

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY

**ANTIOXIDANT ACTIVITY AND POLYPHENOL COMPOSITION OF
SESAME PASTE AND GRAPE MOLASSES BLENDS**

M.Sc. THESIS

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Department of Food Engineering

Food Engineering Programme

JUNE 2014

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

**TAHİN-PEKMEZ KARIŞIMLARININ ANTIOKSİDAN AKTİVİTESİ VE
POLİFENOL İÇERİKLERİNİN İNCELENMESİ**

YÜKSEK LİSANS TEZİ

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Date of Submission : 5 May 2014
Date of Defense : 2 June 2014

To my family,

FOREWORD

Health, the most critical consideration for human is mainly related to nutritional factors. People all consume foods or food blends ordinarily without knowing their health effects. Besides ignoring those effects, there are also misunderstandings in the food field since the interactions within the blends may change the health effects. The purpose of this study was to reveal the effects of consuming a food having high protein content with another food having high polyphenol content, to indicate the best ratio for the blend of grape molasses and sesame paste with respect to antioxidant activity and *in vitro* bioavailability of the products. I hope this study will enhance the current literature on the health characteristics of grape molasses, sesame paste and their blends.

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June 2014

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ABBREVIATIONS

ABTS	: 2,2- azinobis 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt
ANOVA	: Analysis of variance
AOAC	: Association Official of Analytical Chemists
CE	: Catechin Equivalent
CUPRAC	: Copper Reducing Antioxidant Capacity
DF	: Dilution factor
DPPH	: 1,1-Diphenyl-2- picrylhydrazyl
FAO	: Food and Agriculture Organization of the United Nations
FRAP	: Ferric Reducing Antioxidant Capacity
GA	: Gallic acid
GAE	: Gallic Acid Equivalent
GI	: Gastrointestinal
HCA	: Hydroxycinnamic acids
HPLC	: High Performance Liquid Chromatography
PDA	: Photodiode array
SPSS	: Statistical Package for the Social Sciences
TAC	: Total antioxidant capacity
TEAC	: Trolox Equivalent Antioxidant Capacity
TF	: Total flavonoid content
TP	: Total phenolic content
WB	: Wet Basis

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ANTIOXIDANT ACTIVITY AND POLYPHENOL COMPOSITION OF SESAME PASTE AND GRAPE MOLASSES BLENDS

SUMMARY

For long years, sesame paste and grape molasses blends are consumed in traditional breakfasts in Turkey due to their nutritional values and taste. Two products are known with their individual characteristics, *i.e.* grape molasses is considered as an energy or antioxidant source. However, changing characteristics during consumption of foods together is disregarded by consumers although the interactions in food constituents result in significant alterations in food matrix. In the literature, there exist examples to interactions between a variety of food constituents such as proteins, lipids, phenolic compounds and so on.

Grape molasses is rich in phenolic compounds whereas sesame paste has high protein content. Hence, interactions between phenolic compounds and proteins should be paid attention in terms of different characteristics. The studies show that that kind of interactions may result in changes in structural, functional and nutritional properties, and digestibility of proteins. Also these interactions may lead to changes in antioxidant capacity, total phenolic and/or flavonoids content of polyphenols, in addition to bioavailability.

The aim was to gain a better understanding about the changes in total phenolic, flavonoids and antioxidant capacity when sesame paste and grape molasses consumed together compared to individual consumptions. For that purpose, sesame paste, grape molasses and three blends with different percentages (50-50%, 70-30% and 30-70%) of both were analyzed before and after *in vitro* digestion procedure. Furthermore, to gain a better understanding to product characteristics, determination of moisture, protein and lipids as well as rheological analysis were performed.

While sesame paste has 3.9 % moisture, that of grape molasses was found as 82.5 %, and also the blends have moisture contents between the two products.

Protein contents of all samples were devised by Kjeldahl method. Grape molasses was found to have protein content of 0.09% whereas protein content of sesame paste was calculated as 28.5 %.

Lipid content of sesame paste was found as 55.3% while that of grape molasses was in trace amounts. Moreover, lipid contents of blends decrease with increasing grape molasses content.

For rheological characterization, viscometric measurements were conducted at 21 °C. The hysteresis loop was obtained by registering shear stress from 0.01 to 200 s⁻¹ in 120 s, held at 200s⁻¹ for 1 min between the two ramps and down in 120 s. After recording, n and K values for all samples were defined. Power law model was used to describe the non-Newtonian behavior of samples. Grape molasses showed

Newtonian behaviour since linear relationship was found between shear stress and shear rate in the experiments. On the other hand, for sesame paste, at constant shear rate, viscosity decreased by 1% in 1 minutes, helping us to understand the rheological behaviour of sesame paste as thixotropic. For blends, the values of flow behavior index, n , varied between 0.7 and 0.8 indicating shear-thinning behavior. The degree of pseudoplasticity decreases by increasing n value.

Additionally, in analyzed samples prior to gastrointestinal digestion, grape molasses showed higher contents of total phenolics, total flavonoids and antioxidant activity compared to sesame paste and three blends of 50-50%, 70-30% and 30-70%.

Blends had total phenolic contents close to proportional values with respect to two individual foods. However, their total flavonoid contents were found lower than proportional values probably resulted from the interactions between sesame proteins and catechin in the matrix. Antioxidant capacities of blends were between the two products and the results of the assays were similar for initial samples.

Correlation between spectrophotometric assays were calculated in order to relate the results with each other. Highest correlation was found between total phenolic content analysis with DPPH and ABTS assays (R^2 is equal to 0.989, 0.987, respectively) while that of total flavonoid content analysis was with ABTS assay (R^2 is equal to 0.962).

After stomach and pancreatin bile salt digestion, a significant decrease was observed in all dialyzed (IN) samples, half of the phenolic compounds were absorbed in small intestines.

In dialyzed blends, total phenolic contents were close to each other and a little lower than both products. Hence, total phenolic content of sesame paste seems to be absorbed easier than all others, since total phenolic content of dialyzed sesame paste is higher compared to others although it has low phenolic content at the beginning.

Furthermore, total flavonoid content of blends extracts were less than proportional values that may be caused by strongly binding characteristics of catechin to amino acids. By the way, catechin concentration may decrease in blends when being together with amino acids in the environment. Also, it is conducted that flavonoids can be absorbed more easily in blends than in grape molasses individually since even if grape molasses has highest total flavonoid content before pancreatic ingestion it has a total flavonoid content a little higher than that of blends.

Trolox equivalent antioxidant capacities (TAEC) of the two blends containing sesame paste 70% and 30%, respectively are lower than proportional values while half and half blend has shown higher TAEC value. The blends showed lower TAEC values rather than proportional values since binding of phenolic compounds to protein sites or molecules directly may lead to masking of polyphenol contents on the proteins. As well as total phenolic contents of dialyzed blends decreased less than the two products after *in vitro* digestion. Outcomes from assays were quite different. To illustrate, in ABTS assay, dialyzed blends have higher antioxidant activities rather than sesame paste or grape molasses although the masking effect of proteins could be detected easily in this assay as mentioned before. Hence, it can be deduced that after pancreatic ingestion, masking effect decreases and presumably changed adversely.

HPLC results were generally consistent with previous experiments. Dominant phenolic compounds in grape molasses were found as gallic acid, catechin, cinnamic acid and epicatechin; whereas that of sesame paste was sesamin.

TAHİN/PEKMEZ KARIŞIMLARININ ANTIOKSİDAN AKTİVİTESİ VE POLİFENOL İÇERİKLERİNİN İNCELENMESİ

ÖZET

Uzun yıllardır tahin ve pekmez karışımları gerek lezzeti gerekse besinsel değerleri gereği geleneksel Türk kahvaltılarının vazgeçilmezlerindedir.

Hem tahin hem pekmez tüketiciler tarafından tipik özellikleriyle bilinmektedir. Örnek olarak, pekmezin enerji ya da antioksidan kaynağı olarak tanınması gösterilebilir.

Fakat, gıdaların beraber tüketiminde içeriklerindeki bileşenlerin etkileşimleri sonucunda meydana gelen veya gelebilecek olanlar değişimlerden tüketicilerin pek haberi yoktur.

Literatür çalışmaları incelendiğinde, gıdaların yapısında bulunan birçok bileşenin birbiriyle etkileşime girebildiği görülmektedir. Protein, yağ veya fenolik maddelerin etkileşimleri üzerine örnekler bulmak mümkündür.

Tahin-pekmez karışımları göz önünde bulundurulduğunda; öncelikle pekmezin fenolik maddece zengin, tahinin yüksek protein içeriğine sahip bir ürün olduğu göz önünde bulundurulmalıdır. İki ürün karışım olarak tüketildiğinde ise bu iki bileşenin birbirleriyle etkileşimleri gıda matrisinde birçok değişime sebebiyet verebilir.

Çalışmalar gösteriyor ki bu tarz etkileşimler gıdaların yapısal, fonksiyonel ve besinsel değerlerinde değişiklikler ile sonuçlanabilir.

Aynı zamanda, antioksidan kapasitesi, fenolik ve flavonoid miktarlarının da bu etkileşimler ile değişiklik gösterebileceği gözlemlenmiştir.

Tez çalışmasının amacı, tahin ve pekmezin birlikte tüketilmesinin toplam fenolik, flavonoid ve antioksidan kapasitelerinde meydana getirebileceği değişiklikleri incelemek olmuştur.

Bu amaçla, tahin, pekmez ve farklı yüzdelerle sahip üç karışıma (%50-50, %70-30 ve %30-70) için *in vitro* gastrointestinal sindirim sisteminin simülasyonu öncesi ve sonrasında analizler yapılmıştır.

Ayrıca, ürün özelliklerinin daha iyi anlaşılmasını sağlamak amacıyla tahin, pekmez ve karışım numuneleri nem, protein ve lipid içeriği tayininin yanısıra reolojik analizlere de tabii tutulmuşlardır.

Ürün karakterizasyonu için yapılan analizler sonucunda nem oranı tahin için %3.9 iken bu oran pekmezde %82.5 olarak bulunmuştur. Karışımların nem yüzdeleri ise matematiksel olarak tahmin edildiği gibi iki ürünün yüzdelerinin aralığında değerler olarak bulunmuştur..

Bunun yanısıra numunelerin protein içerikleri Kjeldahl metodu ile ölçülmüştür. Protein miktarı pekmez için %0.09 olarak bulunurken tahin için %28.5 olarak hesaplanmıştır.

Yağ oranı tahin için %55.3 olarak bulunmuştur, pekmezde ise yağ eser miktardadır.

Reolojik ölçümler 21°C'de yapılmıştır. Histerezis döngüsü kayma gerilmesi aralığı 0.01 s⁻¹'de 200 s⁻¹ kadar (120 saniye içerisinde), 200 s⁻¹'de 1 dakika ve 200 s⁻¹'den 0.01s⁻¹'e yine 120 saniyede indirilerek elde edilmiştir.

Veriler sisteme kaydedildikten sonra, n ve K değerleri tüm numuneler için ölçülmüştür. Numunelerin Newtonsu olmayan davranışlarını saptamak adına Power law modeli kullanılmıştır.

Reolojik ölçümler sonucunda pekmez Newtonsu davranış kaydetmiştir. Kayma gerilmesi, kayma hızı ile lineer bir ilişki göstermiştir. Fakat, tahinin viskozitesinin sabit kayma hızında azaldığı gözlemlenmiştir ve davranışı tiksotropik olarak tanımlanmıştır. Karışımların n değerleri (0.7-0.8) kaymayla incelen davranışlarını göstermiştir.

Fenolik maddelerin yarıya yakını küçük bağırsakta emilmektedir ve analiz sonuçlarına göre de gastrointestinal sindirimden sonra, diyalize olmuş ürünlerin fenolik/flavonoid madde içeriklerine bakıldığında bir düşüş gözlemlenmektedir.

Bunun yanısıra ekstrakte edilmiş numunelere gastrointestinal sindirim prosedürü uygulanmadan önce yapılan analizlerde görülmüştür ki en yüksek fenolik ve flavonoid içerikleri pekmeze aitken en düşük değerleri tahin göstermiştir. Karışımlar ise iki ürünün verdiği sonuçların arasında değerlere sahip bulunmuştur.

Toplam fenolik analizi dikkate alındığında karışımların iki ürünün matematiksel oranıyla hesaplanmış beklenen değerlere yakın sonuçlar verdiği gözlemlenirken benzer sonuçlara flavonoid analizi sonucunda ulaşamamıştır.

Karışımlarda ise beklenenden daha düşük flavonoid analizlenebilmiştir. Bu durumu kateşinin ortamdaki amino asitler ile kuvvetli bağ yapma eğilimi ile ilişkilendirmek mümkündür.

Spektrofotometrik tahliller arasında ilişki kurabilmek için korrelasyon hesaplaması yapılmıştır. Bu hesapların sonucu göstermiştir ki en yüksek korrelasyon toplam fenolik analizi ile DPPH ve ABTS metotları arasında bulunurken (R² sırasıyla 0.989, 0.987); toplam flavonoid analizi ile en yüksek korrelasyonu ABTS metodu göstermiştir (R² 0.962).

Ayrıca, ekstrakte edilmiş pekmezin flavonoid madde içeriği en yüksekken, diyalize olmuş pekmezinki diğer ürünlerden çok da yüksek değildir. Bu da karışımların tüketiminde flavonoid emiliminin yalnız pekmez tüketimindekinden daha etkili olabileceğini göstermiştir.

Öte yandan, toplam antioksidan kapasiteleri %70 ve %30 tahin içerikli karışımlarda beklenenin altında çıkarken, %50'lik karışımlarda beklenenin üzerinde çıkmıştır. Beklenenin altında çıkan değerleri fenolik bileşenlerin proteince bağlanmasının polifenollerini maskeleymesi ile ilişkilendirmek mümkündür.

Toplam fenolik içeriği gibi, diyalize olmuş karışımların toplam antioksidan kapasiteleri *in vitro* gastrointestinal sindirim sistemi simülasyonundan sonra iki ürüne göre daha az bir düşüş göstermiştir.

Ek olarak, toplam antioksidan kapasitesi ölçme metotlarının sonuçlarının birbirlerinden farklı olduğunu söylemek mümkündür. Örneğin, ABTS sonuçlarına göre diyalize olmuş karışımların antioksidan kapasitelerinin tahin ve pekmezden daha yüksek olduğu gözlemlenmiştir.

Bu da göstermektedir ki ekstrakte olmuş karışımlarda proteinlerin fenolikleri maskeleyen eğilimi ön plana çıkarken bu ürünler gastrointestinal sistemde tam tersi bir etkileşim içindedirler.

HPLC sonuçları ile pekmezdeki baskın fenolik maddeler gallik asit, kateşin, sinamik asit ve epikateşin olarak saptanırken, tahinde sesamin olarak gözlemlenmiştir.

Ayrıca saptanan bu fenolik maddelerin gastrointestinal sindirim sonrasında miktarlarında genellikle bir azalma meydana geldiği gözlemlenmiştir. Bu sonuçlar önceki analizlerle çoğunlukla tutarlılık göstermiştir.

1. INTRODUCTION

1.1 Purpose of Thesis

In this study, the aim is to gain a better understanding about the changes in total phenolic, flavonoids and antioxidant capacity when the two products consumed together compared to individual consumptions. Also, bioavailability of the products was tried to be examined by following *in vitro* digestion procedure. This study is thought to provide a new perspective to sesame paste/ grape molasses blends in addition to contribution to protein/ phenolic interactions. For that purpose, sesame paste, grape molasses and three blends of both (70-30%, 50-50% and 30-70%) were analyzed before and after *in vitro* digestion procedure. To begin with, product characteristics of the blend were determined by analyzing moisture content, protein content, lipid content and finally rheological properties. Second section is composed of analysis related to polyphenols such as total phenolic, flavonoid, antioxidant capacity analyses with four assays that are CUPRAC, ABTS, DPPH and FRAP. Moreover, HPLC analysis was done so as to gain information about major individual components. Additionally, all samples are exposed to *in vitro* digestion and all polyphenol related analyses have been done to the obtained samples.

1.2 Literature Review

Consumption of grape molasses with sesame paste together is indispensable in traditional Turkish breakfasts with different ratios due to its high energy content and good taste. For years, the two products have been available in the market individually; however, nowadays their blends take its place on the shelf.

Grape molasses is one of the various grape products that is widely consumed in Turkey due to its nutritional quality. Dry soluble matter of grape molasses is about 70-80 %, consisting of sugars, mostly glucose and fructose, minerals and organic acids (Karaman *et al.*, 2011, Karababa *et al.*, 2005). Also, it is known with high antioxidant activity and by the way beneficial health effects such as anticancer

properties, and due to its high sugar content molasses can be consumed as an energy source (Goksel *et al.*, 2013).

Moreover, sesame paste is obtained from roasted dehulled sesame seeds by milling. It is a product rich in lipids (54–65%) and proteins (17–27%); moreover, it consists of carbohydrates (6.4–21%) and dietary fiber (9.3%) (Arslan *et al.*, 2005). It is a preferred ingredient to bakery foods, confectionery products and especially halva in Turkey in addition to its consumption with blends with some other products (Altay *et al.*, 2005). Hence, this blend has its own value as being a blend of a food with high protein content and another one with high phenolic content.

Rather than an energy source, this type of blends should be consumed by considering the interactions of proteins with phenolic compounds since their nutritional value is of preference besides taste. Studies in the literature widely focus on rheological characterization of this blend; however, it is hard to find a study examining interactions between protein and phenolic compounds. Interactions of proteins with phenolic compounds are important since structural, functional and nutritional properties, and digestibility of proteins are thought to be affected by these interactions just as by other interactions with lipids or others. Also these interactions result in changes in antioxidant capacity, total phenolic and/or flavonoids content of phenolic compounds, in addition to bioavailability (Ozdal *et al.*, 2013). Consumption of grape molasses with sesame paste together constitutes a good example to these types of products.

Moreover, there are studies examining other food blends with same characteristics such as milk chocolate or tea with milk considering the interactions between milk proteins and some polyphenols. However, a conclusion is hard to be reached because there are contradictory results within the studies.

In this study, the aim is to gain a better understanding about the changes in total phenolic, flavonoids and antioxidant capacity when the two products consumed together compared to individual consumptions. Also, bioavailability of the products was tried to be examined by following *in vitro* digestion procedure. This study is thought to provide a new perspective to sesame paste/ grape molasses blends in addition to contribution to protein/ phenolic interactions.

For that purpose, sesame paste, grape molasses and three blends of both (70-30%, 50-50% and 30-70%) were analyzed before and after *in vitro* digestion procedure. Ratio of grape molasses to sesame paste in this kind of a blend is preferable. The ratio is expected to affect the consequences of the interactions.

1.2.1 Grape molasses

There are different types of molasses produced from a variety of fruits such as grape, watermelon, mulberry, sugar beet containing sugar naturally (Batu, 2005). The most commonly consumed type of molasses is grape molasses that is also named as pekmez in Turkish.

Production steps of grape molasses (Figure 1.1) begin with the selection of grapes. Then stalks are removed by washing and crushing (Arslan *et al.*, 2005).

