SECTION 5: THESIS DETAILS

NUMBER OF PAGES

Page number on the last page of this document: 105

DEDICATION

To my family

ACKNOWLEDGMENTS*

I would like to express my gratefulness to my supervisor Assoc.Prof. Dr. Mecit, Halil Öztop for his endless support, encouragement, assistance and most importantly for his patience from beginning to end.
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Finally, I would like to express my sincere gratitude to my beloved family; my father Mehmet Narin, my mother Fatma Narin, my brothers Ozan Narin and Eren Narin and their beloved wives who are more than sisters to me Dilek Narin and Görkem Narin, my niece Ezgi Leyla Narin for their endless love, invaluable support and their beliefs in me throughout my life. Everything I have accomplished in my life happened thanks to my family

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* If you have received project support from TÜBİTAK, you must mention about it.

ENCAPSULATION OF PEA PROTEIN IN ALGINATE MATRIX BY COLDSET GELATION METHOD AND USE OF THE CAPSULES IN FRUIT JUICES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

CEREN NARİN

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
FOOD ENGINEERING

DECEMBER 2019

Approval of the thesis:

ENCAPSULATION OF PEA PROTEIN IN ALGINATE MATRIX BY COLDSET GELATION METHOD AND USE OF THE CAPSULES IN FRUIT JUICES

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I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

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ABSTRACT

ENCAPSULATION OF PEA PROTEIN IN ALGINATE MATRIX BY COLDSET GELATION METHOD AND USE OF THE CAPSULES IN FRUIT JUICES

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Master of Science, Food Engineering
Supervisor: Assoc. Prof. Dr. Mecit Halil Öztop
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December 2019, 105 pages

Plant based proteins gained importance in recent years due to the increase in the awareness of healthy diet and the increase in the consumption of plant-based foods. However, some features of plant-based proteins like the undesirable odor and flavor affect the sensorial properties of food they are added in. Therefore, encapsulation of these proteins could be a good strategy to tackle with this problem. It is also important to design stable microcapsules which would remain intact in the food they are used. In this study, microcapsules were designed and evaluated in terms of physical and functional properties. The objective of this study is to design microcapsules (beads) consisting of pea protein isolate by using sodium alginate as the coating material and cold gelation method as the encapsulation technique and to investigate the effect of different alginate concentrations (1, 1.5 and 2%) and heating (at 80ºC for 30 mins) of proteins on the protein content, encapsulation efficiency, particle size, bead stability and the morphology of the capsules. Additionally, TD-NMR relaxometry analysis was also conducted to observe the changes in the beads related to change in parameters. Spin-spin relaxation (T_2) time measurements were conducted to extract information on the microstructure of the beads. Microcapsules were also added to the real fruit juices (melon and pomegranate) since the goal was to enhance to protein content of

juices by masking the flavour through encapsulation. To understand the suspension behavior of the beads, pectin was added to the juices (melon and pomegranate) at different ratios (0.5 and 1%) and the effects of pectin on the rheological behavior of the juices were investigated. Effect of pectin on the rheological properties juices was also was investigated since it could affect the release of the proteins from the capsules. Beads formed with 1.5% alginate was found to have the highest particle size for both samples regardless of the heat treatment ($p<0.05$). Heat treatment significantly increased the particle size of the samples ($p<0.05$). Results showed that both heat treatment and change in alginate ratio did not have change the encapsulation efficiency significantly ($p<0.05$). Also, protein content of the beads significantly decreased with heat treatment ($p<0.05$). SEM images showed that both alginate ratio and heat treatment resulted in change of the surface morphology of the beads. NMR relaxometry results demonstrated that as alginate ratio increased, T_2 relaxation time decreased and non-heated samples had longer T_2 values. Denaturation of the proteins with heating had a direct effect on the mobility of the protons thus T_2 values decreased. Difference in pectin ratio was found to affect the viscosity of the juices. As pectin ratio increased, viscosity of both juices increased significantly ($p<0.05$). Melon juice was found to be more suitable in terms of the increase in viscosity and release rate of beads in juices. Results indicated that as alginate ratio increased and pectin ratio decreased, release of pea protein from alginate beads significantly increased ($p<0.05$). Finally, heat treatment was found to be effective on the release of the protein from beads. It significantly increased the release rate of the core material. In overall, it was concluded that, alginate was a suitable coating to encapsulate pea protein isolate and increase the protein content of the juices. As a next step, sensorial analysis would be performed to test the flavor masking power of the capsules on the juices.

Keywords: Pea Protein, Fruit Juice, Alginate, Encapsulation, Cold Set Gelation

ÖZ

SOĞUK JELLEŞME METODU İLE ALJİNAT MATRİSİNDE BEZELYE PROTEİNİNİN KAPLANMASI VE KAPSULLERİN MEYVE SULARINDAKULLANIMI

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Aralık 2019, 105 sayfa

Bitkisel kaynaklı proteinler, sağlıklı beslenme bilincindeki artış ve bitkisel kaynaklı gıda tüketimindeki artış nedeniyle son yıllarda önem kazanmıştır. Ancak istenmeyen koku ve tat gibi bitki bazlı proteinlerin bazı özellikleri, içine katıldıkları ürünlerin duyusal özellikleri olumsuz yönde etkilemektedir. Enkapsülasyon bitkisel kaynaklı proteinlerin bahsedilen olumsuz özelliklerini engellemede etkili bir yöntem olarak düşünülmektedir. Aynı zamanda gıda ile temas halinde bozulmadan kalabilecek, kararlı kapsüllerin oluşturulması da önem arz etmektedir. Bu çalışmada oluşturulan mikrokapsüller fiziksel ve fonksiyonel özellikler açısından değerlendirilmiştir. Bu çalışmanın amacı, kaplama malzemesi olarak sodyum aljinat ve kapsülleme tekniği olarak soğuk jelasyon yöntemi kullanılarak bezelye proteini izolatından mikrokapsüller (boncuklar) tasarlamak ve farklı aljinat konsantrasynları (%1, 1.5 ve 2) ile proteinlere uygulanan ısıl işlemin (80 ° C'de 30 dakika boyunca) protein içeriği, kapsülleme verimliliği, parçacık boyutu, boncuk stabilitesi ve kapsüllerin morfolojisi üzerine etkisini araştırmaktır. Ek olarak, parametrelerdeki değişimin kapsül özellikleri üzerindekini etkisini anlamak amacı ile nükleer manyetik rezonans (TD-NMR) analizleri yapılmıştır. Boncukların mikro yapısı hakkında bilgi elde etmek için gevşeme (T2) zaman ölçümleri yapılmıştır. Mikrokapsüller, gerçek meyve sularına

(kavun ve nar) da eklenmiştir, çünkü amaç, kapsülleme yoluyla istenmeyen koku ve tadın maskelenmesiyle meyve sularının protein içeriğini arttırmaktır. Tanelerin süspansiyon davranışını anlamak için, meyve sularına farklı oranlarda (% 0.5 ve% 1) pektin eklenmiş ve pektinin meyve sularının reolojik davranışı üzerindeki etkileri araştırılmıştır. Proteinlerin kapsüllerden salımını etkileyebileceği düşünülerek, pektinin meyve sularının reolojik özellikleri üzerindeki etkisi de araştırılmıştır. % 1.5 aljinat ile oluşturulan tanelerin, ısıl işlemden bağımsız olarak her iki örnek için en yüksek partikül boyutuna sahip olduğu bulunmuştur ($p < 0.05$). Isıl işlem numunelerin partikül boyutunu önemli ölçüde arttırdığı gözlemlenmiştir ($p < 0.05$). Sonuçlar hem ısıl işlemin hem de aljinat oranındaki değişimin kapsülleme verimliliğini önemli ölçüde değiştirmediğini göstermiştir. Ayrıca, boncukların protein içeriği ısıl işlemle önemli ölçüde azalmıştır ($p < 0.05$). SEM görüntüleri hem aljinat oranının hem de ısıl işlemin taneciklerin yüzey morfolojisinde değişikliğe yol açtığını göstermiştir. NMR gevşetme sonuçları, aljinat oranı arttıkça T2 gevşeme süresinin azaldığını ve ısıtılmayan numunelerin daha uzun T2 değerlerine sahip olduğunu göstermiştir. Proteinlerin ısıtma ile denatürasyonu, protonların hareketliliği üzerinde doğrudan bir etkiye sahiptir, bu nedenle T2 değerleri azalmıştır. Pektin oranındaki farkın meyve sularının viskozitesini etkilediği bulunmuştur. Pektin oranı arttıkça, her iki meyve suyunun viskozitesi önemli ölçüde artmıştır ($p < 0.05$). Kavun suyunun, meyve sularındaki boncukların viskozitesindeki artış ve salım oranı açısından daha uygun olduğu bulunmuştur. Sonuçlar, aljinat oranı arttıkça ve pektin oranı azaldıkça, aljinat boncuklarından bezelye proteini salınmasının önemli ölçüde arttığını göstermektedir ($p < 0.05$). Son olarak, ısıl işlemin proteinin boncuklardan salınmasında etkili olduğu bulunmuştur. Isıl işlem proteinlerin salım oranını önemli ölçüde arttırmıştır. Genel olarak, aljinatın bezelye proteini izolatını kapsüllemek ve meyve sularının protein içeriğini arttırmak için uygun bir kaplama olduğu sonucuna varılmıştır. Bir sonraki adım olarak, kapsüllerin meyve suları üzerindeki lezzet maskeleme gücünü test etmek için duyusal analiz yapılacaktır.

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Anahtar Kelimeler: Bezelye Proteini, Meyve Suyu, Aljinat, Enkapsülasyon, Soğuk Jelleşme

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ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

Fruit is the fleshy and edible part of the plants that contains seeds of the plant. Although it differs according to the region or climate they grow, fruits contain most macronutrients and micronutrients that are important for human health and therefore have a great importance in daily diet.

Fruits support the body function with the beneficial substances like vitamins and minerals, antioxidant or phenolic substances. For example; citrus class fruits help strengthen the immune system since they are high in vitamin C; berry class fruits contain antioxidant substances that helps to prevent the problems caused by free radicals. In recent years, rather than consumption of fruits, consumption of important substances in fruits as a supplement has increased. However, research has shown that instead of consuming as a supplement, consuming it as a fruit is more effective in terms of the effect of nutrients on the body (Kader, 2001). However, the consumption of fruit is not preferred much by the consumers. In today's busy life, consumers prefer foods that they can consume more easily. In this regard, the most preferred way in terms of fruit consumption is fruit juice.

1.1. Fruit Juice

Fruit juice is a type of beverage counted in soft drink category. There is more than one definition about fruit juice, but it is, in the most basic sense, the water inside the cells of fruits. In a more complex and detailed definition, Codex Alimentarius Commission's define fruit juice as "*the unfermented but fermentable liquid, obtained by mechanical extraction processes for single strength juices not from concentrate or by physical processes for all other juice forms*" (The & Nations, 1999). It can be

obtained by directly pressing the edible part of the fresh fruits or applying physical or chemical treatment to extract the juice.

History of fruit juice consumption dates to ancient times. The first scripts found that mentioned about juice are assumed to belong in ancient Greek area (Rajauria & Tiwari, 2017). Over years fruit juices have been a part of people's daily life and it gains more importance year by year. Researches show that between 1999 and 2004, market for fruit juice and juice drinks reached £2.32 billion, with a growth rate of 37% (Caswell H, 2009)**.** One of the reasons for this increase is the increasing perception of '*healthy living*' among the consumers. People's life order has changed and as in everything, they are directed to fast consumption in the diet. Therefore, there has been a rapid increase in consumption of ready-to-eat foods. In recent years, both healthy and ready-to-eat foods are frequently preferred by consumers. At this stage, fruit juices attracted attention with its rich content, being suitable for fast consumption, being a product that every segment can buy, and most importantly being healthy. All these caused the market to grow rapidly.

Healthy and regular diet has been suggested to be effective in preventing and treating some diseases and the researches have shown results to prove this theory (Doyon & Labrecque, 2014). This understanding led to a new trend in the food sector and people started to consume food not only to satisfy hunger, but also to protect the body from diseases. As a result of these trends, functional foods that are suggested to have positive effects on health have started to attract attention.

1.2. Functional Foods

The name of functional food was first mentioned in the 1980s in Japan and it was defined as food products fortified with beneficial ingredients that has positive physiological effects on body (Corbo, Bevilacqua, Petruzzi, Casanova, & Sinigaglia, 2014).

