name of sample	Type of Fruit Added	1st Batch	2nd Batch	Mean Value ± Standard Deviation
AF 1st day	No	32.3	24.0	28.2±5.85[c]
AF 1st day	Pulp	63.1	67.4	65.2±3.05[b]
AF 1st day	Berries	195	196	195±0.39[a]
AF 8th day	No	34.6	29.5	32.1 ±3.60[c]
AF 8th day	Pulp	77.5	71.1	74.3 ±4.51[b]
AF 8th day	Berries	207	207	207 ±0.34[a]
BF 1st day	No	24.4	23.2	23.8 ±0.84[c]
BF 1st day	Pulp	64.5	61.3	62.9 ±2.24[b]
BF 1st day	Berries	186	190	188 ±2.60[a]
BF 8th day	No	23.6	22.5	23.1 ±0.78[c]
BF 8th day	Pulp	64.9	66.8	65.9 ±1.34[b]
BF 8th day	Berries	191	189	190 ±1.42[a]

[1]Data were given as the mean values ± standard deviations of duplicates. AF: Berries or pulp added after fermentation BF: Berries or pulp added before fermentation. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts within each yogurt set ($p < 0.05$).

A significant effect on the total phenolic content was observed depending on the type of fruits added ($p < 0.001$) in the following order: no<pulp<berries.

The 2-way interaction term (type of fermentation x storage days) is significantly different ($p=0.018$) for the total phenolic content, as well as both main factors ($p<0.001$ and $p=0.001$ for fermentation and storage days respectively). Independent if the type of fruit was added before or after fermentation, no significant difference between the

storage days was observed on the total phenolic content ($p>0.05$). Also for the 2 storage days separately, no difference was observed on the total phenolic content when the fruits was added before or after fermentation ($p>0.05$).

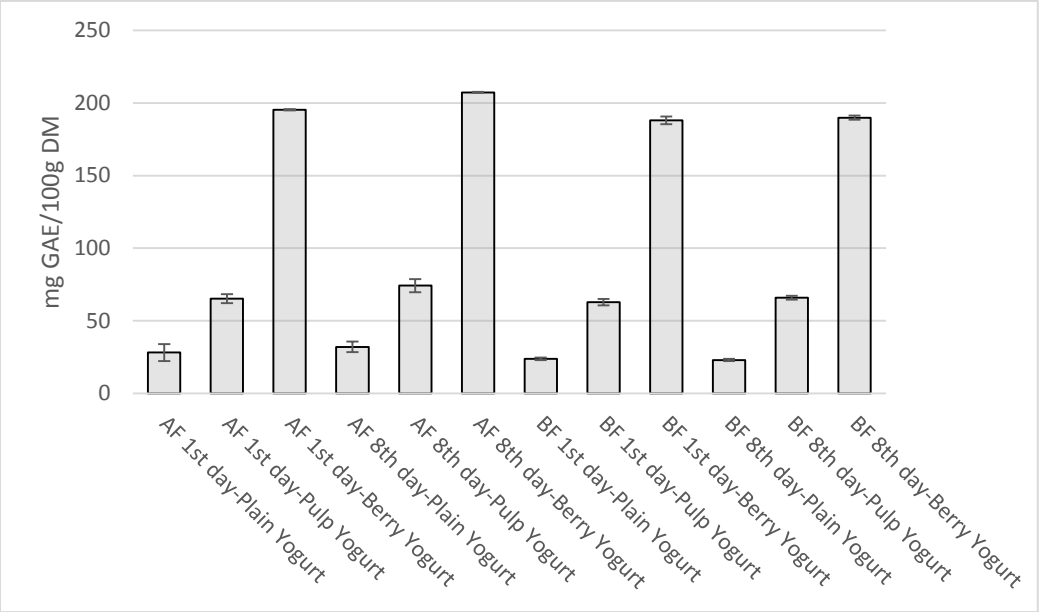

Figure 4.1: Total phenolic content of undigested yogurt samples (n=2)

For the total monomeric anthocyanin content, one can see a similar trend as observed for total phenolic content (Table 4.5 and Figure 4.2). All data are expressed as mg cyanidin-3-glucoside equivalent/100 g on the dry matter basis.

Table 4.5: Total monomeric anthocyanin content of undigested yogurt samples (mg cya-3-glu equivalent/100g DM)[1,2]

Name of sample	Type of Fruit Added	1st Batch	2nd Batch	Mean Value ± Standard Deviation
AF 1st day	No	ND	ND	ND
AF 1st day	Pulp	18.9	26.3	22.6 ±5.22[b]
AF 1st day	Berries	103	124	113 ±14.3[a]
AF 8th day	No	ND	ND	ND
AF 8th day	Pulp	20.7	24.2	22.5 ±2.43[b]
AF 8th day	Berries	110	116	113 ±4.40[a]
BF 1st day	No	ND	ND	ND
BF 1st day	Pulp	17.7	17.3	17.5 ±0.25[b]
BF 1st day	Berries	80.5	80.4	80.4 ±0.03[a]
BF 8th day	No	ND	0.27	0.14 ±0.19[c]
BF 8th day	Pulp	14.6	11.3	13.0 ±2.32[b]
BF 8th day	Berries	76.9	70.4	73.7 ±4.59[a]

[1]Data were given as the mean values ± standard deviations of duplicates. AF: Berries or pulp added after fermentation BF: Berries or pulp added before fermentation. ND= Non Detected. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts within each yogurt set ($p< 0.05$).

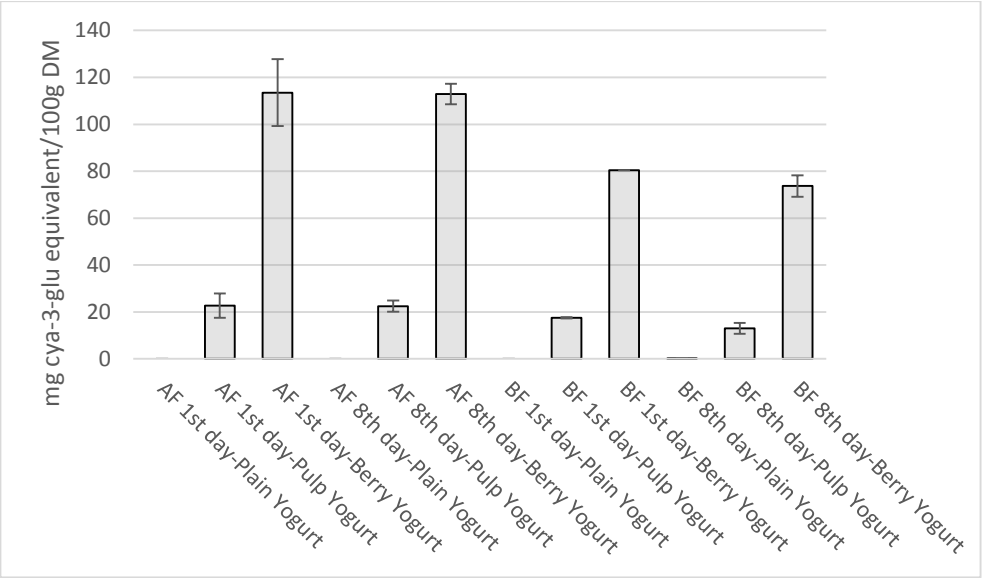

Figure 4.2: Total monomeric anthocyanin content of undigested yogurt samples (n=2)

The 2-way interaction (type of fruit added x type of fermentation) is significant ($p<0.001$), as well as both main factors fruit and fermentation ($p<0.001$). No effect of storage days was observed on the anthocyanin content ($p>0.05$). Anthocyanin content of the yogurt samples were different independent of the fermentation in the order of no<pulp<berries.

The percentage recoveries of anthocyanins are presented in Table 4.6. Both for the berries or the pulp added to the yogurt, a significant difference in anthocyanin content was observed when the fruits were added before or after fermentation ($p<0.001$), also observed in the obtained recoveries.

Table 4.6: Percentage anthocyanin recoveries from undigested yogurt samples

Recoveries, %	AF 1st day	AF 8th day	BF 1st day	BF 8th day
Pulp yogurt	20.1±4.26	20.1±2.08	15.5±0.00	11.3±2.08
Berry yogurt	73.8±9.62	72.6±3.41	52.1±0.51	47.7±3.42

In both cases the amount of anthocyanins measured was lower when the berries or pulp were added before fermentation, compared to adding after fermentation. The lower recovery of anthocyanin added to yogurt before fermentation compared to after fermentation could possibly be linked to the degradation of anthocyanins by lactic acid bacteria. Sasaki & Ohba (2004) observed that, anthocyanin content of sweet potato lactic acid bacteria drink decreased with different levels depending on the lactic acid bacteria species, including the yogurt starters. This decrease was approximately 15% for *Lactobacillus bulgaricus* and 20% for *Streptococcus thermophilus*. Regarding this, 30% less anthocyanin content of the samples in which fruit added before fermentation

compared to added after fermentation is logical if the simultaneous action of the *L. bulgaricus* and *S. thermophilus* is considered. In addition, Vivas, Lonvaud-Funel, & Glories (1997) stated that gallic acid and anthocyanins were metabolized during fermentation, specially by growing cells. On the other hand, even when the fruits were added to the yogurt after fermentation, only 70% of the anthocyanins from the berries were measured, and even not 20% of the pulp anthocyanins. Therefore, we can conclude that not only lactic acid bacteria were involved and the lower amount of anthocyanin found in the final yogurt can be attributed to degradation of the anthocyanins by the mechanisms different from microbial degradation (e.g. temperature or pH change during fermentation), or interaction with other yoghurt components. Kırca, Özkan, & Cemeroğlu (2007) showed that degradation of monomeric anthocyanins increased by increasing solid content during heating. Also, several studies has shown the ability of anthocyanins to complex with proteins (Wiese, Gärtner, Rawel, Winterhalter, & Kulling, 2009) (Mazzaracchio, Pifferi, Kindt, Munyaneza, & Barbiroli, 2004) and lipids (Sengul, Surek, & Nilufer-Erdil, 2014), which could partly explain the lower recovery. However, even though it was aimed to add the same amount of anthocyanins to yogurt and milk, the reason for the 3-4 times lower recovery in anthocyanins observed in the yogurt with pulp compared to berries, is still not clear. Considering the anthocyanin content of fresh pulp was higher than the fresh berries, remarkable lower recoveries were observed in pulp added yogurts compared to berries added yogurts. This notable difference could be attributed to interaction between anthocyanins and food matrix components. Although there is still little knowledge about the binding mechanisms of different types of polyphenols to cellulose and/or other cell wall components, Phan et al. (2015) and Jakobek (2015) indicate that the adsorption of polyphenols comprises the establishment of a number of low energy non-covalent interactions derived from a combination of hydrogen bonds and hydrophobic interactions between polyphenols and proteins or polyphenols and cell wall carbohydrates. In this sense, the probable transformation of these weak linkages into the stronger covalent bonds by several biochemical reactions during the fermentation could be the reason of the lower anthocyanin content observed in pulp added yogurts.

The results of DPPH radical scavenging activity in mg TEAC/100 g DM are shown in Table 4.7 and Figure 4.3. No significant effect of the 2-way interaction terms (type of fruit x storage days; type of fermentation x storage days) was observed ($p>0.05$ for each), as well as no influence of the storage days ($p>0.05$). However, the 2-way interaction of type of fruit x type of fermentation was highly significant on the

antioxidant capacity ($p<0.001$), as well as the main effects, type of fermentation and fruit ($p<0.001$). A slightly higher antioxidant capacity in fruits added after fermentation samples was observed comparing to fruit added before fermentation samples. In addition to that, berry added yogurts have the highest antioxidant capacity while as expected plain yogurts have the lowest.

Table 4.7: DPPH radical scavenging activities of undigested yogurt samples (mM TEAC/100g DM)[1,2]

Name of sample	Type of Fruit Added	1st Batch	2nd Batch	Mean Value ± Standard Deviation
AF 1st day	No	0.17	0.15	0.16 ±0.02[c]
AF 1st day	Pulp	0.36	0.39	0.38 ±0.02[b]
AF 1st day	Berries	1.25	1.22	1.24 ±0.02[a]
AF 8th day	No	0.17	0.16	0.17 ±0.01[c]
AF 8th day	Pulp	0.41	0.41	0.41 ±0.00[b]
AF 8th day	Berries	1.21	1.26	1.23 ±0.03[a]
BF 1st day	No	0.17	0.13	0.15 ±0.03[c]
BF 1st day	Pulp	0.39	0.35	0.37 ±0.02[b]
BF 1st day	Berries	1.03	1.06	1.05 ±0.02[a]
BF 8th day	No	0.17	0.14	0.15 ±0.02[c]
BF 8th day	Pulp	0.34	0.31	0.33 ±0.02[b]
BF 8th day	Berries	1.07	1.04	1.05 ±0.02[a]

[1]Data were given as the mean values ± standard deviations of duplicates. AF: Berries or pulp added after fermentation BF: Berries or pulp added before fermentation. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts within each yogurt set ($p< 0.05$).

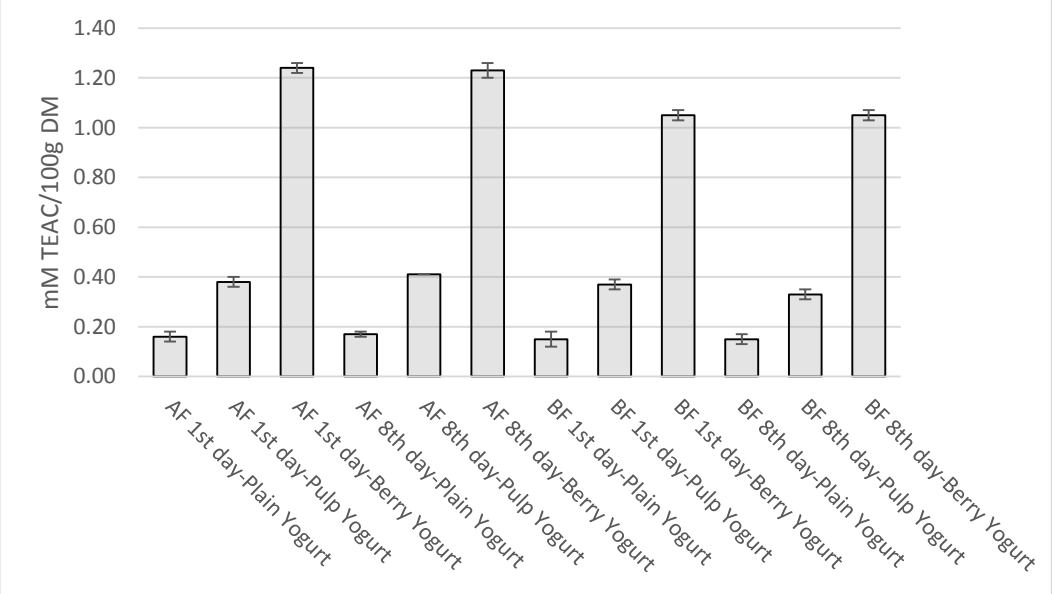

Figure 4.3: DPPH radical scavenging activities of undigested yogurt samples (n=2)

Cupric ion reducing abilities of the undigested yogurt samples are shown in Table 4.8 and Figure 4.4. Same trend was observed between the yogurt varieties as it was observed in DPPH method. According to that, the highest antioxidant activity was observed in berries added yogurts while the lowest one was in the plain yogurts. Similarly, slightly higher antioxidant activities were observed in samples which fruits were added after fermentation.

Table 4.8: Cupric ion reducing antioxidant capacities (CUPRAC) of undigested yogurt samples (mM TEAC/100g DM)[1,2]

Name of sample	Type of Fruit Added	1st Batch	2nd Batch	Mean Value ± Standard Deviation
AF 1st day	No	0.25	0.09	0.17 ±0.11[c]
AF 1st day	Pulp	0.78	0.72	0.75 ±0.05[b]
AF 1st day	Berries	3.24	3.10	3.17 ±0.10[a]
AF 8th day	No	0.20	0.35	0.27 ±0.11[c]
AF 8th day	Pulp	0.95	0.68	0.81 ±0.19[b]
AF 8th day	Berries	2.98	2.88	2.93 ±0.07[a]
BF 1st day	No	0.93	0.98	0.30 ±0.03[c]
BF 1st day	Pulp	1.71	1.47	0.93 ±0.17[b]
BF 1st day	Berries	3.48	3.42	2.79 ±0.04[a]
BF 8th day	No	0.99	1.00	0.34 ±0.01[c]
BF 8th day	Pulp	1.35	1.20	0.60 ±0.11[b]
BF 8th day	Berries	3.12	3.51	2.35 ±0.27[a]

[1]Data were given as the mean values ± standard deviations of duplicates. AF: Berries or pulp added after fermentation BF: Berries or pulp added before fermentation. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts within each yogurt set ($p < 0.05$).

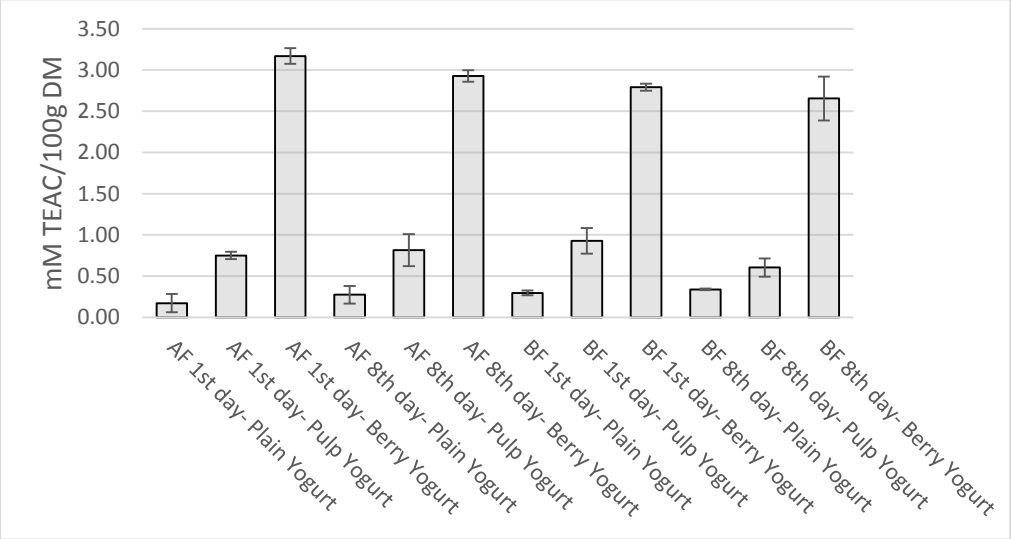

Figure 4.4: Cupric reducing antioxidant capacities (CUPRAC) of undigested yogurt samples (n=2)

4.4 Major Individual Anthocyanin Content of Undigested Yogurts

Major individual anthocyanin content of undigested yogurts were reported in the Table 4.9 and the chromatograms were given in Appendix D, Figure D.1 to D.8. Same anthocyanin profile as chokeberry fruit products obtained when the fruits were added to yogurt. The prevailing anthocyanin was the cyanidin-3-galactoside in chokeberry added yogurts with 65-70% of total anthocyanins. The second most abundant anthocyanin was cyaniding-3-arabinoside, followed by cyaniding-3-xyloside and cyaniding-3-galactoside accounting for approximately 3% and 2% of the total anthocyanins, respectively.

The amount of individual anthocyanins was decreased when the fruits were added before the fermentation. In contrast to that, Sun-Waterhouse, Zhou & Wadhwa (2013) observed an increase in anthocyanin content and a difference in anthocyanin profile when the extracts of blackcurrant polyphenols were added before the fermentation in drinking yogurts. Contrarily, they observed no difference between the purified cyanidin added both before and after the fermentation in drinking yogurts. Chemical and physical effects such a protection by the gel structure of yogurt, binding to yogurt peptides, complexation with proteins and polysaccharides may be the reason of this difference (Sun-Waterhouse, Zhou, & Wadhwa, 2013).

Table 4.9: Major individual anthocyanin content of undigested yogurts[1,2]

Name of sample	Type of Fruit Added	Cya-3-gal	Cya-3-glu	Cya-3-ara	Cya-3-xyl
AF 1st day	No	0.00 ±0.00^{b}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{c}
AF 1st day	Pulp	14.4 ±4.10^{b}	0.37 ±0.39^{b}	6.09 ±1.84^{b}	0.24 ±0.04^{b}
AF 1st day	Berries	85.0 ±10.5^{a}	2.00 ±0.04^{a}	32.9 ±3.95^{a}	3.79 ±0.33^{a}
AF 8th day	No	0.00 ±0.00^{b}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{c}
AF 8th day	Pulp	14.4 ±1.66^{b}	0.22 ±0.00^{b}	5.55 ±0.96^{b}	0.49 ±0.32^{b}
AF 8th day	Berries	82.2 ±1.41^{a}	2.14 ±0.35^{a}	31.4 ±0.08^{a}	3.55 ±0.16^{a}
BF 1st day	No	0.00 ±0.00^{b}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{c}
BF 1st day	Pulp	11.1 ±0.09^{b}	0.33 ±0.05^{b}	4.49 ±0.29^{b}	0.54 ±0.08^{b}
BF 1st day	Berries	51.9 ±2.08^{a}	1.80 ±0.27^{a}	22.3 ±0.22^{a}	2.42 ±0.27^{a}
BF 8th day	No	0.00 ±0.00^{b}	0.00 ±0.00^{c}	0.00 ±0.00^{c}	0.00 ±0.00^{c}
BF 8th day	Pulp	7.39 ±0.92^{b}	0.13 ±0.18^{b}	3.53 ±0.58^{b}	0.23 ±0.33^{b}
BF 8th day	Berries	46.5 ±6.46^{a}	1.80 ±0.28^{a}	20.7 ±2.56^{a}	2.58 ±0.07^{a}

[1]Data were given as the mean values ± standard deviations of duplicates. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts within each yogurt set ($p < 0.05$).

4.5 Total Phenolics, Total Monomeric Anthocyanins and Antioxidant Capacity of Digested Yogurt Samples

The total phenolic content of digested yogurt samples is shown in Appendix E, Table E.1 to E.4 as well as Figure E.1 to E.4. All data are given in terms of mg GAE/100 g on the dry matter basis. The same trend was observed in all type of batches. The highest total phenolic content was measured in berry added yogurt samples following pulp added yogurt and plain yogurt respectively, independent of the gastric stage. Additionally, no difference was observed between BID and OUT samples as well as between the absorbed inner fraction of BID and post gastrointestinal digestion samples.

During the buccal phase of digestion no or slight amount of phenolic compounds were observed in plain yogurts and pulp yogurts. On the other hand, the measured total phenolic compounds in salivary berry samples were much higher (approximately 83-84% of the total phenolic content of undigested yogurts) compared to plain and pulp added yogurts. However, after gastric digestion and intestinal digestion much higher total phenolic content was observed both in plain yogurts and pulp or berry added yogurts, even values that are higher than what was measured in the undigested samples. The Folin-Ciocalteau method strongly relies on the reduction of the Folin-Ciocalteau reagent. As a result of this, any reducing component such as small peptides or reducing sugars formed during fermentation or digestion can interfere in the Folin-Ciocalteau assay. On the other hand, Folin-Ciocalteau assay is also used in the determination proteins (Ikawa, Schaper, Dollard, & Sasner, 2003). From this point of view, it may be assumed that the higher total phenolic content in plain yogurts comes from the other components such as proteins and sugars, while the difference between the total phenolic content of plain yogurt and pulp or berries added yogurt comes from the added pulp or berries. Additionally, it can be seen from the graphs, the total phenolic content of samples increases as the digestion procedure continues reflecting that more proteins are hydrolyzed as the digestion is carried out. This results in a better reaction of protein hydrolysates with Folin-Ciocalteau reagent indicating that the protein digestion was not affected by phenolic compounds, although there are many reports about the inhibitory effects of polyphenols on proteases and associatively protein digestion (McDougall, Kulkarni,, & Stewart, 2008). On the other hand, even though during the digestion there were differences on the total phenolic content of each type of yogurt samples (plain<pulp added<berries added), these differences become much lower in the absorbed fractions (in BID-IN and PG-IN samples). Furthermore, although there were much higher phenolics in the outer

fractions, it is evident that the inner fractions have quite a little total phenolic content. Possible complexations between the phenolic compounds and other matrix components might have an adverse effect on the absorption of the polyphenolic compounds. One of these components could be simple carbohydrates such as lactose, fructose and glucose, which have an adverse effect (about 2 fold decrease) in the IN digested fractions (Sengul, Surek, & Nilufer-Erdil, 2014).

