

ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE
ENGINEERING AND TECHNOLOGY

**SYNERGIST EFFECT OF HYDROCOLLOIDS ON FILLING AND FILLED-
BISCUIT QUALITY**



M.Sc. THESIS

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Department of Food Engineering

Food Engineering Programme

JUNE 2018

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**HİDROKOLLOİDLERİN SİNERJİK ETKİLERİNİN DOLGU ve DOLGULU
BİSKÜVİ KALİTESİ ÜZERİNE ETKİSİ**

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To my spouse and family,



FOREWORD

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ABBREVIATIONS

Aw	: Water Activity
CRR	: Carrageenan
CS	: Corn Starch
DSC	: Differential Scanning Calorimetry
LBG	: Locust bean gum
LMP	: Low methoxyl pectin
MCC	: Microcrystalline cellulose
WT	: Weight total
XG	: Xanthan gum



SYMBOLS

t : Time

α : Alfa

β : Beta

ΔH : The amount of energy differentiation





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SYNERGIST EFFECT OF HYDROCOLLOIDS ON FILLING and FILLED-BISCUIT QUALITY

SUMMARY

In recent years, food contents has become more important for consumers of food industry. Preservatives, artificial ingredients or additives are replaced by more natural ones in those days and the consumes prefer more natural foods. Food industry uses hydrocolloids very commonly for different purposes so that research in this area is still in concern and widespread.

Hydrocolloids improve the stability of food by means of different properties; thickening, suspending, gelling and emulsifying. Often gums are preferred as hydrocolloids. In food legislations there is no limitation for gums. Hydrocolloids are also used for; extending shelf-life, as a fat substitute, for reducing fat migration, reducing the expensive input consumption, providing structural integrity of extruded product or utilizing calorie-reduced diet products without resulting in loss of flavor.

Considering the variety of products in the market, each food has its own different characteristics: Liquid products, juices, spoonable ice creams, garnished cookies, jam on the state deposit, jellies, etc. Hydrocolloids are major ingredients that give different structure and fun experience to food.

Food hydrocolloids when replaced with the use of multiple hydrocolloids can result in totally different structures. For example, some polymers has not gelling property but, if these polymers react with another hydrocolloid, they can show gelling property while mixing. This effect is known as “synergism”. Synergism can take place by formation of covalent bonds between the two polymers. Hydrocolloids have contribution to the texture, stability, color, taste etc. These colloids are added to provide at least one or two of these effects. The desired structure must be well defined and the hydrocolloid that is to be used must be selected properly.

Generally, combinations of hydrocolloids are preferred although it is hard to understand the effect of each hydrocolloid in the food system. There are several reasons to use combination of hydrocolloids in food recipes. Most of hydrocolloids have synergistic effect with each other. They give more strong texture and viscosity to the product which it is added. The gum combinations creates more effective three-dimensional network for water structure.

The purpose of this study is to analyse, evaluate and determine the synergistic effect of different hydrocolloids on filled biscuit quality. Different ratios of starch and xanthan gum, carrageenan and locust bean gum mixtures are analyzed on biscuit quality periodically. In the first step of this study, starch that is to be used was decided. Four different types of starches were added to base filling recipe; wheat, corn, tapioca and rice. starch was at 5% level and filling is prepared and then deposited on biscuit.

Moisture value, water activity and pH was measured for the first day, after one week and two weeks later. Corn starch was selected because of its water retention quality.

After selecting starch type, two different base type fillings were prepared. Starch-xanthan gum, carrageenan -locust bean gum combinations were applied. For the first combination; 5% corn starch, 0.2% and 0.8% xanthan gum were prepared. As the second combination; 0.5% carrageenan, 0.1% and 0.2% locust bean gum fillings were deposited on biscuits. In the fillings at 1., 15., 30., 45., 60., 75. ve 90. days of storage; moisture, water activity (a_w), pH values were measured, and the whole products were analyzed for their hardness and brittles parameters. In addition, DSC was used for determining the gelling behaviour of them.

Xanthan and locust bean gum was found to increase water retention properties besides hardness and brittleness of biscuits. While evaluating DSC parameters samples were prepared at 25% concentrations. At the first day and 14th day of storage samples were analyzed. According to results; addition of xanthan gum decreased starch gelatinization temperature and retrogradation values. Locust bean gum also decreased gelling point of carrageenan. Both of the combinations increased dehydration temperature of samples.

In this study, evaluation of starch-xanthan and carrageenan-locust bean gum combinations according to water retention capacity and their effects on filling structures and filled biscuit quality was performed.

Starches are used in fillings for their thickening or gelling property. But, by the addition of xanthan gum to the filling it effects water retention capability of the filling. Especially this property is important in filled biscuits as problems arise by the migration of moisture from filling to the biscuit.

At the end of shelf life, results showed that water activity of fillings were decreased by the addition of xanthan gum. Starch and xanthan gum combination together give strength and viscosity to the filling. Hardness and brittleness results for biscuits changed by migration of water, after 30 days hardness decreased and then biscuit started to get firmer. The same situation is applicable to carrageenan based filling. Carrageenan and locust bean gum forms a gel in the filling and is more effective than xanthan gum-starch combination according to water activity retaining. Carrageenan and locust bean gum has water retention capability either in combination or alone.

As a result of this study, synergism of hydrocolloids provides advantages to filling such as cost, shelf life extension and desired texture. Carrageenan and locust bean gum combination is found to increase water retention more than the starch and xanthan gum combination. Besides, starch-xanthan gum combination have softer texture for the biscuits they are filled. Thus, this study showed that for both biscuit fillings and other food products hydrocolloid combinations can solve the problems or improve the quality. As there are many hydrocolloids available, new studies are necessary to study their two or even more combinations to understand their synergistic effects.

HİDROKOLLOİDLERİN SİNERJİK ETKİLERİNİN DOLGU ve DOLGULU BİSKÜVİ KALİTESİ ÜZERİNE ETKİSİ

ÖZET

Gıda sektöründe son yıllarda kullanılan içerikler, özellikle tüketici açısından oldukça önemli olmaya başlamıştır. Koruyucu veya yapay bileşenler giderek yerini doğal ve tüketici tarafından anlaşılır doğal bileşenlere bırakmaktadır. Hemen hemen her sektörde, farklı amaçlar için kullanılan hidrokolloidler araştırılmaya ve yaygınlaşmaya devam etmektedir.

Hidrokolloidler, fırıncılık ürünlerinde kıvamlaştırma, süspansiyon halinde tutma, jelleşme ve emülsifiye etme özellikleri ile stabilizasyonu arttırmalar. Genelde gamlar hidrokolloid olarak tercih edilirler. Gıda yasalarında, gamların kullanımında sınırlama yoktur. Ayrıca hidrokolloidler, raf ömrü uzatma, yağ ikamesi olarak kullanılma, yağ migrasyonunu düşürme, pahalı girdi tüketimini düşürme, ekstrüde ürün bütünlüğü sağlamanın yanı sıra, yapısal ve lezzet kaybı olmadan kalorisi düşürülmüş diyet ürünlerin üretiminde de sık kullanılan ürünlerdir.

Markette olan ürün çeşitliliğine bakıldığında, her gıdanın kendine has farklı özellikleri mevcuttur. Akışkan ürünler, akışkan olmayan daha yoğun kıvamda olan meyve suları, kaşıklandırılabilen dondurmalar, içi dolgulu kurabiyeler, depozit haldeki marmelatlar, jöleler, vb. Bu gıdalara yapısal farklılık ve eğlenceli yeme deneyimi kazandıran majör girdilerin başında hidrokolloidler gelir.

Tek başına gıdaya yapı verebilen hidrokolloidler yerlerini birden çok hidrokolloidlerin kullanılması ile gıdaya farklılık kazandırmaya bırakmıştır. Örneğin, jelleşme özelliği göstermeyen bazı polimerler ilgi çekici bir şekilde, karıştırma sırasında farklı bir hidrokolloid ile beraber jelleşme özelliği gösterecektir. Bu etki “sinerjik” olarak adlandırılır. Sinerji etki birçok farklı yolla olabilir. En doğrudan olan mekanizma, iki polimer arasında kovalent bağların oluşması ile gerçekleşir.

Hidrokolloidlerin üründe istenen veya istenmeyen; tekstür, stabilite, renk, lezzet gibi sonuçlara katkısı oldukça yüksektir. Bu girdiler bu etkilerin bir veya en az iki tanesini sağlamak için ilave edilirler. İstenen yapı çok iyi tanımlanmalıdır ve bu tanıma göre kullanılacak hidrokolloid doğru seçilmelidir. Akışkan olan bir yapıdan, katı olan, kaşıklandırılabılır yapıya kadar geniş bir aralığa sahip gıda yapısı tanımlanabilir.

Hidrokolloidler kullanılarak geliştirilecek ürün reçetesinde bazı noktalardan emin olmak gerekmektedir. İlk olarak, gamların tamamen çözünebilen özellikte olması yeterli sıcaklık, katyonların etkisi gibi parametreler maksimum fonksiyonelliği sağlamalıdır. Karıştırma, ısıtma veya soğutma, katyonlar dikkat edilmesi gereken üç ana faktördür. Gamların çözünabilirliğini homojen olarak sağlamak adına sıcaklık ve katyonların etkinliği bilinmelidir. Hidrokolloidler tek başına kullanıldığı ve kombinasyon olarak kullanıldığı zamanlarda farklı etki gösterebilirler. Sıcaklık parametresi bu iki durum için değişkenlik gösterebilir. Düşük sıcaklıkta jelleşme özelliğini hemen gösterebilen bir kombinasyonun sıcaklığını artırmak ve yapıyı takip etmek önemlidir.

Bir gıda ürününde kullanılan tek bir hidrokolloid etkisini anlamak zor olsa da genelde hidrokolloid kombinasyonları tercih edilmektedir. Bu kombinasyon kullanımının birden çok nedeni vardır. En sık sebepler; maliyet, sinerji ve ürün kalitesidir. Ürün maliyetine katkısı incelendiğinde; yüksek maliyetli bir girdi olarak kullanılması gereken bir hidrokolloid aynı etkiyi verebilecek ve maliyet olarak daha avantajlı başka bir gam ile beraber daha yüksek oranda kullanılarak istenen viskozite ve yapı elde edilmiş olur. Çoğu hidrokolloid birbirleriyle sinerjik etkiye sahiptir. İlave edilen reçetede daha güçlü bir yapı ve viskoziteye ulaşılmasını sağlarlar. 2 gam beraber kullanıldığında verdiği etki tek başına kullanılan ve elde edilen etkiden daha güçlü bir yapıya sahiptir. Gam kombinasyonları suyu kullanarak daha etkili 3 boyutlu bir yapı oluştururlar. Örneğin, ksantan gum, guar gum ve keçi boynuzu gamı ile sinerjik etkiye sahiptir. Aynı şekilde, konjac gam karagenan, ksantan ve aljinat ile sinerjik etkiye sahip en yaygın örneklerdir. Birden çok gamın beraber kullanımının kalitesel katkıları ise hem çok yönlü hem de daha pratik bir uygulama alanı yaratmasıdır. Ürün geliştirilmesi sırasında en ideal sonuca ulaşmak için maliyet, sinerji, istenen yapı gibi etkiler ve stabilite düşünülmesi gereken önemli noktalaradır.

Bu çalışmanın amacı, farklı hidrokolloidlerin sinerjik etkilerinin incelenmesi, değerlendirilmesi ve hem dolgu hem de dolgulu bisküvi üzerine olan etkilerinin araştırılmasıdır. Bu çalışmada; gıda endüstrisinde sıklıkla kullanılan nişasta ve ksantan gam ile karagenan ve keçi boynuzu gamının farklı konsantrasyonlarda karıştırılması ile elde edilen karışımların bisküvi üzerine etkisini belirlemek için farklı periyotlarda analitik olarak incelenmiştir.

İlk olarak seçilecek nişasta tipi üzerine çalışma yapılmıştır. Baz dolgu reçetesine buğday nişastası, mısır nişastası, tapyoka nişastası ve pirinç nişastası %5 oranda ilave edilip dolgular hazırlanmış ve bisküvi üzerine depozit edip, paketlenildikten sonra oda sıcaklığında (24-25°C) bekletilmiştir. Nem, su aktivitesi, pH değerlerine bakılmıştır. Ardından 2 haftalık süreçte takip edilip, aynı değerler kontrol edilmiş ve su tutma kapasitesine göre en uygun sonucu veren nişastanın mısır nişastası olduğu belirlenmiştir.

Daha sonrasında mısır nişastası-ksantan gum kombinasyonu ve karagenan-keçi boynuzu gamı kombinasyonu çalışılmıştır. İlk kombinasyonda %5 nişasta, %0.2 ve %0.8 ksantan gum kullanılarak dolgular hazırlanıp ürüne depozit edilmiş; ikinci kombinasyonda %0.5 karagenan, %0.1 ve %0.2 keçi boynuzu gamı kullanılarak dolgular hazırlanmış ve ürüne depozit edilmiştir.

Depoalamanın 1., 15., 30., 45., 60., 75. ve 90. günlerinde dolgudaki nem, su aktivitesi, pH değişimi ve bütün üründe sertlik ve kırılma testleri yapılmıştır. Ayrıca, DSC cihazı ile jelleşme değerleri test edilmiştir. Ksantan gum ve keçi boynuzu ilavesi ile ürün nemi ve su tutma kapasitesinin yükseldiği gözlemlenmiş, pH değişiminde su ile orantılı olarak bir azalma kaydedilmiş ancak, önemli ölçüde değişiklik olmamıştır. Bisküvi sertliğinde de artış ve kırılma test sonuçlarında ürünün tekstürünün sertleştiği görülmüştür.

DSC analizleri için numuneler %25 konsantrasyonda hazırlanmıştır. Aynı oranların karşılaştırması yapılması adına karagenan bazlı numuneler de nişasta bazlı dolgu ile aynı oranda çalışılmıştır. Nişasta %5, nişasta %5 + ksantan %0.2, nişasta %5 + ksantan %0.8 ve karagenan %5, karagenan %5 + keçi boynuzu gamı %0.2, karagenan %5 + keçi boynuzu gamı %0.8 değerlerine göre hazırlanmıştır. İlk gün analiz sonrasında 14. gün tekrar analiz edilmiştir. Analiz sonuçlarına göre, ksantan gum ilavesi nişastanın jelleşme sıcaklığında azalmaya neden olmuş ve retrogradasyon derecesini düşürme

etkisi gözlemlenmiştir. Buna ek olarak da, suyu uzaklaştırma sıcaklığı da artış göstermiştir. Başka bir deyişle suyu tutma özelliği geliştirilmiştir. Karagenan tek başına daha yüksek sıcaklarda jelleşirken, keçiyoynuzu gamı ilavesi ile jelleşme sıcaklığı düşmüş ve su tutma kapasitesi arttığından dolayı, suyu uzaklaştırma sıcaklığı artmıştır.

Özetle bu çalışmada; nişasta-ksantan gam ve karagenan-keçiyoynuzu gamı kombinasyonlarının su tutma kapasiteleri ve dolgu yapısı ve bisküvi kalitesi üzerine etkileri araştırılmıştır.

Nişastalar, kalınlaştırıcı veya jelleşme özellikleri için dolgularda kullanılır. Ancak, dolguya ksantan gamı ilavesi dolgunun su tutma kapasitesini de etkiler. Bu özellik dolgulu bisküvilerde, nemin dolgudan bisküviye geçişi ile ortaya çıkan problemlerden dolayı önemlidir.

Raf ömrünün sonunda, sonuçlar, ksantan sakızının eklenmesiyle dolgunun su aktivitesinin azaldığını göstermiştir. Nişasta ve ksantan gamı kombinasyonu birlikte dolguya güç ve viskozite kazandırmıştır. Suyun migrasyonu ile değişime uğrayan bisküviler için 30 gün sonundaki sertlik ve kırılabilirlik sonuçlarına göre sertlik azalmış ve daha sonra bisküviler sertleşmeye başlamıştır. Aynı durum, karagenan bazlı dolgu için de geçerlidir. Karagenan ve keçiyoynuzu gamı, dolguda bir jel oluşturmuş ve su aktivitesine bağlı olarak ksantan gamı nişastası kombinasyonundan daha etkili olmuştur. Karagenan ve keçiyoynuzu gamının, kombinasyon halinde veya tek başına su tutma özelliğine sahip olduğu görülmüştür.

Bu çalışmanın sonucunda; hidrokolloidlerin sinerjik etkilerinin, maliyet, raf ömrünü uzatmaları ve arzu edilen dokuyu sağlamaları gibi avantajları ile dolguya üstünlük sağladıkları görülmüştür. Karagenan ve keçiyoynuzu gamı kombinasyonunun, nişasta ve ksantan gamı kombinasyonundan daha fazla su tutmayı arttırdığı bulunmuştur. Ayrıca nişasta-ksantan gamı kombinasyonu, dolduruldukları bisküviler için daha yumuşak bir yapıya neden olmuşlardır. Bu nedenle, bu çalışma, hem bisküvi dolguları hem de diğer gıda ürünleri için hidrokolloid kombinasyonlarının problemleri çözebileceğini veya kaliteyi artırabileceğini göstermiştir. Birçok hidrokolloid mevcut olduğundan, sinerjik etkilerini anlamak için iki veya daha fazla kombinasyonlarını incelemek için yeni çalışmalar gereklidir.



1. INTRODUCTION

Food gums and hydrocolloids are added to so many food systems for several reasons. These reasons are thickening, gelling, emulsifying, stabilization, coating and etc. The most important reason for the use of hydrocolloids in foods is their ability to modify the rheology of food systems. Flow behavior (viscosity) and mechanical solid property (texture) are basic properties of these food systems. Hydrocolloids are used in a lot of food formulations such as; soups, gravies, salad dressings, sauces and toppings as additives to achieve the desired viscosity and mouthfeel. In addition, they are also used in many food products like ice creams, jams, jellies, gelled desserts, cakes and candies, to create the preferred texture. Combination of hydrocolloids are used in specific areas of applications such as reduced gelling points or increased viscosity. However, there is limited information available in the literature about their applicability in combinations.

There are many starch types used in food industry for giving different texture and structure properties. Although it may be a challenge to fully understand one hydrocolloid in a food system, it is often desirable to use a combination of gums in a food product. Most of the common reasons of using multiple hydrocolloids in foods are cost, synergy, serendipity and quality. Several gums are synergistic with other gums, if two gums are used together, it will show greater texture for mouthfeel than using single gum. Also, combinations interact with water effectively to form three-dimensional network. Carrageenan is synergistic with locust bean gum but, xanthan gum and starch do not show synergistic effect with each other. Multiple gums are generally more functional and practical to give food the desired texture. While developing a product, synergistic effects, costs, desired textures, and stabilities are all considered besides the approach for an ideal formulation.

The purpose of this study is to analyse, evaluate and determine the synergistic effect of different hydrocolloids on filled biscuit quality. Different ratios of starch and xanthan gum, carrageenan and locust bean gum mixtures are analyzed on biscuit quality periodically.

2. LITERATURE REVIEW

2.1 Hydrocolloids

Hydrocolloids have been used since ancient times of Egypt. One type, which is the exudate of a plant in the genus *Acanthus*, was used as an adhesive in the surrounding of mummies and also as a medium for water-based paints. Additionally, when we look at the food area, many hydrocolloids have been part of the human diet for several thousand years. For example, name of “Saint John’s bread” come from locust bean gum (LBG) and it is still called with the same name even now in several European countries (Hofler, 2004).

Hydrocolloids are widely used in so many formulations to improve quality attributes. They have two main functions; thickening and gelling. They are used in less viscous foods such as in soups, gravies, salad dressings, sauces and toppings as thickening agents and they are typically used in viscous products like jam, jelly, marmalade, restructured foods and low sugar/calorie gels as gelling agents (Saha and Bhattacharya, 2010). Hydrocolloids have more functional properties in foods like thickening, gelling, emulsifying, stabilizing, coating, etc. (Milani and Maleki, 2012). Because of the increasing consumption of ready meals and new processing technologies have led to a rapid rising in the use of hydrocolloids. Also, by changes in modern lifestyles people look for more healthy foods which include high fibre and low fat products. A summary of the hydrocolloids market is given in Figs 2.1(a) and 2.1(b).

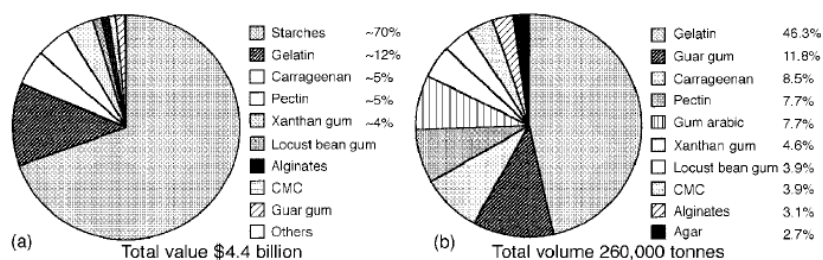


Figure 2.1 : (a) Value of world market for individual hydrocolloids; (b) Volume of World market for individual hydrocolloids (Williams and Philips, 2009).

2.2 Basic Structure

Hydrocolloids are a heterogeneous group of long chain polymers (polysaccharides and proteins). Hydrocolloids have large number of hydroxyl (-OH) groups, for this reason their water binding speciality is rather high. Because of these properties they are preferred in many type of products. Additionally, they produce a dispersion, which is an intermediate between a true solution and a suspension, and exhibits the properties of a colloid. Considering these two properties, they are termed as ‘hydrophilic colloids’ or ‘hydrocolloids’ (Saha and Bhattacharya, 2010). Hydrocolloids are hydrophilic polymers which generally contain many hydroxyl groups and may be polyelectrolytes (Burey, *et al.*, 2008).

“Hydrocolloid,” is the more scientific name for gums, its actual name is hydrophilic colloid. Hydrocolloids are not really colloids, because they are water soluble. Hydrocolloids can have linear or branched molecules Fig. 2.2 (Hofler, 2004).

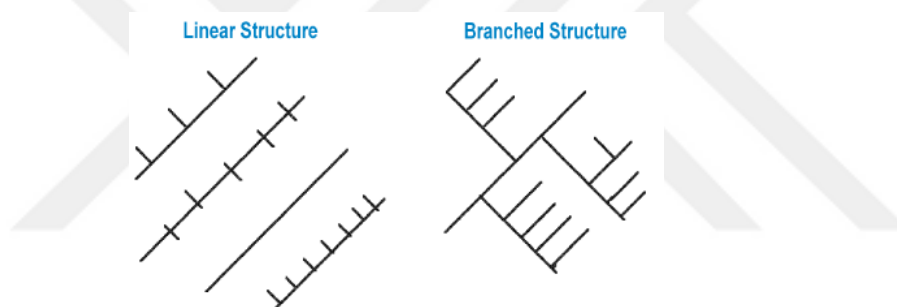


Figure 2.2 : Comparison of linear and branched hydrocolloid molecules.

2.3 Sources

Hydrocolloids are derived from vegetable, animal, microbial or synthetic origins and they are naturally present in foodstuffs or added to control the functional properties of such materials. In most applications, hydrocolloids are used to change food properties for example; rheology and water binding as well as emulsion stabilization, prevention of ice recrystallization and enhancement of sensorial properties. Additional applications include adhesion, suspension, foam stabilization and film formation (Burey, *et al.*, 2008). The commercially important hydrocolloids and their origins are given in Table 2.1 (Philips and Williams, 2009).

Table 2. 1 : Sources of commercially important hydrocolloids.

Botanical	Algal	Microbial	Animal
Trees	Red seaweeds	Xanthan gum	Gelatin
Cellulose	Agar	Curdlan	Caseinate
Tree gum exudates	Carrageenan	Dextran	Whey protein
Gum arabic	Brown seaweeds	Gellan gum	Soy protein
Gum karaya		Cellulose	Egg White protein
Gum ghatti	Alginate		Chitosan
Gum tragacanth			
Plants			
Starch			
Pectin			
Cellulose			
Seeds			
Guar gum			
Locust bean gum			
Tara gum			
Tamarind gum			
Tubers			
Konjac mannan			

2.4 Types of Hydrocolloids

Different types of classification systems based on chemical, functional and physical properties are mentioned. In Table 2.2, hydrocolloids are classified by source or by structure and charge characteristics.

Hydrocolloids are separated different categories depends on their properties. Starch, xanthan, guar gum, locust bean gum, gum karaya, gum tragacanth, gum Arabic and cellulose derivatives use as a thickening; alginate, pectin, carrageenan, gelatin, gellan and agar use as a gelling (Saha and Bhattacharya, 2010).

Table 2. 2 : Classifications of hydrocolloids.

Method	Hydrocolloids
Raw Material Source	
Seeds ^a	Guar, locust bean gum
Root ^a	Konjac
Exudates	Gum arabic, tragacanth, gum karaya
Seaweed extracts	
Red	Agar, carrageenan
Brown	Sodium alginate, propylene glycol alginate
Plant extracts	Pectin, amylose
Animal extracts	Gelatin ^b
Microbiological	Xanthan gum, gellan gum, pullulan
Cellulose derivatives	Cellulose gum, methylcellulose, hydroxypropylcellulose, methylhydroxypropylcellulose, MCC
Other derivatives	Propylene glycol alginate, low ester amidated pectin
Structure	
Linear	
Neutral	MCC, methylcellulose, hydroxypropylcellulose, amylose, guar, LBG, tara, konjac
Charged	Pectin and LMP, alginate and propylene glycol alginate, carrageenan, cellulose gum, agar, xanthan gum, gellan gum
Branched	
Neutral	Amylopectin
Charged	Gum arabic, tragacanth, karaya

^aThe galactomannans and glucomannans.

^bAlthough chemically gelatin is a protein rather than a carbohydrate, it is classified here as a hydrocolloid on the basis of its functionality and behaviour in food systems.

2.4.1 Starch

Polysaccharides are mostly used in food recipes to control texture and structure. Starch is a family of polysaccharides (Taggart and Mitchell, 2009). Starch types are industrial, maize, potato, wheat, tapioca and rice (Taggart and Mitchell, 2009; Sheldrake, 2010). Table 2.3 shows characteristics of starch granules.

Table 2. 3 : Starch granule characteristics.

Starch	Type	Diameter	Morphology
Maize ^a	Cereal	5-30	Round polygonal
Waxy maize	Cereal	5-30	Round polygonal
Tapioca	Root	4-35	Oval truncated ‘kettle drum’
Potato	Tuber	5-100	Oval spherical
Wheat	Cereal	1-45	Round lenticular
Rice	Cereal	3-8	Polygonal spherical compound granules
Sago	Pith	15-65	Oval truncated
Highamylose maize	Cereal	5-30	Polygonal irregular elongated

^a Maize is often referred as ‘corn’, ‘dent corn’ or ‘regular maize’

Starches are used in very different industries and there are many new opportunities when we look up the starch suitability. Also, it has cheaper price (Saha and Bhattacharya, 2010; Sheldrake, 2010) than other hydrocolloid types and even starch level used is low such as 2 to 5 %, and there is no effect on product taste (Saha and Bhattacharya, 2010). 95% of the starch is from corn in the United States, 60% of starch also obtained from corn grown in Europe and 20% is from potato and wheat (Chen et al., 2017). Production volumes are shown at Figure 2.3 (Sheldraker, 2010).