There are multiple definitions for functional foods. Definitions are sometimes referred to as enhanced, fortified or enriched foods, while others are referred to as foods that

help prevent a specific disease or health problem. Functional juices can help maintain the general conditions of the body or may help to improve it. Some types of functional foods are especially designed to have these effects. Probiotic added juices or collagen added juices can be examples of this type. Also, functional foods can be good for decreasing the risk of a certain disease or even curing some diseases. The European Commission's Concerted Action on Functional Food Science in Europe (FuFoSE), coordinated by International Life Science Institute (ILSI) Europe defined functional food as follows: ''a food product can only be considered functional if together with the basic nutritional impact it has beneficial effects on one or more functions of the human organism thus either improving the general and physical conditions or/and decreasing the risk of the evolution of diseases (Siró, Kápolna, Kápolna, & Lugasi, 2008).

Functional foods can be divided into four categories. These categories are fortified foods, enriched foods, altered foods and enhanced commodities (Siró et al., 2008). Fortified products can be defined as a food product that is fortified with additional nutrients. In order to be named as fortification, additional nutrients should be found in the food naturally. Fruit juices that are fortified with vitamins can be an example for this type. Also, protein added fruit juices are counted as fortified products. Enriched products are basically same with fortified products. Enriched products should also contain additional nutrients. However, in this case additional nutrients should be new nutrients and should not be normally found in the food product. Most of the functional foods can be counted in this category. Probiotic added can be examples of the enriched products. Altered foods are the foods that a deleterious component inside the food has been replaced with other substances to have beneficial effects. Finally, enhanced commodities can be defined as a food in which one of the components has been naturally enhanced by generating different growth conditions, or genetic modification (Mocanu & Botez, 2012).

Under these categories, there are various segments of functional juices available in the market. The categories that have the highest share in this market belong to energy

drinks followed by sports drinks, nutrient enhanced drinks, and dairy based drinks (Papolulis & Pillai, 2012) (Corbo et al., 2014).

Dairy-based functional beverages generally include probiotic bacteria. Probiotic are beneficial bacteria living in the human body and are of great importance for human health. The most important effect of probiotic bacteria on human body is regulating the digestive system. They also help to strengthen the immune system. Fermented products such as yogurt and kefir naturally contain probiotic bacteria; therefore, this function is mostly preferred in dairy based drinks. However, in recent years, probiotic bacteria have been used together with different foods, such as juices.

Sports drinks are water products that are often enriched with various vitamins and minerals, designed to maintain the body's electrolyte balance before, during or after sports. Isotonic, hypertonic and hypotonic are types of sport drinks to meet the body's water and sugar needs for different sport activities (Lebensmittelsicherheit, 2001).

Energy drinks are a group of beverages used by consumers to provide an extra boost in energy. In order to provide these functions, mostly caffeine is used as active ingredient. (Ishak, Ugochukwu, Bagot, Khalili, & Zaky, 2012).

Energy drinks and sports drinks are the segments that have the biggest share in functional beverage sales. These two categories account for 82% of the functional beverage sales. However, based on growth rates, it is seen that protein drinks have the strongest growth among other segments. In 2013, the market share of the functional beverage category grew by 7.4%, while the growth rate of the protein drinks segment alone was 7.9% (Papolulis & Pillai, 2012).

Protein is one of the most abundant and important molecules in the body. Protein has several benefits on human body. Help on the growth and maintaining the body mass can be some good examples of the benefits of proteins. Every cell and substance like enzymes or hormones in the body contains protein (Hermann, n.d.). Protein molecules consist of different amino acids. There are 20 different types of amino acid and human body cannot utilize all of them. Nine of these amino acids are named as essential amino

acids since they cannot be synthesized in the body. Therefore, these amino acids should be taken by foods through our diet. Since the deficiency of these amino acids will cause problems in the body, the amount of protein to be taken daily has been determined by the authorities. This amount was determined to be 0.8 g of quality protein / kg (Institute of Medicine, 2002). For athletes or for people who aim to build muscles, this amount increases to 1.4-2 g / kg (Banaszek et al., 2019). Protein supplementation is especially necessary in athletes to ensure enough protein intake during the day. Based on an adult male, average of 70 kg, the daily protein requirement corresponds to 56 grams. For an athlete this amount corresponds to 98-140 grams. It can be hard to reach these amounts only through food during the day. At this stage, protein-supplemented functional foods, especially protein drinks, can be a good alternative.

There are a variety of protein-rich foods that people can consume during the day. Poultry, dairy products, legumes, grains are some of the foods rich in protein. However, the amount and the quality of the protein inside foods can be different. Proteins are divided into two categories as complete proteins and incomplete proteins. Complete proteins are proteins containing all essential amino acids that are needed to support a normal growth and maintain the body conditions. Unlike complete proteins, incomplete proteins do not contain all essential amino acids (Paulsen, 2009). Protein inside the animal-based foods and plant-based foods are different in terms of quality and the protein content. Therefore, proteins are divided into two different categories based on their sources.

Meat, fish, poultry, egg and dairy products constitute the main types of animal-based proteins. Animal based proteins are classified as complete proteins since they contain all the essential amino acids. These types of foods are the foods that people consume most when they want to take protein since they have high protein content. Animal based proteins are the best option to meet daily protein intake. However, animal-based proteins could have some disadvantages. It cannot be consumed by everyone due to the allergenicity reasons (especially milk and egg proteins could be allergic on

infants). In addition, people who follow a diet that prohibits the consumption of animal-based foods such as veganism do not consume animal-based protein.

Plant based proteins have started to attract attention in recent years. With the spread of vegan alimentation, a tendency towards vegetable-based consumption has started among people. Plant based proteins are the most important nutrients in this type of diet. In addition to this feature, it attracts attention with its being a sustainable source.

Plant based proteins are mostly classified as incomplete proteins. Only soybean protein is classified as complete protein amongst the plant based proteins (Paulsen, 2009). Plant based proteins are mostly preferred by people who cannot consume animal-based foods. Most plant-based foods are not considered a good source of protein because they have low protein content but among plant-based foods, legume proteins and grains stand out as high protein foods.

The biggest question about plant protein is whether it has the same effect as animal proteins on the body. Various researches have been made on this subject. People receiving animal-based proteins and plant-based proteins were subjected to the same training program and no significant difference was observed (Banaszek et al., 2019). Likewise, the protein delivery efficiencies of the two protein species were compared (Sabaté & Soret, 2014).

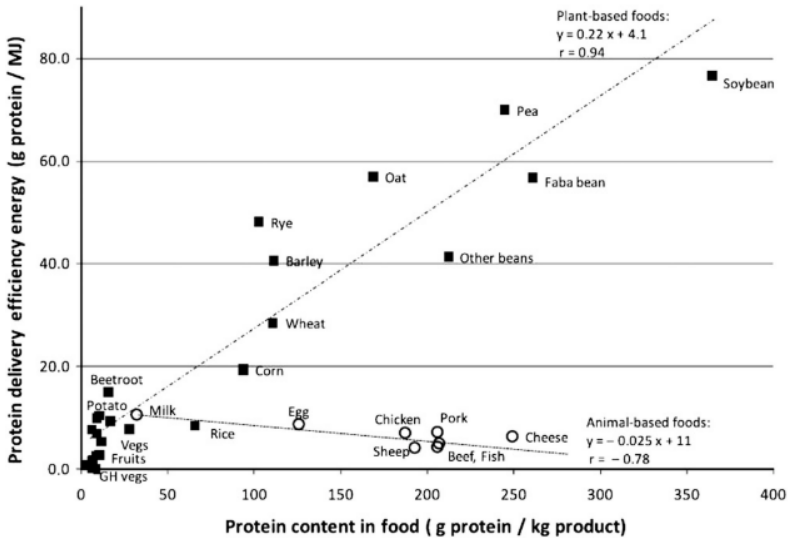


Figure 1.1. Protein delivery efficiency in terms of energy use as a function of food protein content (Sabaté & Soret, 2014)

As can be seen from Figure 1.1, soybean protein and pea protein are the most effective proteins based on protein content and delivery efficiency. Soybean protein is not preferred much because it is known as an allergen. Considering this information, pea protein stands out as an effective source of protein as a plant-based protein.

Pea protein is generally used in plant-based protein containing foods. Pea protein contains a minimum of 80% protein (Sumner, Nielsen, & Youngs, 1981). The high protein content makes pea protein a more suitable option. However, some properties of pea protein make its use in foods undesirable. First, the plant-based proteins have an off odor and off flavor unique to their species. This changes the product's sensorial attributes and creates a negative impression for the consumer. Another problem about pea protein is the texture. This problem especially is observed at low pH foods like fruit juices. The pH range is neutral to basic pH (pH 7.0-8.0), where the pea protein has optimal solubility (Barac et al., 2010). Fruit juices have acidic pH, usually between 3.00-4.00. Since the protein solubility is low at these pH values, it forms a granular structure and prevents smooth texture. Another factor that decreases the solubility of the pea protein is heat treatment. A study showed that solubility of pea protein that

was exposed to heat at 90˚C for 3 min decreased compared to non-treated pea protein sample. Heat treatment parameters used in the study were the parameters used for pasteurization of fruit juices. This showed that pasteurization process had a significant effect on the solubility of pea protein and consequently the texture of the product.

In order to improve the sensorial properties of pea protein added juices, encapsulation can be a good strategy.

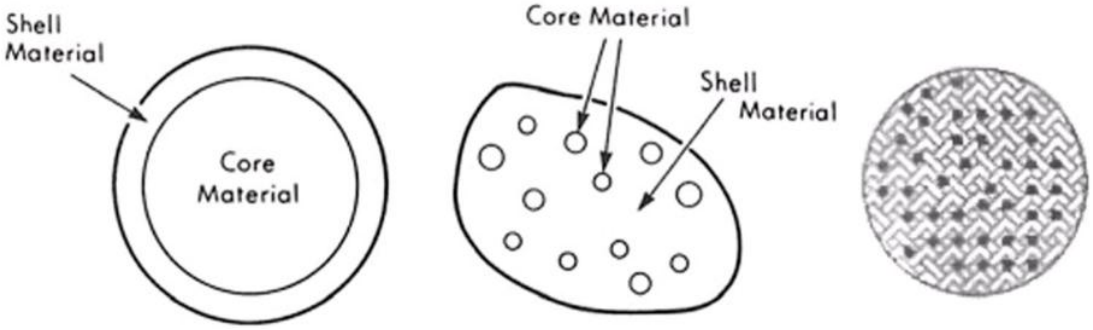
1.3. Encapsulation

Encapsulation is a method that involves the entrapment of an active agent, enzyme or other materials within a small capsule. The material that is coated is named as core material or active material, while the coating material is named as capsule or wall material (Gibbs & Kermasha, 1999). Encapsulation has a wide range of applications. It protects active agents from extreme conditions like high temperature or low pH and hence increase the viability under these conditions. This technology is also effective in improving the delivery of core material into foods (Gibbs & Kermasha, 1999) . Also, in encapsulation the wall material acts as a barrier between the active agent and the environment. This barrier provides to cover some characteristics of active agent such as taste and odor and thus, mask them. Considering these factors, encapsulation technology is mostly used in food, cosmetic and pharmaceutical industry.

In encapsulation technology, capsules are divided into three categories based on the particle size. Macrocapsules or millicapsules are defined as the capsules bigger than 1 mm. Macrocapsules are usually used in cosmetic sector and household products. Since the particle size is large, these capsules are visible inside the product. Microcapsules can be classified as the capsules whose size is between 1 mm and 1000 μm. Microcapsules are used in food, pharmaceutical and cosmetic sector. They are used in most of the encapsulation applications. Nanocapsules are the capsules whose size is between 1nm and 1000 nm. Nanocapsules are used in applications where the capsules not to be detected inside product (Martins, Poncelet, Rodrigues, & Renard, 2017).

The shape and the morphology of the capsules vary depending on core material and

encapsulation method used for capsule formation. Regarding the morphology capsules can be classified into 3 different categories; mononuclear, polynuclear and matrix types (Figure 1.3) (Srivastava, Semwal, & Sharma, 2013). In mononuclear capsules, one core is entrapped within the wall material. They have a spherical shape with continuous core and wall. In polynuclear capsules, more than one core is entrapped within the wall material. This type of capsules has irregular shapes. In matrix capsules, core material should be dispersed homogeneously into wall material. (Das et al., 2011).