The total monomeric anthocyanin content of digested yogurt samples is shown in Appendix E, Table E.5 to E.8 and Figure E.5 to E.8. All data are given in terms of mg cya-3-glu eq/100 g on the dry matter basis. During the digestion, no anthocyanins were observed in the plain yogurts as expected.

If the anthocyanin recoveries viewed (Table 4.10), one can conclude that higher amounts of anthocyanins can be recovered in berry added yogurts compared to pulp added yogurts. The highest recoveries were 3.5% and 32.1% (for IN and OUT sample respectively) for pulp added yogurt as well as 6.3% and 34.3% (for IN and OUT sample respectively) for berries added yogurt.

Table 4.10: Percentage anthocyanin recoveries from digested yogurt samples

	AF 1st day		AF 8th day	
Recoveries, %	**PG IN**	**PG OUT**	**PG IN**	**PG OUT**
Pulp added	2.60±0.50	24.1±1.30	2.60±0.80	32.1±6.80
Berries added	6.30±0.30	28.3±0.70	5.10±2.70	30.1±0.80
	BF 1st day		BF 8th day	
Recoveries, %	**PG IN**	**PG OUT**	**PG IN**	**PG OUT**
Pulp added	3.50±2.80	19.0±0.00	3.10±1.10	26.5±4.90
Berries added	5.00±1.30	34.3±7.40	5.10±0.20	29.3±1.00

During the buccal phase of digestion, in a short span of two minutes, it can be seen that there is a 10% to 26% decrease in anthocyanin content. This can be linked to degradation of anthocyanins by salivary enzymes. According to an ex-vivo study carried out by Kamonpatana et al. (2012) the degradation of anthocyanins in the mouth is structure-dependent and largely mediated by oral microbiota. They suggested that, loss of chokeberry anthocyanins in saliva was primarily enzymatic and dependent on the cellular activity rather than secretions from the salivary glands or binding of chokeberry anthocyanins to salivary proteins. They also reported that anthocyanins can be degraded non-enzymatically by the effect of electrolytes existing in saliva. Since an artificial saliva was used in our study, it can be concluded that the loss of anthocyanins in buccal phase is mainly linked to the electrolyte content.

As the digestion process continues, further losses in anthocyanin content were observed. The results showed that a high stability of anthocyanins during simulated gastric digestion step, as a recovery up to approximately 92% was measured. Additionally, pH was approximately 3.5 at the end of the gastric digestion step which was relatively higher than the pH of the gastric fluid. The observed pH values at the end of the gastric digestion are compatible with existing data (Martini, Bollweg, Levitt, & Savaiano, 1987) (Marteau, Minekus, Havenaar, & Huis In't Veld, 1997). Martini, Bollweg, Levitt, & Savaiano (1987) stated that, the pH of stomach following yogurt ingestion depends upon the interaction of buffering capacity of yogurt, which has excellent buffering properties, and gastric acid secretion. Intestinal digestion caused a further loss in anthocyanin content of BID samples and OUT samples by ranging from 48% to 68% and 68% to 81 % respectively comparing to undigested samples. Also, a study on stability of polyphenols in black chokeberry subjected to an in vitro gastric and pancreatic digestion showed that gastric digestion had no considerable effect on any of the main phenolic compounds present in chokeberry (anthocyanins, flavan-3-ols, flavonols and caffeic acid derivatives). However, these compounds were significantly altered during the pancreatic digestion and this effect was more remarkable for anthocyanins as approximately 43% was lost during the 2h treatment with pancreatin, while flavonols and flavan-3-ols decreased by 26% and 19%, respectively (Bermúdez-Soto, Tomás-Barberán, & García-Conesa, 2007). According to Fossen, Luis Cabrita, & Andersen (1998) color stability of cyanidin-3-glucoside decreases above pH 3.1 and reaches minimum at pH 7. This high amount of loss can be attributed to the pH of the intestinal conditions which was observed during the digestion procedure (approximately pH 7). From another point of view, since the color stability of anthocyanins changed in different pH ranges, this instability may have an adverse effect on the accuracy of the anthocyanin content when it measured spectrophotometrically. On the other hand, during the holding of digestion membranes for 45 min before the addition of intestinal juices, some absorption of anthocyanins was observed. In addition to that, higher absorption of anthocyanins as a buffering effect was observed in pulp added yogurt compared to berry added yogurt. However, at the end of the digestion the highest absorptions were observed in berry added yogurt samples (7 to 9% for pulp added yogurt and 9.7 to 17% for berry added yogurt).

The results of DPPH radical scavenging activity in mM TEAC/100 g DM are presented in Appendix E, Table E.9 to E.12 and Figure E.9 to E.12 and cupric ion reducing

antioxidant capacities (CUPRAC) in mM TEAC/100 g DM are shown in Appendix Table E.13 to E.16 and Figure E.13 to E.16.

When the DPPH and CUPRAC methods are compared, it can be seen that samples showed higher antioxidant capacity when they were analysed with CUPRAC method in comparison to DPPH method. In all berry added yogurt samples a higher DPPH radical scavenging activity and cupric ion reducing ability were observed compared to plain and pulp added yogurts and this higher antioxidant capacity remains stable during the digestion process. However the antioxidant activity substantially decreased in the absorbed inner fractions. Although according to the total phenolic content data indicating that all the phenolic content has lost in the buccal phase, one can see some antioxidant activity in saliva samples, similar to the ones in the undigested yogurts. This observation can be explained by the presence of some bioactive peptides showing antioxidant activity. Indeed, it has been shown that bioactive peptides can be generated either during the fermentation process of milk or from hydrolysis of caseins during digestion (Hafeez, et al., 2014).

4.6 Correlations Between the Methods

No correlation was found between the methods. However, as seen in Figure 4.5, up to a specific anthocyanin content (approximately 40 mg cya-3-glu/100g DM), the DPPH radical scavenging activity increases linearly.

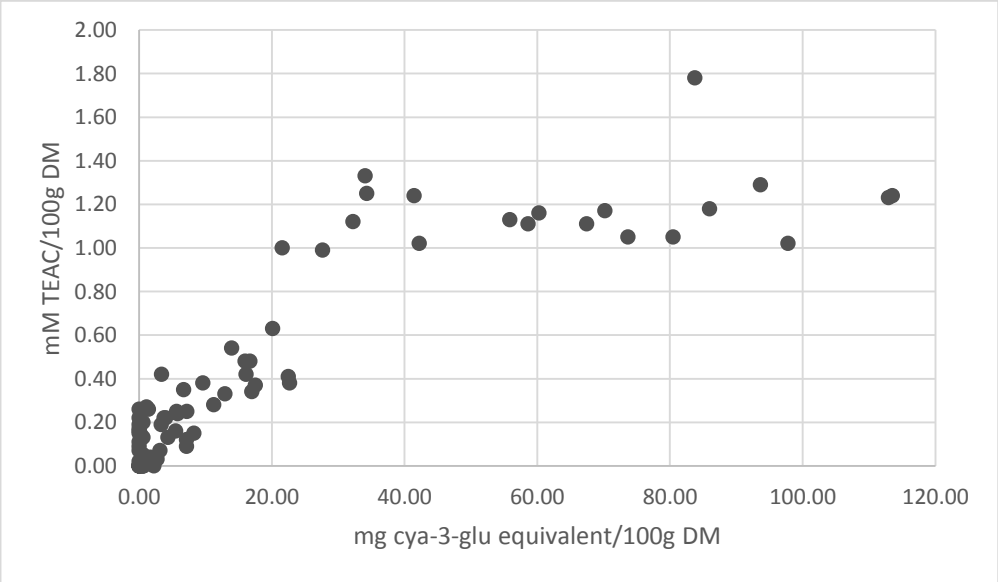

Figure 4.5: Correlation between DPPH radical scavenging activity (mM TEAC/100g DM) and total monomeric anthocyanin content (mg cya-3-glu equivalent/100g DM)[1]

However, after that point the antioxidant capacity remains stable as the anthocyanin content increases. This situation could be attributed to insufficient amount of DPPH to oxidize the existing anthocyanins which might be caused by use of trolox as a standard instead of anthocyanins. In the same vein, a study by Kim, Won Lee, Joo Lee, & Yong Lee (2002) also reveals that the different standards show different vitamin C antioxidant capacity when they evaluated by the same method.

4.7 Evaluation of the Degree of Hydrolysis

The degree of hydrolysis of the two batches of yogurt samples (duplicates) was measured to have a better understanding about the extent of hydrolisation of the proteins during simulated gastrointestinal digestion. In other words, since our above mentioned results showed that the activity and the measurable amount of phenolic compounds of chokeberry fruit changed due to the some interactions when they were added to the yogurt, the aim was to examine whether the phenolic compounds affect the hydrolisation of proteins by comparing the plain yogurt and fruit added yogurts. The results of the degree of hydrolysis are given in miliequivalent (meq)/1 g DM (Table 4.11).

Table 4.11: The degree of hydrolysis of yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) (meq/1 g DM)[1,2]

	BF1-1	BF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	119	116	118±2.25[a]
UD-Pulp yogurt	117	114	115±2.22[a]
UD-berry yogurt	115	115	115±0.68[a]
S-plain yogurt	124	121	122±1.66[b]
S-pulp yogurt	129	131	130±1.44[ab]
S-berry yogurt	129	127	128±1.26[a]
AGD-plain yogurt	145	144	144±0.59[a]
AGD-pulp yogurt	142	138	140±3.06[a]
AGD-berry yogurt	138	136	137±1.18[a]
BID OUT-plain yogurt	231	230	230±0.59[a]
BID OUT-pulp yogurt	240	229	234±7.55[a]
BID OUT-berry yogurt	220	217	219±1.60[a]
PG OUT-plain yogurt	221	221	221±0.00[a]
PG OUT-pulp yogurt	227	212	220±10.8[a]
PG OUT-berry yogurt	222	216	219±4.65[a]

[1]Data were given as the mean values ± standard deviations of duplicates. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

Figure 4.6 shows that the degree of hydrolysis increases as the in vitro gastrointestinal digestion continues. No difference was observed between the yogurt varieties in each digestion step except oral phase. On the other hand, slight increases on the degree of hydrolysis were observed until the end of gastric digestion. In contrast to that, after the addition of duodenal juice and bile juice the degree of hydrolysis has a noticeably increase. It is well known that proteins are firstly reduced into the polypeptides during gastric digestion and then these polypeptides are separated into the smaller polypeptides and amino acids by pancreatic digestion (Ası, 1999). In this sense, our findings correlate with the mechanism of protein digestion.

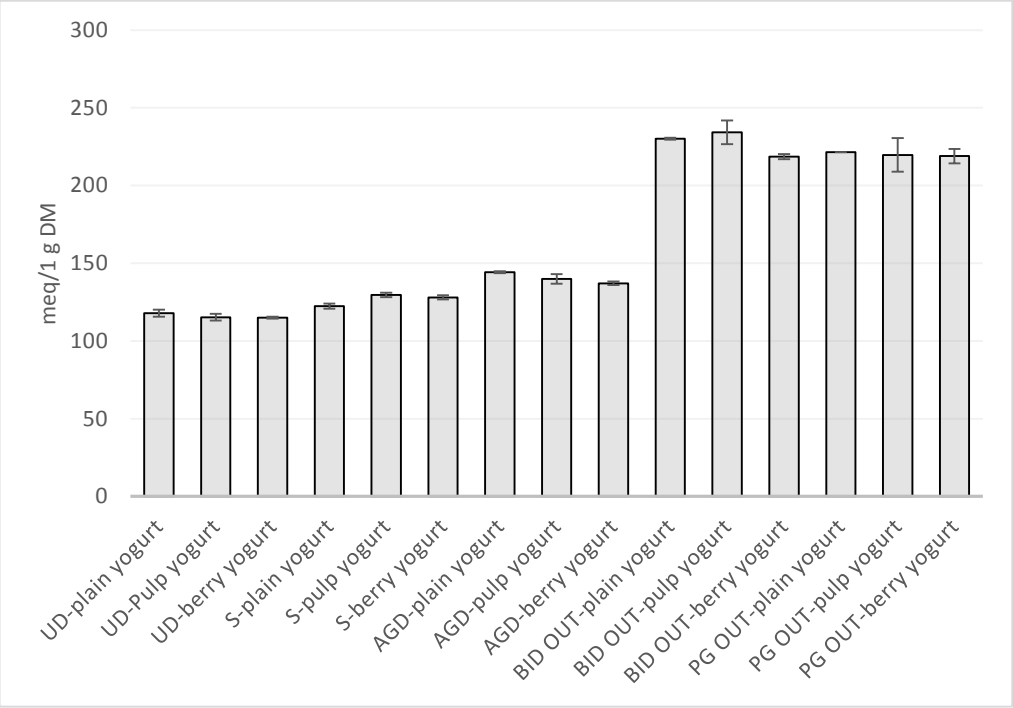

Figure 4.6: The degree of hydrolysis of yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) n=2

4.8 Qualitative Evaluation of Protein Profiles of the Undigested and Digested Yogurts

SDS Page analysis was carried out to evaluate the protein profile of the yogurts qualitatively before, during and after digestion process. The analysis was performed in duplicate and the most representative gels are presented in Figure 4.7 as well as the protein content of bovine milk was given in Table 4.12 (Eigel, et al., 1984) to compare the molecular weight ranges of the gel bands.

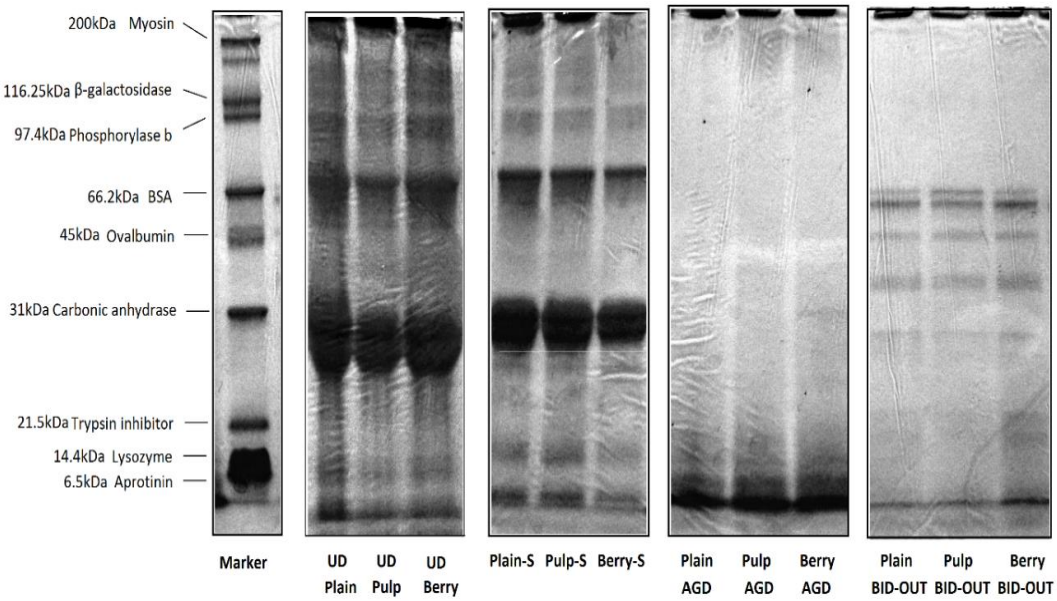

Figure 4.7: SDS-Page analysis of undigested and digested yogurts during buccal, gastric and duodenal phases of *in vitro* gastrointestinal digestion. (Plain: Plain yogurt, Pulp: Pulp added yogurt, Berry: Berries added yogurt, UD: Undigested, S: Saliva added samples, AGD: After gastric digestion, BID-OUT: Before intestinal digestion outer fraction)

Table 4.12: Proteins of bovine milk

Protein and suggested abbreviation	Molecular weight range (Da)
α_{S1}-Casein (α_{S1}-CN)	22 068-23 614
α_{S2}-Casein (α_{S2}-CN)	25 230
β-Casein (β-CN)	23 944-24 092
κ-Casein (κ-CN)	19 007-19 039
β-Lactoglobulin (β-LG)	18 205-18 363
α-Lactalbumin (α-La)	14 147-14 175
Bovine serum albumin (BSA)	66 267
Immunoglobulin G_1 (IgG_1)	153 000-163 000
Immunoglobulin G_2 (IgG_2)	146 000-154 000
Immunoglobulin A^k (IgA^k)	385 000-417 000
Immunoglobulin M (IgM)	960 000-1 000 000
Secretory component (SC)	79 000

According to the SDS Page profiles, there was no difference between the three undigested yogurt samples. A distinct band over the 66.2 kDa molecular weight area was observed in undigested yogurt samples which might be the BSA (bovine serum albumin) whose molecular weight was also suggested as 69 kDa by Abcam (2015). However, according to the observations made by Rinaldi, Gauthier, Britten, & Turgeon (2014) and Dupont, et al. (2010), BSA band in undigested yogurts was quite pale. Additionally, similar to Rinaldi, Gauthier, Britten, & Turgeon (2014) and Dupont, et al. (2010), outstanding bands were observed around 31 kDa molecular weight range which might be the caseins.

After the addition of saliva, the protein bands remained the same with undigested yogurts, likewise a similar trend is previously observed in the degree of hydrolysis of the samples. This finding was expected since there is no protease in the buccal juice.

At the end of the gastric phase, a clear degradation of the large molecular weight proteins to low molecular weight proteins was observed and undefined bands appeared in the low molecular weight region (<6.5 kDa) of the gels. This situation is likely a result of the activity of pepsin enzyme existing in the gastric fluid. On the other hand, the β-Lactoglobulin proteins seemed to be resistant to the pepsinolysis in gastric phase, as indicatied by Dupont, et al. (2010). Furthermore, while the color intensity of the bands in buccal phase was as follow: plain and pulp>berry added yogurt, this order had a reverse situation in gastric digestion and pancreatic digestion stages: plain and pulp<berry added yogurt. This circumstance can be attributed to effect of possible interactions between milk proteins and fruit phenolic compounds.

Similar trends in gel patterns between BID-OUT samples and completely digested samples (PG-OUT) were observed. When the pancreatic solution was introduced, a further degradation of small molecular weight proteins resulting leaner bands in the low molecular weight range was observed and some new bands in the higher molecular weight area, which were indicated as digestive juice proteins by Rinaldi, Gauthier, Britten, & Turgeon (2014), were observed. Both in BID-OUT and PG-OUT samples, the two lanes appearing between 31 and 66.2 kDa were more clear in pulp>berry>plain which are likely to be BSA and other proteins coming from the digestive juices. Some lower molecular weight proteins between 14 and 31 kDa (most probably the proteins given in the range 14-25 kDa in Table 4.12) were more appearing in the berry yogurt compared to plain and pulp yogurt. In short, in the pancreatic digestion stage, low molecular weight proteins appearing below the AGD gels were further degraded and formed very low molecular weight compounds which cannot be held by gels. As a result, the undermost bands in AGD gels faded away in BID-OUT gels and PG-OUT gels.

5 CONCLUSION

In this study, *in vitro* simulated gastrointestinal digestion of anthocyanins from black chokeberry (*Aronia melanocarpa*) added to homemade yogurts was investigated in terms of bioaccessibility, antioxidant activity and the possible fate of anthocyanins. The main findings from this study can be described as follow:

In the undigested yogurts, a significant effect on the total phenolic content, total monomeric anthocyanin content and antioxidant capacity was observed depending on the type of fruits added in the following order: no < pulp < berries. Both for the berries or the pulp added to the yogurt, a significant difference in anthocyanin content was observed when the fruits were added before or after fermentation. Four individual anthocyanins were observed in the extracts of chokeberry juice, berries and pulp as well as chokeberry added yogurt samples. These anthocyanins can be ordered from abundant to rare one as follow: cya-3-gal, cya-3-ara, cya-3-glu, cya-3-xyl.

During the digestion process anthocyanins were found to be more stable in gastric conditions rather than in pancreatic digestion. In addition to that, a marked decrease of anthocyanins in buccal phase was also observed. At the end of the digestion, only a small amount of anthocyanins could be recovered. A remarkable increase in total phenolic content even in the plain yogurts was observed during the digestion. This situation was attributed to the formation of the interfering compounds during the digestion. On the other hand, the total phenolic content of the IN fractions were quite lower than the other samples, which could be linked to the possible complexations, having an adverse effect on the absorption of the polyphenolic compounds, between the phenolic compounds and other matrix components. A higher antioxidant capacity was observed in all berry added yogurts compared to plain and pulp added yogurts. However, the antioxidant activity substantially decreased in the absorbed IN fractions.

Yogurt samples were also evaluated on the degree of hydrolysis of the proteins during the digestion process and it was found that the degree of hydrolysis increases when the samples were subjected to pancreatic digestion. Also, small differences in the degree of hydrolysis were observed between the yogurt varieties in each digestion step. However, this observation was insignificant and reflecting that the digestion of proteins was not affected by the anthocyanins. On the evaluation of protein profiles of the samples during digestion step, no effect of buccal digestion stage on the proteins

was observed. However, in the gastric digestion phase there were a remarkable degradation of high molecular weight proteins. Following that a further hydrolysis of low molecular weight proteins occurred during the duodenal digestion where the low molecular weight range bands pronouncedly became fainter.

Overall, it can be said that food components or food matrices as well as the processing of foods have different effects on anthocyanins. However, future studies, e.g. the investigation of the phenolic compounds by HPLC, are required to examine the exact effect of processing and food matrix. In this study, bioaccessibility of the bioactive compounds were analyzed and evaluated by *in vitro* gastrointestinal digestion model. However, *in vivo* and *in vitro* studies are required to understand the bioavailability of nutritive compounds of chokeberry and chokeberry products in human body.

REFERENCES

Adler-Nissen, J. (1979). Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzene sulphonic acid . *Journal of Agriculture and Food Chemistry, 27*, 1256-1262.

Adolfsson, O., Meydani, S., & Russell, R. M. (2004). Yogurt and gut function. *The American journal of clinical nutrition, 80*(2), 245-256.

Alhaj, O., & Kanekanian, A. (2014). Milk-derived Bioactive Components from Fermentation. In A. Ara Kanekanian (Ed.), *Milk and Dairy Products as Functional Foods* (pp. 237-288). Wiley Blackwell.

Apak, R., Güçlü, K., Özyürek, M., & Karademir, S. (2004). Novel Total Antioxidant Capacity Index for Dietary Polyphenols and Vitamins C and E, Using Their Cupric Ion Reducing Capability in the Presence of Neocuproine: CUPRAC Method. *J. Agric. Food Chem., 52 (26)*, 7970–7981.

Ara, V. (2002). Schwarzfruchtige Aronia: Gesund – und bald “in aller Munde”? *Flüssiges Obst. Vol. 10*, 653-658.

Ası, T. (1999). *Tablolarla Biyokimya Cilt 2.* Ankara: Nobel Tıp Kitapevi.

Benvenuti, S., Pellati, F., Melegari, M., & Bertelli, D. (2004). Polyphenols, Anthocyanins, Ascorbic Acid, and Radical Scavenging Activity of Rubus, Ribes, and Aronia. *Journal of Food Science 69(3)*, 164-169.

Bermúdez-Soto, M., Tomás-Barberán, F., & García-Conesa, M. (2007). Stability of polyphenols in chokeberry (Aronia melanocarpa) subjected to in vitro gastric and pancreatic digestion. *Food Chemistry, 102*(3), 865-874.

Bijak, M., Bobrowski, M., Borowiecka, M., Podsędek, A., Golański, J., & Nowak, P. (2011). Anticoagulant effect of polyphenols-rich extracts from black chokeberry and grape seeds. *Fitoterapia, 82*(6), 811-817.

Bräunlich, M., Økstad, O. A., Slimestad, R., Wangensteen, H., Malterud, K. E., & Barsett, H. (2013). Effects of Aronia melanocarpa Constituents on Biofilm Formation of Escherichia coli and Bacillus cereus. *Molecules, 18*(12), 14989-14999.