Later on, sample is transferred to the mash tank and kept there for 30-45 min prior to pneumatical or chemical pressing (Batu, 2005, Capanoglu *et al.*, 2013). The pressed sample called as grape juice is then treated with a calcareous soil known as 'pekmez earth' containing approximately 90% calcium carbonate which is responsible for causing precipitation of naturally existing tartaric and malic acids as calcium tartarate and calcium malate. So, a sedimentation step is needed to decrease the acidity and to provide clarification.

Also clarification can be done by centrifugation in order to get rid of suspended particles in the juice, then juice is depectinized by the addition of enzymes such as pectinase and amylase before a fining treatment to prevent cloudiness. The juice is then pasteurized to 100-107 C so as to be concentrated to a Brix value of 65-70 (Capanoglu *et al.*, 2013, Arslan *et al.*, 2005).

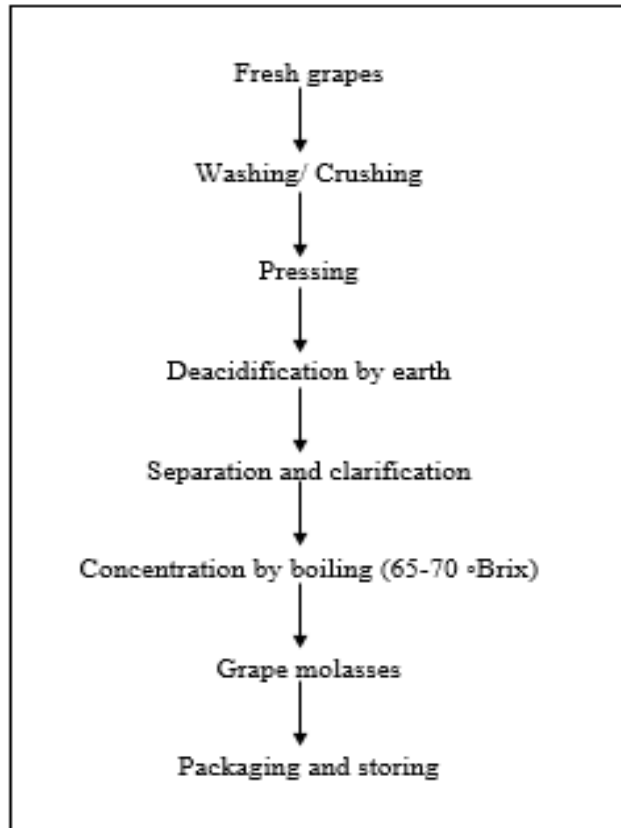


Figure 1.1 : Production process of grape molasses.

Grape molasses is considered as an energy source in Turkey due to its high carbohydrate content mostly fructose and glucose that is shown in Table 1.1 (Simsek *et al.*, 2002). Additionally, it is composed of some minerals such as calcium (0.084-0.086%) and iron (0.005-0.01%).

Also it has riboflavin, thiamin ve niacin vitamins catering for 20% of human need daily (Batu, 1993).

Furthermore, grape molasses has important health effects due to having high antioxidant activity owing to phenolic compounds such as phenolic acids and flavonoids (Ozdal *et al.*, 2013).

Table 1.1: Physical, chemical and mineral content of grape molasses (Batu, 2011).

Component	Total dry matter (%)	77.12
	pH	5.26
	Titratible acidity (%)	0.74
	HMF (mg/kg)	2.11
	Total carbohydrate (%)	64.13
	Glucose (%)	32.38
	Fructose (%)	31.75
	Total ash (%)	1.5
	Phosphor (P)	78
	Iron (Fe)	1.45
	Copper (Cu)	0.39
	Zinc (Zn)	0.12
	Potassium (K)	929
Mineral (mg/100 g)	Sodium (Na)	33
	Magnesium (Mg)	73
	Calcium (Ca)	132
Vitamin (mg/100g)	Riboflavin	0.15
	Thiamin	0.04
	Niacin	1.4

As seen in the table above, carbohydrate content of grape molasses is high and it is composed of mainly monosaccharides named as glucose and fructose. (Batu, 2011). By the way, it can be absorbed in the digestion system; afterward join to blood (Kamiloglu *et al.*, 2013).

1.2.2 Sesame paste

Sesame paste is obtained by milling of roasted dehulled sesame seeds. It is mainly composed of lipids and proteins, mainly sesame proteins. In addition, it is rich in carbohydrates and dietary fiber shown in Table 1.2 (Abu-Jdayil *et al.*, 2002; Altay *et*

al., 2005). Moreover, some minerals such as calcium, phosphorous and iron are present in sesame paste more excessively than grape molasses. Furthermore, sesame paste also includes some vitamins that are niacin and thiamin (Abu-Jdayil *et al.*, 2002).

Table 1.2: Physical, chemical and mineral content of sesame paste (Abu-Jdayil *et al.*, 2002).

Component (%)	Lipid	54-65
	Protein	17-27
	Dietary fiber	9.3
	Carbohydrate	6.4-21
Mineral (mg/100g)	Calcium	429
	Phosphorous	732
	Iron	9
Vitamins (mg/100g)	Niacin	4.5-5.5
	Thiamin	1.1

The main constituent in sesame paste is considered to be sesame that is an important oilseed crop that has antioxidant activity to owing to its content of unique unsaponifiable lignans such as sesamin, sesaminol and sesamol (Achouri *et al.*, 2012). Antioxidant activity in sesame paste is related to oxidative stability in the light of the studies. They may have the potential of inhibiting the process of aging in man and in biological systems (Abou-Gharbia *et al.*, 2000).

Production steps of sesame paste starts with wetting and dehulling sesame seeds. After centrifuging, roasting step takes place, and finally milling is done (Figure 1.2).

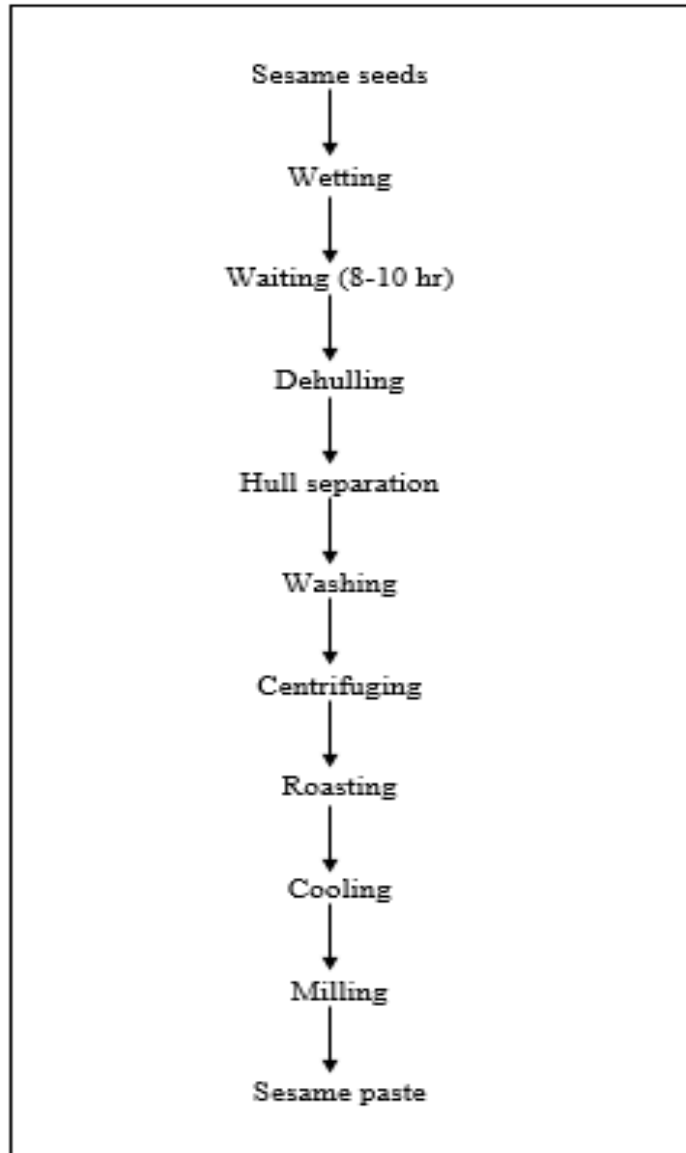


Figure 1.2: Production process of sesame paste (Arslan *et al.*, 2005).

1.2.3 Healthy compounds

In grape molasses, most remarkable compounds are phenolic compounds which are valued as contributing to the resistance of plants to physical stress caused by injuries during mechanized harvesting or biological stress by fungi or bacteria. They can prevent such damages due to being an easy target for free radicals in the nature that is why they are called as antioxidants (Karakaya *et al.*, 2001).

To begin with, phenolic compounds have a structure of a hydroxyl group that is bonded to an aromatic ring (Ozidal *et al.*, 2013). Those compounds are regarded as secondary metabolites since they do not contribute to the growth or energy

metabolism in the body. (Harnly *et al.*, 2007). As far as known, there exist more than 8000 phenolic compounds in some fruits, vegetables and seeds (Cuykens *et al.*, 2004). Phenolic compounds are mainly composed of some polyphenols that are the main sources of antioxidants (Grafet *et al.*, 2005, Vermerris *et al.*, 2006). Moreover, there are subgroups of polyphenols with respect to the carbon skeleton in the structure which can be listed as phenolic acids, flavonoids and lignans (Ozidal *et al.*, 2013).

1.2.3.1 Phenolic acids

There are two subgroups of phenolic acids such as hydroxybenzoic and hydroxycinnamic acids. Phenolic acids having C₆-C₁ structure take place in hydroxybenzoic group such as gallic, *p*-hydroxybenzoic, protocatechuic, vanillic and syringic acids having; whereas. On the other hand, hydroxycinnamic acids that are also known as aromatic compounds with a three-carbon side chain (C₆-C₃) include caffeic, ferulic, *p*-coumaric and sinapic acids (Balasundram *et al.*, 2006). Phenolic acids in grape molasses are basically resveratrol and acid derivatives such as gallic acid (Batu, 2011). Furthermore, a study of Karakaya and co-workers indicates that total phenolic content of grape molasses is 1.25 mg GAE (Gallic acid equivalent)/100 g while it is 1.58 mg/100 g for grape itself (Karakaya *et al.*, 2001, Kamiloglu *et al.*, 2013).

1.2.3.2 Flavonoids

Flavonoids are the phenolic compounds that have low molecular weight and they consist of mainly 15 C atoms arranged in C₆-C₃-C₆ configuration. There are two aromatic rings A and B joined by a 3-carbon bridge, usually in the form of a heterocyclic ring, C in their structures.

Major flavonoid types occur according to their degree of oxidation in heterocycle; that are flavonols, flavones, flavanones, flavanols isoflavones, flavanonols, and anthocyanidins . (Guo *et al.*, 2009). Flavonoids in grape molasses are quercetin, catechin and tannins (Batu, 2011). Quercetin can decrease the formation of free oxygen radicals and inhibit lipid peroxidation under *in vitro* conditions.

1.2.3.3 Bioavailability

Bioavailability is the fraction of a nutrient after being ingested and being available to the body for utilization (Castenmiller *et al.*, 1999). Bioavailability of a constituent depends on mainly its two features that are digestive stability and release from food matrix (Tagliazucchi *et al.*, 2010). Also, it changes with respect to the type of polyphenol since the absorption characteristics are different for all polyphenols. In the literature, studies show that absorption of 17 polyphenols is resulted by passive diffusion across the membranes in the gut epithelial cells. Also, it is not easy to absorb some polyphenols since they exist in the form of esters, glycosides or polymers in the food matrix. (Manach *et al.*, 2005).

Even if there exist both *in vivo* and *in vitro* experiments showing the bioavailability of polyphenols in the literature, *in vitro* methods are more widely used and proven to be well correlated with *in vivo* results. *In vitro* experiments provide information about the stability of them under gastrointestinal (GI) conditions by simulating GI digestion rapidly and safe (Bouyed *et al.*, 2011, Liang *et al.*, 2012). Studies considering *in vitro* digestion of some polyphenols in grape (Tagliazucchi *et al.*, 2010), pomegranate juice (Perez-Vicente *et al.*, 2002), raspberry (McDougall *et al.*, 2005) or apple (Bouyed *et al.*, 2011) are available in the literature.

In the light of the investigations throughout the literature, no previous study was found in which *in vitro* gastrointestinal (GI) digestion of sesame paste or the blends of sesame paste and grape molasses are examined.

The blends have been mostly a part of rheological studies before. These studies indicate that this type of blend exhibit non-Newtonian, shear thinning behavior while each product show different rheological characteristics. Sesame paste shows thixotropic behavior while grape molasses does Newtonian (Arslan *et al.*, 2005).

1.2.3.4 Protein and lipids

Proteins are made up of twenty amino acids that include of an α -carbon atom covalently attached to a hydrogen atom, an amino group, a carboxyl group, and a side-chain R group (Damodaran, 1996). Proteins have a significant role in growth and maintenance in human body. They are major structural components in the body found in all cells, especially in muscles. Proteins are digested into smaller polypeptide chains in the stomach via HCl and protease actions in order to synthesize

essential amino acids that human body could not biosynthesize (Nelson *et al.*, 2005). They may also have a role in formation of complexes with other components in food including polyphenols. Proteins mostly exist in milk, meat, cereals and oilseeds (Ozdal *et al.*, 2013). Sesame paste is rich in proteins that can be listed as methionine, tryptophan and valine (Kahyaoglu *et al.*, 2006).

1.2.3.5 Consumption of the products as blends

Blends of sesame paste and grape molasses exist in the form of emulsions just like mayonnaise or milk butter by showing the characteristics of oil-in water emulsion. (Alparslan *et al.*, 2002). Sesame paste contains the oil phase while grape molasses contain the water phase by the way the blend is composed of two immiscible liquids.

In recent studies, the topic related to the consumption of two foods one of which is rich in protein content while the other one in polyphenol content gain attention. Interactions of proteins with phenolic compounds have become popular since structural, functional and nutritional properties, and digestibility of proteins are thought to be affected by these interactions just as by other interactions with lipids or others. Also these interactions result in changes in antioxidant capacity, total phenolic and/or flavonoids content of polyphenols, in addition to bioavailability (Ozdal *et al.*, 2013).

The interactions between polyphenols and proteins are tried to be clarified in the studies with various types of protein and polyphenol sources. Although the mechanism of how proteins influence polyphenols is still not yet known, but changes in the structure, functional and nutritional value and digestibility have been observed (Ozdal *et al.*, 2013). An example to the interactions between polyphenols and casein molecules can be that tea polyphenols weakly bind to α -casein and β -casein through both hydrophilic and hydrophobic interactions (Hasni *et al.*, 2011). Additionally, there are studies indicating that at alkaline pH, polyphenols can be oxidized by molecular oxygen with side chain amino groups of peptides at alkaline pH to quinines, by the way formation of protein cross-links could be observed (Damodaran, 1996; Prodpran *et al.*, 2012). Afterwards, quinines can irreversibly react with sulfhydryl and amino groups of proteins and undergo condensation reactions leading to formation of a pigment, tannin. This highly reactive tannin can combine with SH and amino groups of proteins, that decrease digestibility and bioavailability of

protein-bound lysine and cysteine (Damodaran, 1996). Furthermore, since phenolic compounds are hydrogen donors, hydrogen bonds could be formed between phenolic compounds and carboxyl group of proteins (Mulaudzi *et al.*, 2012). Moreover, a study of Prigent implies that the protein-phenolic interactions increase the molecular weight of proteins (Prigent *et al.*, 2003).

On the other hand, whether the interactions have positive or negative effects could be hardly concluded since there are contradictory results within the studies. To illustrate, a study of Belščak *et al.* (2009) on total phenolic contents, total flavonoid contents and antioxidant capacities of various chocolate products including milk in different ratios. The outcome of the study indicates that lowest total phenolic and flavonoid content as well as total antioxidant capacities were observed in milk chocolate although it contains higher cocoa solids content (29%) than cocoa bars (16%). This decrease was related to strong catechin-protein interactions. (Belščak *et al.*, 2009). On the contrary, Dubeau *et al.* (2010) concluded that milk decreased the antioxidant capacities of teas according to ABTS and voltammetry methods. However, the results showed that milk enhanced the chain-breaking antioxidant capacity of teas by the lipid peroxidation method. These findings were explained by dual effects of milk proteins on the antioxidant capacity of tea such as an inhibitory effect for reactions taking place in solution or at a solid–liquid interface and an enhancing effect for those in oil-in-water emulsions (Dubeau *et al.*, 2010).

2. MATERIAL AND METHODS

2.1 Materials

2.1.1 Plant materials

Grape molasses and sesame paste produced in 2012 according to the labels were collected from a local market in Istanbul, Turkey that are shown in Figure 2.1.

Three repetitions (n=3) were carried out for molasses, sesame paste and their three blends with different ratios (50-50%, 70-30% and 30-70%). The blends were prepared homogeneously by weighing in precision scales and then agitating. The samples were kept in the refrigerator before using. Also, after the extraction procedure was followed, the extracts were kept at -20 °C prior to analysis as well as the samples exposed to *in vitro* digestion.



Figure 2.1: Sesame paste and grape molasses.

2.1.2 Chemicals

In this study, chemicals with analytical purity were used. For extraction and determination of spectrophotometric assays gallic acid ($\geq 98\%$), (+)-catechin ($\geq 98\%$), acetone ($\geq 99.8\%$), ethanol ($\geq 99.8\%$), hexane ($\geq 95\%$), Folin-Ciocalteu phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) and neocupraine (Nc) from Sigma-Aldrich Chemie GmbH (Steinheim, Germany); methanol ($\geq 99.9\%$), formic acid ($\geq 98\%$) hydrochloric acid (37%), *n*-butanol ($\geq 99.5\%$), sodium carbonate (Na_2CO_3), sodium nitrite (NaNO_2), sodium

hydroxide (NaOH), sodium acetate trihydrate (CH₃COONa.3H₂O), potassium persulfate (K₂S₂O₈), dipotassium hydrogen phosphate (K₂HPO₄), potassium dihydrogen phosphate (KH₂PO₄), copper (II) chloride (CuCl₂) and ammonium acetate (NH₄Ac) from Merck KGaA (Darmstadt, Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and aluminum chloride (AlCl₃) from Fluka Chemie (Buchs, Switzerland); and potassium chloride (KCl) from Riedel-de Haen Laborchemikalien GmbH (Hanover, Germany); from Lachema (Czech Republic) and 2,2'-azinobis(3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt (ABTS) from Applichem GmbH (Darmstadt, Germany) were purchased.

The following standards and reagents were used for the quantification of phenolic compounds: (+)-catechin (≥99%), gallic acid (≥99%) from Extrasynthese (Genay, France); acetonitrile (99.8%) from Sigma-Aldrich (Steinheim, Germany). For simulation of *in vitro* gastrointestinal system, pepsin, pancreatin, bile salts, dialysis bags (Membra-Cel MD34) from Sigma-Aldrich and sodium bicarbonate (NaHCO₃) from BDH Chemicals Ltd. (Poole, UK) were purchased.