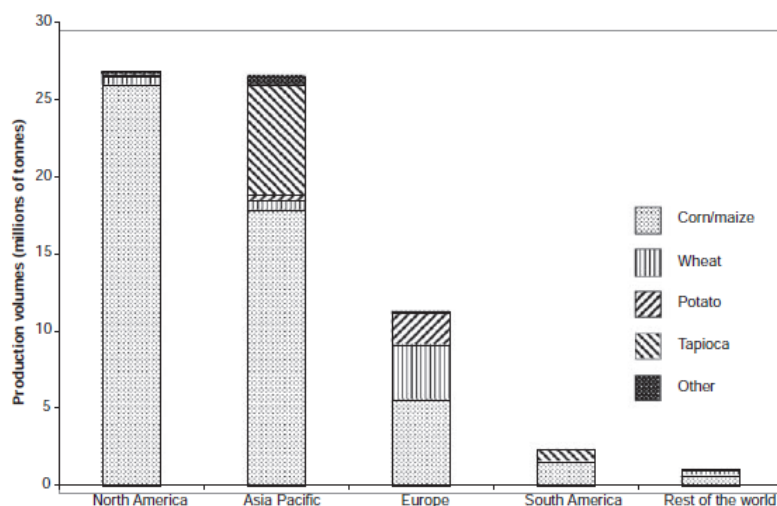


Figure 2.3 : Global starch production volumes by region.

Starch has two main polysaccharide fractions: amylose and amylopectin. In a native starch; amylose content is about 15-30% (Taggart and Mitchell, 2009) amylopectin content is 70-75% (Biliaders, 2009). Those polysaccharides are shown in Figure 2.4. (Jane, 2009).

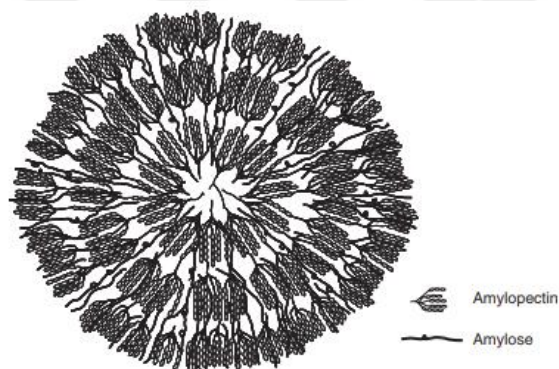


Figure 2.4 : Schematic of the organization of a starch granule.

Amylose is the most common linear polysaccharide consisting of α -1-4 linked D-glucopyranosyl units (Taggarts and Mithcell, 2009; Sheldrake, 2010; Le-Bail et al, 2017). Figure 2.5 shows the structure of amylose. Starch has also β -linked units. Their differences are: α -linked one can be digestible for animal but, β -linked units can not be digested (Sheldrake, 2010). Amylopectin is the predominate polysaccharide. This is a highly branched polymer of glucose, the branch consist of α -1-6 linkages (Taggarts and Mithcell, 2009). This linkage is shown in Figure 2.6 (Sheldrake, 2010).

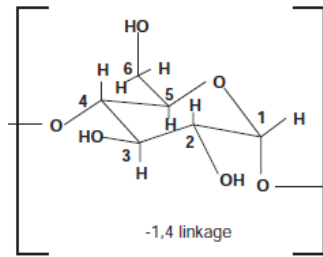


Figure 2.5 : α -1-4 linked part of starch.

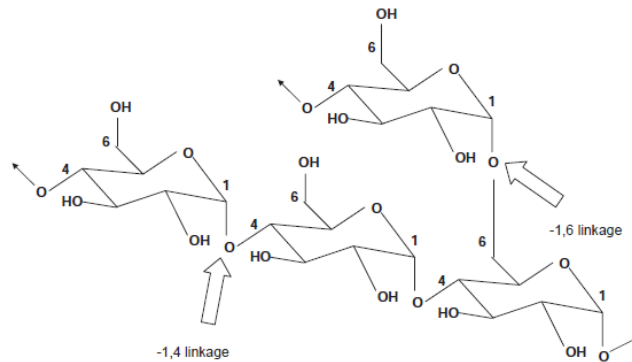


Figure 2.6 : α -1-6 linked part of starch.

Differences in viscosity between some available starch types are shown in Figure 2.7 (Sheldrake, 2010).

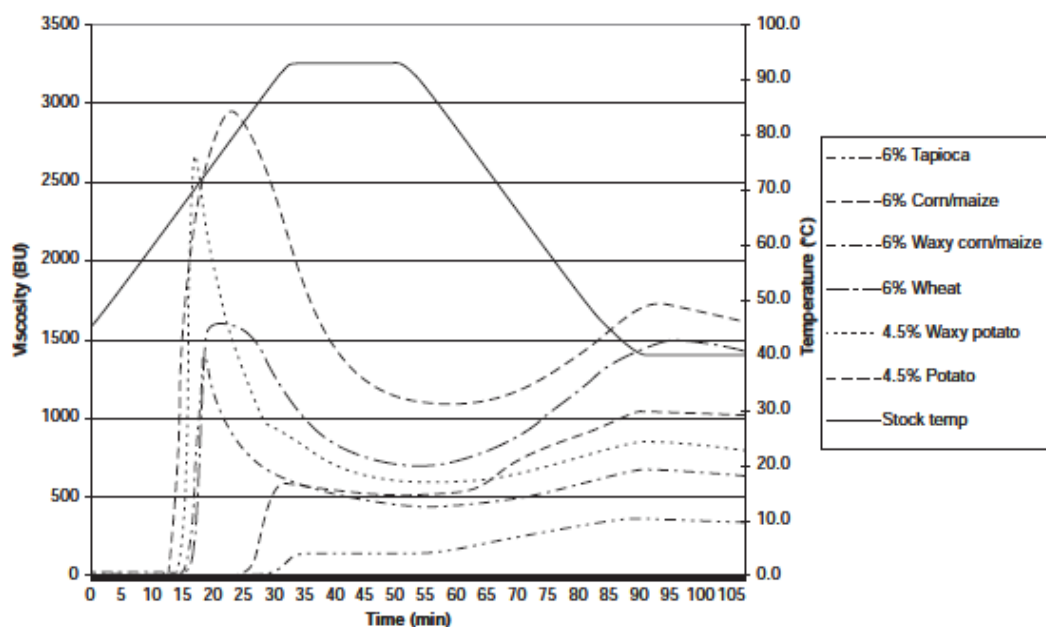


Figure 2.7 : Brabender Viscograph profiles of a range of commercially available starches.

Normally, starch granules are insoluble before heating in water. When heating is applied to starch, starch molecules are swelling and reach the polarize disruption

point (Biliaderis, 2009) or loss of birefringence and of the crystallinity that a radical changes (Le-Bail et al., 2017). These steps are shown in Figure 2.8 (Luallen, 2017).

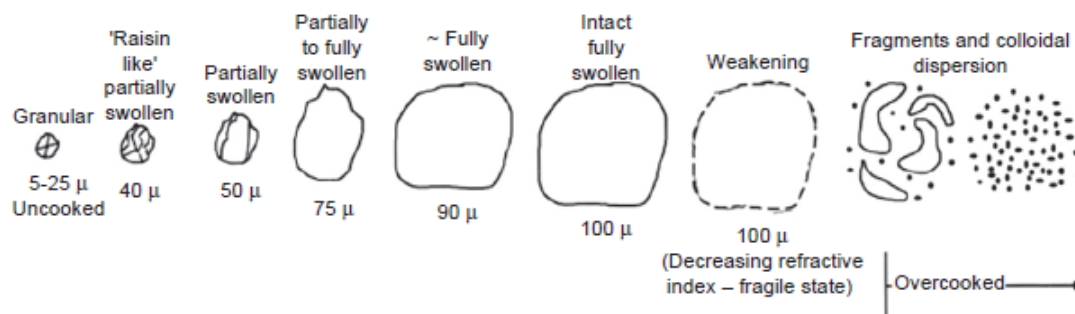


Figure 2.8 : Morphology of cooked starch microscopic evaluation.

Steps of starch granules during heating and cooling are swelling of granule, gelatinization, pasting and retrogradation (Biliaderis, 2009; Taggarts and Michelle, 2009; Le-Bail et al.; 2017).

2.4.2 Xanthan gum

Xanthomonas campestris is main source to obtain xanthan gum which is a member of polysaccharide class (Sworn, 2009) and xanthan is produced by aerobic fermentation of *X. Campestris* (Morris, 2017). Xanthan gum has water-solubility both in cold and hot water media (Habibi and Darani, 2017; Kumar et al., 2018). Chemical composition of xanthan gum is given in Figure 2.9 (Sworn, 2009).

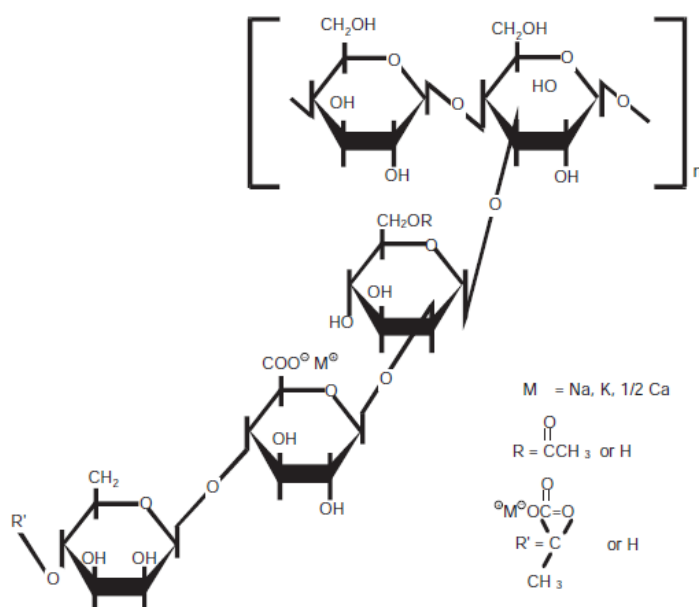


Figure 2.9 : Chemical structure of xanthan gum.

Xanthan gum has advantages in some of its properties like pseudoplasticity, products that contain this gum have fluidity, good mouthfeel, and adhesion. Because of those properties xanthan gum is used as a thickener, stabilizer and suspending agent in many food formulations, such as, bakery products (Sworn, 2010), meat and seafood products, as edible coating for fruits, dairy products, salad dressings (Sworn, 2010), dry mixes and frozen foods, canned foods, sauces, syrups, soups, beverages, chocolate, encapsulated nutrients, desserts, and in the structure of biodegradable food packaging (Habibi, 2017).

There are many situations to effect xanthan functionality;

- Temperature

Xanthan solutions are more stable during heat application even in the presence of salts and acids. Figure 2.10 shows viscosity – temperature graphs. The viscosity is decreasing if temperature increases, but the viscosity turns back upon cooling (Sworn, 2009).

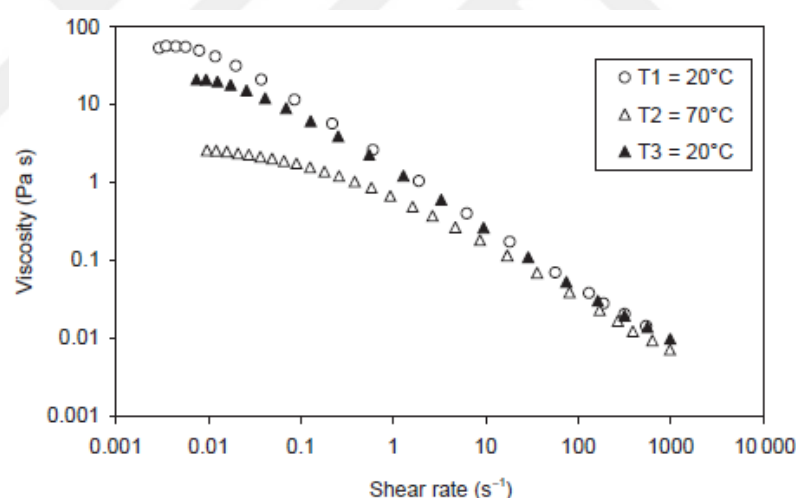


Figure 2.10 : Temperature graph viscosity of xanthan gum.

- pH

pH range is not so effective on xanthan gum solution. When pH value is under 3, viscosity of xanthan solution is decreasing. pH changes is given in Figure 2.11.

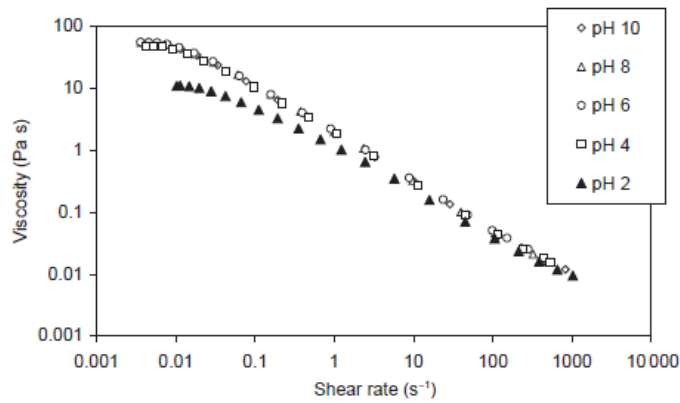


Figure 2.11 : pH changes effect.

- Salts

Salt amount depends on xanthan solution concentration. If xanthan solution is below 0.3%, viscosity is decreasing with addition of salt. However, if xanthan solution is above 0.3%, addition of salts increase viscosity. Figure 2.12 shows salts amount and xanthan concentrations (Sworn, 2009).

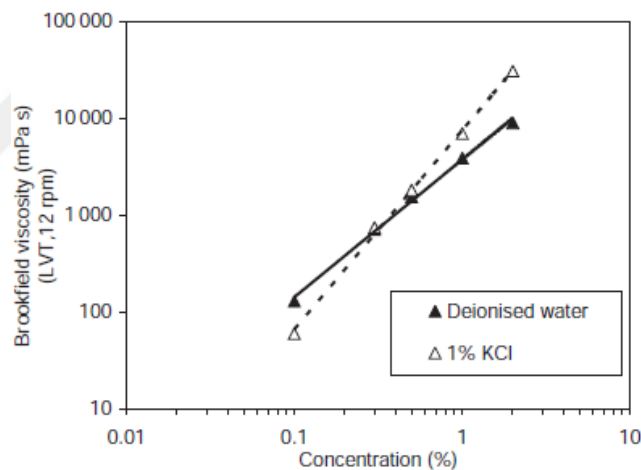


Figure 2.12 : Salts and xanthan concentration effect on viscosity.

2.4.3 Locust bean gum

Locust bean gum is obtained from carob tree that's why locust bean is also known as carob gum. It is a creamy white powder, and known as *Ceratonia siliqua* L. (botanically) which is found in Mediterranean regions (Higiro et al., 2007; Barak and Mudgil, 2014). Structure of locust bean gum is a family of galactomannan contain (1-4)- β -D-mannopyranosyl backbone with attachment of (1-6)- α -D-galactose single units (Dunstan, 2001). Figure 2.13 shows its chemical structure.

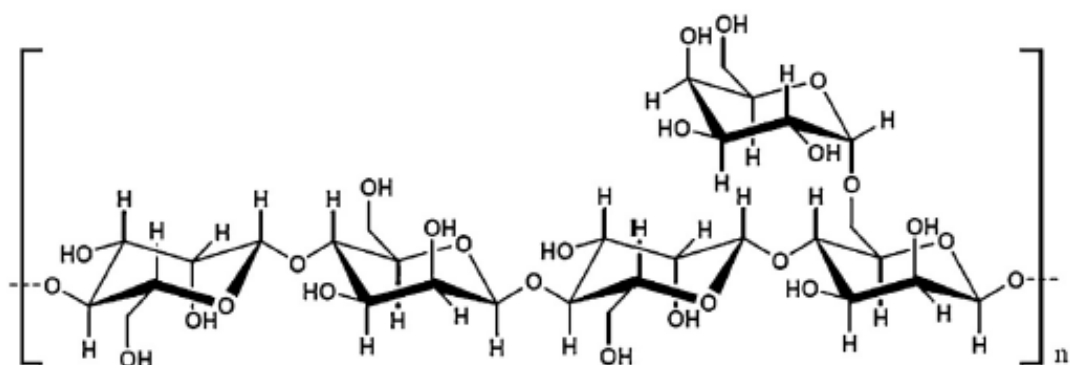


Figure 2.13 : Structure of locust bean gum.

Galactose content is important for physico-chemical properties of galactomannans and the distribution of the galactose units along the main chain. Locust bean gum has stronger synergistic effect and greater functionality with polymer because of longer galactose side chains yield (Higiro et al., 2007).

Galactomannan is in higher amount in locust bean gum composition about 80-85% followed by moisture 10-12%, protein content 5-6%, fat content 0.5-0.9%, crude fiber 0.8-1%, ash 0.5-1%, respectively (Barak and Mudgil, 2014).

Locust bean gum is mostly used as a thickening agent and also it is well known to form a gel after freeze/thaw cycles (Richardson and Norton, 1998). Locust bean gum needs temperatures above 80°C for complete solubility. pH stability is in the range of 4-9. If pH reach to 9, viscosity of this gum is decreasing (Barak and Mudgil, 2014).

In general, functional properties of galactomannans which include locust bean gum has wide range;

- Improving shelf life by binding water, the freeze-thaw behaviour,
- Preventing syneresis, the retrogradation of starch products,
- Maintaining turbidity in soft drinks and juices, acting as a dietary fibre (Wielinga, 2010).

Locust bean gum is mostly used in food industry as a thickening and stabilizing agent. In beverage applications, locust bean gum is preferred because of its stability in a wide range of pH value. In bakery products, LBG gives a good texture and viscosity to the dough. Also, dough development time increases and water absorption capacity improves. For low-fat yogurt locust bean gum was found to improve texture. If LBG

is added at 0.02% (w/w), yogurt firmness, water holding capacity and viscosity was improved and syneresis was reduced (Barak, 2014).

2.4.4 Carrageenan

Carrageenan is obtained from red seaweeds which contain different type of polysaccharides like carrageenan, furcelleran and gum (Imeson, 2009; Blakemore and Harpell, 2010). Also, red alga has different species about carrageenan; kappa and iota. Kappa carrageenan which is anionic sulphated polysaccharide (Baeza et al, 2002) is mostly used in food industry as a gelling, thickening and stabilizing agent (Blakemore and Harpell, 2010).

Carrageenan has linear polysaccharide comprising galactose units and 3,6-anhydrogalactose (3,6-AG), both sulphated and non-sulphated, joined by alternating α -(1,3) and β -(1,4) glycosidic links and Figure 2.14 shows its chemical structure (Imeson, 2009). Kappa, lambda and iota carrageenan has different ester sulphate groups and because of this differentiation, types of carrageenan show different rheological properties like hydration, gel strength, texture, syneresis, synergism, melting and setting temperature (Sadar, 2004).

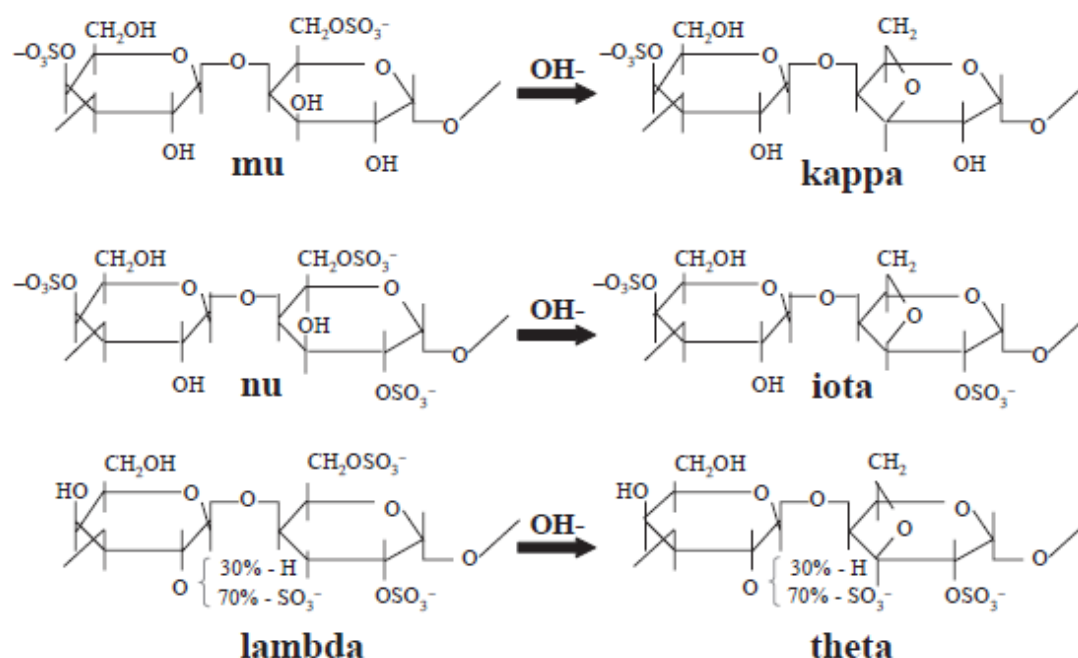


Figure 2.14 : Structures of carrageenans.

All types of carrageenan species show different specialities especially gelation properties. Table 2.4 shows gelation properties (Blackmore and Harpell, 2010)

Table 2. 4 : Summary of carrageenan gelation properties.

Gelation	Kappa	Iota	Lambda
Effect of cations	Strongest with K ⁺	gels Strongest with Ca ⁺⁺	gels Non-gelling
Gel texture	Brittle	Elastic	-
Syneresis	Yes	No	-
Hysteresis	10-20C	5-10C	-
Freeze-thaw stable	No	Yes	Yes
Synergy with LBG	Yes	No	No
Synergy with konjac flour	Yes	No	No
Synergy with starch	No	Yes	No
Shear-reversible	No	Yes	Yes
Stability in acid	Hydrolysis, accelerated by heat, low pH and time and Gels are stable		Hydrolysis
Protein reactivity	Specific reaction with kappa casein	Strong protein interaction in acid	

Carrageenans are soluble in hot water but sodium salts of kappa and iota types are soluble in cold water. And also temperature is an important factor about hydration of carrageenans (Imeson, 2009), kappa type is hydrated at about 40°C and hydration graph is given at Figure 2.15.

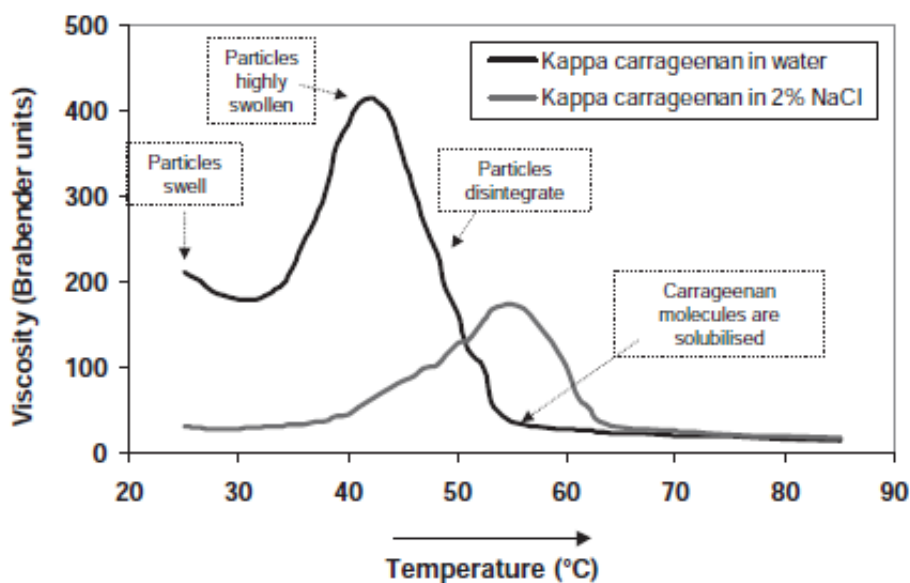


Figure 2.15 : Hydration property of kappa carrageenan in water and salt solution.

According to Baeza et al, kappa carrageenan hydrates above 60°C and adopts a random coil conformation. Between 30-70°C temperature which is the below set point, hot solutions of kappa and iota carrageenans sets (Blackmore and Harpell, 2010). When stability of carrageenans about pH is evaluated, these solutions lose viscosity and gel strength at pH below 4.3 (Imeson, 2009).

In food applications, carrageenan is used mostly in dairy and water based fillings. Because kappa carrageenan and kappa casein has specific interaction that gives stable network and prevent syneresis (Imeson, 2009). In milk products kappa carrageenan is the most important ingredient because of gelation properties in the presence of milk proteins, micellar caseins and these interaction effect of modified textural characterization of protein gels (Baeza et al, 2002). Uses of carrageenan in milk products are given Table 2.5 (Sadar, 2004).

Table 2. 5 : Milk (dairy) applications of carrageenan.

Use	Function	Product	Use level (%)
Frozen Desserts	whey prevention control	kappa	0.01-0.03
Ice cream, ice milk	meltdown		

Pasteurized milk			
Products	suspension, bodying	kappa	
Chocolate, egg-nog, fruit-flavored	bodying	kappa, iota	0.025-0.035
Fluid skimmilk	emulsion stabilization	kappa, iota	
Filled milk	bodying		
Creamy mixture for cottage cheese	cling	kappa	
Sterilized Milk Products			
Chocolate, etc.	suspension, bodying	kappa	0.01- 0.035
Controlled calorie	suspension, bodying	kappa	0.01- 0.035
Evaporated	emulsion stabilization	kappa	0.005- 0.015
Infant formulations	fat and protein stabilization	kappa	0.02- 0.040
Milk Gels			
Cooked flans or custards	gelation	kappa,iota	0.02- 0.030
Cold prepared custards	thickening, gelation	kappa,iota,lam	0.02- 0.050
Pudding an pie fillings	level starch gelatinization	bda kappa	0.01- 0.02
	syneresis control, bodying	iota	0.01- 0.02
Whipped Products			
Whipped cream	stabilize overrun, stabilize	lambda	0.05-0.15
Aerosol whipped cream	stabilize overrun	kappa	0.02-0.05
Cold Prepared Milks			
Instant breakfast	suspension, bodying	lambda	0.1-0.2
Shakes	suspension,bodying stabilize overrun	lambda	0.1-0.2
Acidified milks			
Yogurt	bodying, fruit suspension	kappa, lbg	0.2-0.5

Another application is water gels which is water dessert gels and cake glazes for controlling syneresis and give brittle gel formation (Blackmore and Harpell, 2010). Also, carrageenan improves water retention, mouthfeel and slicing properties of especially hard gels. In this application, the most important factor is pH for gelling properties. Table 2.6 shows water based applications (Sadar, 2004).

Table 2. 6 : Water based applications of carrageenan.