Bräunlich, M., Slimestad, R., Wangensteen, H., Brede, C., Malterud, K., & Barsett, H. (2013). Extracts, anthocyanins and procyanidins from Aronia melanocarpa as radical scavengers and enzyme inhibitors. *Nutrients, 5*(3), 663-678.

Buttriss, J. (2005). Dairy Products. In B. Caballero, L. Allen, & A. Prentice (Eds.), *Encyclopedia of Human Nutrition* (pp. 511-517). London, UK: Elsevier Academic Press.

Cano, P. G., Agüero, G., & Perdigon, G. (2002). Immunological effects of yogurt addition to a re-nutrition diet in a malnutrition experimental model. *Journal of Dairy Research, 69*, 303-316.

Carbonell-Capella, J. M., Buniowska, M., Barba, F. J., Esteve, M. J., & Frigola, A. (2014). Analytical Methods for Determining Bioavailability and Bioaccessibility of Bioactive Compounds from Fruits and Vegetables: A Review. *Comprehensive Reviews in Food Science and Food Safety, 13*, 155-171.

Caroli, A., Poli, A., Ricotta, D., Banfi, G., & Cocchi, D. (2011). Invited review: Dairy intake and bone health: A viewpoint from the state of the art. *Journal of dairy science, 94*(11), 5249-5262.

Chabance, B., Marteau, P., Rambaud, J., Migliore-Samour, D., Boynard, M., Perrotin, P., . . . Fiat, A. (1998). Casein peptide release and passage to the blood in humans during digestion of milk or yogurt. *Biochimie, 80*(2), 155-165.

Chandan, R., & O'Rell, K. (2006). Manufacture of various types of yogurts. In R. Chandan, C. White, A. Kilara, & Y. Hui (Eds.), *Manufacturing Yogurt and Fermented Milks* (pp. 211-235). Oxford, UK: Blackwell Publishing.

Chandra, A., Rana, J., & Li, Y. (2001). Separation, Identification, Quantification, and Method Validation of Anthocyanins in Botanical Supplement Raw Materials by HPLC and HPLC -MS. *J. Agric. Food Chem., 49*, 3515−3521.

Chouchouli, V., Kalogeropoulos, N., Konteles, S., Karvela, E., Makris, D., & Karathanos, V. (2013). Fortifi cation of yoghurts with grape (Vitis vinifera) seed extracts. *LWT - Food Science and Technology,53*, 522-529.

Ciocoiu, M., Badescu, L., Miron, A., & Badescu M. (2013). The involvement of a polyphenol-rich extract of black chokeberry in oxidative stress on experimental arterial hypertension. *Evidence-Based Complementary and Alternative Medicine*.

Correa-Betanzo, J., Allen-Vercoe, E., McDonald, J., Schroeter, K., Corredig, M., & Paliyath, G. (2014). Stability and biological activity of wild blueberry (Vaccinium angustifolium) polyphenols during simulated in vitro gastrointestinal digestion. *Food Chemistry, 165* , 522–531.

Cossu, M., Juliano, M., Pisu, R., & Alamanni, M. C. (2009). Effects of Enrichment with Polyphenolic Extracts from Sardinian Plants on Physico-Chemical, Antioxidant and Microbiological Properties of Yogurt. *Ital. J. Food Sci. n. 4, vol. 21*, 447-459.

D'Alessandro, L., Vauchel, P., Przybylski, R., Chataigné, G., Nikov, I., & Dimitrov, K. (2013). Integrated process extraction–adsorption for selective recovery of antioxidant phenolics from Aronia melanocarpa berries. *Separation and Purification Technology, 120*, 92–101.

D'Archivio, M., Filesi, C., Vari, R., Scazzocchio, B., & Masella, R. (2010). Bioavailability of the polyphenols: status and controversies. *International Journal of Molecular Sciences, 11*(4), 1321-1342.

De Oliveira, M. N. (2014). Fermented Milks and Yogurt. In C. Batt, & M. L. Tortorello (Eds.), *Encyclopedia of Food Microbiology* (2nd ed., pp. 784-791). London, UK: Elsevier.

Denev, P. N., Kratchanov, C. G., Ciz, M., Lojek, A., & Kratchanova, M. G. (2012). Bioavailability and Antioxidant Activity of Black Chokeberry (Aronia melanocarpa) Polyphenols: in vitro and in vivo Evidences and Possible Mechanisms of Action: A Review. *Comprehensive Reviews in Food Science and Food Safety, 11*, 471-489.

Donovan, S. M., & Shamir, R. (2014). Introduction to the Yogurt in Nutrition Initiative and the First Global Summit on the Health Effects of Yogurt. *The American journal of clinical nutrition, 99*(5), 1209S-1211S.

Dupont, D., Mandalari, G., Molle, D., Jardin, J., Leonil, J., Faulks, R., . . . Mackie, A. (2010). Comparative resistance of food proteins to adult and infant in vitro digestion models. *Mol. Nutr. Food Res., 54*, 767–780.

Dupont, D., Mandalari, G., Molle, D., Jardin, J., Rolet-Repecaud, O., Duboz, G., . . . Mackie, A. (2010). Food processing increases casein resistance to simulated infant digestion. . *Molecular Nutrition & Food Research, 54*, 1677-1689.

Early, R. (2012). Dairy products and milk-based food ingredients. In D. Baines, & R. Seal, *Natural Food Additives, Ingredients and Flavourings* (pp. 417-444). Cambridge: Woodhead Publishing.

Eheart, J., & Massey Jr., P. (1962). Chemical Constituents of Plants, Factors Affecting the Oxalate Content of Spinach. *Journal of Agricultural and Food Chemistry, 10*(4), 325-327.

Eigel, W., Butler, J., Ernstorm, C., Farrell Jr., H., Harwalkar, W., Jenness, R., & Whitney, R. (1984). Nomenclature of Proteins of Cow's Milk: Fifth Revision. *Journal of Dairy Science, 67(8)*, 1599-1631.

Eskin, N. (1990). Biochemistry of Foods. London, UK: Academic Press Limited.

Fossen, T., Luis Cabrita, L., & Andersen, O. (1998). Colour and stability of pure anthocyanins infuenced by pH including the alkaline region. *Food Chemistry, 63(4)*, 435-440.

Galván D'Alessandro, L., Dimitrov, K., Vauchel, P., & Nikov, I. (2013). Kinetics of ultrasound assisted extraction of anthocyanins from Aronia melanocarpa (black chokeberry) wastes. *Chemical Engineering Research and Design*, 1818-1826.

Gasiorowski, K., Szyba, K., Brokosa, B., Kolaczyliska, B., Jankowiak-Wlodarczyk, M., & Oszmianski, J. (1997). Antimutagenic Activity of Anthocyanins Isolated from Aronia Melanocarpa Fruits. *CancerLetters, 119*, 37-46.

Hafeez, Z., Cakir-Kiefer, C., Roux, E., Perrin, C., Miclo, L., & Dary-Mourot, A. (2014). Strategies of producing bioactive peptides from milk proteins to functionalize fermented milk products. *Food Research International, 63*, 71–80.

Harbers, L. (1998). Ash Analysis. In S. S. Nielsen (Ed.), *Food Analysis* (2nd ed.). Gaithersburg, Maryland, US: Aspen Publishers.

Hill, A. R., & Kethireddipalli, P. (2013). Dairy Products: Cheese and Yogurt. In M. N. Eskin, & F. Shahidi (Eds.), *Biochemistry of Foods* (3rd ed., pp. 319-362). Waltham, UK.

Holst, B., & Williamson, G. (2008). Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. *Current opinion in biotechnology, 19*(2), 73-82.

Ikawa, M., Schaper, T., Dollard, C., & Sasner, J. (2003). Utilization of Folin−Ciocalteu Phenol Reagent for the Detection of Certain Nitrogen Compounds. *J. Agric. Food Chem.,51*, 1811−1815.

Jakobek, L. (2015). Interactions of polyphenols with carbohydrates, lipids and proteins. *Food Chemistry, 175*, 556–567.

Kamonpatana, K., Failla, M., Purnima, K. S., & Giusti, M. M. (2014). Anthocyanin structure determines susceptibility to microbial degradation and bioavailability to the buccal mucosa. *Journal of agricultural and food chemistry, 62*, 6903-6910.

Kamonpatana, K., Giusti, M., Chitchumroonchokchai, C., Moreno Cruz, M., Riedl, K., Kumar, P., & Failla, M. (2012). Susceptibility of anthocyanins to ex vivo degradation in human saliva. *Food Chem. 135(2)*, 738–747.

Kapci, B., Neradová, E., Čížková, H., Voldřich, M., Rajchl, A., & Çapanoğlu, E. (2013). Investigating The Antioxidant Potential Of Chokeberry (Aronia Melanocarpa) Products. *Journal of Food and Nutrition Research (Slovak Republic)*.

Kay, C. D., Mazza, G., Holub, B., & Wang, J. (2004). Anthocyanin metabolites in human urine and serum. *British Journal of Nutrition, 91*(06), 933-942.

Kim, D., Won Lee, K., Joo Lee, H., & Yong Lee, C. (2002). Vitamin C Equivalent Antioxidant Capacity (VCEAC) of Phenolic Phytochemicals. *J. Agric. Food Chem., 50(13)*, 3713–3717.

Kırca, A., Özkan, M., & Cemeroğlu, B. (2007). Effects of temperature, solid content and pH on the stability of black carrot anthocyanins. *Food Chemistry, 101*, 212–218.

Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: Production and functionality. *International Dairy Journal*, 945-960.

Kulling, S. E., & Rawel, H. M. (2008). Chokeberry (Aronia melanocarpa) - A review on the characteristic components and potential health effects. *Planta Med, 74*(13), 1625-1634.

Kumaran, A., & Karunakaran, R. (2006). Antioxidant and free radical scavenging activity of an aqueous extract of Coleus aromaticus. *Food Chemistry, 97*(1), 109-114.

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International, 88*(5), 1269-1278.

Liang, L., Wu, X., Zhao, T., Zhao, J., Li, F., Zou, Y., . . . Yang, L. (2012). In vitro bioaccessibility and antioxidant activity of anthocyanins from mulberry (Morus atropurpurea Roxb.) following simulated gastro-intestinal digestion. *Food Research International*, 76-82.

Marette, A., & Picard-Deland, E. (2014). Yogurt consumption and impact on health: focus on children and cardiometabolic risk. *The American journal of clinical nutrition, 99*(5), 1243S-1247S.

Marteau, P., Minekus, M., Havenaar, R., & Huis In't Veld, J. (1997). Survival of Lactic Acid Bacteria in a Dynamic Model of the Stomach and Small Intestine: Validation and the Effects of Bile. *J Dairy Sci 80*, 1031–1037.

Martini, M., Bollweg, G., Levitt, M., & Savaiano, D. (1987). Lactose digestion by yogurt β-galactosidase: influence of pH and microbial cell integrity. *Am J Clin Nutr 45* , 432-436.

Matsumoto, M., Hara, H., Chiji, H., & Kasai, T. (2004). Gastroprotective Effect of Red Pigments in Black Chokeberry Fruit (Aronia melanocarpa Elliot) on Acute Gastric Hemorrhagic Lesions in Rats. *Journal of agricultural and food chemistry, 58*(2), 2226-2229.

Mayer-Miebach, E., Adamiuk, M., & Behsnilian, D. (2012). Stability of Chokeberry Bioactive Polyphenols during Juice Processing and Stabilization of a Polyphenol-Rich Material from the By-Product. *Agriculture 2*, 244-258.

Mazzaracchio, P., Pifferi, P., Kindt, M., Munyaneza, A., & Barbiroli, G. (2004). Interactions between anthocyanins and organic food molecules in model systems. *International Journal of Food Science and Technology, 39*, 53–59.

McDougall, G., K. N., & Stewart, D. (2008). Current developments on the inhibitory effects of berry polyphenols on digestive enzymes. *BioFactors,33* , 1–8.

Merdzhanov, P., Angelov-Romova, M., Nenov, N., Zlatanov, M., Antov, G., Atasanov, T., . . . Stoyanova, A. (2013). Low temperature extraction of plants by liquificate gases waste of chokeberry fruits (Aronia melanocarpa (Michx) Elliott.). *Journal of EcoAgriTourism, 9*(1), 23-27.

Mladin, P., Mladin, G., Oprea, E., Radulescu, M., & Nicola, C. (2011). Variability of the Anthocyanins and Tannins in Berries of Some Lonicera caerulea var. kamchatica, Aronia melanocarpa and Berberis thunbergii var. atropurpurea Genotypes. *Scientific Papers, 27*.

Najda, A., & Łabuda, H. (2013). Content of Phenolic Compounds and Antioxidant Properties of Fruits of Selected Orchard Shrub Species. *Modern Phytomorphology 3*, 105-109.

Naruszewicz, M., Łaniewska, I., Millo, B., & Dłużniewski, M. (2007). Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infraction (MI). *Atherosclerosis, 194*(2), e179-e184.

Nawirska, A., & Uklanska, C. (2008). Waste Products From Fruit And Vegetable Processing As Potential Sources For Food Enrichment In Dietary Fibre. *Actat Sci Pol Technol Aliment, 7*(2), 35-42.

Ochmian, I., Grajkowski, J., & Smolik, M. (2012). Comparison of Some Morphological Features, Quality and Chemical Content of Four Cultivars of Chokeberry Fruits (Aronia melanocarpa). *Notulae Botanicae Horti Agrobotanici 40(1), 40*(1), 253-260.

Olsen, H., Aaby, K., & Borge, G. (2009). Characterization and quantification of flavonoids and hydroxycinnamic acids in curly kale (Brassica oleracea L. Convar. acephala Var. sabellica) by HPLC-DAD-ESI-MSn. *J. Agric. Food Chem., 57*, 2816-2825.

Ornelas, I. J., Galvan-Potrillo, M., & López-Carrillo, L. (2007). Protective effect of yoghurt consumption on Helicobacter pylori seropositivity in a Mexican population. *Public Health Nutrition, 10*(11), 1283-1287.

Patel, S. (2011). Evaluating the Effect of Milk Protein Concentrates (MPC) Fortification on Rheological Properties of Nonfat Set Yogurt Using Vane Rheometry. *Master Thesis. The Graduate School, University of Wisconsin-Stout, Menomonie, WI.*

Phan, A., Netzel, G., Wang, D., Flanagan, B., D'Arcy, B. R., & Gidle, M. (2015). Binding of dietary polyphenols to cellulose: Structural and nutritional aspects. *Food Chemistry, 171*, 388–396.

Racedo, S., Villena, J., Salva, S., & Alvarez, S. (2009). Influence of yogurt consumption on the respiratory immune response. *Food and Agricultural Immunology, 20*(3), 231-244.

Ramchandran, L., & Shah, N. P. (2011). Yogurt Can Beneficially Affect Blood Contributors of Cardiovascular Health Status in Hypertensive Rats. *Journal of Food Science, 76*(4), 131-136.

Ramić, M., Vidović, S., Zeković, Z., Vladić, J., Cvejin, A., & Pavlić, B. (2015). Modeling and optimization of ultrasound-assisted extraction of polyphenolic compounds from Aronia melanocarpa by-products from filter-tea factory. *Ultrasonics sonochemistry, 23*, 360-368.

Rein, M. J., Renouf, M., Cruz-Hernandez, C., Actis-Goretta, L., Thakkar, S. K., & Da Silva Pinto, M. (2013). Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. *British journal of clinical pharmacology, 75*(3), 588-602.

Richelle, M., Sabatier, M., Steiling, H., & Williamson, G. (2006). Skin bioavailability of dietary vitamin E, carotenoids, polyphenols, vitamin C, zinc and selenium. *British Journal of Nutrition, 96*, 227-238.

Rinaldi, L., Gauthier, S. F., Britten, M., & Turgeon, S. L. (2014). In vitro gastrointestinal digestion of liquid and semi-liquid dairy matrixes. *LWT-Food Science and Technology, 57*(1), 99-105.

Rizzoli, R. (2014). Dairy products, yogurts, and bone health. *The American journal of clinical nutrition, 99*(5), 1256S-1262S.

Robinson, R. K. (2002). Yogurt: Role of Starter Culture. In H. Roginski, J. Fuquay, & P. Fox (Eds.), *Encyclopedia of Dairy Sciences* (pp. 1059-1063). London, UK: Academic Press.

Rop, O., Jiri Mlcek, J., Jurikova, T., Valsikova, M., Jiri Sochor, J., Reznicek, V., & Kramarova, D. (2010). Phenolic Content, Antioxidant Capacity, Radical Oxygen Species Scavenging and Lipid Peroxidation Inhibiting Activities of Extracts of Five Black Chokeberry (Aronia Melanocarpa (Michx.) Elliot) Cultivars. *Journal of Medicinal Plants Research 4(22)*, 2431 -2437.

Sasaki, Y., & Ohba, R. (2004). Antioxidant Activity and Optimal Manuf acturing Conditions of Purple Sweet Potato Lactic Acid Bacteria Drink . *Food Sci. Technol. Res. 10 (4)*, 447-452.

Savaiano, D. A. (2014). Lactose digestion from yogurt: mechanism and relevance. *The American journal of clinical nutrition, 99*(5), 1251S-1255S.

Selvamuthukumaran, M., & Farhath, K. (2014). Evaluation of shelf stability of antioxidant rich seabuckthorn fruit yoghurt. *International Food Research Journal 21(2)*, 759-765.

Sengul, H., Surek, E., & Nilufer-Erdil, D. (2014). Investigating the effects of food matrix and food components on bioaccessibility of pomegranate (Punica granatum) phenolics and anthocyanins using an in-vitro gastrointestinal digestion model. *Food Research International, 62*, 1069–1079.

Serafeimidou, A., Zlatanos, S., Kritikos, G., & Tourianis, A. (2013). Change of fatty acid profile, including conjugated linoleic acid (CLA) content, during refrigerated storage of yogurt made of cow and sheep milk. *Journal of Food Composition and Analysis, 31*, 24-30.

Serafeimidou, A., Zlatanos, S., Laskaridis, K., & Sagredos, A. (2012). Chemical characteristics, fatty acid composition and conjugated linoleic acid (CLA) content of traditional Greek yogurts. *Food Chemistry, 134*(4), 1839-1846.

Shah, N. P. (2003). Yogurt: The product and its manufacture. In B. Caballero, L. Trugo, & P. Finglas, *Encyclopedia of Food Sciences and Nutritions* (pp. 6252-6260). London, UK: Academic Press.

Shi, J., Wang, J., Zhu, Y., & Chen, J. (2014). Characterization of intermolecular interaction between cyanidin-3-glucoside and bovine serum albumin: Spectroscopic and molecular docking methods. *Luminescence, 29*, 522-530.

Singh, G., Singh Kapoor, I. P., & Singh, P. (2011). Effect of Volatile Oil and Oleoresin of Anise on The Shelf Life of Yogurt. *Journal of Food Processing and Preservation, 35*(6), 778-783.

Singleton, V., Orthofer, R., & Lamuela-Raventos, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol. 299*, 152-178.

Skoczyñska, A., Jêdrychowska, I., Porêba, R., Affelska-Jercha, A., Turczyn, B., Wojakowska, A., & Andrzejak, R. (2007). Influence of chokeberry juice on arterial blood pressure and lipid parameters in men with mild hypercholesterolemia. *Pharmacological Reports, 59*(1), 177-182.

Šnebergrová, J., Čížková, H., Neradová, E., Kapci, B., Rajchl, A., & Voldřich, M. (2014). Variability of Characteristic Components of Aronia. *Czech J. Food Sci., 32(1)*, 25-30.

Sójka, M., Kołodziejczyk, K., & Milala, J. (2013). Polyphenolic and basic chemical composition of black chokeberry industrial by-products. *Industrial Crops and Products, 51*, 77-86.

Sun-Waterhouse, D., Zhou, J., & Wadhwa, S. (2013). Drinking yoghurts with berry polyphenols added before and after fermentation . *Food Control, 32*, 450-460.

Sunyecz, J. A. (2008). The use of calcium and vitamin D in the management of osteoporosis. *Therapeutics and clinical risk management, 4*(4), 827-836.

Surono, I., & Hosono, A. (2002). Fermented Milks: Types and Standarts of Identity. In H. Roginski, J. Fuquay, & P. Fox (Eds.), *Encyclopedia of Dairy Sciences* (pp. 470-475). London, UK: Academic Press.

Tong, X., Dong, J., Wu, Z., Li, W., & Qin, L. (2011). Dairy consumption and risk of type 2 diabetes mellitus: a meta-analysis of cohort studies. *European journal of clinical nutrition, 65*(9), 1027-1031.

Toydemir, G., Capanoglu, E., Gomez Roldan, M., R.C.H., D. V., Boyacioglu, D., . . . Beekwilder, J. (2013). Industrial Processing Effects on Phenolics Compounds in Sour Cherry (Prunus cerasus L.) Fruit. *Food Res Int, 53*, 218-225.

Tseng, A., & Zhao, Y. (2013). Wine grape pomace as antioxidant dietary fibre for enhancing nutritional value and improving storability of yogurt and salad dressing. *Food Chemistry, 138*(1), 356-365.

Van Hecke, T., Vossen, E., Bussche, J. V., Raes, K., Vanhaecke, L., & De Smet, S. (2014). Fat Content and Nitrite-Curing Influence the Formation of Oxidation Products and NOC-Specific DNA Adducts during In Vitro Digestion of Meat. *PLoS ONE, 9*(6), e101122.

Versantvoort, C. H., Oomen, A., Van de Kamp, E., Rompelberg, C., & Sips, A. (2005). Applicability of an in vitro digestion model in assessing the bioaccessibility of mycotoxins from food. *Food and Chemical Toxicology, 43*(1), 31-40.

Vivas, N., Lonvaud-Funel, A., & Glories, Y. (1997). Effect of phenolic acids and anthocyanins on growth, viability and malolactic activity of a lactic acid bacterium. *Food Microbiology 14*, 291–300.

Walstra, P., Wouters, J., & Geurts, T. (2006). *Dairy Sciences and Technology* (2nd ed.). (Taylor, & Francis, Eds.) Florida: Boca Roton.

Wang, H., Livingston, K. A., Fox, C. S., Meigs, J. B., & Jacques, P. F. (2013). Yogurt consumption is associated with better diet quality and metabolic profile in American men and women. *Nutrition Research*, 18-26.

Wangensteena, H., Braunlicha, M., Nikolica, V., Malteruda, K. E., Slimestadb, R., & Barsett, H. (2014). Anthocyanins, Proanthocyanidins and Total Phenolics in Four Cultivars of Aronia: Antioxidant and Enzyme Inhibitory Effects. *Journal of Functional Foods 7*, 746-752.

Wawer, I., Wolniak, M., & Paradowska, K. (2006). Solid state NMR study of dietary fiber powders from aronia, bilberry, black currant and apple. *Solid state nuclear magnetic resonance, 30*(2), 106-113.

Wiczkowski, W., Romaszko, E., & Piskula, M. K. (2010). Bioavailability of cyanidin glycosides from natural chokeberry (Aronia melanocarpa) juice with dietary-relevant dose of anthocyanins in humans. *Journal of agricultural and food chemistry, 58*(23), 12130-12136.

Wiese, S., Gärtner, S., Rawel, H. M., Winterhalter, P., & Kulling, S. E. (2009). Protein interactions with cyanidin-3-glucoside and its influence on α-amylase activity. *Journal of the Science of Food and Agriculture, 89*(1), 33-40.

Worsztynowicz, P., Napierala, M., Bialas, W., Grajek, W., & Olkowicz, M. (2014). Pancreatic α-amylase and lipase inhibitory activity of polyphenolic compounds present in the extract of black chokeberry (Aronia melanocarpa L.). *Process Biochemistry, 49*(9), 1457-1463.

Xiao, J., Mao, F., Yang, F., Zhao, Y., Zhang, C., & Yamamoto, K. (2011). Interaction of dietary polyphenols with bovine milk proteins: Molecular structure–affinity relationship and influencing bioactivity aspects. *Molecular Nutrition & Food Research, 55(11)*, 1637-1645.

Zheng, W., & Wang, S. Y. (2003). Oxygen Radical Absorbing Capacity of Phenolics in Blueberries, Cranberries, Chokeberries, and Lingonberries. *J. Agric. Food Chem. 51(2)*, 502-509.