I. II. III.

Figure 1.2. Morphology of capsules: I. Mononuclear, II. Polynuclear; III. Matrix (Das et al., 2011)

1.3.1. Encapsulation Methods

There are various number of methods used for encapsulation of active agents. Some of these methods are spray drying, spray cooling, freeze drying, fluidized bed coating, emulsification, coacervation and extrusion methods. These methods are divided into two categories: chemical techniques and mechanical techniques (Table 1.1) (Srivastava et al., 2013).

Table 1.1. Classification of encapsulation techniques

	Encapsulation techniques
Chemical techniques	- Coacervation
	- Emulsification
Mechanical techniques	- Spray drying/cooling
	- Freeze drying
	- Fluidized bed coating
	- Extrusion

1.3.1.1. Spray Drying

Spray drying is an easy to apply and an economical technique. It provides high production rate. Therefore, spray drying is the most widely used encapsulation technique. This method is used in approximately 80-90% percent of the encapsulation applications in food industry (Nedovic, Kalusevic, Manojlovic, Levic, & Bugarski, 2011). By spray drying, capsules are obtained in powder form with small particle size (less than 40 μm), so this method is usually preferred when sensorial properties and texture of final product are not desired to change (Nedovic et al., 2011). This method is suitable for large scale productions. Moreover finished product encapsulated by spray drying method has good stability (Srivastava et al., 2013).

The principle of spray drying is based on evaporating the excess water and obtaining the desired product in powder form and it is only applied on water-based solutions (Nedovic, Kalusevic, Manojlovic, Petrovic, & Bugarski, 2013). Therefore, wall material should be highly soluble in water. Hydrophilic carbohydrate molecules such as gum, modified starch etc. are usually chosen as wall material for encapsulation purposes in spray drying applications. In spray drying method, first wall material is dissolved in water and an emulsion or a suspension is obtained by dispersing the core material into wall material matrix. Then drying chamber is fed by the mixture. In

chamber, mixture is dissociated into small particles with a nozzle. Water inside the particles evaporates by hot air and powder forms.

1.3.1.2. Spray-Chilling

Spray chilling is used to encapsulate active agents with a lipid-based coating material. In this method, wall material is usually vegetable oil or hydrogenated vegetable oil which has a melting point at 32-42ºC (Gibbs & Kermasha, 1999). The principle of spray chilling method involves atomization of the mixture to cool air, enabling wall material adhering onto the active agent and forming a capsule by solidifying. After encapsulation, capsules should be kept at low temperature to prevent melting.

1.3.1.3. Freeze Drying

Freeze drying is a method in which water inside the product is removed by sublimation (Nireesha et al., 2013). In contrast to spray drying method, freeze drying operates at low temperature, below freezing point. Therefore, this method is more suitable for encapsulating heat-sensitive active agents (Nedovic et al., 2013) However, freeze drying method has a few disadvantages. High energy consumption and long operation time are main disadvantages of the method. Another disadvantage of freeze-drying method is wall material forms a porous structure around the active agent. Therefore, capsules could have a poor stability (Nedovic et al., 2011).

1.3.1.4. Fluidized Bed Coating

In this method, active agent or core material is usually used in powder form and aqueous solution of wall material is usually used for coating. Core material is put into the humidity and temperature-controlled chamber and fluidized by high velocity air. Then wall material which is in aqueous form is sprayed into the chamber (Gibbs & Kermasha, 1999). Droplets of wall material form a barrier around the core material and excess water is evaporated by the hot air inside the chamber (Srivastava et al., 2013). This technique is sometimes used to create an additional coating to capsules that is already coated by spray drying method (Nedovic et al., 2013).

1.3.1.5. Emulsification

An emulsion is created by two immiscible liquids. Usually oil and water are used as the immiscible liquids. To form an emulsion one of these liquids should be dispersed into the other liquid in form of droplets (Serdaroğlu, Öztürk, & Kara, 2015). There are several types of emulsions. Water-oil emulsions, oil-water emulsions and water-oil-water double emulsions are types of emulsions (Nedovic et al., 2013).

These systems enhance the bioavailability of products which have poor water solubility. Also, it offers controlled delivery of hydrophilic and hydrophobic agents in one system (Kakran & Antipina, 2014). Therefore, emulsification is a highly used technique for encapsulation especially in pharmaceutical and cosmetic industry.

There are different ways to encapsulate materials by emulsification. Layer by layer, solvent removal and emulsion polymerization technique are just some ways used for encapsulation (Kakran & Antipina, 2014).

1.3.1.6. Coacervation

Coacervation can be defined as the phase separation of colloidal systems (Eghbal & Choudhary, 2018). For coacervation to occur, there should be two oppositely charged hydrocolloids in the system (Korma et al., 2016). That is why proteins and surfactants are widely used in coacervation.

Coacervation technique is usually used to encapsulate lipophilic agents like vitamins, flavors, vegetable oils etc. (Korma et al., 2016)(Nedovic et al., 2013). This method can be divided into two: simple coacervation and complex coacervation (Srivastava et al., 2013). While simple coacervation includes one type of polymer, complex coacervation includes more types of polymer. Electrostatic interactions between the oppositely charged polymers cause a layer formation around the active agent and hence core material is encapsulated.

1.3.1.7. Extrusion

Extrusion is the most common used technique for encapsulation. It is a simple method that has a low operation cost. There are several extrusion methods: *simple extrusion* and *concentric extrusion* are some of the commonly used ones. In simple extrusion, usually an aqueous solution of a polymer is prepared, and the core material is dispersed into this solution. Then the dispersion is dripped into a gelling bath (Martins et al., 2017). Dripping can be done by several tools like syringe, pipette, nozzle etc. Alginate is generally used as the coating material and calcium chloride solution is used as the gelling bath because alginate is able to form a hydrogel in the presence of calcium ions (Nedovic et al., 2011). Capsules obtained by this method is known as polynuclear capsules. In concentric extrusion, core material and polymer solution are extruded with concentric cylinders. This method provide high active agent loading into capsules (Martins et al., 2017).

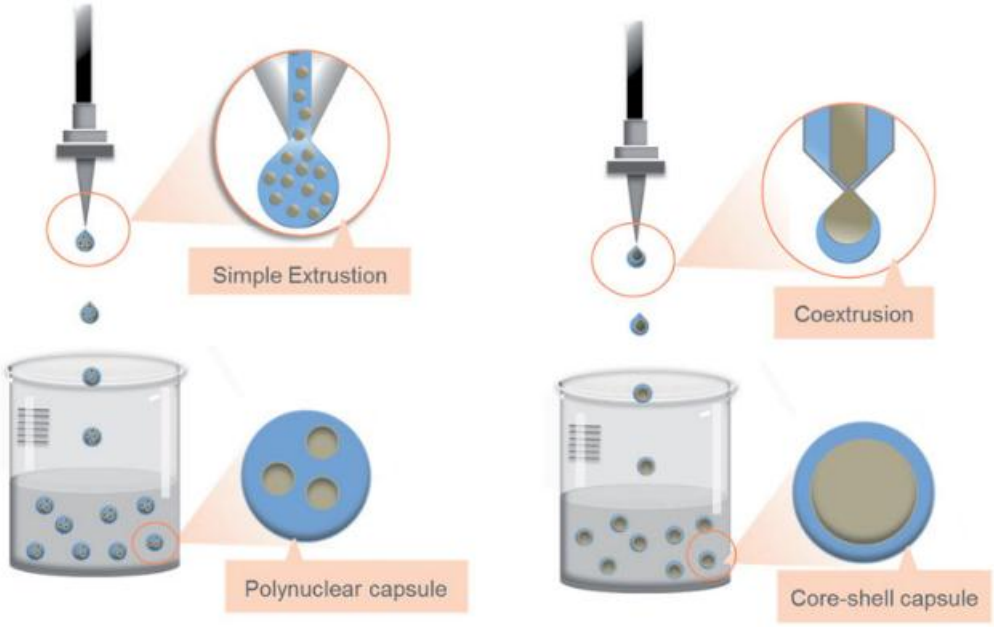


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Figure 1.3. Simple and Concentric Extrusion Methods (Martins et al., 2017).

Beads diameter usually changes between 2-3 mm when beads are formed by extrusion method. After bead formation, beads can be dried by air and shelf stable beads can be obtained. Since encapsulation process and drying process do not require high temperature, extrusion method can be used to encapsulate heat-sensitive materials (Mortazavian, Razavi, Ehsani, & Sohrabvandi, 2007).

1.3.2. Coating Materials

Coating is the barrier around the bioactive compound following encapsulation. It provides protection against external factors and improves the quality of the active agent. Wall material choice is very important in encapsulation because it affects the efficiency of the process and the stability of the end products (Lucy et al., 2014). Types of wall materials used in encapsulation can differ according to the aim of use. In food industry, selected encapsulant must be food-grade, biodegradable and most importantly classified as GRAS (Nedovic et al., 2011). Wall materials used in food applications can be divided into three categories: protein-based coatings, lipid-based coatings and carbohydrate-based coatings. Some of the materials in these categories are listed in Table 1.2.

Table 1.2. Wall Material Types

	Wall Materials	References
Protein based coatings	Whey protein	(Khan, Wang, Sun, Killpartrick, & Guo, 2019)
	Soy protein	(Y. Zhang et al., 2015)
	Pea protein	(Varankovich, Khan, Nickerson, Kalmokoff, & Korber, 2015)
	Gelatin	(Shaddel et al., 2018)
	Sodium caseinate	(Hogan, McNamee, Dolores O'Riordan, & O'Sullivan, 2001)
Lipid based coatings	Waxes	(Mellema, Van Benthum, Boer, Von Harras, & Visser, 2006)
	Phospholipids	(Fricker et al., 2010)
Carbohydrate based coatings	Maltodextrin	(Watson, Lea, & Bett-Garber, 2017)
	Chitosan	(Caetano, Almeida, & Gonçalves, 2016)
	Xanthan Gum	(Ravichandran et al., 2014)
	Guar Gum	(Pieczykolan & Kurek, 2019)
	Gum Arabic	(Santana, Cano-Higuita, De Oliveira, & Telis, 2016)
	Locust Bean Gum	(Totosaus, Ariza-Ortega, & Pérez-Chabela, 2013)
	Pectin	(Cabrera, Cambier, & van Cutsem, 2011)
	Alginate	(Z. Zhang, Zhang, Zou, & McClements, 2016)
	Starch	(Wang, Yuan, & Yue, 2015)

1.3.2.1. Protein Based Coating Materials

Protein is a macromolecule and it is the most abundant molecule in human body after water (Hermann, n.d.). Proteins consists of linear chain of amino acids. They have crucial roles in the human body since every cell inside the body includes protein molecules.

Proteins are also used as coating materials in encapsulation. Proteins are usually used in encapsulation of oil or oil soluble components like flavor compounds (Nedovic et al., 2013). They are good to inhibit oxygen and carbon dioxide permeability and hence a good barrier to these compounds (Quirós-Sauceda, Ayala-Zavala, Olivas, & González-Aguilar, 2014). However, they have poor water barrier properties. Because of their hydrophilic nature, in the presence of moisture, protein based capsules tend to solubilize in water and this causes the release of the active agent quickly (Khanvilkar, Ranveer, & Sahoo, 2016). Therefore, they are used with other coating materials, usually with carbohydrates, to increase the stability of the capsules.

Some of the most commonly used protein-based coating materials are whey protein and gelatin. Gelatin is a unique protein obtained by hydrolysis of collagen. Gelatin is dissolved in water when aqueous solution is heated up to approximately 40ºC and form a thermoreversible gel when this solution is cooled below 30 ºC (Djabourov, Leblond, & Papon, 1988). Gelatin is suitable for encapsulation of oil phase active agents or core materials with low moisture content (Khanvilkar et al., 2016).

Whey protein, which is the soluble fraction of the milk protein, is a by-product of cheese process. It includes β-lactoglobulin which is responsible for gelation of whey protein (Wandrey, Bartkowiak, & Harding, 2010). Whey protein forms hydrogel and mechanical properties of this gel can be adjusted by changing the pH or concentration (Gunasekaran, Ko, & Xiao, 2007)

1.3.2.2. Lipid Based Coating Materials

In contrast to barrier properties of proteins, lipid based coatings has excellent water barrier properties since they have a hydrophobic nature (Lee & Wan, 2005). It is also good to inhibit gas permeability. However, hydrophobic nature of the lipids has also a disadvantage; it forms brittle coatings. Therefore, when lipids are used as coatings, they are usually mixed with other coating materials in order to increase the flexibility of films (Quirós-Sauceda et al., 2014).

