

**İSTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND
TECHNOLOGY**

**CHIA SEED ADDED STRAWBERRY MARMELADE
WITH NO ADDED SUGAR**



M.Sc. THESIS

Tuğba ÖZBEK

Department of Food Engineering

Food Engineering Programme

JUNE 2016

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

ÇİYA TOHUMU İLAVELİ, ŞEKER İLAVESİZ ÇİLEK MARMELADI

YÜKSEK LİSANS TEZİ

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To my husband and son,



FOREWORD

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May 2016

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ABBREVIATIONS

ANOVA	:Analysis of Variance
SMWC	:Strawberry Marmalade with Chia
PUFA	: Polyunsaturated Fatty Acids
EPA	: Eicosapentaenoic Acid
DHA	: Docosahexaenoic Acid
GC	: Gas Chromatography
TDF	: Total Dietary Fiber
HMF	: Hydroxymethyl furfural



SYMBOLS

G'	: Storage modulus
G''	: Complex modulud
Tan δ	: Loss tangent



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CHIA SEED ADDED STRAWBERRY MARMALADE WITH NO ADDED SUGAR

SUMMARY

There is a demand on increasing the nutritional value and variety of diabetic foods in the market in our country. In this study, we aimed to enrich a diabetic marmalade by means of dietary fiber, omega-3 polyunsaturated fatty acids (PUFAs) and antioxidant contents by using chia seed.

Chia seed is an ancient grain but a new superfood becoming popular in the recent years. In this study, composition of chia seed was analyzed and it is found that chia seed is composed of 7.42 % moisture, 3.93 % ash, 19.59 % protein, 29 % fat, 38.94 % total dietary fiber. According to these values, we can say that, chia seed is very rich in nutritional value compared to other seeds.

Chia seed was analyzed for its composition, phenolic compounds and antioxidant capacity. Its composition was found as moisture 7.42 %, ash 3.93 %, protein 19.59, fat 29.01 %, total dietary fiber 38,94 %. Sugar free , chia added marmalade formula was optimized by a two step sensory analysis. First step was for selecting the sweetener type, the second was for selecting the percentage of chia. The sweeteners tried in the formula were izomalt, stevia, sorbitol and izomalt-sorbitol mixture. Sorbitol was the nearest one to the control, marmalade with sucrose, by means of sweetness, strawberry odor, strawberry flavor and spreadability. The percentage of chia was selected as 5 %. Its thickness, cereal taste, strawberry flavor were more consumable according to consumers. The composition analyses were performed on the formula: 48 % strawberry, 47 % sorbitol and 5 % chia seed. This jam had the composition; moisture 35.16 %, ash 0.46 %, protein 1.30 %, fat 2.46 %, total dietary fiber 2.7 % and carbohydrate 61.26 %. Soluble solid of the marmalade was 64.3 % and acidity was 0.5 %, which were same with control marmalade. pH value of the marmalade was 4.43, while the control marmalade had 3.43 pH value. This could make the new marmalade more favorable to microbial growth, and further analyses may focus on optimizing the pH value of chia added marmalade without changing the sensory parameters. The sugar free chia added marmalade was compared to control marmalade by means of phenolic content, energy and total dietary fiber. 5 % chia additon increased the phenolics by 15.45 %, increased the dietary fiber content by 168 % and decreased the caloric value by 48 %. Rheological parameters of the nem formulated sugar free chia added marmalade showed that the jam was pseudoplastic, resulted a gretaer G' value than G'' value indicating a viscoelastic behaviour and a tan δ smaller than 1 indicating a gel-like behaviour.

The sugar free chia added strawberry marmalade may be a new functional food serving functional food ingredients such as antioxidants, dietary fiber and omega-3 PUFAs. Since Turkish people consume a signficiant amount of marmalade, especiallly in breakfast, it may be a good way to serve these functional foods in jam, by not dramatically changing the nutrition habits of Turkish people.



ÇİYA TOHUMU İLAVELİ, ŞEKER İLAVESİZ ÇİLEK MARMELADI

ÖZET

Son yıllarda insanların kanser, diyabet, kalp hastalıkları gibi ölümcül hastalıklara olan farkındalığı artmış; bu farkındalık fonksiyonel gıdaları tüketmeye yöneltmiştir. Bu fonksiyonel gıdalar, bazı esansiyel yağ asitlerinden bağırsak düzenleyici prebiyotik gıdalara kadar çeşitlenmektedir. Şeker ilavesiz ürünler de şekerin zararlı etkilerini ortadan kaldırmaları sebebiyle fonksiyonel gıda sınıfında yer alabilir. Ancak ülkemizde şeker ilavesiz ürünlerin çeşitliliği sınırlıdır. Şeker ilavesiz ürünlerin tek fonksiyonel özellikleri şekerin zararlı etkilerini barındırmamasıdır. Bu çalışmada da çiya tohumu ilavesi ile omega-3 çoklu doymamış yağ asitleri, toplam diyet lifi ve antioksidanlar bakımından zenginleştirilmiş şeker ilavesiz marmelat yapmak hedeflenmiştir. Çiya tohumu ilavesi ile marmelat, bazı fonksiyonel gıda bileşenlerince zenginleştirilmiş olurken; şeker ilavesiz olması sebebiyle de şekerin zararlı etkileri ortadan kaldırılmış olacak, diyabet hastaları ve kilo kaybetmeyi hedefleyen bireyler için besin değeri yüksek bir kahvaltılık ürün geliştirilmiş olacaktır.

Çiya tohumu tarih öncesinden gelen, ancak son yıllarda “süper gıda” olarak popülerleşen bir tohum çeşididir. Çiya tohumunun “süper gıda” olarak anılması içeriğinde yüksek oranda faydalı besin bileşenleri bulunmasından dolayıdır. Çiya *Lamiaceae* bitki ailesinden, 1 m uzunluğunda, yapraklı bir bitkidir. Çiya bitkisinin tohumları 1,5 – 2 mm çapında siyah ve gri renklerde yuvarlak tohumlardır (Coates, 2011). Çiya bitkisi ılıman iklimde yetişen, donmaya karşı dayanıklı olmayan bir bitkidir ve en fazla oranda ekvator çevresinde yetişir. İlk üreticilerinden olan Meksika, günümüzde de en büyük üretici ve ihracatçı durumundadır. Meksika’ yı Avusturalya, Peru, Paraguay ve Ekvator takip etmektedir (Coates, 2011). Literatür çalışmaları çiya tohumunun yaklaşık % 40 diyet lifi, % 30 yağ ve % 20 protein içerdiğini göstermektedir. Yağ içeriğinin % 60 kadarı insan vücudunda EPA ve DHA yağ asitlerinin sentezlenmesini sağlayan omega- 3 yağ asididir. EPA ve DHA insan gözü ve beyinde yüksek oranda bulunan yağ asitleridir. Ayrıca kalp rahatsızlıkları, kanser türleri ve şizofreni üzerine olumlu etkileri bulunmaktadır. (Koletzko ve ark., 2001; Lora ve ark., 2007). Çiya tohumunda yaklaşık % 40 oranında diyet lifi bulunmaktadır. Amerikan Diyetisyenler Birliği, sağlıklı bireylerin günde 20 – 35 gr diyet lifi tüketmesinin bağırsak faaliyetlerini düzenlediğini, kanser türlerinden korunmaya yardımcı olduğunu bildirmiştir. Çiya tohumu vitamin ve minerallerce de zengindir. Sütten 6 kat daha fazla kalsiyum, 11 kat daha fazla fosfor, 4 kat daha fazla

potasyum içerir (Munoz ve ark., 2013). Çiya tohumunda yüksek oranda bulunan antioksidanlar olan kamferol ve kuersetinin yağ oksidasyonunu önleme kapasitesi vitamin C' nin yağ oksidasyonunu önleme kapasitesinden daha yüksek bulunmuştur (Caudillo ve ark., 2008). Çiya tohumu tüketiminin kolesterol düşürme (Ayerza ve Coates, 2007), diyabetle mücadele (Vuksan ve ark., 2007), kanserle mücadele (Espada ve ark., 2007) etkileri bilimsel araştırmalara konu olmuştur. Bazı çalışmalarda çiya tohumunun hayvansal kaynaklı gıdalar üzerinde etkileri incelenmiş; yumurta (Ayerza ve Coates, 2010) ve domuz etinin (Ayerza ve Coates, 2009) omega-3 miktarlarını artırdığı, ancak müşteri beğenilerinin değişmediği gözlenmiştir. Çiya tohumu ayrıca müsiliye oluşturmaya jelleşme özelliğine sahiptir. Kendisinin 27 katı kadar su tutma kapasitesine sahiptir (Munoz ve ark., 2012). Çiya tohumunun emülgatör olma özelliği bazı çalışmalara konu olmuştur (Pintado ve ark., 2015). Son yıllarda doğal hidrokolloidler üzerine araştırmalar yapılması, çiya tohumunun jelleşme özelliğini daha ön plana çıkarmıştır. Çiya tohumun su ve yağ tutma kapasitelerinin ticari kıvam artırıcılardan daha yüksek olduğu gözlenmiştir (Coorey ve ark., 2013). Çiya tohumu ve unu birçok gıdada fonksiyonel bileşen olarak denenmiştir. Buğday unu yerine ekmekte denenmiş ve besin değeri artarken bayatlama hızının düştüğü, dilim hacminin arttığı gözlenmiştir (Justo ve ark., 2007).

Bu çalışma metodolojik olarak 3 ayrı bölümde tamamlanmıştır. Öncelikle çiya tohumu karakterize edilmiş; kompozisyon, antioksidan kapasitesi ve jelleşme kapasitesi incelenmiştir. Kompozisyon analizlerinde AOAC metodları kullanılmıştır. Yağ analizi otomatik Soxhlet cihazı kullanılarak yapılmış; yağ kompozisyonu gaz kromatografi ile incelenmiştir. Çiya tohumunun kompozisyonun % 7.42 nem, % 3.93 kül, % 19.59 protein, % 29 yağ, % 38.94 toplam diyet lifinden oluştuğu tespit edilmiştir. Bu değerler literatür ile uyum göstermektedir (Munoz ve ark., 2013). Bu sonuçlara göre, çiya tohumunun birçok tahıl türünden zengin olduğunu söylemek mümkündür. Antioksidan kapasitesi serbest radikal yakalama aktivitesi ve toplam fenolik bileşen içeriği ile değerlendirilmiştir. Çiya tohumu ve ununun jelleşme özellikleri diferansiyel tarama kalorimetresi ile incelenmiş; camsı geçirgenlik dereceleri kıyaslanmıştır. Çiya tohumunun jelleşme kapasitesinin, ununkinden daha fazla olduğu gözlenmiştir. Çiya tohumunun jelleşme özelliklerinin modifiye pektin türlerine çok yakın olduğu gözlenmiştir (Karaki ve ark., 2016). Bu sonuç, ticari reçeller veya soslarda pektin yerine çiya tohumunun kullanılabileceğini göstermektedir. Çiya tohumu ve ununun jelleşme kapasiteleri kıyaslandığında ise, tohumun daha güçlü jel oluşturduğu gözlenmiştir. İkinci aşamada çilek marmeladı formülü iki adımlı duyusal analiz ile optimize edilmiştir. İlk adım tatlandırıcı tipini seçmek için, ikinci adım ise çiya tohumu yüzdesini seçmek için yapılmıştır. İlk adımda denenilen tatlandırıcılar izomalt, sorbitol, stevia ve izomalt-sorbitol karışımıdır. Kontrol olarak sukroz içeren marmelat kullanılmıştır. Sorbitol içeren formül, tatlılık, çilek tadı, çilek kokusu ve sürülebilirlik parametreleri açısından kontrole en yakın bulunmuştur. Çiya tohumu yüzdesi ikinci adım duyusal analizde % 5 olarak seçilmiştir. Kompozisyon analizlerine % 48 çilek, % 47 sorbitol ve % 5 çiya tohumu içeren formül ile devam edilmiştir. Üçüncü adımda bu marmelat; kompozisyon, antioksidanlar, fiziksel parametreler, toplam şeker içeriği, hidroksi metil furfural (HMF) içeriği ve reoloji açılarından karakterize edilmiştir. Çiya ilaveli, şeker ilavesiz çilek marmeladı; tüm bu parametrelerce kontrol ile kıyaslanmıştır. Tüm kompozisyon analizleri AOAC metodları ile yapılmıştır. Yağ miktarı otomatik Soxhlet ile analiz edilmiş; yağ kompozisyonu gaz kromatografi ile incelenmiştir. Çiya ilaveli, şeker ilavesiz marmeladın % 35.16 nem, % 0.45 kül, % 1.3 protein, % 2.46 yağ, % 2.7 toplam diyet lifi ve % 61.26 karbonhidrat içerdiği tespit edilmiştir.

Suda çözünen kuru madde miktarı % 64.3, asitliği % 0.49 bulunmuştur. Bunlar kontrol marmeladın değerleri ile aynıdır. Ancak, kontrolün pH değeri 3.43 iken, çiya tohumu ilaveli marmeladın pH değerinin 4.43 olduğu tespit edilmiştir. Bu pH değeri, yeni marmeladın mikrobiyal gelişim açısından daha elverişli olduğu sonucunu verebilir. İlerleyen çalışmalarda duyuusal parametreleri etkilemeden, pH düşürülmeye çalışılabilir. Şeker ilavesiz, çiya ilaveli marmelat fenolik bileşenler, enerji ve toplam diyet lifi bakımından kontrol marmelat ile kıyaslanmıştır. Fenolik bileşenlerin % 15.45 arttığı, diyet lifinin % 168 arttığı, enerji değerinin % 48 düştüğü tespit edilmiştir. Reolojik parametreler, şekersiz çiya tohumu ilaveli marmeladın kayma incelme özelliği taşıdığını göstermiştir. G' değeri, G'' değerinden yüksek bulunmuş, viskoelastik bir yapıda olduğu tespit edilmiştir. Tan δ değeri 1' den küçük bulunduğu için jelimsi bir yapıda olduğu tespit edilmiştir. Sukroz içeren kontrol marmeladın viskozitesinin çok daha düşük olduğu gözlenmiş; çiya tohumu ilavesinin viskoziteyi artırdığı tespit edilmiştir.

Çiya ilaveli, şeker ilavesiz çilek marmeladı için örnek etiket oluşturulmuş; etikette yapılabilecek deklarasyonlar belirtilmiştir. Türk Gıda Kodeksi Etiket Yönetmeliği' ne (29.12.2011; 28157) göre bu ürün üzerinde "*Tatlandırıcı içerir*"; "*Fazla tüketildiğinde laksatif etki gösterebilir*" ibareleri bulundurmak zorundadır. Ayrıca benzer ürünlere göre enerjisi % 50 daha az olduğu için "*Düşük enerjili*" denilebilir. % 0,3' den daha fazla omega-3 içerdiği için "*Omega-3 kaynağı*" ibaresini ; % 5' den daha az şeker içerdiği için "*Düşük şeker*" ibaresini taşıyabilir.

Çiya tohumu ilaveli, şeker ilavesiz çilek marmeladı içerdiği antioksidan, diyet lifi ve omega-3 değerleri yüksek olduğundan dolayı yeni bir fonksiyonel gıda olabilir. Türk toplumu, özellikle kahvaltıda, reçel ve marmelat ürünlerini tüketmektedir. Bu fonksiyonel bileşenleri reçel veya marmelat içinde sunmak, Türk toplumunun beslenme alışkanlıklarını dramatik bir şekilde değiştirmeden, besin değeri yüksek, enerji değeri düşük bir gıda ile tanıştırmamızın iyi bir yolu olacaktır.



1. INTRODUCTION

In recent years, the interest on functional foods and the components of functional foods have increased by the increase in awareness of people for more healthy life. In the developed countries, the most mortal diseases are coronary heart diseases, cancer and diabetes. By the increasing risk of these diseases, people tend to consume foods known for their health labels. By considering from this point of view, sugar-free foods, foods with high amount of dietary fiber and omega-3 polyunsaturated fatty acids (PUFAs) have come into prominence. Because of this interest of the consumers, a need for decreasing sugar amount, increasing dietary fiber and omega-3 PUFAs in the diet has risen.

The experts on nutrition and health advise intake of omega-3 PUFAs by 500 – 2000 mg/day and dietary fiber by 25 – 38 g/day (American Dietetic Association, 2008). In spite of this knowledge on the health effects of dietary fiber and omega-3 PUFAs, the consuming amounts of them are lower than the advises.

American Dietetic Association suggest dietary fiber intake for its health protection properties and advises an intake of 20 – 35 g/day. They also indicate that dietary fiber intake by 10 – 29 g/day affects the glycemic index and control diabetes. Dietary fiber is also indicated by its cancer controlling properties.

American Heart Association advises intake of unsaturated fatty acids by 300 mg/day. This amount is 1 g/ day for coronary heart patients (Coupland, 2008). The nutrition and health specialists advise omega-3 intake of 500 – 2000 mg/day, although it alters according to different needs of body. Omega-3 PUFAs are also important for babies and adults because of being the pre molecules of the biomolecules of prostaglandin, thromboxan and leukotriene.

Chia seed contains approximately 16 g protein, 30 g fat, 34 – 40 g dietary fiber and 17 – 18 g omega-3 PUFAs in 100 gr (Munoz et al, 2014). It also contains high amount of alpha linolenic acid (ALA), an essential fatty acid. Other omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesized

from ALA in the body. EPA helps some functions of the immune system, and also is efficient of the treatments of coronary heart diseases, cancer types and schizophrenia (Koletzko et al, 2001; Lora et al, 2007). DHA has an important role since it is found in brain and eye in high amount. EPA and DHA prevent arrhythmias, decrease blood pressure level, decrease plasma triglycerides, help treatment of cardiovascular illnesses by decreasing inflammation (Koletzko et al, 2001).