Use	Function	Product	Use level (%)
Desserts Gels	gelation	kappa+iota kappa+iota+lbg	0.5-1.0
Low calorie jellies	gelation	kappa+iota kappa+galactomannas	0.5-1.0 0.5-1.0
Pet foods (canned)	fatstabilization, thickening,suspending, gelation	kappa +locust bean	
Fish gels	gelation suspension, bodying	kappa+locustbean kappa, iota kappa, lambda	0.5-1.0 0.3-0.5
Syrups			
Fruit drink powders	bodying	Na+kappa, lambda	0.1-0.2
Frozen concentrates	pulping effects	K+/Ca+ kappa	
Relishis, pizza, BBQ sauce	bodying	kappa	0.1-0.2 0.2-0.5
Imitation milk	bodying, fat stabilization	iota, lambda	
Imitation coffee cream	emulsion stabilization	lambda	0.03-0.06
Whipped topping	stabilize emulsion,overrun	kappa,iota	0.1-0.2 0.1-0.3
Puddings (nondairy)	emulsion stabilization	kappa	0.1-0.3

2.4.5 Guar gum

Guar gum is obtained from ground endosperm halves, that are guar splits and it takes place on the seeds of the guar plant *Cyamopsis tetragonoloba* L. In Indian, guar has been grown for centuries and it is used as animal and human food. Sanskrit word “Gau-ahar” gives name to guar and ‘gau’ means cow and ‘ahar’ indicates food (Wielinga, 2000).

Guar gum is water-soluble non-ionic polysaccharide from the ground endosperm of guar (*Cyamopsis tetragonoloba*) seeds. Figure 2.16 is showing guar gum seed shape. Guar gum has a main chain of (1-4)-linked b-D-mannopyranosyl units, bearing single a-D-galactopyranosyl units attached to O-6 of the main-chain units (Achayuthakan and Suphantharika, 2007).

Guar seeds composition is; 20±22 % of hull, 44±46% of germ and 32±36% of endosperm on an absolute dry basis. Table 3 shows that analysis of guar seed components with regard to protein content, ash and moisture content, which is defined as [100 wt% - (wt% protein + wt% moisture + wt% AIR)] (Wielinga, 2000).

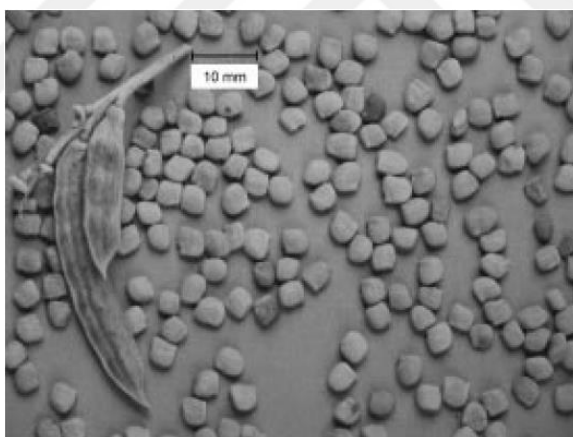


Figure 2.16 : Guar pods and seeds.

Table 2. 7 : Composition and chemical analysis of guar seed components.

Seed component	Composition of each portion						
	Weigh fraction, dry matter	Protein %	Ether soluble %	Ash %	Moisture %	AIR %	Gum content %
Hull	20-22	5.0	0.3	4.0	10.0	36.0	49.0
Endosperm	32-36	5.0	0.6	0.6	10.0	1.5	83.5
Germ	44-46	55.3	5.2	4.6	10.0	18.0	16.7

Guar gum obtained from these three seeds and these seeds are composed entirely of linear (1-4)- β -D-mannan chains with varying amounts of single D-galactose substituents linked to the main backbone by (1-6)- α -glycosidic bonds (Figure 2.17 and 2.18)

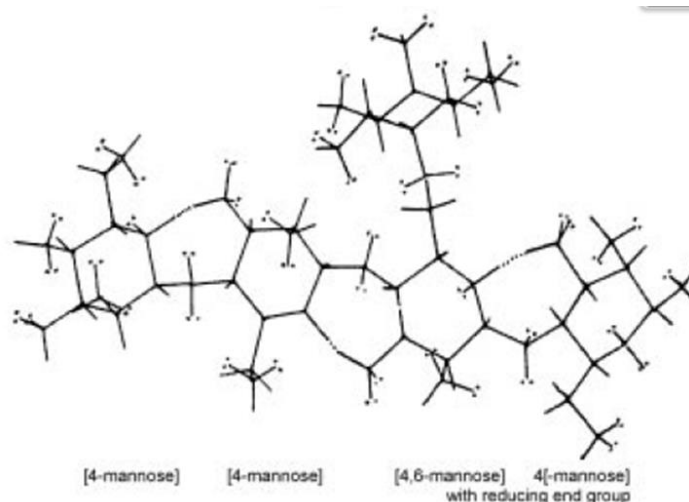


Figure 2.17 : Chemical scheme of guar gum.

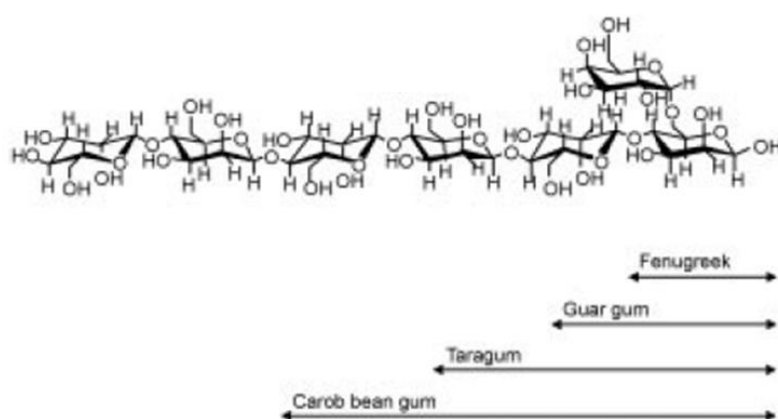


Figure 2.18 : Chemical structure comparison

Guar and locust bean gum are part of galactomannan family and also they are used as thickening agents like starch and xanthan gum. Guar and locust bean gum have different galactose substitution, therefore their water solubilities are different from each other. For example, guar gum has higher galactose content and it swells and disperse completely in cold and hot water (Saha and Bhattacharya, 2010).

Because of this water solubility characterization, guar gum is used as a thickening and stabilizing agent in a wide range of water based products. Apart from water based

components it is also used in cheese products because of preventing syneresis (Sadar, 2004). Solution properties even in cold water is a big advantage and also guar gum is stable in wide range values of pH.

2.5 Synergism of Hydrocolloids

When two components interact with each other and give advantages when compared to either separate component, synergy occurs (Blakemore and Harpell, 2010).

In food systems especially hydrocolloids are used in combinations with other components. Previously, hydrocolloids used partly increase viscosity, gel structure and stability of particles. But, in mixed systems it is not easy to give characterization with interactions of each hydrocolloid (Foster and Wolf, 2010). Synergism of hydrocolloids have been described for mixture of galactomannas with xanthan and kappa carrageenan and locust bean gums have a weak gel formation with xanthan gum. However, kappa carrageenan make a stronger gel with locust bean gum. There are many ways to perform synergistic gel formation and the most direct mechanism is covalent bonds formed between two polymers (Morris, 1990).

Advantages for applications of polysaccharide blends are observed. There are many factors like freeze-thaw stability, thickening, improving water retention, increasing the heat penetration and other effects (Sikora et al, 2006).

Hydrocolloid mixtures are usually used to increase rheological characteristic of food products. Typical examples; kappa carrageenan and locust bean gum together activate gel formation. If the two hydrocolloids associate then precipitation or gelation can occur. According to studies, some stiff polysaccharide molecules (xanthan gum, carrageenan, etc.) have galactomannas and glucomannas. They will associate to gel formation (Williams and Philips, 2009). Hydrocolloids are used to give different texture and modifications on food systems, hence, these food additives helps to modify also the sensory properties. Food industry can apply hydrocolloids for specific purposes because of their different specifications (Saha and Bhattacharya, 2010).

There are many application studies about synergistic effects of hydrocolloids; kappa-carrageenan+xanthan, lbg+xanthan, xanthan+xyloglucan, alginate+high methoxy pectin, etc.

2.5.1 Starch and xanthan gum

Starch is mostly used in food industry because of its low cost and availability. Also, it is used as a gelling, bulking and water retention agent (Nawab et al, 2016). In food systems, addition of gum to starch is known to modify and control the rheological properties of starch. Molecular interactions between starches and gums are important. The most common result is that by the addition of gum viscosity increases and gelatinization and retrogradation of starch is affected (Kim and Yoo, 2006). Starch has strong interaction with other hydrocolloids, for example starch and xanthan is a well-known combination. Hydrocolloid mixing with starch has advantages about mouthfeel and taste perception however this mixing can give rapid dilution in the mouth (Taggart and Michelle, 2009).

There are studies about different polysaccharide combinations like potato starch-xanthan gum and oat starch-xanthan gum. This study's evaluation criteria is thickener performance on the basis of its effect on sensory and rheology characterization. Oat combination had better thickening property than potato starch (Gibinski et al., 2006).

Gum addition to starch is effecting both physical property and time-temperature course of gelatinization and also viscosity property (Morris, 1990; Kim and Yoo, 2004) and retrogradation of starch (Kim and Yoo, 2004). It is shown in Figure 2.19 (Morris, 1990).

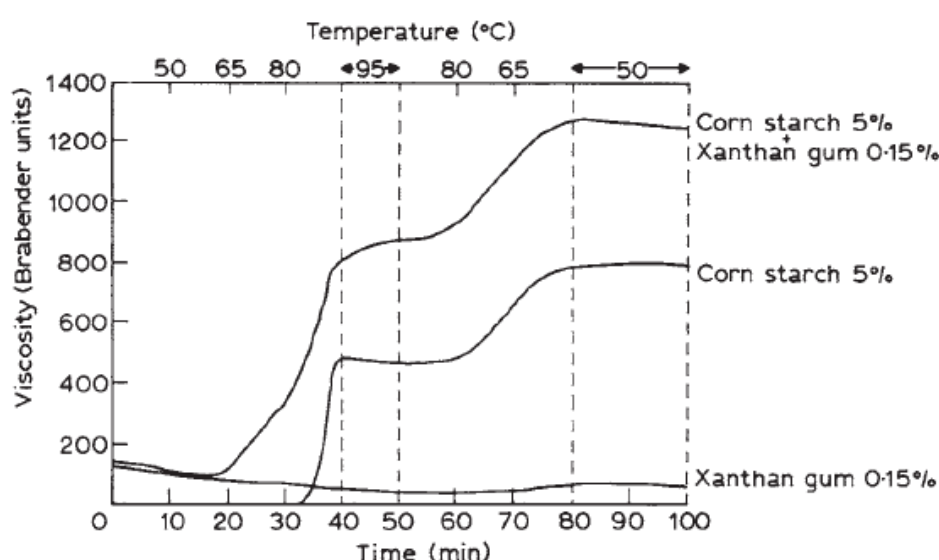


Figure 2.19 : Viscosity graph of corn starch with xanthan gum.

Starch-xanthan combination is found to be as a thickener in dessert sauces like strawberry sauce. It provides stable sensory and textural properties of the sauces for

at least three months. While starch thickener used sauces are most stable during storage time or during shelf life; potato starch with 0.12% xanthan gum is a superior thickener (Sikora et al. 2007; Saha and Bhattacharya, 2010).

The addition of hydrocolloids changes the structure and rheological features of starch without starch gelatinization temperature (onset, peak and final temperature). But, swelling characteristic is affected from hydrocolloid addition (Mandala and Bayas, 2004). Xanthan gum uses in starch –based product to improve its properties even at low level. Xanthan gum effect stability of starch positively and inhibit retrogradation however, does not effect gelatinization of starch (Sworn, 2010).

2.5.2 Carrageenan and locust bean gum

Combination of hydrocolloids are mostly used to improve rheological properties of food products and also it decreases the cost value. Kappa carrageenan and locust bean gum interaction is a typical example to give product with more transparent gel formation (Williams and Philips, 2009). This typical combination increase gel strength about 8% and is much more modest than the important increases obtained (Imeson, 2009).

Kappa carrageenan and locust bean gum systems studied and LBG is the most effective about improving apparent gel strength. This means ‘synergistic’ effects was first detected when LBG is added to diluted carrageenan solution (Dunstan, 2001). Syneresis is the water coming out from gel as the gel structure harden. Kappa carrageenan gels have high syneresis levels, iota gels exhibit no syneresis. Freeze-thaw stability is related with these syneresis property. Therefore, syneresis control is necessary for applications (Blackmore and Harpell, 2010). Addition of locust bean gum is a solution for syneresis problem. About gel structure, kappa carrageenan and locust bean gum give strong, elastic gels with low syneresis even they are cooled below 50-60°C (Imeson, 2009).

There is a study about low-fat and reduced-sodium meat products. If inclusion uses in salt mixture some anionica hydrocolloids can be use also like kappa carrageenan and cation type is important for water binding capacity ($K^+ > Ca^{2+} > Na^+$). Locust bean gum is other important additive to improve texture of kappa carrageenan gels and also reduce syneresis (Garcia-Garcia and Totosa, 2008).

In dairy application, an example for ice-cream which include kappa carrageenan and locust bean gum mixed with milk proteins. Study showed that carrageenan stayed in protein phase which has skimmed milk protein. The existence of carrageenan in milk thickens and slow down ‘wheying off’ which means separation to two layers (Foster and Wolf, 2010).

2.6 Hydrocolloids in Bakery

In processed foods hydrocolloids are mostly used for their properties like thickening, suspending, gelling and emulsifying and these characterizations improve stabilization and give integrity to most of the processed foods. Additionally, they are often used for other properties like; shelf life extension, fat replacement, oil migration reduction, decreasing high cost ingredient usage, freeze-thaw stability and especially moisture control (Cruz, 2011). Some gums also have effects on bakery applications:

Acaica gum gives many benefits to bakery products especially to extruded products like breakfast cereals and snacks. Addition of 2-5% levels improves shape of the last product and increase crispiness and crunchiness because of its greasing property (Thevenet, 2010).

Alginate is used in bakery products such as bread, muffins, cup cakes and soft cookies for their ability to extend shelf life. It has a very hydrophilic characteristic so that it binds water and keeps product humid. This property is important for gluten-free type bakery products. Gluten-free pre-mixes usually include alginate in their formula for bread, crisp bread and chocolate cakes (Helgerud et al., 2010).

Gellan gum is an important gum especially in bakery dry mixes. It can hydrate even in cold water and unlike other gums mostly found in such products. Gellan gum hydrates during baking, hence it has a good moisture retention and shelf life extension in the final bakery products. It is preferred in brownie type products and it is used at 0.08-1% levels in dry mixes (Valli and Clark, 2010).

Low shear viscosity of xanthan gum helps to improve cake batter which includes fruit and chocolate pieces. Its water retention property gives long shelf life and keeps texture soft and also it controls recrystallization of amylose. Because of this aspects, cake’s staling rate is retarded. Additionally, xanthan gum increase volume and reduce crumblines (Sworn, 2010). In frozen dough, xanthan increases its stability during

freeze-thaw circle and also guar and locust bean gum improve fresh bread quality (Guarda et al., 2004).

2.7 Hydrocolloids in Filling

Fillings include different formulations depending on the type; such as fruit-based, cream based or fat based. Formulations can change depending on levels of acidity (pH), water activity (a_w) and also amount of solids that are included. Some fillings can have short shelf life but their water activity must be low in order to protect from microbial growth (Nieto and Akins, 2011). Thus, selection of gums is important according to desired texture and shelf life.

Alginate is mostly used in fruit fillings because of its stability during baking. Also, alginate decreases moisture transfer from filling to the baked product. Normally, pectin and calcium are present in the fruit so they provide gel forming between alginate and pectin. During production, alginate gives good deposition control in production machinery (Helgerud et al., 2010).

Starch is important for its thickening and texturising properties especially for filling type of products. The most difficult point is keeping the starch granules in their overall form if they are to be used in acidic fruit fillings (Taggart and Michelle, 2009). Native starches are mostly used in industry to increase viscosity, texture and stability. Also, food industry can use 'clean' label in their products. However, there is a reason to limit the use of starches which show break down with prolonged heating or under high shear or acidic conditions. In addition, starches are prone to severe retrogradation and syneresis upon cooling (Agudelo et al., 2014).

Carrageenan is used in dessert gels and fillings to give firm and brittle gel properties with the texture modified if necessary cohesiveness and syneresis is controlled using iota carrageenan or other hydrocolloids which is locust bean gum or konjac (Williams and Philips, 2009). Also, it is used as an alternative to gelatin in vegan products and its properties are similar to gelatin.



3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Sugars

For the preparation of filling syrup different sugars were used. Granulated sugar was obtained from Konya Şeker, glucose syrup and fructose syrup from Cargill.

3.1.2 Starches

First step of the study aimed choosing the proper starch type, hence, there were 4 different types of starch. Wheat starch, corn starch, tapioca starch, rice starch were supplied from National Starch Company.

3.1.3 Hydrocolloids

Hydrocolloids that we used are shown in Table 3.1 with their suppliers.

Table 3.1 : Hydrocolloids used for the trials.

Hydrocolloids	Company
Corn, Wheat, Tapioca, Rice	National Starch
Xanthan gum	Cargill
Carrageenan	Tate & Lyle
Locust bean gum	Danisco

3.2 Methods

3.2.1 Preparation of starch based syrup

Firstly, 4 different mixtures were prepared and then each one deposited one by one on the biscuits. For the reference recipe, all ingredients were weighed but for the trials 10% of the crystal sugar was separated and mixed with 5% of each starch and then added in the formulation. After that, the mixture is heated and when syrup temperature reached to about 104-105°C, Brix value was measured. The value was about 75°Bx.

After cooking step, syrup was deposited on biscuits. Syrup to biscuit ratio was 32:68. Syrup reference and trial formulations are given in Table 2.9.

Table 3.2 : Reference and trial formulations for syrup.

Ingredients	Reference (%)	Trial A (%)	Trial B (%)	Trial C (%)	Trial D (%)
Crystal Sugar	16	16	16	16	16
Glucose Syrup	31.5	31.5	31.5	31.5	31.5
Fructose Syrup	31.5	31.5	31.5	31.5	31.5
Water	21	21	21	21	21
Wheat Starch	-	5	-	-	-
Corn Starch	-	-	5	-	-
Tapioca Starch	-	-	-	5	-
Rice Starch	-	-	-	-	5

Reference formulation contains sugar syrup types without hydrocolloids. Trial A, syrup with wheat starch (5%). Trial B, syrup with corn starch (5%). Trial C, syrup with tapioca (5%) and the Trial D, syrup with rice starch (5%).

3.2.2 Preparation of hydrocolloid combinations

There are two different base recipes, one of them is starch base and the other is carrageenan base. First group is starch base: only starch (5%), starch + xanthan gum (0.2%), starch + xanthan gum (0.8%). Syrup recipe is the same; crystal sugar (16%), glucose syrup (31.5%), fructose syrup (31.5%), water (21%) and starch (5%). When starch or starch + xanthan gum mixture is added to mix they are added to crystal sugar at about two fold of the amount of starch. It is about 10% sugar. Second group is carrageenan base; only carrageenan (0.5%), carrageenan (0.5%) + locust bean gum (0.1%), and carrageenan (0.5%) + locust bean gum (0.2%).

3.2.3 Moisture content analysis

Moisture contents of all were measured using Mettler Toledo moisture analyzer and that analysis was 3 repeats. It works along the thermogravimetric principle. An infrared radiator (halogen lamp) starts to heating after the sample is weighed. The loss in weight

is continuously recorded and drying is terminated when a defined criterion is reached. From the difference in the mass of samples the moisture content is automatically calculated.

3.2.4 Water activity analysis

Water activity is a relative value of product equilibrium humidity. Water activity is measured with Novasina measurement device and it was 3 repeats. The principle is differentiation between air and sample pressure. The test sample is placed in the measuring area, the sample can humidify or dehumidify the air inside the chamber until the equilibrium humidity is reached. Samples were weighed about 5 gr and after 5 min result is digitally given on screen of the instrument.

3.2.5 pH analysis

pH is a measure of the acidity and alkalinity of a water or water based products. Hydrogen (H^+) or hydroxyl ion (OH^-) numbers define the acidity or alkalinity of water solutions. pH measurement is based on the use of a pH sensitive electrode (usually glass), a reference electrode, and a temperature element to provide a temperature signal to the pH analyzer. The pH measuring electrode is a hydrogen ion sensitive glass bulb. The reference electrode output does not vary with the activity of the hydrogen ion. Sample is prepared for pH measurement, the probe is connected to main box. There are two electrodes inside the probe that measure voltage. One of them measures fixed pH (buffer), other measures the acidity of the sample through the amount of H^+ ions. A voltmeter in the probe measures difference between the voltages of two electrodes. Most pH sensors are designed to produce a 0 mV signal at 7.0 pH, with a (theoretically ideal) slope (sensitivity) of $-59.16 \text{ mV} / \text{pH}$ at 25°C .

3.2.6 Texture analysis

Texture involves sensory perception of food structure and when force is applied structure of food gives response to the force. Texture analyzer systems draw a graphics to interpret some values like hardness or brittleness, etc. Mostly, hardness, brittleness, firmness and/or crispiness is analyzed in biscuit industry. There are different type of probes according to which analyze is preferred. In this study, hardness value was measured three point bending rig with 5 kg force and break samples with flexure unit. In Figure 3.1. texture analyzer picture is given.



Figure 3.1 : Texture analyzer (hardness).

3.2.7 Differential Scanning Calorimeter analysis

Differential scanning calorimetry is a technique that measures thermal transitions of compounds in a controlled temperature/time programs. In starch starch or other gums mostly gelatinization or gelling properties are carried out with measurements of enthalpy (ΔH) for the energy transformation that occur during gelling or melting of crystals in the gum granules besides temperature values. DSC measurements were made with a DSC instrument (TA Q10, TA Instruments, ABD). DSC analysis was performed according to the study of Yoshimura et al. (1996). Each 5 mg of powdered sample and their combinations and 15 mg distilled water were weighed into about 30 mg alluminum pans that were hermetically sealed. Reference pan was empty. At the first step the samples were equilibrated at 25°C and then temperature was raised to 130°C at 1.0°C/min. after the first run heating, the samples was cooled from 130°C to 5°C at 10°C/min. After analysis, samples were stored at 5°C for 14 days. After 14 days samples were rerun using the same method.

3.2.8 Statistical analysis

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

4. RESULTS AND DISCUSSION

4.1 Moisture Contents

4.1.1 Starch type decision

For deciding the starch type, moisture contents are given in Table 4.1 and results are the average of triplicate analysis. One of the most important selection criteria was moisture content. As a reference, only syrup recipe was prepared without any hydrocolloids. Other syrups involving 5% wheat starch (WS), corn starch (CS), tapioca starch (TS) or rice starch (RS) were prepared and then their moisture contents were monitored for 2 weeks. Differentiation between starch types helped us in deciding the type of starch by looking at the one having more moisture retention property. When the results were evaluated using univariate analysis method, there was statistically significant difference both between the samples and between the storage days ($p < 0.05$). At the beginning of storage corn starch and wheat starch had the highest moisture contents. Rice starch and tapioca starch were not successful in water retention at the beginning. Wheat starch, corn starch and rice starch had the highest moisture retentions by the end of 14 days. All starch samples accept for tapioca starch significantly increased water retention when compared to syrup.

Table 4.1 : Moisture contents of starches during storage.

Samples	Moisture contents %		
	1. day	7. day	14. day
Syrup	21.54 ^{aA}	13.17 ^{bA}	10.17 ^{cB}
Syrup + WS	20.59 ^{aB}	10.80 ^{bC}	11.23 ^{bA}
Syrup + CS	20.92 ^{aAB}	12.32 ^{bAB}	10.79 ^{cA}
Syrup + TS	19.53 ^{aC}	11.66 ^{bB}	10.00 ^{bcB}
Syrup + RS	19.79 ^{aC}	12.14 ^{bAB}	11.11 ^{bcA}

Data presented in this table consist of average values $\pm$ standard deviation of five independent batches. Different small letters in the rows represent statistically significant differences ($p < 0.05$). Different capital letters in the columns represent statistically significant differences ($p < 0.05$).

4.1.2 Hydrocolloid combinations

Moisture contents of different hydrocolloid combinations are given in Table 4.2. All samples were measured in every 15 days for 3 months as 3 replicates.

In starch base syrups, xanthan gum combination with starch was found to effect moisture value during shelf life. At the beginning xanthan gum was not so much effective, but during shelf life, especially after 30 days, moisture content significantly decreased as the level of xanthan gum (0.8%) increases.

For carrageenan based syrups, locust bean gum addition did not effect moisture content of fillings as much as xanthan gum. Syrup moisture contents were more stable during storage.

In Table 4.3 when the average evaluation during storage is evaluated for moisture contents all samples were found to be similar except for xanthan gum (0.8%) and locust bean gum (0.2%) additions with higher levels which showed lower moisture contents. Also, in graph; a, b, c.. letters shows difference between each other of average results depends on statistical analysis. Some studies suggest about addition of galactomannas type hydrocolloids at about 0,1-0,5% levels. Corn starch (4%) and added galactomannas (0.1-0.5%) were tested for gelation process in the study of Biliaderis (2009).

According to Gibinski et. al, (2006) potato and oat starch (3%) and xanthan gum (0.1-0.2%) combinations were possible. Water retention ability of corn starch + xanthan gum combination during storage time was reported to increase with increasing gum ratio. That means this collaboration prevents syneresis.

Average moisture content of starch with gum (13.34%) and without gum (9.88%) were determined ($p < 0.05$). Also, water activity of starch with gum (0.597), and without gum (0.558) were determined ($p < 0.05$).

Both xanthan gum and locust bean gum (LBG) had effects on syneresis; LBG 16.22% and xanthan gum 7.69% thus xanthan gum showed higher reduction effect on syneresis than LBG (Arocas et. al, 2009).

Table 4.2 : Moisture values of hydrocolloid combinations during shelf life.

Samples	Moisture content (%)						
	1.day	15.day	30.day	45.day	60.day	75.day	90.day
Starch (5%)	24.46 ^a	14.80 ^b	13.07 ^c	12.50 ^c	11.11 ^c	9.60 ^d	7.80 ^e
Starch (5%) + xanthan gum (0,2%)	26.46 ^a	15.99 ^b	12.59 ^c	10.6 ^d	8.5 ^d	7.20 ^e	5.70 ^f
Starch (5%) + xanthan gum (0,8%)	23.86 ^a	11.83 ^b	9.12 ^c	8.7 ^c	7.00 ^d	5.11 ^e	3.50 ^f
Carrageenan (0,5%)	24.12 ^a	13.51 ^b	12.85 ^b	11.80 ^b	9.13 ^c	8.50 ^c	6.83 ^d
Carrageenan (0,5%) + locust bean gum (0,1%)	25.67 ^a	12.72 ^b	11.11 ^b	9.40 ^{bc}	7.86 ^c	5.80 ^d	4.69 ^d
Carrageenan (0,5%) + locust bean gum (0,2%)	23.10 ^a	10.20 ^b	9.31 ^b	7.76 ^c	5.60 ^{cd}	3.80 ^d	3.10 ^d

Data presented in this table consist of average values $\pm$ standard deviation of six independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$).