The most common lipid used as coatings are natural waxes and phospholipids. Natural waxes are one of the oldest coatings (Garcia, Martino, & Zaritzky, 2000). It is used to coat fresh fruits to protect the fruit from water loss etc. It is also suitable for encapsulation of aroma compounds (Nedovic et al., 2013).

Phospholipids are fatty acids which includes a phosphor-containing group. It has amphiphilic properties which means it has both hydrophobic and hydrophilic parts (Wandrey et al., 2010). When phospholipids are mixed with water, hydrophilic parts interact with water and hydrophobic parts form a bilayer. This structure is called as liposomes. Liposomes are suitable for encapsulation of water soluble molecules (Gomaa, Martinent, Hammami, & Fliss, 2017).

1.3.2.3. Carbohydrate Based Coating Materials

Carbohydrates are most abundant molecules found in nature. They constitute almost 90% of the biomass (Wandrey et al., 2010). Carbohydrates are widely used in encapsulation process because they are economical compared to other materials and they have desirable chemical and mechanical properties.

Starch is obtained from the roots and tubers. It is composed of amylose and amylopectin. Starch and starch derivatives are commonly used for encapsulation of sensitive core materials especially for encapsulation of oils (Khanvilkar et al., 2016). It forms tasteless, odorless, flexible coatings. Starch that is rich in amylose provides

better oxygen and carbon dioxide barrier properties than proteins (Division, Venkateswara, & Pradesh, 2019) .

Chitosan is a polysaccharide which is found in the exoskeleton of crustaceans. Aqueous solutions of chitosan forms clear and flexible coatings which is impermeable to oxygen (Khanvilkar et al., 2016). However, this coating is not a good water barrier.

Gums are usually used as texture modifiers in food industry because they increase the viscosity of the solutions. Most gums are soluble in both cold and hot water. The viscosity of the solution depends on pH and ionic strength (Wandrey et al., 2010). Gum based coatings are very effective to inhibit water vapor diffusion when used with lipids (Khanvilkar et al., 2016).

Pectin is a water-soluble polysaccharide which is usually used to increase the viscosity. Based on esterification degree (DE), pectin is classified as high methoxy pectin (HMP) and low methoxy pectin (LMP) (Wandrey et al., 2010). Depending on the DE gelation mechanisms differ. Due to galacturonic acid units , pectin is an anionic molecule and it forms an excellent coating when used with a cationic molecule like chitosan (Khanvilkar et al., 2016).

Alginate is a polysaccharide obtained mostly from marine brown algae. Unlike other coating materials, unique gelation properties in the presence of multi-covalent ions of alginate makes it more suitable for encapsulation (Draget, Smidsrod, & Skjåk-bræk, 2005).

Alginate molecules are composed of two different monomers which are β-D-mannuronic (M-block) acid and α-L-guluronic acid (G-Block) (Ching, Bansal, & Bhandari, 2017).

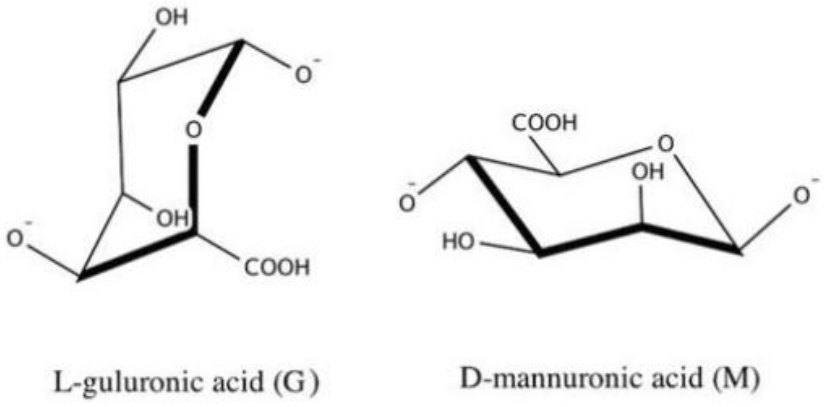


Figure 1.4. Chemical Structure of G and M blocks (Ching et al., 2017)

These blocks bind in different sequences and create three different blocks. One of the blocks consist of only L-guluronic acid and called G block (Figure 1.4). Likewise, M block is composed of D-mannuronic acid only and MG block includes equal proportion of both molecules. Distribution of these blocks in the polymer can vary depend on the origin of the alginate molecule (Brunetti & St. Martin, 2006).

In the presence of multi-covalent cations, alginate molecules are cross-linked with these cations and forms hydrogels. Alginate molecules form gels independent from temperature and this distinguishes alginate from other materials (Draget et al., 2005).

Gel formation of alginate molecules depends on its specific ion binding properties. Alginate affinity towards multivalent cations can be listed as Mn < Zn, Ni, Co < Fe < Ca < Sr < Ba < Cd < Cu < Pb (Ching et al., 2017). However, considering the toxicity of the materials and utilization in food applications most of the ions like Pb, Cd etc. are not used in gelation. Calcium is a non-toxic substance and therefore used for cross-linking of alginate molecules. In gel formation, calcium ions are linked to G-Blocks and form a three-dimensional hydrogel. These model is called as "*Egg-Box Model*" (Figure 1.5) (Leick, Henning, Degen, Suter, & Rehage, 2010). Gel formation and strength of alginate gels are directly related with the length of the G-Blocks.

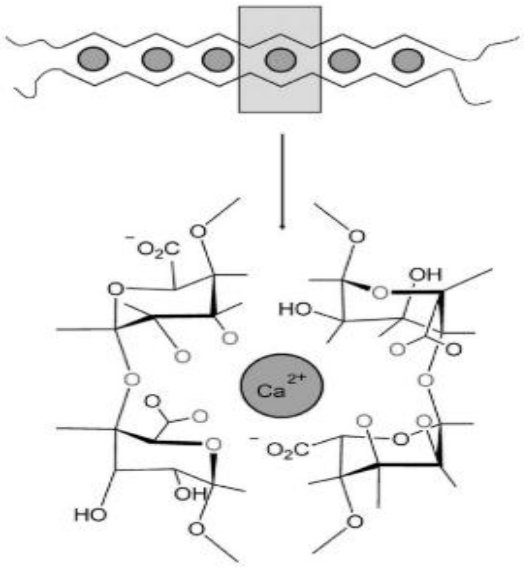


Figure 1.5. Egg-Box Model (Leick et al., 2010)

Alginate forms gel particles in two different ways: external gelation and internal gelation (Ching et al., 2017)**.** In external gelation**,** alginate solution with or without core material are extruded into calcium chloride solution. Calcium ions inside the solution diffuses into alginate droplets and forms hydrogels (Quong, Neufeld, Skjåk-Bræk, & Poncelet, 1998). In this method, gel formation is rapid at the outer surface, but gelation does not occur at the center of the droplet. Therefore, gel particles obtained by external gelation could not be homogenous (Ching et al., 2017). For most of the alginate bead preparation, external gelation method is used. In internal gelation method, alginate solution is mixed with inactive Calcium complex such as $CaSO_4$ or $CaCO_3$. Then, by changing pH or adding mineral acid to the solution, Calcium ions are released from the source. Since Calcium from an internal source interacts with alginate, gel formation occurs simultaneously and homogenous gels are obtained (Ching et al., 2017; Quong et al., 1998).

Alginate beads are usually prepared by extrusion of alginate-core material solution into calcium chloride solution. Diameter of beads can vary depend on the size of the

needle used for extrusion and alginate concentration. Calcium alginate beads have small pore size which is ranging between 5-200 nm. This makes alginate a suitable wall material for large molecules like protein. Since calcium-alginate beads have small pores, release of the large molecules such as protein from the capsule can be slower than small molecules depending on the molecular weight of the alginate matrix (Brunetti & St. Martin, 2006). Therefore, calcium alginate offers more stable beads for large molecules.

1.4. Objective of the Study

Encapsulation is a widely used application in different areas like food, pharmaceutical or cosmetic industry**.** In food industry, encapsulation of phenolic compounds, aroma compounds etc. are well-known applications and in the same way protein encapsulation and immobilization studies are performed in drug industry. However, the studies on encapsulation of protein and the use of encapsulated protein in food industry are limited. Protein is a vital molecule for human life and with the increase in demand for healthy foods, especially plant-based proteins gained importance. Due to undesirable sensorial properties of plant-based proteins such taste and odor, insolubility resulting in sandy texture these proteins are not preferred to be used as direct ingredients in food formulations. The main objective of this study is to encapsulate pea protein within calcium-alginate beads (microcapsules) to eliminate the undesirable characteristics of the pea protein. In the study, encapsulation of pea protein with different alginate ratios at different conditions and the behavior of beads inside the fruit juices as functional drinks were investigated. Extrusion method was used to encapsulate the proteins. Protein content of the beads were measured to understand the effectiveness of encapsulation process. To investigate the bead characteristics, SEM, particle size and NMR relaxometry experiments were performed. To observe the behavior of the beads inside the fruit juices, rheology, swelling and release analysis were performed.

CHAPTER 2

MATERIAL & METHODS

2.1. Materials

The pea protein isolate from Nature's Ingredients (Finland) was used for the protein encapsulation (Protein content >80%). Sodium alginate was purchased from Alfasol and calcium chloride dihydrate was purchased from Interlab (Turkey). Distilled water was used to prepare the solutions.

Behavior of the capsules were investigated in the fruit juices in which they are supposed to be added. Two different fruit juices; melon ad pomegranate that were prepared at Elite Naturel Organik Gıda San. Tic. A.Ş. (Ankara, Turkey) under aseptic conditions were used for the experiments.

2.2. Methods

2.2.1. Encapsulation of Pea Protein in Alginate Beads

The method of the Zhang, (2016) was used with slight modifications for encapsulation of protein (Z. Zhang et al., 2016). 2% w/v pea protein solution was prepared by adding powder pea protein to distilled water and stirring by using magnetic stirrer for 30 minutes for complete hydration. Alginate solutions at different ratios 2%, 1.5% and 1% w/v were prepared by dissolving sodium alginate powder in distilled water and stirring for 30 mins.

Native and denatured pea protein were used for encapsulation. In previous studies, it was shown that pea protein could be denatured at approximately 80ºC (Mession, Sok, Assifaoui, & Saurel, 2013). Since it is known that digestibility of proteins increases with denaturation it was aimed to encapsulate denatured proteins as well also to see the effect of using denatured protein on encapsulation.

2% w/v protein solution and 2% w/v alginate solution were mixed at a ratio of 1:1 v/v and further stirred to have a homogenous solution for 20 min. To induce gelation, calcium chloride (10% w/v) was used. To prepare pea protein-alginate beads, pea protein-alginate aqueous solution was injected into 10% calcium chloride solution by using a syringe having a diameter of 1mm. After injection process, gel beads were kept at calcium chloride solution for 30 min to let crosslink be completed with the calcium ions. When the beads were hardened, they were filtered and washed with distilled water to remove the excess calcium on the surface of the beads. Filtered beads were dried at an incubator (55˚C) overnight. To prepare the heated samples, first distilled water was heated up to 80˚C and protein powder was added at this temperature. Then the mixture was stirred for 30 minutes. During stirring temperature was kept constant at 80˚C.

2.3. Analysis of Pea Protein-Alginate Beads

2.3.1. Particle Size Measurement

Particle size of the protein loaded bead samples was measured by a digital caliper. To better understand the particle size distribution and to determine the mean particle size, measurement was performed for 100 beads for all samples. Mean and standard errors were reported.