Zlatanov, M. D. (1999). Lipid composition of Bulgarian chokeberry, black currant and rose hip seed oils. *Journal of the Science of Food and Agriculture, 79*(12), 1620-1624.

Abcam. (2015, 03 06). *Abcam.* Retrieved from www.abcam.com: http://www.abcam.com/bovine-serum-albumin-full-length-protein-ab172196.html

Anon. (2015, 06 10). Retrieved from http://acaiberrykorea.com/127

BioRad. (2015, 03 06). *Bio Rad.* Retrieved from http://www.bio-rad.com/: http://www.bio-rad.com/webroot/web/pdf/lsr/literature/4006035.pdf

Danone. (2014, 11 16). *nutrijournal.danone.com*. Retrieved from The Nutrijournal: http://nutrijournal.danone.com/en/articles/stories/global-yoghurt-consumption-per-capita-and-per-year/

***FAO Corporate Document Repository*.** (2014, 11 19). Retrieved from Milk and Milk Products: http://www.fao.org/wairdocs/x5434e/x5434e0d.htm

APPENDICES

Appendix A. Black Chokeberry and Yogurt Samples

Figure A.1. Black Chokeberry (*Aronia melanocarpa*) (Anon., 2015)

Figure A.2. Fruits added after fermentation samples (In order: Minced berries added yogurt, pulp added yogurt, plain yogurt)

Figure A.3. Fruits added before fermentation samples (In order: Plain yogurt, pulp added yogurt, minced berries added yogurt)

Appendix B. Calibration Curves

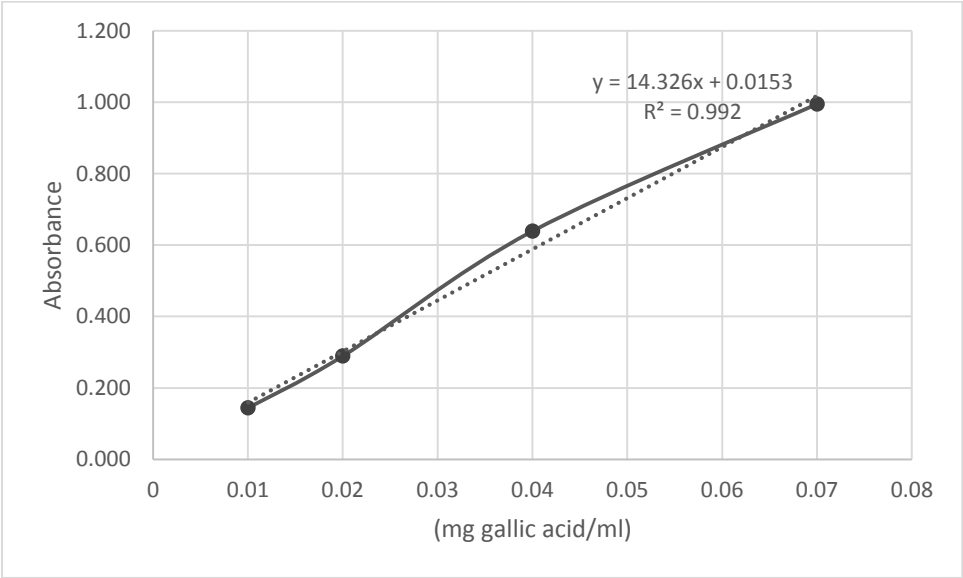

Figure B.1. Calibration curve for total phenolics in 90% aqueous-methanol.

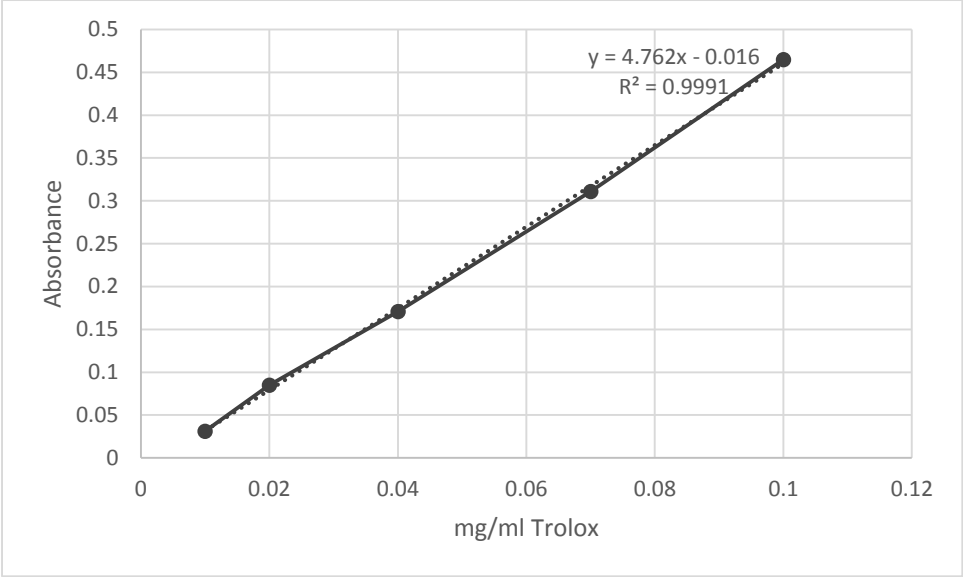

Figure B.2. Calibration curve for DPPH assay in 90% aqueous-methanol.

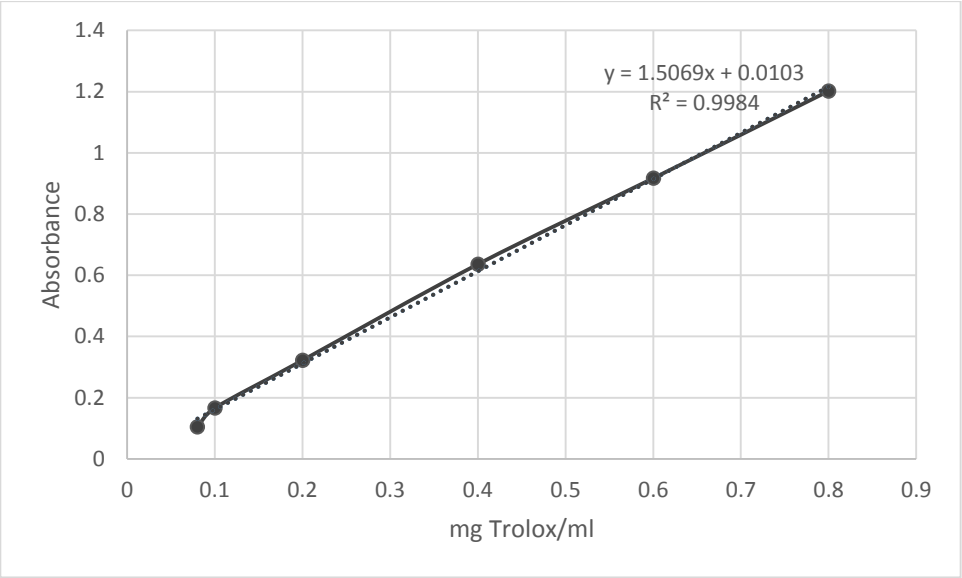

Figure B.3. Calibration curve for CUPRAC assay in 90% aqueous-methanol.

Appendix C. HPLC Chromatograms of Chokeberry Juice, Berries and Pulp

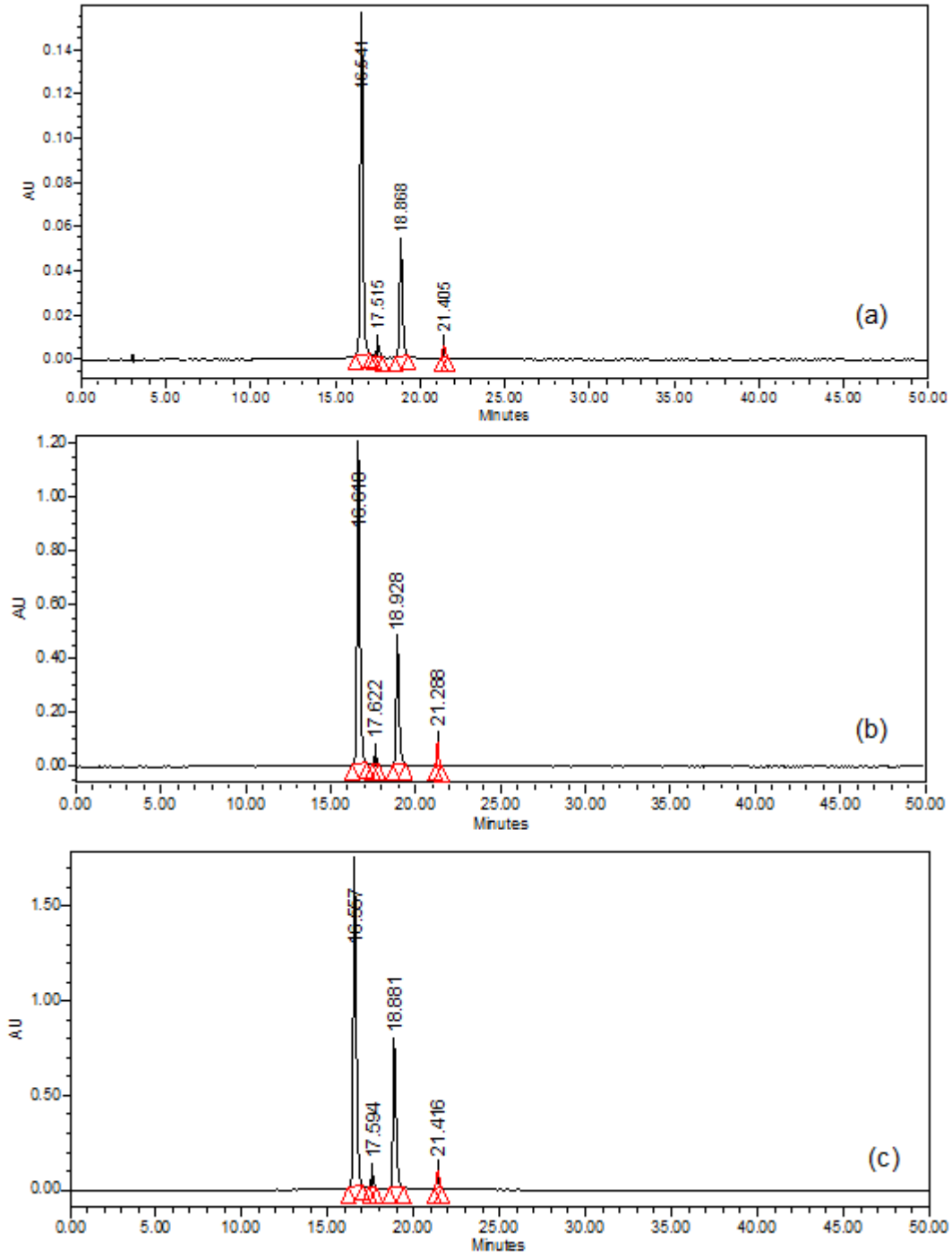

Figure C.1: HPLC chromatograms (recorded at 520 nm) of black chokeberry (a) juice, (b) pulp and (c) berry extracts

Appendix D. HPLC Chromatogramps of Undigested Pulp and Berries Added Yogurts

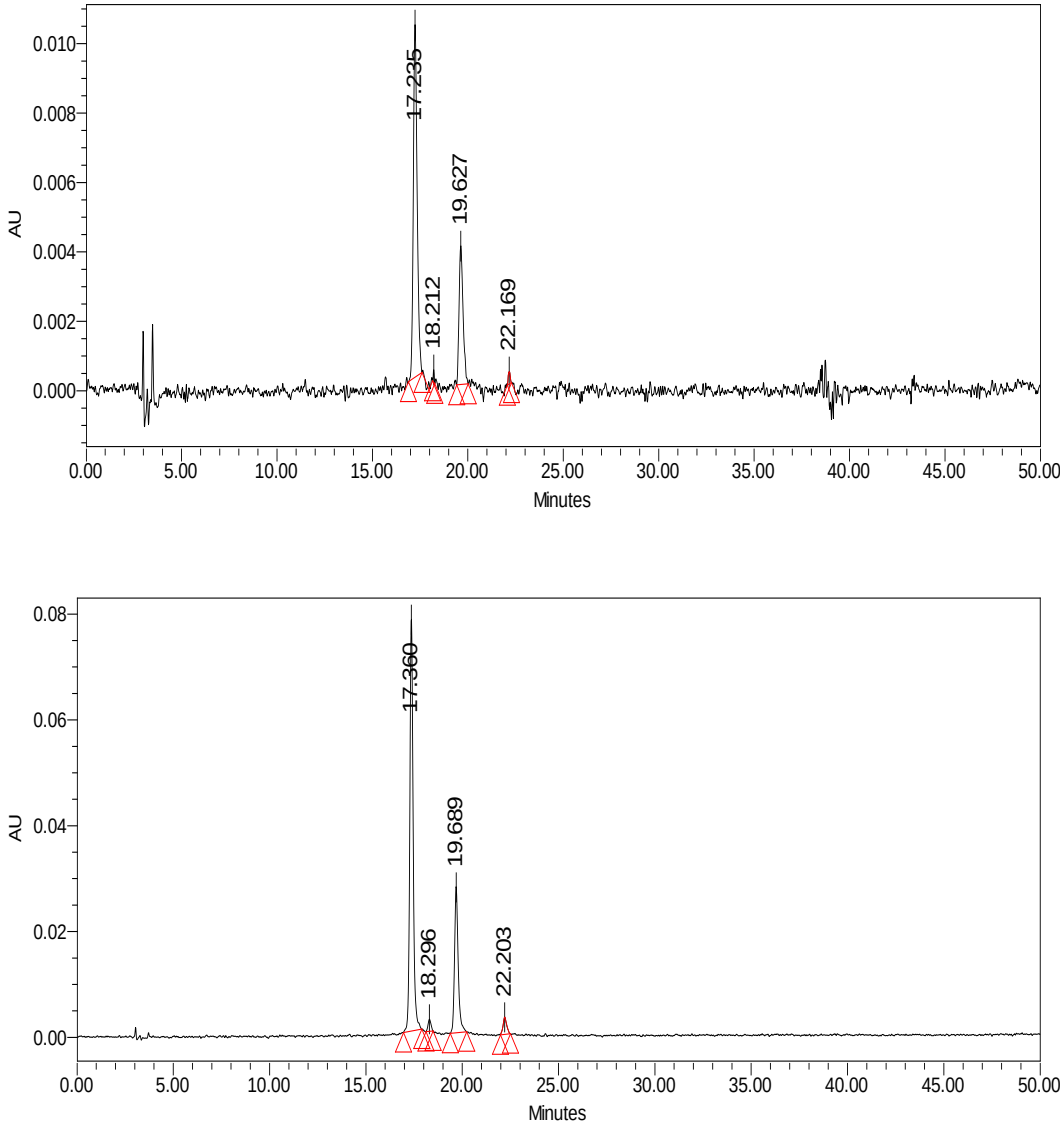

Figure D.1: HPLC chromatograms (recorded at 520 nm) of undigested yogurt samples. Pulp (upper panel) or berry (lower panel) added after fermentation, 1st batch, 1 day stored

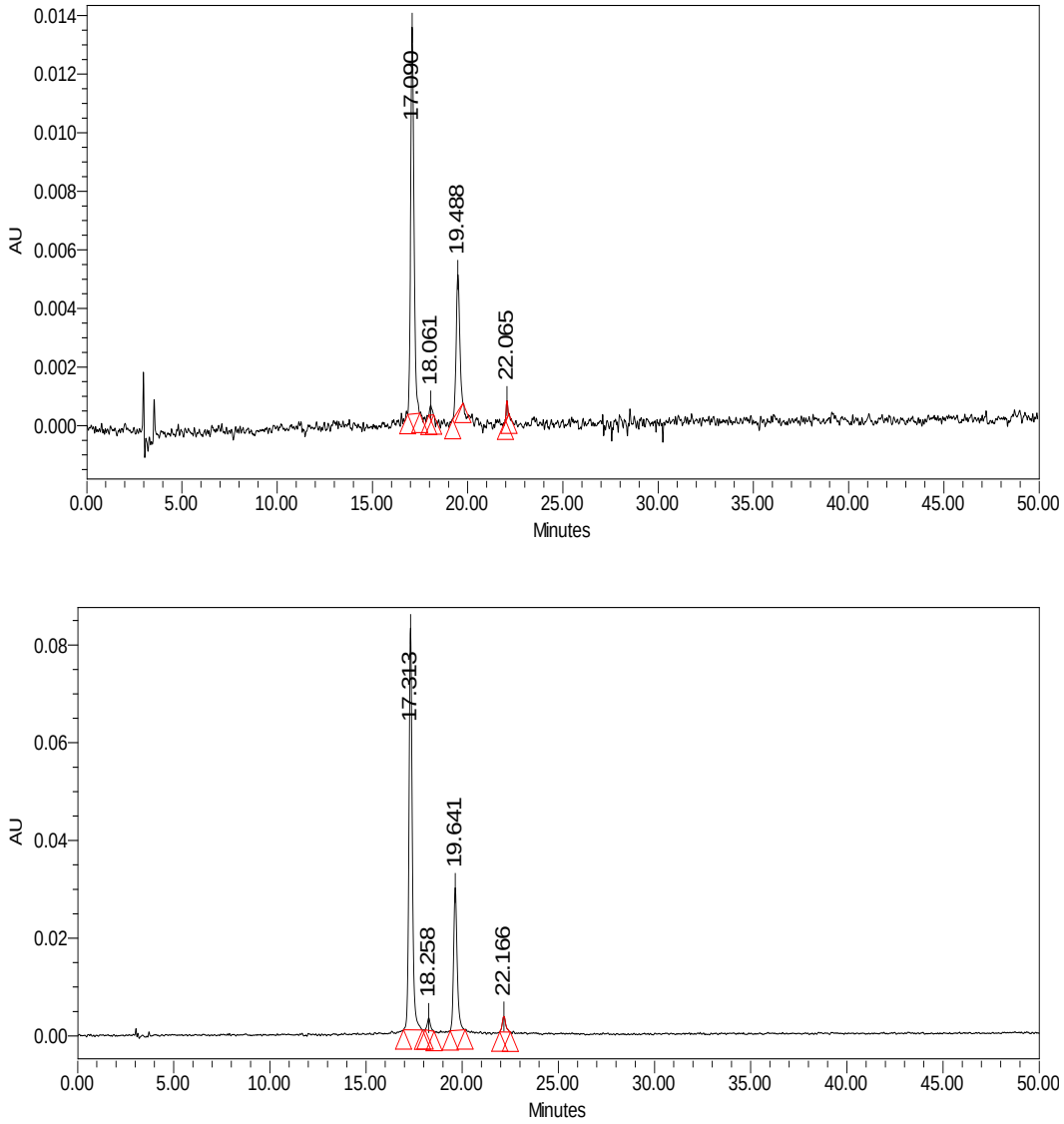

Figure D.2: HPLC chromatograms (recorded at 520 nm) of undigested yogurt samples. Pulp (upper panel) or berry (lower panel) added after fermentation, 1st batch, 8 days stored

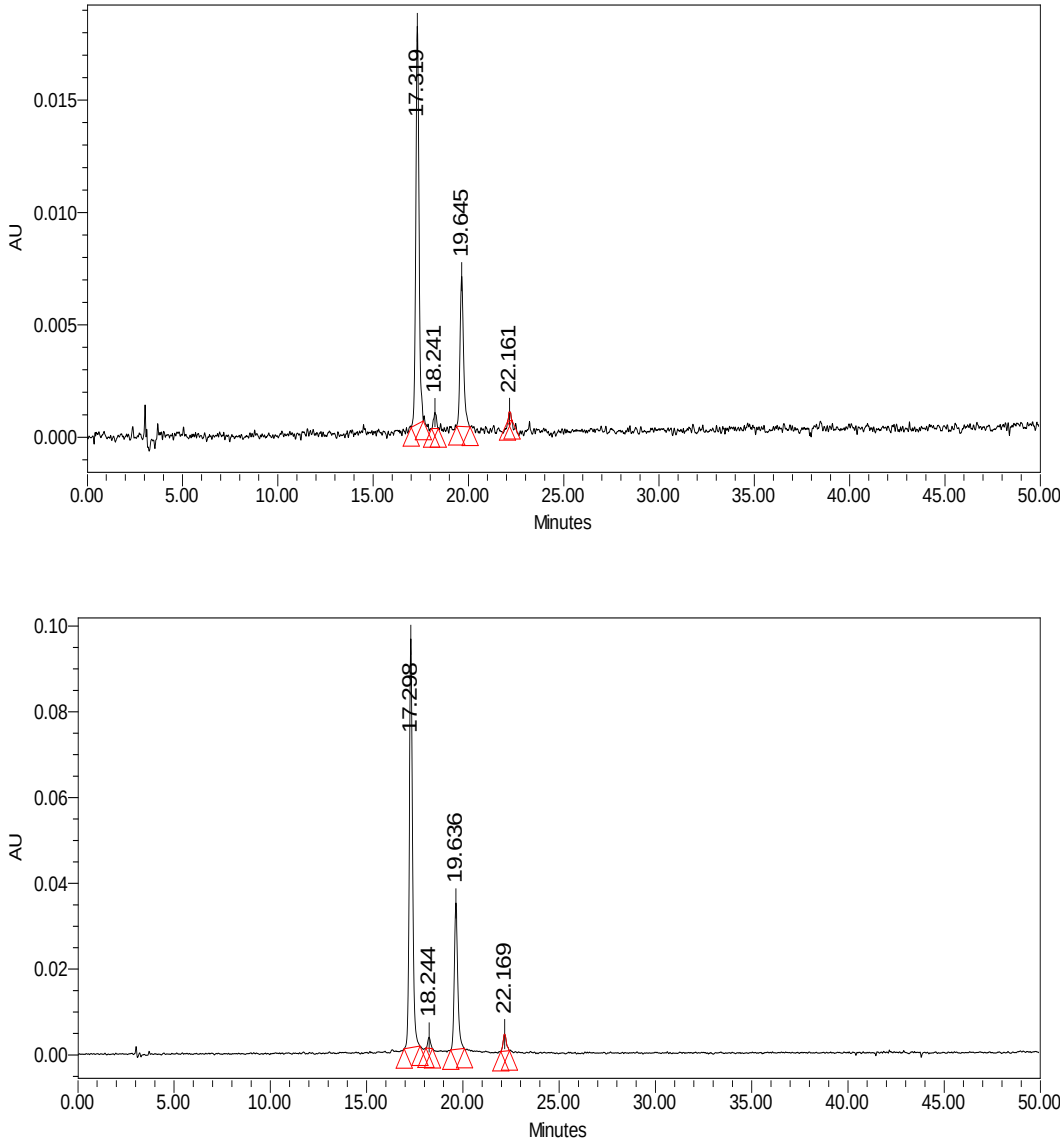

Figure D.3: HPLC chromatograms (recorded at 520 nm) of undigested yogurt samples. Pulp (upper panel) or berry (lower panel) added after fermentation, 2nd batch, 1 day stored