Water that is distilled and purified with the water purification system was used for all analysis and *in vitro* digestion (TKA GenPure, Germany) shown in Figure 2.2.



Figure 2.2: TKA GenPure water purification system.

2.2 Methods

2.2.1 Determination of moisture content

So as to determine moisture content of samples; 1 g of grape molasses, sesame paste and three blends were placed into the electronic moisture analyzer instrument

(Denver, IR) shown in Figure 2.3, respectively and allowed for their moisture content to be calculated via evaporation in the instrument.



Figure 2.3: Electronic moisture analyzer.

2.2.2 Determination of protein content by Kjeldahl method

Protein content was determined by Kjeldahl method according to AOAC (1990) methods by automatic Kjeldahl analyzer (BUCHI, K 360) shown in Figure 2.4. Initially, grape molasses, sesame paste and three blends were weighed as 2 grams each. 0.3 g of copper sulphate and 15 g of potassium sulphide were added to the samples. Then, 25 mL of H₂SO₄ was added. Later on, the samples were burnt for 2 hours. After being burnt, they were allowed to be cooled down for 30 min. Afterwards, they were placed in the distillation unit of the instrument (Figure 3.2.2) respectively and at the same time erlen mayer flask filled with 25 mL of boric acid and 2 drops of methylene red and 3 drops of methylene blue was placed in the distillate unit. After distillation, the distillate was titrated with 0.2 N HCl and protein content of the samples was calculated with the equation below (2.1) (AOAC, 1990).

$$\text{Protein(\%)} = \frac{\text{mL HCl} * 0.2(\text{Normality of HCl}) * 0.014 * 100}{\text{g of sample}} \quad (2.1)$$



Figure 2.4: Automotic Kjeldahl analyzer.

2.2.3 Determination of lipid content

Lipid content of samples were determined via automated Soxtherm analyzer (Gerthard) that is shown in Figure 2.5. Analysis begins with weighing 3 grams of sample and putting it to flask. Then, 150 mL of hexane is added to the sample before it is placed into the equipment. $T_{\text{classification}}$ is set to 200 °C and the analysis is waited for about 2.5 h. Later on, the sample is placed into volumetric flask and hexane is added, at 50 °C the solute is evaporated and the rest is weighed and calculated as lipid content.



Figure 2.5: Automated Soxtherm analyzer.

2.2.4 Rheological characterization

Rheological characteristics of foods are important in terms of food quality and consumer acceptance. In this study, rheological properties of grape molasses, sesame paste and their blends were analyzed at 21°C, with two replicates. Rheological measurements were conducted using a rheometer Haake Rheostress 1 coupled with external DC 10 circulator (Haake GmbH, Karlsruhe) using cone and platesystem (d: 35 mm, angle = 2DEG) that is shown in Figure 2.6. The flow curves of sesame paste samples were measured. The samples were blended and allowed to rest for 2-3 min after loading, before measurement. The method of Ciftci *et al.* (2008) was followed. The hysteresis loop was obtained by registering shear stress from 0.01 to 200 s⁻¹ in 120 s, held at 200s⁻¹ for 1 min between the two ramps and down in 120 s. The power law equation (2.2) (Ciftci *et al.*,2008), which is the most frequently used for engineering application, was applied to describe the steady shear flow data:

$$\tau = K * \dot{\gamma}^{n-1} \quad (2.2)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s⁻¹), K is the consistency index (Pa.s), and n is the exponent, the flow behavior index.

The relationship between shear stress and viscosity of samples are shown in Appendix C, Figures C.1-C.5.



Figure 2.6: HAAKE Rheostress Equipment.

2.2.5 Extract Preparation

According to the method described by Capanoglu *et al.*(2008) five independent extractions for each sample were carried out with slight modifications. To begin

with, 2 ± 0.01 g of grape molasses and sesame paste was extracted with 5 ml of 75% aqueous-methanol containing 0.1% (v/v) formic acid for 15 min in a cooled ultrasonic bath (Azakli, Turkey) shown in Figure 2.7.

Same procedure was applied to a 2 ± 0.01 g of three blends with 50-50%, 70-30% and 30-70%. Then, treated samples were centrifuged (Hettich Zentrifugen Universal 32R, UK) (Figure 2.8) for 10 min at 4000 rpm and the supernatant was collected. Later on, another 5ml 75% aqueous-methanol containing 0.1% (v/v) formic acid was added to the pellet and this extraction procedure was repeated two more times. All six supernatants were combined and adjusted to a final volume of 20 ml; sesame paste and blends were filtrated. Prepared extracts were stored at -20 °C until analysis.



Figure 2.7: Ultrasonic bath (Azaklı).



Figure 2.8: Hettich Zentrifugen Universal 32R centrifuge.

2.2.6 Determination of total phenolic content (TP)

According to the procedure given by Velioglu *et al.*(1998), the TP of extracts was determined using Folin-Ciocalteu reagent. To begin with, 0.75 mL of freshly prepared Folin-Ciocalteu reagent (1:10, v/v with distilled water) was added to 100 μ L of extracts of all samples. Then the blends were allowed to stand for 5 min prior

to addition of 0.75 mL of 6% sodium carbonate solution. Later on, the samples were incubated for 90 min at room temperature, and their absorbance was read at 725 nm using a UV-Vis spectrophotometer (Shimadzu UV-1700, Tokyo, Japan) (Figure 2.9). The TP of extracts was expressed as milligrams of gallic acid equivalent (GAE) per 100 g sample in wet basis. Samples of each extract were analyzed in triplicate. The calibration curve is shown in Appendix, Figure A.1 while statistical results are in Appendix D, Table D.1 for initials and D.2-D.4 for samples after *in vitro* digestion.



Figure 2.9: Shimadzu UV-1700 spectrophotometer.

2.2.7 Determination of total flavonoid content (TF)

The TF procedure of Kim *et al.* (2003) was followed and TF was measured colorimetrically. The experiment started with addition of 0.3 mL of 5% NaNO₂ solution to 1 mL of sample at time zero. After 5 min, 0.3 mL of 10% AlCl₃ was added. Then, at the 6th min, 2 mL of 1 M NaOH was also added. Immediately, 2.4 mL of distilled water was added and the blends were vortexed. The standard curve was drawn with respect to (+)-catechin and expressed as milligrams of (+)-catechin equivalent (CE) per g of sample. Samples were analyzed for each extract in triplicate. The calibration curve is shown in Appendix, Figure A.2.

2.2.8 Determination of total antioxidant capacity (TAC)

The total antioxidant capacities for all samples were estimated by four different assays that are ABTS, DPPH, FRAP and CUPRAC. In all assays, trolox was used as a standard and results were expressed in terms of milligrams of trolox equivalent

antioxidant capacity (TEAC) per 100 g sample in wet basis. Analyses for all samples were in triplicate for each assay. The calibration curves obtained by each assay are shown in the Appendix, Figures A.3-A.6.

CUPRAC (Copper Reducing Antioxidant Capacity) assay procedure given in the article of Apak *et al.*(2004) was followed in order to determine total antioxidant capacity of the extracts. Firstly, 100 μ L of extract was Blended with 1 mL of 10 mM CuCl_2 , 7.5 mM neocuproine and 1 M NH_4Ac (pH:7). Then, 1 mL of distilled water was added to the blend rapidly so as to make the final volume 4.1 mL. Absorbance was read at 450 nm against a reagent blank after 60 min of incubation at room temperature.

The ABTS (2,2- azinobis 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt) assay was performed as described by Miller and Rice-Evans (1997). To begin with, ABTS and potassium persulfate solutions were Blended and kept at room temperature in the dark for overnight. Then, ABTS stock solution was diluted in 50 mM potassium phosphate buffer (pH 8.0) to an absorbance of 0.90 (± 0.05) at 734 nm to prepare the ABTS-working solution. Later on, 100 μ L of sample extract was Blended with 1 mL of ABTS-working solution and the absorbance was measured at 734 nm exactly 1 min after initial Blending.

The DPPH (1,1-diphenyl-2- picrylhydrazyl) assay was carried out according to Kumaran and Karunakaran (2006). At first, 100 μ L of each sample extract was Blended with 2 mL of 0.1 mM DPPH in methanol. Samples were incubated for 30 min at room temperature prior to measurement of absorbance at 517 nm against methanol.

The FRAP (Ferric Reducing Antioxidant Power) assay was performed accoring to the procedure of Benzie and Strain (1996). Initially, 900 μ L aliquot of freshly prepared FRAP reagent (a blend of acetate buffer (pH 3.6), 10 mM TPTZ solution and 20 mM ferric chloride in proportions of 10:1:1 (v/v/v), respectively) was added to 100 μ L of fruit extract. The absorbance of the reaction blend was then recorded at 593 nm after 4 min.

2.2.9 HPLC analysis of major individual phenolic compounds

The method of of Capanoglu *et al.* (2008) was used so as to determine major individual phenolic compounds in all samples. To begin with, extracts were filtered

through a 0.45- μm membrane filter and analyzed by the HPLC system comprised a Waters 600 control unit, a Waters 996 photodiode array (PDA) detector, and a Waters 2475 fluorescence detector. Luna 3 C18 150x4.60 mm column (Phenomenex, Torrance, CA, USA) was used. Solvent A, Milli-Q water with 0.1% (v/v) trifluoroacetic acid (TFA) the mobile phase consisted of) and solvent B, acetonitrile with 0.1% (v/v) TFA are the constituents of mobile phase. A linear gradient was used as follows: at 0 min, 95% solvent A and 5% solvent B; at 45 min, 65% solvent A and 35% solvent B; at 47 min, 25% solvent A and 75% solvent B; and at 54 min returns initial conditions. The flow rate was 1 ml/min. Detection was done at 254 nm. Identification was based on the retention times and characteristic UV spectra and quantification was done by external standard curves. Chromatograms are given in the Appendix B, Figures B.1-B. 20.

2.3 *In vitro* gastrointestinal (GI) digestion

In vitro digestion method in which the physiochemical and biochemical changes that occur in the upper gastrointestinal tract are tried to be mimicked was followed as described by McDougall *et al.* (2005).

To mimic the stomach, pepsin was added while pancreatin was added in order to mimic intestines.

In gastric phase, 5 grams of sesame paste, grape molasses and three blends were weighed in three beakers for each and 20 ml of distilled water was added into all beakers. Later on, 1.5 ml of pepsin solution was added to all, 5 N HCl was used to adjust pH to 1.7. Beakers were covered by parafilms and waited for 2 hours in a Memmert shaking water bath (Nürnberg, Germany) (Figure 2.10) at 37 °C and 100 rpm.

After 2 hours 2 mL aliquots of postgastric digestion (PG) samples were collected and stored at -20 °C until further analysis. Also, a blank was prepared without food matrix and exposed to the same procedure.



Figure 2.10: Memmert shaking water bath.

Then, the intestinal phase began with addition of 4.5 ml of 4 mg/mL pancreatin solution and bile salt to beakers consisting of PG samples. Dialysis bags were filled with sufficient NaHCO_3 (20 mL) to neutralize the sample's titratable acidity and then the beaker was again sealed with parafilm and were waited in Memmert shaking water bath at 37°C , 100 rpm for 2 hours again.

After 2 hours, IN (dialyzed) and OUT (nondialyzed) samples of sesame paste, grape molasses and blend were collected. In order to have enough PG samples, the first part was repeated and all PG, IN and OUT samples were centrifuged (Hettich Zentrifugen Universal 32R, UK) for 10 min at 18000 rpm and the supernatant was collected. For sesame paste and blend, filtration process was required in order to get rid of particulates and sesame oil in PG and OUT samples, and after filtration the samples were stored at -20°C before analysis. TF, TP and TAC analyses were done for all PG, IN and OUT samples with the same procedure done to extracts.

2.4 Statistical Analysis

For statistical analysis, data were collected from three independent extractions for each fraction and reported as mean \pm SD. Data were subjected to statistical analysis using SPSS software (version 16.0 for Windows, SPSS Inc.) for the analysis of variance (ANOVA) for multiple comparisons. Tukey's Test was used to analyze differences between treatments ($p < 0.05$), tables are available in Appendix D.

3. RESULTS AND DISCUSSION

3.1 Moisture Content

Moisture contents of sesame paste, grape molasses and three blends were determined in triplicates; results are given in the Table 3.1. According to the table below; while sesame paste has 3.9 % moisture, that of grape molasses was found as 82.5 %.

Moreover, in literature studies, moisture content of sesame paste was determined as 3.4 % that is consistent with our findings (Kahyaoglu *et al.*, 2006). Moreover, for grape molasses, moisture content was found as 84.2 % that is close to 82.5 % (Batu, 2011).

Table 3.1: Moisture contents of sesame paste, grape molasses and three blends¹.

Sample	Moisture Content (%)
Sesame paste	3.9
Blend1 (70-30%)	22.3
Blend2 (50-50%)	42.3
Blend3 (30-70%)	69.3
Grape molasses	82.5

¹Data represent average quantities standard deviation of 3 independent samples.

3.2 Protein Content

Protein contents of all samples were devised by Kjeldahl method. According to the experimental results in Table 3.2 below, protein content of grape molasses was 0.09% whereas that of sesame paste was calculated as 28.5 %. Also, blends have protein contents in between two individuals, proportionally.

Furthermore, a study of Kahyaoglu *et al.* (2006) implies that 27.2% of sesame paste is sesame protein. Moreover, protein amount in grape molasses was found in trace amounts in a study of Batu (2011).

Table 3.2: Protein content of sesame paste, grape molasses and three blends¹.

Sample	Protein Content (%)
Sesame paste	28.5
Blend1 (70-30%)	20.1
Blend2 (50-50%)	11.3
Blend3 (30-70%)	3.2
Grape molasses	-

¹Data represent average quantities standard deviation of 3 independent samples.

3.3 Lipid Content

Lipid content of sesame paste was found as 55.3% (Table 3.3) which is in the range given in a study of Abu-Jdayil *et al.*(2002).They suggested that lipid content of sesame paste is between 54 and 65%. Moreover, grape molasses was found to have trace amounts of lipid content in the experiment. A study of Batu (2011) supports the result. Additionally, lipid contents of blends decreases with increasing grape molasses content.

Table 3.3: Lipid contents of sesame paste, grape molasses and three blends¹.

Sample	Lipid content (%)
Sesame paste	55.3
Blend1 (70-30%)	33.2
Blend2 (50-50%)	26.7
Blend3 (30-70%)	12.3
Grape molasses	-

¹Data represent average quantities standard deviation of 3 independent samples.

3.4 Rheological Characterization

According to the literature, the most frequently used equation for modelling of fluids having non-Newtonian behavior is a power-law. This model is used extensively to describe the non-Newtonian flow behavior both in theoretical analysis and in practical engineering calculations (Bourne, 1982).

In order to determine the rheological characterization of the samples, viscometric measurements were conducted at 21 °C for 1 min. After recording, n and K values for all samples were defined (Table 3.4).

Table 3.4: Power-law parameters for sesame paste, grape molasses and blends¹.

	T (°C)	n	K(Pa.sⁿ)	R²
Sesame paste	21	0.83	7.78	0.99
Blend1 (70-30%)	21	0.76	42.50	0.98
Blend2 (50-50%)	21	0.73	40.86	0.97
Blend3 (30-70%)	21	0.79	14.88	0.99
Grape molasses	21	0.97	1.83	0.99

¹Data represent average quantities standard deviation of 3 independent samples.

In the light of the literature, for Newtonian fluids; shear stress is linear function of shear rate. By the way flow behaviour index, n, is nearly equal to 1. (Yogutcu & Kamisli, 2005). Here in this study, for grape molasses, the linear relationship between shear stress and shear rate is calculated with R² equals to 0.99. Hence, also as supported by a study of Kaya and Belibagli (2002), grape molasses possess Newtonian fluid characteristics.

For sesame paste, at constant shear rate, viscosity decreased by 1% in 1 minutes, helping us to understand the rheological behaviour of sesame paste as thixotropic. In a study of Abu-Jdayil *et al.* (2002), sesame paste was found to have shear thinning non-Newtonian behavior with decreasing viscosity as a function of time at constant shear rate. They found the decay in viscosity of sesame paste at T= 25 °C as 4% in 5 minutes.

For blends, the values of flow behavior index, n, varied between 0.7 and 0.8 indicating shear-thinning behavior. The degree of pseudoplasticity decreases by increasing n value. (Grigelmo *et al.*, 1999). Hence, degree of pseudoplasticity of half and half blend is higher than the others. Moreover, viscosity of half and half blend is higher than the others showing that it is harder to make it flow than the others. Moroset *al.* (2002) reported that the viscosities of the emulsions increased with an increasing oil concentration while Alparslan & Hayta (2002) indicated that increasing

pekmez concentration resulted in an increase in viscosity. Those two studies help to explain the highest viscosity value of half and half blend in this research.

3.5 Total Phenolic Content

Total phenolic contents of extracts are given in Figure 3.1. Among 5 extracts with 3 replicates that are sesame paste, grape molasses and three blends, grape molasses has shown highest TP content while sesame paste has lowest. Extracts of blends have TP between sesame paste and grape molasses. Blend1 has shown a TP value closest to sesame paste and the TP is increasing as the content of grape molasses increases and the value is close to that of grape molasses. Replicates have given parallel results.

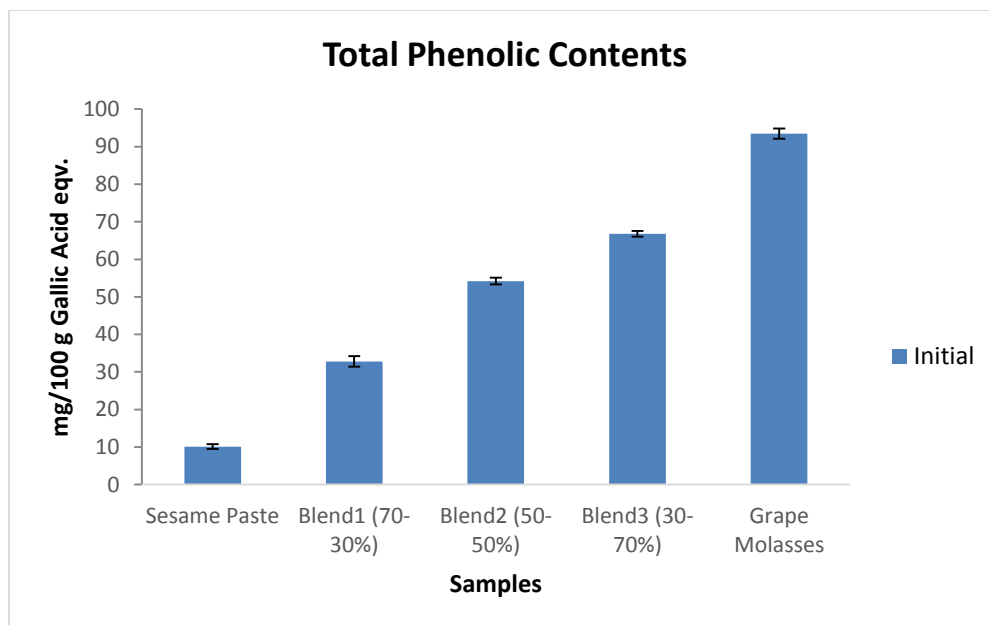


Figure 3.1: Total phenolic contents of initial samples.