Table 4.3 : Moisture value comparison of two hydrocolloid combinations during shelf life*

Samples	Moisture %
Starch (5%)	13.34 ± 5.42 ^a
Starch (5%) + xanthan gum (0,2%)	12.42 ± 7.09 ^{ab}
Starch (5%) + xanthan gum (0,8%)	9.88 ± 6.74 ^b
Carrageenan (0,5%)	12.39 ± 5.72 ^a
Carrageenan (0,5%) + lbg (0,1%)	11.04 ± 7.04 ^{ab}
Carrageenan (0,5%) + lbg (0,2%)	8.98 ± 6.78 ^b

*Data presented in this table consist of average values ± standard deviation of six independent batches. Different letters in the columns represent statistically significant differences ($p < 0.05$).

4.2 Water Activiy

4.2.1 Starch type decision

As seen from Table 4.4, all samples showed similar decreasing trend during storage. Corn starch had the highest water activity level at the end of storage period.

When the average values during storage is evaluated (Table 4.5) between the samples using one-way Anova, it was seen that all samples were same ($p > 0.05$)

Table 4.4 : Water activity values for different type of starches during storage.

Samples	Water Activity		
	1. day	7. day	14. day
Syrup	0.756 ^a	0.567 ^b	0.426 ^c
Syrup + WS	0.747 ^a	0.520 ^b	0.440 ^c
Syrup + CS	0.743 ^a	0.549 ^b	0.500 ^b
Syrup + TS	0.740 ^a	0.506 ^b	0.420 ^{bc}
Syrup + RS	0.726 ^a	0.578 ^b	0.470 ^c

Data presented in this table consist of average values ± standard deviation of five independent batches. Different small letters in the rows represent statistically significant differences ($p < 0.05$).

Table 4.5 : Comparison of water activity values of samples.

Samples	Water activity
Syrup	0.583 ± 0.17^a
Syrup + WS	0.569 ± 0.16^a
Syrup + CS	0.597 ± 0.13^a
Syrup + TS	0.555 ± 0.17^a
Syrup + RS	0.591 ± 0.13^a

Data presented in this table consist of average values $\pm$ standard deviation of five independent batches. Same letters in the column means that samples are statistically same ($p > 0.05$).

4.2.2 Hydrocolloid combinations

Water activity values of different hydrocolloid combinations are given in Table 4.6. All samples were measured in every 15 days of 3 months storage. Starch is not effective in decreasing aw in filling, at the beginning, it's values were about 0.78, but carrageenan was about 0.65, addition of xanthan gum was found to be less effective than locust bean gum.

Comparison of all samples by the average value during storage is shown in Table 4.7. According to those results; except for locust bean gum (0.2%) and carrageenan combination all samples were found to be statistically similar.

For selection of the sample with the highest stability, locust bean gum (0.2%)+carrageenan combination can be the choice. In Figure 4.1 shows locust bean gum and carrageenan combination picture.

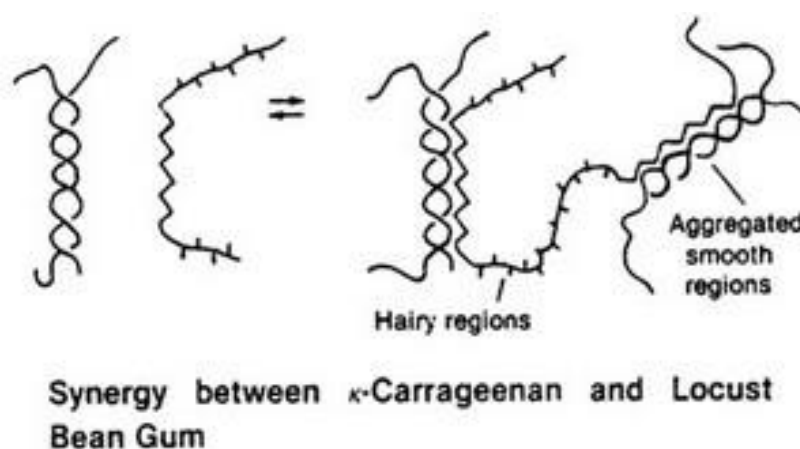


Figure 4.1 : Carrageenan and locust bean gum combination

According to Necas and Bartosiko (2013) carrageenan should be used at a ratio about 0.005- 2% by weight in products. About the combination with locust bean gum, there are so many researches.

Carrageenan and locust bean gum was reported to have synergistic effect and one of them said addition of locust bean gum to carrageenan, texture characterization was improved and syneresis was increased, additionally, when only carrageenan is used total moisture; 75.37% with lbg; 71.75% thus found to be more effective in retaining water (Garcia-garcia and Totosaous, 2008).

Results of statistical analysis determining by ($p < 0.05$), moisture content carrageenan solutions are different to each other during shelf life. Average of moisture contents; carrageenan (0.5%) is 12.39%, carrageenan (0.5%) + locust bean gum (0.1%) is 11.04% and carrageenan (0.5%) + locust bean gum (0.2%) is 8.98%.

The main differences of values were started after 30 days. locust bean gum amount was effective for retention of water. However, in water activity analysis there were not big differences between first and second type of crr solutions.

First one's aw value was 0.54, second one's 0.53, but the carrageenan (0.5%) + locust bean gum (0.2%) was different from others 0.49. Thus, it shows when use of locust bean gum water activity capacity is decreased. And also, the more locust bean gum is increasing water acitivity value of last product.

In one of study about crr and lbg effect on cooked ham when carrageenan used alone, the most syneresis was shown by it however, use of locust bean gum reported to decrease syneresis of product.; syneresis percentage of carrageenan was 1.37, carrageenan and locust bean gum (75/25) was 1.02 in 14 days. Also, carrageenan was 4.53, carrageenan and locust bean gum (75/25) was 3.56 in 28 days (Cossio et al., 2013).

Table 4.6 : Water activity values of hydrocolloid combinations during shelf life.

Samples	Water activity						
	1.day	15.day	30.day	45.day	60.day	75.day	90.day
Starch (5%)	0.783 ^a	0.648 ^b	0.623 ^{bc}	0.581 ^c	0.551 ^c	0.512 ^{cd}	0.481 ^d
Starch (5%) + xanthan gum (0,2%)	0.806 ^a	0.659 ^b	0.604 ^{bc}	0.550 ^c	0.530 ^c	0.504 ^c	0.450 ^d
Starch (5%) + xanthan gum (0,8%)	0.794 ^a	0.593 ^b	0.575 ^b	0.547 ^b	0.523 ^{bc}	0.472 ^c	0.410 ^{cd}
Carrageenan (0,5%)	0.683 ^a	0.610 ^{ab}	0.583 ^b	0.530 ^b	0.457 ^c	0.454 ^c	0.435 ^c
Carrageenan (0,5%) + locust bean gum (0,1%)	0.651 ^a	0.585 ^{ab}	0.578 ^{ab}	0.520 ^b	0.508 ^{bc}	0.477 ^c	0.401 ^{cd}
Carrageenan (0,5%) + locust bean gum (0,2%)	0.611 ^a	0.563 ^b	0.558 ^b	0.504 ^{bc}	0.480 ^c	0.422 ^{cd}	0.359 ^d

*The data presented in this table consist of average values $\pm$ standard deviation of six independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$).

Table 4.7 : Water activity values comparing two hydrocolloid combinations during shelf life (one-way anova).

Samples	aw
Starch (5%)	0.597 ± 0.10 ^a
Starch (5%) + xanthan gum (0,2%)	0.586 ± 0.12 ^a
Starch (5%) + xanthan gum (0,8%)	0.558 ± 0.12 ^{ab}
Carrageenan (0,5%)	0.536 ± 0.09 ^{ab}
Carrageenan (0,5%) + lbg (0,1%)	0.531 ± 0.08 ^{ab}
Carrageenan (0,5%) + lbg (0,2%)	0.499 ± 0.09 ^b

Data presented in this table consist of average values ± standard deviation of six independent batches. Different letters in the column represent statistically significant differences ($p < 0.05$).

4.3 pH

4.3.1 Starch type decision

Besides moisture content and water activity, changes in pH value are important for product quality. pH values were measured in fillings directly.

Table 4.8 shows values during 2 weeks of storage. pH values were in the range of 6.10-6.86. Wheat starch had the highest pH both at the beginning and at the end of storage. While rice starch had the lowest at the end of storage.

In Table 4.9 the mean value of pH for samples is evaluated statistically by one-way Anova method. According to those results; wheat starch was significantly different than corn starch and rice starch samples ($p < 0.05$).

Table 4.8 : pH values of samples during storage

Samples	pH		
	1.day	7.day	14.day
Syrup	6.73 ^a	6.44 ^{ab}	6.36 ^b
Syrup + WS	6.86 ^a	6.53 ^{ab}	6.40 ^b
Syrup + CS	6.38 ^a	6.36 ^a	6.34 ^a
Syrup + TS	6.52 ^a	6.38 ^{ab}	6.37 ^{ab}
Syrup + RS	6.75 ^a	6.11 ^b	6.10 ^b

Data presented in this table consist of average values ± standard deviation of five independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$).

Table 4.9 : Comparing pH values of samples* (one-way anova).

Samples	pH
Syrup	6.51 ± 0.19 ^{ab}
Syrup + WS	6.59 ± 0.24 ^a
Syrup + CS	6.36 ± 0.02 ^b
Syrup + TS	6.42 ± 0.08 ^{ab}
Syrup + RS	6.32 ± 0.37 ^b

*Data presented in this table consist of average values ± standard deviation of five independent batches. Same letters in the column means statistically same values ($p > 0.05$).

4.3.2 Hydrocolloid combinations

Table 4.10 shows measured pH values of fillings during shelf life. pH changes correlate with moisture and water activity. If moisture values decreases, pH values also decrease. And also, when water activity is decreased, pH is decrease at the same time.

But, pH value is sensitive the other ions' existence. Some ions like Ca, Na etc. can change of pH values. So, water which is use in recipe is important because of existence of ions.

It depends even on used water and syrups as they provide other anion or cations. Interestingly, xanthan gum additions significantly decreased the pH values of starch based fillings. Other gums are not so effective on pH values.

However, by the end of storage only xanthan gum added fillings had increased pH contents. It links with at the beginning of values, and it is stable during shelf life.

Starch and carrageenan based fillings have different pH values, individually they are shown in Table 4.11. Starch (5%) + xanthan gum (0.8%) and carrageenan (0.5%) + locust bean gum (0.2%) combinations had significantly lowest pH values. As writes before, pH value is correlate with water content or water activity of filling includes.

Table 4.10 : pH values of hydrocolloids during shelf life

Samples	pH						
	1.day	15.day	30.day	45.day	60.day	75.day	90.day
Starch (5%)	4.24 ^a	4.00 ^{ab}	4.16 ^a	4.06 ^{ab}	3.98 ^b	3.78 ^b	3.60 ^{bc}
Starch (5%) + xanthan gum (0,2%)	3.52 ^{cd}	3.98 ^c	4.79 ^a	4.56 ^{ab}	4.45 ^b	4.20 ^{bc}	3.95 ^c
Starch (5%) + xanthan gum (0.8%)	3.28 ^{cd}	3.50 ^c	4.62 ^a	4.3 ^{ab}	3.98 ^b	3.54 ^c	3.43 ^c
Carrageenan (0.5%)	4.50 ^a	4.43 ^a	4.48 ^a	4.2 ^{ab}	4.00 ^b	3.92 ^b	3.80 ^b
Carrageenan (0.5%) + locust bean gum (0.1%)	4.20 ^a	4.18 ^a	4.35 ^a	4.22 ^a	4.05 ^{ab}	3.80 ^b	3.65 ^b
Carrageenan (0.5%) + locust bean gum (0.2%)	4.12 ^{ab}	4.21 ^a	4.32 ^a	3.98 ^b	3.65 ^b	3.50 ^{bc}	3.20 ^c

*Data presented in this table consist of average values $\pm$ standard deviation of six independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$).

Table 4.11 : Comparison of pH values for samples* (one-way anova).

Samples	pH
Starch (5%)	3.98 ± 0.22 ^{ab}
Starch (5%) + xanthan gum (0,2%)	4.21 ± 0.43 ^a
Starch (5%) + xanthan gum (0,8%)	3.81 ± 0.50 ^b
Carrageenan (0,5%)	4.19 ± 0.29 ^a
Carrageenan (0,5%) + lbg (0,1%)	4.06 ± 0.25 ^{ab}
Carrageenan (0,5%) + lbg (0,2%)	3.85 ± 0.41 ^b

*Data presented in this table consist of average values ± standard deviation of six independent batches. Different letters in the columns represent statistically significant differences ($p < 0.05$).

4.4 Texture Analysis

4.3.1 Hardness

Hardness values were measured during shelf life and they are shown in Table 4.12. Biscuit hardness is dependent on the amount of water migrated to the biscuit. Thus, it can change at the beginning, in the middle and at the end of the shelf life. Hardness of biscuits significantly increased during storage.

Especially, it changes day by day and the important point is which time it starts to hardness or softness. Because that day gives information about when it can be consume in right time.

In filled biscuits it is a desired phenomena as it means that water is not migrated to biscuits from the fillings. Up to 45 days of storage softening takes place but after that retrogradation might be the cause for hardening.

Statistical analysis of comparison between starch and carrageenan samples' hardness values are given in Table 4.13. When the average values are compared it is obvious that hydrocolloid additions to fillings significantly increases the hardness values of the biscuits.

Table 4.12 : Hardness values of filled biscuits during shelf life.

Samples	Hardness (g/F)						
	1.day	15.day	30.day	45.day	60.day	75.day	90.day
Starch (5%)	303.8 ^{bc}	340.4 ^b	325.5 ^b	271.8 ^c	474.9 ^{ab}	501.7 ^a	530.4 ^a
Starch (5%) + xanthan gum (0,2%)	350.0 ^b	386.2 ^b	362.3 ^b	387.8 ^b	495.8 ^{ab}	586.7 ^a	607.7 ^a
Starch (5%) + xanthan gum (0,8%)	469.9 ^b	510.8 ^{ab}	455.2 ^b	416.9 ^c	575.3 ^a	610.8 ^a	652.0 ^a
Carrageenan (0,5%)	309.7 ^{cd}	406.9 ^{bc}	370.8 ^c	346.2 ^c	490.0 ^b	633.1 ^a	650.8 ^a
Carrageenan (0,5%) + locust bean gum (0,1%)	361.5 ^c	468.4 ^b	426.3 ^{bc}	364.8 ^c	510.9 ^b	658.6 ^a	712.6 ^a
Carrageenan (0,5%) + locust bean gum (0,2%)	482.4 ^{bc}	540.3 ^b	510.3 ^b	491.5 ^{bc}	643.1 ^{ab}	703.5 ^a	752.2 ^a

*Data presented in this table consist of average values $\pm$ standard deviation of six independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$).

Table 4.13 : Comparing hardness values for filled biscuits during shelf life* (one-way anova).

Samples	Hardness (g/F)
Starch (5%)	392.7±105.9 ^c
Starch (5%) + xanthan gum (0.2%)	453.8±108.9 ^{ab}
Starch (5%) + xanthan gum (0.8%)	527.3±87.3 ^{ab}
Carrageenan (0.5%)	458.2±137.6 ^{bc}
Carrageenan (0.5%) + lbg (0.1%)	500.4±138.1 ^b
Carrageenan (0.5%) + lbg (0.2%)	589.1±109.6 ^a

*Data presented in this table consist of average values ± standard deviation of six independent batches. Different letters in the column represent statistically significant differences ($p < 0.05$).

4.3.2 Brittleness

Brittleness values were also measured by the same analyzer. It indicates the first bite of the consumer. The values are given in Table 4.14. Trend during storage was similar to that obtained for hardness.

Statistical analysis of comparison between starch and carrageenan samples' brittleness values are given in Table 4.15. When the average values are compared it is obvious that hydrocolloid additions to fillings significantly increases the brittleness values of the biscuits.

Hardness and brittle analysis results are relevant to each other. If hardness increases, brittleness also increase. The hardest composition was carrageenan (0.5%)+lbg (0.2%) and the softest one is corn starch (5%) during shelf life ($p < 0.05$).

When we evaluate values each one in 15 days, hardness and brittles were decreasing in 30 days and 45 days because of water migration from filling to biscuit. However, it started to get harder after 45 days.

Cossio et. al, (2013) showed that carrageenan when used alone was less hard that when it used in combination with locust bean gum for both 14 and 28 days of storage.

Table 4.14 : Brittleness values of filled biscuits during shelf life.

Samples	Brittle (g/F)						
	1.day	15.day	30.day	45.day	60.day	75.day	90.day
Starch (5%)	622.3 ^{bc}	661.5 ^b	616.8 ^{bc}	572.7 ^c	770.6 ^{ab}	812.2 ^a	848.1 ^a
Starch (5%) + xanthan gum (0.2%)	731.2 ^c	757.6 ^c	726.2 ^c	692.6 ^{cd}	774.3 ^c	886.8 ^a	912.4 ^a
Starch (5%) + xanthan gum (0.8%)	827.8 ^b	840.4 ^b	840.9 ^b	847.2 ^b	944.1 ^a	954.6 ^a	994.4 ^a
Carrageenan (0.5%)	619.8 ^{bc}	669.9 ^b	673.4 ^b	622.9 ^{bc}	832.4 ^a	851.8 ^a	883.3 ^a
Carrageenan (0.5%) + locust bean gum (0.1%)	747.6 ^c	765.4 ^c	773.7 ^c	693.9 ^d	850.2 ^b	962.5 ^a	983.1 ^a
Carrageenan (0.5%) + locust bean gum (0.2%)	830.9 ^b	846.9 ^b	779.2 ^c	748.4 ^c	979.1 ^a	1043.5 ^a	1085.4 ^a

*Data presented in this table consist of average values $\pm$ standard deviation of six independent batches. Different letters in the rows represent statistically significant differences ($p < 0.05$)

Table 4.15 : Brittleness values compasion of two hydrocolloid combination during shelf life* (one-way anova).

Samples	Brittle (g/F)
Starch (5%)	700.6±108.1 ^e
Starch (5%) + xanthan gum (0.2%)	783.0±84.0 ^{cd}
Starch (5%) + xanthan gum (0.8%)	892.8±69.0 ^{ab}
Carrageenan (0.5%)	736.2±114.7 ^{de}
Carrageenan (0.5%) + lbg (0.1%)	825.2±111.0 ^{bc}
Carrageenan (0.5%) + lbg (0.2%)	901.9±133.1 ^a

*Data presented in this table consist of average values ± standard deviation of six independent batches. Different letters in the column represent statistically significant differences (p < 0.05).

4.5 Differential Calorimetric Analysis (DSC)

DSC analysis results are shown in Table 4.16. In DSC curves, first transition is gelation step, and there are 2 important points. T₀ is the beginning of the gelation and T_p is the first peak point in gelatinization/gelation. ΔH value is the enthalpy value between starting point until end point of transition. Glass transition is the other phase transition and material is becoming glassy structure in T_g point.

Water evaporation temperature gives information about which combination hydrocolloid system hold water better. By the end of 14 days of storage, retrogradation crystal melting transition is becoming available.

Beginning of the gelatinization temperature of starch solution is about max 53°C and gelation is about 60°C and needed enthalpy for gelation is 34.9 J/g. Gelatinization enthalphy of native starches are 5-20 J/g and ΔH has two different terminology; granule swelling and crystallite melting (endothermic), hydration and chain reordering (exothermic). Also, different genetical characterization of corn, rice, barley, amaranth, etc. shows wide range variation in DSC melting parameters (ΔH, transition temperature) (Biliaderis, 2009). Xanthan gum decreased starch gelatinization temperature. And also, initial temperature (T₀) of gelatinization was decreased from 47°C to about 41°C. Gelation point was 61°C for starch, by the addition of xanthan gum it was about 45°C. There is a study about addition of xanthan to wheat starch and results; initial gelation temperature of starch is about 80°C, addition of xanthan (0,45%) decreased temperature to 53°C (Christianson et. al, 1981).

Water retention characterization of hydrocolloids is different from each other. When starch based filling is evaluated, addition of xanthan gum increased water retention capability. Starch based filling (5%) had a water evaporation temperature of 104°C, xanthan addition increased the temperature to 111°C. However, for carrageenan based filling same situation about water retention temperature was not evident. Carrageenan based filling (5%) had a water evaporation temperature of 109°C, locust bean gum of 102°C, but their combination crr (5%) + lbg (0.1%) had 103°C, crr (5%) + lbg (0.2%) had 106°C. Results showed that xanthan gum increases water retention of starch but locust bean gum increased water retention capability of carrageenan when used at levels above 0.2%. DSC aparative is given in Figure 4.2.

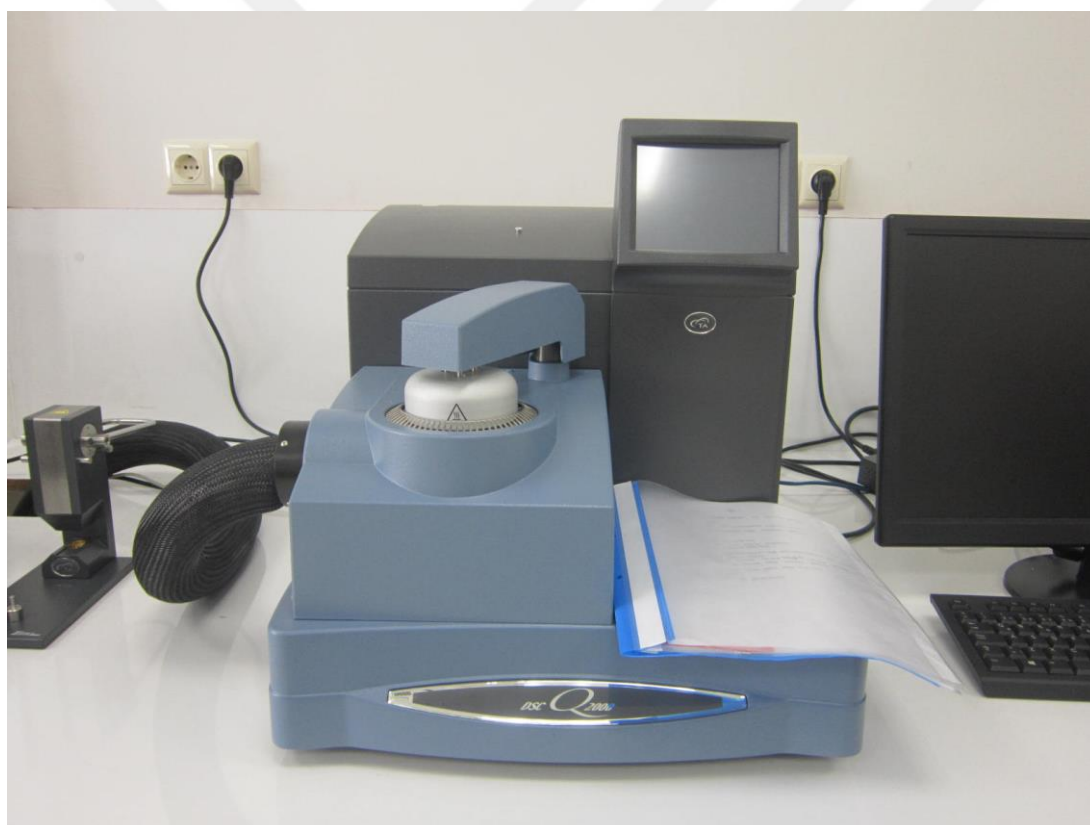


Figure 4.2 : DSC device

Table 4.16 : DSC analysis of hydrocolloids used in the study

Samples	Gelation			Glass transition	T _{water} evaporation	14 days of storage
	T ₀ (°C)	T _p (°C)	ΔH (J/g)	T _g (°C)	T _{we} (°C)	T _c (°C)
Starch (5%)	52.8 ^b	58.3 ^{ab}	34.9 ^a	72.1	104.1 ^b	70.8
Xanthan gum (5%)	25.3 ^b	27.0 ^c	3.2 ^c	28.4	103.4 ^b	26.3
Starch (5%) + xanthan gum (0.2%)	41.7 ^b	46.1 ^{bc}	28.6 ^{ab}	64.0	104.2 ^b	59.3
Starch (5%) + xanthan gum (0.8%)	42.1 ^b	44.2 ^c	0.18 ^d	92.8	111.3 ^a	25.8
Carrageenan (5%)	88.9 ^a	90.6 ^a	2.5 ^c	95.6	108.8 ^a	93.1
Locust bean gum (5%)	25.2 ^b	26.0 ^c	1.8 ^a	27.5	101.8 ^b	26.1
Carrageenan (5%) + locust bean gum (0.2%)	25.0 ^b	25.9 ^{bc}	12.3 ^b	45.9	103.4 ^b	87.0
Carrageenan (0.5%) + locust bean gum (0.8%)	26.9 ^b	28.9 ^{bc}	2.6 ^c	38.2	106.0 ^a	80.7

Different letters in the columns represent statistically significant differences ($p < 0.05$).



5. CONCLUSION AND RECOMMENDATIONS

Previous studies are mostly about carrageenan and locust bean gum synergistic effects. In recent studies there are studies about synergistic effect of different starch types but, their purpose is mostly for their thickening effects. In this study, evaluation of starch-xanthan and carrageenan-locust bean gum combinations according to water retention capacity and their effects on filling structures and filled biscuit quality was performed.

Starches are used in fillings for their thickening or gelling property. But, by the addition of xanthan gum to the filling it effects water retention capability of the filling. Especially this property is important in filled biscuits as problems arise by the migration of moisture from filling to the biscuit.

At the end of shelf life, results showed that water activity of fillings were decreased by the addition of xanthan gum. Starch and xanthan gum combination together give strength and viscosity to the filling. Hardness and brittleness results for biscuits changed by migration of water, after 30 days hardness decreased and then biscuit started to get firmer. The same situation is applicable to carrageenan based filling. Carrageenan and locust bean gum forms a gel in the filling and is more effective than xanthan gum-starch combination according to water activity retaining. Carrageenan and locust bean gum has water retention capability either in combination or alone.

As a result of this study, synergism of hydrocolloids provides advantages to filling such as cost, shelf life extension and desired texture. Carrageenan and locust bean gum combination is found to increase water retention more than the starch and xanthan gum combination. Besides, starch-xanthan gum combination have softer texture for the biscuits they are filled. Thus, this study showed that for both biscuit fillings and other food products hydrocolloid combinations can solve the problems or improve the quality. As there are many hydrocolloids available, new studies are necessary to study their two or even more combinations to understand their synergistic effects.