2.3.2. Determination of Protein Content

Protein content of the beads were analyzed by Kjeldahl method. Kjeldahl method is composed of three main parts: digestion, distillation and titration. In the digestion part, the purpose is to decompose the nitrogen in the samples. To do that, concentrated sulfuric acid solution was used. 3-4 g of bead sample was grinded to reduce the particle size. The purpose of this step is to have a more homogenous sample and to obtain better results. Then 10 ml concentrated H_2SO_4 solution and potassium sulfate (K_2SO_4+Se) tablets were added. K_2SO_4+Se tablets were used as catalyst to increase

the temperature of the mixture during boiling. Samples were boiled in concentrated sulfuric acid for 30 minutes at 420ºC. Equation 1 shows the chemical reaction takes place during digestion. After digestion ammonium sulfate was obtained. When digestion was completed, digestion mixture was cooled down to 50-60ºC and 50 ml distilled water was added. Then 50 mL of 35% (w/v) NaOH solution was added to increase the pH of the mixture. Increase of pH causes conversion of ammonium (NH_4^+) ions to ammonia (NH_3). After NaOH addition, sample was distilled. In distillation, 25 mL of 4% (w/v) boric acid (H_3BO_3) was used to trap the distillate. Distillation process ended when 100 mL of distillate was collected. Finally, for titration step, 2-3 drops of indicator were added to distillate and the mixture was titrated with 0.1 M HCl. Titration was performed until color change was observed. Protein content was calculated by using equation 1 and 2 (Kurowski, Buffler, & Labortechnik, 2010) :

$$\%N = \frac{[V(1) - V(Bl)]\text{x F x c x f } x\ M(N)}{m\ x\ 1000} \quad (1)$$

$$\%P = \%N\ x\ PF \quad (2)$$

Where;

% N: percent of nitrogen, V(1): volume of HCl used in titration of sample (mL), V(Bl): volume of HCl required for blank (mL), F: molar reaction factor (for HCl F=1), c: concentration of titrant (mol/L), f: factor of HCl, M(N): molecular weight of nitrogen (14.007 g/mol), m: sample weight, 1000: conversion factor (mL in L), %P: percent of protein and PF: protein factor (6.25).

To understand the effectiveness of encapsulation process, percent of protein recovery was calculated. For calculation of the protein recovery in alginate beads equation 3 (Chandy, Das, Wilson, & Rao, 2002) was used:

$$Protein\ Recovery\ (\%) = \frac{Amount\ of\ protein\ in\ beads}{Amount\ of\ protein\ fed\ in\ the\ system}\ x100 \quad (3)$$

2.3.3. Morphology Analysis

Morphology of the alginate beads was analyzed by using Scanning electron Microscopy (SEM) (JEOL, Japan). For SEM analysis, beads were fixed on an aluminum stub. Then beads were coated with gold for analysis. Coated beads samples were analyzed at an voltage of 20 kV (Mustafa, 2017).

2.3.4. Release Experiments

The release of protein from alginate beads was observed in two different fruit juices at different pH values. To observe whether pectin has an effect on protein release rate, release of protein was also tested in juices that includes 0.5% pectin and 1% pectin. 3 g dried alginate beads were tested for protein release in 200 ml fruit juice for 1 month. During this period, samples were kept at constant conditions at 37ºC to accelerate the release. After 1 month, beads were removed from the juices and the protein content of the juices were measured. To calculate the amount of the protein released from the beads, protein content of the juices at initial condition were also measured.

2.3.5. Rheological Properties

Rheological properties were analyzed by using a rheometer (Kinexus, Malvern Instruments). To understand the effect of added pectin amount, rheological properties of two fruit juices with different pH values (pH 3.3 and pH 3.8) were mixed with 0.5% pectin and 1% pectin. As the control group, rheological behavior of juice without pectin was also analyzed. For the analysis, cup and bob geometry was filled with 20 milliliters of samples and the shear stress vs shear rate (0.1 s^{-1} to 100 s^{-1} profiles were recorded. Measurements were conducted at 25ºC. The values obtained were fitted to a Newtonian model. Following equations were used for this analysis:

$$\tau = \mu\gamma \quad (4)$$

where;

τ: shear stress (Pa) and γ: shear rate(s^{-1}), μ: Newtonian viscosity (Pa.s).

2.3.6. NMR Relaxometry Measurements

NMR relaxometry experiments were conducted to understand the hydration behavior of the samples. The preparation of samples was performed by mixing 0.5 g sample with 0.75 g distilled water at room temperature. T_2 (spin-spin relaxation) times were measured by using 0.32 T Spin Track instrument with a frequency of 13.63 MHz (Russia). CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence was used to obtain the data. To acquire the data, number of echo and echo time were set to 1000 and 500 ms respectively.

2.3.7. Statistical Analysis

Analysis of variance (ANOVA) was performed by MINITAB to observe if there are significant differences between the results of the analysis. In order to compare the means of each analysis Tukey's Multiple Comparison test was used. Probability level was considered less than 0.05 ($p<0.05$). All experiments were done in triplicate.

CHAPTER 3

RESULTS AND DISCUSSIONS

3.1. Particle size

The mean diameter values of capsules are given in Table 3.1. As seen in the Table, different alginate ratios had a significant effect (Table A.10 and Table A.11) on mean particle diameter of the gel beads ($p<0.05$). Results showed that heat treatment did not have a significant effect on mean diameter of the beads ($p<0.05$). Capsules prepared with 1.5% alginate ratio showed in the highest particle size in both heated and non-heated samples.

Alginate is a polysaccharide consisting of guluronic and mannuronic acid and thus has carboxyl groups and these carboxyl groups cause gelation by cross-linking with calcium ions. Increase in alginate concentration results in an increase in the concentration of carboxyl groups in the environment. While these groups create a complex gel structure with calcium ions, more alginate layers are formed around the core materials and therefore particle size of the capsules increases (Gomathi, Susi, Abirami, & Sudha, 2017). When the results were analyzed, it was seen that particle size of beads with 1.5 % alginate ratio was larger than beads with 2% alginate ratio for non-heated samples. The reason behind this could be explained by coalescence of the particles during extrusion. It was reported that increase in core material to coating material ratio could result in increase in particle size of capsules (Hogan et al., 2001). As coating material amount decreased, coating material could not encapsulate the core material properly and thus particles became integrated, forming larger particles.

It was reported that flow rate and the distance between the needle and calcium chloride solution had also an effect on particle size (Ramos et al., 2018). It was shown that there was a direct proportion between particle size and these factors. As flow rate or

the distance between the solution and the needle increased, particle size of the alginate beads increased. Since beads were produced manually at lab conditions without using a syringe pump, despite the efforts to keep the distance as same as possible there could have occurred some deviations. Ramos et al, (2018) also stated that these effects were directly related with viscosity and might not be observed at alginate ratio below 1% (w/v) (Ramos et al., 2018). However, viscosity of the solution with 1.5% alginate was high enough as will be explained later and, therefore these factors (distance and flow rate) could be the reason that capsules with 1.5% alginate ratio had the highest particle size.

Mean diameter of heated samples ranged between 1.72 mm to 1.85 mm while mean diameter of non-heated samples ranged between 1.61 mm to 1.85 mm. The results showed that heat treatment did not have a significant effect on the particle size ($p<0.05$).

Table 3.1. Particle size of capsules with different alginate ratio

Alginate Ratio	**Heat Treatment**	**Mean Diameter (mm)**
1.0%	Non-heated	1.61 ± 0.04^{c}
1.5%	Non-heated	1.85 ± 0.04^{a}
2.0%	Non-heated	1.68 ± 0.04^{bc}
1.0%	Heated	1.72 ± 0.05^{abc}
1.5%	Heated	$1.85 \pm 0.04^{a\,*}$
2.0%	Heated	1.79 ± 0.03^{ab}

* Values with different letters are significantly different ($p < 0.05$). Values are expressed as mean ± SE.

3.2. Protein Content & Protein Recovery

Protein content and protein recovery of capsules are given in Table 3.2 and 3.3 respectively. There was a significant effect (Table A.4 and Table A.5) of alginate ratio on the protein content of capsules. Capsules with 2% alginate had the lowest protein content while the capsules with 1% alginate had the highest. Results showed that contrary to protein content, alginate concentration did not have a significant effect (Table A.9) on protein recovery ($p<0.05$). Protein recovery and protein content were expected to increase with increase in alginate concentration. There are several studies found in literature stating that encapsulation efficiency increased proportionally with alginate concentration and this was explained with the increase in the degree of cross-linking with calcium ions (Mandal, Senthil Kumar, Krishnamoorthy, & Basu, 2010). In this study, it was observed that increase in alginate concentration did not significantly change the protein recovery. A similar result was also observed by Silva et al., (2006) (Silva, Ribeiro, Figueiredo, Gonçalves, & Veiga, 2006). This case could be explained by the effectiveness of alginate on the encapsulation. Several studies showed that use of alginate alone for protein encapsulation might not be sufficient enough due to the low viscosity of alginate (Norudin, Mohamed, & Yahya, 2018). In order to increase the encapsulation efficiency, viscosity of the alginate solution should be high enough to encapsulate large molecules like protein. It can be interpreted that in order to observe the effect of alginate concentration on encapsulation efficiency, alginate should be used at a concentration higher than 2%.

On the other hand, increase in alginate concentration had a reverse effect on protein content of the capsules. This result was obtained due to the higher coating to core material ratio (Silva et al., 2006). Since loading efficiency of all samples were similar, as coating material ratio increased, core material ratio inside the beads decreased. Therefore, highest protein content was obtained from the beads with 1% alginate ratio.

In this study, heat treatment was also applied to denature the proteins to see its effect on encapsulation. The results showed that heat treatment did not have a significant

effect (Table A.6) on protein content and protein recovery ($p<0.05$). Pea protein consists of approximately 70% of globulins (Mession et al., 2013). When pea protein solution was heated to denaturation temperature, globulins unfolded. However, after unfolding denatured globulins were rearranged into soluble aggregates in contrast to many other proteins which loses its solubility (Mession et al., 2013). But, formation of soluble aggregates neither changed the protein recovery nor efficiency of the encapsulation. Low interaction between alginate and pea protein could have been the reason of this stable case.

Table 3.2. Protein content of capsules with different alginate ratio

Alginate Ratio	**Heat Treatment**	**Protein Content**
1.0%	Non-heated	$30.26 \pm 1.03^{a*}$
1.5%	Non-heated	25.24 ± 0.77abc
2.0%	Non-heated	20.30 ± 2.37^{c}
1.0%	Heated	28.12 ± 0.95^{ab}
1.5%	Heated	23.19 ± 0.85^{bc}
2.0%	Heated	19.28 ± 1.06^{c}

* Values with different letters are significantly different ($p < 0.05$). Values are expressed as mean ± SE mean.

Table 3.3. Protein recovery of capsules with different alginate ratio

Alginate Ratio	Heat Treatment	Protein Recovery
1.0%	Non-heated	49.36 ± 5.64^{a} *
1.5%	Non-heated	45.91 ± 1.61^{a}
2.0%	Non-heated	44.17 ± 1.67^{a}
1.0%	Heated	48.33 ± 2.52^{a}
1.5%	Heated	45.87 ± 1.61^{a}
2,00%	Heated	44.17 ± 1.55^{a}

* Values with different letters are significantly different ($p < 0.05$). Values are expressed as mean ± SE mean.

3.3. Rheological Measurements

Alginate beads were suspended in different fruit juices as explained in Chapter 2. Pectin was added to the juices as a stabilizer. Rheological characterization experiments were performed on the fruit juices to interpret the behavior of alginate beads in the juices.

The relationships between shear stress and shear rate of melon juice and pomegranate juice with 0.5% pectin and 1% pectin are given in Figure 3.1 and Figure 3.2 respectively. When the results were analyzed, it was shown that melon juice and pomegranate juice exhibited Newtonian behavior without the addition of pectin as expected. Viscosities of both melon and pomegranate juice samples remained steady while shear rate increased. Newtonian behavior of the juices was not affected from pectin addition, but viscosity values of both juices increased. The results were consistent with the literature. Studies showed that rheological properties of pectin solutions at concentration that did not exceed 0.5% followed Newtonian behavior (Chiba, 2003). However, rheological properties of the solution could shifts to

pseudoplastic behavior, if the pectin ratio was higher than 1% (Chiba, 2003). In our case, Newtonian behavior remained event at 1% concentration.

Pectin has been used in beverage industry for many years to provide stability in colloidal systems as a thickening and gelling agent (Dambal, Padaki, Herur, Kashinakunti, & Manjula, 2013). Pectin is capable of forming gels under suitable conditions and gelation depends on the methoxylation degree. In this study high methoxy pectin was used. Pectin is a water-soluble polysaccharide and its solubility is a factor that affects the gel formation (Gawkowska, Cybulska, & Zdunek, 2018). Solubility of pectin is inversely proportional with the increase in viscosity. When pectin solubilizes in water, intermolecular distance increases and hence viscosity decreases.