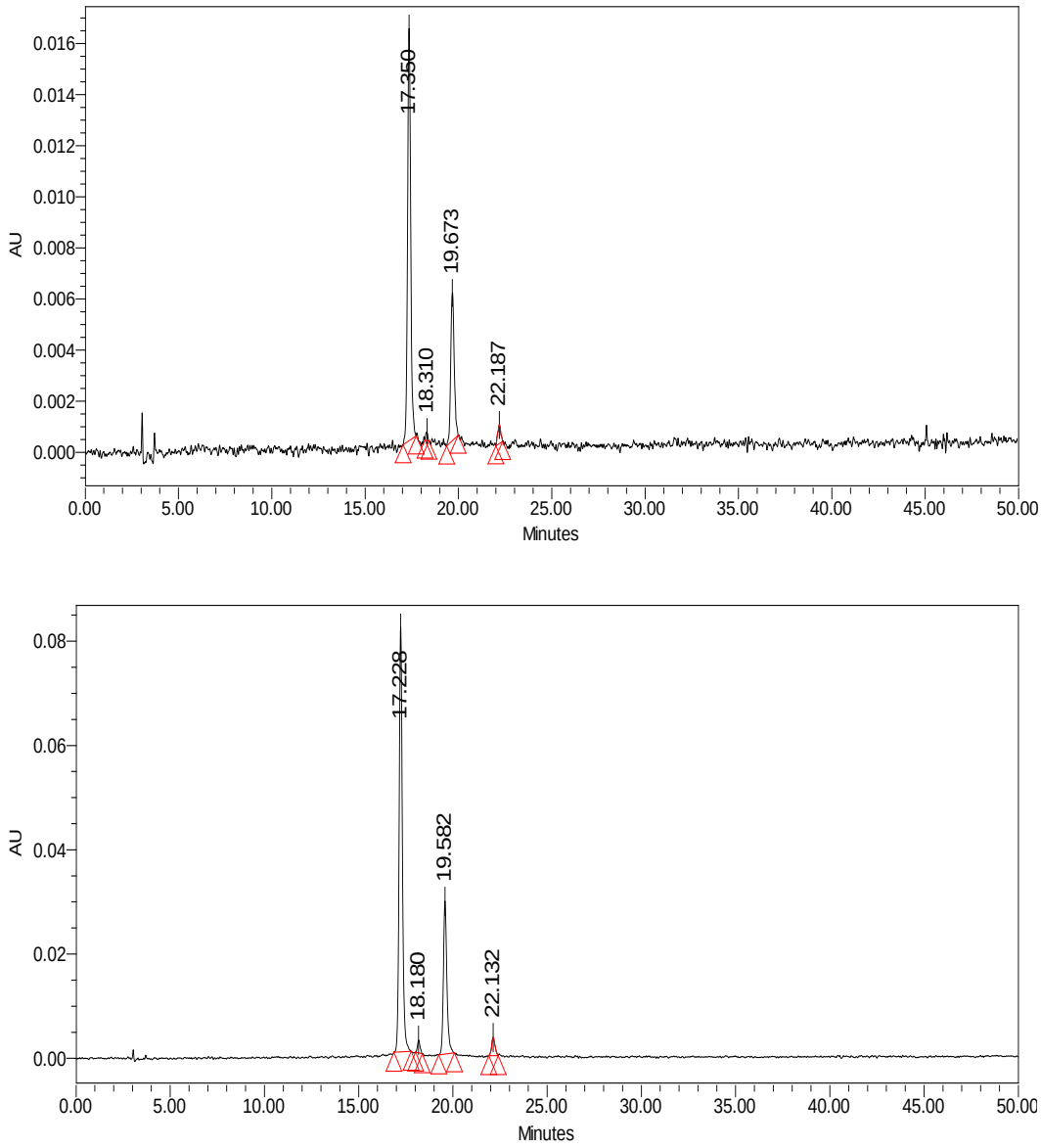

Figure D.4: HPLC chromatograms (recorded at 520 nm) of undigested yogurt samples. Pulp (upper panel) or berry (lower panel) added after fermentation, 2nd batch, 8 days stored

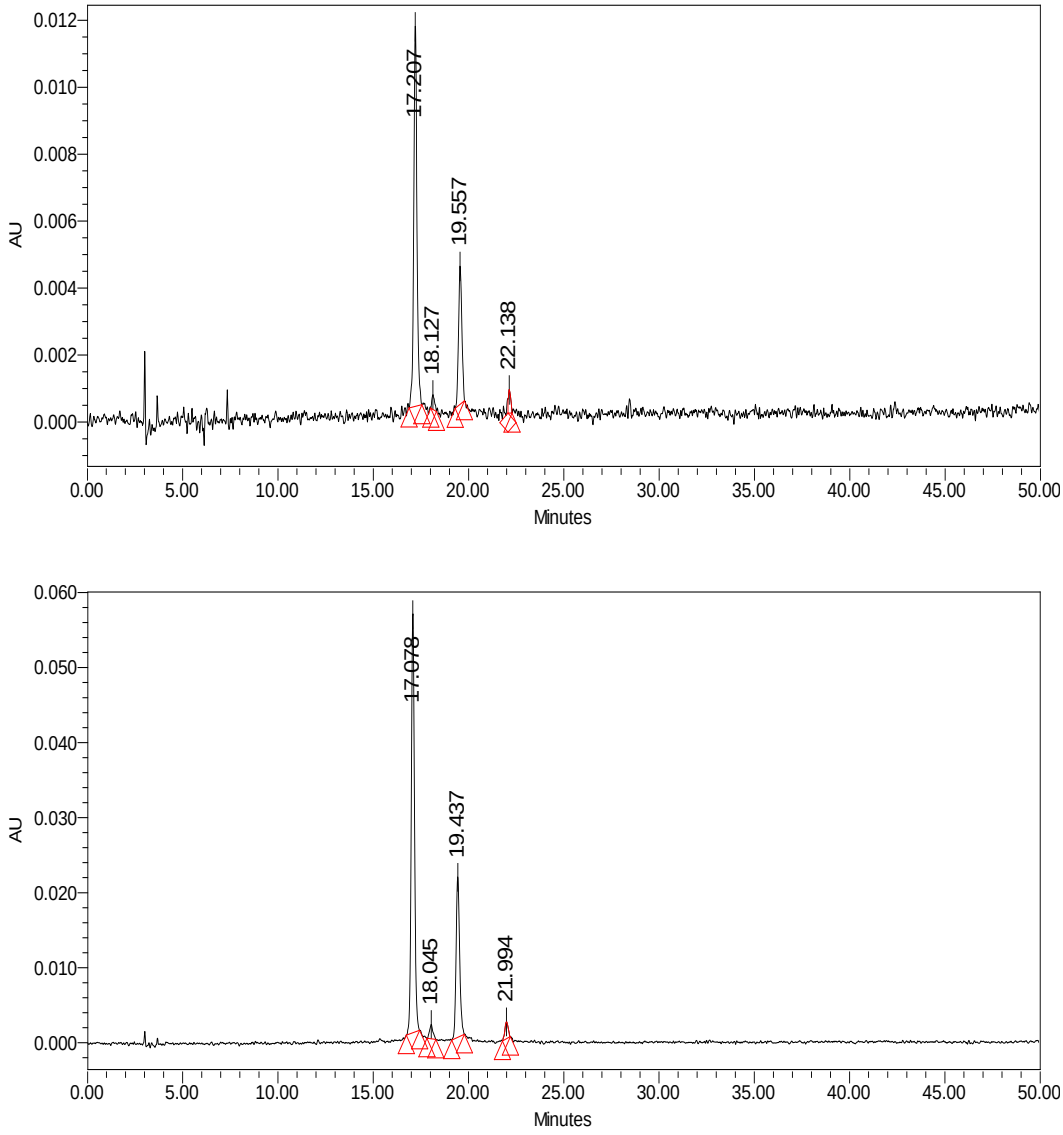

Figure D.5: HPLC chromatograms (recorded at 520 nm) of undigested yogurt samples. Pulp (upper panel) or berry (lower panel) added before fermentation, 1st batch, 1 day stored

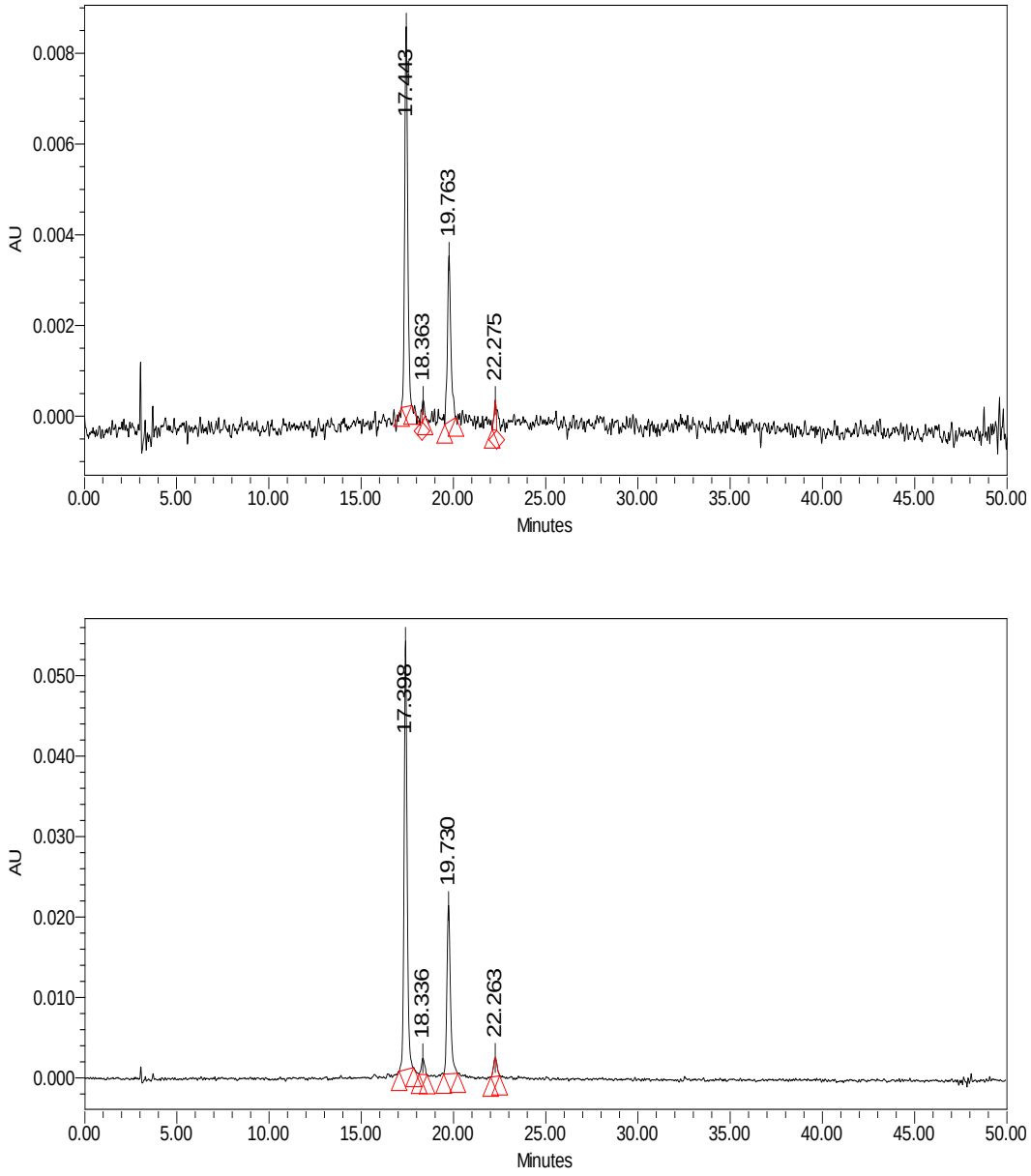

Figure D.6: HPLC chromatograms (recorded at 520 nm) of undigested yogurt samples. Pulp (upper panel) or berry (lower panel) added before fermentation, 1st batch, 8 days stored

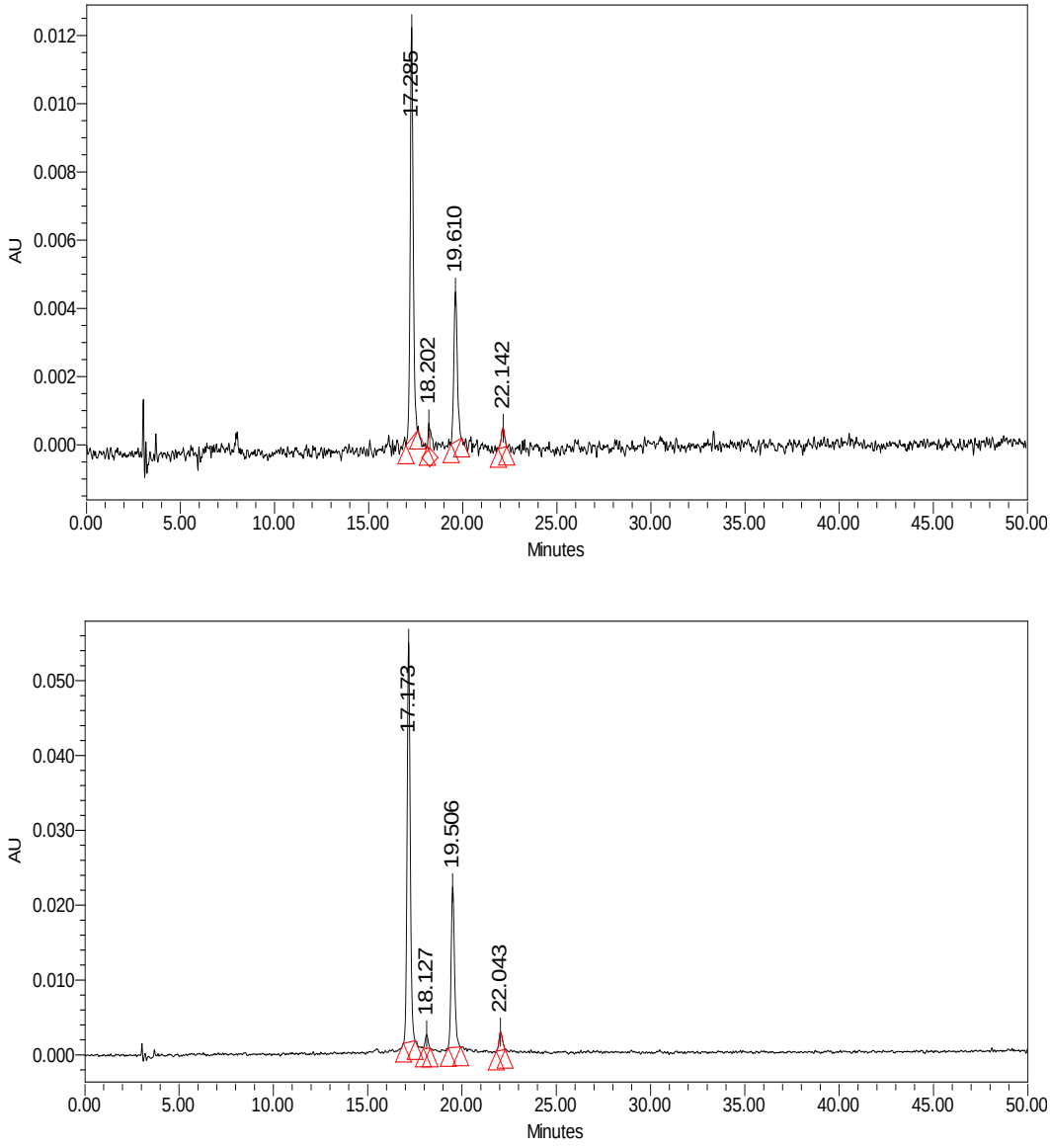

Figure D.7: HPLC chromatograms (recorded at 520 nm) of undigested yogurt samples. Pulp (upper panel) or berry (lower panel) added before fermentation, 2nd batch, 1 day stored

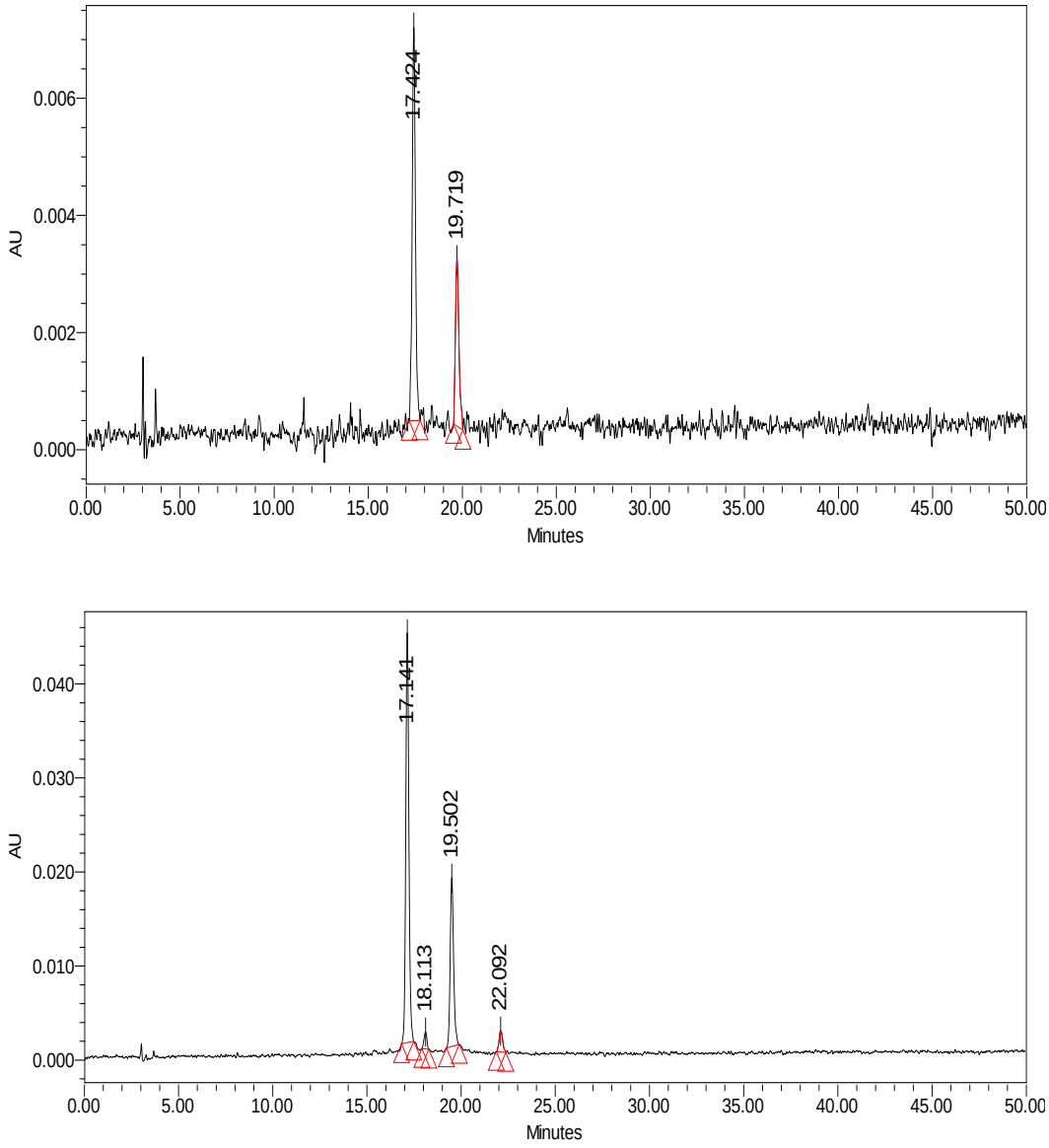

Figure D.8: HPLC chromatograms (recorded at 520 nm) of undigested yogurt samples. Pulp (upper panel) or berry (lower panel) added before fermentation, 2nd batch, 8 days stored

Appendix E. DPPH Radical Scavenging Activities and Cupric Reducing Antioxidant Capacities (CUPRAC) of Digested Yogurt Samples

Table E.1: Total phenolic content of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 1 day stored) (mg GAE/100g DM)[1,2]

Type of sample	Type of Fruit Added	AF1-1	AF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	No	32.3	24.0	28.2±5.80[c]
UD-pulp yogurt	Pulp	63.1	67.4	65.2±3.10[b]
UD-berry yogurt	Berries	195	196	195±0.00[a]
S-plain yogurt	No	ND	ND	ND
S-pulp yogurt	Pulp	ND	ND	ND
S-berry yogurt	Berries	178	147	162±22.1[a]
AGD-plain yogurt	No	298	316	307±12.5[c]
AGD-pulp yogurt	Pulp	318	355	336±25.8[b]
AGD-berry yogurt	Berries	416	411	413±4.00[a]
BID-plain yogurt	No	431	445	438±9.70[b]
BID-pulp yogurt	Pulp	471	534	502±45.1[ab]
BID-berry yogurt	Berries	465	545	505±57.0[a]
OUT-plain yogurt	No	451	455	453±2.80[a]
OUT-pulp yogurt	Pulp	503	518	511±10.7[a]
OUT-berry yogurt	Berries	535	509	522±18.5[a]
BID IN-plain yogurt	No	19.3	45.6	32.5±18.6[c]
BID IN-pulp yogurt	Pulp	55.3	49.4	52.4±4.20[b]
BID IN-berry yogurt	Berries	65.0	66.4	65.7±1.00[a]
PG IN-plain yogurt	No	70.8	70.8	70.8±0.00[c]
PG IN-pulp yogurt	Pulp	72.4	73.0	72.7±0.40[b]
PG IN-berry yogurt	Berries	77.4	76.8	77.1±0.40[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

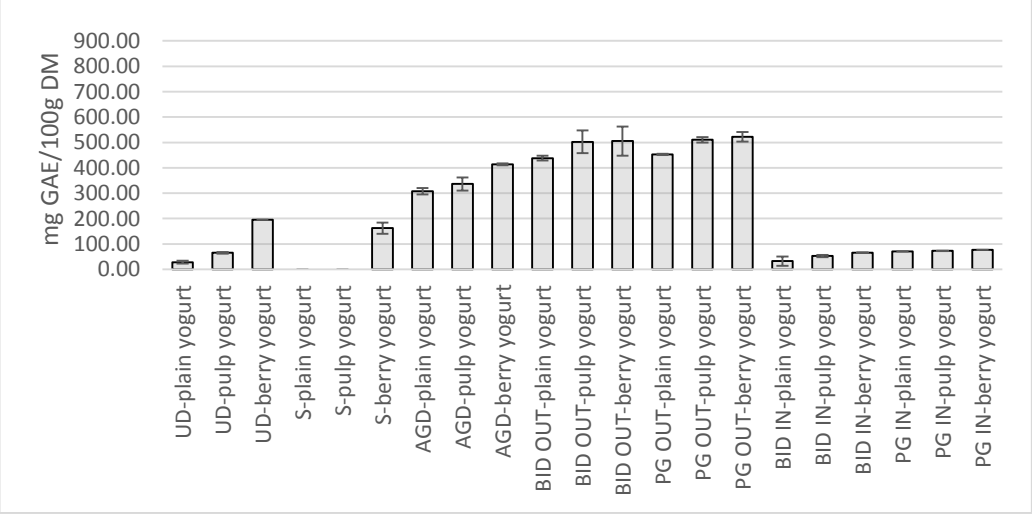

Figure E.1: Total phenolic content of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 1 day stored) n = 2

Table E.2: Total phenolic content of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 8 days stored) (mg GAE/100g DM)[1,2]

Type of sample	Type of Fruit Added	AF1-8	AF2-8	Mean Value ± Standard Deviation
UD-plain yogurt	No	34.6	29.5	32.1±3.60^{c}
UD-pulp yogurt	Pulp	77.5	71.1	74.3±4.50^{b}
UD-berry yogurt	Berries	207	207	207±0.30^{a}
S-plain yogurt	No	ND	ND	ND
S-pulp yogurt	Pulp	54.0	3.0	28.5±36.1^{b}
S-berry yogurt	Berries	216	131	174±60.7^{a}
AGD-plain yogurt	No	323	330	326±5.00^{c}
AGD-pulp yogurt	Pulp	381	373	377±5.90^{b}
AGD-berry yogurt	Berries	456	481	468±17.6^{a}
BID-plain yogurt	No	470	500	485±21.8^{b}
BID-pulp yogurt	Pulp	551	542	546±6.50^{ab}
BID-berry yogurt	Berries	566	585	576±12.9^{a}
OUT-plain yogurt	No	433	466	449±23.1^{a}
OUT-pulp yogurt	Pulp	464	493	478±21.0^{a}
OUT-berry yogurt	Berries	538	570	554±22.2^{a}
BID IN-plain yogurt	No	43.5	45.2	44.3±1.20^{c}
BID IN-pulp yogurt	Pulp	55.4	58.0	56.7±1.80^{b}
BID IN-berry yogurt	Berries	64.4	61.3	62.8±2.20^{a}
PG IN-plain yogurt	No	73.3	71.2	72.3±1.50^{c}
PG IN-pulp yogurt	Pulp	76.1	76.1	76.1±0.10^{b}
PG IN-berry yogurt	Berries	78.8	77.7	78.2±0.80^{a}