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APPENDICES

APPENDIX A: DSC Curves for the 1st day of storage

APPENDIX B: DSC Curves for the 14th day of storage

APPENDIX C: Photos of hydrocolloid solutions

APPENDIX D: Anova tables

APPENDIX A: DSC Curves (1st day)

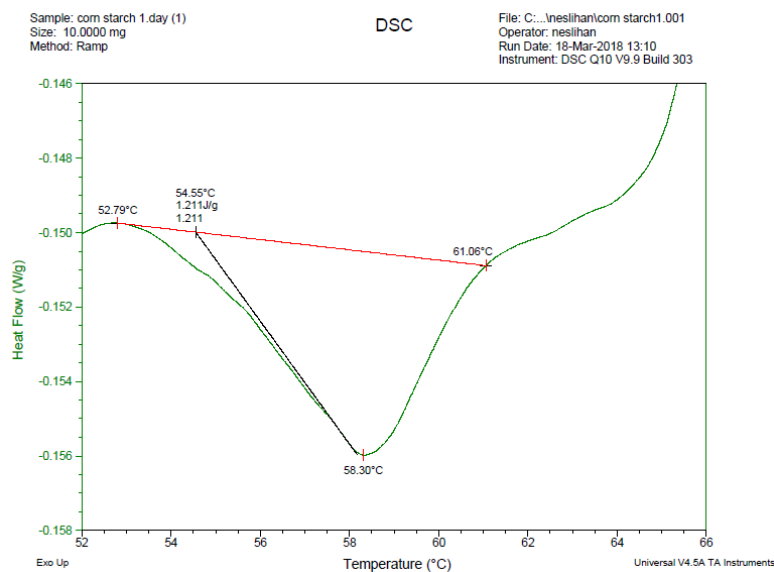


Figure A.1 : Gelation transition for corn starch (parallel 1)

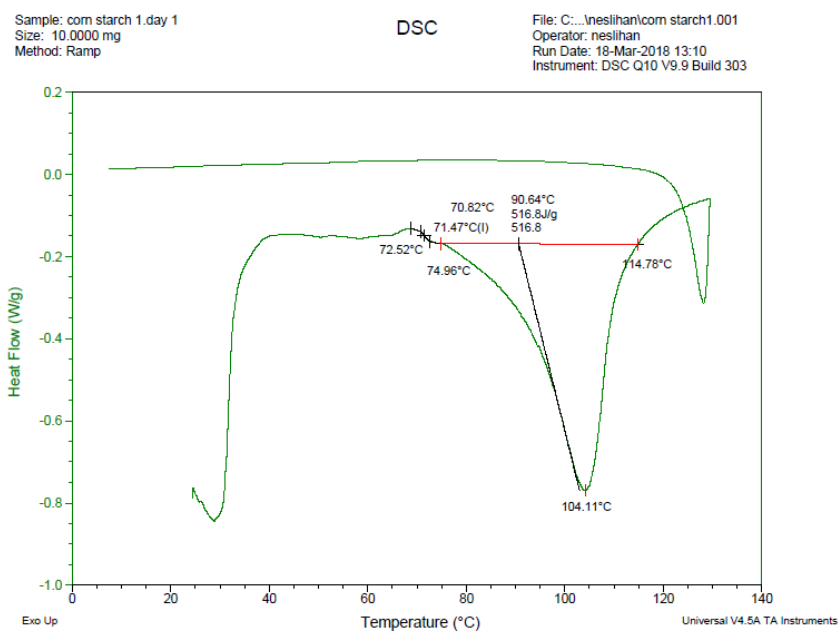


Figure A.2 : Thermogram for corn starch (parallel 1)

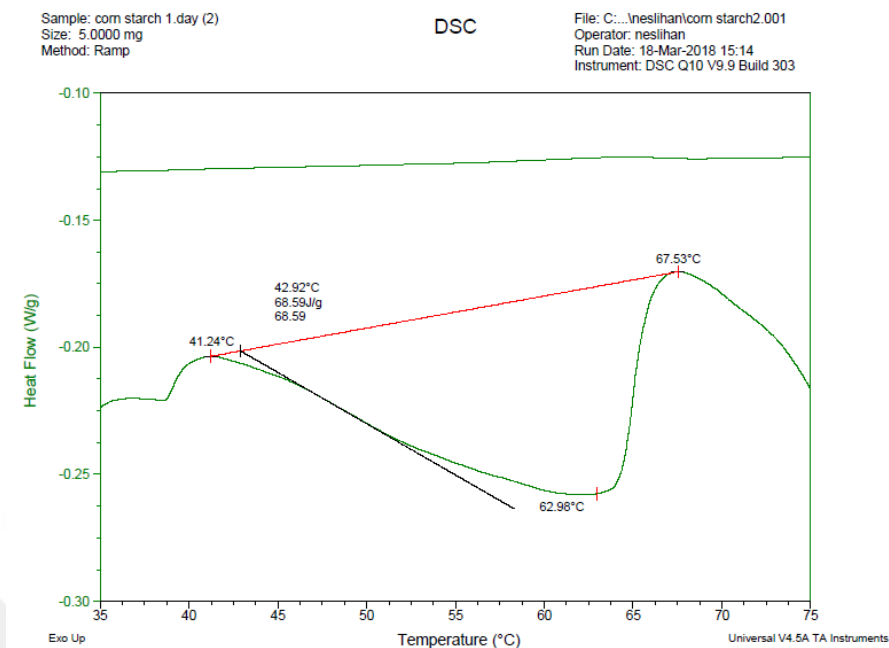


Figure A.3 : Gelation transition for corn starch (parallel 2)

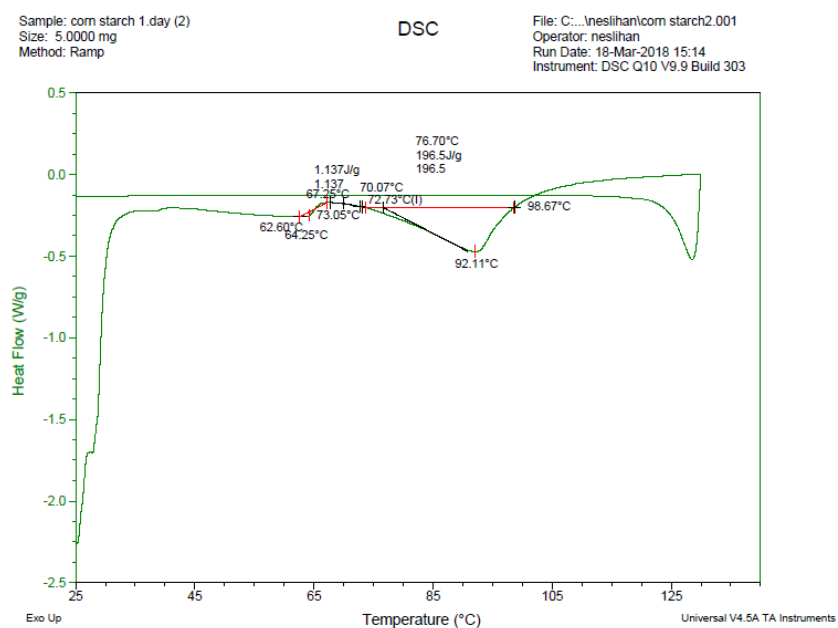


Figure A.4 : Thermogram for corn starch (parallel 2)

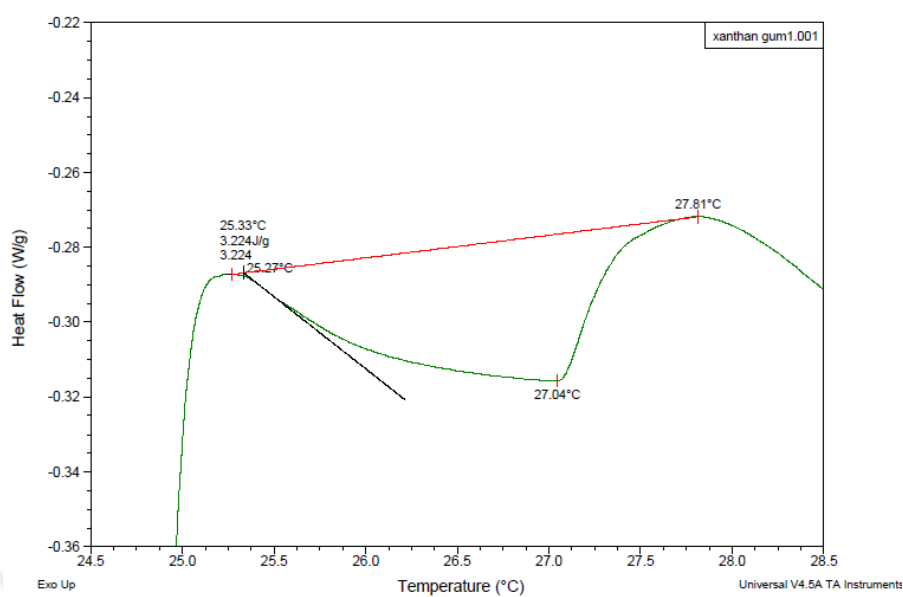


Figure A.5 : Gelation transition for xanthan gum

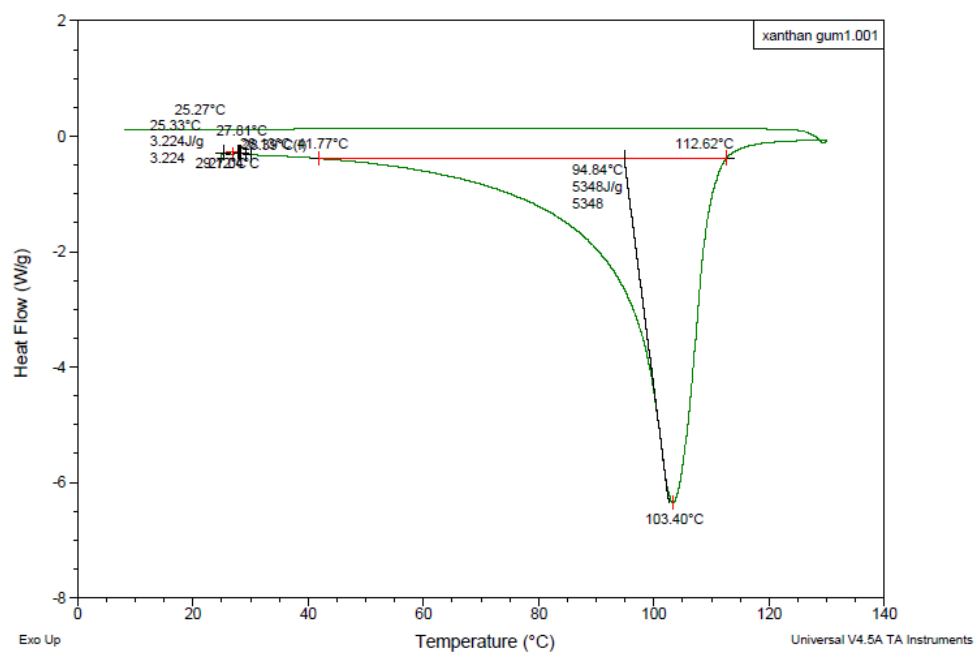


Figure A.6 : Thermogram for xanthan gum

Sample: cstarch + xanthan(0,2%) 1.day 1
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...starch + xanthan gum(0,2%)1.001
Operator: neslihan
Run Date: 31-Mar-2018 14:37
Instrument: DSC Q10 V9.9 Build 303

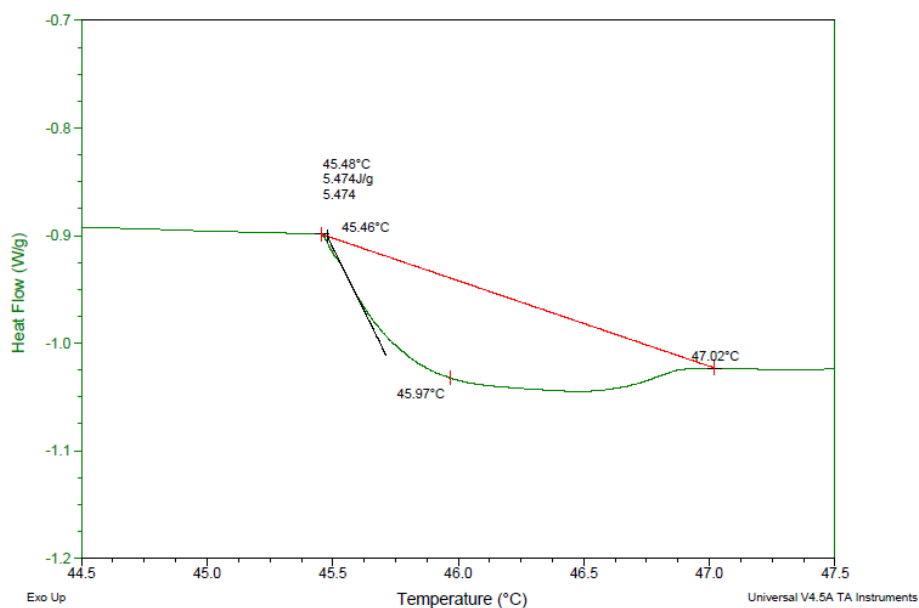


Figure A.7 : Gelation transition for corn starch (5%)+xanthan gum (0,2%) (parallel 1)

Sample: cstarch+xanthan(0,2%)1.day 2
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...starch + xanthan gum(0,2%)2.001
Operator: neslihan
Run Date: 31-Mar-2018 16:37
Instrument: DSC Q10 V9.9 Build 303

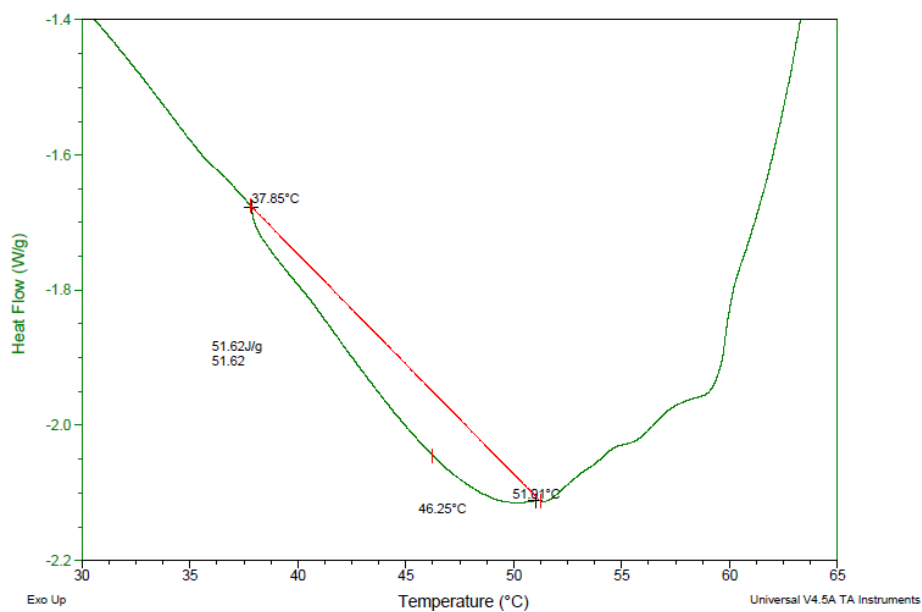


Figure A.8 : Gelation transition for corn starch (5%)+xanthan gum (0,2%) (parallel 2)

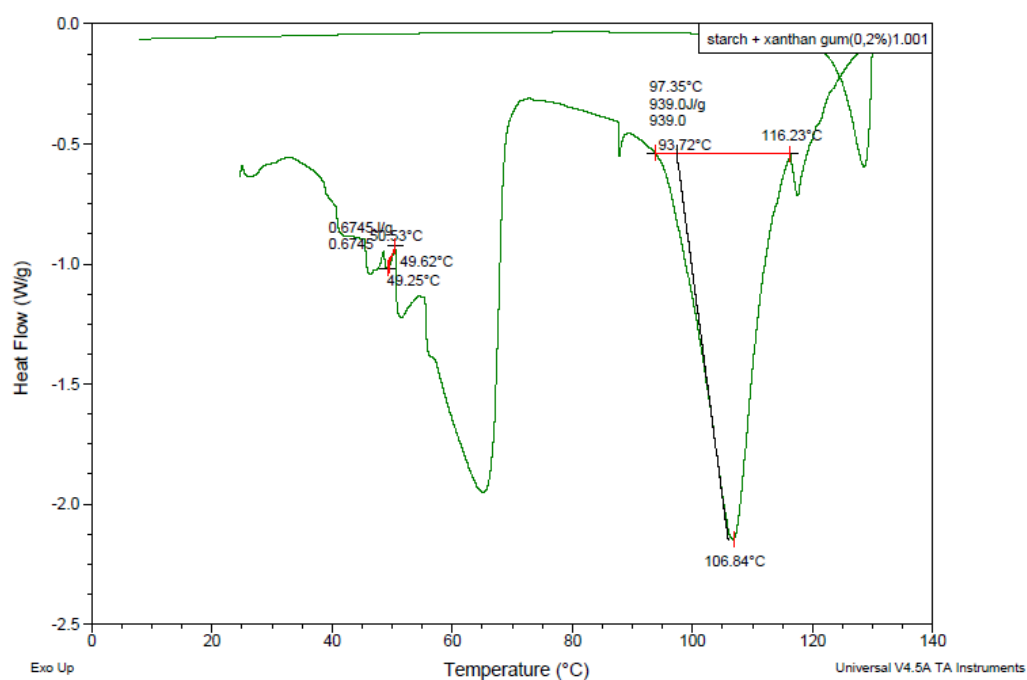


Figure A.9 : Thermogram for corn starch (5%)+xanthan gum (0,2%) (parallel 1)

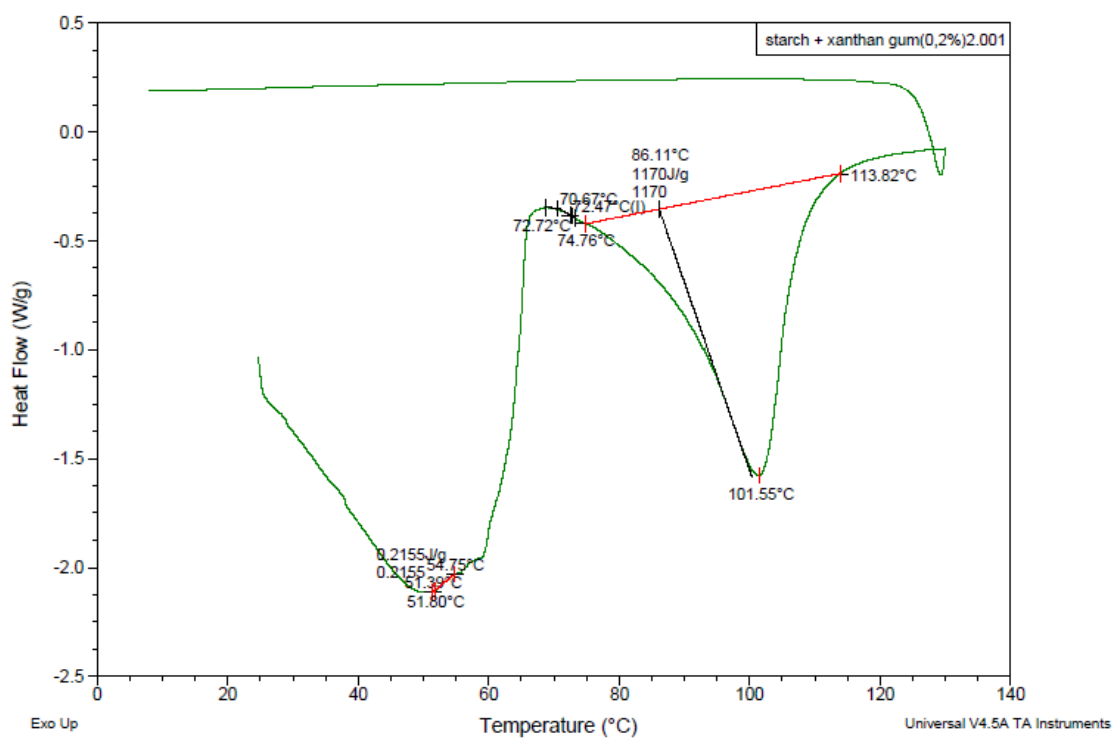


Figure A.10 : Thermogram for corn starch (5%)+xanthan gum (0,2%) (parallel 2)

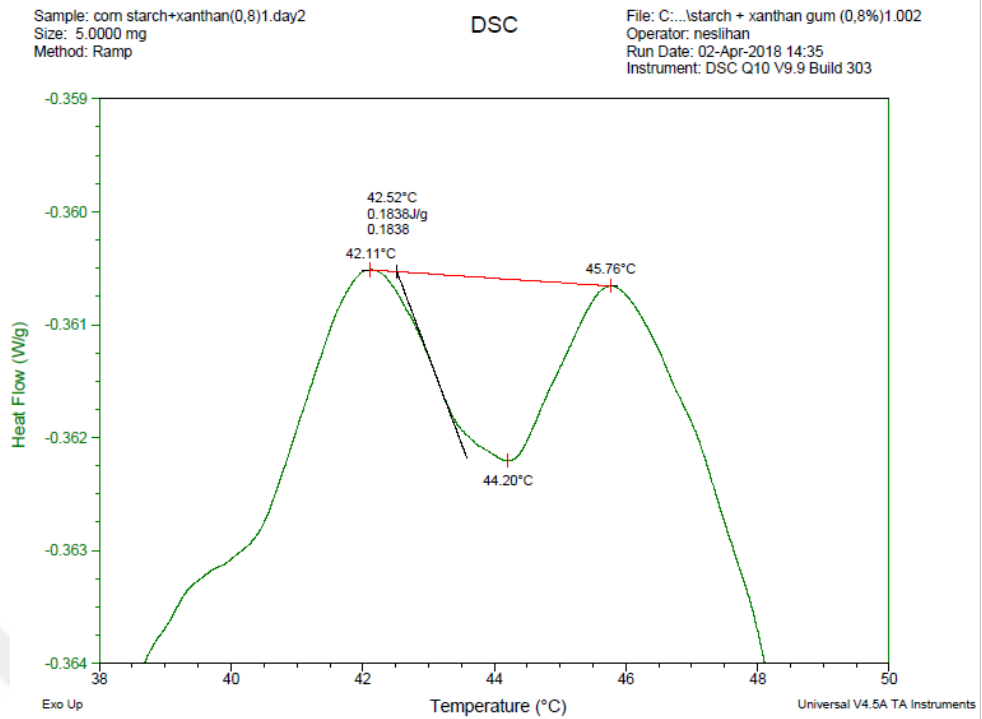


Figure A.11 : Gelation transition for corn starch (5%)+xanthan gum (0,8%)

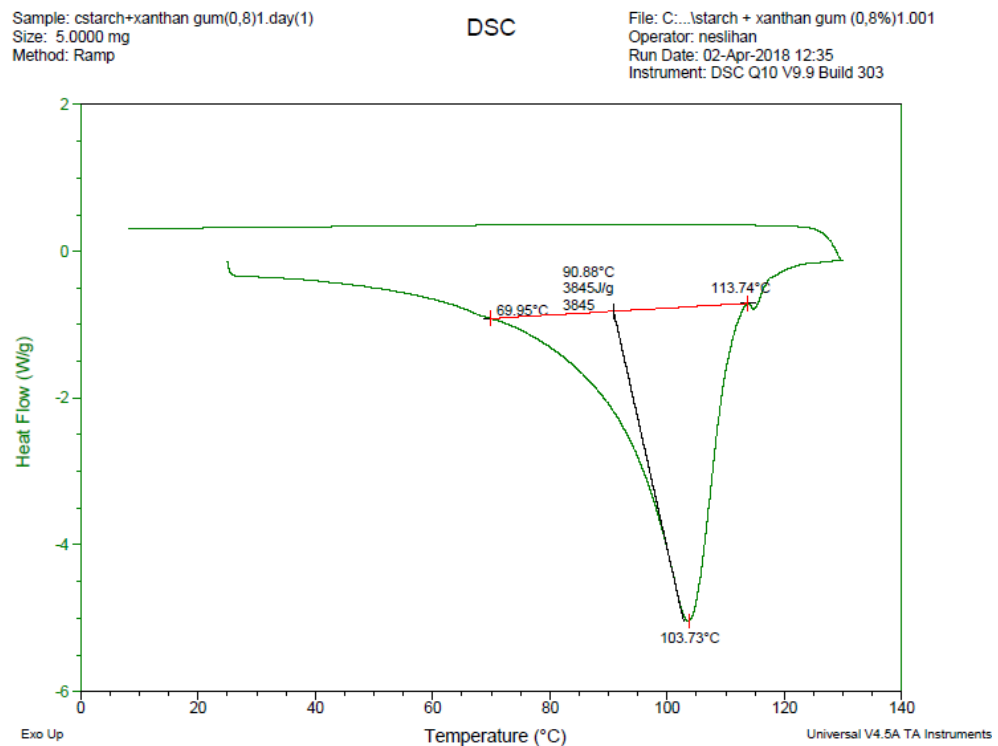


Figure A.12 : Thermogram for corn starch (5%)+xanthan gum (0,8%) (parallel 1)

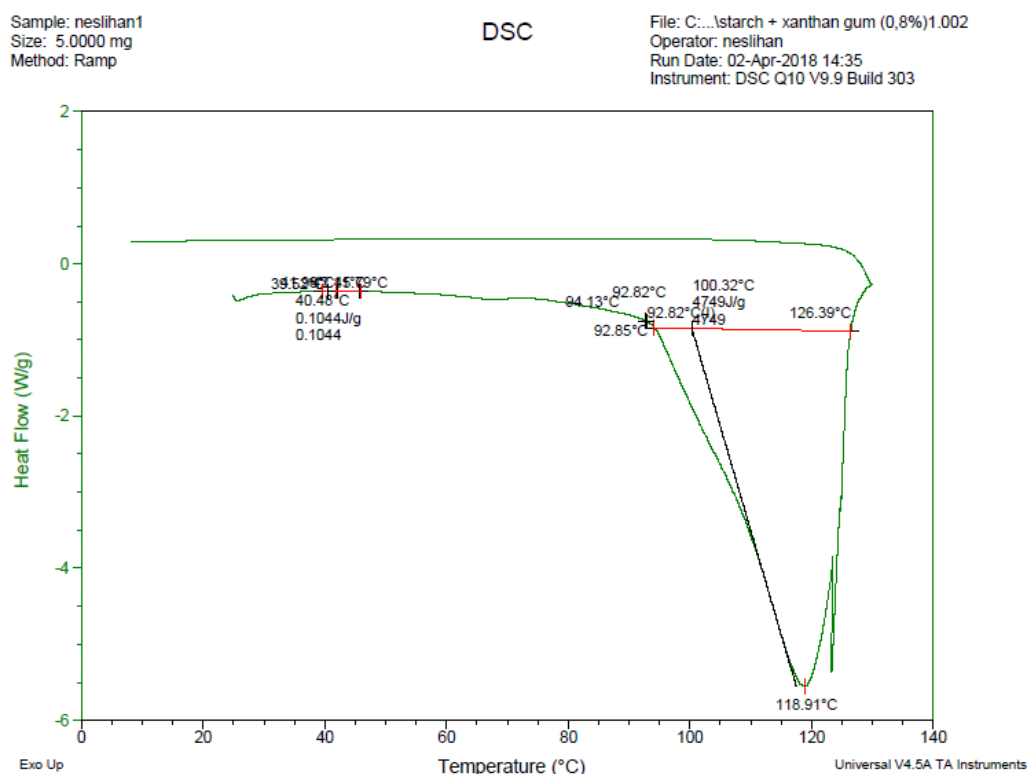


Figure A.13 : Thermogram for corn starch (5%)+xanthan gum (0,8%) (parallel 2)