Low-calorie, sugar free foods involve sugar alcohols which are not absorbed in small intestine and do not cause increase in level of sugar in blood. Sugar alcohols release 65 % of the calorie released by sucrose.

Besides these, the variety of the jams for diabetes is very poor in our country. The thickness, color and taste are very poor compared to normal jams. Also some additional thickening agents have to be used to obtain the same thickness with the other jams. The sugar free jams in the market do not involve any functional component other than the ones coming from the fruit. There is no jam enriched with dietary fiber and omega-3 in Turkish market.

It is not so easy to modify the nutritional habits of societies. Increasing the functional components in the most consumed foods, is the best way to increase the functional food component intake. Turkish people consume high amount of jams, especially at breakfast. A sugar-free, omega-3 fatty acid and dietary fiber involving jam may help Turkish people for providing these healthy components.

Eventually, chia seed added strawberry marmalade with no added sugar is a functional food, which will help to provide the healthy components of Turkish people, by these properties of it: sugar free, rich in dietary fiber, low in glycemic index, rich in omega-3 PUFAs, rich in antioxidants, low calorie, suitable for diabetics, helpful to loss weight by its appetite control property.

This study aims to optimize the best formula of strawberry marmalade with chia without no additional sugar, to analyse the supplement of chia to strawberry marmalade and to compare the nutritional values of a marmalade with sugar and without chia to a marmalade sugar free chia added marmalade.

2. LITERATURE REVIEW

In pre-Columbian times, chia (*Salvia hispanica L.*) was one of the four basic foods of Central American civilizations. It was less important than corn and beans, but more important than amaranth. Chia seed was not just a food, it was also used for medical purposes and regarded as an offering to Aztec gods (Coates, 2011).

There is evidence that chia seed was first used as food as early as 3500 BC, and was a cash crop in the center of Mexico between 1500 and 900 BC. Chia was cultivated in the center of Mexico between 2000 and 2600 BC by the Teotihuacan and Toltecs, and was one of the four main components of the Aztec diet (Coates, 2011).

Chia was used by the Aztecs as food, mixed in water and consumed as a beverage, ground into flour, induced in medicines, fed to birds, and pressed for oil for use as a base for face and body paints and to protect religious statues and painting from the elements. Chia seed and flour could be stored for many years, explained by the high percentage of antioxidants in it. It was considered to be a highly energetic grain, hence it was an essential food when conducting military exercises (Coates, 2011).

Figure 2.1 shows a chia plant, Figure 2.2 shows the flowers of chia.



Figure2.1 Chia plant



Figure 2.2 Chia flower

2.1 BOTANICAL DESCRIPTION OF CHIA

Chia belongs to *Labiatae* or *Lamiaceae* family (mint family).

It is an annual herb, normally about 1 m high, having branched quadrangular stems, and opposite leaves with a 40 mm petiole. The leaves are oval to oblong, almost smooth, with few pubescens that are whitish and very short. The leaves have serrated edges and are 80-100 mm long by 40-60 mm wide. The flowers are produced in terminal or axillary spikes; the seed are present in four groups of four, and are smooth, shiny, grayish-black color with irregular spots measuring 2 mm by 1.5 mm (Coates, 2011).

2.2 CHIA SEED DESCRIPTION

The seeds are small (1.87 ± 0.1 mm length, 1.21 ± 0.08 mm width, and 0.88 ± 0.04 mm thickness), with an oval, flattened shaped and ranged in color from dark coffee to begie with small darker spots as shown in Figure 2.3, the white seeds are greater in weight, with and thickness than the darker ones.

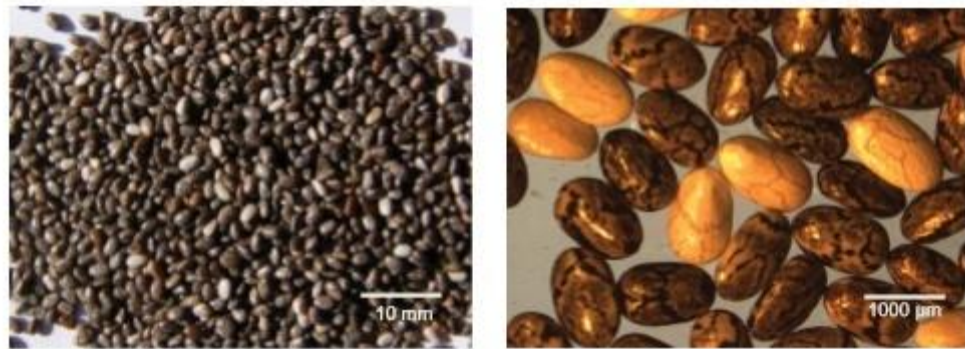


Figure 2.3 Chia seeds optical images (Munoz, 2013)

In Figure 2.4, chia seed microstructure is given.

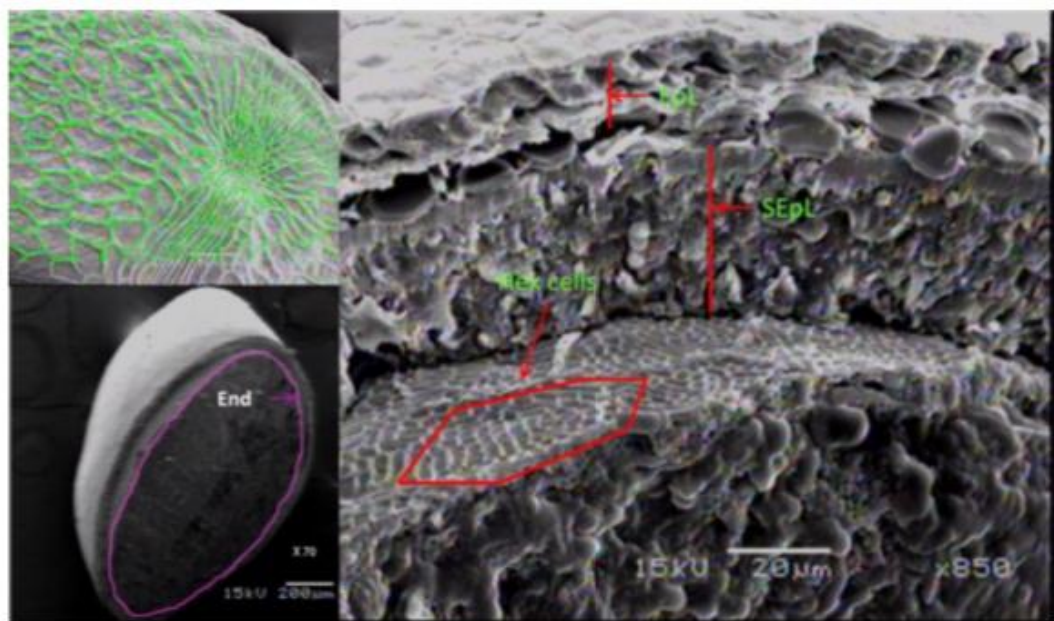


Figure 2.4 Chia seed microstructure; A: seed epidermis; B: view of seed external layers; C: seed transversal cut; EpL: epidermal layer or exotesta cells; SEpL: second layer or subepidermal cells; End: third layer or core endosperm; Hex cells: polygonal isodiametric cells. (Cruz, et al., 2013)

2.3 CHIA PRODUCTION

Historically, chia has been cultivated in tropical and subtropical environments, from frost-free areas to regions where frost occur every year, from sea level up to 2500 m. Today chia is commercially grown in Argentina, Ecuador, Peru, Paraguay, Australia, Mexico, Nicaragua, Guatemala and Bolivia. In Argentina and Mexico it is a summer – autumn crop, similar to corn, soybeans and beans. In Bolivia, chia is a autumn – winter crop, sown following the harvest of other crops, and competes with winter wheat and sunflower. In Ecuador the crop can be grown all year round, with three to four harvest possible, depending upon location (Coates, 2011).

Chia is not frost tolerant. It develops best in sandy loam soils; however, it can be grown in clay loam soils with adequate drainage. Generally, chia grows well in soils containing a range of nutrients, however, a low nitrogen content appears to be a barrier to good seed yield (Coates, 2011).

Chia is sown at a rate 5 – 6 kg/ha, with row spacings of 0.7 or 0.8 m. It requires wet soil to germinate, but once seedlings are established chia does well with limited water. It can be grown either dry land or irrigated (Coates, 2011).

2.4 CHIA SEED COMPOSITION

The lipid, protein, fiber and antioxidant contents of chia are significantly higher than in many other crops. Although chia serves mainly as a source of omega-3 fatty acids, it also contains a number of other components that are important for human nutrition. Table 2.1 shows the nutritional composition of chia seed.

Table 2.1 Nutritional composition of chia seed (Munoz et al., 2014)

Nutrient	Amount in 100 g chia seed
Energy (kcal)	486.00
Proteins (g)	16.54
Total fat (g)	30.74
Saturated fatty acids (g)	3.33
Monounsaturated fatty acids (g)	2.31
Polyunsaturated fatty acids (g)	23.67
Trans fatty acids (g)	0.14
Omega-3 fatty acids (g)	17.83
Cholesterol (g)	0
Carbohydrate (g)	42.12
Fiber, total dietary (g)	34.4

2.4.1 Fiber

Dietary fiber has demonstrated benefits for health maintenance and disease prevention. The recommendation for adult dietary fiber intake generally is in the range of 20-35 g/day. Chia seed contains between 34 and 40 g dietary fiber per 100 g, equivalent to 100 % of the daily recommendations for the adult population; the defatted flour possesses 40 % fiber, 5-10 % of which is soluble and forms part of the mucilage. Therefore, chia seed can be used in the prevention of many cardiovascular diseases and diabetes, among others as demonstrated by a number of epidemiological studies (American Dietetic Association, 2009).

Alfredo et al. (2009) analysed the nutritional content of fiber-rich fraction of defatted chia flour. The total dietary fiber was quantified to be 56.46 g/100g out of which 53.45 g /100 g was soluble fiber. Emulsifying activity was 53.26 % and emulsion stability was 94.84 %. Antioxidant activity was higher than wine, tea, coffee and orange juice.

2.4.2 Lipids and Lipid Components

One of the most important characteristic of this seed is its high content in polyunsaturated fatty acids. Chia seed contains the highest known percentage of α -

linolenic acid fatty (ALA) acid of any plant source. This kind of fatty acid is the dietary precursor for the long chain omega-3 polyunsaturated fatty acids (Munoz et al., 2013). Lipid content and fatty acid composition of chia and several seeds are given in Table 2.2.

Table 2.2. Lipids and lipid components in foods (per 100 g)
(Munoz et al., 2014; Lemahieu et al., 2015)

Lipid and lipid component	Chia Seed	Quinoa	Amaranth	Flaxseed
Total fat (g)	30.74	5.3-6.2	9.1	41
Saturated fatty acids (g)	3.33	Nr	2.45	Nr
Monounsaturated fatty acids (g)	2.31	Nr	2.17	Nr
Polyunsaturated fatty acids (g)	23.67	Nr	4.47	23

(Nr: not reported)

Çiftçi et al. (2012) determined the fatty acids, tocopherols, sterols and triacylglycerols of chia seeds. Predominance of ALA in lipid, 446 mg/kg tocopherol and 4132 mg/kg phytosterol was reported.

2.4.3 Proteins

The protein content of chia seed varies between 15 – 23 % depending on the geographic location of the crop and growing conditions, exceeding that of traditional cereals such as wheat, corn, rice, oats and barley and other seeds such as amaranth and quinoa as seen in Figure 2.5 (Munoz et al., 2014).

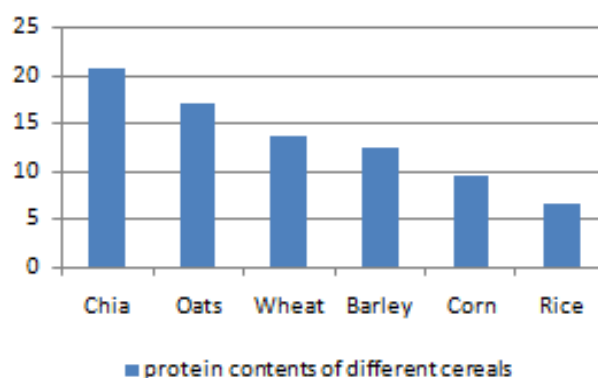


Figure 2.5 Protein content comparisons between chia seed and cereals (%)
(Coates et al., 2005)

The seed does not contain gluten, so any food prepared using chia as its basis can be digested by celiac disease patients. Table 2.3 shows the amino acid content and demonstrates that chia seed possesses the nine essential amino acids.

Table 2.3 Amino acid contents of chia seed (per 100 g) (Munoz et al., 2013)

Amino acid	g / 100 g seed
Aspartic acid	1.689
Serina	1.049
Glutamic acid	3.500
Glycine	0.943
Alanine	1.044
Cysteine	0.407
Tyrosine	0.563
Arginine	2.143
Proline	0.776
Threonine*	0.709
Valine*	0.950
Methionine*	0.588
Isoleucine*	0.801
Leucine*	1.371
Tryptophane*	0.436
Phenylalanine*	1.016
Lysine*	0.970
Histidine*	0.531

*Essential amino acids

Olivos-Lugo et al. (2010) determined the nutritional profiles of protein fractions of chia seeds. The protein isolates demonstrated good emulsifier characteristics as water holding and oil-retention capacities. Further high amounts of glutamic acid, arginine and aspartic acid were reported. However essential amino acid lysine was lacking thus supplementation with a suitable protein seemed to be a requisite for wholesome protein source.

2.4.4 Vitamins

Chia seed is a good source of vitamin B. Compared with other cereals, the seed has higher niacin content than corn, soybeans and rice, As for its thiamine and riboflavin contents, they are similar to those found in rice and corn. Table 2.4 shows the vitamin content of chia seeds.

Table 2.4. Vitamin content of chia seed (per 100 g) (Munoz et al., 2013)

Vitamins	mg / 100 g
Vitamin C (total ascorbic acid)	1.60 mg
Thiamine	0.62 mg
Riboflavin	0.17 mg
Niacin	8.83 mg
Folate	49.0 µg
Vitamin A	54 IU
Vitamin E (α-tocopherol)	0.50 mg

2.4.5 Minerals

Chia seed is an excellent source of minerals, and contains 6 times more calcium, 11 times more phosphorus and 4 times more potassium than 100 g milk, besides possessing iron, magnesium, zinc and copper. Chia contains 13 – 354 times more calcium, 2 – 12 times more phosphorus, 1.6 – 9 times more potassium than 100 g of wheat, rice, oats and corn. The iron content of chia is also quite high compared with most other seeds; it has more iron than spinach, 1.8 times more than lentils and 2.4 times more than liver (Munoz et al., 2013). Table 2.5 shows the mineral content of chia seed.

Table 2.5. Mineral content of chia seed (per 100 g) (Munoz et al., 2013)

Nutrient	mg / 100 g
<i>Macroelements</i>	mg
Calcium	631
Potassium	407
Magnesium	335
Phosphorus	860
<i>Microelements</i>	
Selenium(μ g)	55.2
Copper	0.924
Iron	7.72
Manganase	3.723
Molybdenum	0.2
Sodium	16
Zinc	4.58

2.4.6 Antioxidants

Chia seed has number of compounds that can act as antioxidants. Among the most important are the phenolic compounds and the tocopherols. These compounds are primary and synergetic antioxidants and make a proportionally greater contribution to the antioxidant capacity of chia. Total amount of tocopherols in chia seed is similar to that in peanut oil, but lower than flaxseed, sunflower and soybean. The major phenolic compounds found in chia seeds are chlorogenic and caffeic acid, followed by, quercetin and kaemperol. The antioxidant concentrations of chia seed is given in Table 2.6.

Table 2.6. Concentration of antioxidants in chia seed (Caudillo et al., 2008)

Compound	mg / g seed
Kaempherol	0.360
Total phenols	0.757
Quercetin	0.15

analyzed the nutritional content of hydrolyzed crude extracts of chia seeds. Quercetin, kaempherol and myrcetin were quantified in substantial proportions while caffeic acid and chlorogenic acid were detected in lesser amounts. The antioxidants have been shown to inhibit lipid peroxidation better than vitamin C.

2.5 NUTRITIONAL IMPORTANCE OF CHIA SEED

Modern diet is loaded with calories and trans fats, leading the consumers towards cardiovascular and other chronic diseases. The quest for wholesome food sources has catapulted chia seeds to prominence.

Beneficial roles of chia seeds are gradually being validated. The most promising applications are summarized below.

2.5.1 Endurance Enhancement

Illian et al. (2011) compared two high dietary carbohydrates, the traditional Gatorade (a sports drink) and chia seed based drink for sports performance augmentation. After a treadmill and track running trials of six subjects, no difference was observed in the recovery from the intense exercise. From the results, omega-3 chia loading appears a viable option for enhancing performance for endurance events lasting more than 90 min. It slashes the dietary intake of sugar and increases that of omega-3 fatty acids.

Nieman et al. (2015) evaluated the effects of chia beverage on human running performance. They observed that chia beverage supplementation elevated plasma ALA by 337 %. Runtime to exhaustion did not differ. No difference observed for respiratory exchange ratio, oxygen consumption, ventilation, ratings of perceived exertion, plasma glucose and blood lactate.

2.5.2 Cholesterol Reduction

A skewed ratio ω -3 and ω -6 fatty acids causes rise in cholesterol level. Chia seeds have been discovered to be an ample source of ALA, known to be the precursor of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA). These ω -3 fatty acids are known to reduce inflammation in the heart and liver.