From Figure 3.1, mg/100 g gallic acid equivalent (GAE), by the way, total phenolic content of grape molasses was found as 93.46 that is close to 96.25 mg found in a study of Ozkan *et al.*(2004) done for grape pomace. TP values differs with respect to grape types. In that study, total phenolic contents of two grape pomaces were examined; one of which is Kalecik karası while the other one is Emir cultivars. TP of the first one is close to our findings while second one was lower and calculated as 68.77 mg/100 g GAE.

On the other hand, it is hard to find a study focusing on TP of sesame paste to compare with our findings as 10.13 mg/ 100 g GAE that is significantly lower than grape molasses.

Moreover, TP contents of blends were calculated as 32.79, 54.19 and 66.79 mg/ 100g GAE, respectively. Proportional values for blends by calculating from the ratio of two products are close to the findings. Hence, it can be said that blends have increasing TP contents with grape molasses in the shelf.

3.6 Total Flavonoid Content

Total flavonoid contents of extracts were measured colorimetrically and are given in Figure 3.2. As well as TP values, grape molasses has given the highest TF content and the blends have TF values between the two products. However, TF of blends are closer to that of sesame paste.

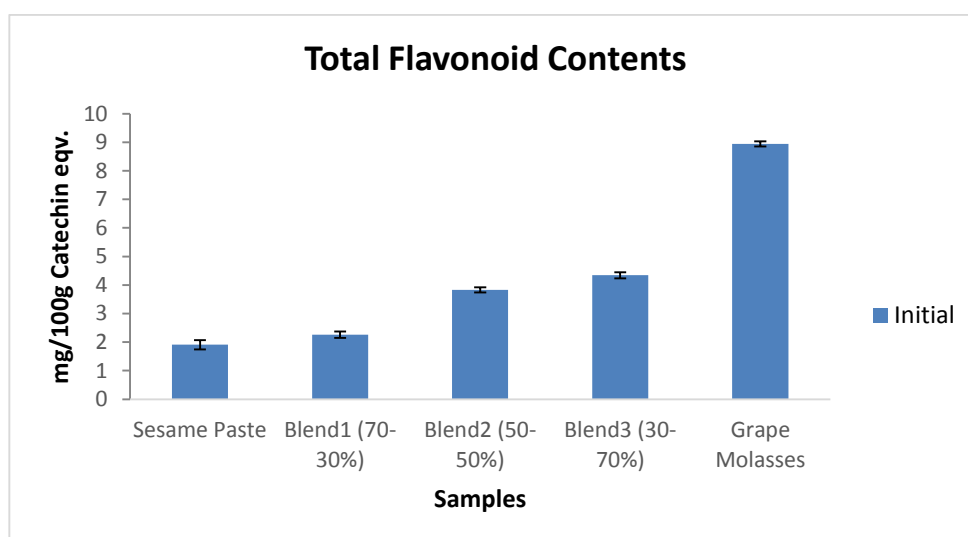


Figure 3.2: Total flavonoid contents of initial samples.

TF of sesame paste was calculated as 1.91, whereas that of grape molasses was 8.94 mg/ 100g CE (Figure 3.2). In a study TF of grape molasses was found as 14.3 mg/100 g CE in dry matter which is a little higher than our findings (Kamiloglu & Capanoglu, 2014). This is probably because our findings are grounded on wet basis. However, there are studies showing greatly different results in the literature. To illustrate, Selcuk *et al.* (2011) implies that TF of grape seeds from grape molasses has a TF of 49.2 mg/g CE that is a greatly higher value compared to our results.

Alasalvar *et al.*, (2005) explain the difference of TF between molasses and seeds by effect of production steps of grape molasses leading to lower catechin content.

Moreover, TF of blends was found as 2.26, 3.83 and 4.34 mg/ 100g CE, respectively. For blends, proportional TF values are higher than findings. This can be explained by a study of Kammarrer *et al.* (2011) indicating that catechin concentration may decrease when being together with amino acids in the environment since it strongly binds to amino acids. Here, in this research, catechin may have shown this characteristic of itself and TF of blends was found lower than proportional value. Hence, it can be concluded that a decay in polyphenol content such as flavonoids may be observed as of blends.

3.7 Total Antioxidant Capacity

Studies on antioxidant activity of molasses, *i.e.* mulberry molasses, imply that phenolics and flavonoids are the major contributors to the antioxidant capacity. Even if there exist some polyphenols in sesame paste, its antioxidant activity is low just as proportional while grape molasses has great antioxidant activity (Mahattanatawe *et al.*, 2006).

TEAC values were measured by CUPRAC assay which is given in Figure 3.3. Grape molasses has shown highest TEAC values while sesame paste has lowest that are consistent with both TP and TF analysis. Besides, extracts of blends have TEAC between two.

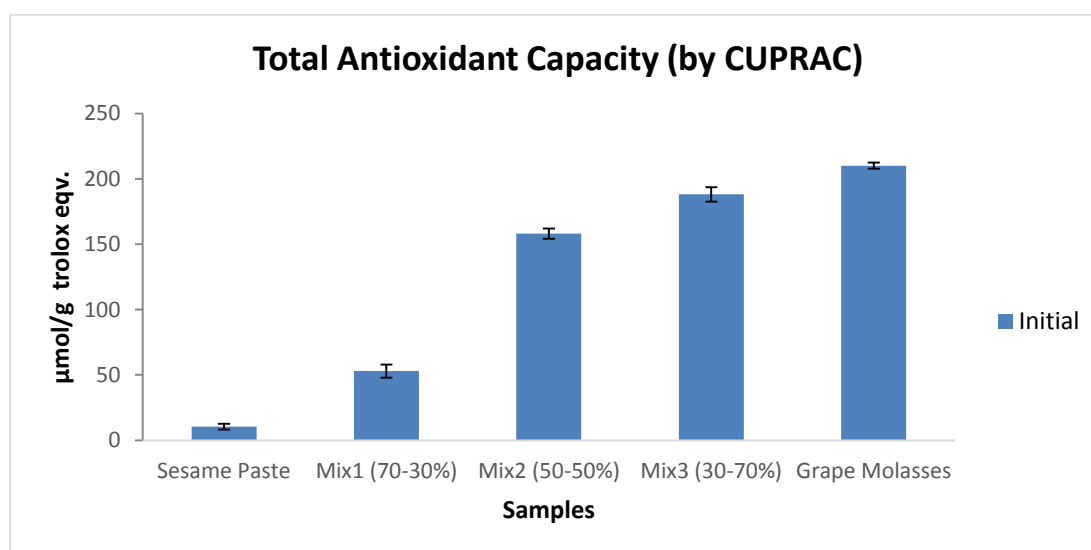


Figure 3.3: Total antioxidant capacity (by CUPRAC) of initial samples.

The figure above indicates that copper reducing antioxidant capacities the blends are between that of grape molasses and sesame paste. Blend having 70% sesame paste has an antioxidant capacity closest to it. On the other hand, the other two blends have closest antioxidant capacity to grape molasses. Grape molasses has 210.9 $\mu\text{mol}/100\text{ g}$ trolox equivalent of grape molasses extracts. TAEC of grape molasses was found as 220.7 $\mu\text{mol}/100\text{ g}$ as in wet basis in a study of Kamiloglu & Capanoglu (2014) that is consistent with our findings.

On the other hand, in a study of Mahattanatawee *et al.* (2006), $\mu\text{mol}/100\text{ g}$ trolox equivalent of grape puree was found as 151 which is a lower value when compared to the findings. This may be a result of measurement of TAC by a different assay called ORAC in that study and due to multiple reaction characteristics and mechanism, application of more assays and comparison of them provide an estimate of antioxidant activity (Li *et al.*, 2009).

Moreover, when the TAEC values of blends are examined, they show differences with respect to proportional values. TAEC values of the two blends containing sesame paste 70% is lower than proportional ones. On the other hand, half and half blend has shown higher TAEC value than proportional value. Lower TEAC values could be explained by a study of Sanchez-Gonzalez *et al.* (2005) considering that when milk is added to the coffee; with the effect of interactions between phenolic compounds and proteins; antioxidant activity was observed as decreasing by increasing amounts of milk.

However, half and half blend shows increasing antioxidant activity rather than consuming the products individually according to copper reducing antioxidant capacities. The increase in antioxidant activity was also observed in a study on peas after immersion with five phenolic compounds. The researchers extracted superoxide dismutase (SOD) enzyme from peas, and allowed SOD to form protein-phenolic interaction complex. Later on, they measured SOD activity and binding capacity of this complex with pea protein and an increase in antioxidant capacity occurred as a result of protein-phenolic interactions in peas which stabilized the protein, generally SOD (Tsai *et al.*, 2006).

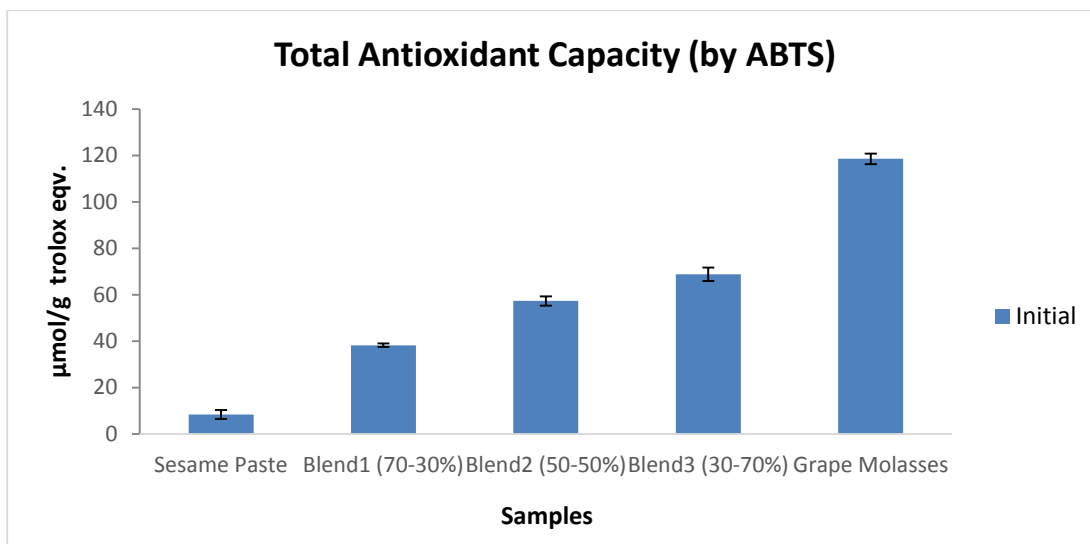


Figure 3.4: Total antioxidant capacity (by ABTS) of initial samples.

Figure 3.4 indicates that TAEC evaluated from ABTS assay, parallel results with CUPRAC assay were observed that grape molasses has shown highest TEAC values while sesame paste has lowest. In addition, extracts of blends have TEAC between two.

According to this assay, grape molasses has 118.6 $\mu\text{mol}/100\text{ g}$ TAEC that is found as 127.2 $\mu\text{mol}/100\text{ g}$ wet basis in the literature (Kamiloglu & Capanoglu, 2014). Moreover, that of sesame paste was examined as 8.3 $\mu\text{mol}/100\text{ g}$.

The blends showed lower TAEC values rather than proportional since binding of phenolic compounds to protein sites or molecules directly may lead to masking of polyphenol contents on the proteins (Arts *et al.*, 2002). Also Dubeau *et al.* (2010) measured the antioxidant capacities of teas consumed with milk and found a decrease in the antioxidant capacities by ABTS assay. Milk is suggested as having dual effects on the antioxidant capacity of tea and its inhibitory effect for reactions taking place in solution or at a solid–liquid interface and an enhancing effect for those in oil-in-water emulsions was used as an explanation to the decline in antioxidant capacity in the study mentioned. Most probably, in ABTS assay, it is easier to detect the masking effect of interactions between phenolic compounds and proteins to antioxidant capacity by observing lower results than proportional values.

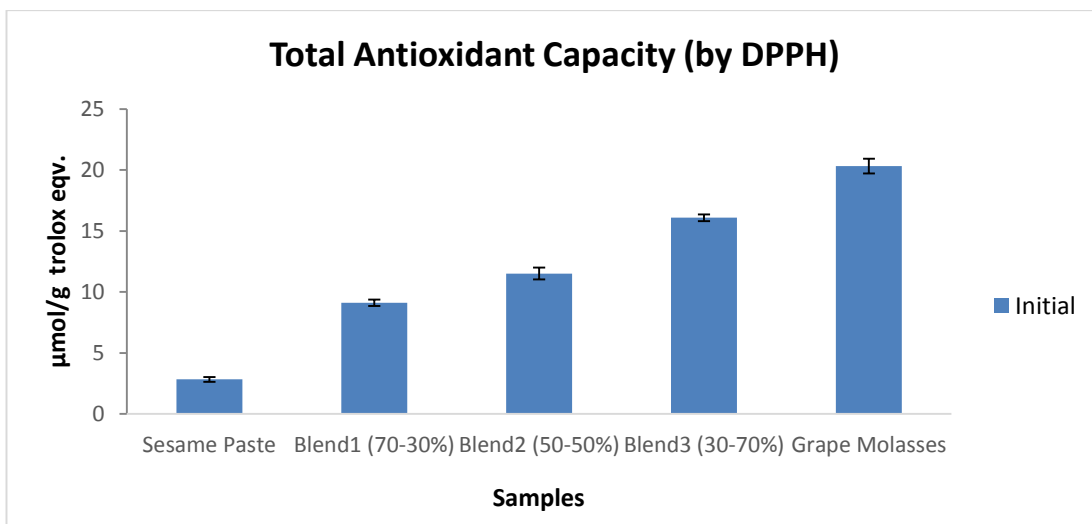


Figure 3.5: Total antioxidant capacity (by DPPH) of initial samples.

From Figure 3.5, blends have antioxidant capacities with respect to DPPH assay between that of grape molasses and sesame paste, respectively. Sesame paste shows least antioxidant capacity rather than the blends and grape molasses as proportional and consistent with the results found in previous assays. DPPH and ABTS show proportional results, that could be related to the fact that they both are radical scavenging assays.

Here, in this assay, first blend showed a bit lower TAEC value than proportional, whereas others showed close values.

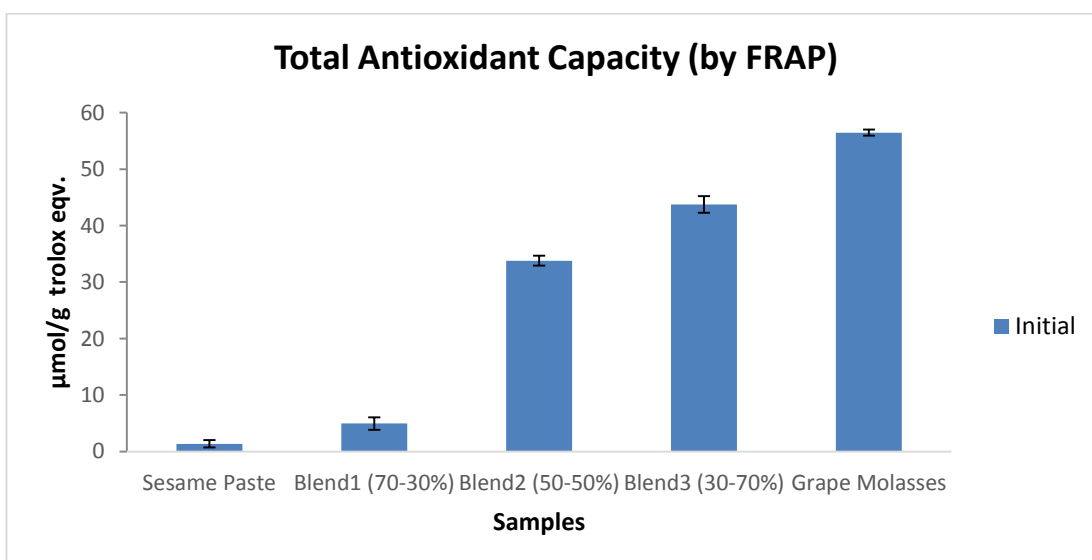


Figure 3.6: Total antioxidant capacity (by FRAP) of initial samples.

Figure 3.6 indicates that antioxidant capacities of the blends are between that of grape molasses and sesame paste according to FRAP assay. Sesame paste and blend having 70% sesame paste have antioxidant capacities that are lowest and closest to each other. On the other hand, the other two blends have closest antioxidant capacity to grape molasses that is consistent with CUPRAC assay.

3.8 Correlation Between Spectrophotometric Assays

The correlation coefficients (R^2) for spectrophotometric assays ranged from 0.815 to 0.989 (Table 3.5). TP and TF showed a linear relationship with a high correlation coefficient of $R^2=0.923$. Among all four TAC assays, the highest correlation was demonstrated between TP and DPPH ($R^2=0.989$), followed by TP and ABTS ($R^2=0.987$), CUPRAC and FRAP ($R^2=0.984$) and ABTS and DPPH ($R^2=0.972$). These results imply that phenolics and flavonoids were the major contributors to the antioxidant capacity of the investigated samples.

Table 3.5: The correlation coefficients (R^2) for spectrophotometric assays.

	TP¹	TF²	CUPRAC	ABTS	DPPH	FRAP
TP	-	0.923	0.960	0.987	0.989	0.969
TF	0.923	-	0.815	0.962	0.888	0.886
CUPRAC	0.960	0.815	-	0.905	0.943	0.984
ABTS	0.987	0.962	0.905	-	0.972	0.932
DPPH	0.989	0.888	0.943	0.972	-	0.943
FRAP	0.969	0.886	0.984	0.932	0.943	-

1 TP: Total phenolic content, 2 TF: Total flavonoid content.

3.9 *In Vitro* Gastrointestinal (GI) Digestion

3.9.1 Total phenolic content

Total phenolic content of initial samples and IN (dialyzed) and their comparison with TP of extracts are given in Figure 3.7.