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

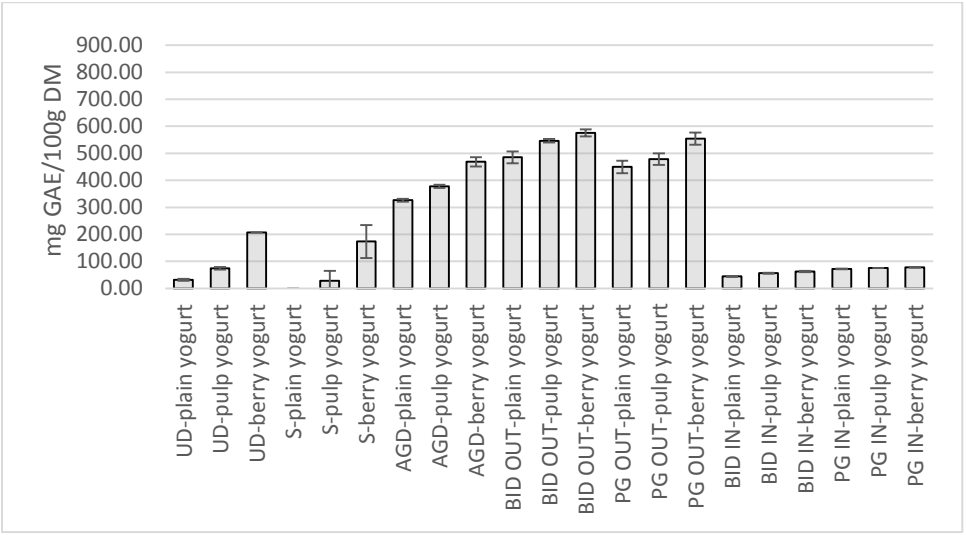

Figure E.2: Total phenolic content of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 8 days stored) n=2

Table E.3: Total phenolic content of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) (mg GAE/100g DM)[1,2]

Type of sample	Type of Fruit Added	BF1-1	BF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	No	24.4	23.2	23.8±0.80[c]
UD-pulp yogurt	Pulp	64.5	61.3	62.9±2.20[b]
UD-berry yogurt	Berries	186	190	188±2.60[a]
S-plain yogurt	No	ND	ND	ND
S-pulp yogurt	Pulp	ND	ND	ND
S-berry yogurt	Berries	145	172	158±19.1[a]
AGD-plain yogurt	No	271	272	271±0.60[c]
AGD-pulp yogurt	Pulp	344	292	318±36.8[b]
AGD-berry yogurt	Berries	428	384	406±31.7[a]
BID-plain yogurt	No	502	446	474±40.0[b]
BID-pulp yogurt	Pulp	528	483	506±31.7[ab]
BID-berry yogurt	Berries	833	486	659±245[a]
OUT-plain yogurt	No	704	472	588±164[a]
OUT-pulp yogurt	Pulp	745	487	616±182[a]
OUT-berry yogurt	Berries	799	504	651±208[a]
BID IN-plain yogurt	No	41.0	33.2	37.1±5.50[c]
BID IN-pulp yogurt	Pulp	44.1	46.6	45.3±1.80[b]
BID IN-berry yogurt	Berries	56.0	49.1	52.5±4.90[a]
PG IN-plain yogurt	No	72.5	73.3	72.9±0.60[c]
PG IN-pulp yogurt	Pulp	71.9	76.1	74.0±2.90[b]
PG IN-berry yogurt	Berries	75.8	78.4	77.1±1.80[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

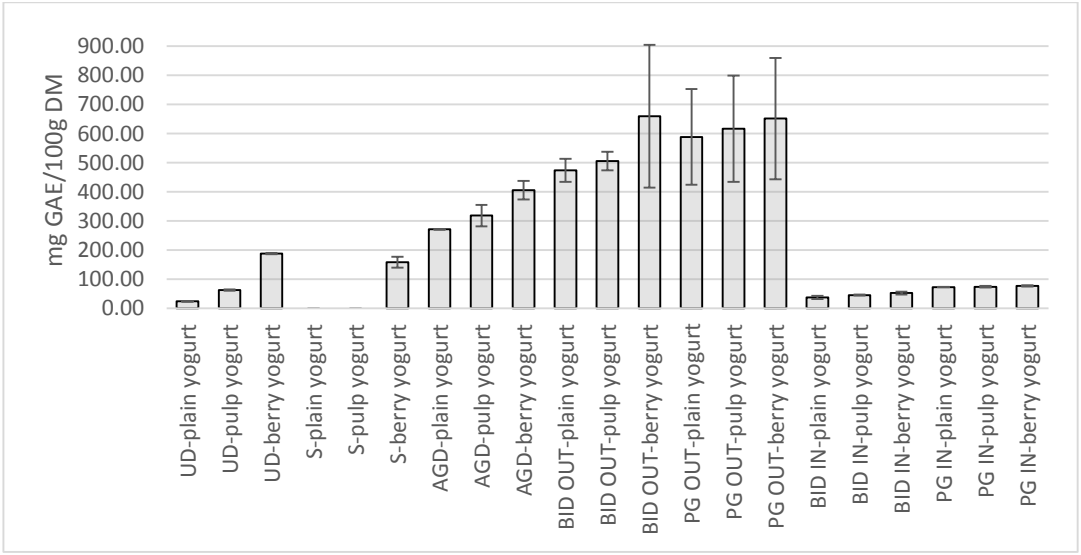

Figure E.3: Total phenolic content of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) n=2

Table E.4: Total phenolic content of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 8 days stored) (mg GAE/100g DM)[1,2]

Type of sample	Type of Fruit Added	BF1-8	BF2-8	Mean Value ± Standard Deviation
UD-plain yogurt	No	23.6	22.5	23.1±0.80[c]
UD-pulp yogurt	Pulp	64.9	66.8	65.9±1.30[b]
UD-berry yogurt	Berries	191	189	190±1.40[a]
S-plain yogurt	No	ND	ND	ND
S-pulp yogurt	Pulp	3.10	24.5	13.8±15.1[b]
S-berry yogurt	Berries	156	159	158±2.20[a]
AGD-plain yogurt	No	271	292	282±14.8[c]
AGD-pulp yogurt	Pulp	310	331	321±15.2[b]
AGD-berry yogurt	Berries	390	397	393±5.00[a]
BID-plain yogurt	No	454	400	427±38.2[b]
BID-pulp yogurt	Pulp	466	449	458±12.2[ab]
BID-berry yogurt	Berries	499	503	501±3.30[a]
OUT-plain yogurt	No	409	430	420±15.5[a]
OUT-pulp yogurt	Pulp	417	444	431±19.2[a]
OUT-berry yogurt	Berries	486	497	492±7.30[a]
BID IN-plain yogurt	No	42.6	38.3	40.4±3.10[c]
BID IN-pulp yogurt	Pulp	44.6	41.9	43.3±1.90[b]
BID IN-berry yogurt	Berries	57.8	58.8	58.3±0.70[a]
PG IN-plain yogurt	No	72.9	73.8	73.4±0.70[c]
PG IN-pulp yogurt	Pulp	77.0	75.1	76.1±1.30[b]
PG IN-berry yogurt	Berries	77.6	78.6	78.1±0.70[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

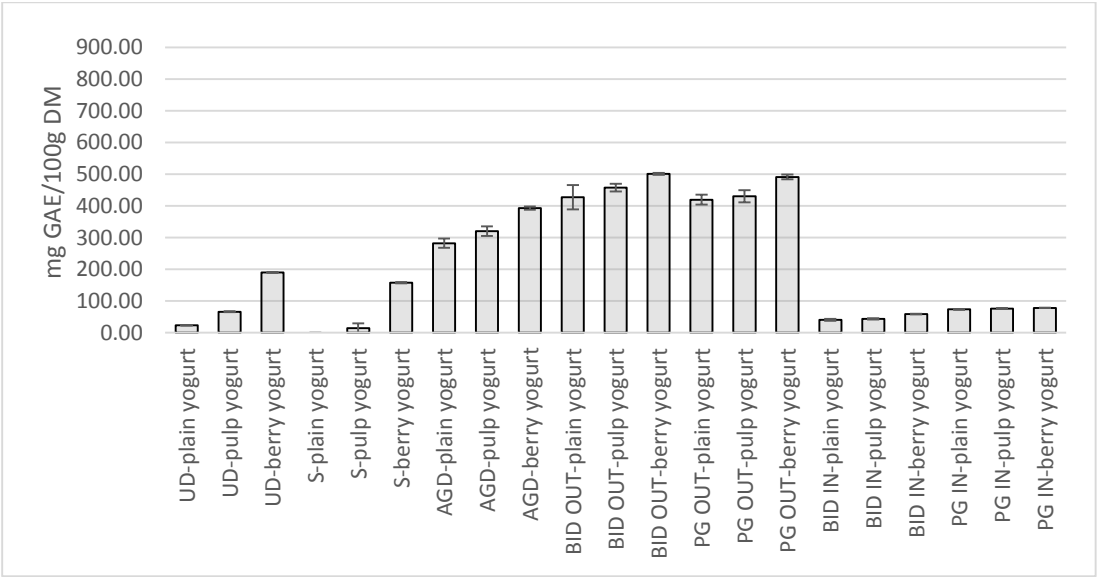

Figure E.4: Total phenolic content of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 8 days stored) n=2

Table E.5: Total monomeric anthocyanin content of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 1 day stored) (mg cya-3-glu equivalent/100g DM)[1,2]

Type of sample	Type of Fruit Added	AF1-1	AF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	No	ND	ND	ND
UD-pulp yogurt	Pulp	18.9	26.3	22.6 ±5.22[b]
UD-berry yogurt	Berries	103	123.6	113 ±14.3[a]
S-plain yogurt	No	ND	ND	ND
S-pulp yogurt	Pulp	16.8	16.5	16.7±0.26[b]
S-berry yogurt	Berries	86.2	81.3	83.7 ±3.49[a]
AGD-plain yogurt	No	ND	ND	ND
AGD-pulp yogurt	Pulp	17.6	14.3	15.9 ±2.33[b]
AGD-berry yogurt	Berries	88.5	83.5	86.0 ±3.52[a]
BID-plain yogurt	No	ND	ND	ND
BID-pulp yogurt	Pulp	8.79	7.69	8.24 ±0.78[b]
BID-berry yogurt	Berries	28.7	55.7	42.2 ±19.0[a]
OUT-plain yogurt	No	ND	ND	ND
OUT-pulp yogurt	Pulp	4.39	6.59	5.49 ±1.55[b]
OUT-berry yogurt	Berries	28.7	35.6	32.2 ±4.86[a]
BID IN-plain yogurt	No	ND	ND	ND
BID IN-pulp yogurt	Pulp	0.26	0.68	0.47 ±0.30[b]
BID IN-berry yogurt	Berries	3.70	4.94	4.32 ±0.87[a]
PG IN-plain yogurt	No	ND	ND	ND
PG IN-pulp yogurt	Pulp	0.43	0.77	0.60 ±0.24[b]
PG IN-berry yogurt	Berries	6.28	8.05	7.17 ±1.25[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion (p< 0.05).

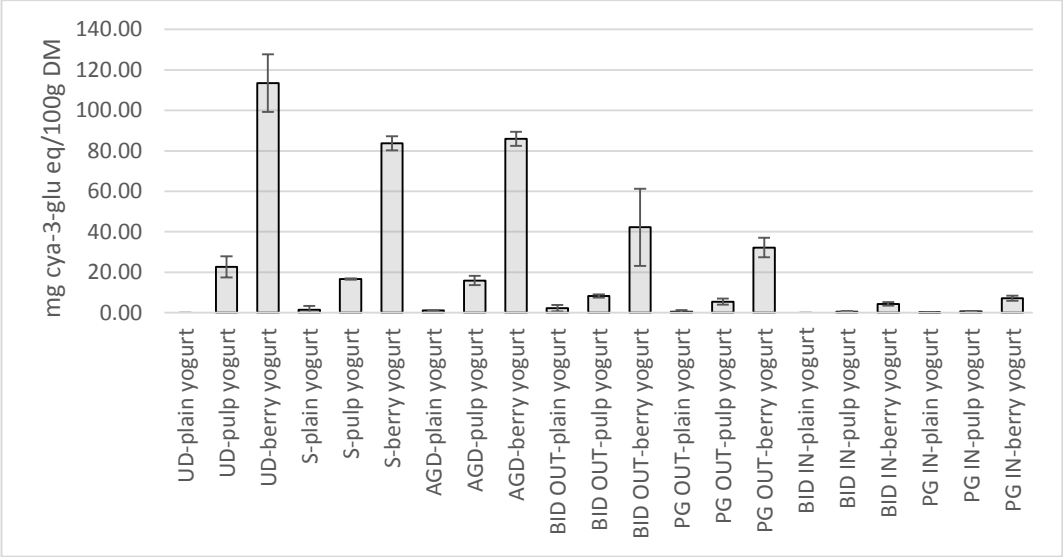

Figure E.5: Total monomeric anthocyanin content of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 1 day stored) n = 2

Table E.6: Total monomeric anthocyanin content of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 8 days stored) (mg cya-3-glu equivalent/100g DM)[1,2]

Type of sample	Type of Fruit Added	AF1-8	AF2-8	Mean Value ± Standard Deviation
UD-plain yogurt	No	ND	ND	ND
UD-pulp yogurt	Pulp	20.7	24.2	22.4 ±2.43[b]
UD-berry yogurt	Berries	110	116	113 ±4.40[a]
S-plain yogurt	No	ND	ND	ND
S-pulp yogurt	Pulp	23.7	16.5	20.1 ± 5.11[b]
S-berry yogurt	Berries	106	81.3	93.6 ±17.5[a]
AGD-plain yogurt	No	ND	ND	ND
AGD-pulp yogurt	Pulp	19.7	14.3	17.0 ±3.79[b]
AGD-berry yogurt	Berries	112	83.5	98.0 ±20.2[a]
BID-plain yogurt	No	ND	ND	ND
BID-pulp yogurt	Pulp	6.55	7.69	7.12 ±0.81[b]
BID-berry yogurt	Berries	56.0	55.7	55.8 ±0.26[a]
OUT-plain yogurt	No	ND	ND	ND
OUT-pulp yogurt	Pulp	7.64	6.59	7.12 ±0.74[b]
OUT-berry yogurt	Berries	32.5	35.6	34.1 ±2.21[a]
BID IN-plain yogurt	No	ND	ND	ND
BID IN-pulp yogurt	Pulp	0.42	0.68	0.55 ±0.18[b]
BID IN-berry yogurt	Berries	1.31	4.94	3.12 ±2.57[a]
PG IN-plain yogurt	No	ND	ND	ND
PG IN-pulp yogurt	Pulp	0.42	0.77	0.60 ±0.24[b]
PG IN-berry yogurt	Berries	3.49	8.05	5.77±3.23[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

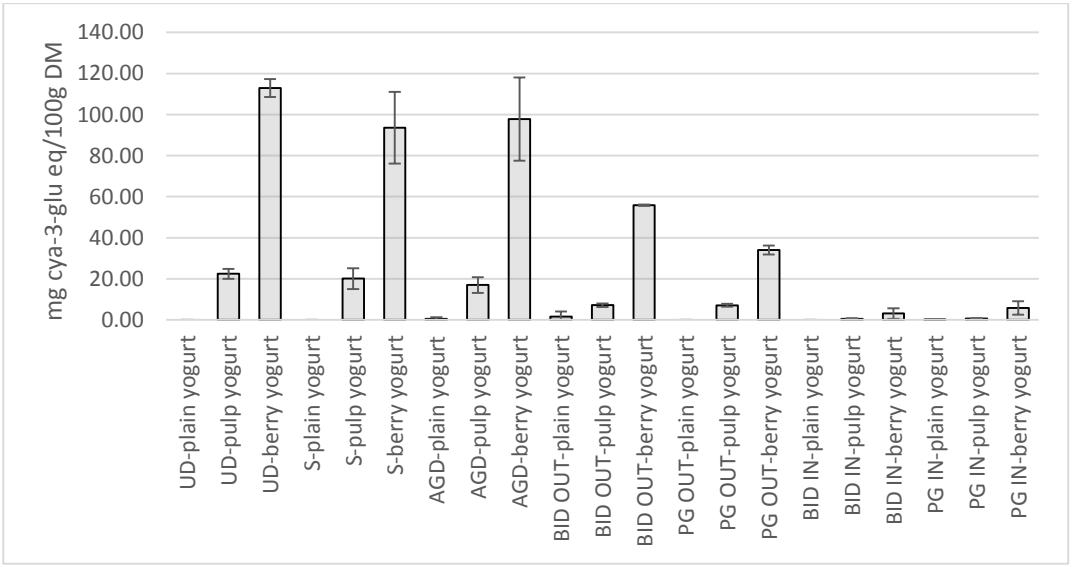

Figure E.6: Total monomeric anthocyanin content of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 8 days stored) n = 2

Table E.7: Total monomeric anthocyanin content of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) (mg cya-3-glu equivalent/100g DM)[1,2]

Type of sample	Type of Fruit Added	BF1-1	BF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	No	ND	ND	ND
UD-pulp yogurt	Pulp	17.6	17.3	17.5 ±0.25[b]
UD-berry yogurt	Berries	80.5	80.4	80.4 ±0.03[a]
S-plain yogurt	No	ND	ND	ND
S-pulp yogurt	Pulp	15.7	12.1	13.9 ±2.59[b]
S-berry yogurt	Berries	75.8	64.6	70.2 ±7.94[a]
AGD-plain yogurt	No	ND	ND	ND
AGD-pulp yogurt	Pulp	16.8	15.4	16.1 ±1.01[b]
AGD-berry yogurt	Berries	70.3	64.6	67.4 ±4.06[a]
BID-plain yogurt	No	ND	ND	ND
BID-pulp yogurt	Pulp	8.97	4.39	6.68 ±3.23[b]
BID-berry yogurt	Berries	49.4	33.4	41.4 ±11.3[a]
OUT-plain yogurt	No	ND	ND	ND
OUT-pulp yogurt	Pulp	3.36	3.30	3.33 ±0.05[b]
OUT-berry yogurt	Berries	31.9	23.4	27.6 ±6.00[a]
BID IN-plain yogurt	No	ND	ND	ND
BID IN-pulp yogurt	Pulp	0.35	0.94	0.64 ±0.42[b]
BID IN-berry yogurt	Berries	2.65	2.68	2.67 ±0.02[a]
PG IN-plain yogurt	No	ND	ND	ND
PG IN-pulp yogurt	Pulp	0.26	0.94	0.60 ±0.48[b]
PG IN-berry yogurt	Berries	3.25	4.76	4.00 ±1.07[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion (p< 0.05).

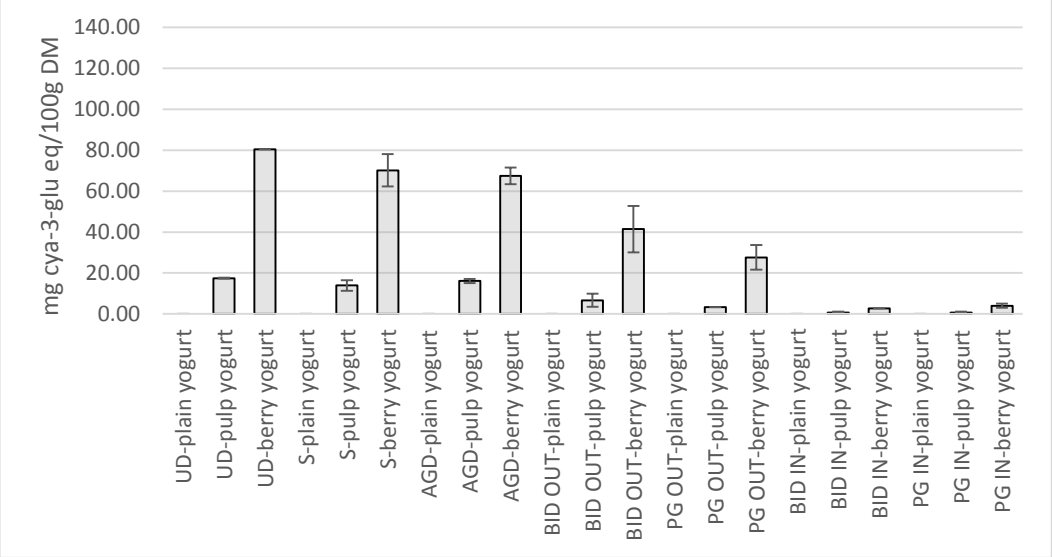

Figure E.7: Total monomeric anthocyanin content of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) n=2

Table E.8: Total monomeric anthocyanin content of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 8 days stored) (mg cya-3-glu equivalent/100g DM)[1,2]

Type of sample	Type of Fruit Added	BF1-8	BF2-8	Mean Value ± Standard Deviation
UD-plain yogurt	No	ND	ND	ND
UD-pulp yogurt	Pulp	14.6	11.3	13.0±2.32[b]
UD-berry yogurt	Berries	76.9	70.4	73.7±4.59[a]
S-plain yogurt	No	ND	ND	ND
S-pulp yogurt	Pulp	10.1	9.03	9.56±0.75[b]
S-berry yogurt	Berries	61.5	55.7	58.6±4.14[a]
AGD-plain yogurt	No	ND	ND	ND
AGD-pulp yogurt	Pulp	12.3	10.1	11.2±1.54[b]
AGD-berry yogurt	Berries	64.8	55.7	60.2±6.47[a]
BID-plain yogurt	No	ND	ND	ND
BID-pulp yogurt	Pulp	1.12	10.1	5.64±6.39[b]
BID-berry yogurt	Berries	33.0	35.6	34.3±1.89[a]
OUT-plain yogurt	No	ND	ND	ND
OUT-pulp yogurt	Pulp	3.36	3.38	3.37±0.02[b]
OUT-berry yogurt	Berries	22.0	21.1	21.7±0.58[a]
BID IN-plain yogurt	No	ND	ND	ND
BID IN-pulp yogurt	Pulp	0.26	0.44	0.35±0.13[b]
BID IN-berry yogurt	Berries	2.31	0.35	1.33±1.39[a]
PG IN-plain yogurt	No	ND	ND	ND
PG IN-pulp yogurt	Pulp	0.35	0.44	0.39±0.06[b]
PG IN-berry yogurt	Berries	4.02	3.46	3.74±0.39[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

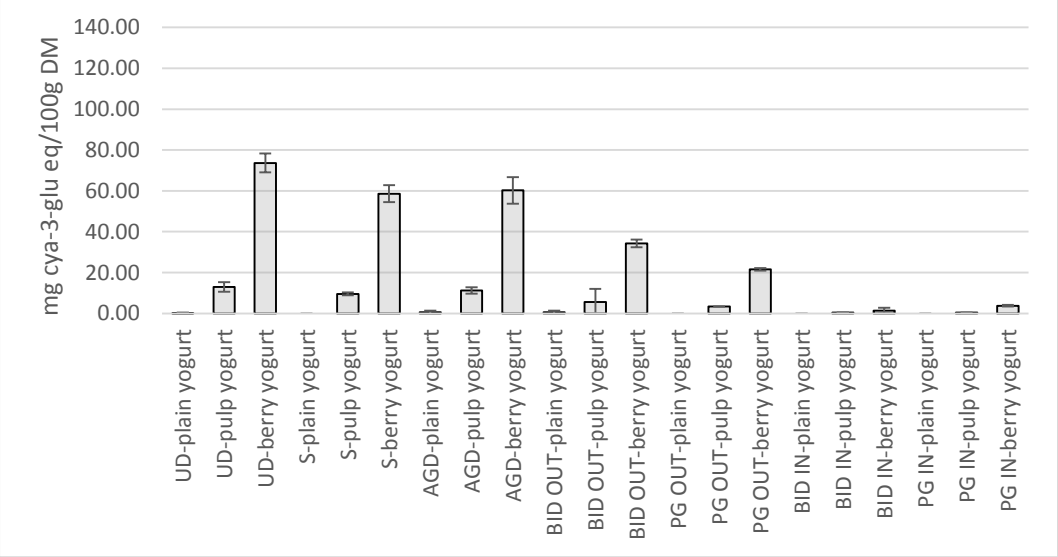

Figure E.8: Total monomeric anthocyanin content of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 8 days stored) n=2

Table E.9: DPPH radical scavenging activities of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 1 day stored) (mM TEAC/100 g DM)[1,2]