When the results were analyzed, it was observed that juices with 0.5 and 1% pectin had higher viscosity values than the no pectin containing samples. So, pectin definitely showed its desirable effect as explained above.

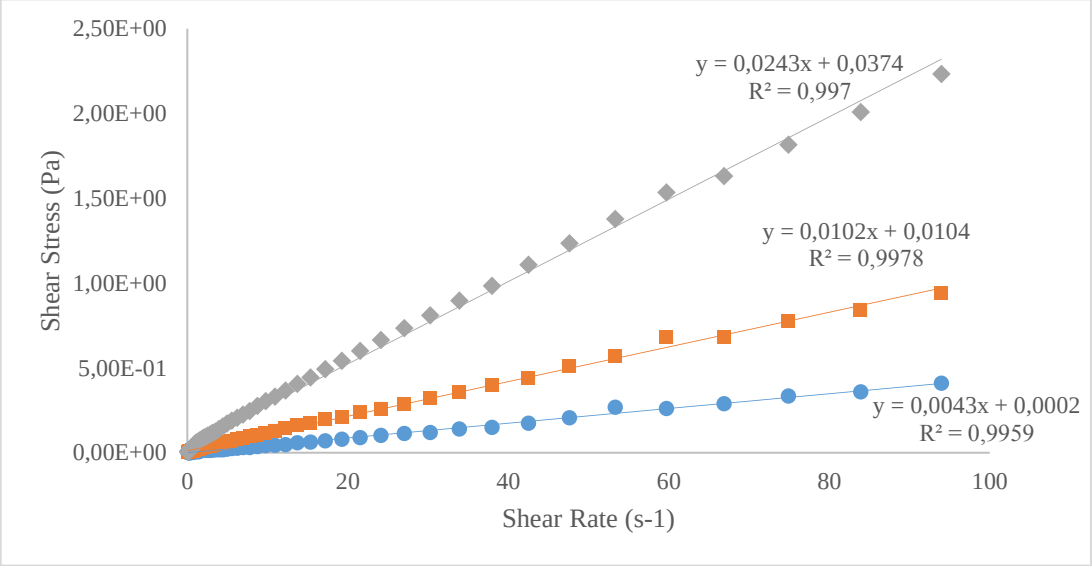


Figure 3.1. Variation of shear stress with shear rate of melon juice: ◦ Control, □ 0.5% pectin, ◊ 1%pectin

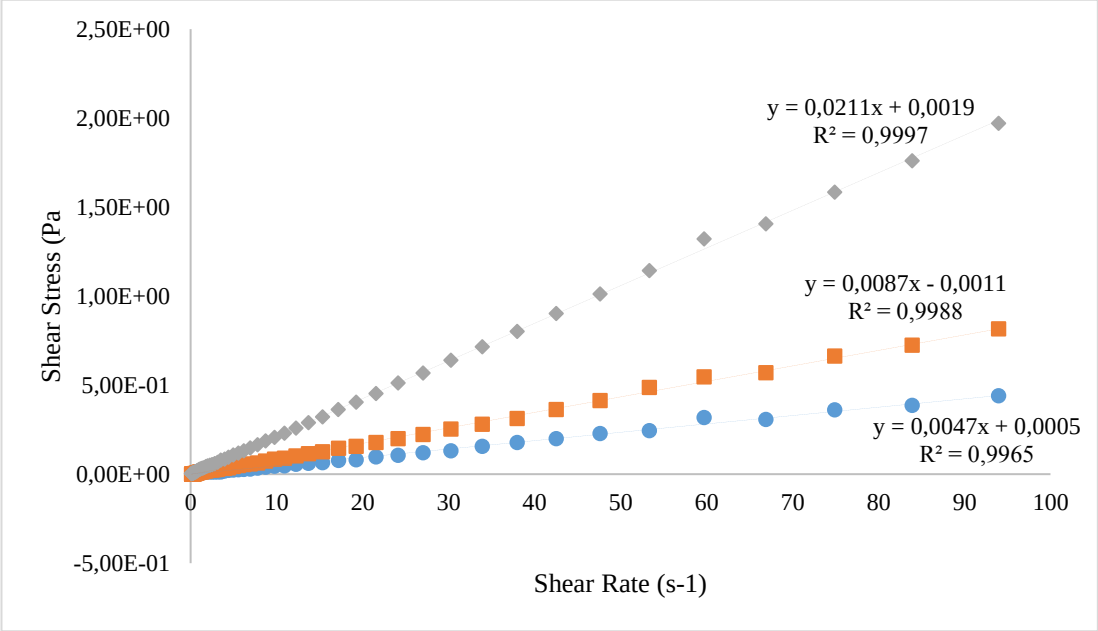


Figure 3.2. Variation of shear stress with shear rate of pomegranate juice: ◦ Control, □ 0.5% pectin, ◊ 1%pectin

Viscosity values of melon and pomegranate juices with different pectin ratios are given in Table 3.4. It was observed that at their native state, both pomegranate and melon juice had similar viscosity values. Addition of pectin increased the viscosity of both juices significantly (Table A.3) ($p<0.05$). It was observed that increase in viscosity of melon juice was significantly different (Table A.3) than pomegranate juice. And when the concentration dependence of the viscosities was examined it was observed that the effect was more prominent on melon juice. Concentration dependence of the viscosities were fitted to an exponential model and viscosity increase rate was found significantly larger in melon juice ($p<0.05$) (Fig 3.3)

Table 3.4. Viscosity values of melon and pomegranate juices with different pectin ratios

Pectin Ratio	Juice Type	Viscosity (Pa.s)	R^2
Control	Melon	0.0041 ± 0.00^{e}	0.99
0.5%	Melon	0.0103 ± 0.00^{c}	0.99
1.0%	Melon	0.0241 ± 0.00^{a} *	0.99
Control	Pomegranate	0.0047 ± 0.00^{e}	0.99
0.5%	Pomegranate	0.0086 ± 0.00^{d}	0.99
1.0%	Pomegranate	0.0219 ± 0.00^{b}	0.99

* Values with different letters are significantly different ($p < 0.05$). Values are expressed as mean ± SE mean.

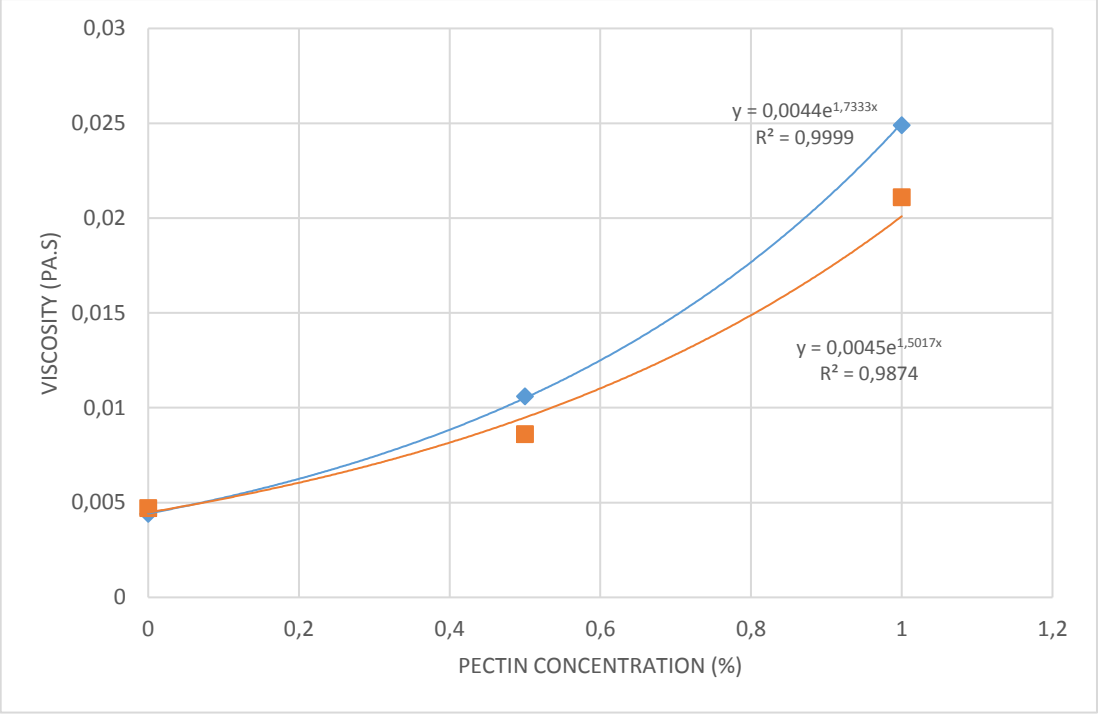


Figure 3.3. Dependence of viscosity with respect to pectin concentration: □ Melon juice, ◇ Pomegranate juice

Pectin was added to increase the viscosity and thus provide the solid-liquid suspension stability after encapsulated protein beads were added to fruit juice. Viscosity increase due to pectin is usually expected to occur through gelation or entrapping the water in the juice. Gelation is affected by the pH and sugar content. Hydrogen bonding and hydrophobic interactions are the two main mechanisms behind the pectin gelation. Pectin includes free carboxyl groups which are responsible for the gelation. If the carboxylic acid groups are in unprotonated form ($-COO^-$) galacturonic acid units repel each other and prevents gelation since hydrogen bonding is prevented. At low pH, carboxyl groups become protonated and converts into carboxylic acid (-COOH) and this causes a decrease on the repulsive forces between pectin molecules (Dambal et al., 2013). Therefore, low pH is required to induce gelation. In our case, pH of the juices was measured as 3.3 and 3.8 for pomegranate and melon juice respectively and they were found to be significantly different from each other ($p<0.05$). According to the gelation theory, higher gelation was expected to occur in pomegranate juice. But this was not the case observed. Pomegranate juice's viscosity increased but it increased more in melon juice. For pectin gelation hydrogen bonding between protonated carboxyl acid groups is facilitated when pectin does not make H bond with water. Methoxy groups of pectin (methoxyl groups) , aggregate in the presence of water to decrease the contact surface with water and with the contribution of hydrogen bonds between protonated carboxyl groups, junction zones are formed (El-Nawawi & Heikel, 1997). These junction zones allow the independent pectin molecules to bind to form gel structure. And for that situation to be satisfied, there should be sufficient sugar in the environment which would do hydrogen bond with sugar. In order to decrease water activity, usually sugar is added to the solution because sugar molecules in the environment decrease the water activity and prevents the hydration of the pectin. Therefore, pectin could not stay in dispersed phase and forms a gel. In our case pomegranate juice had a $Brix^o$ of 15 whereas melon juice had a brix of 10. Thus, sugar concentration was not high enough. So, the added pectin would do hydrogen bond

with water and it would do more in the case of melon due to lower brix values and thus more hydrogen bond with pectin would increase the viscosity more.

Another factor that plays important role in pectin gelation is water content. In solutions that have high water content, hydrophobic interactions cannot be achieved sufficiently. Hydrophobic interactions are important for gelation because of ester groups.

Another factor that could affect pectin's behavior in a fruit juice is the sugar type. It is also effective in developing hydrophobic interactions between pectin molecules (Chiba, 2003). Bulone et al. (2010) , found that, sucrose concentration had a positive effect on pectin gelation (Bulone, Giacomazza, Manno, Martorana, & San Biagio, 2010). Figure 3.3 shows the gelation of pectin in the presence of sucrose at low pH environment. When the sucrose content of melon and pomegranate was analyzed, it was shown that melon includes 5.3 g sucrose/100g but pomegranate includes trace amount of sucrose (Chayut et al., 2015; Melgarejo, Salazar, & Artés, 2000). Because of its sucrose content, pectin gelation could have occurred more in melon juice than pomegranate juice and therefore viscosity increase of melon juice became higher than pomegranate juice when pectin was added. However as mentioned before due to high water content this effect was not though to dominate the increase in the viscosity.