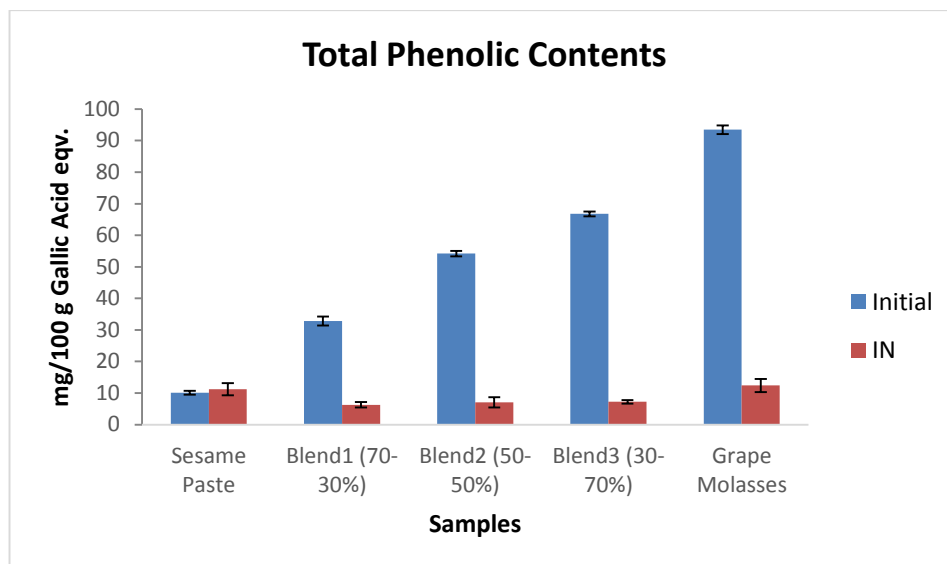


Figure 3.7: Total phenolic content of samples after *in vitro* digestion.

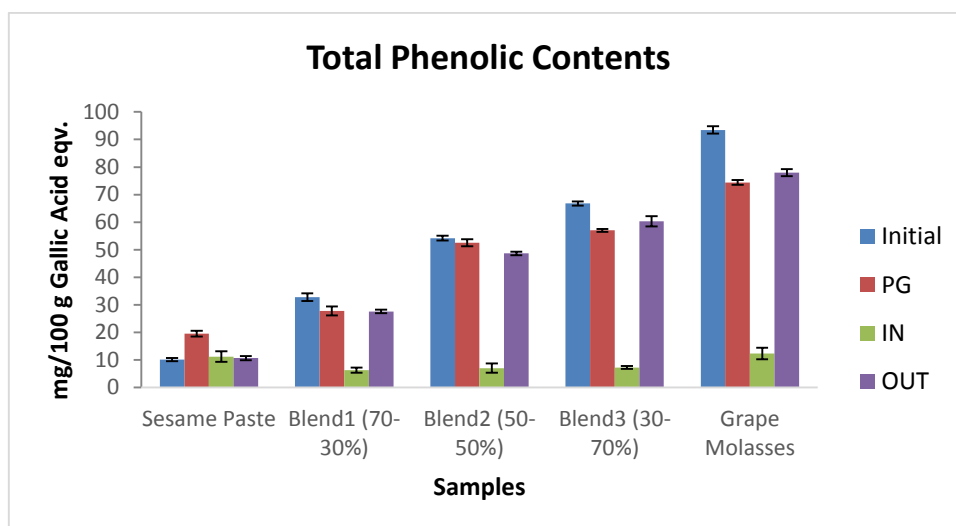


Figure 3.8: Total phenolic content of samples before and after *in vitro* Digestion.

In the light of Figure 3.8, after stomach and pancreatin bile salt digestion, a significant decrease was observed in all dialyzed (IN) samples. That can be explained in terms of a study on cane molasses, where more than half of the phenolic compounds were absorbed in small intestines (Guimarães *et al.*, 2007). Moreover, Perez-Vicente *et al.* (2002) explained this decrease by observing the fact that phenolic compounds are stable in acidic solutions, and pH 7.5 is an inappropriate condition for them. Moreover, the decrease in TP of blend samples after gastric ingestion may be explained by another claim of Perez-Vicente *et al.*(2002). Gallic acid may bind amino acids in sesame paste due to its high affinity to functional

groups at pH 7; hence a major decrease could be observed in mg/g gallic acid in dialyzed blend samples. Additionally, a study of Tagliazucchi *et al.*(2010) indicates that TP after pancreatic ingestion did not show a significant change due to the fact that gallic acid is stable and unlikely to be bound; by the way sum of phenolic contents in IN and OUT samples are almost equal to that of PG. However, most important consideration here is phenolic contents of dialyzed samples representing the phenolic amounts absorbed by the body. The dialyzed blends have a little lower TP content than both products. TP of sesame paste seems to be absorbed easier than all others, since figure shows that TP content of dialyzed sesame paste is higher compared to others although it has low TP at the beginning. In contrast, TP of grape molasses could not be absorbed well. Moreover, highest decrease was observed in third blend with 70% grape molasses, and at the end, TP of all blends was found to be close to each other, by effect of absorbance capacity seen in results belonging to sesame paste. Hence, it can be concluded that no matter sesame paste or grape molasses is highest in ratio, dialyzed amount of TP is nearly same.

3.9.2 Total flavonoid content

Total flavonoid content of initial samples and IN (dialyzed) and their comparison with TF of extracts are given in Figure 3.9.

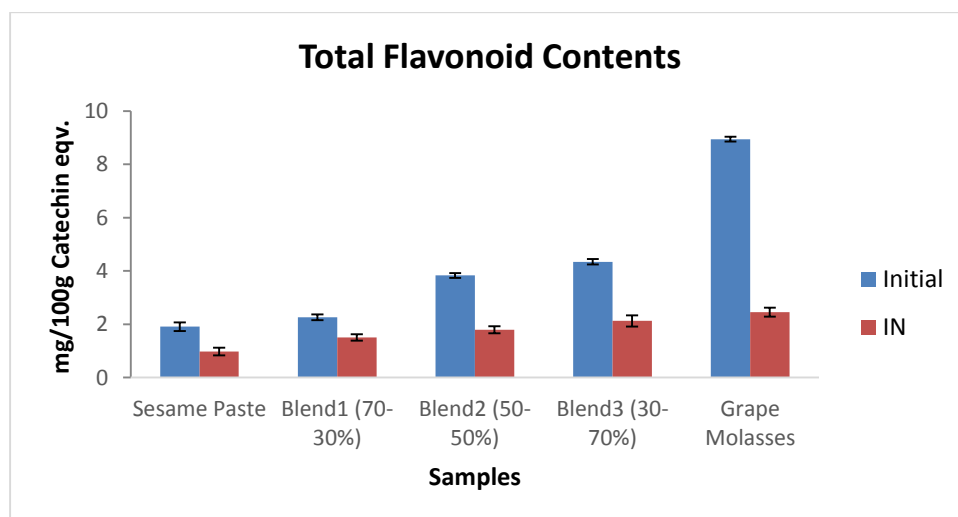


Figure 3.9: Total flavonoid content of samples after *in vitro* digestion.

Figure 3.9 implies that TF values of blends decreased less than the two products after *in vitro* digestion which can be based on a study conducted *in vivo*, in which the

interaction of milk increases the bioaccessibility of catechins in tea. (Burg-Koorevaar *et al.*, 2011).

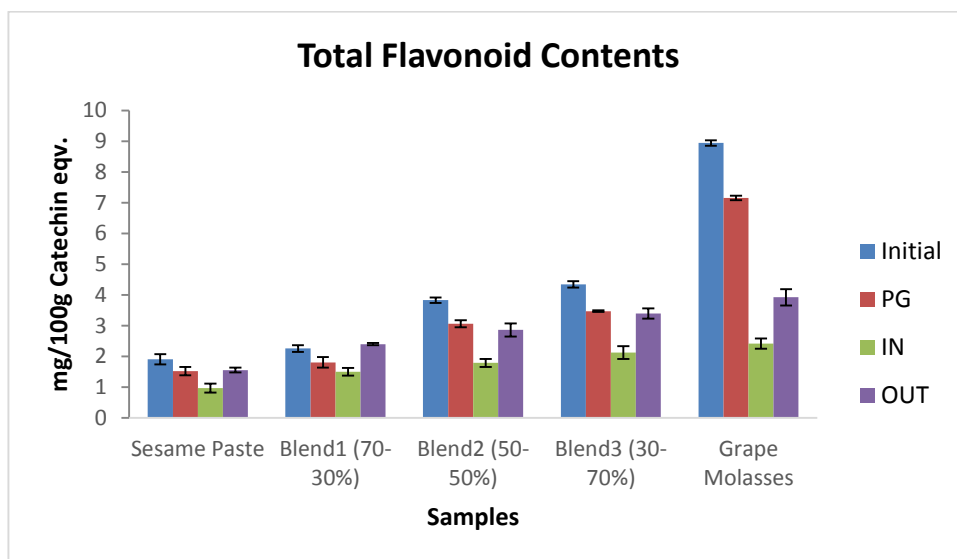
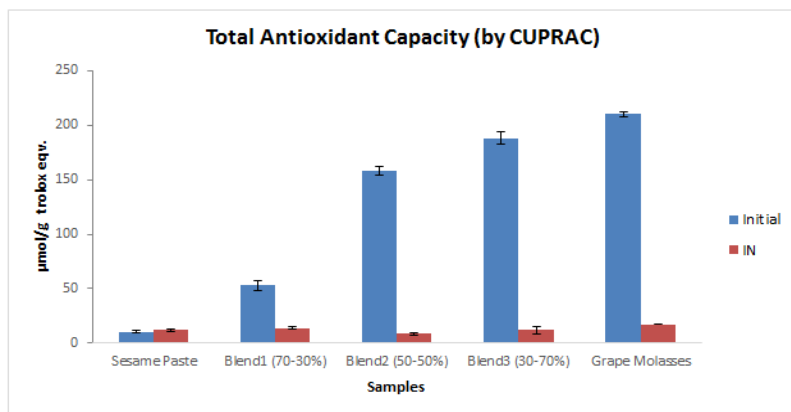


Figure 3.10: Total flavonoid content of samples before and after *in vitro* Digestion.

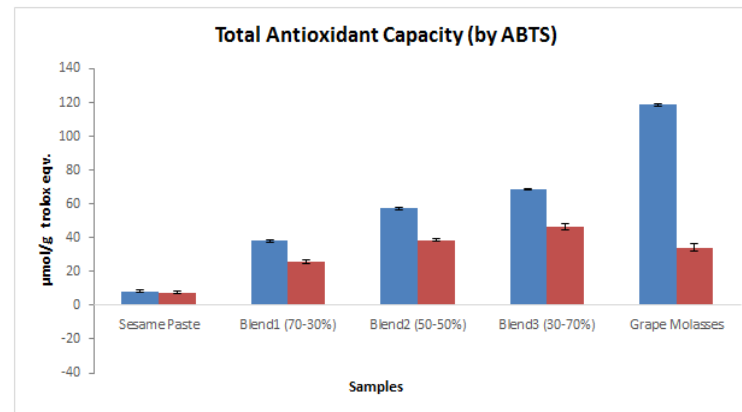
PG, IN and OUT samples and their comparison with TF of extracts are given in Figure 3.10. After gastric ingestion (PG), TF values of all samples have shown little amount of decrease except grape molasses that lost more than half of TF amount using catechin as standard. Here, again after pancreatic ingestion, dialyzed (IN) samples have shown lowest TF content. As well as TP, TF contents of dialyzed blends are close to that of grape molasses and higher than sesame paste. From the figures above, it is clear that flavonoids can be absorbed more easily in blends than in grape molasses individually since even if grape molasses has highest TF content before pancreatic ingestion it has a TF content a little higher than that of blends. This little difference may be explained by a study of Serafini *et al.* (2003) suggesting that interaction between proteins and flavonoids is the reason for inhibition of the absorption of catechin into the bloodstream.

3.9.3 Total Antioxidant Capacity

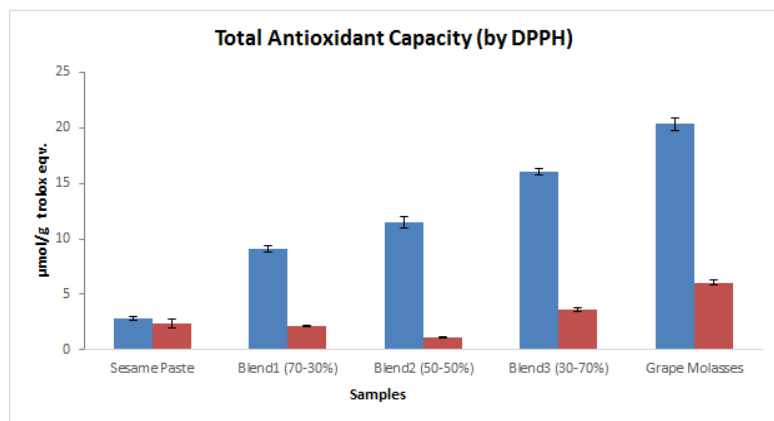
TEAC values of PG, IN and OUT samples were also measured by CUPRAC, ABTS, DPPH and FRAP assays. The results and their comparison with TEAC of extracts are given in Figure 3.11, respectively.



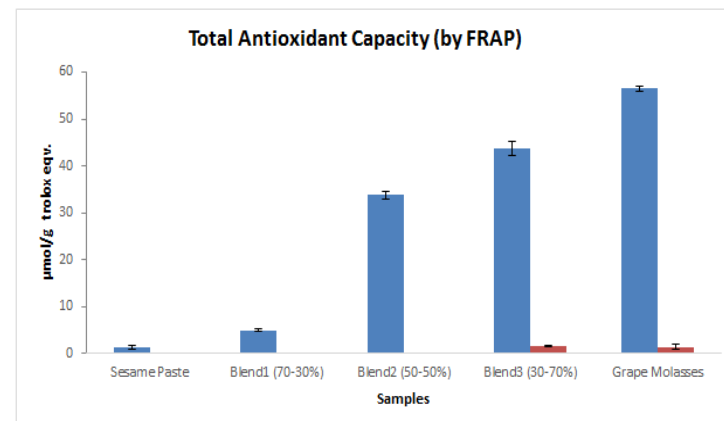
(a)



(b)



(c)



(d)

Figure 3.11: Total antioxidant capacity after *in vitro* digestion (a) by CUPRAC assay (b) by ABTS assay (c) by DPPH assay (d) by FRAP assay.

From the figure above, TAEC values obtained by ABTS assay differ from the other assays. According to that, dialyzed blends have higher antioxidant activities rather than sesame paste or grape molasses although the masking effect of proteins could be detected easily in this assay as mentioned before. Hence, it can be deduced that after pancreatic ingestion, masking effect decreases and presumably changed adversely. Serafini *et al.* (2009) clarified that in a study related to the bioavailability of phenolics and *in vivo* antioxidant capacity of blueberries consumed with and without milk by conducting the experiments. The researchers concluded that interactions of milk proteins and blueberry polyphenols impair the *in vivo* antioxidant properties of blueberries. On the other hand, other three assays point out that dialyzed blends do not own a better TAEC value than the two products individually. The differences between results of analyses may come from distinctions of the methods followed. Even if TAC assays were proven to correlate with Folin-Ciocalteu assays (TP) in herbals and apricots (Apak *et al.*, 2007), this correlation may support our results except for pancreatic digestion part. Moreover, Park *et al.* (2006) claimed that there is a low correlation between TF assays and some TAC assays, highest of all was between TF and ABTS as mentioned in Table 3.5.

Furthermore, as far as known, there are also contradictory results about the consequences of interactions in terms of antioxidant activity. To illustrate, Lotito *et al.* (2006) suggest that linkage of polypeptide chains with phenolic compounds probably leads to decay in the accessibility of phenolic compounds to the colonic microbiota and thus degradation of them takes place. Also, it is possible to come across to studies supporting the idea that interactions between polyphenols and proteins do not significantly affect antioxidant activity (Leenen *et al.*, 2000).

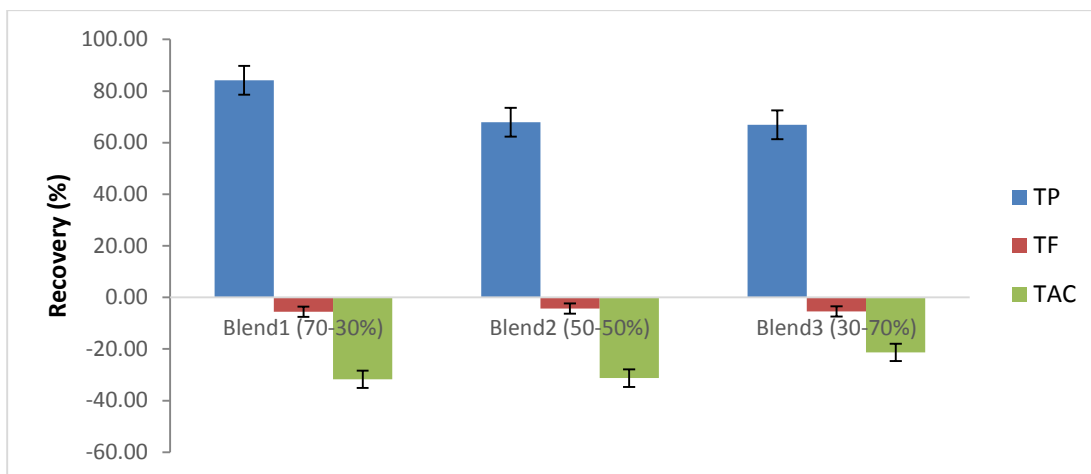


Figure 3.12: Recovery (%) of samples in TP, TF and TAC assays.

As shown in Figure 3.12. TP of blends shows positive recovery in contrast to TF and TAC. By the way, phenolic contents of blends are said to be higher than proportional while this is opposite for flavonoids and antioxidant capacities.

3.10 Major Individual Phenolic Compounds

According to HPLC analysis, Figure 3.13 lightens that predominant phenolic compounds were found as gallic acid, catechin, epicatechin and cinnamic acid in grape molasses.

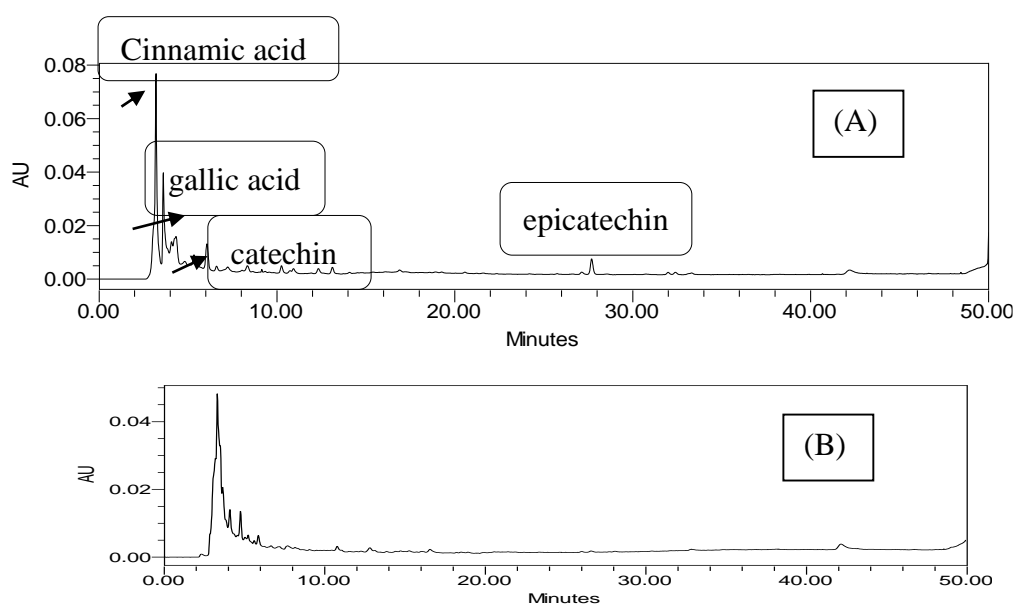


Figure 3.13: (A) HPLC chromatograms (PDA, recorded at 254 nm) of grape molasses extracts. (B) HPLC chromatograms (PDA, recorded at 254 nm) of dialyzed grape molasses.