Type of sample	Type of Fruit Added	AF1-1	AF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	No	0.17	0.15	0.16 ±0.02[c]
UD-pulp yogurt	Pulp	0.36	0.39	0.38 ±0.02[b]
UD-berry yogurt	Berries	1.25	1.22	1.24 ±0.02[a]
S-plain yogurt	No	0.18	0.34	0.26 ±0.12[b]
S-pulp yogurt	Pulp	0.14	0.82	0.48 ±0.48[b]
S-berry yogurt	Berries	2.10	1.46	1.78 ±0.46[a]
AGD-plain yogurt	No	0.08	0.46	0.27 ±0.27[b]
AGD-pulp yogurt	Pulp	0.30	0.66	0.48 ±0.25[b]
AGD-berry yogurt	Berries	1.04	1.32	1.18 ±0.19[a]
BID-plain yogurt	No	ND	0.22	0.11 ±0.00[b]
BID-pulp yogurt	Pulp	ND	0.46	0.23 ±0.33[b]
BID-berry yogurt	Berries	0.51	1.52	1.02 ±0.72[a]
OUT-plain yogurt	No	ND	0.13	0.06 ±0.00[b]
OUT-pulp yogurt	Pulp	ND	0.39	0.20 ±0.28[b]
OUT-berry yogurt	Berries	0.88	1.36	1.12 ±0.34[a]
BID IN-plain yogurt	No	ND	ND	ND
BID IN-pulp yogurt	Pulp	0.01	ND	0.01 ±0.01[b]
BID IN-berry yogurt	Berries	0.15	0.10	0.13 ±0.03[a]
PG IN-plain yogurt	No	ND	ND	ND
PG IN-pulp yogurt	Pulp	0.02	0.03	0.03 ±0.01[b]
PG IN-berry yogurt	Berries	0.24	0.26	0.25 ±0.01[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

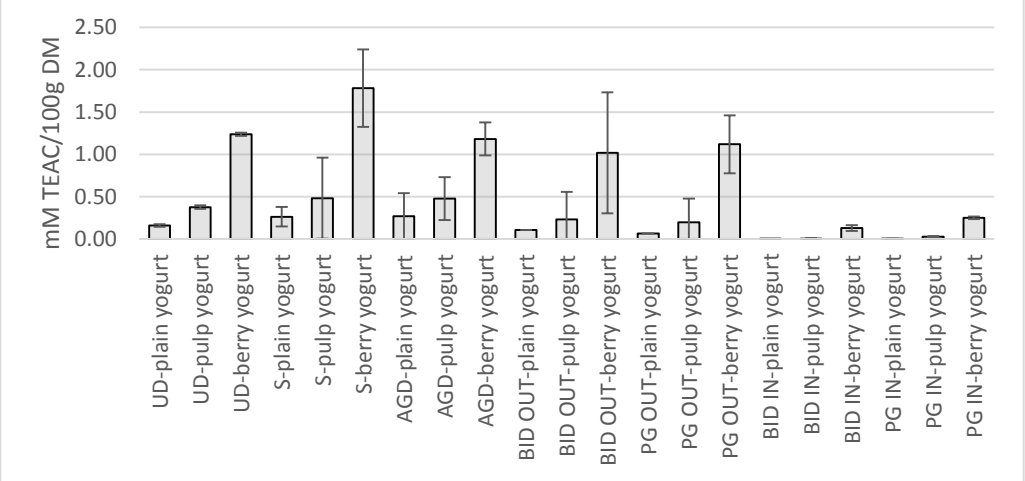

Figure E.9: DPPH radical scavenging activities of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 1 day stored) n=2

Table E.10: DPPH radical scavenging activities of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 8 days stored) (mM TEAC/100 g DM)[1,2]

Type of sample	Type of Fruit Added	AF1-8	AF2-8	Mean Value ± Standard Deviation
UD-plain yogurt	No	0.17	0.16	0.17 ±0.01[c]
UD-pulp yogurt	Pulp	0.41	0.41	0.41 ±0.00[b]
UD-berry yogurt	Berries	1.21	1.26	1.23 ±0.03[a]
S-plain yogurt	No	ND	0.25	0.12 ±0.00[b]
S-pulp yogurt	Pulp	0.66	0.59	0.63 ±0.05[b]
S-berry yogurt	Berries	1.25	1.33	1.29 ±0.05[a]
AGD-plain yogurt	No	ND	0.48	0.24 ±0.34[b]
AGD-pulp yogurt	Pulp	0.12	0.57	0.34 ±0.32[b]
AGD-berry yogurt	Berries	0.90	1.14	1.02 ±0.17[a]
BID-plain yogurt	No	ND	0.29	0.15 ±0.21[b]
BID-pulp yogurt	Pulp	ND	0.31	0.16 ±0.22[b]
BID-berry yogurt	Berries	0.91	1.34	1.13 ±0.30[a]
OUT-plain yogurt	No	ND	0.20	0.10 ±0.00[b]
OUT-pulp yogurt	Pulp	ND	0.31	0.16 ±0.22[b]
OUT-berry yogurt	Berries	1.05	1.61	1.33 ±0.40[a]
BID IN-plain yogurt	No	ND	ND	ND
BID IN-pulp yogurt	Pulp	ND	ND	ND
BID IN-berry yogurt	Berries	0.08	0.05	0.07 ±0.02[a]
PG IN-plain yogurt	No	0.01	0.02	0.01 ±0.01[c]
PG IN-pulp yogurt	Pulp	0.04	0.05	0.05 ±0.01[b]
PG IN-berry yogurt	Berries	0.23	0.25	0.24 ±0.02[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

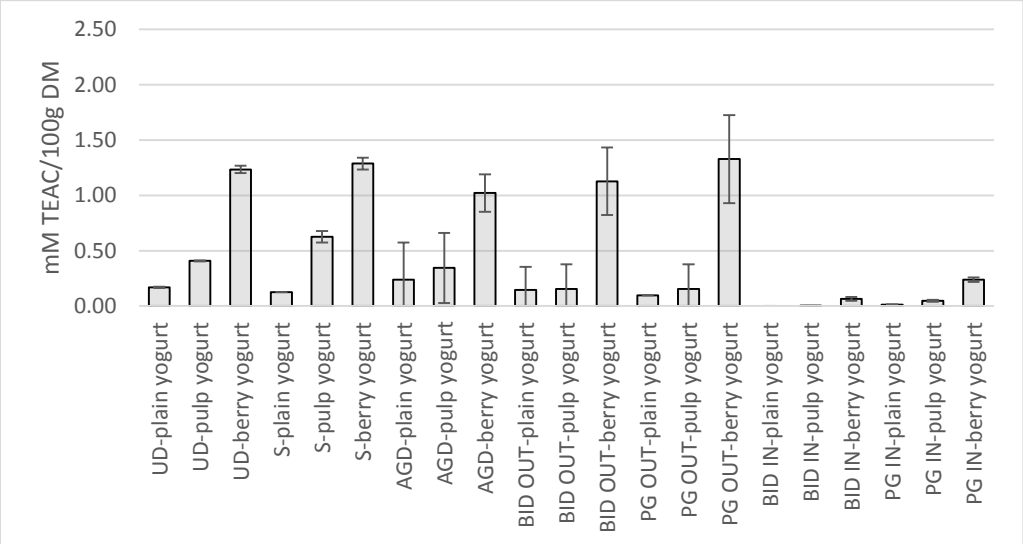

Figure E.10: DPPH radical scavenging activities of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 8 days stored) n=2

Table E.11: DPPH radical scavenging activities of digested yogurt samples (Berry or pulp before after fermentation, 1st and 2nd batch, 1 day stored) (mM TEAC/100 g DM)[1,2]

Type of sample	Type of Fruit Added	BF1-1	BF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	No	0.17	0.13	0.15 ±0.03[c]
UD-pulp yogurt	Pulp	0.39	0.35	0.37 ±0.03[b]
UD-berry yogurt	Berries	1.03	1.06	1.05 ±0.02[a]
S-plain yogurt	No	0.18	0.27	0.22 ±0.07[b]
S-pulp yogurt	Pulp	0.64	0.43	0.54 ±0.15[b]
S-berry yogurt	Berries	1.00	1.34	1.17 ±0.24[a]
AGD-plain yogurt	No	0.14	0.25	0.19 ±0.07[b]
AGD-pulp yogurt	Pulp	0.35	0.48	0.42 ±0.09[b]
AGD-berry yogurt	Berries	0.94	1.28	1.11 ±0.24[a]
BID-plain yogurt	No	0.01	0.16	0.09 ±0.10[b]
BID-pulp yogurt	Pulp	0.35	0.34	0.35 ±0.00[b]
BID-berry yogurt	Berries	1.13	1.36	1.24 ±0.16[a]
OUT-plain yogurt	No	ND	0.20	0.10 ±0.14[b]
OUT-pulp yogurt	Pulp	0.14	0.23	0.19 ±0.06[b]
OUT-berry yogurt	Berries	0.78	1.20	0.99 ±0.30[a]
BID IN-plain yogurt	No	ND	ND	ND
BID IN-pulp yogurt	Pulp	ND	ND	ND
BID IN-berry yogurt	Berries	0.05	0.02	0.03 ±0.02[a]
PG IN-plain yogurt	No	0.02	0.01	0.02 ±0.01[c]
PG IN-pulp yogurt	Pulp	0.03	0.05	0.04 ±0.02[b]
PG IN-berry yogurt	Berries	0.20	0.23	0.22 ±0.02[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

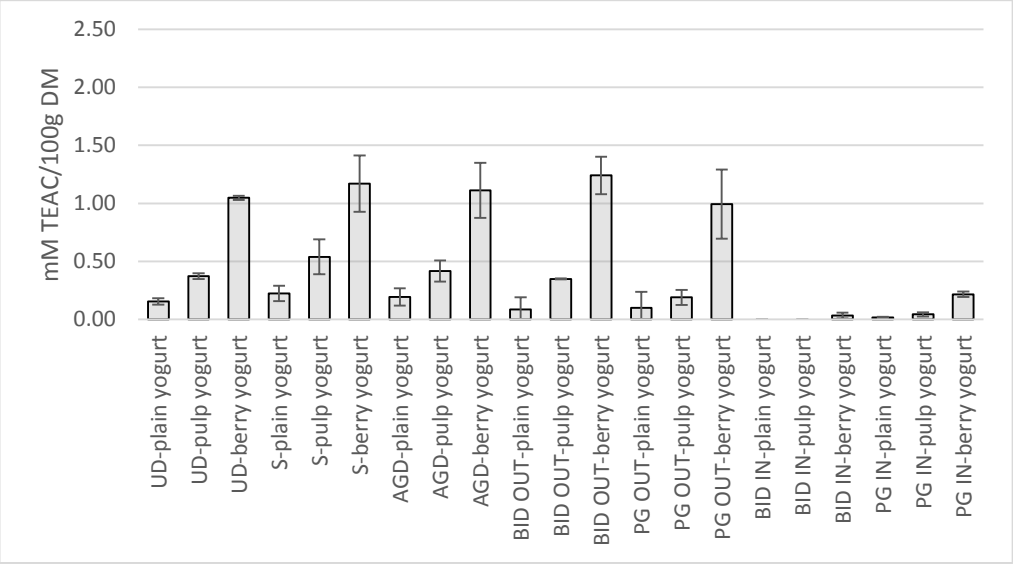

Figure E.11: DPPH radical scavenging activities of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) n=2

Table E.12: DPPH radical scavenging activities of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 8 days stored) (mM TEAC/100 g DM)[1,2]

Type of sample	Type of Fruit Added	BF1-8	BF2-8	Mean Value ± Standard Deviation
UD-plain yogurt	No	0.17	0.14	0.15 ±0.02[c]
UD-pulp yogurt	Pulp	0.34	0.31	0.33 ±0.02[b]
UD-berry yogurt	Berries	1.07	1.04	1.05 ±0.02[a]
S-plain yogurt	No	0.17	0.34	0.26 ±0.12[b]
S-pulp yogurt	Pulp	0.34	0.42	0.38 ±0.06[b]
S-berry yogurt	Berries	1.00	1.22	1.11 ±0.16[a]
AGD-plain yogurt	No	0.06	0.21	0.13 ±0.11[b]
AGD-pulp yogurt	Pulp	0.29	0.28	0.28 ±0.01[b]
AGD-berry yogurt	Berries	1.08	1.23	1.16 ±0.10[a]
BID-plain yogurt	No	ND	0.15	0.07 ±0.10[b]
BID-pulp yogurt	Pulp	0.10	0.41	0.25 ±0.22[b]
BID-berry yogurt	Berries	1.15	1.34	1.25 ±0.14[a]
OUT-plain yogurt	No	0.06	0.16	0.11 ±0.07[b]
OUT-pulp yogurt	Pulp	0.45	0.39	0.42 ±0.04[b]
OUT-berry yogurt	Berries	0.84	1.17	1.00 ±0.23[a]
BID IN-plain yogurt	No	ND	ND	ND
BID IN-pulp yogurt	Pulp	ND	ND	ND
BID IN-berry yogurt	Berries	0.04	0.02	0.03 ±0.01[a]
PG IN-plain yogurt	No	ND	0.01	0.00 ±0.01[c]
PG IN-pulp yogurt	Pulp	0.03	0.04	0.03 ±0.01[b]
PG IN-berry yogurt	Berries	0.22	0.21	0.22 ±0.01[a]

[1]Data were given as the mean values ± standard deviations of duplicates. ND: Non Detected. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

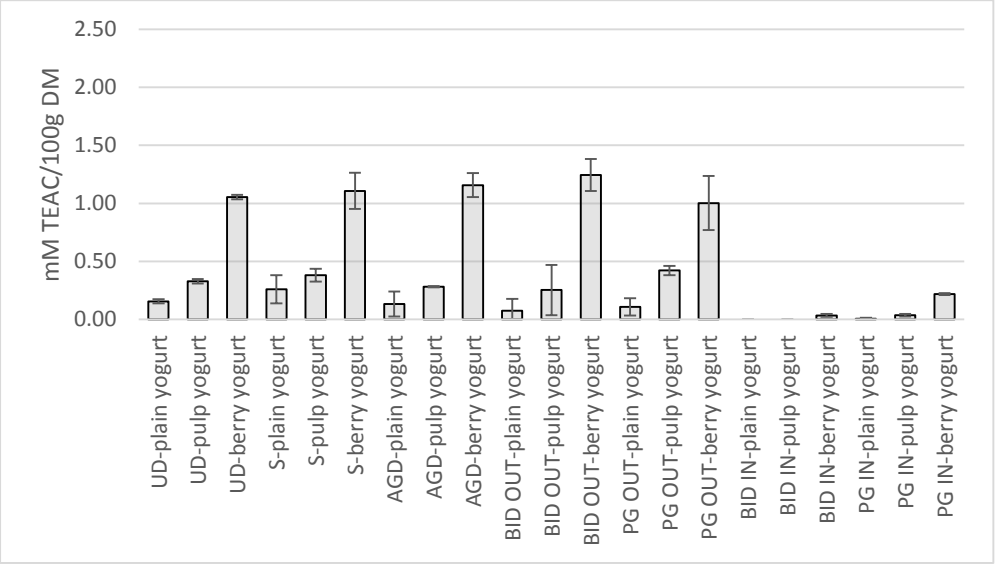

Figure E.12: DPPH radical scavenging activities of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 8 days stored) n=2

Table E.13: Cupric reducing antioxidant capacities (CUPRAC) of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 1 day stored) (mM TEAC/100 g DM)[1,2]

Type of sample	Type of Fruit Added	AF1-1	AF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	No	0.25	0.09	0.17 ±0.11[c]
UD-pulp yogurt	Pulp	0.78	0.72	0.75 ±0.05[b]
UD-berry yogurt	Berries	3.24	3.10	3.17 ±0.10[a]
S-plain yogurt	No	6.08	3.51	1.35 ±0.64[a]
S-pulp yogurt	Pulp	5.68	3.76	1.25 ±0.22[a]
S-berry yogurt	Berries	9.33	5.54	3.97 ±1.49[a]
AGD-plain yogurt	No	4.32	4.91	2.00 ±0.42[b]
AGD-pulp yogurt	Pulp	5.01	5.11	2.42 ±0.03[b]
AGD-berry yogurt	Berries	6.87	7.35	4.49 ±0.35[a]
BID-plain yogurt	No	8.99	2.82	6.34 ±0.05[b]
BID-pulp yogurt	Pulp	10.9	10.7	8.15 ±0.16[b]
BID-berry yogurt	Berries	11.5	10.4	8.32 ±0.78[a]
PG OUT-plain yogurt	No	9.76	8.61	6.57 ±0.81[c]
PG OUT-pulp yogurt	Pulp	11.9	11.2	8.92 ±0.50[b]
PG OUT-berry yogurt	Berries	12.1	11.6	9.23 ±0.34[a]
BID IN-plain yogurt	No	0.25	0.23	0.04 ±0.01[b]
BID IN-pulp yogurt	Pulp	0.29	0.43	0.16 ±0.09[b]
BID IN-berry yogurt	Berries	0.78	0.61	0.49 ±0.12[a]
PG IN-plain yogurt	No	0.59	0.26	0.22 ±0.23[b]
PG IN-pulp yogurt	Pulp	0.33	0.55	0.24 ±0.15[ab]
PG IN-berry yogurt	Berries	0.67	0.70	0.48 ±0.02[a]

[1]Data were given as the mean values ± standard deviations of duplicates. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

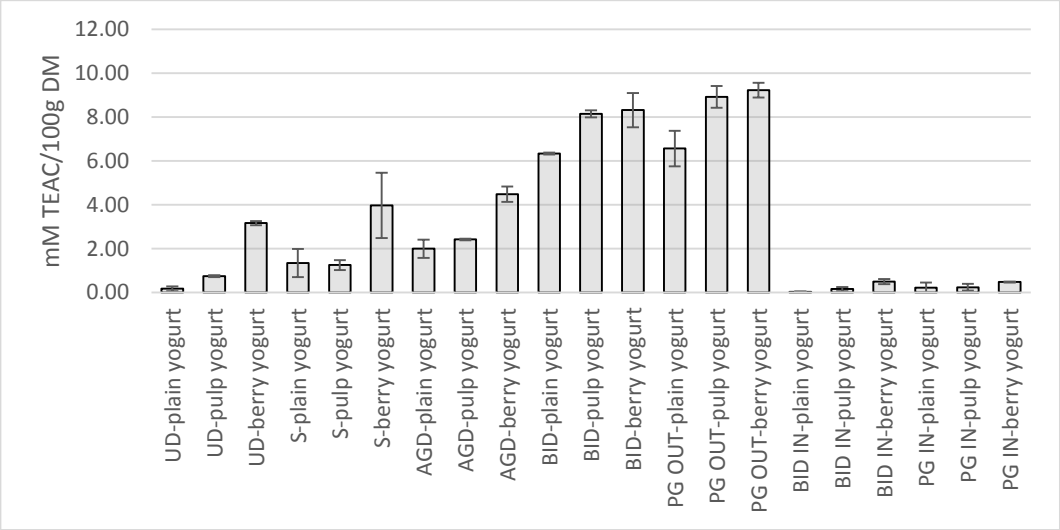

Figure E.13: Cupric reducing antioxidant capacities (CUPRAC) of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 1 day stored) n=2

Table E.14: Cupric reducing antioxidant capacities (CUPRAC) of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 8 days stored) (mM TEAC/100 g DM)[1,2]

Type of sample	Type of Fruit Added	AF1-8	AF2-8	Mean Value ± Standard Deviation
UD-plain yogurt	No	0.20	0.35	0.27 ±0.11[c]
UD-pulp yogurt	Pulp	0.95	0.68	0.81 ±0.19[b]
UD-berry yogurt	Berries	2.98	2.88	2.93 ±0.07[a]
S-plain yogurt	No	4.14	3.71	0.54 ±0.76[a]
S-pulp yogurt	Pulp	5.87	4.32	1.66 ±0.05[a]
S-berry yogurt	Berries	8.52	5.40	3.46 ±1.00[a]
AGD-plain yogurt	No	4.73	3.89	1.69 ±0.61[b]
AGD-pulp yogurt	Pulp	4.63	4.59	2.01 ±0.04[b]
AGD-berry yogurt	Berries	6.29	6.53	3.75 ±0.18[a]
BID-plain yogurt	No	7.98	9.47	6.10 ±1.04[b]
BID-pulp yogurt	Pulp	8.38	8.57	5.87 ±0.13[b]
BID-berry yogurt	Berries	12.0	13.1	9.90 ±0.78[a]
PG OUT-plain yogurt	No	9.34	9.58	6.83 ±0.16[c]
PG OUT-pulp yogurt	Pulp	9.59	10.5	7.43 ±0.62[b]
PG OUT-berry yogurt	Berries	10.8	11.5	8.48 ±0.49[a]
BID IN-plain yogurt	No	0.31	0.39	0.15 ±0.05[b]
BID IN-pulp yogurt	Pulp	0.41	0.42	0.21 ±0.00[b]
BID IN-berry yogurt	Berries	0.54	0.53	0.33 ±0.01[a]
PG IN-plain yogurt	No	0.57	0.46	0.31 ±0.08[b]
PG IN-pulp yogurt	Pulp	0.61	0.37	0.29 ±0.17[ab]
PG IN-berry yogurt	Berries	0.87	0.85	0.65 ±0.01[a]

[1]Data were given as the mean values ± standard deviations of duplicates. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

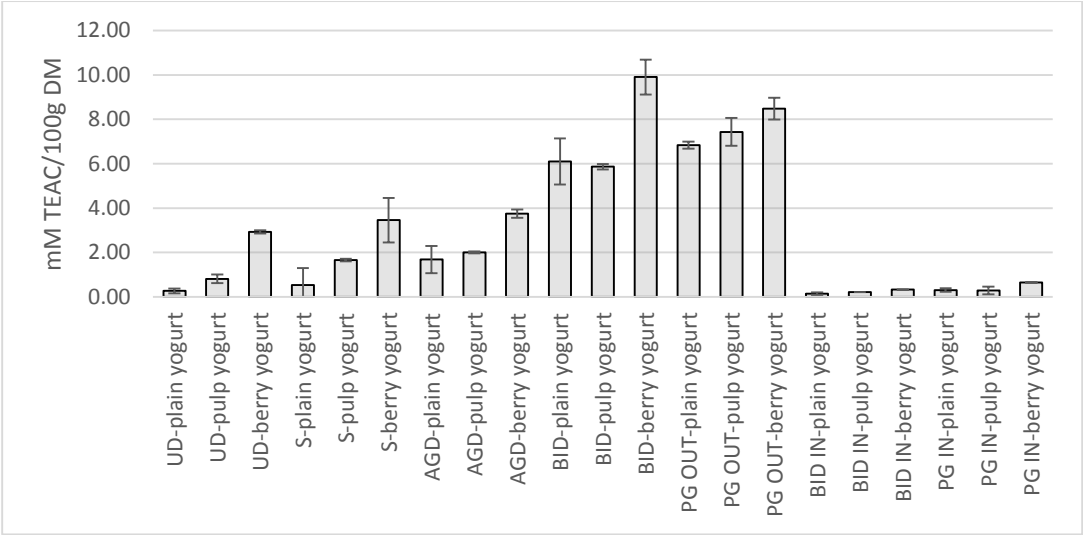

Figure E.14: Cupric reducing antioxidant capacities (CUPRAC) of digested yogurt samples (Berry or pulp added after fermentation, 1st and 2nd batch, 8 days stored) n=2

Table E.15: Cupric reducing antioxidant capacities (CUPRAC) of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) (mM TEAC/100 g DM)[1,2]