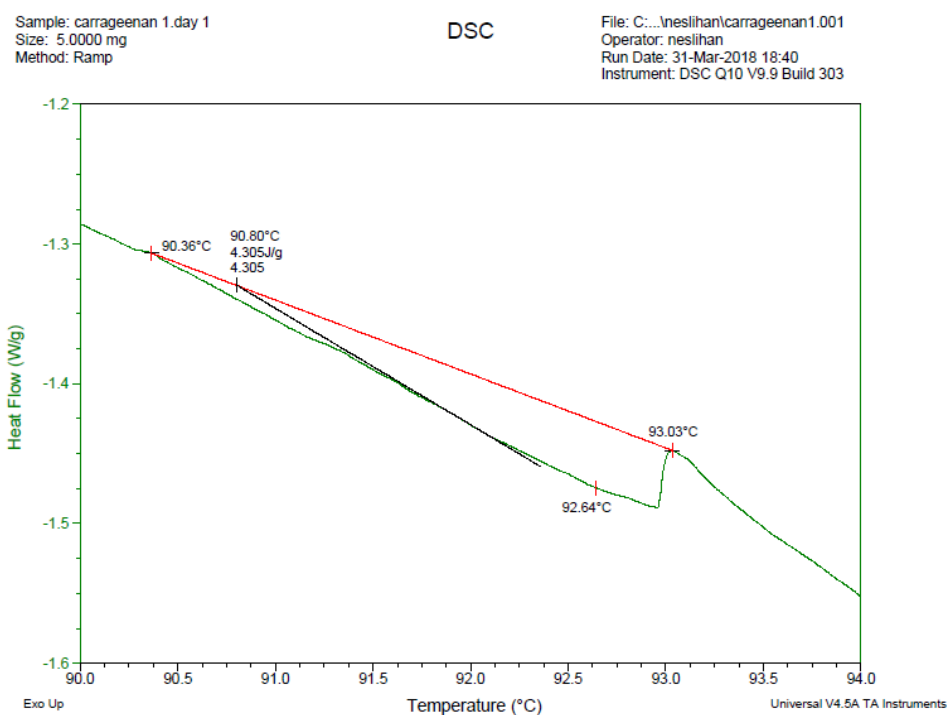


Figure A.14 : Gelation transition for carrageenan (5%) (parallel 1)

Sample: carrageenan 1.day 2
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\carrageenan2.002
Operator: neslihan
Run Date: 02-Apr-2018 10:36
Instrument: DSC Q10 V9.9 Build 303

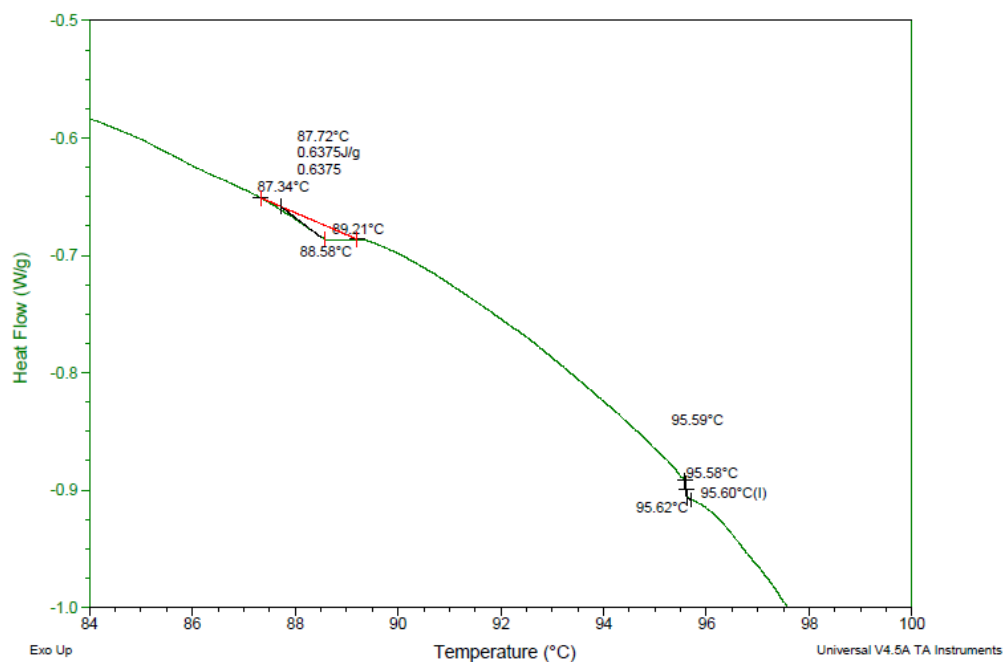


Figure A.15 : Gelation transition for carrageenan (5%) (parallel 2)

Sample: carrageenan 1.day 1
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\carrageenan1.001
Operator: neslihan
Run Date: 31-Mar-2018 18:40
Instrument: DSC Q10 V9.9 Build 303

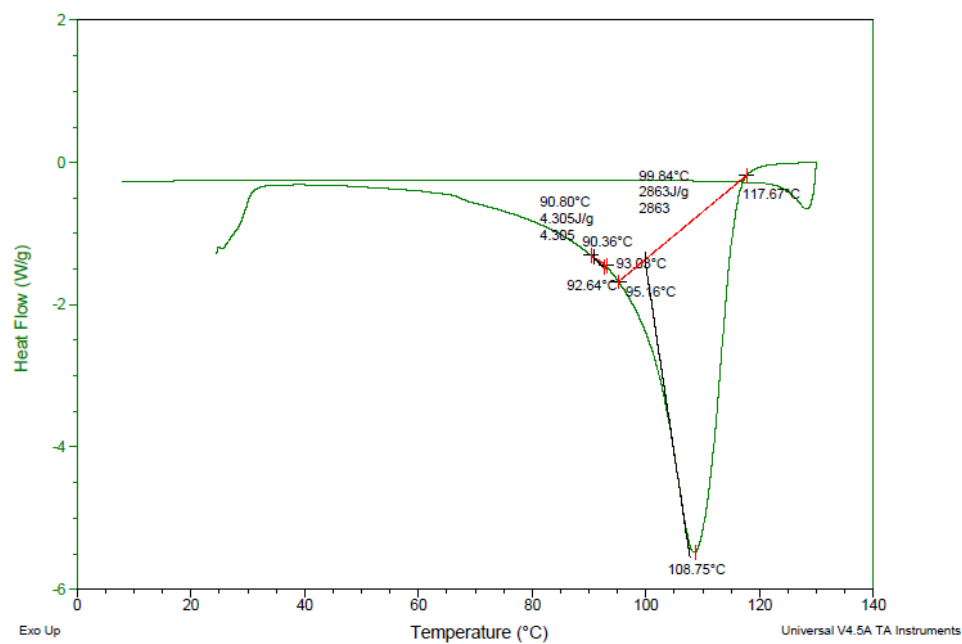


Figure A.16 : Thermogram for carrageenan (5%) (parallel 1)

Sample: carrageenan 1.day 2
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...\neslihan\carrageenan2.002
Operator: neslihan
Run Date: 02-Apr-2018 10:36
Instrument: DSC Q10 V9.9 Build 303

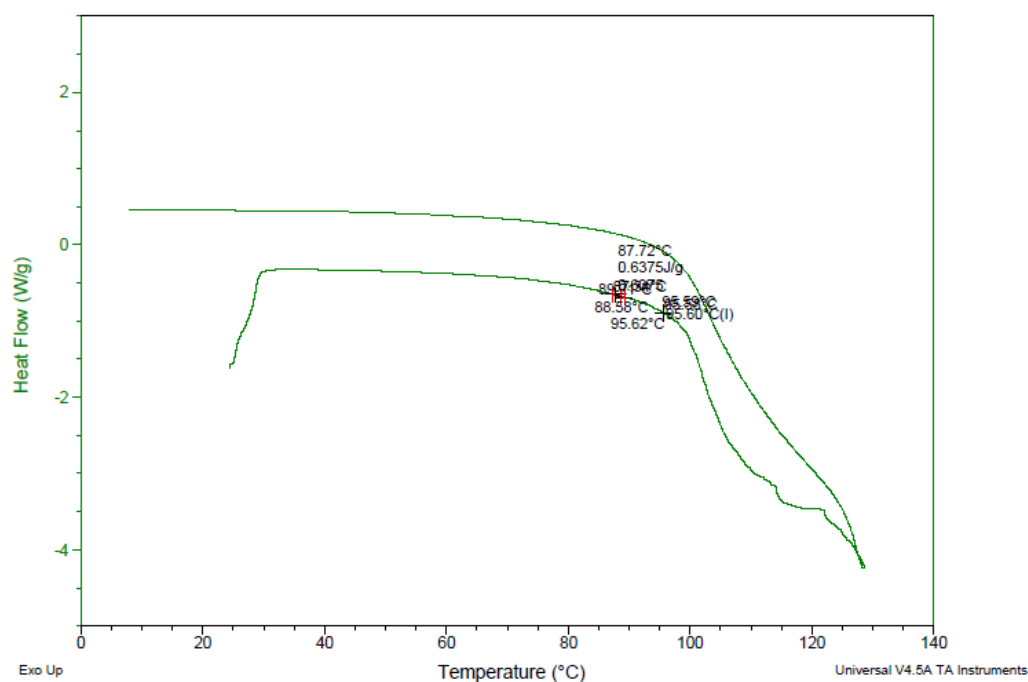


Figure A.17 : Thermogram for carrageenan (5%) (parallel 2)

Sample: lbg 1.day (1)
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...\NalanDemir\neslihan\lbg 1.001
Operator: neslihan
Run Date: 07-Apr-2018 10:21
Instrument: DSC Q10 V9.9 Build 303

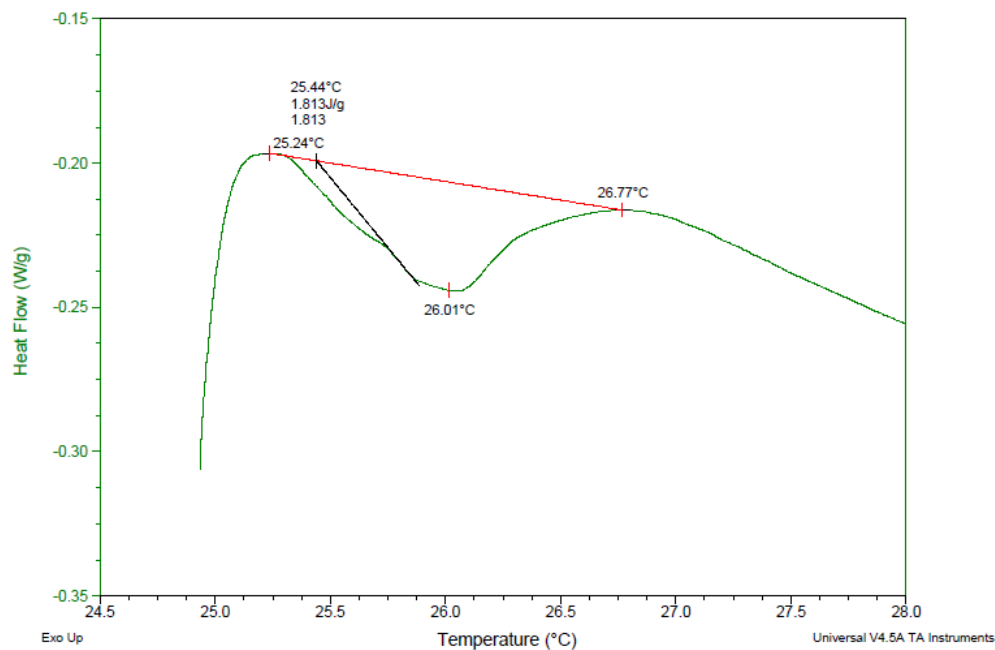


Figure A.18 : Gelation transition for locust bean gum (5%)

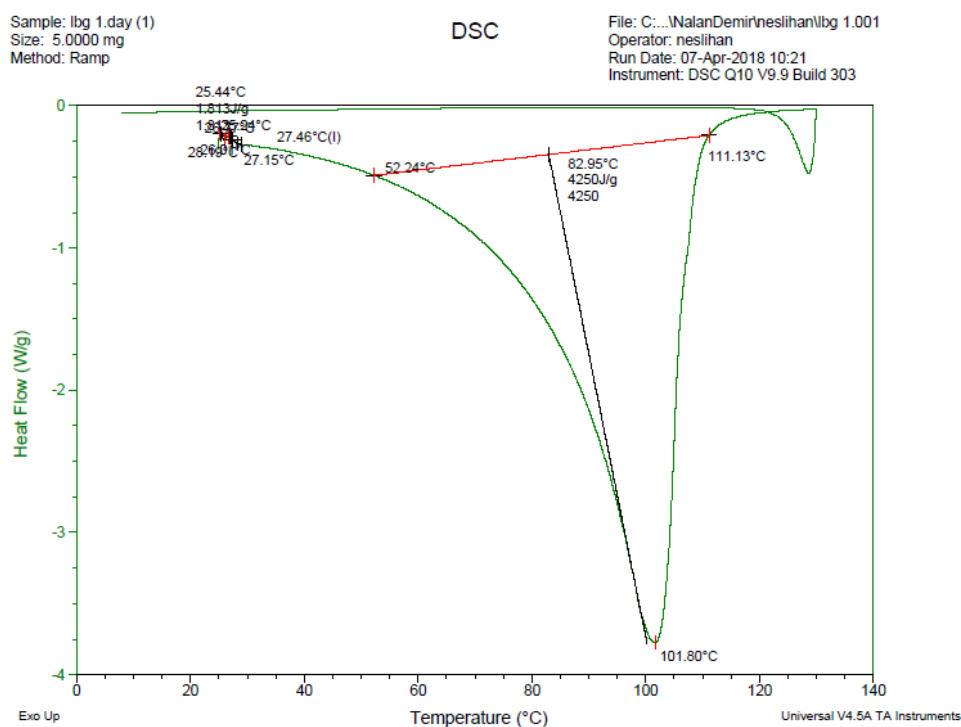


Figure A.19 : Thermogram for locust bean gum (5%) (parallel 1)

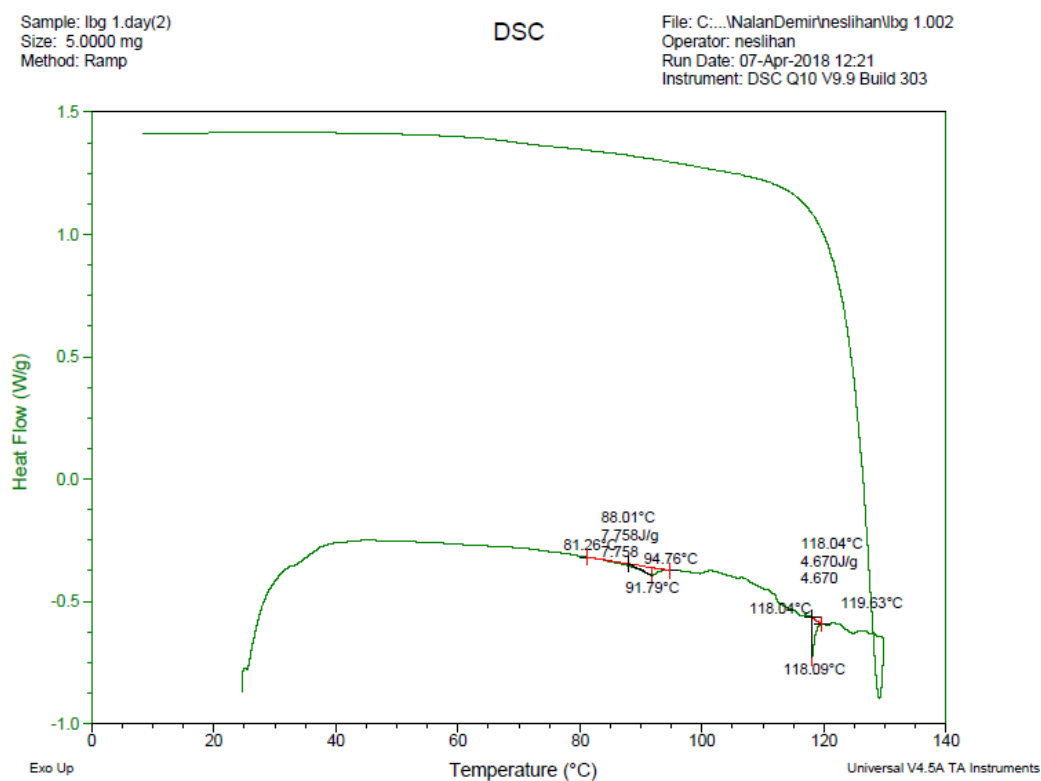


Figure A.20 : Thermogram for locust bean gum (5%) (parallel 2)

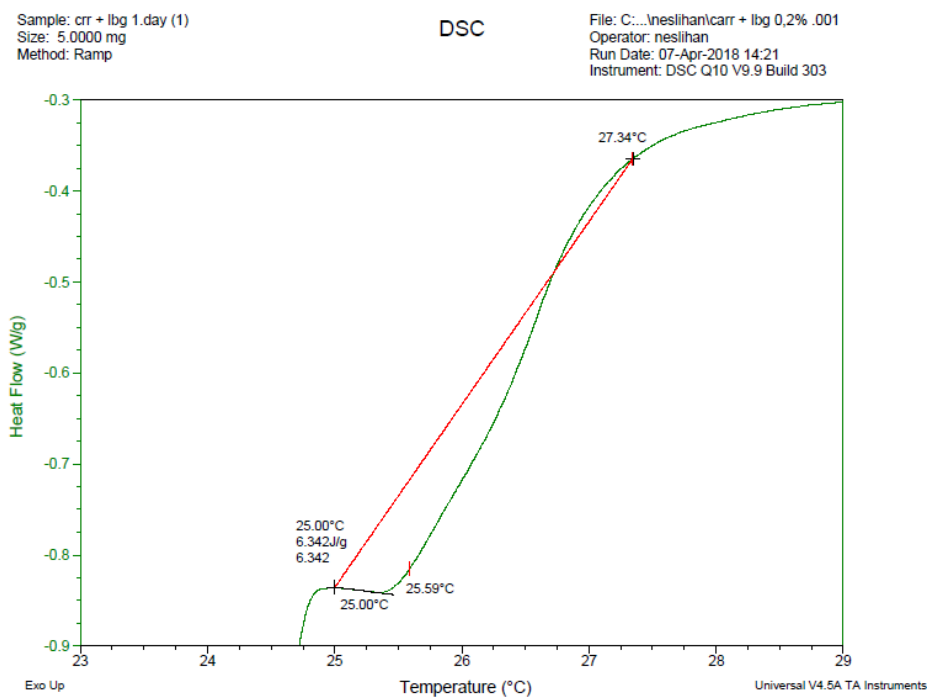


Figure A.21 : Gelation transition for carrageenan (5%)+locust bean gum (0,2%) (parallel 1)

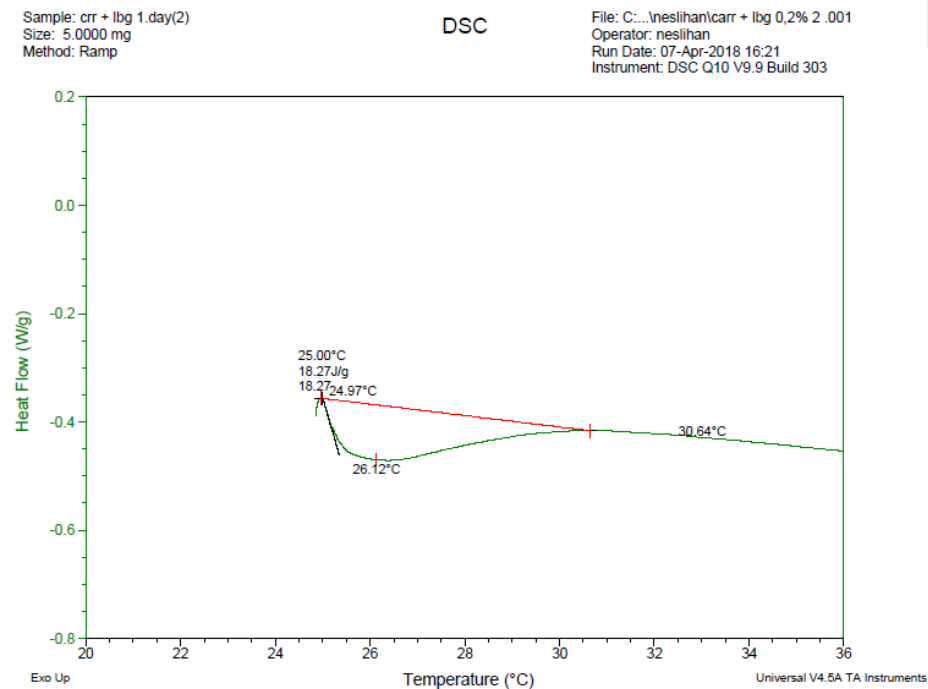


Figure A.22 : Gelation transition for carrageenan (5%)+locust bean gum (0,2%) (parallel 2)

Sample: carr + lbg 1.day (1)
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...\neslihan\carr + lbg 0,2% .001
Operator: neslihan
Run Date: 07-Apr-2018 14:21
Instrument: DSC Q10 V9.9 Build 303

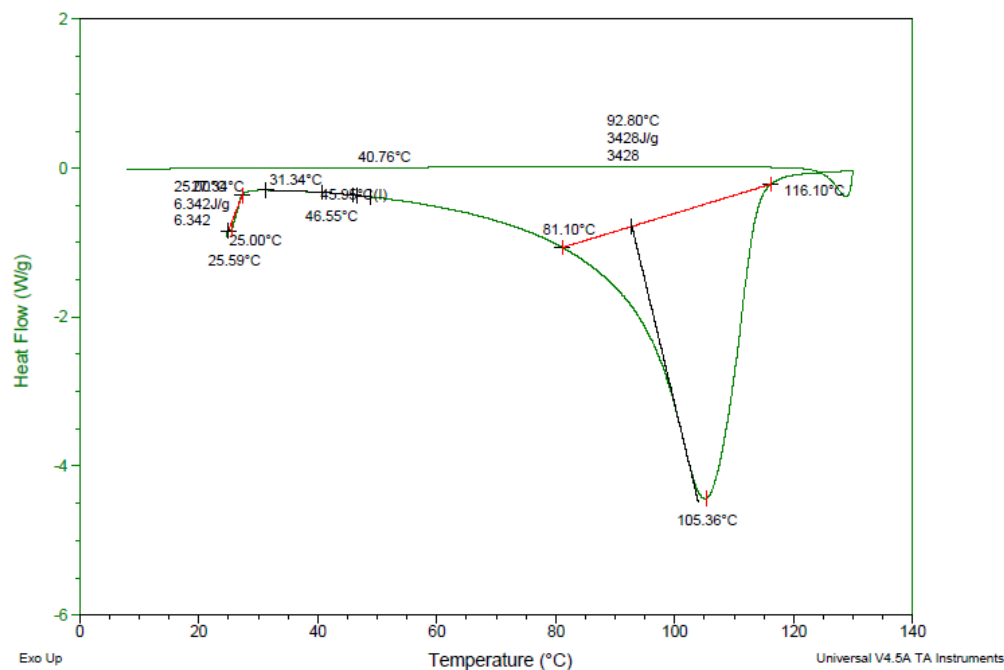


Figure A.23 : Thermogram for carrageenan (5%)+locust bean gum (0,2%) (parallel 1)

Sample: carr + lbg 1.day(2)
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...\neslihan\carr + lbg 0,2% 2 .001
Operator: neslihan
Run Date: 07-Apr-2018 16:21
Instrument: DSC Q10 V9.9 Build 303

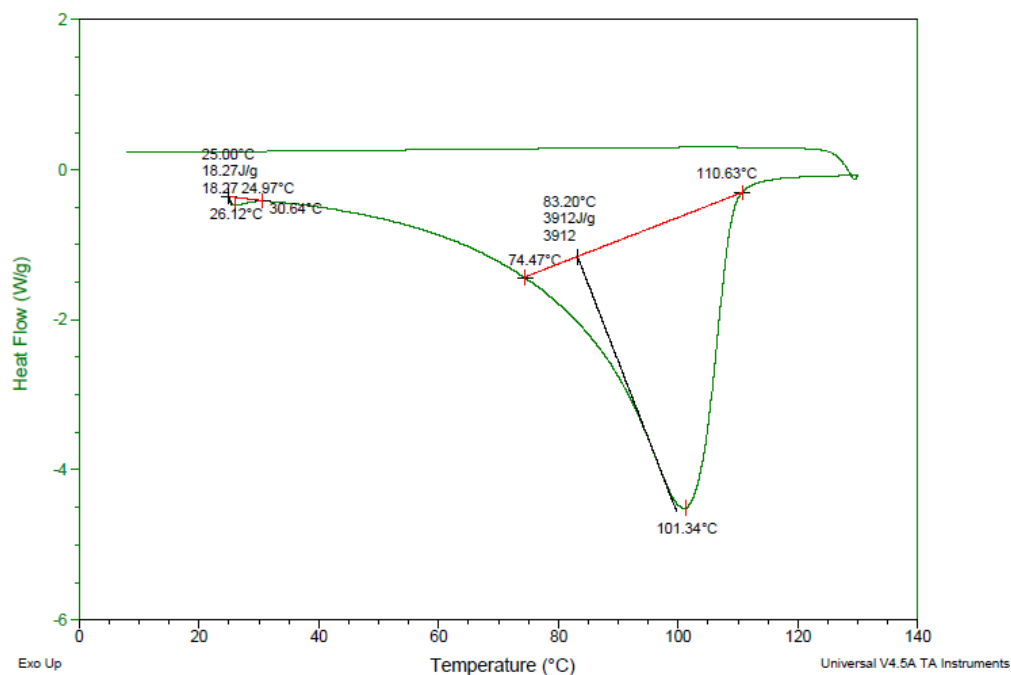


Figure A.24 : Thermogram for carrageenan (5%)+locust bean gum (0,2%) (parallel 2)

APPENDIX B: DSC Curves (14st day)

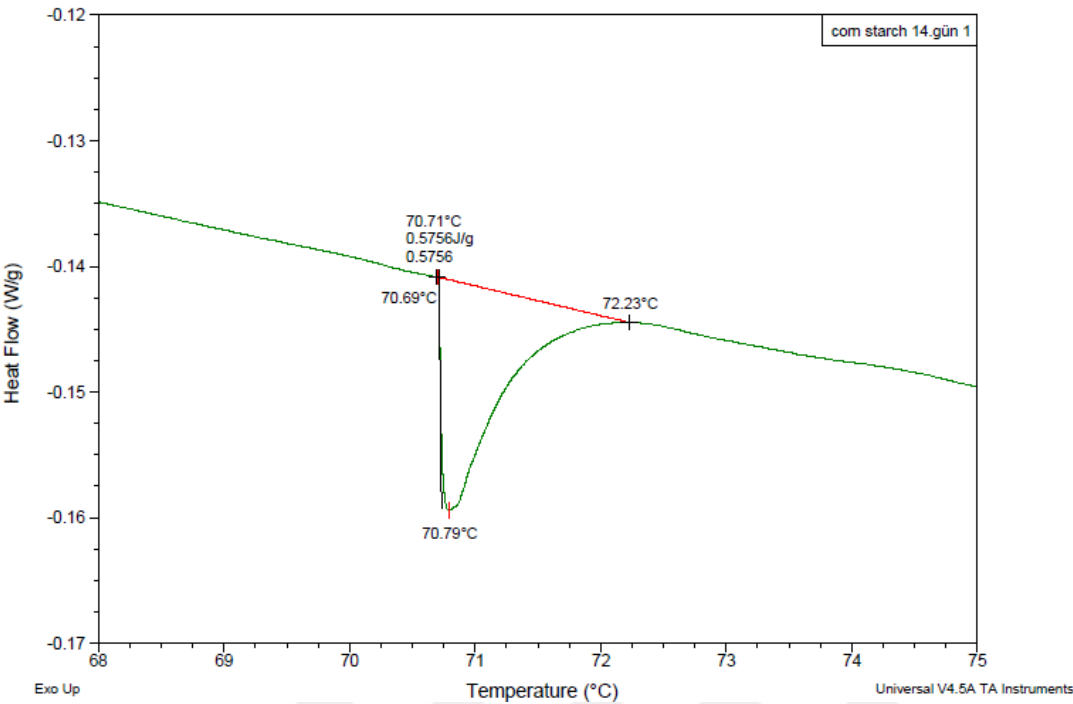


Figure B.1 : Gelation transition for corn starch (5%) (parallel 1)