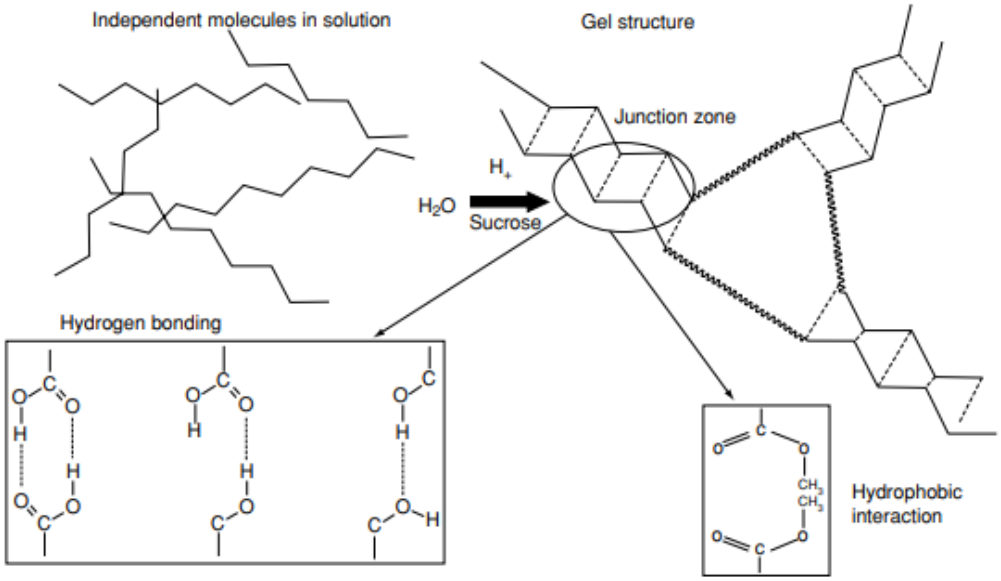


Figure 3.4. Gelation of pectin in acidic environment and sugar matrix (Chiba, 2003)

3.4. Release of Proteins from the Capsules

Once the prepared beads are put into the juices, protein content of the juices were measured at the end of 1 month after the beads are harvested. Percentages of protein released from the beads with respect to alginate ratio, pectin ratio, juice type and heat treatment are given in Table 3.5 and Table 3.6. Alginate and pectin concentrations, heat treatment and juice type had all significant effect on protein release ($p<0.05$) (Table A.14). In terms of alginate ratio, beads with 1% alginate had the lowest release percent and beads with 2% alginate had the highest percent independent of other factors. When the results were analyzed, it was shown that pectin ratio affected the release percent inversely. In other words, as pectin ratio increased release decreased significantly. While suspending the beads in the solution, viscosity increase created a barrier for the release.

Heat treatment was also effective on the amount of protein released from the beads. It was observed that release rate of the heated samples was higher than non-heated samples. When juice types were compared, it was seen that protein release from beads

was more in pomegranate juice. This may be explained with the viscosity of juice. As discussed before pomegranate juices had lower viscosity values. This was consistent with the previous results.

Table 3.5. Percentages of the protein released from the beads with respect to alginate ratio, pectin ratio and heat treatment in melon juice

Alginate Ratio	**Pectin Ratio**	**Heat Treatment**	**Protein release (%)**
1.0	Control	Non-Heated	11.00 ± 0.01^{v}
1.5	Control	Non-Heated	14.51 ± 0.02^{s}
2.0	Control	Non-heated	18.08 ± 0.02^{p}
1.0	Control	Heated	15.40 ± 0.02^{r}
1.5	Control	Heated	$21,55 \pm 0.02^{m}$
2.0	Control	Heated	27.68 ± 0.02^{f}
1.0	0.5	Non-Heated	5.51 ± 0.06^{aa}
1.5	0.5	Non-Heated	6.60 ± 0.07^{z}
2.0	0.5	Non-Heated	9.88 ± 0.07^{w}
1.0	0.5	Heated	11.87 ± 0.06^{u}
1.5	0.5	Heated	15.80 ± 0.06^{q}
2.0	0.5	Heated	22.49 ± 0.05^{l}
1.0	1.0	Non-Heated	2.20 ± 0.06^{ad}
1.5	1.0	Non-Heated	2.62 ± 0.06^{ac}
2.0	1.0	Non-Heated	8.21 ± 0.06^{y}
1.0	1.0	Heated	4.75 ± 0.06^{ab}
1.5	1.0	Heated	8.62 ± 0.06^{x}
2.0	1.0	Heated	12.10 ± 0.06^{u}

Table 3.6. Percentages of the protein released from the beads with respect to alginate ratio, pectin ratio and heat treatment in pomegranate juice

Alginate Ratio	**Pectin Ratio**	**Heat Treatment**	**Protein release (%)**
1.0	Control	Non-Heated	13.16 ± 0.01^{t}
1.5	Control	Non-Heated	18.50 ± 0.02^{o}
2.0	Control	Non-heated	26.29 ± 0.02^{hı}
1.0	Control	Heated	20.08 ± 0.02^{n}
1.5	Control	Heated	27.30 ± 0.02^{g}
2.0	Control	Heated	34.54 ± 0.02^{b}
1.0	0.5	Non-Heated	18.71 ± 0.06^{o}
1.5	0.5	Non-Heated	23.74 ± 0.07^{k}
2.0	0.5	Non-Heated	32.82 ± 0.07^{c}
1.0	0.5	Heated	26.09 ± 0.05^{ı}
1.5	0.5	Heated	31.66 ± 0.05^{d}
2.0	0.5	Heated	39.78 ± 0.05^{a}
1.0	1.0	Non-Heated	14.31 ± 0.06^{s}
1.5	1.0	Non-Heated	18.50 ± 0.06^{o}
2.0	1.0	Non-Heated	26.54 ± 0.06^{h}
1.0	1.0	Heated	17.80 ± 0.05^{p}
1.5	1.0	Heated	24.42 ± 0.06^{j}
2.0	1.0	Heated	31.11 ± 0.06^{e}

Swelling behavior of the beads, in other words, penetration of the release medium into beads and the dissociation of alginate matrix can also affect the release rate (Mandal et al., 2010). The higher fluid uptake capability of the beads means more disintegration of calcium-alginate matrix and hence dissolution of alginate matrix and dissolution of alginate matrix leads to increase in release rate of the core material. In literature, it was found that increase in alginate concentration results in increase in water uptake of

beads (Del Gaudio, Colombo, Colombo, Russo, & Sonvico, 2005). In this study, beads with highest alginate ratio (2%), had the highest release rate. In the light of these information, it can be interpreted that the results were compatible with the previous findings.

Another factor that could affect the release was pectin ratio of the juices. Decrease in release rate was observed with increase in pectin ratio. Pectin is used as wall material in encapsulation applications since it can form a gel structure under suitable conditions (Khanvilkar et al., 2016). There are many studies in which pectin was used as encapsulant to enhance the stability of alginate capsules (Perez-Gago, Serra, & Río, 2006). Since calcium was also releasing out to the juices gel structure could have formed by the pectin in juices which could help to obtain more stable beads by coating the alginate bead and forming an additional layer around the capsule. It was observed from the results that release rate of the beads in melon juice was significantly lower than in pomegranate juice (Table A.14). As discussed in rheological measurement, increase in viscosity with pectin addition was higher in melon juice. Increase in viscosity could have limited the movement of the capsules and thus restricted the convection effects on the system. Such convective effects could be more prominent in pomegranate juice resulting in higher release rates.

When the results were analyzed, it was observed that there was a significant difference between the release rate of heated and unheated samples (Table B14). Release rate of heated samples was higher than unheated samples. The reason behind this can be explained by denaturation of protein. Kumagai et al., (2014) stated that water sorption behavior of protein could be changed by heat treatment (Kumagai, Seto, Sakurai, Ishii, & Kumagai, 1997). It was stated that heat treatment caused a decrease in the amount of disulfide bonds and therefore resulted in significant decrease in surface hydrophobicity and increase in solubility of pea protein. When beads are added in fruit juice, they were swollen, and certain amount of juice was penetrated into beads. Since the solubility of pea protein increased due to the denaturation, protein dissolution

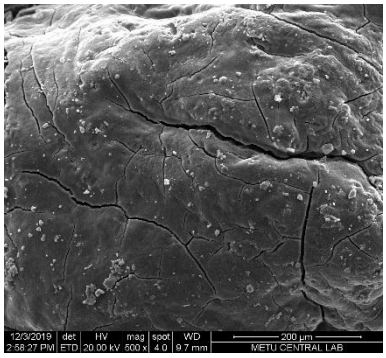
could have occurred, and protein molecules would be released from the beads. Therefore, it was reasonable to obtain higher release rate at heated samples.

3.5. Morphological Analysis by SEM

Scanning electron micrographs of the beads with 1%, 1,5% and 2% alginate ratios are given Figure 3.4 for heated and non-heated samples. Non -heated samples (Figure 3.3-I, Figure 3.3-III and Figure 3.3-V) were characterized by rough surfaces while the heated samples (Figure 3.4-II, Figure3.4-IV and Figure3.4-VI) were characterized by smooth surfaces. It can be definitely interpreted that heat treatment had an important effect on the surface morphology. When heat treatment is applied, protein denaturation and correspondingly protein gelation occurred. Gelation resulted in a more uniform and homogeneous structure. Similar results were obtained by Long et al., 2015. They stated that wet heat treatment of soy glycinin caused gelation and therefore resulted in more uniform structure.

It was also observed that sphericity of the beads was changed with alginate concentration. At higher alginate concentration more, spherical beads were obtained but as the alginate ratio decreased, beads in irregular shape were obtained. Sphericity of the samples can be affected by the degree of cross-linking of calcium ions. Smrdel et al., (2008) stated that as the degree of cross-linking increased, strength of gel increased and hence more regular shape beads were obtained (Smrdel, Bogataj, & Mrhar, 2008). It was also stated that degree of cross-linking was related with the temperature and concentration of calcium bath. However, in this study, both factors were same for all samples. So, increase in degree of cross-linking could be explained by the increase in alginate concentration.

(I)



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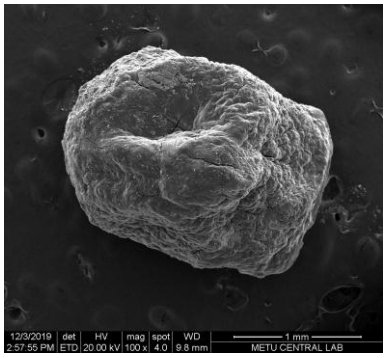

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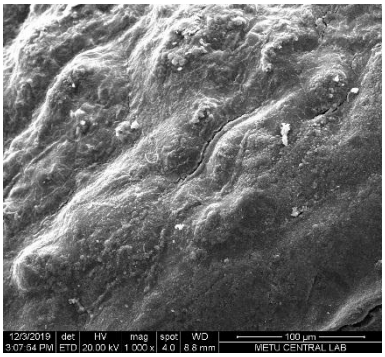
(II)



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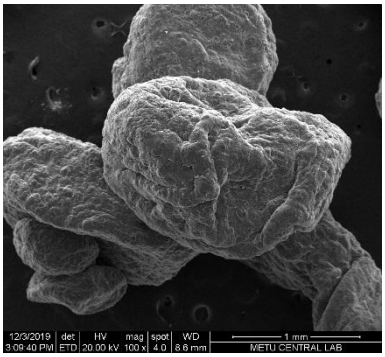

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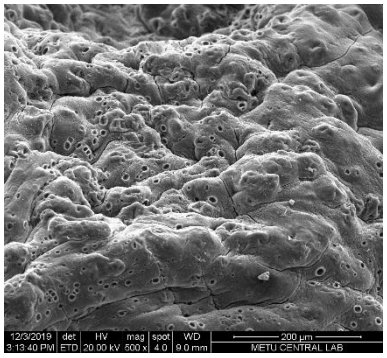
(III)



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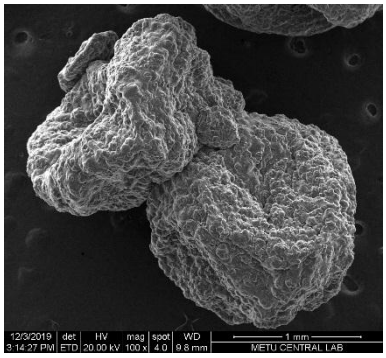