Ayerza and Coates (2007) investigated that chia decreased serum triacylglycerol content and increased HDL content of wistar rats. It remarkably increased the plasma ALA, EPA and DHA contents compared to control diet.

Rossi et al. (2012) investigated that the replacement of maize oil by chia normalized the rise in dyslipidaemia, liver triacylglycerols, fatty acid synthase, acetyl-CoA

carboxylase and glucose-6-phosphate dehydrogenase activities, and increased fatty acid oxidase and CAT-I activities of rats.

Nieman et al. (2012) observed that the ingestion of 25 g/day milled chia seed compared to whole seed or placebo for 10 weeks by overweight women increased plasma ALA and EPA by 58 and 39 %, respectively.

Poudyal et al. (2012) observed that chia seed supplementation increased whole body lean mass and lipid redistribution from the abdominal area and normalized the chronic low-grade inflammation.

Guevara – Cruz et al. (2012) observed that chia based diet provided a greater decline and rise in serum adipoectin concentration after 2 month of dietary treatment, compared to oat, soy protein and nopal, a type of fig, based diets.

Poudyal et al. (2012) observed that chia seed- supplemented rats demonstrated reduced visceral adiposity, decreased hepatic steatosis and reduced cardiac and hepatic inflammation and fibrosis without changes in plasma lipids or blood pressure.

2.5.3 Diabetes Management

Vuksan et al. (2007) investigated through a single-blind cross-over design whether Salba (a variety of chia) minimizes cardiovascular risk factors in individuals with type 2 diabetes.

Further, Vuksan et al. (2010) made subjects feed breads made by salba. Decrease in postprandial glycemia provides rationale for improvement in blood pressure, coagulation and inflammatory markers previously observed after 12 week Salba supplementation in type 2 diabetes.

Chicco et al. (2009) observed that the dietary chia seed prevented the onset of dyslipidaemia and insulin resistance in the rats fed the high-sucrose diet for 3 weeks.

Jeong et al (2010) applied a typical formulation containing 4 % chia seed oil for 8 weeks on patients and healthy volunteers. They observed significant improvements in skin hydration, lichen simplex chronicus and prurigo nodularis in all patients and healthy volunteers.

Poudyal et al. (2012) also observed that chia seed supplemented rats had improved insulin sensitivity and glucose tolerance.

2.5.4 Cancer Mitigation

Espada et al. (2007) observed that chia oil diet decreased the tumor weight and metastasis number. Apoptosis and T-lymphocyte infiltration were higher and mitosis decreased with respect to other diets. Cancerous growth and metastasis inhibition of chia seeds became evident.

2.5.5 Hypertensive Reduction

Toscano et al. (2014) evaluated blood pressure, systolic blood pressure, ambulatory blood pressure, lipid peroxidation and plasma nitrite levels of hypertensive subjects. The subjects were divided into three groups: hypertensive –drug treated (chia+MD), hypertensive – untreated (chia + NM), placebo (PLA+MD). Chia flour is said to have the ability to reduce ambulatory and clinical blood pressure in both treated and untreated hypertensive individuals.

2.5.6 ACE Inhibition, Antimicrobial and Antioxidant Activities

ACE (angiotensin I-converting enzyme) is a functional zinc containing enzyme found in different tissues. It plays an important role in regulating blood pressure. ACE inhibition mainly produces a hypotensive effect, but can also influence regulatory systems involved in immune defence and nervous system activity. Commercial ACE inhibitors can have serious effects. That's why scientists research for natural ones (Ullah et al., 2015).

Chia protein hydrolysates have been mentioned to be a natural ACE inhibitor. Campos et al (2012) supplemented chia protein hydrolysates to white bread and carrot cream. Addition of chia protein hydrolysates improved ACE inhibitory effect of both white bread and carrot cream, had no effect on antimicrobial activity of both food, had no effect on antioxidant activity of white bread but a slight difference of carrot cream.

Marinelli et al (2015) aimed to investigate the effects of dietary chia seed oil on plasma and liver oxidative status in diet-induced obese rats. There were no difference in plasma and liver superoxide dismutase activity among chia diets and high-fat high-fructose (HFF) group. Chia seed and oil intake did not modify liver lipid peroxidation, but was able to reduce plasma thiobarbituric acid reactive substances. Plasma and hepatic antioxidant capacity values were increased in chia seed and oil

groups by 35 and 47 %. Chia group presented similar antioxidant potential, regardless of treatment time. Dietary chia seed and oil reduced oxidative stress in vivo, since it improved antioxidant status and reduced lipid peroxidation in diet-induced obese rats.

2.5.7 Food Sensitives

Chia seeds are free of gluten. Many people are allergic to that protein. Chia seed is being considered as an alternative to produce gluten-free products, also with high nutritional value (Seema, 2015)

Chia seed can also be considered as alternative to fish and fish products, for the people who are allergic to that products, as a source of ω -3 (Seema, 2015).

Chia seeds are conveniently free of any allergic response towards consumers. Studies on adverse effects of chia did not result in any significant adverse effect (Seema, 2015).

2.6 EFFECTS OF CHIA SEED ON ANIMAL ORIGIN FOODS

Chia is very rich in ω -3 fatty acid. It is experienced to enhance ω -3 levels of eggs .

Ayerza (2010) compared chia seed oil as a ω -3 source to enhance ω -3 levels of eggs of hens with flaxseed, fish oil /meal or marine alga. All the ω -3 sources were fed to hens. ω -3 content of chia eggs could not be reached by the other sources without negatively affecting the eggs or the hens.

Ayerza and Coates (2010) observed that chia significantly improved the lipidic profiles of the products produced as well as the serum of rats, with no evidence of a decrease in product quality or animal health. This is unlike flaxseed and fish meal/oil which have been shown to give off-flavors (fishy flavor) to eggs, and in the case of flaxseed, reduced egg production. Adding chia to bread and other bakery products could give consumers with an easily obtainable, low cost food that would significantly increase their ω -3 intake without having to change their normal diet, or be faced with an allergic reaction caused by fish.

Peiretti and Meineri (2008) evaluated the effects of three levels of chia-fortified diet (0, 10, 15 %) on the growth performance, carcass characteristics and fatty acid profiles of rabbit meat and perirenal fat. The polyunsaturated fatty acid concentration

in the longissimus dorsi muscle and perirenal fat was significantly increased with increasing chia inclusion, while the saturated fatty acid decreased from 4.55 in the control group to 1.03 in the 15 % chia fed group.

Coates and Ayerza (2009) studied the effect of feeding chia seeds a fatty acid composition of the meat, internal fats, growth performance and meat sensory characteristics of meat of pig. When fed with 10 and 20 % chia-amended fed to finishing pigs, it modified the fatty acid composition of the meat fat, significantly less palmitic, stearic, arachidic acids were found. ALA content increased proportionally with chia content of the diet. Also the panel members showed preference for the aroma and flavor of the chia-fed meat. Chia seems to be a viable feed that can produce healthier pork for human consumption.

2.7 CHIA SEED AS A FUNCTIONAL FOOD INGREDIENT

Besides its functional components, chia seed has been evaluated by its functional properties such as gelling capacity, emulsifier and stabilizer properties, its capacity to yield chia gum and also its mucilage properties.

Chia seeds possess high hygroscopicity and turn into mucilaginous mass on hydration. Studies reveal that they can imbibe 27 times their weight in water (Munoz et al, 2012).

Chia could also be a good source of formation of gel. The characteristics of chia gel were studied by Coorey et al. in 2013, they compared chia gel with guar gum and gelatin which are commonly used as thickening agent in food industry. Both chia seed gel and chia flour gel were compared to the industrial thickening agents. The chia seeds or flour were mixed with deionized water using an overhead mixer RW 20 DZM.n at 1920 rpm for 3 h. The mixture was then centrifuged using an Eppendorf 5810R centrifuge at 3220 * g for 50 min at 20 °C to separate the gel. The top layer (excess water) was removed, and the gel (middle layer) was collected, the seeds settled at the bottom. In the chia flour, there was no clear separation of gel, hence some chia flour remained within the gel. The gel characteristics were evaluated. Chia seed gel performed a better water holding capacity, oil holding capacity and emulsion stability than commercial guar gum. Some omega-3 and omega-6 were recovered to the chia seed gel and chia flour gel, with a greater amount in chia flour

gel. Due to the better functional properties of chia seed gel, it has the potential to be used as a thickening agent in food formulations. Chia seed gel can also be used as emulsifying agent. Chia seed gel can be applied in frozen food product due to its freeze-thaw stability. Since some of the omega-3 and omega-6 were recovered to the gel providing additional health benefits.

Pintado et al. (2015) evaluated emulsion stabilization of chia with some other cold gelling agents such as transglutaminase, alginate or gelatin. Chia was found to be a promising emulsion stabilizer, besides its health beneficial properties by involving a variety of bioactive compounds (α -linolenic fatty acids, valuable proteins, antioxidants and dietary fiber).

Guiotto et al. (2015) studied the stability of oil-in-water emulsions with sunflower lecithin and chia mucilage. Optical characterization and droplet size distribution of oil-in-water emulsions formulated with different types and concentrations of modified sunflower lecithin (phosphatidylcholine enriched lecithin - PC enriched lecithin), with or without chia mucilage (0.75 % wt/wt) were evaluated as a function of storage time at 4 °C. Chia mucilage presented a good performance as a functional ingredient to be used as stabilizer or thickener agent in emulsions. The addition of chia mucilage contributed to emulsion stability against creaming during refrigerated storage due to reduction of mobility of oil particles by the formation of tridimensional network. Modified sunflower lecithin and chia mucilage interaction would favor the production of stable emulsions.

Campos et al. (2014) obtained gum from chia from its dietary fiber fractions to be used as an additive to control viscosity, stability, texture and consistency in food systems. The gum was obtained by submitting chia seeds to gum extraction with water using a 1:20 ratio (w/v) for 30 min at 50 °C temperature. After that the suspension was milled in a mixer and then boiled again at 50 °C under stirring for 15 min. The crude mixture, containing water, gum and seeds was centrifuged at 9460 * g at 15 °C for 3 h. The recovered gum was dried at – 40 °C for 24 h and milled. One portion of the recovered gum was partly defatted in a Soxhlet. The partly defatted chia gum (PDCG) had higher water holding and water binding capacities. The fatty chia gum (FCG) had greater oil holding capacity and water absorption capacity. In dispersion trials, the gums exhibited a non-Newtonian fluid behaviour, specifically shear thinning or pseudoplastic type. PDCG had more viscosity than FCG. Chia seed

was regarded as an excellent natural source of gum with good physicochemical and functional qualities

Dick et al. (2015) considered chia flour as an edible film barrier. Chia flour was used together with maize starch in different ratios. The transparency of the films increased as a function of maize starch. Moreover chia flour based films presented a low solubility and a satisfactory water vapor barrier and showed a UV-radiation-shielding properties. The presence of maize starch in the composite film elevated the molecular interactions and led to a more compact structure; this resulted in improved strength, although the elongation still remained low. Chia flour based films would be a promising material for the formulation of edible films with adequate physicochemical and mechanical properties and a high nutritional quality.

2.8 CHIA SEED AS A FOOD INGREDIENT IN VARIOUS FOOD PRODUCT FORMULATIONS

Chia seeds are promising to be a good replacer for so many food ingredients being used in food industry.

Justo et al. (2007) developed bread from whole-wheat flour, chia seed flour and flaxseed flour by mixing at various ratios. Due to their high levels of protein, unsaturated fatty acids, dietary fiber and folic acid, these breads had high nutritional value. Glucose dialysis retardation index values were between 89.1 – 98.1 % and folic acid was in the range 699.44-991.3 mg /100 g dry matter. Also the taste was approved by the sensory panel.

Iglesias-Puig and Haros (2013) aimed to develop a new cereal based product with increased nutritional quality by using whole chia flour, semi-defatted chia flour and low fat chia flour in order to evaluate their potential as a bread maker. The samples with chia addition significantly increased the levels of protein, lipids, ash and dietary fibre in the final product. Breads with seeds or ground seeds showed similar technological quality to the control bread, except for the increase in specific bread volume, decrease in crumb firmness and change in crumb color. Sensory analysis showed that the inclusion of chia increased overall acceptability. The thermal properties of the starch did not alter substantially with the inclusion of chia. However, the incorporation of chia inhibited the kinetics of amylopectin

retrogradation during storage, which would be directly related to the delay in bread staling.

Pizarro et al. (2014) aimed to obtain a functional bread with n-3 alpha linolenic acid from whole chia flour (WCF). They used WCF and vital gluten (VG) to investigate nutritional and sensory qualities of bread. WCF decreased the specific volume, lightness and hue angle of bread loaves, but did not affect the chroma values. WCF and VG contributed to maintenance of the moisture content of loafs during storage period. The increased firmness found with the addition of high levels of WCF (more than 10 %) was countered by larger amounts of VG (more than 2%). The optimum loaf (10% WCF and 2% VG) showed 26 % more lipids, 19 % more protein and 11 % more ash than the standard loaf (0 % WCF and 0 % VG). A better lipid profile was also found (higher ω -3 fatty acid content and a better ω -6 / ω -3 ratio). Both breads were positively rated in sensory analysis.

Coelho and Mellado (2014) reduced hydrogenated vegetable fat content and added chia seeds or flour to the formulation of wheat bread. They generated two type of breads: bread with 7.8 g/100 g chia flour + 0.9 g/100 g fat and bread with 11.0 g/100 g chia seeds and 1.0 g/100 g fat, resulting in a reduction of 27 and 24 % , respectively, in the level of saturated fat compared to that of bread prepared with wheat flour. The ratio of polyunsaturated and saturated fats which was 1.01 in the control bread, was increased to 3.1 and 3.9, respectively. The content of fiber and ω -3 fatty acid was increased in the final products. These new formulations might be used on an industrial scale to prepare products that could contribute to reducing the intake of saturated fatty acids and increasing that of essential fatty acids.

These new formulations might be used on an industrial scale to prepare products that could contribute to reducing the intake of saturated fatty acids and increasing that of essential fatty acids.

Zettel et al. (2014) investigated the characteristics of chia gel in wheat bread dough. To avoid competition between ground chia and starch, chia was incorporated as gel. The gel was prepared from ground chia with 5 and 10 g/g water, respectively. The doughs were prepared with 1 – 3 % chia related to the amount of wheat flour. Farinograph, Rheofermenter and Kieffer dough rig were performed. Rheological characteristics, baking experiments, staling and crumb firmness were evaluated.

Dough analyses showed that the doughs with added chia gel have a softer consistency. Dough stability during fermentation and volume yield of the bread loafs increased with added chia gel to a certain extent. These changes are visible with already 1 % chia content. The bread quality was improved with respect to storage as the crumb firmness was reduced compared to the breads without chia gel.

Steffolani et al. (2014) aimed to investigate the effect of addition of chia seeds or flour with and without pre-hydration on the technological and sensory characteristics of the bread. Chia flour significantly increased the water absorption during kneading, and the effect was more pronounced when the flour was pre-hydrated. Chia flour addition also increased the tenacity and reduced the extensibility of doughs. Adding a 10 g/100 g chia seeds produced a decrease in bread volume and increase in crumb firmness, but this effect was lower when seeds were pre-hydrated. With the addition of chia flour at levels of 10 g/100g, bread volume remained unchanged in pre-hydrated flour. However, a decrease in the volume of breads made by flour without pre-hydration was also observed. In general, an evaluation of the loafs containing by potential consumers, the global score was above 6 out of 9, with no significant differences compared to the control bread. Chia flour without pre-hydration was the only exception where lower values of consumer acceptance were observed.

Steffolani et al. (2013) made gluten free bread by using chia seeds and flour. Rice breads were elaborated with the addition of 15 g of chia flour or seed, either dry or pre-hydrated, per 100 g of rice flour. The addition of chia reduced specific volume and increased the hardness of breads, and the effect was more evident with the flour than with the seed. Chia addition minimized weight loss during baking in all cases. The addition of chia flour produced a darker crust and crumb. There were no significant differences between the breads in the overall acceptability, but chia seed breads presented better values in terms of texture than the control.

Inglett et al. (2014) investigated physical properties of sugar cookies containing chia - oat composites. Nutrim, oat bran concentrate (OBC) and whole oat flour (WOF) were composited with finely ground chia, used in cookies at 20 % replacement of wheat flour for improved nutritional and physical quality. These composites had improved water-holding capacities compared to starting material. The geometrical properties and texture properties of the cookies were not greatly influenced by a 20

% flour replacement using chia – OBC or chia – WOF composites. There was a decrease in the cookie diameter, and increases in the height of cookies and dough hardness using chia-nutrim composite.

Felisberto et al. (2014) studied chia seed as a fat replacer in pound cakes. They investigated the effects of 25, 50, 75 and 100 g/100 g of vegetable fat by chia mucilage gel (CMG) on the technological properties of pound cakes. CMG was produced by rehydration with water of lyophilized chia mucilage. Replacement of vegetable fat by CMG did not significantly alter the specific volume, symmetry, uniformity, moisture and water activity of pound cakes. Color parameters, as well as crumb firmness of cakes, were influenced by higher levels of the replacement. The replacement of up to 25 g/100 g of fat by CMG is technologically feasible in pound cakes.

Borneo et al. (2010) investigated the overall acceptability, sensory characteristics, functional properties, nutrient content of cakes baked using chia gel as a substitute of oil or eggs. Sensory panel indicated that the 25 % chia gel cakes were similar to the control in terms of color, smell, taste and texture.