From the figure above, it can be observed that total phenolic compounds reduced by almost half in content after gastric ingestion. In TP experiment, mg/100g GAE was lowered more than half in Figure 3.7. On the other hand, catechin content is seen to decrease a little compared to gallic acid. That is also consistent with the experiment done before indicating that decay in TF content was found as lower than that of TP in Figure 3.9. Additionally, since TP and TF experiments are done with the standard of gallic acid and catechin, respectively; the other phenolic compounds may not be established and counted on during the analysis.

Figure 3.14 shows the major individual phenolic compound found in sesame paste that is sesamin which contributes to antioxidant activity of sesame paste (Williamson *et al.*, 2008). Sesamin content also shows a decrease after gastric ingestion. Although sesamin is in high amounts, that does not show a significant contribution in the experiments done before most probably due to standard variation.

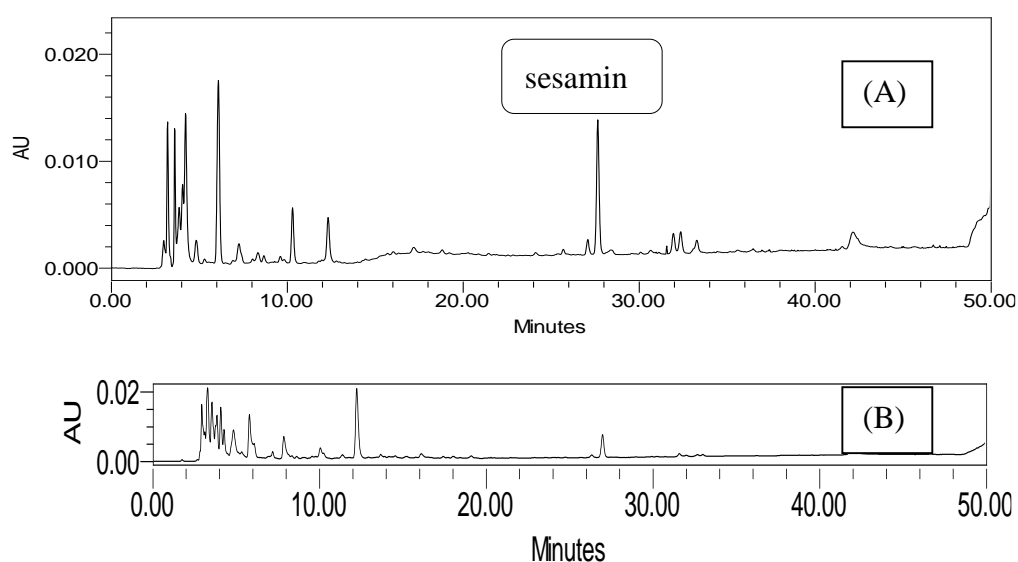


Figure 3.14: (A) HPLC chromatograms (PDA, recorded at 254 nm) of sesame paste extracts. (B) HPLC chromatograms (PDA, recorded at 254 nm) of dialyzed sesame paste.

From the three figures below (Figure 3.15-3.17), detected phenolic compounds are increasing with grape molasses.

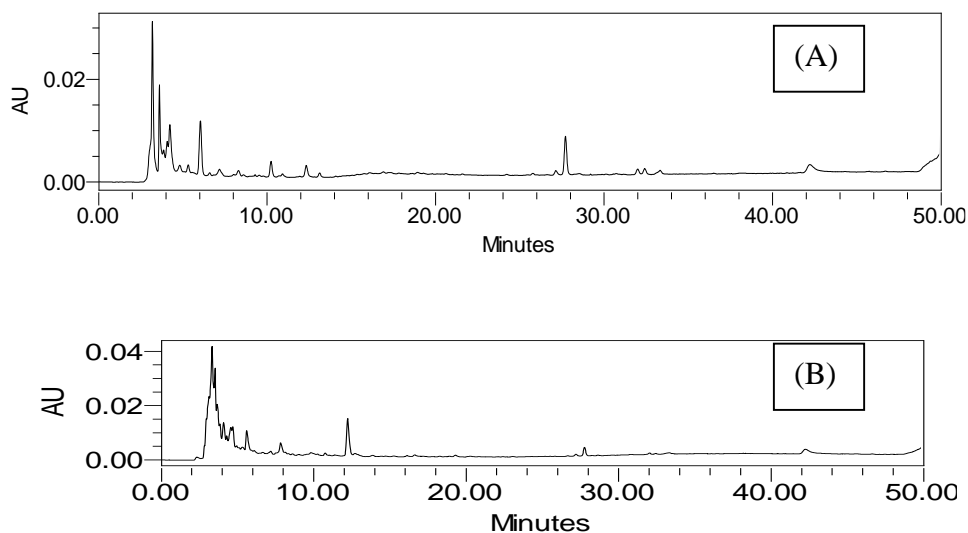


Figure 3.15: (A) HPLC chromatograms (PDA, recorded at 254 nm) of extracts of blend with 70% sesame paste and 30% grape molasses. (B)HPLC chromatograms (PDA, recorded at 254 nm) of dialyzed blend with 70% sesame paste and 30% grape molasses.

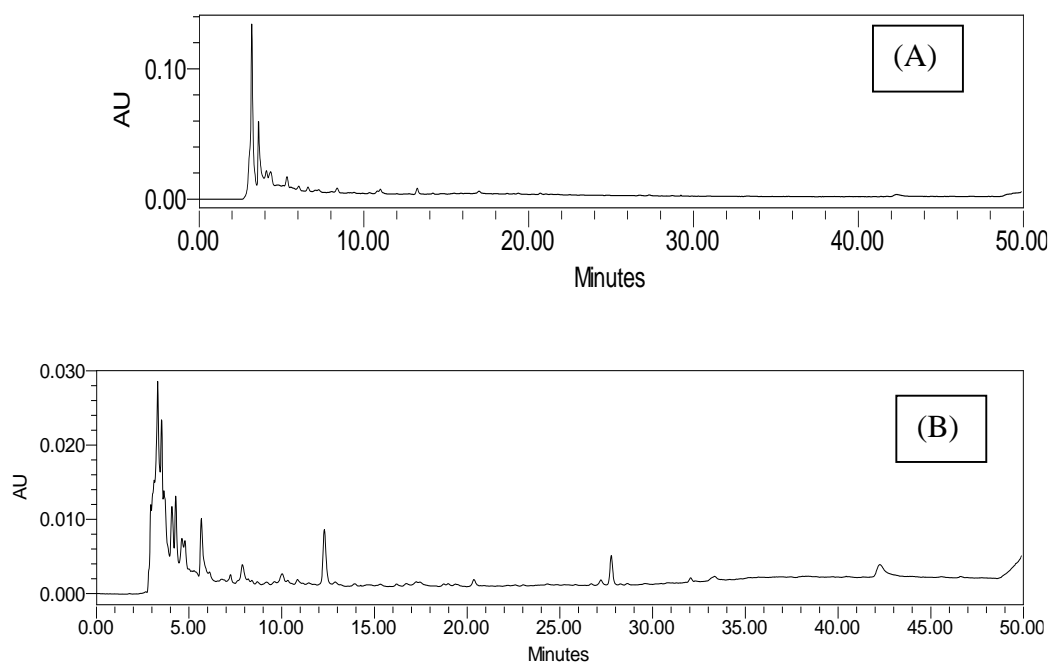


Figure 3.16: (A) HPLC chromatograms (PDA, recorded at 254 nm) of extracts of half and half blend.(B)HPLC chromatograms (PDA, recorded at 254 nm) of dialyzed half and half blend.

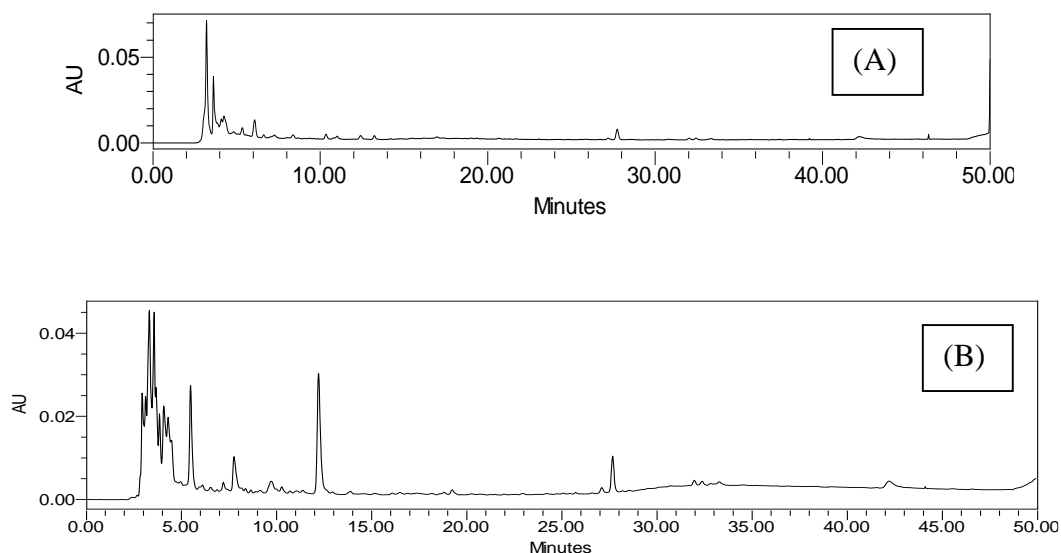


Figure 3.17: (A) HPLC chromatograms (PDA, recorded at 254 nm) of extracts of blend with 30% sesame paste and 70% grape molasses. (B) HPLC chromatograms (PDA, recorded at 254 nm) of dialyzed blend with 30% sesame paste and 70% grape molasses.

In the light of those three figures, after gastric ingestion, gallic acid content show a decrease in all three blends just as in the experiments. However, catechin content does not exhibit a great decrease for first and third blends. This outcome is also consistent with the experiments. Also, from the experiments, it was concluded that catechin contents of blends were higher than that of individual products after pancreatic ingestion. From the chromatograms, this consequence could be supported by the decrease observed in catechin contents of individual products while that is remained nearly same for two blends. However, for half and half blend, catechin content decreased just as gallic acid while there is not a significant decrease in observed phenolic compounds totally.

Moreover, half and half blend shows highest phenolic compound content at the beginning and lowest at the end. That outcome could not be explained by one assay. The highest beginning shows its effect on CUPRAC and ABTS assays, whereas lowest end does on DPPH assay.

4. CONCLUSION AND RECOMMENDATIONS

Grape products such as molasses have high nutritional value and beneficial health effects with respect to polyphenol contents and antioxidant activities. Sesame paste is traditionally consumed together with grape molasses. When consumed them as a blend, the interactions between proteins and phenolic compounds may take place. There are studies examining those kind of interactions between mostly milk proteins and polyphenols from different sources in the literature; however, there are limited available studies in the literature. Hence, further studies focusing on interactions between plant proteins and polyphenols are needed just as it is tried in the research. In this study, main focus was to examine changes in total phenolic, flavonoids and antioxidant capacity when sesame paste and grape molasses consumed together compared to individual consumptions.

In this study, the reason for highest TP, TF and TEAC values of OUT samples was explained by the idea that only 5 % of polyphenols enters IN samples and the majority of polyphenols passes intact of the colon fraction and may be degraded to phenolic compounds, by the way the values for OUT samples are higher than that of IN samples. In TP or TF analyses, it is possible to only detect some polyphenols, but in TAC analyses, a general view to antioxidant capacity may be provided which includes the effects of most polyphenols and by the way, increase in TAEC values after *in vitro* digestion is observed much more than other analyses.

With respect to outcomes of TP and TF analyses, it was observed that sesame paste ease the absorbance of phenolic compounds in grape molasses since blends have TP or TF contents close to each other.

In the light of the experimental results, changes in TF, TP and TAC values may be related to interactions between phenolic compounds especially from grape molasses and proteins from sesame paste. This issue is tried to be supported by literature. However, there are limited examples to studies on interactions between plant derived proteins and phenolics. Moreover, in this type of studies, focus is usually on effects

of interactions on protein characteristics. More often than not, studies are based on the interactions of milk proteins with phenolics Ozdal *et al.*, 2013).

Certainly, interactions between proteins and phenolics continue to get attention via scientists. More studies should be done in this area, especially for plant proteins which consider not only protein characteristics but also polyphenol activities and contents, and also effects on rheology of blends to have desired products with best sensory and nutritional quality.

In conclusion, this study was investigated to examine effects of interactions between sesame proteins and polyphenols of grape molasses. Furthermore, in order to gain information about product characteristics, analyses related to moisture, protein and lipid content as well as rheological measurements were done. Although the results obtained with simulated *in vitro* GI digestion do not directly predict the human *in vivo* conditions, still this model is considered as helpful for investigating the bioavailability of polyphenols. In further studies, the consequences of interactions in different fields would be interesting to focus on.

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APPENDICES

APPENDIX A: Calibration Curves

APPENDIX B: HPLC Chromatograms

APPENDIX C: Rheological Graphs

APPENDIX D: ANOVA Tables

APPENDIX A

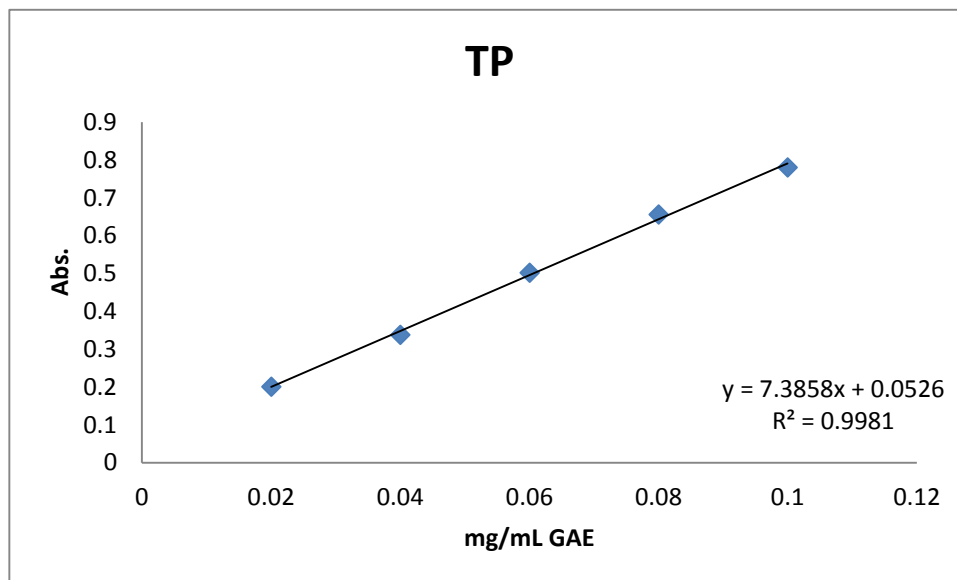


Figure A.1 : Calibration curve for total phenolics in 75% aqueous-methanol containing 0.1% (v/v) formic acid.

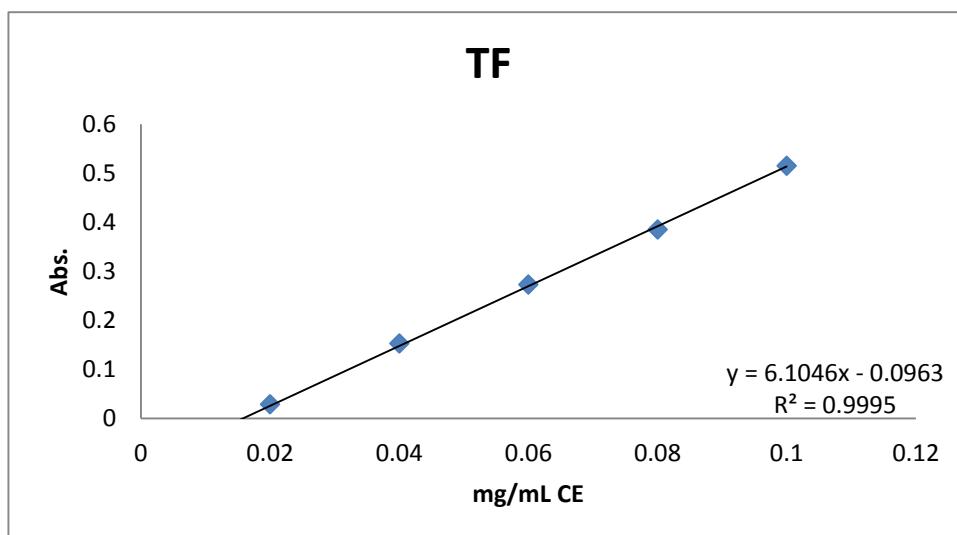


Figure A.2 : Calibration curve for total flavonoids in 75% aqueous-methanol containing 0.1% (v/v) formic acid.

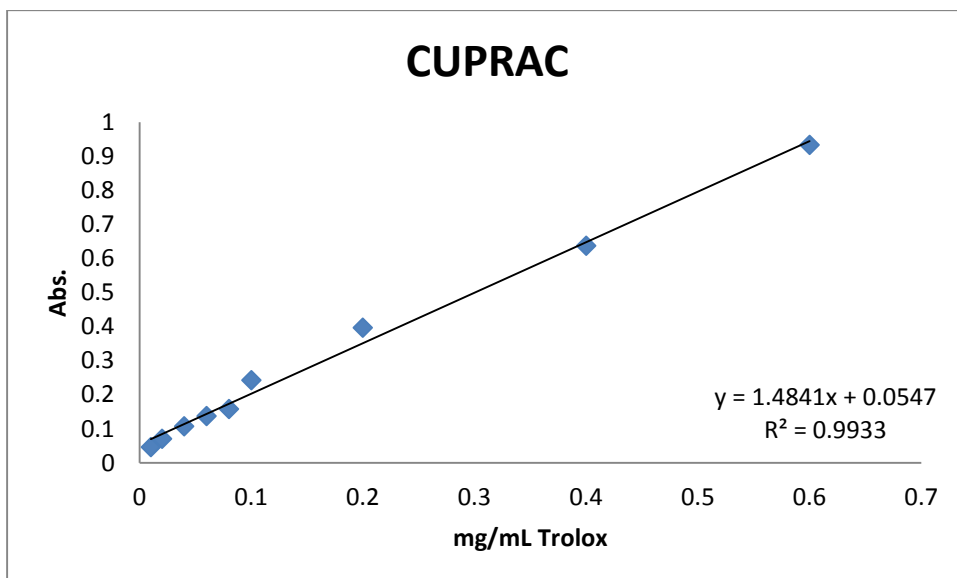


Figure A.3 : Calibration curve for CUPRAC assay in 75% aqueous-methanol containing 0.1% (v/v) formic acid.