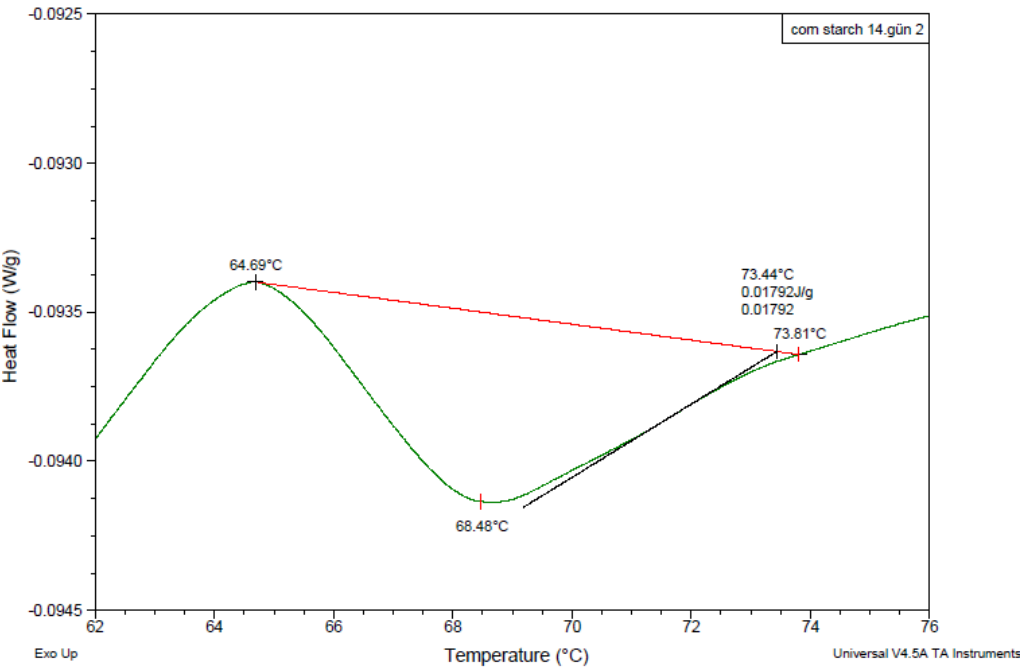


Figure B.2 : Gelation transition for corn starch (5%) (parallel 2)

Sample: corn starch 14day (1)
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\corn starch 14.gün 1
Operator: neslihan
Run Date: 01-Apr-2018 15:16
Instrument: DSC Q10 V9.9 Build 303

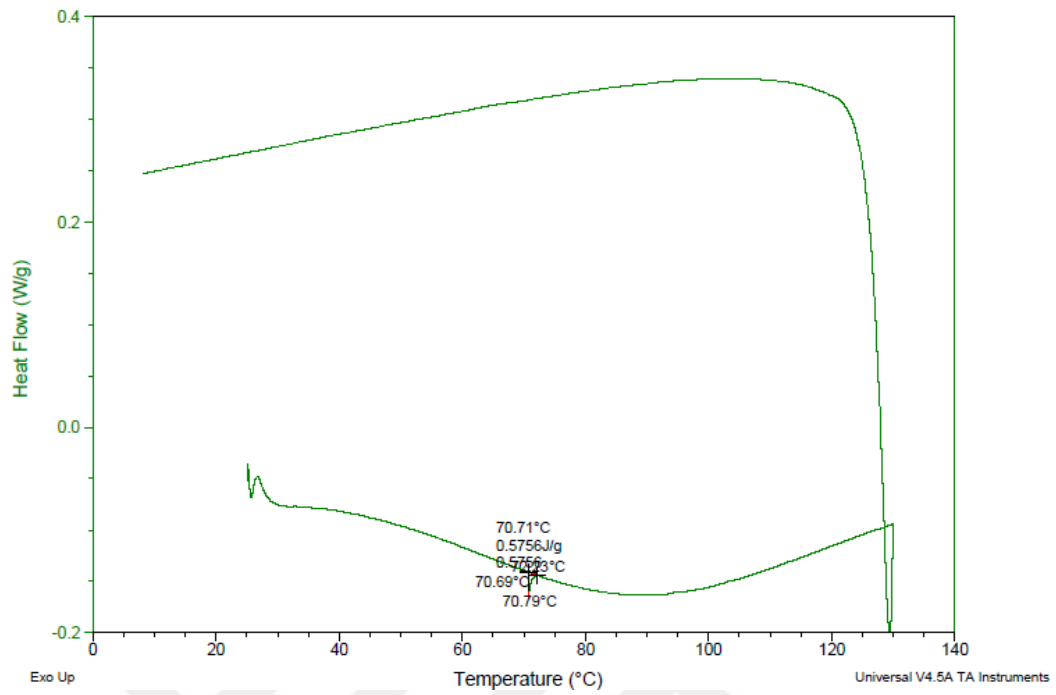


Figure B.3 : Thermogram for corn starch (5%) (parallel 1)

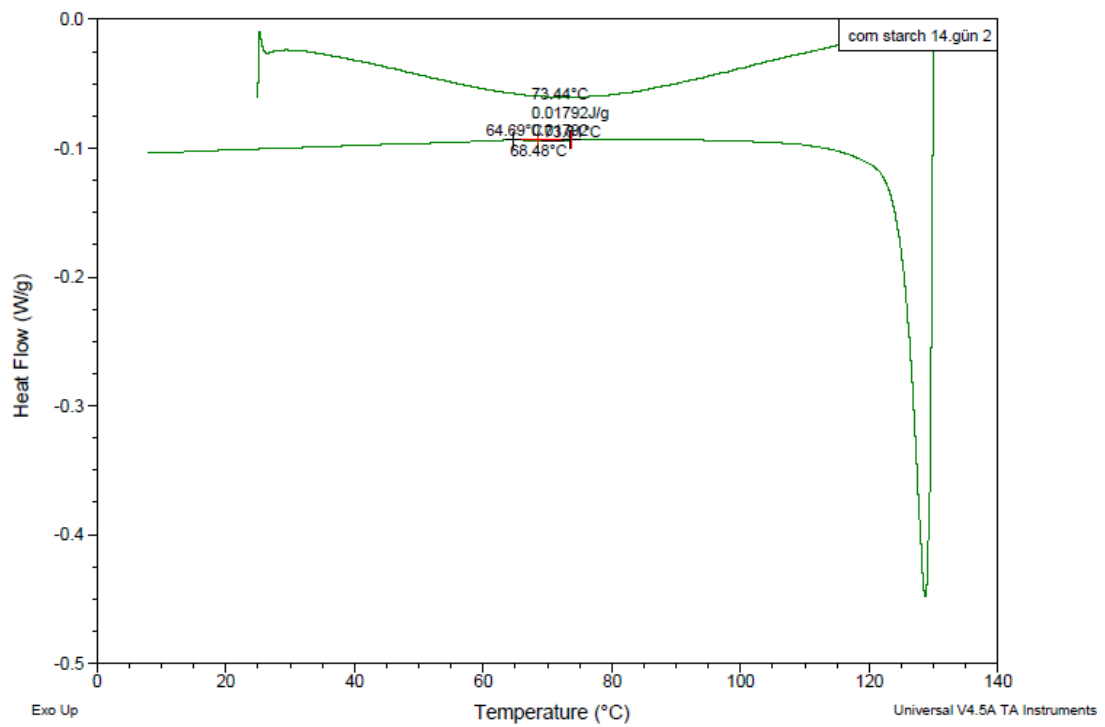


Figure B.4 : Thermogram for corn starch (5%) (parallel 2)

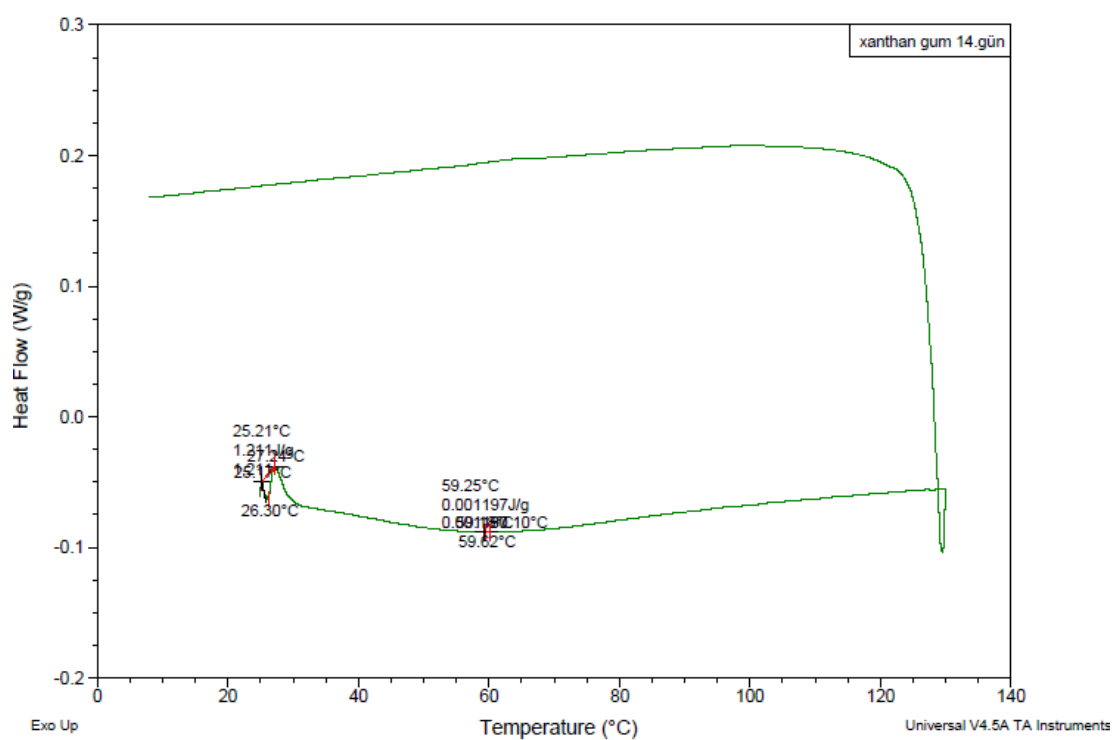


Figure B.5 : Thermogram for xanthan gum (5%)

Sample: cs + xanthan gum1 14.gün
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\cs+ xanthan gum1.001
Operator: neslihan
Run Date: 14-Apr-2018 19:35
Instrument: DSC Q10 V9.9 Build 303

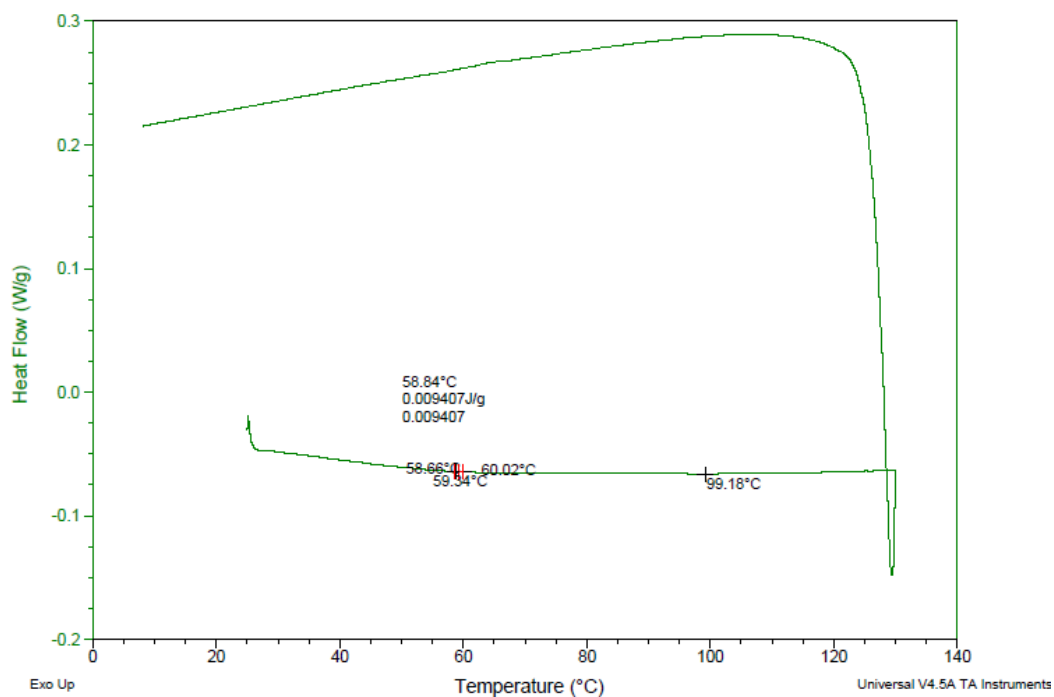


Figure B.6 : Thermogram for corn starch (5%)+xanthan gum(0,2%)

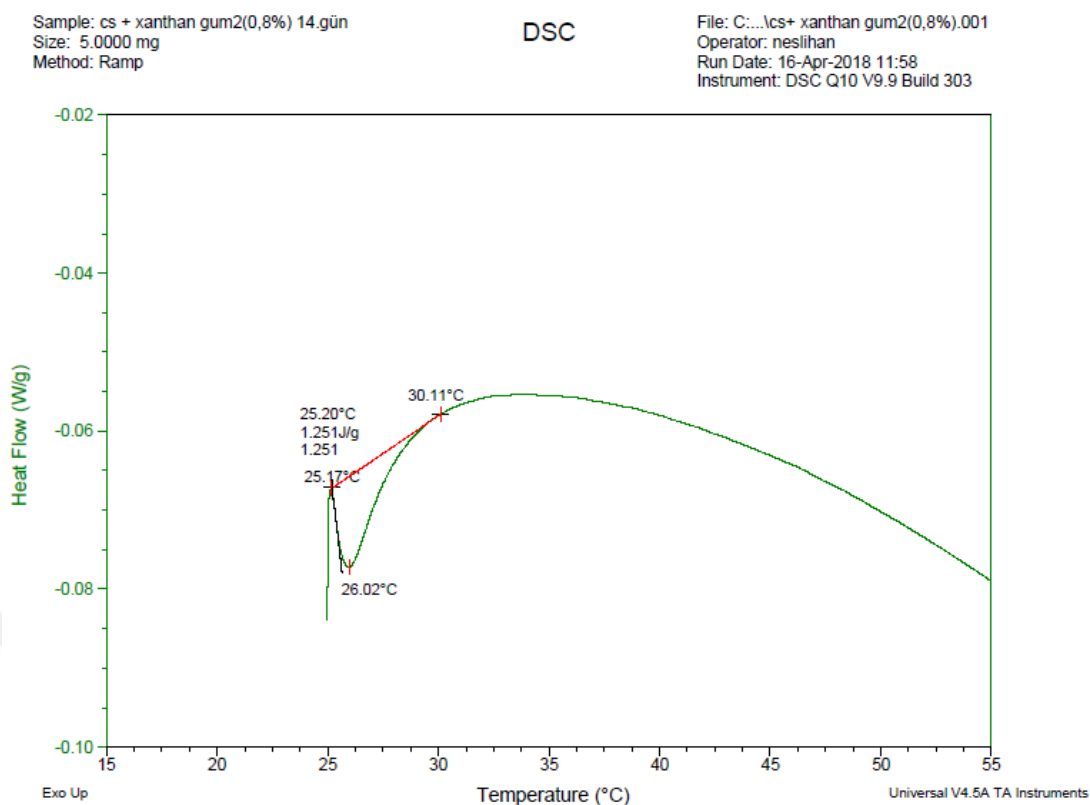


Figure B.7 : Gelation transition for corn starch (5%)+xanthan gum(0,8%)

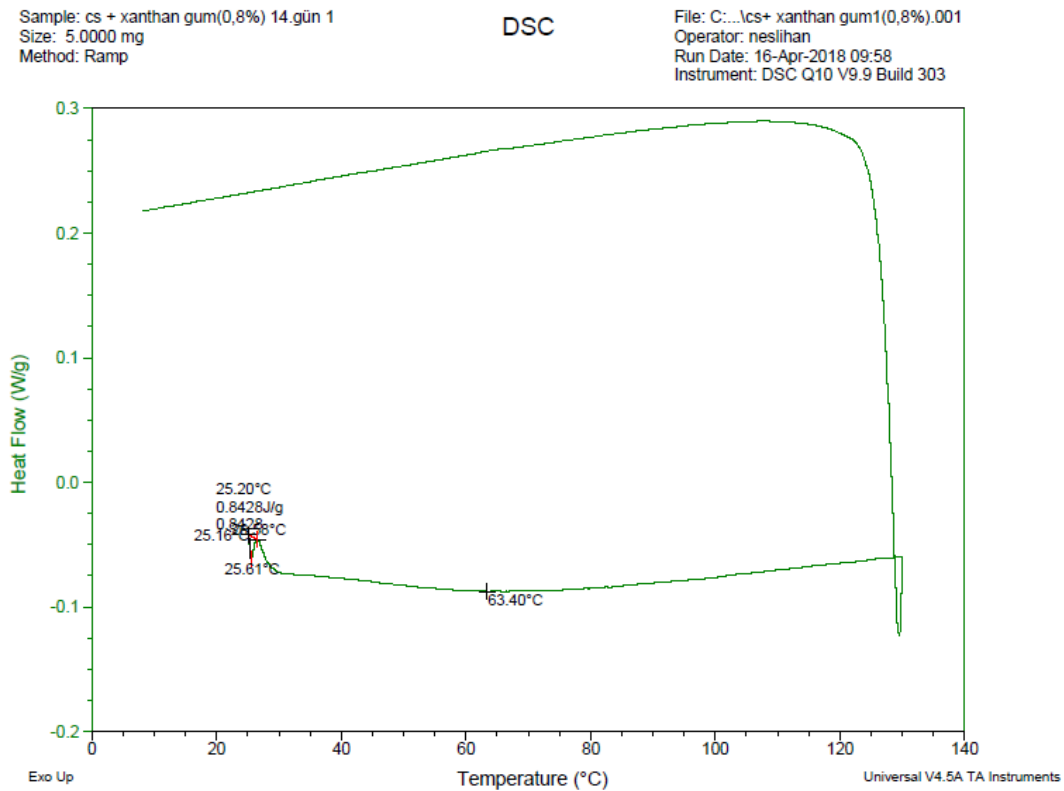


Figure B.8 : Thermogram for corn starch (5%)+xanthan gum(0,8%)

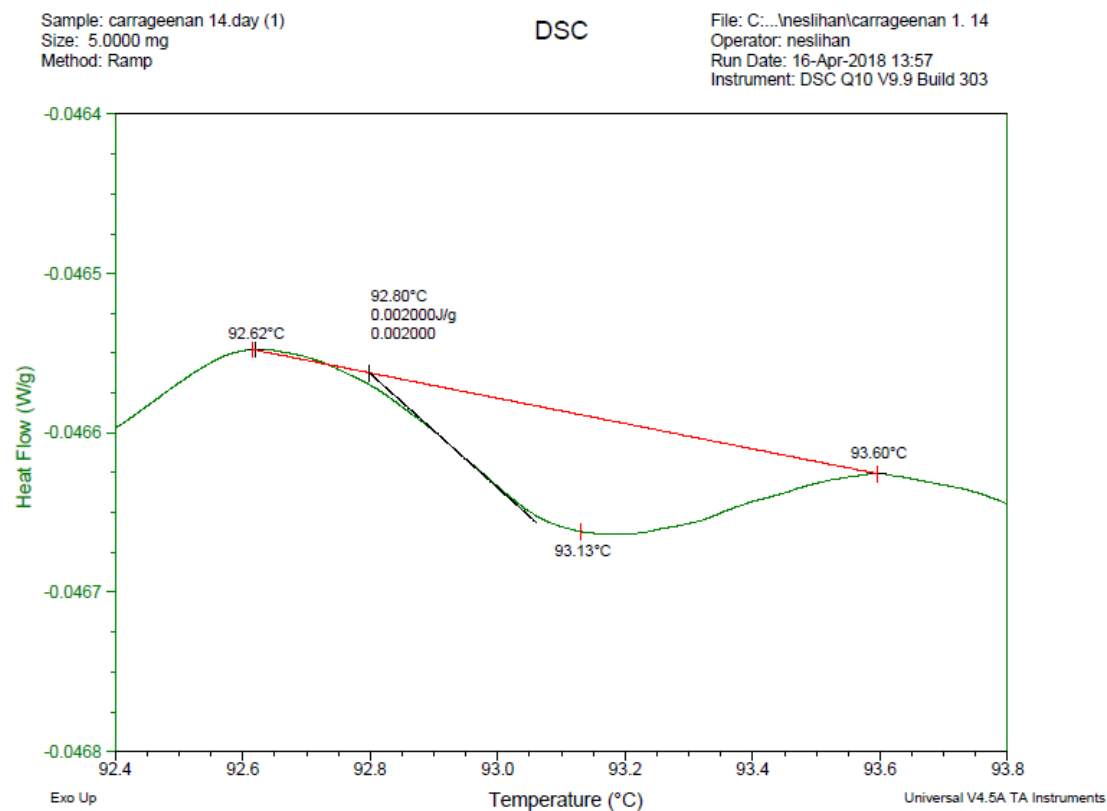


Figure B.9 : Gelation transition for carrageenan (5%)

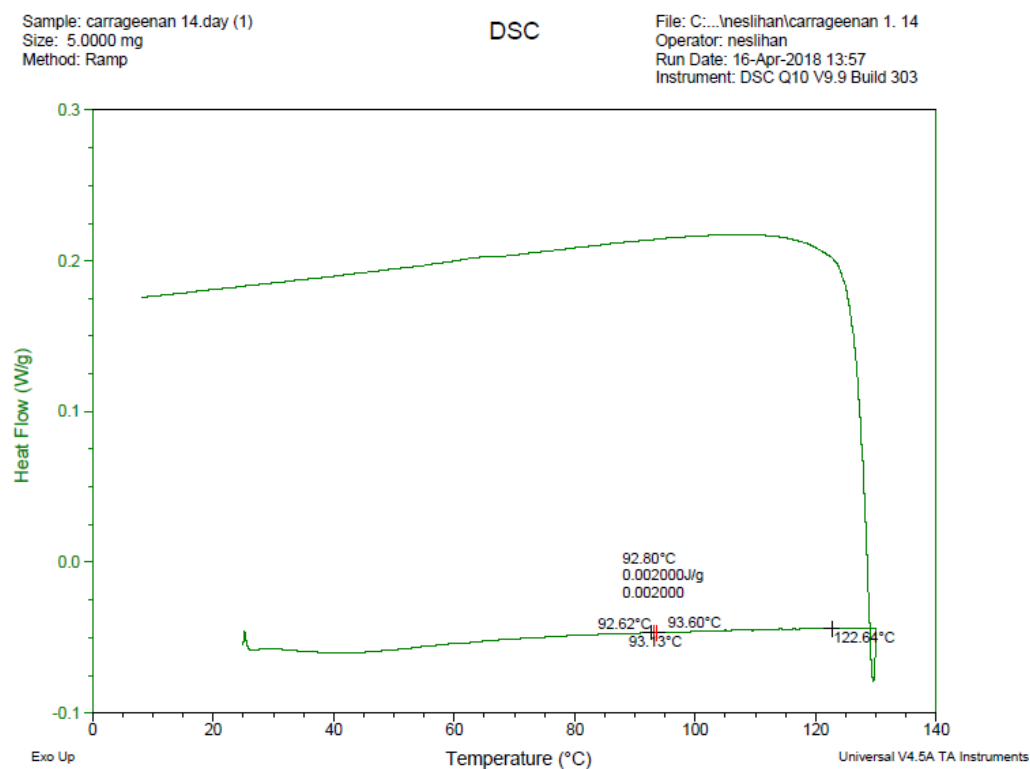


Figure B.10 : Thermogram for carrageenan (5%)

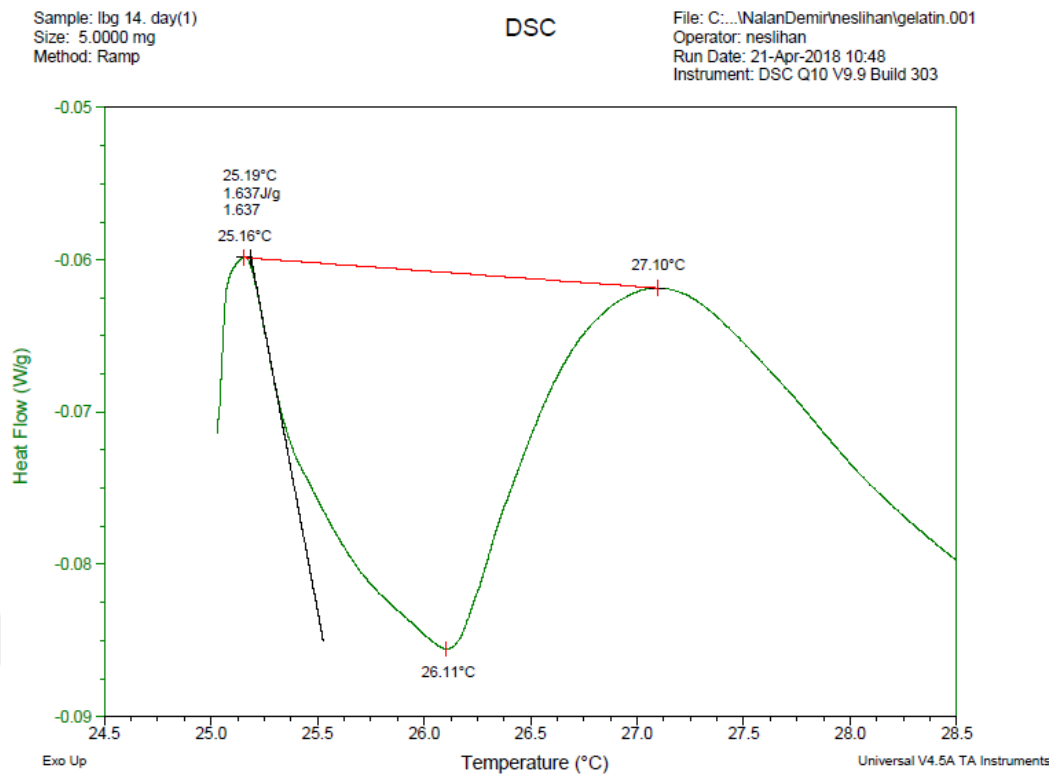


Figure B.11 : Gelation transition for locust bean gum (5%) (parallel 1)