Souza et al. (2014) investigated the effects of replacement of partially defatted chia flour (CFPD) with a second factor, concentration of textured soy proteins (TSP) for hamburger meat. The factors TSP and CFPD were significant, and the increased values contributed to improve the composition of fatty acids, crude protein and ash. Principal components analysis distinguished the samples with a higher content of chia. In desirability analysis, the highest level of TSP and CFPD was described as the optimal region, and it was not necessary to make another experimental point.

2.9 GELATINIZATION PROPERTIES OF CHIA SEED AND FLOUR

In the recent years, the demand for hydrocolloids from plant sources has increased because they are most notable ingredient in liquid and semisolid foods (Koochelki, Reza, Bostan, 2013). Mucilage and gums are widely used in the food, pharmaceutical and cosmetic industries as thickeners, water retention agents, emulsion stabilizers, suspending agents, binders, coating agents, packing films, etc. When the chia seeds are soaked in water, they exude a clear mucilaginous gel high in uronic acid content that remains tightly bound to the seed (Daniel and Whistler, 1994).

Coorey, Tjoe and Jayasena (Australia, 2013) compared chia seeds and chia flour by means of their compositions and their abilities to form gel. They also compared the gelling properties of them with commercially used thickeners such as gelatin and guar gum. Table 2.7 shows the comparison of chemical compositions of chia seed, chia flour, chia seed gel and chia flour gel.

Table 2.7 Chemical compositions of chia seed, chia flour, chia seed gel and chia flour gel (Munoz, 2013)

Component (dry basis %)	Chia seed	Chia flour	Chia seed gel	Chia flour gel
Ash	5.09	7.66	3.78	8.61
Protein	21.4	31.95	3.54	19.44
Crude fiber	25.55	29.17	57.84	21.86
Oil	33.65	8.75	0.55	8.85
ALA	11.52	2.59	0.26	2.33
LA	2.55	0.81	0.1	0.81

Chia seed gel and chia flour gel were obtained by same way: mixing with water, centrifuging and freeze-drying, respectively.

Chia seed gel performed better water holding and oil holding capacities than commercial guar gum. Some omega-3 (ALA) and omega-6 (LA) were recovered to the chia seed gel and chia flour gel, with a greater amount in chia flour gel. Due to the better functional properties of the CSG it has the potential to be used as thickening agent in food formulations. Chia seed gel can also be used as an emulsifying agent, due to its higher capacity of emulsifying and stabilizing. Chia seed gel can be applied in frozen product due to its freeze-thaw stability. Since some of the ALA and LA were recovered to the gel providing additional health benefits. Table 2.8 gives the comparison between water and oil holding capacities of chia seed gel, chia flour gel, gelatin and guar gum.

Table 2.8 Water and oil holding capacities of chia seed gel, chia flour gel, gelatin and guar gum (Coates, 2012).

Sample	Water holding capacity (g water retained / g sample)	Oil holding capacity (g oil retained / g sample)
---------------	---	---

Chia seed gel	266.55	58.61
Chia flour gel	12.42	19.18
Guar gum	24.83	0.87
Gelatin	7.2	1.05

Figure 2.6 gives the viscosity values by temperature of chia seed gel, chia flour gel, guar gum and gelatin. Chia seed gel yields the highest viscosity values at any temperature; while the viscosity of chia flour gel is negligible.

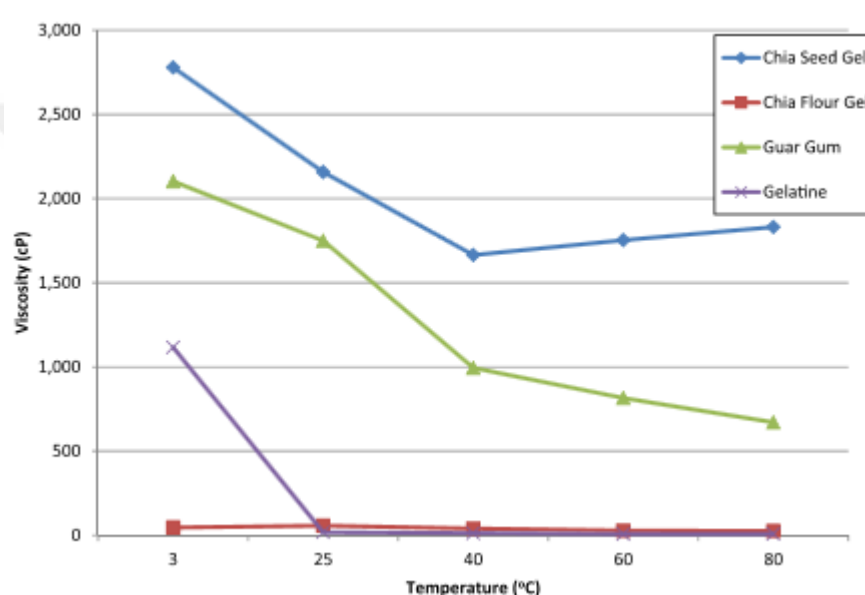


Figure 2.6 Effects of temperature on viscosity of chia seed gel, chia flour gel, guar gum and gelatin (Coorey et al.,2014)

Table 2.9 gives the emulsion activity and emulsion stability of chia seed gel, chia flour gel, guar gum and gelatin. Guar gum has the highest emulsion activity, while chia seed gel is very close to it. The highest emulsion stability is yielded by chia seed gel .

Table 2.9. Emulsion activity and stability of chia seed gel, chia flour gel, guar gum and gelatin (Coorey et al.,2014)

Sample	Emulsion activity (%)	Emulsion stability (%)
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Chia seed gel	61.50	69.83
Chia flour gel	53.67	47.67
Guar gum	62.33	35.17
Gelatin	57.00	54.67

2.10 DIETARY FIBER AND HEALTH BENEFITS

Dietary fiber is defined as “carbohydrate polymers with 10 or more monomeric units which are not hydrolysed by the endogeneous enzymes in the small intestine of humans” by Codex Alimentarius Commission in 2009 (Kristensen and Jensen, 2011).

Dietary fiber is not digested in small intestine, but can be fermented in large intestine. In 2008, the EU directive 2008/100/EC on food labeling stated that “dietary fiber” term should be included fermentation in large intestine, provides about 8 kj (2 kcal) per gr of dietary fibre (Westenbrink et al., 2013).

Dietary fiber is divided into two groups according to solubilities of them: as soluble and insoluble dietary fiber. The soluble one forms a viscous gel structure by binding water. The insoluble one binds water to 20 times of its amount without forming a viscous gel. (Burdurlu and Karadeniz, 2002).

The recommendation about consumption of dietary fiber is as below:

- American Dietetic Association: 14 g dietary fiber per 100 kcal, or 25 g for adult woman and 38 g for adult man.
- National Health and Medicinal Research Council of Australia: 25 g / day for woman, 30 g / day for man.
- Institue of Medicine in USA: 19 g / day for children between 1 – 3 years.

Dietary fiber is well known for its appetite regulator property. There are some possible mechanisms explaining the effects of fiber on appetite. The first is energy dilution, as only around 40 % fiber is fermented in the colon. Its energy contribution per unit of weight is very slow, so including fiber in your diet can lower energy density. It has also been reported that the capacity of fiber to bind water can lead to an

additional lowering of the energy-to-weight ratio in foods, resulting with an increased satiety and decreased energy intake.

The second is chewing. Foods higher in dietary fiber usually require more chewing. This is thought to slow down the rate of ingestion of foods and promote satiation. The greater chewing required for higher fiber foods may also result in an increased production of saliva and gastric acid and may lead to gastric distension. The distension may also be enhanced through the ability of fiber to absorb water and form gels. Gastric distension is believed to trigger vagal signals of fullness and impact on satiety.

Soluble fiber has been shown to delay gastric emptying by forming a viscous gel matrix resulting in a slower rate of nutrient absorption. This has been linked with increased satiety.

The impact of dietary fiber on gut hormones also promote satiety as dietary fiber can decrease the rate of absorption of nutrients, leading to a reduction in the rate of fat absorption from the small intestine resulting in an increased contact time between the intestine and dietary fat. This may enhance the release of hormones such as cholecystokinin. Cholecystokinin is a hormone that is secreted in the small intestine after fat ingestion and has a role in appetite regulation. Accordingly an increased release of cholecystokinin may contribute to the satiating effect of fiber.

Dietary fiber has also been linked to alterations to the postprandial secretion of other hormones, more specifically a decrease in acylated ghrelin and total ghrelin. Ghrelin plays a role in the control of appetite, with increased levels leading to the initiation of eating. (Belski, 2011).

While gastric satiety is mechanical origin, internal satiety is typically nutrient-dependent, where satiety signals are released upon close interaction between the intestinal wall and nutrients. Exposure of the intestinal mucosa to the nutrients induces release of appetite regulating peptides which either function as hormones or active neutral pathways. Viscous dietary fibres increase the viscosity of digesta in the small intestine and therefore prolong small intestine transit time and absorption rate of nutrients. The modified absorption rate of nutrients in the small intestine affects the release of peptide which in turn affects gastric emptying and signalling to the central nervous system. A study in 2012 also emphasized this mechanism of dietary

fibre on appetite regulation (Rasoamanana et al., 2012). Mice were fed with low viscous, viscous and high viscous guar gum mixtures. It appeared that viscous and high viscous preloads reduced the daily energy intake by 14% and 21%, respectively. High viscous mixture load decreased meal size by 57 % and meal duration by 65%.

There are various benefits of adequate intake of dietary fiber. Intake of 12 to 33 g dietary fiber per day from whole foods or 42.5 g fiber per day from supplements lower blood pressure, improve serum lipid levels and reduce indicators of inflammation (American Dietetic Association, 2008).

Various risk factors including age, gender, hypercholesterolemia, diabetes, hypertension, smoking, obesity and diet play critical roles in coronary artery, which is the main cause of coronary heart diseases (CHD). Higher fiber intake had a significantly inverse association with CHD risk, both of incidence (7% reduction) and mortality (17% reduction) (Wu et al, 2014).

The inverse relationship between dietary fiber intake and CHD risk could be explained by many mechanisms. First, fiber can modify blood lipid profiles such as reducing serum total cholesterol, triglyceride and oxidized low density lipoprotein, which plays a critical role in the formation of atherosclerotic plaques. Second, fiber can reduce blood glucose concentrations by slowing the intestinal absorption and improving the sensitivity of insulin, thereby reducing inflammation and enhancing antioxidant process. Furthermore, fiber can also reduce blood pressure and increasing viability of coronary artery endothelial cells and reducing intracellular ROS generation. (Wu et al, 2014).

Interestingly, although cereal and fruit fiber has strong inverse associations with CHD risk, no such associations are observed for vegetable fiber. There is a possibility that the common vegetables may lead to high glycemic load, which has been shown to increase risk of CHD (Wu et al, 2014).

10 to 29 g / day fiber intake can also control diabetes by glycemic control. Fiber may control risk of cancer such as breast cancer, large-bowel cancer, colon and prostate cancer (American Dietetic Association, 2008).

Fibers also serve as prebiotics. Prebiotics are selectively fermented ingredients that allow specific changes, both in the composition and / or activity in the gastrointestinal microflora that confers benefits upon host well being and health.

Functional fibers are known to alter the intestinal microflora and be deemed as prebiotics. (American Dietetic Association, 2008).

Fibers have also been found to affect mineral absorption, bone mineral content and bone structure (American Dietetic Association, 2008).

2.11 OMEGA 3 PUFA AND HEALTH BENEFITS

Over the past 20 years many studies and clinical investigations have been carried out on the metabolism of polyunsaturated fatty acids (PUFAs) in general and on n-3 fatty acids in particular. Today we know that n-3 fatty acids are essential for normal growth and development.

Essential fatty acids refer to fatty acids which are required in the body but cannot be synthesized in mammals and therefore have to be provided by diet. The omega-6 polyunsaturated fatty acid (n-6 PUFA), linoleic acid (LA,18:2n-6) followed by the omega-3 polyunsaturated fatty acid (n-3 PUFA), alpha-linolenic acid (ALA,18:3n-3) are the primary essential PUFAs. They play various roles in the body including blood pressure regulation, modulation of inflammatory and immune responses and play critical roles in chemotaxis, platelet aggregation, cellular growth and cell differentiation (Dunbar et al., 2014). In Figure 2.7, metabolic pathway of ω -3 PUFAs and food sources of these fatty acids are given.

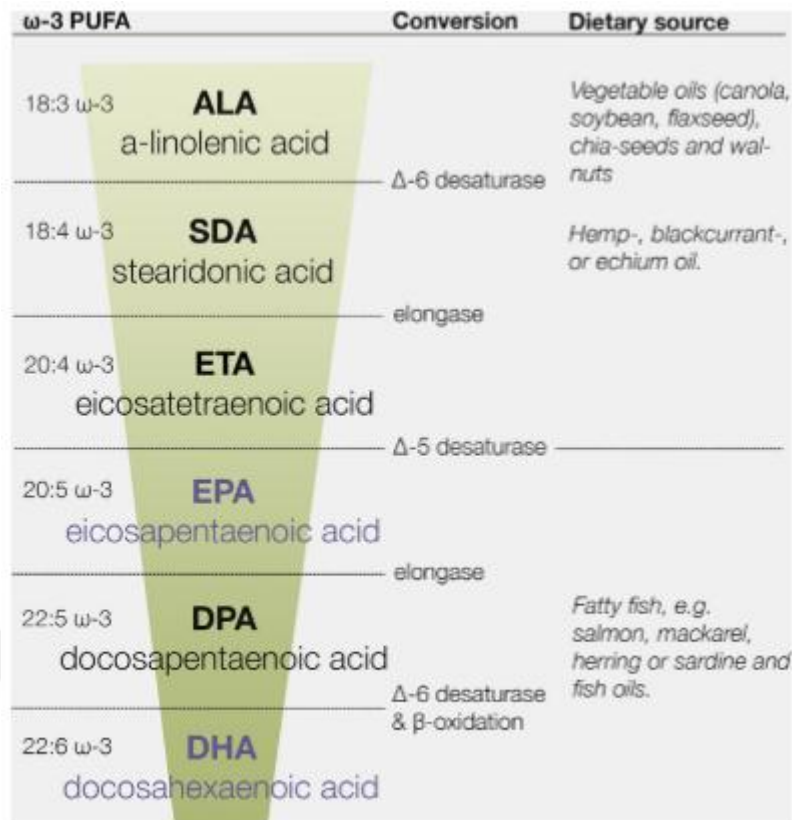


Figure 2.7 Metabolic pathway of ω -3 PUFAs and food sources of these fatty acids (Bos et al., 2016)

2.11.1 Omega – 3 Brain Function

The two most common types of omega-3 PUFAs in the human brain are eicosapentaenoic acid (EPA:20:5) and docosahexaenoic acid (DHA:22:6). DHA is abundantly present in the mammalian brain, comprising up to 40% of the total brain fatty acids. In contrast, EPA represents only less than 1 % of the total fatty acids in brain (Bos et al., 2015).

Luchtman and Song (2013) summarized the effects of PUFAs as:

1. modulate the structure function of lipid rafts and neuronal membranes, including raft and membrane incorporated proteins, such as channels, receptors and signaling proteins;
2. act as direct ligands to transcription factors of genes that play a role in a wide variety of processes, including fatty acid metabolism, neurogenesis and synaptogenesis, differentiation, inflammation and oxidative stress;

3. act as precursors to biosynthesis of lipid mediators which are involved in regulation of many cells and tissue responses, particularly aspects of inflammation.

It has been hypothesized that ω -3 PUFAs contribute to the modulation of the structure and function of biological membranes, including elasticity, membrane organization and ion permeability and may there facilitate brain glucose uptake, neurotransmission and neuronal function (Gorjao et al., 2009; Freemantle et al., 2006; Luchtman and Song, 2012).

EPA has been specifically suggested to have important neuroprotective properties; such as antioxidative activity and regulation of anti-inflammatory processes (Cunnane et al., 2009). Preclinical studies have shown that removing ω -3 PUFAs from the diet can result in cognitive deficits, such as impairments in attention and learning ability (Bourre et al., 1989; Carrie et al., 2000).

Alzheimer's disease (AD), accounting for more than 70 % of all cases of dementia, is the most dreaded disease and the fifth leading cause of death in persons aged 65 and older. The role of nutrition in prevention of dementia and AD arouses increasing hope with particular interest in the dietary intake of ω -3 fatty acids, for brain tissue membranes are rich in ω -3 fatty acids, including DHA. Higher intakes of ω -3 fatty acids from dietary sources are related to reduced risk of dementia and AD (Wu et al., 2015)

Omega-3 fatty acids are well known with their effects on human brain development especially in the pregnancy period. The pregnant are advised to consume omega-3 containing foods or supplements during pregnancy.

McNamara et al., (2010) showed in healthy boys (8-10 years old) that only 8 weeks of supplementation with 400 mg (low, normal dose) or 1200 mg (high dose) of DHA per day dose dependently increased the erythrocyte membrane DHA composition, which was positively correlated with functional activity in the dorsolateral prefrontal cortex and inversely correlated with reaction time during performance on attention task.

2.11.2 Omega – 3 and Cardiovascular Health

High blood pressure (BP) is a major risk factor for cardiovascular disease and mortality. The prevalence of hypertension is more than doubled in the elderly than in

the young population. The progressive increase in BP over time is directly linked to vascular aging, characterized by endothelial dysfunction, arterial stiffening and inflammation.

Epidemiological and clinical data suggest that higher intake of ω -3 PUFAs could have beneficial effects on cardiovascular health and is associated with lower systolic and diastolic BP.

Omega-3 PUFA administration is associated with improved vascular function and blunted inflammation in at risk population indicating an antihypertensive effect over time (Taggetti et al., 2015).