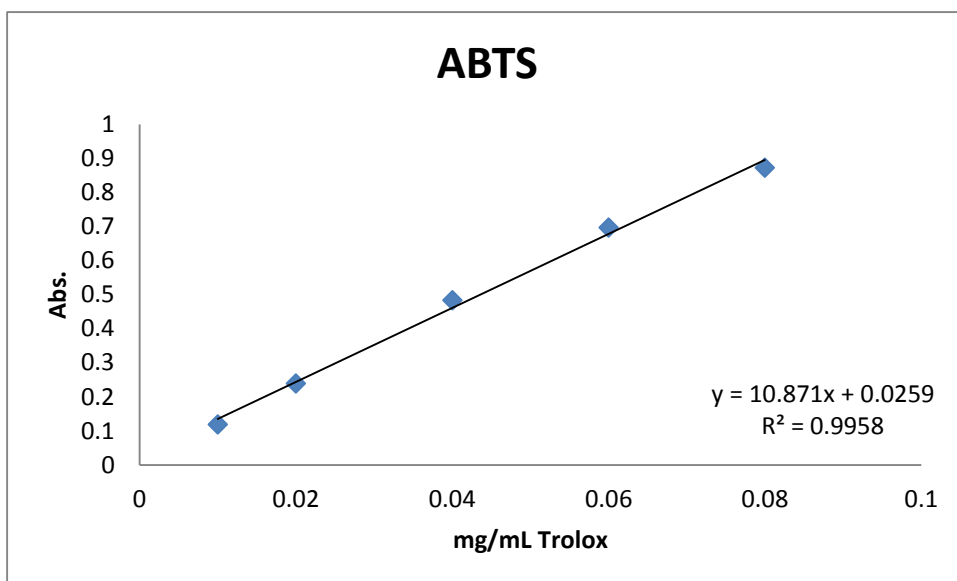


Figure A.4 : Calibration curve for ABTS assay in 75% aqueous-methanol containing 0.1% (v/v) formic acid..

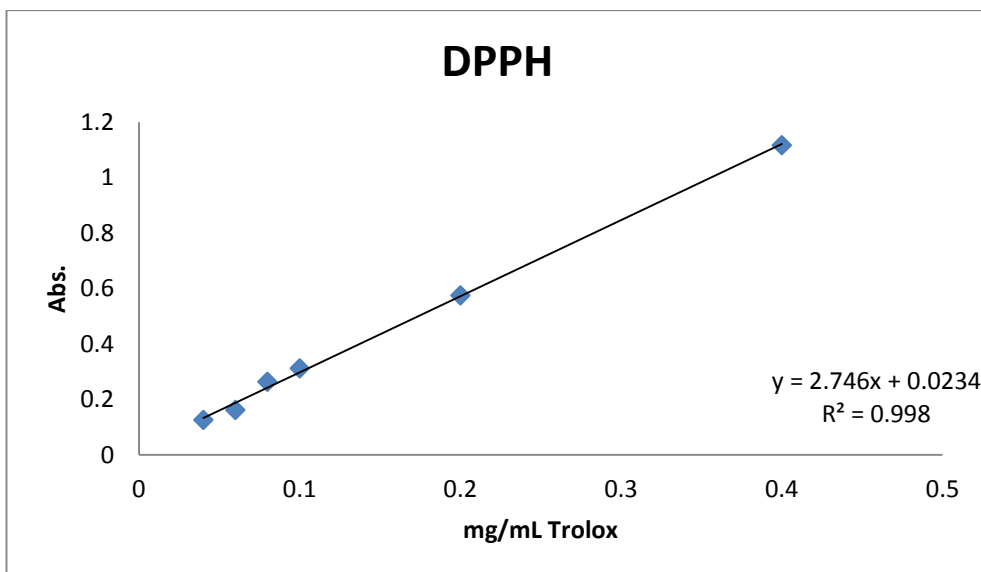


Figure A.5 : Calibration curve for DPPH assay in 75% aqueous-methanol containing 0.1% (v/v) formic acid.

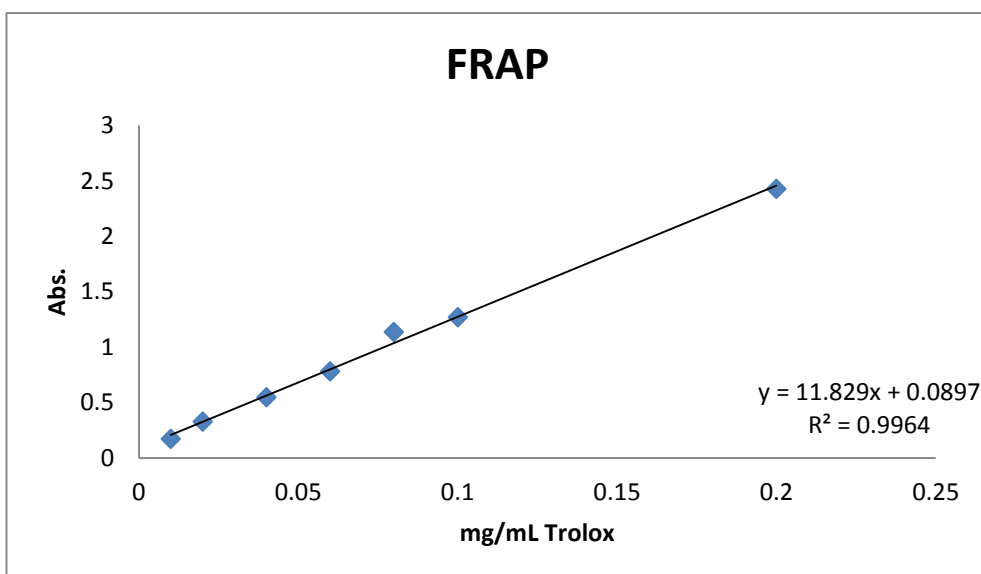


Figure A.6 : Calibration curve for FRAP assay in 75% aqueous-methanol containing 0.1% (v/v) formic acid.

APPENDIX B

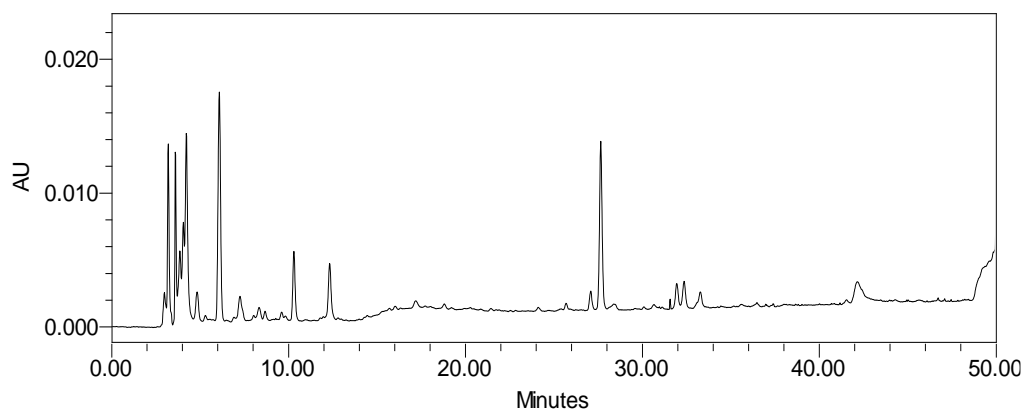


Figure B.1. HPLC chromatogram (recorded at 254 nm) of sesame paste extracts.

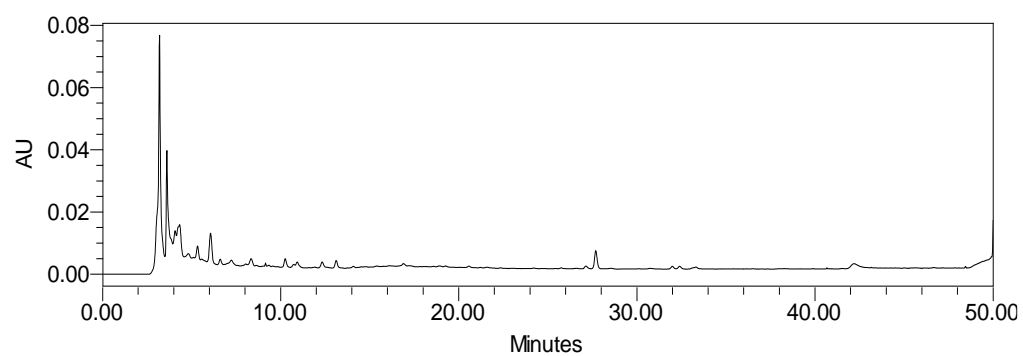


Figure B.2: HPLC chromatogram (recorded at 254 nm) of grape molasses extracts.

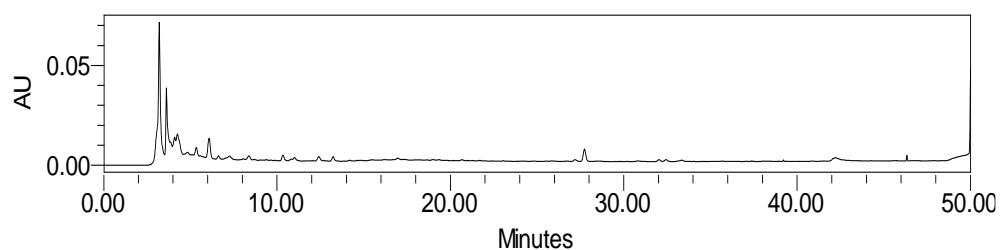


Figure B.3: HPLC chromatogram (recorded at 254 nm) of extracts of blend with 30% sesame paste and 70% grape molasses.

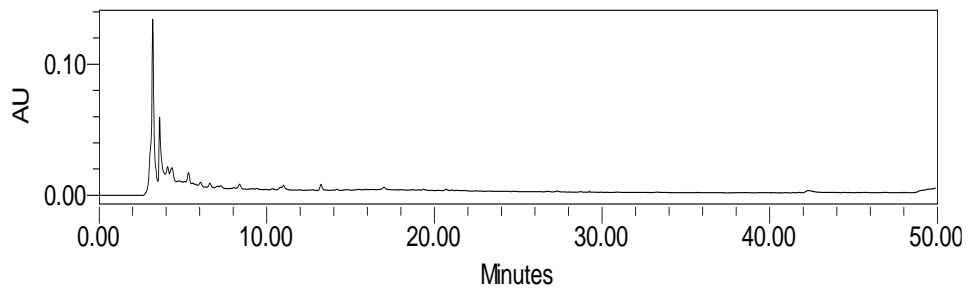


Figure B.4: HPLC chromatogram (recorded at 254 nm) of extracts of half and half blend.

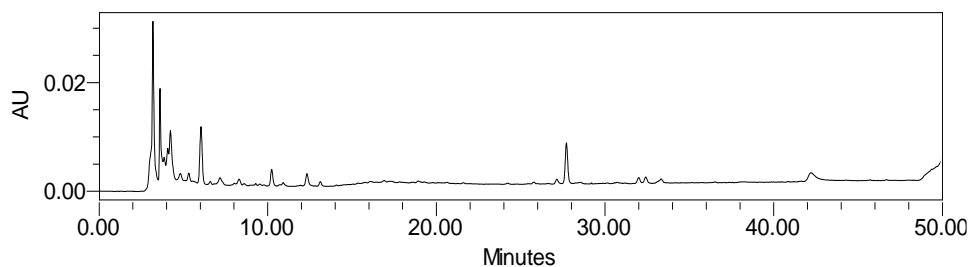


Figure B.5: HPLC chromatogram (recorded at 254 nm) of extracts of blend with 70% sesame paste and 30% grape molasses.

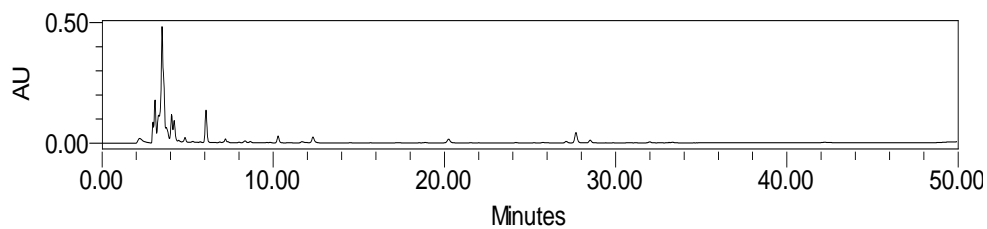


Figure B.6: HPLC chromatogram (recorded at 254 nm) of sesame paste (PG).

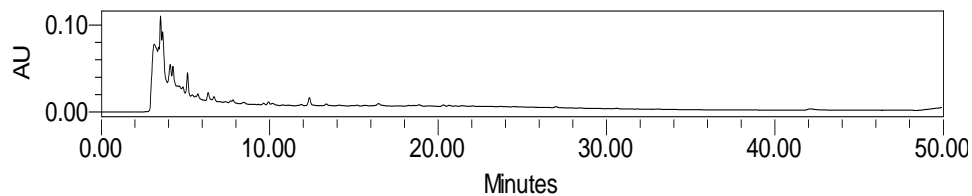


Figure B.7: HPLC chromatogram (recorded at 254 nm) of grape molasses (PG).

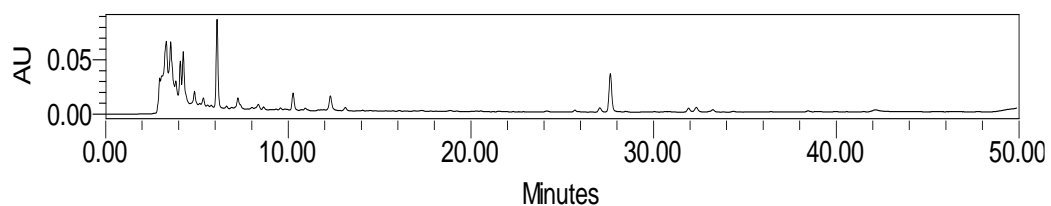


Figure B.8: HPLC chromatogram (recorded at 254 nm) of blend with 30% sesame paste and 70% grape molasses (PG).

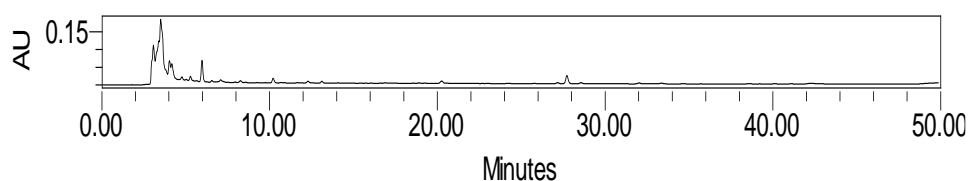


Figure B.9: HPLC chromatogram (recorded at 254 nm) of half and half blend (PG).

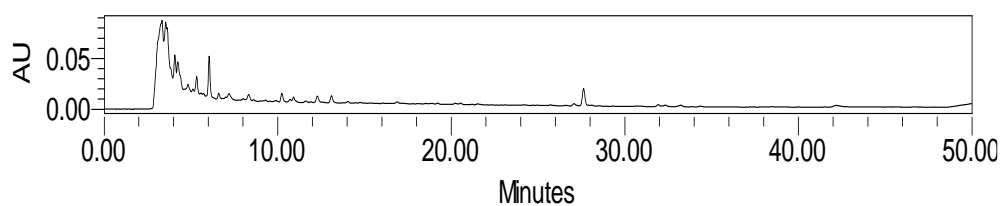


Figure B.10: HPLC chromatogram (recorded at 254 nm) of blend with 70% sesame paste and 30% grape molasses (PG).

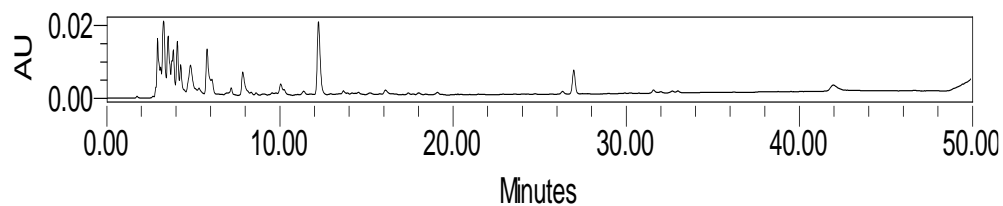


Figure B.11: HPLC chromatogram (recorded at 254 nm) of dialyzed sesame paste.

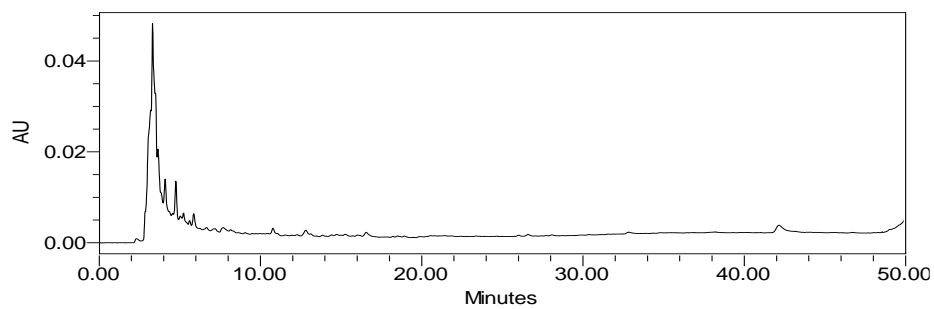


Figure B.12: HPLC chromatogram (recorded at 254 nm) of dialyzed grape molasses.

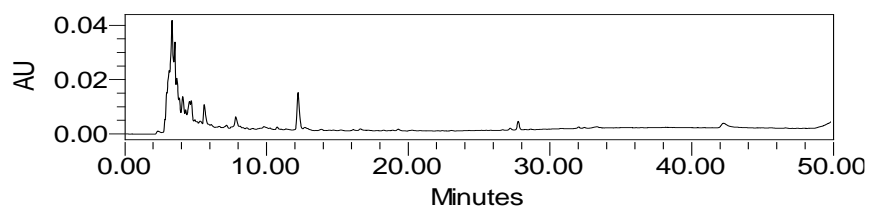


Figure B.13: HPLC chromatogram (recorded at 254 nm) of dialyzed blend with 30% sesame paste and 70% grape molasses.

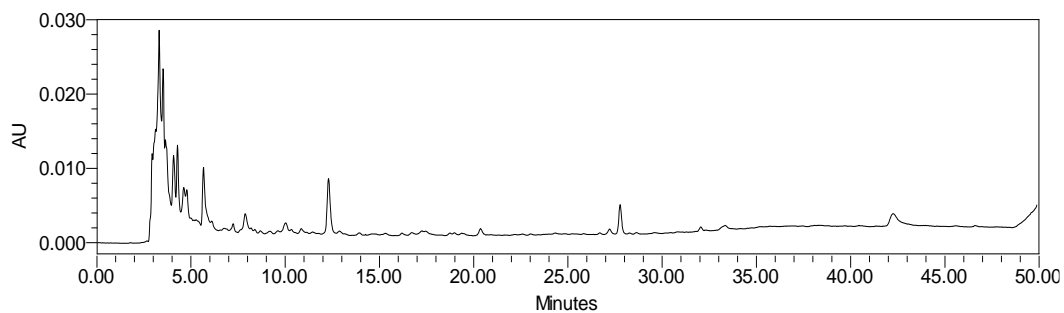


Figure B.14: HPLC chromatogram (recorded at 254 nm) of dialyzed half and half Blend.