Type of sample	Type of Fruit Added	BF1-1	BF2-1	Mean Value ± Standard Deviation
UD-plain yogurt	No	0.25	0.09	0.30 ±0.03[c]
UD-pulp yogurt	Pulp	0.78	0.72	0.93 ±0.16[b]
UD-berry yogurt	Berries	3.24	3.10	2.79 ±0.04[a]
S-plain yogurt	No	6.08	3.51	0.13 ±0.19[a]
S-pulp yogurt	Pulp	5.68	3.76	1.10 ±0.97[a]
S-berry yogurt	Berries	9.33	5.54	3.67 ±0.54[a]
AGD-plain yogurt	No	4.32	4.91	1.56 ±0.25[b]
AGD-pulp yogurt	Pulp	5.01	5.11	2.00 ±0.78[b]
AGD-berry yogurt	Berries	6.87	7.35	3.32 ±0.22[a]
BID-plain yogurt	No	8.99	2.82	6.91 ±1.36[b]
BID-pulp yogurt	Pulp	10.9	10.7	6.44 ±0.14[b]
BID-berry yogurt	Berries	11.5	10.4	8.40 ±0.59[a]
PG OUT-plain yogurt	No	9.76	8.61	6.57 ±0.22[c]
PG OUT-pulp yogurt	Pulp	11.9	11.2	7.23 ±0.02[b]
PG OUT-berry yogurt	Berries	12.1	11.6	8.40 ±0.30[a]
BID IN-plain yogurt	No	0.25	0.23	0.26 ±0.20[b]
BID IN-pulp yogurt	Pulp	0.29	0.43	0.20 ±0.05[b]
BID IN-berry yogurt	Berries	0.78	0.61	0.29 ±0.02[a]
PG IN-plain yogurt	No	0.59	0.26	0.23 ±0.09[b]
PG IN-pulp yogurt	Pulp	0.33	0.55	0.30 ±0.16[ab]
PG IN-berry yogurt	Berries	0.67	0.70	0.35 ±0.13[a]

[1]Data were given as the mean values ± standard deviations of duplicates. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

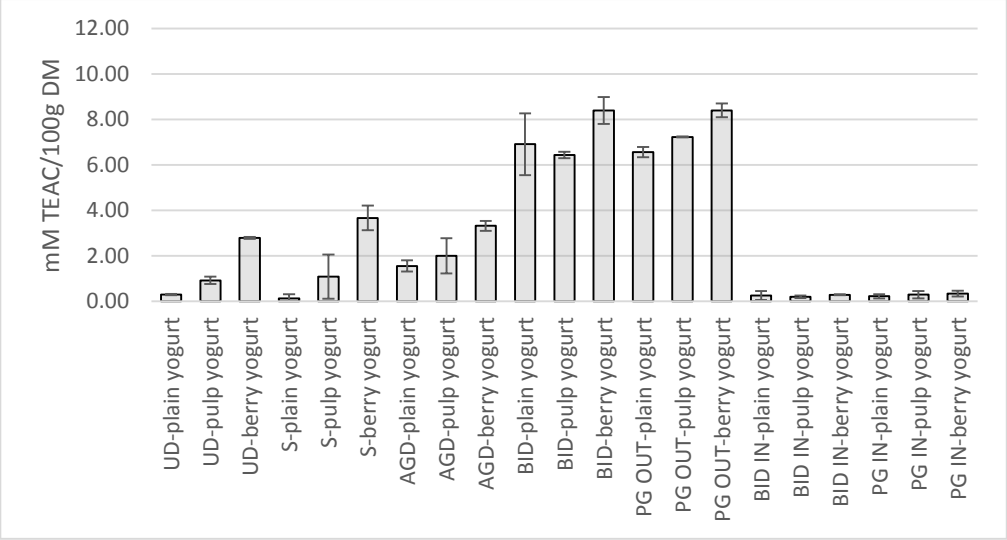

Figure E.15: Cupric reducing antioxidant capacities (CUPRAC) of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 1 day stored) n=2

Table E.16: Cupric ion reducing antioxidant capacities (CUPRAC) of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 8 days stored) (mM TEAC/100 g DM)[1,2]

Type of sample	Type of Fruit Added	BF1-8	BF2-8	Mean Value ± Standard Deviation
UD-plain yogurt	No	0.99	1.00	0.34 ±0.01[c]
UD-pulp yogurt	Pulp	1.35	1.20	0.60 ±0.11[b]
UD-berry yogurt	Berries	3.12	3.51	2.65 ±0.27[a]
S-plain yogurt	No	3.99	3.41	1.08 ±0.40[a]
S-pulp yogurt	Pulp	3.80	3.25	0.85 ±0.40[a]
S-berry yogurt	Berries	5.05	5.75	2.77 ±0.47[a]
AGD-plain yogurt	No	3.78	3.97	1.25 ±0.14[b]
AGD-pulp yogurt	Pulp	5.01	5.01	2.33 ±0.01[b]
AGD-berry yogurt	Berries	7.63	7.20	4.78 ±0.33[a]
BID-plain yogurt	No	8.87	9.27	6.45 ±0.28[b]
BID-pulp yogurt	Pulp	9.03	11.0	7.31 ±1.35[b]
BID-berry yogurt	Berries	11.0	11.4	8.59 ±0.28[a]
PG OUT-plain yogurt	No	9.54	9.51	6.90 ±0.01[c]
PG OUT-pulp yogurt	Pulp	9.92	9.84	7.20 ±0.07[b]
PG OUT-berry yogurt	Berries	10.4	10.8	7.93 ±0.27[a]
BID IN-plain yogurt	No	0.26	0.33	0.09 ±0.05[b]
BID IN-pulp yogurt	Pulp	0.32	0.35	0.13 ±0.02[b]
BID IN-berry yogurt	Berries	0.50	0.57	0.33 ±0.05[a]
PG IN-plain yogurt	No	0.35	0.39	0.17 ±0.03[b]
PG IN-pulp yogurt	Pulp	0.77	0.39	0.37 ±0.27[ab]
PG IN-berry yogurt	Berries	0.45	0.47	0.25 ±0.01[a]

[1]Data were given as the mean values ± standard deviations of duplicates. [2]Different letters in the column represent statistically significant differences between plain, pulp added and berries added yogurts for each fraction of digestion ($p < 0.05$).

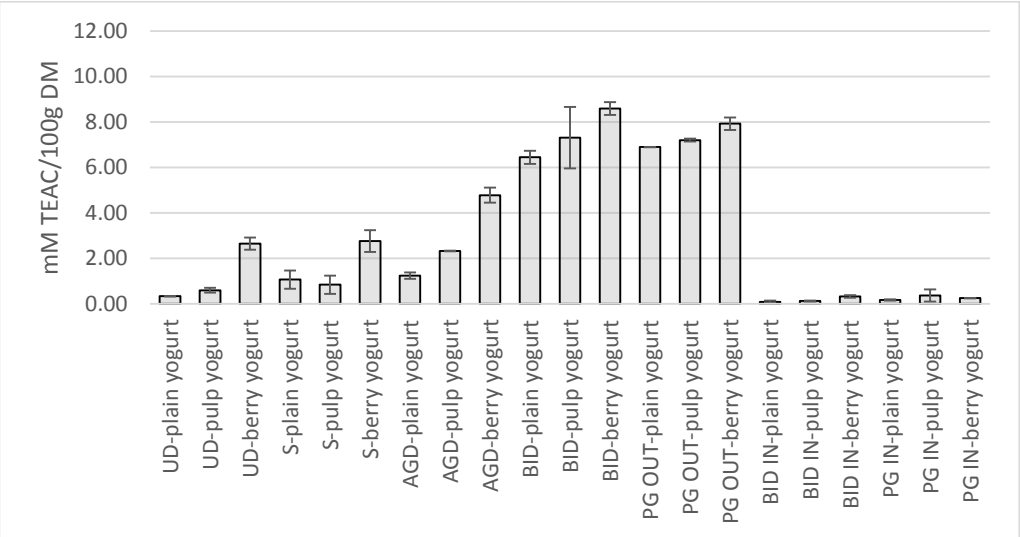

Figure E.16: Cupric reducing antioxidant capacities (CUPRAC) of digested yogurt samples (Berry or pulp added before fermentation, 1st and 2nd batch, 8 days stored) n=2

Appendix F. Results of Statistical Analysis

Table F.1: Statistical analysis results of undigested and digested yogurt samples for total phenolic content

		Sum of Squares	df	Mean Square	F	Sig.
UD	Between Groups	123608.033	2	61804.016	1622.287	.000
	Within Groups	800.034	21	38.097		
	Total	124408.066	23			
S	Between Groups	133062.510	2	66531.255	186.115	.000
	Within Groups	7506.950	21	357.474		
	Total	140569.460	23			
AGD	Between Groups	63313.781	2	31656.890	35.292	.000
	Within Groups	18836.739	21	896.988		
	Total	82150.520	23			
BID_OUT	Between Groups	43541.102	2	21770.551	3.942	.035
	Within Groups	115989.038	21	5523.288		
	Total	159530.140	23			
PG_OUT	Between Groups	24112.013	2	12056.007	1.227	.313
	Within Groups	206355.496	21	9826.452		
	Total	230467.510	23			
BID_IN	Between Groups	1808.576	2	904.288	18.473	.000
	Within Groups	1027.978	21	48.951		
	Total	2836.553	23			
PG_IN	Between Groups	113.276	2	56.638	26.607	.000
	Within Groups	44.703	21	2.129		
	Total	157.978	23			

Table F.2: Post Hoc Test for undigested (UD) yogurt samples for total phenolic content

UD

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	26.7625		
	2.00	8		67.0750	
	3.00	8			195.1000
	Sig.		1.000	1.000	1.000

Table F.3: Post Hoc Test for yogurt samples taken from oral phase (S) of digestion for total phenolic content

S

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.0000	
	2.00	8	10.5750	
	3.00	8		162.9750
	Sig.		.514	1.000

Table F.4: Post Hoc Test for yogurt samples taken from after gastric digestion (AGD) for total phenolic content

AGD

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	296.7250		
	2.00	8		338.1875	
	3.00	8			420.3250
	Sig.		1.000	1.000	1.000

Table F.5: Post Hoc Test for yogurt samples taken from before intestinal digestion (BID-OUT) for total phenolic content

BID_OUT

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	456.1375	
	2.00	8	503.0625	503.0625
	3.00	8		560.3000
	Sig.		.431	.293

Table F.6: Post Hoc Test for yogurt samples taken from post gastrointestinal digestion (PG-OUT) for total phenolic content

PG_OUT

	Fruit	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	1.00	8	477.5875
	2.00	8	509.0375
	3.00	8	554.7875
	Sig.		.286

Table F.7: Post Hoc Test for dialysed fraction of yogurt samples taken from before intestinal digestion (BID-IN) for total phenolic content

BID_IN

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	38.5875		
	2.00	8		49.4125	
	3.00	8			59.8500
	Sig.		1.000	1.000	1.000

Table F.8: Post Hoc Test for dialysed fraction of yogurt samples taken from post gastrointestinal digestion (PG-IN) for total phenolic content

PG_IN

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	72.3250		
	2.00	8		74.7125	
	3.00	8			77.6375
	Sig.		1.000	1.000	1.000

Table F.9: Statistical analysis results of undigested and digested yogurt samples for total anthocyanin content

		Sum of Squares	df	Mean Square	F	Sig.
UD	Between Groups	40574.017	2	20287.009	138.570	.000
	Within Groups	3074.445	21	146.402		
	Total	43648.462	23			
S	Between Groups	26130.441	2	13065.220	139.346	.000
	Within Groups	1968.976	21	93.761		
	Total	28099.417	23			
AGD	Between Groups	27015.384	2	13507.692	122.871	.000
	Within Groups	2308.613	21	109.934		
	Total	29323.997	23			
BID_OUT	Between Groups	8426.802	2	4213.401	83.854	.000
	Within Groups	1055.190	21	50.247		
	Total	9481.992	23			
PG_OUT	Between Groups	3797.845	2	1898.923	145.161	.000
	Within Groups	274.712	21	13.082		
	Total	4072.557	23			
BID_IN	Between Groups	37.993	2	18.997	21.413	.000
	Within Groups	18.630	21	.887		
	Total	56.624	23			
PG_IN	Between Groups	128.373	2	64.187	46.204	.000
	Within Groups	29.173	21	1.389		
	Total	157.546	23			

Table F.10: Post Hoc Test for undigested (UD) yogurt samples for total anthocyanin content

UD

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.0338		
	2.00	8		18.8750	
	3.00	8			95.1363
	Sig.		1.000	1.000	1.000

Table F.11: Post Hoc Test for yogurt samples taken from oral phase (S) of digestion for total anthocyanin content

S

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.3513		
	2.00	8		15.0588	
	3.00	8			76.5325
	Sig.		1.000	1.000	1.000

Table F.12: Post Hoc Test for yogurt samples taken from after gastric digestion (AGD) for total anthocyanin content

AGD

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.5538		
	2.00	8		15.0575	
	3.00	8			77.8600
	Sig.		1.000	1.000	1.000

Table F.13: Post Hoc Test for yogurt samples taken from before intestinal digestion (BID-OUT) for total anthocyanin content

BID_OUT

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	1.1050	
	2.00	8	6.9188	
	3.00	8		43.4413
	Sig.		.251	1.000

Table F.14: Post Hoc Test for yogurt samples taken from post gastrointestinal digestion (PG-OUT) for total anthocyanin content

PG_OUT

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.1375		
	2.00	8		4.8263	
	3.00	8			28.8563
	Sig.		1.000	1.000	1.000

Table F.15: Post Hoc Test for dialysed fraction of yogurt samples taken from before intestinal digestion (BID-IN) for total anthocyanin content

BID_IN

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.0000	
	2.00	8	.4355	
	3.00	8		2.8600
	Sig.		.631	1.000

Table F.16: Post Hoc Test for dialysed fraction of yogurt samples taken from post gastrointestinal digestion (PG-IN) for total anthocyanin content

PG_IN

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.0225	
	2.00	8	.5475	
	3.00	8		5.1700
	Sig.		.652	1.000

Table F.17: Statistical analysis results of undigested and digested yogurt samples for DPPH assay

		Sum of Squares	df	Mean Square	F	Sig.
UD	Between Groups	4.299	2	2.150	551.158	.000
	Within Groups	.082	21	.004		
	Total	4.381	23			
S	Between Groups	5.423	2	2.712	45.410	.000
	Within Groups	1.254	21	.060		
	Total	6.677	23			
AGD	Between Groups	3.709	2	1.854	63.876	.000
	Within Groups	.610	21	.029		
	Total	4.319	23			
BID_OUT	Between Groups	5.230	2	2.615	52.110	.000
	Within Groups	1.054	21	.050		
	Total	6.283	23			
PG_OUT	Between Groups	4.847	2	2.423	60.763	.000
	Within Groups	.838	21	.040		
	Total	5.684	23			
BID_IN	Between Groups	.021	2	.011	16.087	.000
	Within Groups	.014	21	.001		
	Total	.035	23			
PG_IN	Between Groups	.233	2	.116	599.488	.000
	Within Groups	.004	21	.000		
	Total	.237	23			

Table F.18: Post Hoc Test for undigested (UD) yogurt samples for DPPH assay

UD

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.1575		
	2.00	8		.3700	
	3.00	8			1.1425
	Sig.		1.000	1.000	1.000

Table F.19: Post Hoc Test for yogurt samples taken from oral phase (S) of digestion for DPPH assay

S

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.2163		
	2.00	8	.5050		
	3.00	8		1.3375	
	Sig.		.069	1.000	

Table F.20: Post Hoc Test for yogurt samples taken from after gastric digestion (AGD) for DPPH assay

AGD

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.2100	
	2.00	8	.3813	
	3.00	8		1.1163
	Sig.		.134	1.000

Table F.21: Post Hoc Test for yogurt samples taken from before intestinal digestion (BID-OUT) for DPPH assay

BID_OUT

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.1038	
	2.00	8	.2463	
	3.00	8		1.1575
	Sig.		.426	1.000

Table F.22: Post Hoc Test for yogurt samples taken from post gastrointestinal digestion (PG-OUT) for DPPH assay

PG_OUT

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.0938	
	2.00	8	.2388	
	3.00	8		1.1113
	Sig.		.334	1.000

Table F.23: Post Hoc Test for dialysed fraction of yogurt samples taken from before intestinal digestion (BID-IN) for DPPH assay

BID_IN

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.0000	
	2.00	8	.0013	
	3.00	8		.0638
	Sig.		.995	1.000

Table F.24: Post Hoc Test for dialysed fraction of yogurt samples taken from post gastrointestinal digestion (PG-IN) for DPPH assay

PG_IN

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.0088		
	2.00	8		.0363	
	3.00	8			.2300
	Sig.		1.000	1.000	1.000

Table F.25: Statistical analysis results of undigested and digested yogurt samples for CUPRAC assay

		Sum of Squares	df	Mean Square	F	Sig.
UD	Between Groups	30.816	2	15.408	526.185	.000
	Within Groups	.615	21	.029		
	Total	31.431	23			
S	Between Groups	2407.506	2	1203.753	.923	.413
	Within Groups	27381.979	21	1303.904		
	Total	29789.484	23			
AGD	Between Groups	26.634	2	13.317	55.138	.000
	Within Groups	5.072	21	.242		
	Total	31.706	23			
BID_OUT	Between Groups	24.678	2	12.339	15.709	.000
	Within Groups	16.495	21	.785		
	Total	41.173	23			
PG_OUT	Between Groups	12.924	2	6.462	17.222	.000
	Within Groups	7.880	21	.375		
	Total	20.804	23			
BID_IN	Between Groups	.238	2	.119	13.573	.000
	Within Groups	.184	21	.009		
	Total	.422	23			
PG_IN	Between Groups	.170	2	.085	3.865	.037
	Within Groups	.462	21	.022		
	Total	.632	23			

Table F.26: Post Hoc Test for undigested (UD) yogurt samples for CUPRAC assay

UD

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.2708		
	2.00	8		.7746	
	3.00	8			2.8865
	Sig.		1.000	1.000	1.000

Table F.27: Post Hoc Test for yogurt samples taken from oral phase (S) of digestion for CUPRAC assay

S

	Fruit	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	1.00	8	.7763
	3.00	8	3.4675
	2.00	8	23.2400
	Sig.		.441

Table F.28: Post Hoc Test for yogurt samples taken from after gastric digestion (AGD) for CUPRAC assay

AGD

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	1.6213		
	2.00	8	2.1888		
	3.00	8		4.0850	
	Sig.		.076	1.000	

Table F.29: Post Hoc Test for yogurt samples taken from before intestinal digestion (BID-OUT) for CUPRAC assay

BID_OUT

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	6.4488	
	2.00	8	6.9425	
	3.00	8		8.8038
	Sig.		.516	1.000

Table F.30: Post Hoc Test for yogurt samples taken from post gastrointestinal digestion (PG-OUT) for CUPRAC assay

PG_OUT

	Fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	6.7163		
	2.00	8		7.6963	
	3.00	8			8.5113
	Sig.		1.000	1.000	1.000

Table F.31: Post Hoc Test for dialysed fraction of yogurt samples taken from before intestinal digestion (BID-IN) for CUPRAC assay

BID_IN

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.1350	
	2.00	8	.1763	
	3.00	8		.3638
	Sig.		.658	1.000

Table F.32: Post Hoc Test for dialysed fraction of yogurt samples taken from post gastrointestinal digestion (PG-IN) for CUPRAC assay

PG_IN

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.2313	
	2.00	8	.2988	.2988
	3.00	8		.4338
	Sig.		.640	.187

Table F.33: Statistical analysis results of undigested and digested yogurt samples for individual anthocyanins determined by HPLC analysis

		Sum of Squares	df	Mean Square	F	Sig.
cya_3_galactoside	Between Groups	20086.158	2	10043.079	79.547	.000
	Within Groups	2651.321	21	126.253		
	Total	22737.478	23			
cya_3_glucoside	Between Groups	17.595	2	8.797	265.214	.000
	Within Groups	.697	21	.033		
	Total	18.291	23			
cya_3_arabinoside	Between Groups	3266.792	2	1633.396	129.221	.000
	Within Groups	265.447	21	12.640		
	Total	3532.240	23			
cya_3_xyloside	Between Groups	45.281	2	22.641	140.414	.000
	Within Groups	3.386	21	.161		
	Total	48.667	23			
total_anthocyanin	Between Groups	43890.145	2	21945.073	95.426	.000
	Within Groups	4829.379	21	229.970		
	Total	48719.524	23			

Table F.34: Post Hoc Test for cyanidin-3-galactoside

cya_3_galactoside

	fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.0000		
	2.00	8	11.8363		
	3.00	8		66.4250	
	Sig.		.113	1.000	

Table F.35: Post Hoc Test for cyanidin-3-galactoside

cya_3_glucoside

	fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.0000		
	2.00	8		.2637	
	3.00	8			1.9338
	Sig.		1.000	1.000	1.000

Table F.36: Post Hoc Test for cyanidin-3-arabinoside

cya_3_arabinoside

	fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.0000		
	2.00	8		4.9138	
	3.00	8			26.8375
	Sig.		1.000	1.000	1.000

Table F.37: Post Hoc Test for cyanidin-3-xyloside

cya_3_xyloside

	fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	8	.0000	
	2.00	8	.3765	
	3.00	8		3.0838
	Sig.		.171	1.000

Table F.38: Post Hoc Test for cyanidin-3-xyloside

total_anthocyanin

	fruit	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1.00	8	.0000		
	2.00	8	17.3838		
	3.00	8		98.1500	
	Sig.		.079	1.000	

Table F.39: Statistical analysis results of undigested and digested yogurt samples for degree of hydrolysis assay

		Sum of Squares	df	Mean Square	F	Sig.
UD	Between Groups	7.000	2	3.500	1.167	.422
	Within Groups	9.000	3	3.000		
	Total	16.000	5			
S	Between Groups	60.333	2	30.167	10.647	.043
	Within Groups	8.500	3	2.833		
	Total	68.833	5			
AGD	Between Groups	57.000	2	28.500	8.143	.061
	Within Groups	10.500	3	3.500		
	Total	67.500	5			
BID_OUT	Between Groups	277.333	2	138.667	6.351	.084
	Within Groups	65.500	3	21.833		
	Total	342.833	5			
PG_OUT	Between Groups	4.333	2	2.167	.050	.952
	Within Groups	130.500	3	43.500		
	Total	134.833	5			

Table F.40: Post Hoc Test for undigested (UD) yogurt samples for degree of hydrolysis assay

UD

	Fruit	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	3.00	2	115.0000
	2.00	2	115.5000
	1.00	2	117.5000
	Sig.		.427
Duncan[a]	3.00	2	115.0000
	2.00	2	115.5000
	1.00	2	117.5000
	Sig.		.243

Table F.41: Post Hoc Test for yogurt samples taken from oral phase (S) of digestion for degree of hydrolysis assay

S

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1.00	2	122.5000	
	3.00	2	128.0000	128.0000
	2.00	2		130.0000
	Sig.		.092	.535

Table F.42: Post Hoc Test for yogurt samples taken from after gastric digestion (AGD) for degree of hydrolysis assay

AGD

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	3.00	2	137.0000	
	2.00	2	140.0000	
	1.00	2	144.5000	
	Sig.		.056	

Table F.43: Post Hoc Test for yogurt samples taken from before intestinal digestion (BID-OUT) for degree of hydrolysis assay

BID_OUT

	Fruit	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	3.00	2	218.5000	
	1.00	2	230.5000	
	2.00	2	234.5000	
	Sig.		.082	

Table F.44: Post Hoc Test for yogurt samples taken from post gastrointestinal digestion (PG-OUT) for degree of hydrolysis assay

PG_OUT

	Fruit	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	3.00	2	219.0000
	2.00	2	219.5000
	1.00	2	221.0000
	Sig.		.951

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Çatalkaya, G., Çapanoğlu, E., Raes, K., 2015; *In vitro* Bioaccessibility of Anthocyanins in Black Chokeberry (*Aronia Melanocarpa*) Added Yogurts. 4th International Conference of Food Digestion, March 2015, Naples, Italy (Poster presentation).

Other Publications, Presentations and Patents:

Ceylan, F. D., **Çatalkaya, G.,** Çapanoğlu, E., 2014; Biogenic Amines in Traditional Fermented Turkish Foods. International Food Congress on Novel Approaches in Food Industry, May 2014, Kuşadası, Turkey (Poster presentation).

Çatalkaya, G., Ceylan, F.D., Yüce, H., Eskicioğlu, V., Çapanoğlu, E., Erdil, D., 2014; A Review on Multi-Residue Pesticide Analysis of Fruits and Vegetables by Gas Chromatography (GC) and Liquid Chromatography (LC). International Food Congress on Novel Approaches in Food Industry, May 2014, Kuşadası, Turkey (Poster presentation).