2.11.3 Omega – 3 and Obesity

Obesity is a global health problem responsible for 2.8 million deaths each year and the prevalence has almost doubled in the last thirty years. This condition characterized by an excessive fat accumulation and accompanied by chronic low grade inflammation, is related to metabolic diseases including type 2 diabetes, dyslipidemia, atherosclerosis or hypertension.

N-3 PUFAs have been shown to modulate adipocyte number by regulating adipocyte proliferation and differentiation as well as apoptosis. Moreover, n-3 PUFAs also regulates the pathways controlling fat storage and fat mobilization, decreasing lipid accumulation processes and favoring adipocyte oxidative metabolism by prompting mitochondrial biogenesis and fatty acid oxidation (Fernandez, et al., 2015). Figure 2.8 shows the summary of mechanisms by which EPA and DHA regulate adipose tissue mechanism and functions.

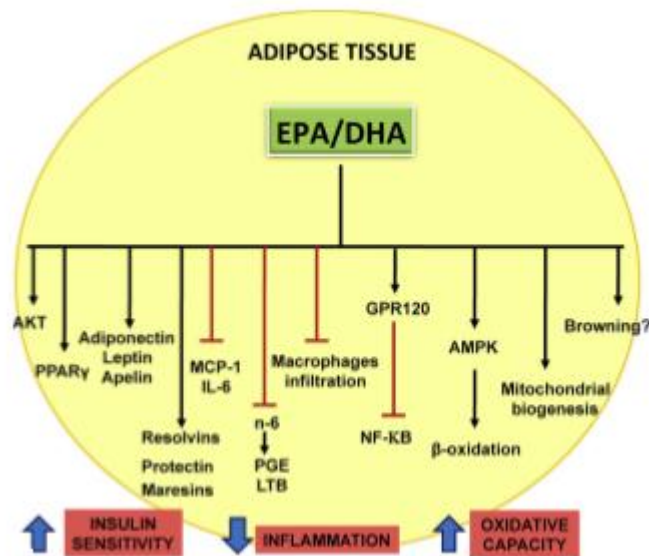


Figure 2.8 Summary of mechanisms by which EPA and DHA regulate adipose tissue mechanism and functions (Fernandez, et al., 2015)

2.11.4 Other Health Benefits of Omega - 3

- Stroke: ALA is studied to decrease stroke risk and protect brain from stroke (Blondeau, 2016).
- HIV / AIDS: ω -3 PUFAs are considered candidates for causing HIV / AIDS pleiotropic effects on immunologic and metabolic systems of the patient (Singh and Watson, 2015).
- Antioxidant activity: PUFAs are prime targets for oxidation, linkage with antioxidant such as phenolic compounds would also limit auto-oxidation to prevent harmful effect of lipid oxidation (Crauste et al., 2015).
- Anticancer activity: n-3 PUFAs are involved in various cellular processes that may help to lower tumor progression and protect healthy tissues during chemotherapy (Crauste et al., 2015).

2.12 SWEETENERS

The term “sweetener” refers to a food additive that imparts sweet flavor and usually provides no or very low energy (EC Regulation No 1333/2008). It is used to sweeten foods, medicines and food supplements with no nutritional purposes. For years, non-calorie sweeteners have been used as substitutes for all part of the sugar content in

foods and beverages. In recent decays its consumption has risen to prevent tooth decay, or as an aid in weight control, obesity and diabetes, and in general, to achieve an optimum energy balance.

Sugars are used to sweeten or enhance the flavor of foods, to modify the freezing and melting point and to preserve them. In the twentieth century, questions began to be raised as to whether excessive consumption of sugars, particularly associated with processed foods, might be related to diabetes or obesity.

Low or no calorie sweeteners have been used for years to replace all part of the sugar content in foods and drinks, but in the last few decades their consumption has increased both in adults and children.

2.12.1 Types of Sweeteners

There are various ways of classifying sweeteners. Using the glycemic index (GI), we can also make a classification of foods based on postprandial blood sugar response, comparing them with a reference food (GI=100). Depending on their energy contribution, they are classified as “caloric” or “low calorie/non-caloric” or as “nutritive” or “non-nutritive” (US FDA). In some cases the term “natural origin” is used in the consumer information, as with stevia glycoside, or, “artificial” origin when they are synthetic. Tables 2.10 and 2.11 gives classification of sweetness according to their nature, degree of sweetness and caloric contribution.

Table 2.10 Classification of sugars and sweeteners (Rapaille et al., 2003)

Natural	Caloric	Sugars	sucrose, galactose, glucose, fructose, lactose, maltose, trehalose
		Others	honey, maple syrup, palm sugar, coconut sugar
	Noncaloric	Lua han guo, stevia, thaumatin, pentadin, monellin, brazzein	
Artificial	Caloric	Modified sugars	high fructose corn syrup, inverted sugar
		Sugar alcohols	Soyclamic acid, sorbitol, xylitol, mannitol, lactitol
	Noncaloric	Artificial Sweeteners	Aspartame, sucralose, saccharin, neotame, acesulfame K, cyclamic acid, alitame, advantame

Table 2.11 Classification of sweeteners according to US FDA and the EU
(Sweetening power is relative to sucrose) (Rapaille et al., 2003)

Sweeteners	Classification by caloric contribution	Classification by sweetening power
<i>Polyols</i>		
Sorbitol E 420	Nutritive	0,5 – 1
Mannitol E 421	Nutritive	0,7
Isomalt E 953	Nutritive	0,45 – 0,65
Maltitol E 965	Nutritive	1
Lactitol E 966	Nutritive	0,5
Xylitol E 967	Nutritive	1
Erythritol E 968	Non-nutritive	0,7
<i>Acesulfame K E 950</i>	Nutritive	Intense (200)
<i>Aspartame E 951</i>	Nutritive	Intense (150 – 200)
<i>Cyclamic acid E 952</i>	Non-nutritive	Intense (50 – 100)

2.12.2 Sugar Alcohols

The polyols (sugar alcohols) derived from sugar and also regarded as carbohydrates are produced, in small quantities, in plants and cereals. Sugar alcohols are artificially obtained by substituting an aldehyde group with a hydroxyl one. As most of the sugar alcohols are produced from the corresponding aldose sugars, they are called alditols. Among sugar alcohols can be listed hydrogenated monosaccharides (sorbitol, mannitol), hydrogenated disaccharides (isomalt, lactitol, maltitol) and mixtures of hydrogenated mono-di-and/or oligosaccharides (hydrogenated starch hydrolates) (Grembecka, 2014).

They generally contain fewer calories than sugar, with very low GI, and have not been associated with the development of tooth decay, since they are non-fermentable; they are used as sweeteners. Their intestinal adsorption is generally very low, and in moderate quantities they can produce flatulence, colic or diarrhea, as they are fermented by the colonic microbiota (Campos et al., 2015). These compounds have a lower nutritional value than sugars, due to slower and incomplete absorption from the intestine, which results in indirect metabolism via fermentative degradation by the intestinal flora (Grembecka, 2014).

Islam et al. (2007), fed 42 rats with sorbitol and lactitol for 16 days. It was observed that body fat and blood fat levels of the rats decreased compared with control group.

Sugar alcohols are usually used in foods for diabetes because the polyols are not readily absorbed in the intestine and do not cause an increase in blood glucose levels postprandially. They also have lower caloric intake, but not calorie free. Sorbitol generates almost 65% of the calories of sucrose. Lactitol and isomalt generate 50% of sucrose's calories (Rapaille et al., 2003).

Sugar alcohols are white, crystalline, non-to slightly hygroscopic powders and clear syrups. They generally stable to heat and do not take part in Maillard-type browning reactions (Rapaille et al., 2003).

They produce a characteristic mouth cooling effect which is directly related to heats of solution and their solubility in water.

They are not degraded by saliva enzymes (amylase) upon oral ingestion. In the stomach slight acid hydrolysis of the di- and oligosaccharide polyols may occur. Once the small intestine is reached, extensive hydrolysis by α -amylases of the mucosa occurs up to 60-80 % of the maltitol and isomalt hydrolyzed. A 50 % glucose / 50 % sorbitol or a 50 % glucose / 25 % sorbitol / 25 % mannitol mixture is obtained (Rapaille et al., 2003). Table 2.12 gives the sweetness of sugar alcohols relative to sucrose.

Table 2.12 Sweetness values of sugar alcohols and sucrose (10 % solution in H₂O at 20 °C) (Rapaille et al., 2003)

Compound	Relative Sweetness
Polyol	
Xylitol	80 - 100
Sorbitol	50 – 60
Mannitol	50 – 60
Maltitol	80 – 90
Lactitol	30 – 40
Isomalt	40 – 50
Erythritol	60 – 70
Sucrose	100

Because of the reduced digestibility of polyols, their intake level is recommended to be restricted 40-50 g/day for adults and 30 g/day for children in order to avoid gastrointestinal discomfort. Higher doses may cause osmotic diarrhea or increased

flatulence due to slow adsorption and extensive fermentation, respectively, but tolerance levels improve upon adaptation.

2.12.3 Sorbitol

Sorbitol, which has a systematic name D-glucol is a 6-carbon sugar alcohol that was discovered by a French chemist in the berries of the mountain ash in 1872. In Figure 2.9, the molecular shape of sorbitol is given.

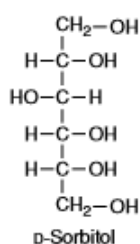


Figure 2.9 Molecular shape of sorbitol (Rapaille et al., 2003)

Sorbitol is produced from glucose or sucrose, by a catalytic hydrogenation with hydrogen gas and nickel catalyst at high temperatures. However it can be also produced by electrochemical reduction of dextrose in alkaline conditions. Although there are known several industrial processes which are used for the production of sorbitol, only a few microorganisms, including three yeast strains and bacteria such as *Zymomonas mobilis* and *Candida boidini* have been suggested as potential sorbitol producers. Production steps of sorbitol is given in Figure 2.10.

Sorbitol supplies fewer calories than sugars, and its sweetness amount is about 60 % of the one assigned to sucrose. It also characterizes with a 20-fold higher solubility than water in mannitol. It is manufactured in both liquid and crystalline form. It has a negative heat of solution, and thus it gives a cooling sensation in mouth. Sorbitol has a sweet, cool and pleasant taste. Besides acting as a sweetener, it is also an excellent humectant, softener, texturizing and anti-crystallizing agent. This polyol is chemically inert and stable, even in high temperatures does not undergo Maillard reactions. Sorbitol can combine well with other food components such as sugars, gelling agents, proteins and fats. It may also reveal synergistic effect with other sweeteners that result in greater sweetness and better taste (Grembecka, 2014).

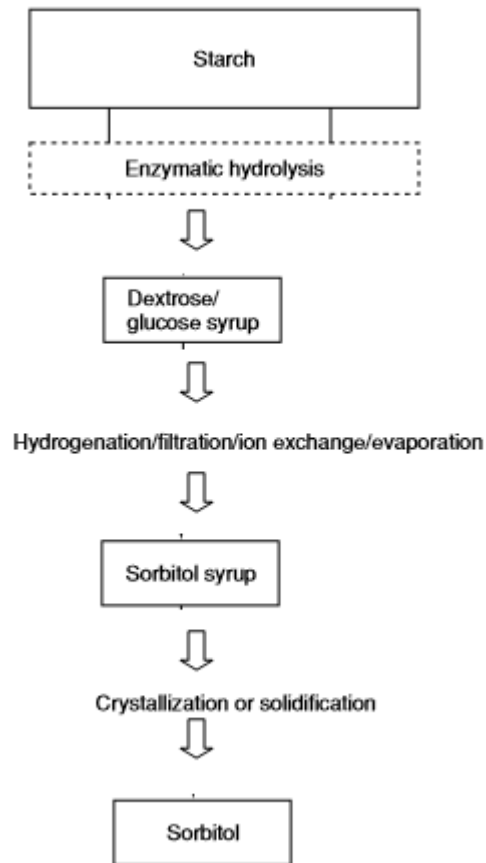


Figure 2.10 Production steps of sorbitol (Rapaille et al., 2003)

Sorbitol is resistant to digestion by oral bacteria which break down the sugars and starches to release acid that may lead to cavities or erode the enamel.

Due to its non-cariogenic properties, it is used in products for special nutritional purposes designed for people with diabetes, but also finds application in pharmaceutical and cosmetics. Sorbitol can be added to a wide variety of products, including sugar-free candies, chewing gums and sugar-free foods such as frozen desserts and bakery goods. As it prevents loss of moisture content, it is used in the production of confectionery, baked foods and chocolate, which have a tendency to become dry or harden during storage. Sorbitol is also used as an important precursor of the vitamin C production (Grembecka, 2014).

Sorbitol is the most commonly used polyol in US because of its low cost. The worldwide production of sorbitol is estimated to be higher than 500,000 tons/year, and the markets continuously increasing, and about 25 % of that production is used for the synthesis of vitamin C.

Its safety has been supported by many scientific studies and JECFA has defined an acceptable daily intake (ADI) for sorbitol as “not specified”, and thus, no limits are placed on its use. Similar to other polyols, it might reveal laxative effect when eaten in excess. (Grembecka, 2014).





3. MATERIALS AND METHODS

The chia seed used for this study was purchased from Malatya Pazarı, Turkey. Its origin is Peru. The sweeteners sorbitol, izomalt and stevia sugar were purchased from ZAG Chemistry Company, Artisan Food Company and Sukrax Sweeteners. All sweeteners were from Turkey. The frozen strawberry was purchased from Özgörkey Frozen Food Company, Turkey.

3.1 ANALYSES FOR CHARACTERIZATION OF CHIA SEED

Chia seeds were firstly milled by a laboratory type miller having 300 – 500 W power before the composition analysis.

3.1.1 Moisture Analysis

The moisture of chia seeds was determined according to AOAC 925.09, direct oven method. Approximately 2 gr of milled chia was exposed to 105 °C in an air oven (Memmert), in aluminium dishes, for 2 hours. The sample was continued to be exposed to hot air till a constant weight was obtained. This analysis was experienced for three parallels. Percentage of moisture was calculated according to Eq.(3.1).

$$\% \text{ moisture} = \frac{(\text{weight of dish before oven} - \text{weight of dish after oven})}{\text{Weight of initial sample}} * 100 \quad (3.1)$$

3.1.2 Protein Analysis

Defatted chia flour was used for the protein analysis to eliminate the possible errors that may result from high amount of fat. The protein content of chia seeds was determined according to AOAC 979.09 Kjeldahl method. The instruments used were Buchi Speed Digestor K-425 (Switzerland) as the digestor and Buchi K-350

(Switzerland) as the distillator. The results are expressed for three parallel measurements. Protein content was calculated by multiplying the % N with 6.5. The % N was calculated according to Eq. (3.2) and (3.3).

$$\% \text{ N} = \frac{(\text{ml HCl consumed for sample} - \text{ml HCl consumed for the blank}) * \text{Normality of HCl} * 1.4007}{\text{gr sample}}$$

$$\% \text{ protein} = \% \text{ N} * 6.5 \quad (3.2), (3.3)$$

3.1.3 Ash Analysis

The ash content of chia seeds was determined according to AOAC 923.03 direct method. Approximately 1 g of sample was put in predried crucible. Preashing was applied by using ethanol. The ash content was calculated after exposing the sample to 550 °C for 8 hours. The results are expressed for three parallel measurements. Percentage of ash was calculated according to Eq. (3.4).

$$\% \text{ ash} = \frac{(\text{weight of crucible before oven} - \text{weight of crucible after oven})}{\text{Weight of initial sample}} * 100 \quad (3.4)$$

3.1.4 Total Dietary Fiber Content

Defatted chia flour was used total dietary fiber analysis to eliminate the possible errors that may result from high amount of fat. Total dietary fiber (TDF) content was determined according to AOAC 985.29 Enzymatic Gravimetric Method, and modified as described by Caudillo, et al (2007). 0.1 gr of sample was used due to the presence of mucilage in the samples. The protein and ash amounts in the residue was then determined by AOAC 979.09 and 923.03, respectively. The analysis was performed in triplicate. TDF amount was calculated by subtracting protein and ash in the residue, as shown in Eq. (3.5).

$$(\text{weight of residue}) - (\text{weight of protein} + \text{weight of ash})$$

$$\text{TDF \%} = \frac{\text{Weight of fat extracted}}{\text{Weight of initial sample}} * 100 \quad (3.5)$$

3.1.5 Fat Content Analysis

Total fat content of chia seed was determined by using an automatic Soxhlet instrument, Gerhardt Soxtherm (Germany). Pure hexane was used as the solvent. The fat content was extracted at 180 °C for 135 minutes. The hexane was evaporated from the fat content by a rotary evaporator under vacuum, Rotavapor, Buchi, RII (Switzerland). The hexane evaporation was achieved at 50°C under vacuum. The percentage of total fat was calculated by Eq. (3.6)

$$\text{Total fat \%} = \frac{\text{Weight of fat extracted}}{\text{Weight of initial sample}} * 100 \quad (3.6)$$

3.1.6 Fatty Acid Composition

Extracted oil was firstly methylated by saponification of glycerid and phospholipids in the presence of BF₃ to extract the fatty acids. The fatty acids were methylated to be readable by the Gas Chromatography.

Fatty acid composition of chia seed was analyzed by using Thermo Quest Trace GC 2000 gas-liquid chromatography (GLC) (Milan, Italy) equipped with a flame-ionization detector and DB-Wax capillary column (30m x 0.32mm ID x 0.25 µm film thickness) (J&W Scientific Folsom, CA) was used for the analysis. The injector and detector temperatures were held at 250°C and 260°C, respectively. The oven temperature was initially held at 150°C for 3 min, and was then programmed to 225°C for 10 min at a rate of 10°C/min, and helium carrier gas flow rate was 1.5 mL/min. A 1 µL sample was injected into the GLC and relative amounts of FAME were calculated as area % by computer. Average values from the results of duplicate analyses were reported.