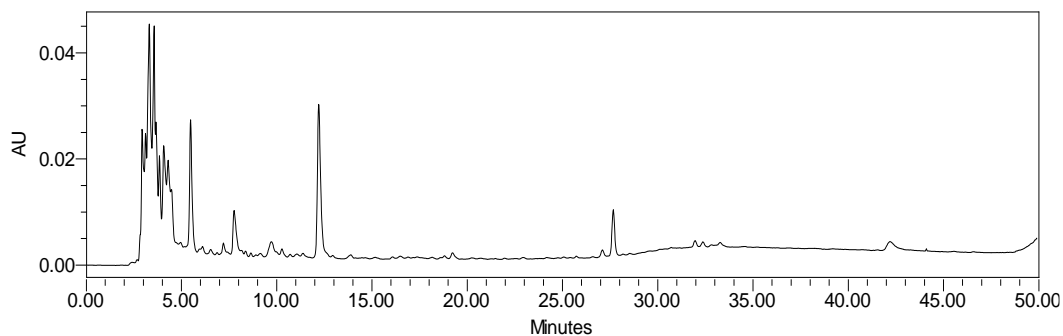


Figure B.15: HPLC chromatogram (recorded at 254 nm) of dialyzed blend with 70% sesame paste and 30% grape molasses.

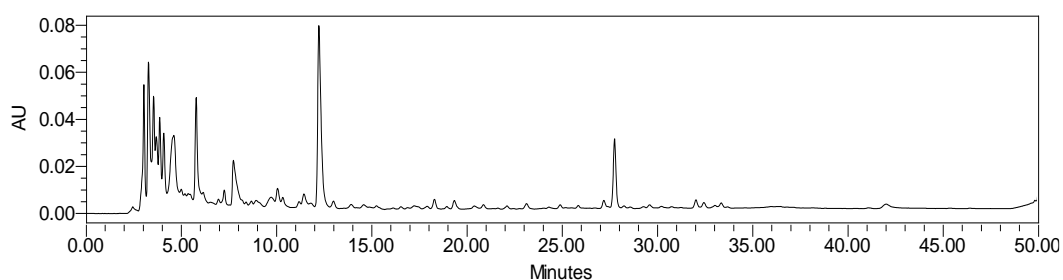


Figure B.16: HPLC chromatogram (recorded at 254 nm) of sesame paste (OUT).

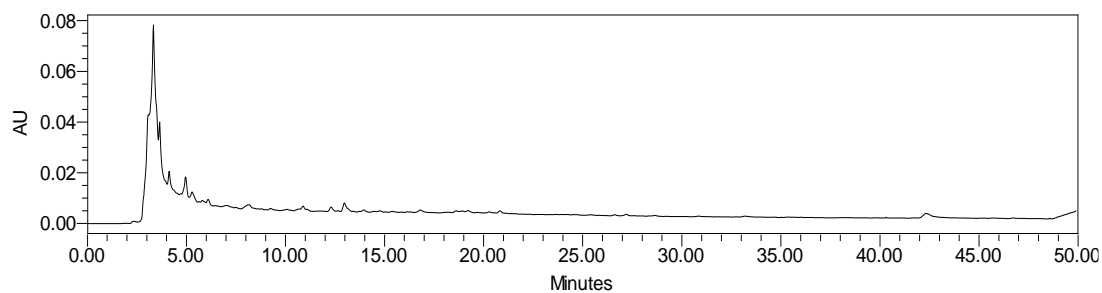


Figure B.17: HPLC chromatogram (recorded at 254 nm) of grape molasses (OUT).

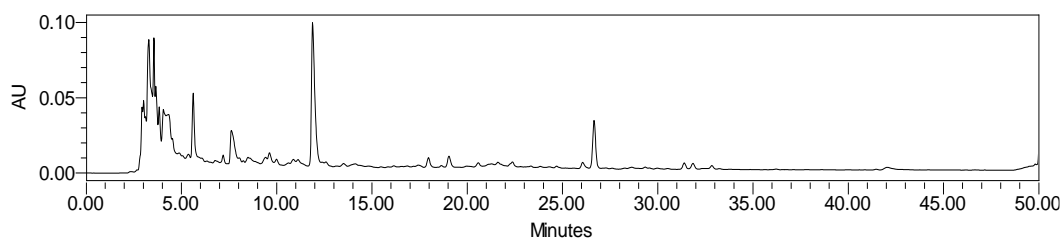


Figure B.18: HPLC chromatogram (recorded at 254 nm) of blend with 30% sesame paste and 70% grape molasses (OUT).

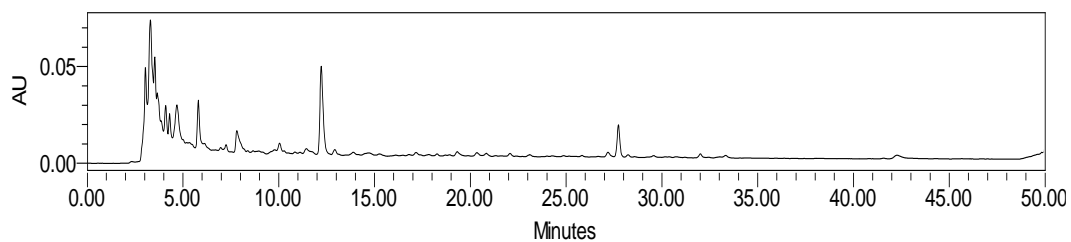


Figure B.19: HPLC chromatogram (recorded at 254 nm) of half and half blend (OUT).

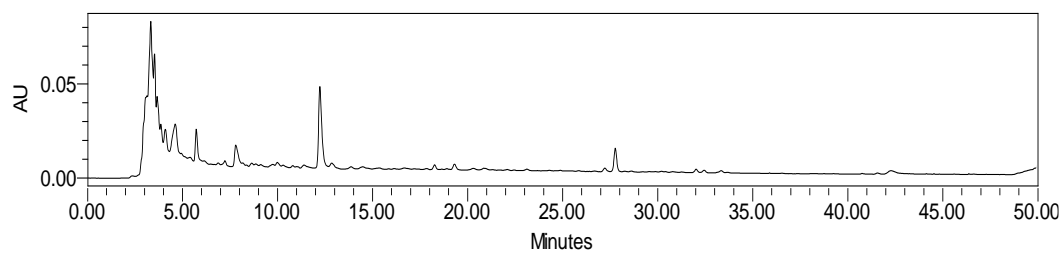


Figure B.20: HPLC chromatogram (recorded at 254 nm) of blend with 70% sesame paste and 30% grape molasses (OUT).

APPENDIX C

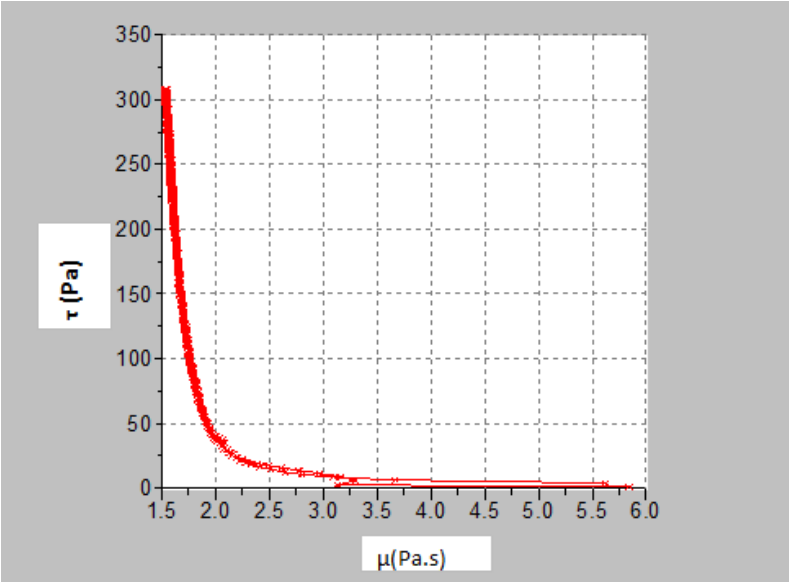


Figure C.1: Shear stress vs. viscosity graph of sesame paste.

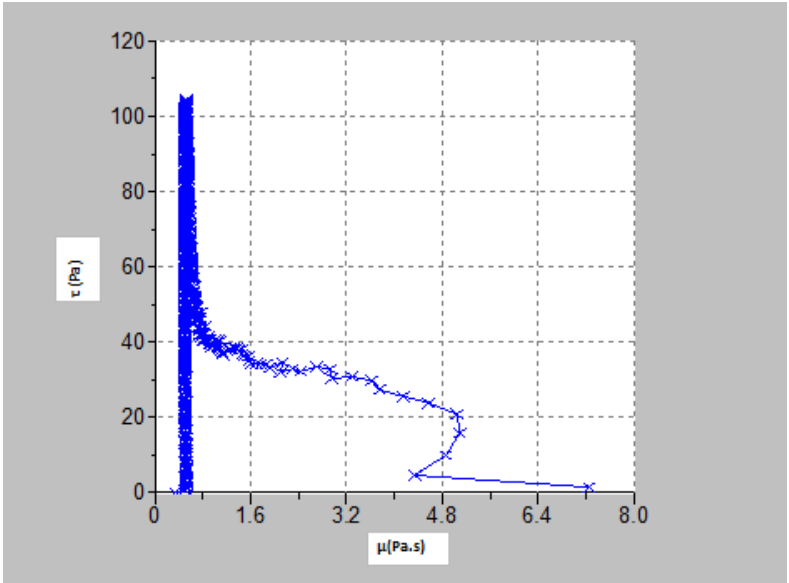


Figure C.2: Shear stress vs. viscosity graph of grape molasses.

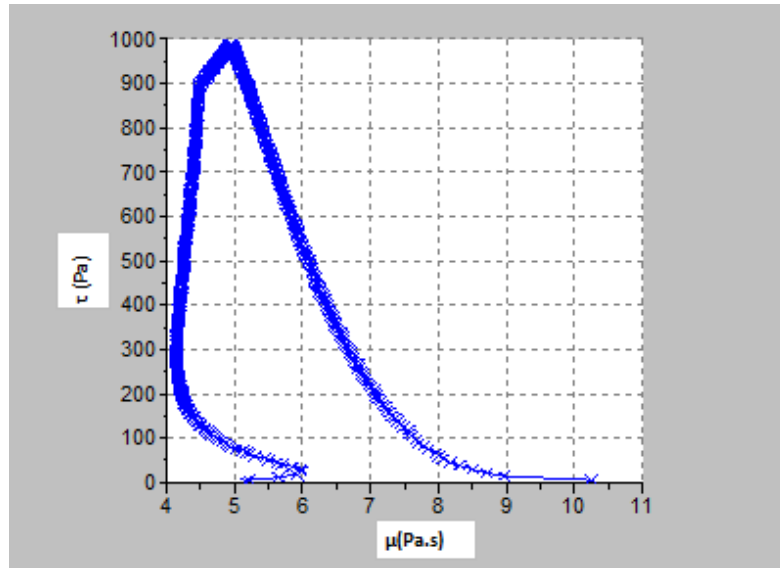


Figure C.3: Shear stress vs. viscosity graph of blend with 70% sesame paste and 30% grape molasses.

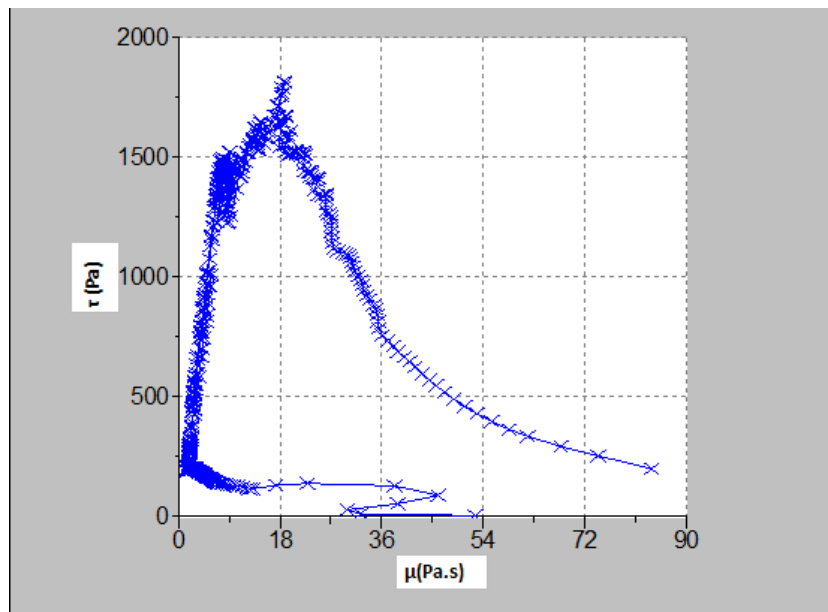


Figure C.4: Shear stress vs. viscosity graph of half and half blend.

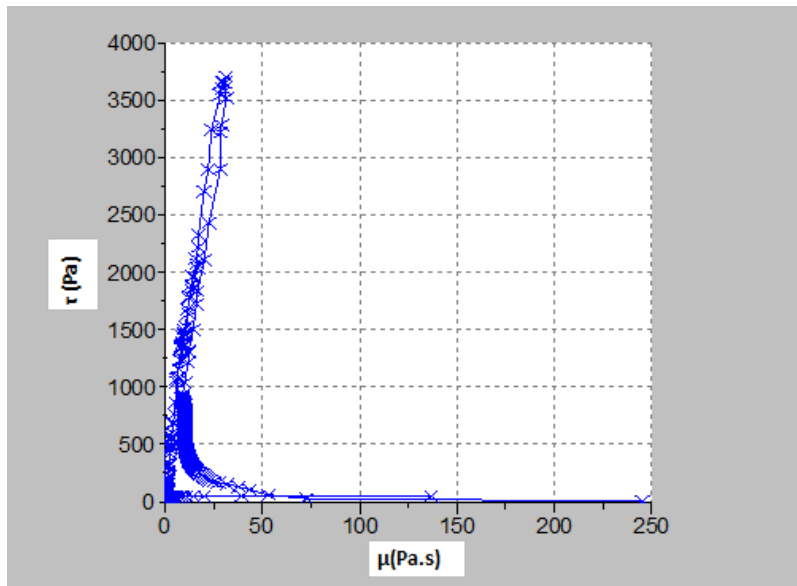


Figure C.5. Shear stress vs. viscosity graph of blend with 30% sesame paste and 70% grape molasses.

APPENDIX D**Table D.1.** Statistical analysis of Initials.

		ANOVA TABLE				
		Sum of	df	Mean	F	Sig.
		Squares		Square		
TP	Between	12190,411	4	3047,603	3144,019	,000
	Groups					
	Within Groups	9,693	10	,969		
	Total	12200,104	14			
TF	Between	94,729	4	23,682	1869,658	,000
	Groups					
	Within Groups	,127	10	,013		
	Total	94,856	14			
CUPRA C	Between	91939,017	4	22984,754	1619,101	,000
	Groups					
	Within Groups	141,960	10	14,196		
	Total	92080,977	14			
ABTS	Between	19931,943	4	4982,986	24267,787	,000
	Groups					
	Within Groups	2,053	10	,205		
	Total	19933,996	14			
DPPH	Between	535,147	4	133,787	912,182	,000
	Groups					
	Within Groups	1,467	10	,147		
	Total	536,613	14			
FRAP	Between	6990,443	4	1747,611	3645,919	,000
	Groups					
	Within Groups	4,793	10	,479		
	Total	6995,236	14			

Table D.2. Statistical analysis of PG samples

		ANOVA TABLE				
		Sum of Squares	df	Mean Square	F	Sig.
TP	Between Groups	5998,351	4	1499,588	1147,644	,000
	Within Groups	13,067	10	1,307		
	Total	6011,417	14			
TF	Between Groups	60,047	4	15,012	2814,687	,000
	Within Groups	,053	10	,005		
	Total	60,100	14			
CUPR AC	Between Groups	79201,593	4	19800,398	13360,593	,000
	Within Groups	14,820	10	1,482		
	Total	79216,413	14			
ABTS	Between Groups	17523,263	4	4380,816	227,378	,000
	Within Groups	192,667	10	19,267		
	Total	17715,929	14			
FRAP	Between Groups	4730,893	4	1182,723	476,520	,000
	Within Groups	24,820	10	2,482		
	Total	4755,713	14			

Table D.3. Statistical analysis of IN samples

		ANOVA TABLE				
		Sum of	df	Mean	F	Sig.
		Squares		Square		
TP	Between	93,531	4	23,383	369,200	,000
	Groups					
	Within	,633	10	,063		
	Groups					
	Total	94,164	14			
TF	Between	3,647	4	,912	195,357	,000
	Groups					
	Within	,047	10	,005		
	Groups					
	Total	3,693	14			
CUPRA C	Between	131,149	4	32,787	184,199	,000
	Groups					
	Within	1,780	10	,178		
	Groups					
	Total	132,929	14			
ABTS	Between	2640,971	4	660,243	26766,595	,000
	Groups					
	Within	,247	10	,025		
	Groups					
	Total	2641,217	14			
DPPH	Between	42,869	4	10,717	211,526	,000
	Groups					
	Within	,507	10	,051		
	Groups					
	Total	43,376	14			
FRAP	Between	,027	1	,027	4,000	,116
	Groups					
	Within	,027	4	,007		
	Groups					
	Total	,053	5			

Table D.4. Statistical analysis of OUT samples

		ANOVA TABLE				
		Sum of	df	Mean	F	Sig.
		Squares		Square		
TP	Between	8458,457	4	2114,614	1749,543	,000
	Groups					
	Within	12,087	10	1,209		
	Groups					
	Total	8470,544	14			
TF	Between	9,983	4	2,496	143,981	,000
	Groups					
	Within	,173	10	,017		
	Groups					
	Total	10,156	14			
CUPRA C	Between	14762,847	4	3690,712	210,914	,000
	Groups					
	Within	174,987	10	17,499		
	Groups					
	Total	14937,833	14			
ABTS	Between	15133,797	4	3783,449	13909,740	,000
	Groups					
	Within	2,720	10	,272		
	Groups					
	Total	15136,517	14			
DPPH	Between	419,813	4	104,953	266,379	,000
	Groups					
	Within	3,940	10	,394		
	Groups					
	Total	423,753	14			
FRAP	Between	6728,997	4	1682,249	904,435	,000
	Groups					
	Within	18,600	10	1,860		
	Groups					
	Total	6747,597	14			

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