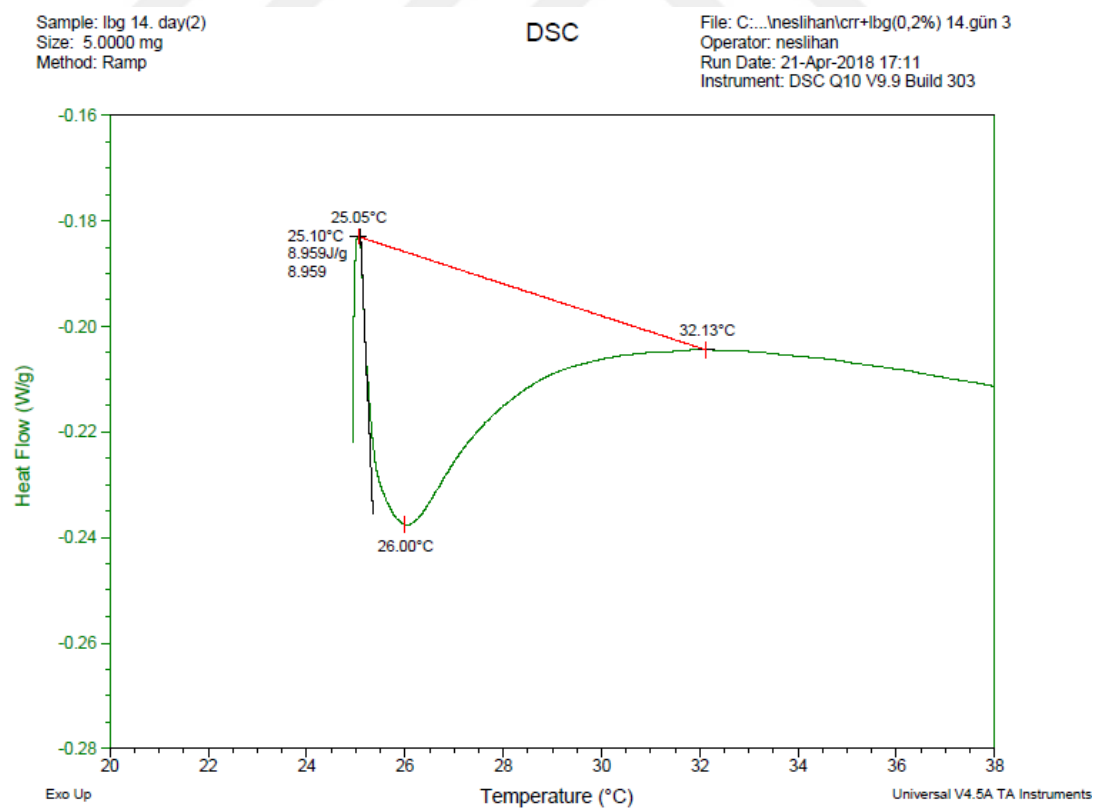


Figure B.12 : Gelation transition for locust bean gum (5%) (parallel 2)

Sample: lbg 14. day(1)
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...NalanDemir\neslihan\gelatin.001
Operator: neslihan
Run Date: 21-Apr-2018 10:48
Instrument: DSC Q10 V9.9 Build 303

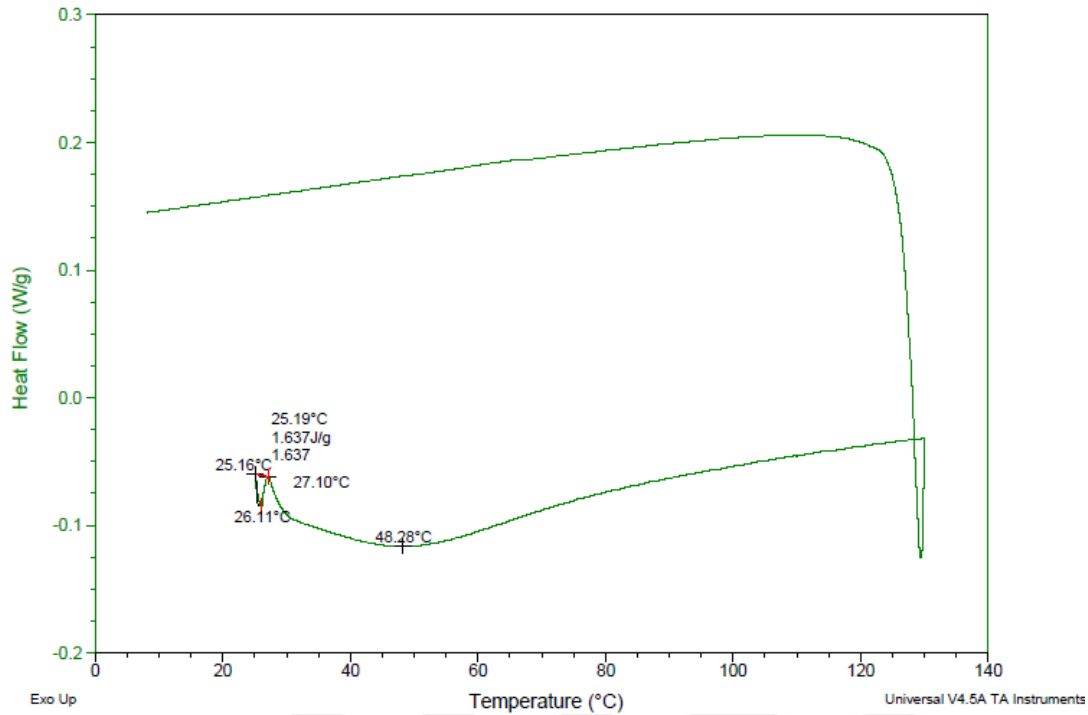


Figure B.13 : Thermogram for locust bean gum (5%) (parallel 1)

Sample: lbg 14. day(2)
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\crr+lbg(0,2%) 14.gün 3
Operator: neslihan
Run Date: 21-Apr-2018 17:11
Instrument: DSC Q10 V9.9 Build 303

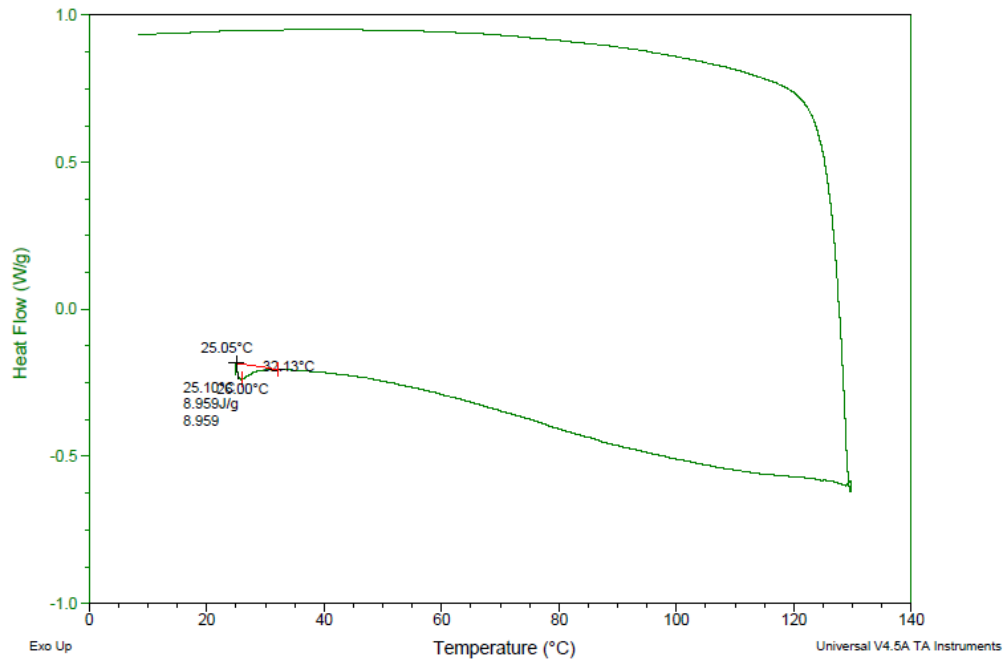


Figure B.14 : Thermogram for locust bean gum (5%) (parallel 1)

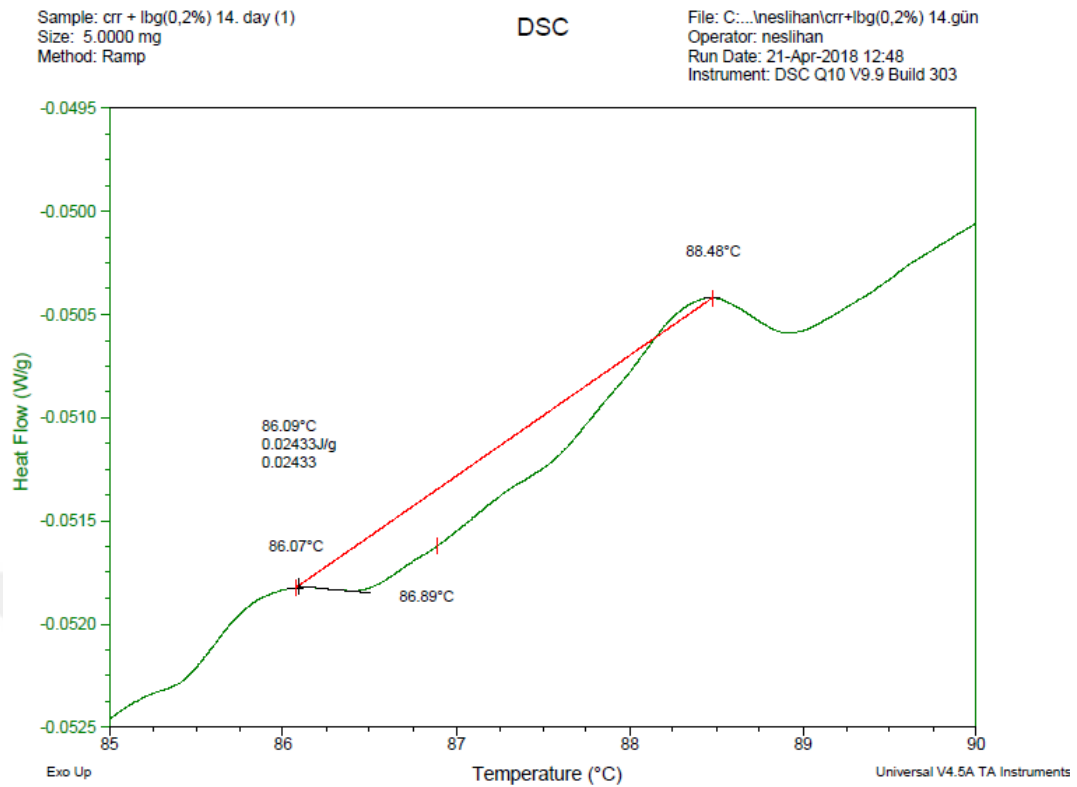


Figure B.15 : Gelation transition for carrageenan (5%)+locust bean gum (0,2%) (parallel 1)

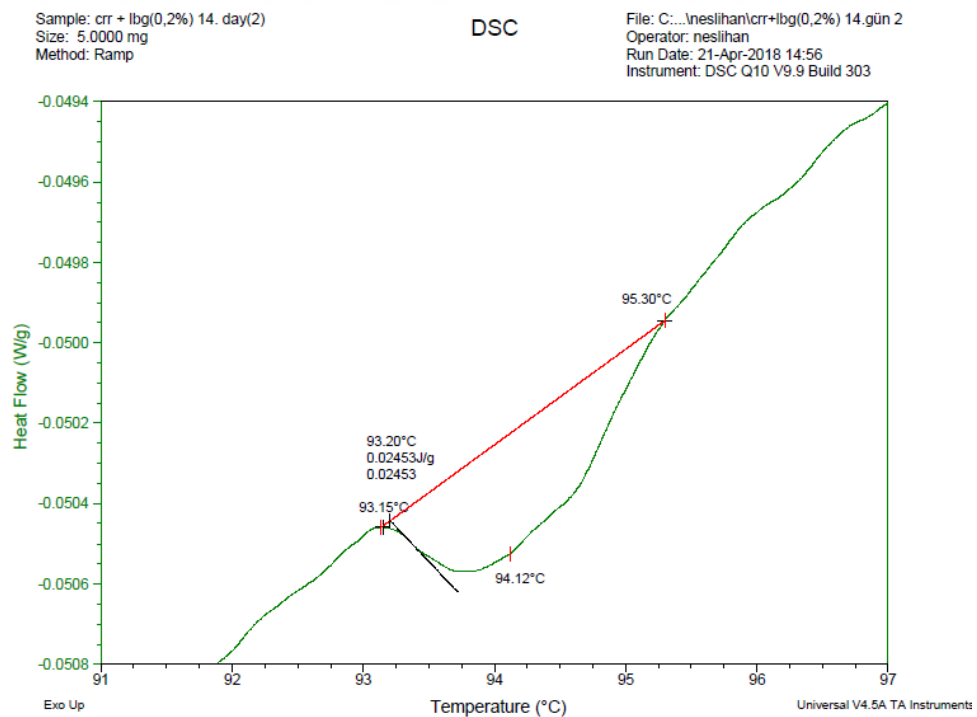


Figure B.16 : Gelation transition for carrageenan (5%)+locust bean gum (0,2%) (parallel 2)

Sample: crr + lbg(0,2%) 14. day (1)
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\crr+lbg(0,2%) 14.gün
Operator: neslihan
Run Date: 21-Apr-2018 12:48
Instrument: DSC Q10 V9.9 Build 303

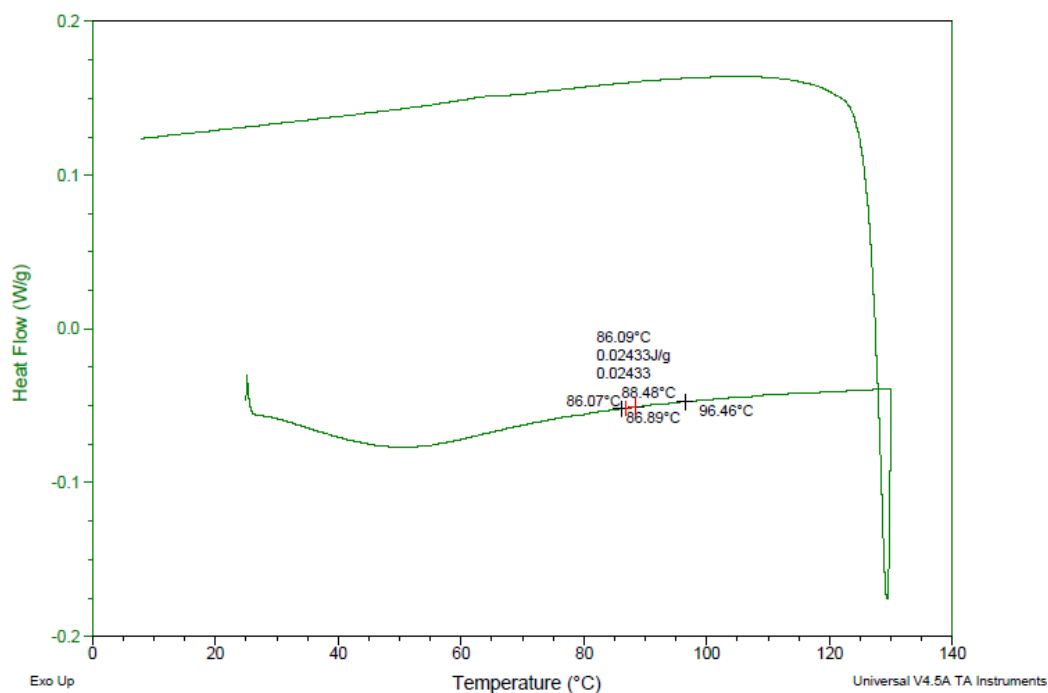


Figure B.17 : Thermogram for carrageenan (5%)+locust bean gum (0,2%) (parallel 1)

Sample: crr + lbg(0,2%) 14. day(2)
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\crr+lbg(0,2%) 14.gün 2
Operator: neslihan
Run Date: 21-Apr-2018 14:56
Instrument: DSC Q10 V9.9 Build 303

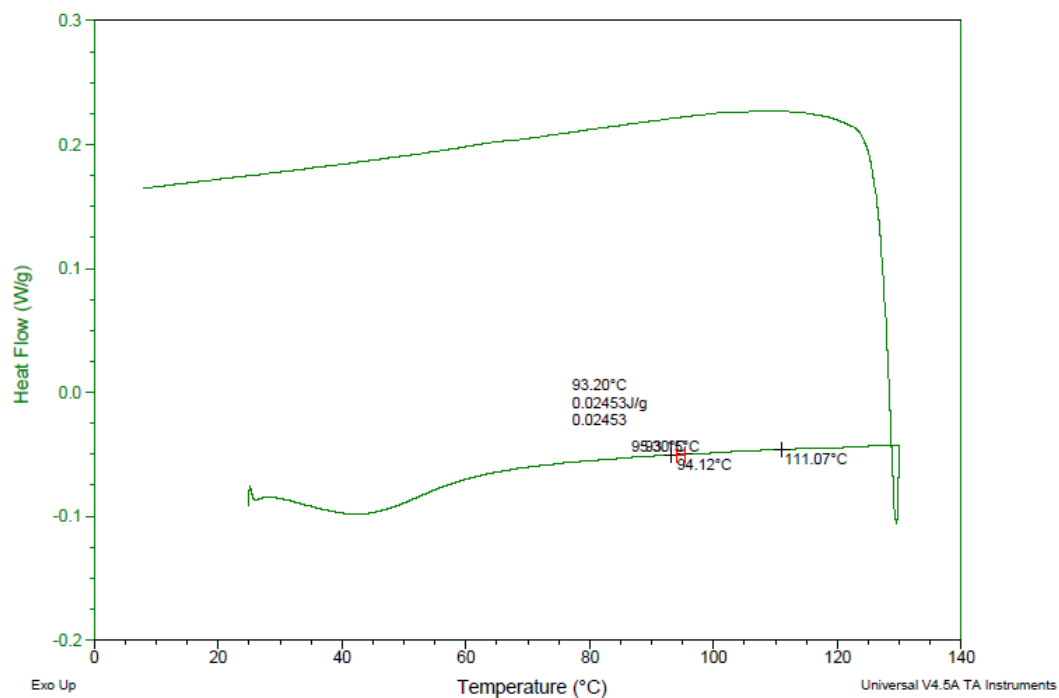


Figure B.18 : Thermogram for carrageenan (5%)+locust bean gum (0,2%) (parallel 2)

Sample: crr + lbg(0,8%) 14.day 1
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\crr + lbg(0,8%) 14.gün 1
Operator: neslihan
Run Date: 22-Apr-2018 11:04
Instrument: DSC Q10 V9.9 Build 303

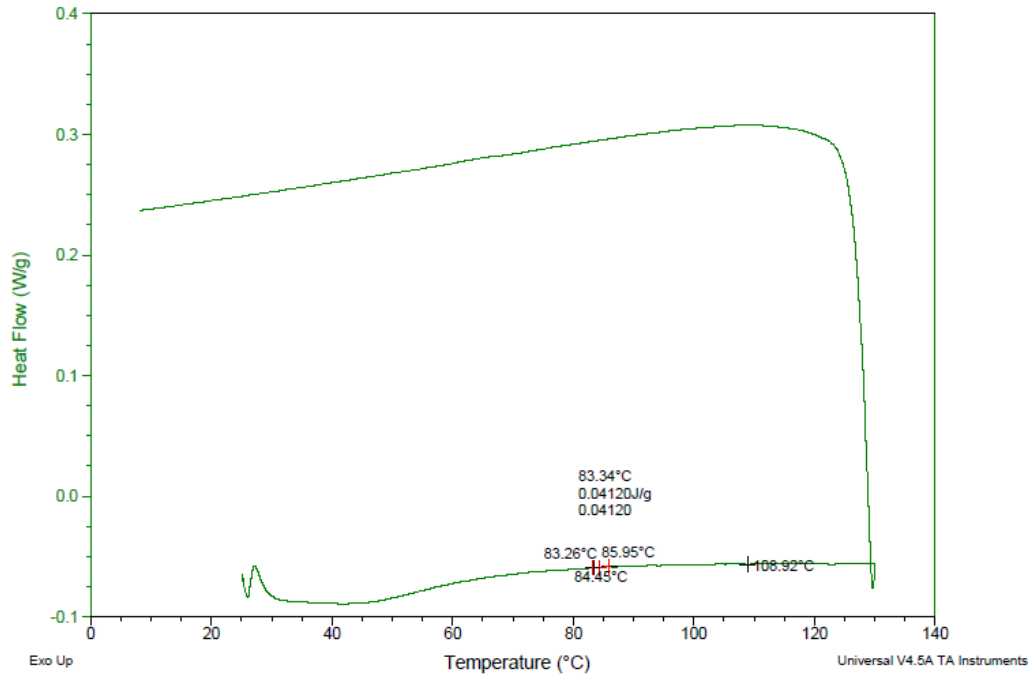


Figure B.19 : Thermogram for carrageenan (5%)+locust bean gum (0,8%) (parallel 1)

Sample: crr + lbg(0,8%) 14.gün 2
Size: 5.0000 mg
Method: Ramp

DSC

File: C:\...neslihan\crr + lbg(0,8%) 14.gün 2
Operator: neslihan
Run Date: 22-Apr-2018 13:04
Instrument: DSC Q10 V9.9 Build 303

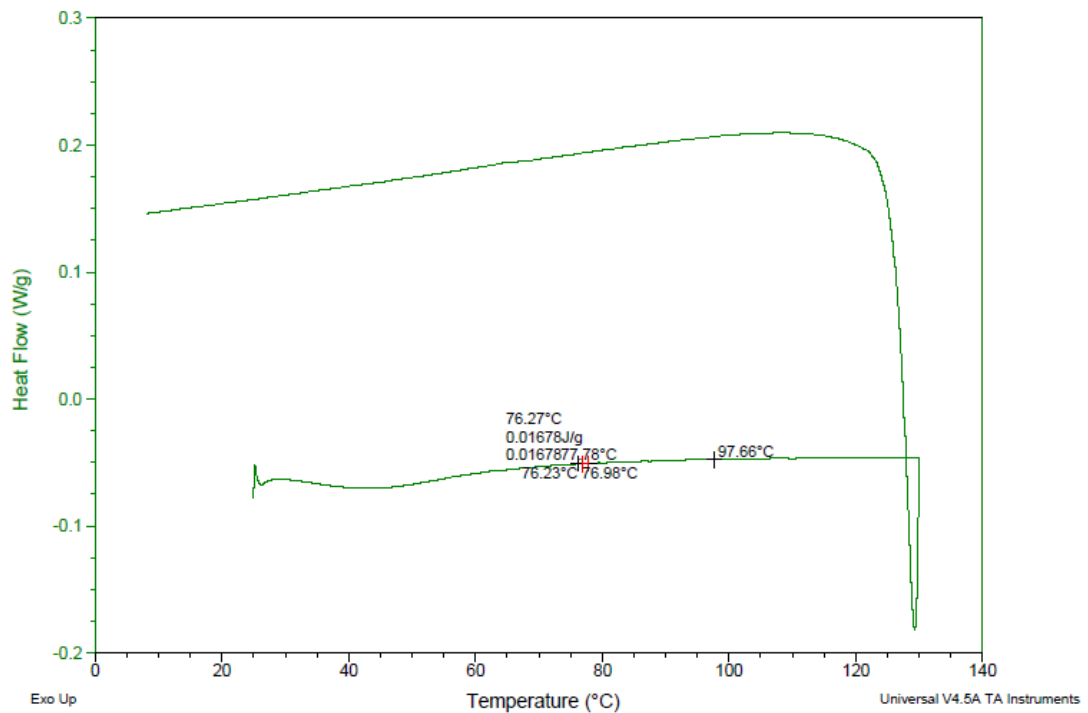


Figure B.20 : Thermogram for carrageenan (5%)+locust bean gum (0,8%) (parallel 2)

APPENDIX C: Photos of Hydrocolloids (25% solutions)



Figure C.1 : Starch (5%)



Figure C.2 : Xanthan gum (5%)



Figure C.3 : Starch (5%)+xanthan gum (0,2%)



Figure C.4 : Starch (5%)+xg(0,8%)



Figure C.5 : Carrageenan (5%)



Figure C.6 : Locust bean gum (5%)



Figure C.7 : Carrageenan + locust bean gum (0,2%)



Figure C.8 : Figure C.8: Carrageenan + locust bean gum (0,8%)

APPENDIX D: Statistical Analysis

Table D.1 : Statistical analysis results for starch types

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
aw	Between Groups	0,011	4	0,003	0,147	0,963
	Within Groups	0,714	40	0,018		
	Total	0,725	44			
moisture	Between Groups	7,985	4	1,996	0,091	0,985
	Within Groups	881,010	40	22,025		
	Total	888,995	44			
pH	Between Groups	0,444	4	0,111	2,453	0,061
	Within Groups	1,811	40	0,045		
	Total	2,255	44			

Table D.2 : Statistical analysis results of starch and xanthan gum

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
aw	Between Groups	0,016	2	0,008	0,67	0,5
	Within Groups	0,722	6	0,012		
	Total	0,739	6			
moistur	Between Groups	134,682	2	67,341	1,77	0,1
	Within Groups	2280,759	6	38,013		
	Total	2415,442	6			
pH	Between Groups	1,694	2	0,847	4,51	0,0
	Within Groups	11,259	6	0,188		
	Total	12,953	6			
hardnes	Between Groups	190806,465	2	95403,232	6,51	0,0
	Within Groups	879203,002	6	14653,383		
	Total	1070009,467	6			
brittlen	Between Groups	390433,081	2	195216,541	20,2	0,0
	Within Groups	578420,361	6	9640,339		
	Total	968853,442	6			

Table D.3 : Statistical analysis results of carrageenan and locust bean gum

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
aw	Between Groups	0,016	2	0,008	1,0	0,3
	Within Groups	0,454	60	0,008		
	Total	0,470	62			
moist	Between Groups	123,881	2	61,940	1,5	0,2
	Within Groups	2349,106	60	39,152		
	Total	2472,987	62			
pH	Between Groups	1,204	2	0,602	4,6	0,0
	Within Groups	7,700	60	0,128		
	Total	8,905	62			
hardn	Between Groups	187363,127	2	93681,564	4,8	0,0
	Within Groups	1168743,380	60	19479,056		
	Total	1356106,508	62			
brittle	Between Groups	288821,770	2	144410,885	9,5	0,0
	Within Groups	903250,112	60	15054,169		
	Total	1192071,883	62			

Table D.4 : Statistical analysis of DSC values.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
T0	Between groups	8893,740	7	1270,534	6,25	0,01
	Within Groups	1624,805	8	203,101		
	Total	10518,545	15			
Tp	Between Groups	10119,753	7	1445,679	6,80	0,00
	Within Groups	1700,448	8	212,556		
	Total	11820,200	15			
delt	Between Groups	2662,986	7	380,427	0,88	0,55
	Within Groups	3426,704	8	428,338		
	Total	6089,690	15			
Tw	Between Groups	10439,597	7	1491,371	0,71	0,66
	Within Groups	16649,825	8	2081,228		
	Total	27089,423	15			
Tc	Between Groups	12560,936	7	1794,419	2,07	0,16
	Within Groups	6916,825	8	864,603		
	Total	19477,761	15			

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