3.1.7 Carbohydrate Content

Total carbohydrate amount was determined by subtracting all the components from 100 according to Eq. (3.7).

$$\% \text{ carbohydrate} = 100 - (\text{moisture} + \text{fat} + \text{protein} + \text{ash}) \quad (3.7)$$

3.1.8 Energy Calculation

Total energy of 100 g chia seed was calculated by using Eq. (3.8) and (3.9).

$$\text{Total energy (kcal/ 100 g)} = (\% \text{ protein} * 4) + (\% \text{ fat} * 9) + (\% \text{ TDF} * 2) + (\% \text{ carbohydrate} * 4) \quad (3.8)$$

$$\text{Total energy (kj/100 g)} = (\% \text{ protein} * 17) + (\% \text{ fat} * 37) + (\% \text{ TDF} * 8) + (\% \text{ carbohydrate} * 17) \quad (3.9)$$

3.1.9 Antioxidant Capacity

3.1.9.1 Extraction with Ethanol

Extraction of the phenolic compounds was carried out according to Marinelli et al., (2014). One gram of defatted chia flour was extracted with 10 mL of ethanol by continuous shaking at room temperature for 3 hours. The mixture was then centrifuged at 3000 rpm for 15 minutes. This extract was used for the analysis of phenolic compounds and free radical scavenging activity.

3.1.9.2 DPPH Free Radical Scavenging Activity

The free radical scavenging activity of chia seed was determined according to Marinelli et al. (2014). 33 µL of the extract was mixed with 1.3 mL DPPH solution prepared in methanol at 0.024 mg/mL concentration, shaken and incubated at room temperature for 30 minutes. The absorbance values were read at 515 nm. A standard curve was prepared using Trolox solution of 5 – 600 µmol concentrations. Results were expressed in Trolox equivalent (TE/g).

3.1.10 Total Phenolic Content

The total phenolic content of chia seed was determined according to Marinelli et al. (2014), by using Folin-Ciocalteu's method. 50 μ L of the extract, 800 μ L distilled water and 25 μ L 0.25 N of Folin-Ciocalteu's reagent were mixed and incubated at room temperature for 3 minutes. Then 100 μ L of 1 N Na_2CO_3 solution was added and further incubated at room temperature for 2 hours. The absorbance was read at 725 nm by Sinergy_HT Biotek (USA). A standart curve was drawn using gallic acid with concentrations of 16 – 500 μ g gallic acid / mL water and the results were expressed in gallic acid equivalent (GAE/g).

3.1.11 Gelatinization Properties of Chia Seed and Flour

Mucilage extraction was performed according to the method proposed by Munoz, Cobos, Diaz and Aguilera, 2012. 40 g of chia seeds were placed in 1 L beaker and distilled water was added in a 1:20 weight ratio. The pH was adjusted to 8 using NaOH and HCl solutions. The mixture was stirred with a magnetic stirrer and hydrated for 2 h at a constant temperature at 80° C. The mucilage was seperated from the seeds by centrifuging for 8 min at 524 * g.

The supernatant was decanted and the glass transition temperature was determined by differential scanning calorimeter in a TA-DCS Q10 calorimeter (USA), equipped with a mechanical refrigeration system (RSC-refrigerated cooling accesory). Thermal program consisted in a two cycle scan model, with the temperature ranging from -50 to 150 ° C using a heat ramp of 2 ° C / min. An empty aluminium hermetic pan was used as reference. The instrument was purged with nitrogen at a flow rate of 100 cm³ / min. The data was analysed using Universal Analysis 2000 software.

3.2 OPTIMIZING MARMALADE FORMULA

In this study, we investigated the effects of chia seed on strawberry marmalade with chia without additional sugar and tried to optimize the formula of a sugar free, chia-aded strawberry marmalade. The reason for selecting strawberry as the fruit was the similarity between the seeds of strawberry and chia. Their color, shape and dimensions were more similar than seeds of other berries such as blackberry and blueberry which were also tried for marmalade formulation.

The formula of marmalade was optimized by two step sensory analysis. First step was for deciding the sweetener type. The other was for deciding the percentage of chia in the formula.

The marmalades were cooked by a home type heater. The strawberries and sweeteners were put in a dish, waited for the strawberries to release water and wet the sweetener at room temperature, put on heater and heated by continuous stirring until the mixture boiled, thereafter they were heated for 1 more minute, chia seeds were added, heated for 1 minute more, 2-3 drops lemon juice were added and heated for 1 minute more.

3.2.1 Two Step Sensory Analysis

Difference test panel for the marmalade types was carried out. The panelists were asked to number the samples according to a 7 point hedonic scale. (1: terrible, 2: very weak, 3: weak, 4: medium, 5: good, 6: very good, 7: perfect). All evaluations were held in Istanbul Technical University Sensory Analysis Laboratory by female and male panelists. The marmalades were prepared three days before the sensory analysis, held in 4°C and were taken out 3 hours before serving. All samples were represented at room temperature and under normal lighting conditions. The samples were coded by three digit numbers. Biscuits, spoons, drinking water and knife, to test spreadibility, were also served during tests.

3.2.1.1 Sensory Analysis For Type Of Sweetener

5 types of marmalade samples were prepared by different formulas with different sweetener types. A control sample, marmalade with sucrose, was also served to see the differences or similarities of other sweeteners from sucrose. The formulas of jams served in first sensory analysis are shown in Table 3.1.

Table 3.1 Marmalade formulas prepared for the first sensory analysis

Formula Number	Strawberry	Sweetener Type	Sweetener Amount	Chia	Total
1 (control)	50,5	<i>Sucrose</i>	47	2,5	100
2	50,5	<i>Sorbitol</i>	47	2,5	100
3	50,5	<i>Izomalt</i>	47	2,5	100
4	50,5	<i>Izomalt</i>	23,5	2,5	76,5
		<i>Sorbitol</i>	23,5		23,5
5	50,5	<i>Stevia</i>	47	2,5	100

The panelists were asked to evaluate the samples according to a 7 point hedonic scale. (1: terrible, 2: very weak, 3: weak, 4: medium, 5: good, 6: very good, 7: perfect). Evaluated parameters were numbered; color, sweetness, strawberry odor, strawberry flavor, thickness in mouth, thickness by spoon and spreadability.

3.2.1.2 Sensory Analysis For Percentage Of Chia Seed

The formulas of marmalades served in the second sensory analysis are seen in Table 3.2.

Formula Number	Amount of Strawberry (g)	Sweetener Type	Amount of Sweetener (g)	Amount of Chia (g)	Total
1	50.5	<i>Sorbitol</i>	47	2.5	100
2	48	<i>Sorbitol</i>	47	5	100

Table 3.2 Marmalade formulas prepared for the second sensory analysis

The panelists were asked to evaluate the marmalades according to the parameters; color, sweetness, strawberry odor, strawberry flavor, cereal taste, thickness in mouth,

thickness in spoon, spreadability and amount of grain. The panelists were asked to evaluate the samples according the 7 point hedonic scale, same with the first sensory analysis. In the second part preference to purchase was asked by a 5 point hedonic scale (1: I never purchase, 2: I don't think I will purchase, 3: I'm not sure, 4: I may purchase, 5: I exactly purchase).

3.3 ANALYSES FOR CHARACTERIZATION OF STRAWBERRY MARMALADE

3.3.1 Strawberry Marmalade Composition Analyses

Strawberry marmalade was firstly homogenized and blended with a laboratory type blender before the composition analyses. Composition analyses were also carried out with control sample, marmalade with sucrose and without chia, to compare the overall energy values.

3.3.1.1 Moisture Analysis

The moisture analysis of strawberry marmalade was determined according to Naeem et al. (2015) using AOAC. 10 g sample was weighed in aluminium dishes, put in 105 °C air oven for one night. The weight was recorded. Exposing to hot air was continued till a constant weight was obtained. The analysis was performed in triplicate. Moisture content of marmalade was calculated by Eq. (3.1).

3.3.1.2 Protein Analysis

The protein content of marmalade was determined according to AOAC 979.09, Kjeldahl method. The instruments used were Buchi Speed Digestor K-425 (Switzerland) as the digester and Buchi K-350 (Switzerland) as the distillator. The results are expressed for three parallel measurements. The protein content was calculated by Eq. (3.2) and (3.10).

$$\% \text{ protein} = \% \text{ N} * 6,25 \quad (3.10)$$

3.3.1.3 Ash Analysis

The ash content of marmalade was determined according to AOAC 940.26. 10 gr sample was put in dry and clean crucibles, preashing was made with some ethanol by burning, then the crucibles were put in 525 °C oven for one night. This method was modified to prevent sudden splashing in oven by increasing the temperature with 50 °C intervals. The ash content was calculated according to Eq. (3.4).

3.3.1.4 Fat Content Analysis

Total fat content of chia seed was determined by using an automatic Soxhlet instrument, Gerhardt Soxtherm, Germany. Pure petroleum ether was used as the solvent. The fat content was extracted at 40 – 60 °C (Naeem et al., 2015). The petroleum ether was evaporated from the fat content by a rotary evaporator under vacuum, Rotavapor, Buchi, RII (Switzerland). The hexane evaporation was achieved at 40°C under vacuum. The results are expressed for three parallel measurements. The fat content was calculated by using Eq. (3.6).

3.3.1.5 Total Dietary Fiber Content Analysis

Total dietary fiber (TDF) content was determined according to AOAC 985.29 Enzymatic Gravimetric Method. 1 gr of sample was used. The protein and ash amounts in the residue was then determined by AOAC 979.09 and 923.03, respectively. TDF amount was calculated by subtracting protein and ash in the residue. The analysis was experienced in triplicate. Total dietary fiber content was calculated by the Eq. (3.5).

3.3.1.6 Carbohydrate Content

Total carbohydrate amount was determined by subtracting all the components from 100 according to Eq. (3.7).

3.3.1.7 Energy Calculation

Total energy of 100 gr chia seed was calculated by Eq. (3.11) and (3.12).

$$\text{Total energy (kcal/ 100 g)} = (\% \text{ protein} * 4) + (\% \text{ fat} * 9) + (\% \text{ TDF} * 2) + (\% \text{ carbohydrate} * 4) + (\% \text{ polyol} * 2.4) \quad (3.11)$$

$$\text{Total energy (kJ/100 g)} = (\% \text{ protein} * 17) + (\% \text{ fat} * 37) + (\% \text{ TDF} * 8) + (\% \text{ carbohydrate} * 17) + (\% \text{ polyol} * 10) \quad (3.12)$$

3.3.2 Soluble Solid Analysis

The soluble solid amount of jam was determined according to AOAC 932.12, with refractometer, at 20⁰ C. The measurement was repeated for three samples.

3.3.3 Titration Acidity and pH

The pH was determined by pH-meter, Hanna HI 2211 (Island).

The acidity was determined according to AOAC 942.15. 10 g sample was diluted to 100 mL with distilled water, a few drops of 1 % phenolphthalein indicator was added and titrated with 0.1 N NaOH. The experiment was repeated for three replicates. The percentage of citric acid in the sample was calculated according to Eq. (3.13).

$$\begin{aligned} 1 \text{ mL of } 0.1 \text{ N NaOH} &= 0.0064 \text{ g citric acid} \\ \text{citric acid, \%} &= 10 * \text{citric acid in sample} \end{aligned} \quad (3.13)$$

3.3.4 Invert Sugar Analysis

Since the marmalade sample did not contain sucrose, only invert sugar was determined. This invert sugar was determined according to AOAC 923.09 with a modification. The dilution was not 1 %, it was 10 %. Because the titration waste was very high, when the solution was diluted to 1 %. So the result was divided to this dilution factor in the calculation. Titration of the sample solution with the Fehling solution gives the invert sugar amount. The experiment was repeated for three replicates. The percentage of invert sugar in the sample was calculated by the Eq. (3.14) and (3.15).

$$\text{Invert sugar} = \frac{\text{Lane-Eynon factor for the titration} * 100}{\text{mL spent}} \quad (3.14)$$

$$\text{Invert sugar in sample solution (\%)} = \text{Invert sugar} / 10 * 100 \quad (3.15)$$

3.3.5 Hydroxymethyl Furfural (HMF) Analysis

HMF content of marmalade was determined by AOAC 980.23. This method depends on removing the impurities in sample by the Carez solutions and measuring the absorbance at 284 and 336 nm in sodium bisulphide solution. The experiment was repeated for three replicates. The percentage of HMF in the sample was calculated by the Eq. (3.16).

$$\text{mg HMF / 100 g sample} = (A_{284} - A_{336}) * 14.97 * 5 \quad (3.16)$$

3.3.6 Antioxidant Capacity

Antioxidant capacity was determined for both strawberry marmalades with and without chia addition to evaluate the effect of chia on antioxidant capacity of marmalade.

3.3.6.1 Extraction With Ethanol

Extraction of the phenolic compounds was carried out according to Marinelli et al. (2014). One gram marmalade sample was extracted with 10 ml of ethanol by continuous shaking at room temperature for 3 hours. The mixture was then centrifuged at 3000 rpm for 15 minutes. This extract was used for the analysis of phenolic compounds and free radical scavenging activity.

3.3.6.2 DPPH Free Radical Scavenging Activity

The free radical scavenging activity of marmalade was determined according to Marinelli et al, 2014. 33 μ L of the extract was mixed with 1.3 ml DPPH solution diluted in methanol 0.024 mg/ml, shaken and incubated at room temperature for 30 minutes. The absorbances were read at 515 nm. A standard curve was drawn using Trolox solution of 5 – 600 μ mol concentrations. Results were expressed in Trolox equivalent (TE/g).

3.3.7 Total Phenolic Content

The total phenolic content of marmalade was determined according to Marinelli et al. (2014), by using Folin-Ciocalteu's method. 50 μL of the extract, 800 μL distilled water and 25 μL 0.25 N of Folin-Ciocalteu's reagent were mixed and incubated at room temperature for 3 minutes. Then 100 μL of 1 N Na_2CO_3 solution was added and further incubated at room temperature for 2 hours. The absorbance was read at 725 nm by Sinergy_HT Biotek (USA). A standart curve was drawn using gallic acid with concentrations of 16 – 500 μg gallic acid / mL water and the results were expressed in gallic acid equivalent (GAE/g).

3.3.8 Rheological Measurements

Both marmalade with chia and control marmalade were analysed by means of rheological paramters. The rheological measurements were performed on blended marmalade sample because of the presence of chia seeds. The chia seeds are higher than 1 mm diameter and the gap between plates of the rheometer reaches maximum 1 mm.

Viscoelastic measurements were performed using a controlled strain rheometer, Haake Rheostress 1 (USA) with temperature control. Parallel plate geometric configuration was used with a plate diameter of 35 mm and a gap between plates 1 mm. Dynamic rheological measurements; frequency sweep and shear sweep were analysed in triplicate at 25 $^{\circ}\text{C}$.

Shear sweep measurement was performed from 0.01 – 100 Hz for 100 s according to Basu et al., 2011. Apperant viscosity values (η^*) were obtained.

Frequency sweep test was performed from 0.01 to 10 Hz according to Basu et al. (2011). The oscillatory rheological parameters, storage modulus (G'), loss modulus (G''), complex modulus (G^*) and loss tangent ($\tan \delta$) were also determined.

3.4 STATISTICAL ANALYSES

All the statistical analyses were performed using Minitab Release 17 programme (Turkey). The difference between the data were evaluated by ANOVA with a confidence interval at $\alpha=0.05$. The mean values were compared by Tukey test.

4. RESULTS AND DISCUSSION

4.1 CHIA SEED CHARACTERIZATION

4.1.1 Chia Seed Composition

Chia seed was characterized for its moisture, ash, protein, total fiber and carbohydrate contents. Proximate analysis together with energy value of chia seed are shown in Table 4.1.

Table 4.1 Composisiton data of chia seed

Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	Total fiber (%)
7.4 ± 0.11	3.9 ± 0.25	19.6 ± 0.54	29 ± 1.33	38.9 ± 0.5

These findings are in accordance with the data in the literature Munoz et al. (2013).

According to the composition of chia seed, caloric value of 100 g chia seed was determined by direct calculation. The energy values provided by composition materials are given in Table 4.2.

Table 4.2 Energy calculation for chia seed

	Metabolizable Energy Factor (FAO, 1998)	Energy in 100 g
Protein	17 kj / g - 4 kcal / g	333.0 kj – 78.4 kcal
Fat	37 kj / g – 9 kcal /g	1073.4 kj – 261.1 kcal
Carbohydrate	17 kj / g - 4 kcal / g	680.8 kj – 160.2 kcal
Dietary Fiber	8 kj / g – 2 kcal / g	311.5 kj – 77.9 kcal

Fatty acid composition of chia seed was analyzed and the contents of the fatty acids are shown in Table 4.3. Omega-3 (α linolenic acid) was found 18.45 g in 100 g chia seed.

Table 4.3 Fatty acid composition of chia seed

PUFA	g in 100 g chia oil
Myristic acid (C14:0)	0.05
Palmitic acid (C16:0)	7.6
Palmitoleic acid (C16:1)	0.01
Stearic acid (C18:0)	3.4
Oleic acid (C18:1)	7.3
Linoleic acid (C18:2)	19.8
α linolenic acid (C18:3n-3)	61.5

4.1.2 Gelatinization Properties of Chia Seed and Flour

Glass transition temperature is related to chain stiffness and polymer chain structure; it increases as cross linking density increases. A lower Tg implies more free volume, presence of shorter chains and more hydrophilic groups causing higher hygroscopicity. Certain physicochemical and structural processes like stickiness, crispness, collapse and the rates of non-enzymatic browning, are correlated to molecular mobility, therefore these deteriorative processes are related to Tg, which determines these changes begin to occur.

In Figure 4.1 and 4.2, the glass transition temperatures of chia seed and chia flour are seen. Tg for seed was 44.6°C while it was 34.6°C for flour. This indicates a higher gel strength obtained by seed. Karaki et al. (2011) reported that modified pectin had a Tg of 48°C. It can be concluded that chia seed can be used instead of pectin for some formulas.

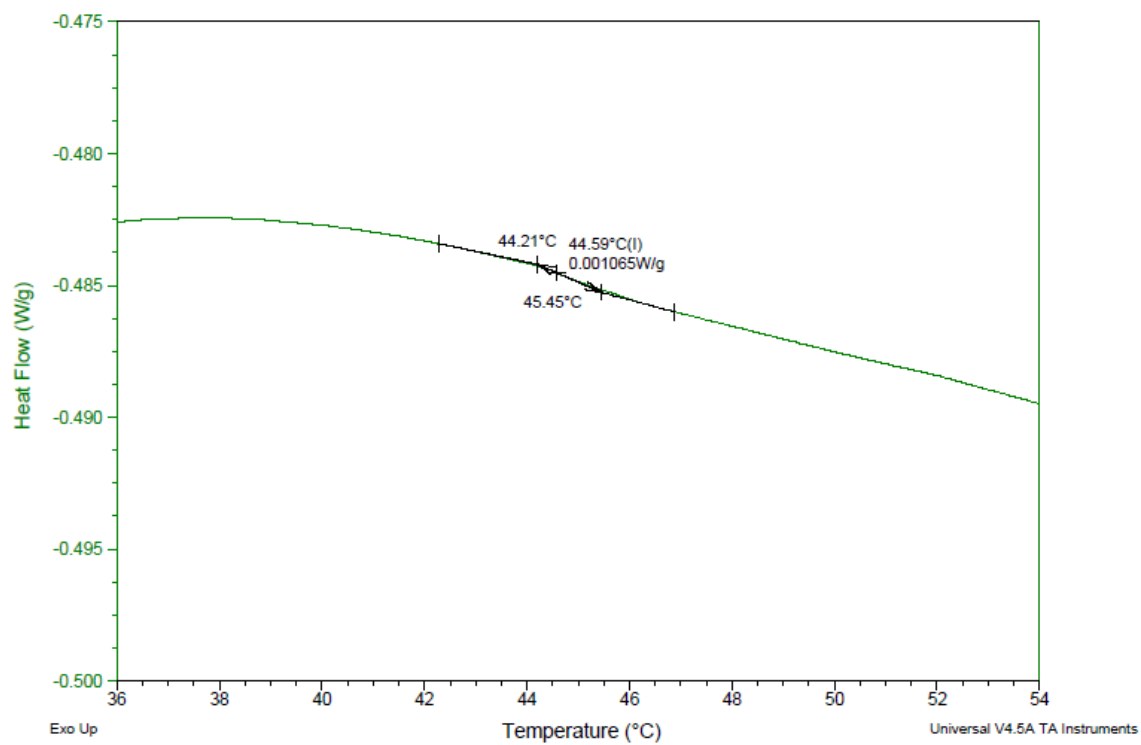


Figure 4.1 Glass transition temperature of chia seed

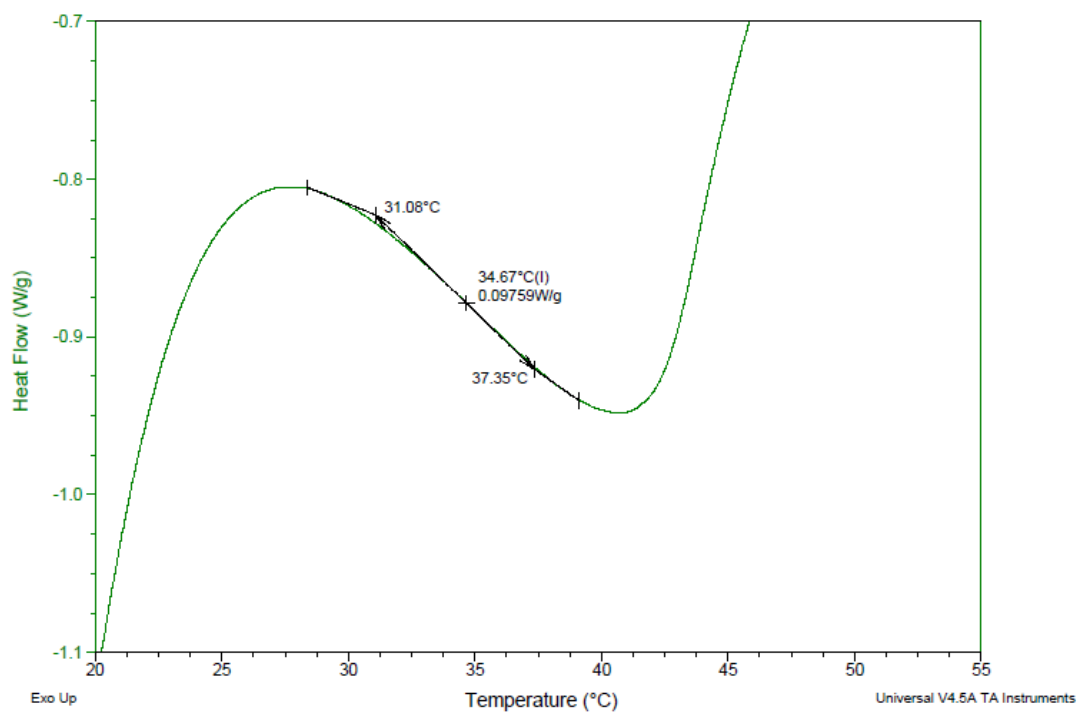


Figure 4.2 Glass transition temperature of chia flour

4.2 OPTIMIZING MARMALADE FORMULA

In our study, sucrose in strawberry marmalade was substituted with sweeteners to decrease caloric value and glycemic index of the marmalade. The marmalade formula was optimized by two step sensory analysis. The Table 4.4 shows the statistical results of the first sensory analysis, experienced for selecting the type of sweetener. The letters show the results “A-not A” test.

Table 4.4 Statistical results of first sensory analysis

Sweetener type	Color	Sweetness	Strawberry flavor	Strawberry odor	Thickness in mouth	Thickness by spoon	Spreadability
Control	4.7 ± 1.20 ^a	5.2 ± 1.01 ^a	4.7 ± 1.71 ^a	4.3 ± 1.73 ^a	5.3 ± 1.00 ^a	5.3 ± 0.86 ^a	4.7 ± 1.39 ^a
Sorbitol	5.2 ± 0.97 ^a	4.5 ± 0.88 ^{ab}	4.6 ± 1.00 ^a	4.8 ± 1.05 ^a	4.6 ± 0.86 ^a	4.5 ± 1.01 ^{ab}	5.4 ± 1.00 ^a
Stevia	5.2 ± 1.39 ^a	3.0 ± 1.5 ^b	3.4 ± 1.50 ^a	4.7 ± 1.39 ^a	3.0 ± 1.11 ^b	3.2 ± 0.97 ^b	3.2 ± 0.83 ^b
Izomalt	5.3 ± 0.86 ^a	4.3 ± 1.41 ^{ab}	4.6 ± 1.58 ^a	5.3 ± 1.41 ^a	4.8 ± 0.92 ^a	5.3 ± 0.86 ^a	5.2 ± 1.20 ^a
Izomalt+Sorbitol	4.8 ± 1.26 ^a	4.1 ± 1.36 ^{ab}	4.6 ± 1.11 ^a	4.6 ± 1.11 ^a	5.0 ± 1.00 ^a	5.4 ± 1.33 ^a	5.4 ± 1.13 ^a

Control sample was made with sucrose and our aim was to select the nearest one to the control. Our most important parameters were the parameters which are most important buying reasons for customers. These parameters were sweetness, strawberry flavor, strawberry odor and spreadability. Sorbitol was the nearest to the control as seen in Table 4.3. So we decided to continue with sorbitol involving formula to further analyses.

In the second sensory analysis we decided the percentage of the chia seed in the formula. The statistical results of the second sensory analysis are given in Table 4.5.

As it is seen on Table 4.5, formula with 5 % chia seed was the one having the highest points for all parameters. This indicates that chia addition up to 5% makes the marmalade favorable by means of consuming parameters. Chia seed makes more compact the structure because of its capability to retain water and to form a viscous gel by mucilage formation.

The further analyses was applied for the formula; 48 % strawberry, 47 % sorbitol and 5 % chia seed.

Table 4.5 Statistical results of second sensory analysis

	Chia seed %	
	2.5	5
Color	4.7 ± 1.09 ^a	5.7 ± 0.83 ^a
Sweetness	4.4 ± 1.33 ^a	5.4 ± 0.72 ^a
Strawberry flavor	5.1 ± 1.36 ^a	5.1 ± 1.16 ^a
Strawberry odor	4.6 ± 1.11 ^a	5.1 ± 1.05 ^a
Cereal taste	3.4 ± 1.23 ^a	3.7 ± 1.64 ^a
Thickness in mouth	4.6 ± 1.00 ^a	5.0 ± 1.00 ^a
Thickness by spoon	4.8 ± 1.16 ^a	5.5 ± 0.72 ^a
Spreadability	4.4 ± 1.23 ^a	5.3 ± 0.70 ^a
Grain amount	4.8 ± 0.92 ^a	5.3 ± 1.23 ^a
Preference to purchase*	2.7 ± 1.30 ^a	3.8 ± 0.92 ^a

*Evaluated by 5 point hedonic scale.

^{a, b}: The letters give the differences between the results

4.3 CHARACTERIZATION OF STRAWBERRY MARMALADE

4.3.1 Strawberry Marmalade Composition and Energy Calculation

The composition analysis were performed for the strawberry marmalade with chia (SMWC) and strawberry marmalade with sugar and without chia as the control to compare the energy values. Table 4.6 shows the composition of our no sugar added chia added marmalade and the control marmalade.

Table 4.6 Composition data of strawberry marmalades, SMWC and control

	SMWC	Control	% increase
Moisture (%)	35.16 ± 0.29	31.23 ± 2.83	12.6
Protein (%)	1.30 ± 0.11	0.32 ± 0.087	306.56
Fat (%)	2.46 ± 0.4	0.14 ± 0.07	1600
Ash (%)	0.46± 0.0	0.23 ± 0.03	99.56
Total Fiber (%)	2.7 ± 0.25	1.00± 0.10	168
Carbohydrate (%)	61.22 ± 1.15	67.65 ± 2.91	-10

The most significant effects of chia addition were seen on protein, fat, fiber and ash contents. Protein amount of control sample increased to 4 times of itself, fat increased to 17 times of itself, total fiber increased to approximately 3 times of itself and ash increased to approximately 2 times of itself. The increase in these contents makes our product more nutritious than the control sample. Higher ash amount may be related with higher amount of minerals. The increase in fiber provides our product a lower glycemic index, higher capability to retain water.

Caloric value of the marmalades were determined by direct calculation method. The energy values for SJWC and control marmalade are seen in Table 4.7.

Table 4.7 Energy calculation of SMWC and control

	Metabolizable Energy Factor (FAO, 1998)	Energy in 100 g of SMWC	Energy in 100 g of control marmalade
Protein	17 kj / g - 4 kcal / g	22.22 kj – 5.23 kcal	5.46 kj – 1.2864 kcal
Fat	37 kj / g – 9 kcal / g	91.02 kj – 22.14 kcal	5.328 kj – 1.296 kcal
Carbohydrate	17 kj / g - 4 kcal / g		1150 kj – 270.6 kcal
Dietary Fiber	8 kj / g – 2 kcal / g	21.6 kj – 5.4 kcal	8.032 kj – 2.008 kcal
Polyol, sorbitol	10 kj / g – 2.4 kcal / g	470 kj – 112.8 kcal	
Total Energy		604.84 kj – 145.57 kcal	1168.82 kj – 275.19 kcal

The energy decrease by sorbitol substituting and chia addition was % 48.25. It can be interpreted from the Table 3.6 that chia, itself, increases the caloric value by its high

amount of fat, protein and dietary fiber. But substituting sucrose with sorbitol decreases the overall energy density.

4.3.2 Soluble Solids, pH and Acidity of Strawberry Marmalade

The Table 4.8 shows the percentage of soluble solid, pH and percentage of acidity. Chia addition and sorbitol substitution did not significantly change the soluble solid amount and acidity, but changed the pH value. This could make our product more favorable for microbial spoilages than the control. Further studies may focus on the preservation techniques and microbial control of chia added, sorbitol substituted marmalade.

Table 4.8 Soluble solids, pH and acidity of SMWC and control

	SMWC	Control
Soluble Solid %	64.3 ± 1.20	65.4 ± 1.11
pH	4.43 ± 1.02	3.28 ± 0.08
Acidity %	0.494 ± 0.95	0.48 ± 0.65

4.3.3 Invert Sugar and HMF Analysis

Invert sugar amount in marmalades and jams is important by means of crystallization. Invert sugars are monosaccharides such as glucose and fructose, inverted from disaccharides such as sucrose. Disaccharides are favorable to crystallize by temperature fluctuations, while monosaccharides does not cause any crystallization even at high concentrations such as 65 % (Cemeroğlu et al., 2003).

Since our marmalade formula does not contain sucrose, we only determined the invert sugar by titration method. The invert sugar amount was calculated by the Eq. (3.14) and (3.15).

$$\text{Invert sugar} = \frac{124 \text{ mg} * 100}{25 \text{ ml}} = 500 \text{ mg} / 100 \text{ ml} = \% 0.496$$

$$\text{Invert sugar in the sample} = 0.496 / 10 * 100 = \% 4.96$$

This 5 % sugar amount makes our product “*low sugar*” according to Turkish Food Codex.

Hydroxymethyl furfural (HMF) is a compound formed by dehydration of sugar in Maillard reaction. HMF gives information about the heat treatment of process and causes the formation of brown pigments. HMF is an important quality parameter since Maillard reaction causes loss of nutritional value of proteins in food (Buldurlu and Karadeniz, 2002). % HMF gives information about degree of Maillard reactions.

The HMF amount was calculated by the Eq. (16).

$$\begin{aligned}\text{mg HMF} / 100 \text{ gr sample} &= (A_{284} - A_{336}) * 14.97 * 5 \\ &= (0.327 - 0.265) * 14.97 * 5 \\ &= 4.64 \text{ mg/100 g sample}\end{aligned}$$

TS 12985, Low Energy Jam Turkish Standart, April 2003 defines the maximum HMF value as 50 mg/kg. The product, SJWC, is found suitable for the standart. This explains that the process we obtained the marmalade is suitable by means of heating time, heating temperature and cooling time. Further analysis may care on the process parameters for higher amounts of marmalade production.

4.3.4 Antioxidant Capacity

Antioxidant capacity of chia seed, control marmalade without chia and chia added marmalade were evaluated by means DPPH free radical scavenging activity. The antioxidant capacities of chia seed and SMWC are seen in Table 4.9.

Table 4.9 Antioxidant capacities of chia seed, SMWC

	Chia Seed	SMWC
DPPH (μmol TE/g)	436.6 ± 2.54	21.9 ± 1.20

4.3.5 Total Phenolic Content

Table 4.10 shows the phenolic compound concentration for chia, marmalade without chia and chia added marmalade. These data are in accordance with Marinelli et al. (2014). Chia increased the phenolic content of the marmalade by 15.4 %. Phenolic content of the control marmalade was also noticeable because of the high phenolic content of strawberry itself. As reported by Rababah et al, strawberry has a phenolic content of 8,503 mg GAE/kg, which is higher than cherry, grape, fig and orange (2012)

Table 4.10 Phenolic contents of chia seed, control jam and SMWC (mg GAE/g)

	Chia Seed	Control Jam	SMWC
mg GAE / g	1.1 ± 0.00	3.5 ± 0.00	4.1 ± 0,06

4.3.6 Rheological Measurement

The viscoelastic behaviour of the blended marmalade sample is pseudoplastic – shear thinning. The viscosity values decreased when subjected to shear strain. Figure 4.3 shows the viscoelastic behavior of marmalade under increasing shear stress and shear values.

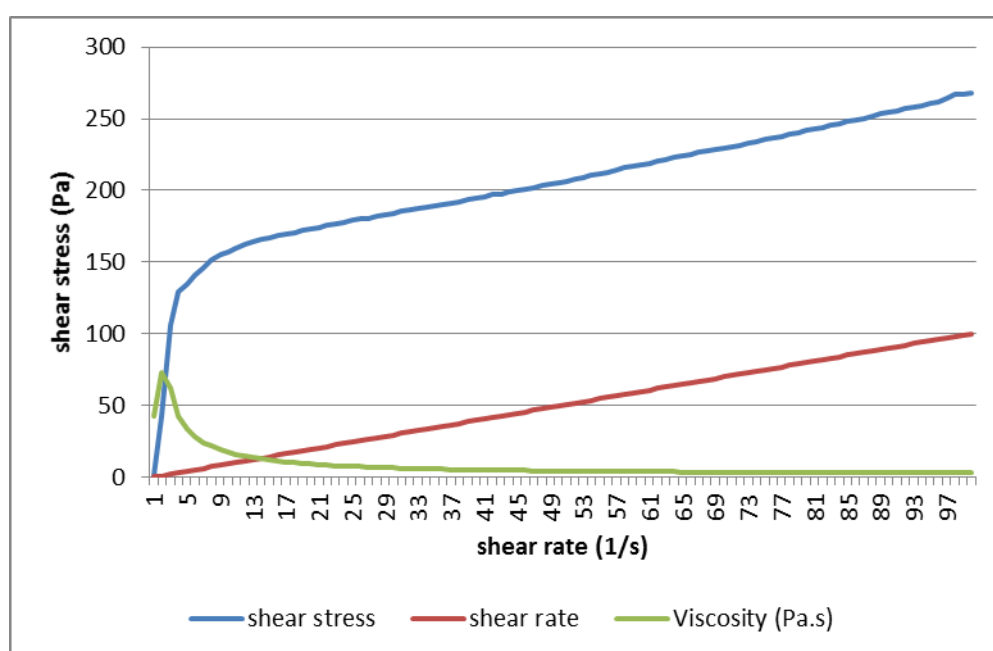


Figure 4.3 Viscoelastic behaviour of SMWC

At low shear values, the viscosity seem to be higher, but when the shear increases and the viscosity begins to decrease. This indicates that our marmalade sample needs a high shear strain before flowing, like ketchup.

The loss modulus (G'') was greater than storage modulus (G') at any given point of Figure 4.4. This indicated a dominant contribution of the elastic component to the viscoelasticity of strawberry marmalade, a behaviour typical for viscoelastic solid.

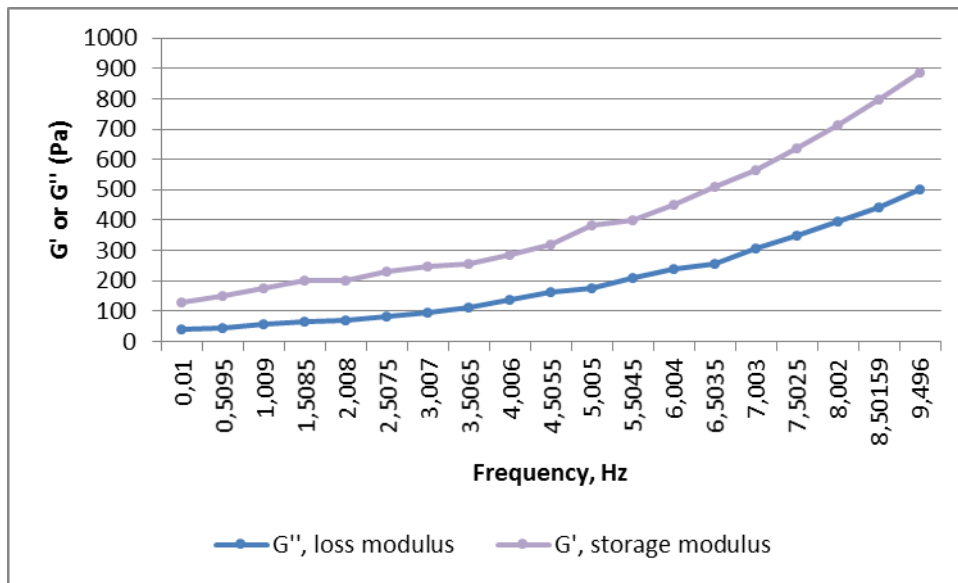


Figure 4.4 G' and G'' of SMWC

The loss tangent ($\tan \delta$) is a direct measure of the relative importance of viscous and elastic effects in the sample. Loss tangent was smaller than 1 at any given point as seen in Figure 4.5, which indicated a gel-like behaviour.

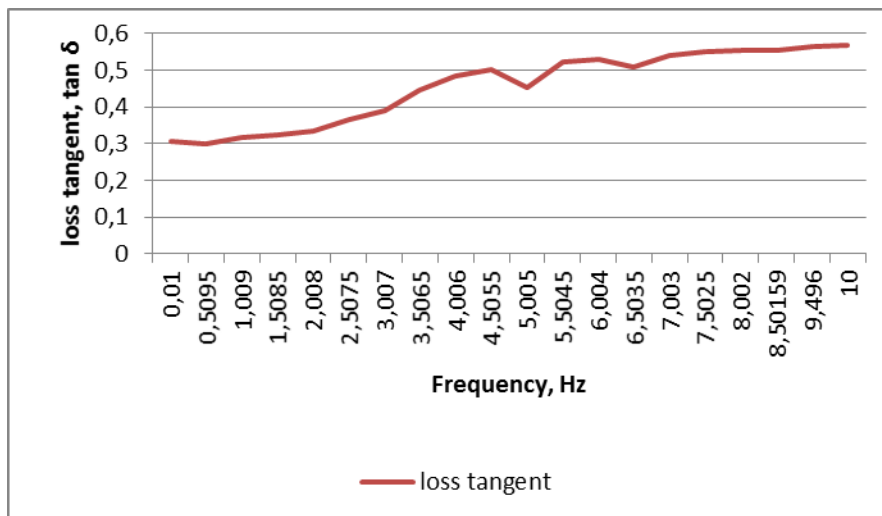


Figure 4.5 tangent, $\tan \delta$, versus frequency data of SMWC

Figure 4.6 shows the comparison of storage and loss modulus of chia added marmelade and the control. The rheological parameters of the control indicates a less viscosity than the chia added marmelade. Chia seed increased the viscosity parameters.

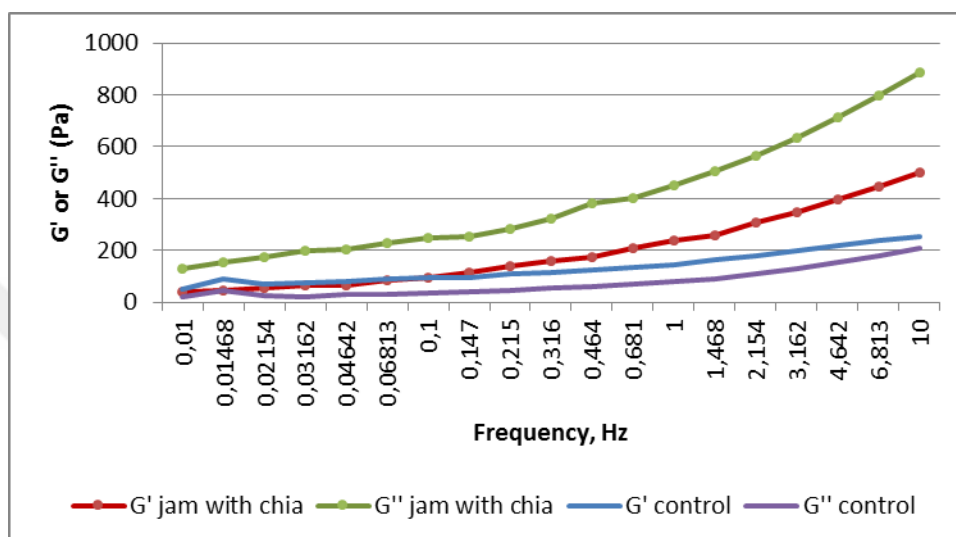


Figure 4.6 G' and G'' versus frequency data of chia added marmelade and the control

Figure 4.7 gives the comparison of the loss tangent values of SMWC and the control. Loss tangent of control is below 1 at any given point of the graph; which indicates a gel like behaviour. Loss tangent of control is below that of SMWC, which indicates control has less viscosity than SMWC.

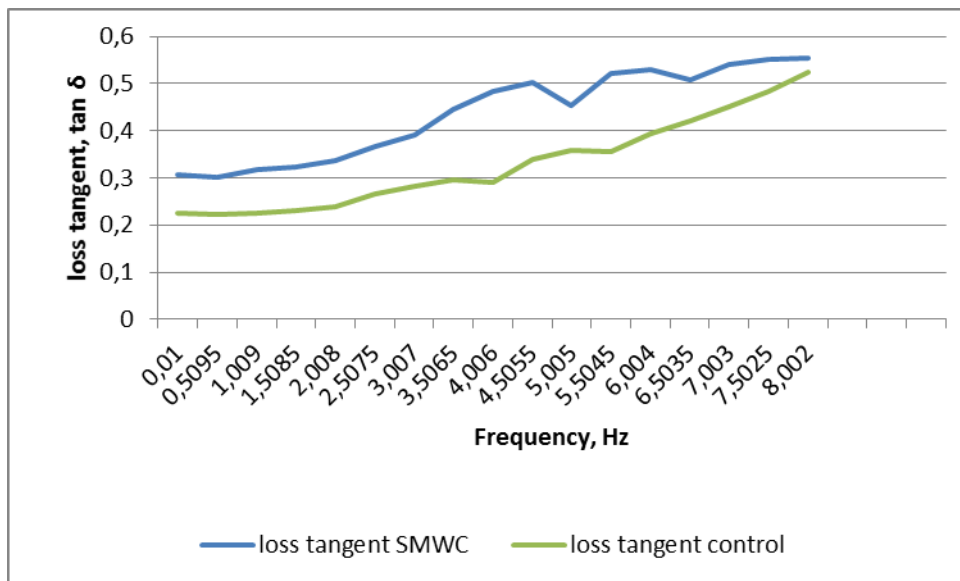


Figure 4.7 Loss tangent, $\tan \delta$, versus frequency data of SMWC and control

Figure 4.8 shows the viscoelastic behaviour of the control marmalade. The viscoelastic behaviour of the control is pseudoplastic – shear thinning, similar with the chia added marmalade. The viscosity values decreased when subjected to shear strain. Figure 4.8 shows the viscoelastic behavior of marmalade under increasing shear stress and shear values. Viscosity values for chia added marmalade was higher at any shear strain. This indicates the positive effect of chia seed to viscosity increase. It can be concluded that chia seed addition made the marmelade more viscous and it can be replaced by any thickening agent in marmalade formula.

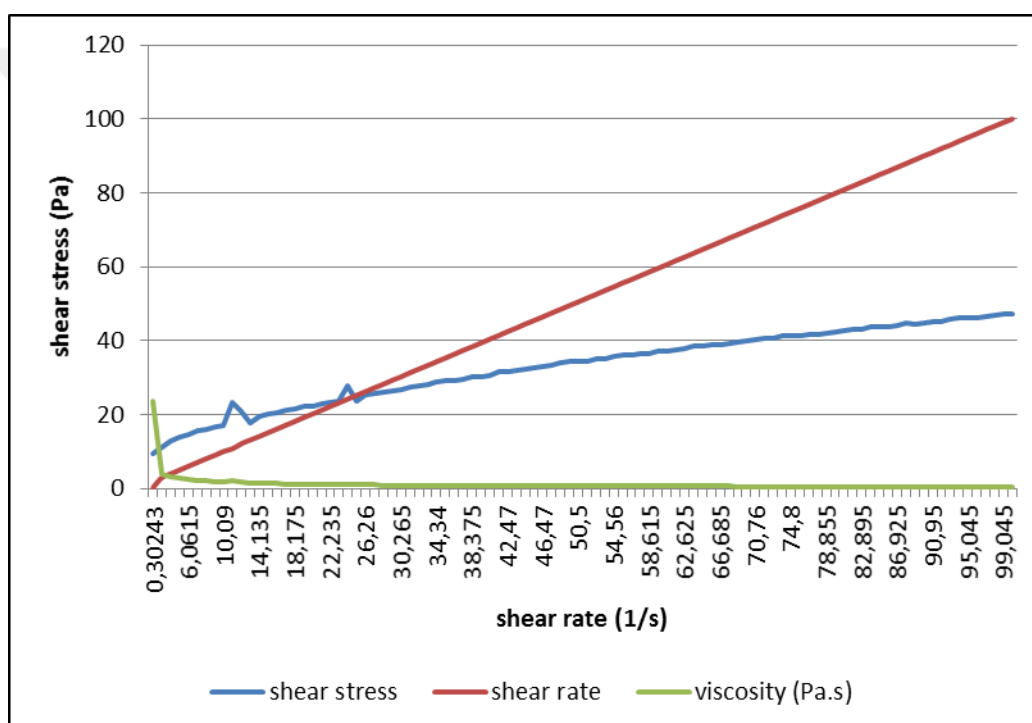


Figure 4.8 Viscoelastic behaviour of control



5.LABELING

5.1 DECLARATIONS

According to Turkish Food Codex Labeling Regulations (29.12.2011, 28157), following declarations can be made to strawberry marmalade with chia product:

- “*Involves sweetener*” : Product has 56.3 % sugar alcohol and according to Turkish can be declared as “involves sweetener”.
- “*May cause laxative effect when eaten in excess*”: Product has 56.3 % sugar alcohol and according to Turkish Food Codex Labeling Regulations, Addition 2, Item 2.4 foods having sugar alcohol higher than 10 % should be declared as “May cause laxative effect when eaten in excess”.
- “*Low energy*”: Product has a caloric value decreased by 48.2 % compared to a sugar jam and according to Turkish Food Codex Labeling Regulations, Addition 14 foods providing at least 30 % less energy than a similar product should be declared as “low energy”.
- “*Source of omega-3*”: Product has 1.5 % omega-3 fatty acid and according to Turkish Food Codex Labeling Regulations Addition 14 food products having at least 0.3 % omega-3 should be labeled as “source of omega-3”.
- “*Low sugar*”: Product has 4.9 % total sugar and according to Turkish Food Codex Labeling Regulations Addition 14 foods having less sugar than 5 % should be labeled as “low sugar”.

5.2 NUTRITIONAL LABELING

According to Turkish Food Codex Labeling Regulations (29.12.2011, 28157), the nutritional label should be as shown in Table 5.1.

Table 5.1 Nutritional and energy value of SMWC

Energy and Nutrients	per 100 g product
Energy	604.8 kj / 145.5 kcal
Fat	2.4 g
<i>Saturated fat</i>	0.3 g
<i>Monounsaturated fat</i>	0.2 g
<i>Polyunsaturated fat</i>	2 g
Carbohydrate	61.2 g
<i>Sugar</i>	4.9 g
<i>Sugar alcohol</i>	56.3 g
Dietary fiber	2.7 g
Protein	1.3 g

5.3 EXAMPLE TO LABEL

5.3.1 Front Face of Label



5.3.2 Back Face of Label

Energy and Nutrients	per 100 g product
Energy	604.8 kJ / 145.5 kcal
Fat	2.4 g
Saturated fat	0.3 g
Monounsaturated fat	0.2 g
Polyunsaturated fat	2 g
Carbohydrate	61.2 g
Sugar	4.9 g
Sugar alcohol	56.3 g
Dietary fiber	2.7 g
Protein	1.3 g

Involves sweetener
May cause laxative effect when eaten in excess



CONCLUSIONS AND RECOMMENDATIONS

A diabetic sugar free strawberry marmalade was enriched by addition of chia seed, which is an ancient grain, but a superfood nowadays. Two separate sensory analysis were performed to decide the strawberry marmalade with chia and no added sugar. The sweetener was decided to be sorbitol, while the chia amount was decided to be 5 %. The effects of 5 % chia addition was evaluated by means of composition, caloric value, antioxidant capacity, fatty acid composition and rheology. By making strawberry marmalade, with sorbitol, as the sweetener, and 5 % chia; we achieved to increase antioxidant capacity by 15 %, increase total dietary fiber content by 168 %, omega -3 PUFA by 18 % and decrease caloric value by 48 %. Since chia seed would make favorable the medium of the marmalade for spoilage microorganisms because of the high nutrient capacity, further studies may focus on the stability of this marmalade controlling the microbial growth and increasing the shelf life of jam. Chia seed addition made the marmalade more viscous compared to control, this can lead to replacement of chia seed with pectin or any thickening agent for marmalade formula. According to Turkish Food Codex, this product can be labeled as “*low sugar*”, “*low energy*”, “*involves sweetener*” and “*may cause laxative effect*”.



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APPENDICES

APPENDIX A: Sensory Analysis 1 Panel Form



Verilen reçel numunelerini 1 – 7 skalasında, aşağıda belirtilen özellikler açılarından değerlendiriniz.

ÖZELLİK	ÖRNEK KODU 158	ÖRNEK KODU 759	ÖRNEK KODU 836	ÖRNEK KODU 427	ÖRNEK KODU 318
Renk					
Tatlılık					
Çilek Aroması					
Çilek Kokusu					
Ağızdaki Kıvam					
Kaşıktaki Kıvam					
Sürülebilirlik*					

*Bıçak ile sürülebilirliğini test ediniz.

APPENDIX B: Sensory Analysis 2 Panel Form

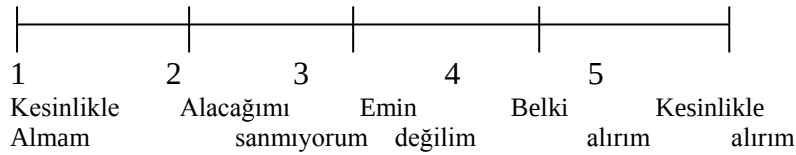
1. Verilen reel numunelerini 1 – 7 skalasında, ařağıda belirtilen zellikler aılarından deęerlendiriniz.



ZELLİK	RNEK KODU 473	RNEK KODU 169
Renk		
Tatlılık		
ilek Aroması		
ilek Kokusu		
Tahıl Tadı		
Ağızdaki Kıvam		
Kaşıktaki Kıvam		
Srlebilirlik*		
Tanecik Miktarı		

*Bıak ile srlebilirliğini test ediniz.

2. Verilen reel numunelerini 1 – 5 skalasında, satın alma tercihiniz aısından deęerlendiriniz.



ZELLİK	RNEK KODU 473	RNEK KODU 169
Satın Alma Tercihi		

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