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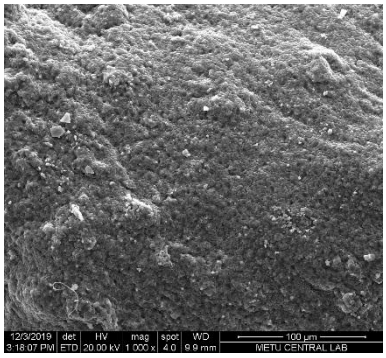
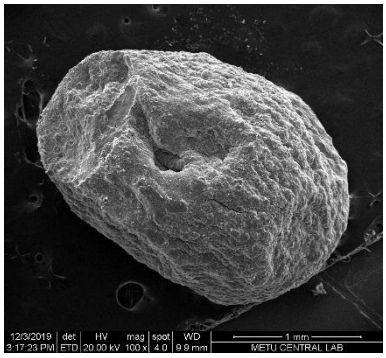
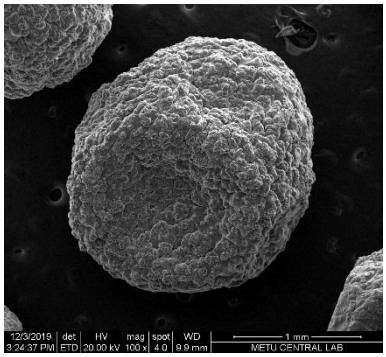
(IV)





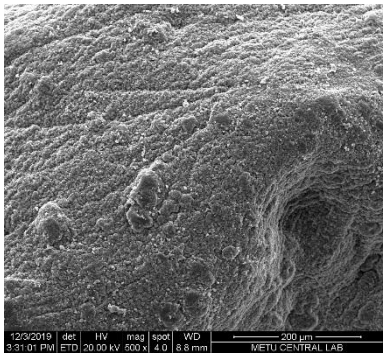
(a) **(b)**

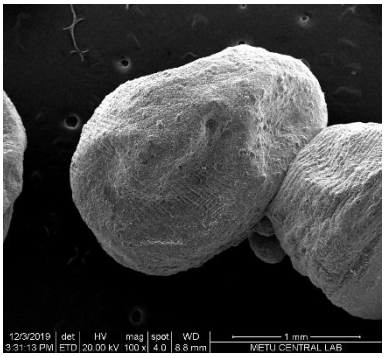
(V)



(a) **(b)**

(VI)





(a) **(b)**

Figure 3.5. SEM images of the beads: (I) 1% alginate non-heated, (II) 1% alginate heated, (III) 1.5% alginate non-heated, (IV) 1.5% alginate heated, (V) 2% alginate non-heated, (VI) 2% alginate non-heated. (a) Magnification level 500x, (b) Maginifation level 100x.

3.6. NMR Relaxometry

The application of nuclear magnetic resonance (NMR) in food systems has gained popularity as it provides considerable information about the foods due to its non-destructive nature and not requiring any pretreatments (Kırtıl, Dag, Guner, Unal, & Oztop, 2017).

In this study, for the formulated alginate beads NMR Relaxometery experiments were performed to assess the hydration ability of the capsules.

The mobility of the protons in the food system is the key to understand the hydration behavior of the food. Although there are two main predictable results which are T_1 and T_2 relaxation times, T_2 relaxation time is the focus of interest due to being a more rapid and robust method to observe the distinctive properties of food systems and polymers (Moraes, Monaretto, & Colnago, 2016). Therefore, in this study T_2 relaxometry measurements were conducted on beads prepared at different alginate concentrations. The beads were mixed with distilled water, melon juice and pomegranate juice to observe the hydration of the beads in different solvents. Table shows the T_2 relaxation times of the samples obtained by NMR Relaxometry.

Table 3.7. T_2 values of the beads with different alginate ratios

Alginate Ratio	Heat Treatment	T2 Results (ms) [DW]	T2 Results (ms) [M]	T2 Results (ms) [P]
1%	Non-heated	44.45 ± 1.67^{a}	29.26 ± 0.94^{de}	36.84 ± 2.55^{bcde}
1.5%	Non-Heated	37.31 ± 0.56^{b}	33.42 ± 1.58^{bcde}	32.32 ± 2.18^{bcde}
2%	Non-Heated	35.97 ± 1.02^{bc}	31.52 ± 1.79^{bcde}	33.1 ± 0.11^{bcde}
1%	Heated	31.17 ± 1.46^{bcde}	34.77 ± 2.33^{bcde}	28.44 ± 1.86^{cde}
1.5%	Heated	31.36 ± 0.67^{bcde}	33.05 ± 2.1^{bcde}	27.32 ± 1.25^{ef}
2%	Heated	35.37 ± 0.99^{bcd}	21.4 ± 1.25^{f}	30.17 ± 0.98^{cde}

* Values with different letters are significantly different ($p < 0.05$). Values are expressed as mean ± SE.

DW= Distilled water, M=Melon Juice, P=Pomegranate Juicepe

According to the ANOVA results, it was found that as alginate concentration increased, T_2 values decreased ($p < 0.05$). Moreover, heat treated protein containing samples had lower T_2 values than the unheated ones ($p < 0.05$). Among the three different solvents, distilled water samples had higher T_2 values and the usage of two juices had similar effect on T_2 values which were lower than distilled water ($p < 0.05$). It was not surprising since fruit juices had sugars and fibers which could have decreased the relaxation times significantly.

Since the working principal of NMR Relaxometry lies under the mobility of the free protons inside the samples, the reduction in T_2 values indicates a decrease in free protons that are hydrogen ions (H^+) coming from water. Therefore, the reduction in T_2 values indicated an increase in hydration of the samples.

The decrease in the T_2 values with increasing alginate concentration can be explained by the decrease in the amount of mobile water in the system. When beads were mixed

with water, the highest T_2 value belonged to beads with 1% alginate concentration. It can be said that less interaction of water resulted in higher T_2 values. Therefore, T_2 values of 1% alginate containing beads were higher due to less interaction of beads and more mobile water in the system. As explained before degree of crosslinking also decreased with decreasing alginate concentration and consequently, the contribution of mobile water become more dominant at that alginate concentration.

The decrease in free water in the system could also be the reason of the decrease in the T_2 values of juices mixed with the beads. Since same amount of solvent (distilled water, melon juice and pomegranate juice) was used during the sample preparation, the mobile water amount can be less in juices when they were compared with same amount of distilled water.

In this study, heated protein containing beads had shorter T_2 values than unheated ones. The reason behind this was explained by the denaturation of pea protein under heat treatment. As stated, before with heating solubility of the pea proteins could have increased. According to a study, it was found that applying heat at 90^{o}C for 5 minutes with the help of the shear increased the pea proteins dispersion in the system (Bogahawaththa, Chau, Trivedi, Dissanayake, & Vasiljevic, 2019). Therefore, the amount of protein that was bound to the water was higher in heated protein containing beads resulting in shorter relaxation times.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this study, characterization of calcium-alginate beads and use of these beads in fruit juices as a result of change in alginate concentration, heat treatment and change in pectin concentration were investigated. Heat treatment was found to be negatively effective on all parameters that were observed, whereas change in alginate ratio was found to have a significant effect on improving bead characteristics. Regarding the results of protein, release and TD-NMR analysis, decrease in alginate ratio, had a positive effect on improving the beads properties. SEM results showed that increase in alginate ratio, positively affected the bead shape and surface morphology. Also, increase in pectin ratio had a positive effect on viscosity of the juices and bead stability by decreasing the release rate.

The study showed that alginate as a wall material and cold set gelation method was effective in encapsulating pea protein. Beads that were obtained by encapsulation with 1% alginate solution was found to be the most effective regarding protein content, particle size and bead stability. Melon juice mixed with 1% pectin had the most desirable properties in terms of viscosity and bead stability in juice. Samples that were not exposed to heat treatment was found to have more desired properties in regards of all parameters.

For future studies, alginate concentrations of higher than 2% is suggested to be used for encapsulation to observe the effect of alginate ratio an encapsulation efficiency. Sensory analysis should also be performed to see the masking effect of the capsules. Different wall materials could be used with alginate to understand the effect of coating material on effectiveness of protein encapsulation and properties of encapsulated beads. Stability of the capsules could be examined in a time dependent way by using

Magnetic Resonance Imaging. Based on the obtained results it is also believed that this type of encapsulation could be widened up to encapsulate other plant-based protein types such as rice protein, chickpea or fava bean protein since they have begun to gain attention lately as alternative protein sources.

REFERENCES

Barac, M., Cabrilo, S., Pesic, M., Stanojevic, S., Zilic, S., Macej, O., & Ristic, N. (2010). Profile and functional properties of seed proteins from six pea (Pisum sativum) genotypes. *International Journal of Molecular Sciences*, *11*(12), 4973–4990. https://doi.org/10.3390/ijms11124973

Bogahawaththa, D., Chau, N. H., Trivedi, J., Dissanayake, M., & Vasiljevic, T. (2018). Impact of selected process parameters on solubility and heat stability of pea protein isolate. LWT, 246-253.

Bogahawaththa, D., Chau, N. H., Trivedi, J., Dissanayake, M., & Vasiljevic, T. (2019). Impact of controlled shearing on solubility and heat stability of pea protein isolate dispersed in solutions with adjusted ionic strength. Food Research International.

Brunetti, M., & St. Martin, A. (2006). Alginate Polymers for Drug Delivery. *Thesis*, 1–69.

Bulone, D., Giacomazza, D., Manno, M., Martorana, V., & San Biagio, P. L. (2010). Sucrose pectin interaction from solution to gels. *Food Hydrocolloids: Characteristics, Properties and Structures*, (January), 225–241.

Cabrera, J. C., Cambier, P., & van Cutsem, P. (2011). Drug encapsulation in pectin hydrogel beads- a systematic study of simulated digestion media. *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(SUPPL. 5), 292–299.

Caetano, L. A., Almeida, A. J., & Gonçalves, L. M. D. (2016). Effect of experimental parameters on alginate/chitosan microparticles for BCG encapsulation. *Marine Drugs*, *14*(5). https://doi.org/10.3390/md14050090

Caswell H. (2009). The role of fruit juice in the diet: an overview. *Nutrition Bulletin*, *34*(1), 273–288.

Chandy, T., Das, G. S., Wilson, R. F., & Rao, G. H. R. (2002). Development of polylactide microspheres for protein encapsulation and delivery. *Journal of Applied Polymer Science*, *86*(5), 1285–1295. https://doi.org/10.1002/app.11139

Chayut, N., Yuan, H., Ohali, S., Meir, A., Yeselson, Y., Portnoy, V., … Tadmor, Y. (2015). A bulk segregant transcriptome analysis reveals metabolic and cellular processes associated with Orange allelic variation and fruit β-carotene accumulation in melon fruit. *BMC Plant Biology*, *15*(1). https://doi.org/10.1186/s12870-015-0661-8

Chiba, T. (2003). SPECTROSCOPY | Visible Spectroscopy and Colorimetry. *Encyclopedia of Food Sciences and Nutrition*, 5454–5459. https://doi.org/10.1016/b0-12-227055-x/01121-4

Ching, S. H., Bansal, N., & Bhandari, B. (2017). Alginate gel particles–A review of production techniques and physical properties. *Critical Reviews in Food Science and Nutrition*, *57*(6), 1133–1152. https://doi.org/10.1080/10408398.2014.965773

Corbo, M. R., Bevilacqua, A., Petruzzi, L., Casanova, F. P., & Sinigaglia, M. (2014). Functional Beverages: The Emerging Side of Functional Foods. *Comprehensive Reviews in Food Science and Food Safety*, *13*(6), 1192–1206. https://doi.org/10.1111/1541-4337.12109

Dambal, A., Padaki, S., Herur, A., Kashinakunti, S., & Manjula, R. (2013). High Sensitivity C-Reactive Protein in Patients of Acute Myocardial Infarction with Type-2 Diabetes Mellitus-A Cross-Sectional Study, *1*(12), 10–13. https://doi.org/10.4172/scientificreports.5

Das, S. K., David, S. R. N., Rajabalaya, R., Mukhopadhyay, H. K., Halder, T., Palanisamy, M., … Nanda, A. (2011). Microencapsulation Techniques and its Practices. *International Journal of Pharmaceutical Science and Technology*, *6*(2), 1–23.

Del Gaudio, P., Colombo, P., Colombo, G., Russo, P., & Sonvico, F. (2005). Mechanisms of formation and disintegration of alginate beads obtained by prilling. *International Journal of Pharmaceutics*, *302*(1–2), 1–9. https://doi.org/10.1016/j.ijpharm.2005.05.041

Dissanayake, M., & Vasiljevic, T. (2009). Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. *Journal of Dairy Science*, *92*(4), 1387–1397. https://doi.org/10.3168/jds.2008-1791

Division, F. T., Venkateswara, S., & Pradesh, A. (2019). Influence of composite edible coating systems on preservation of fresh meat cuts and products : a brief review on their trends and applications, *26*(April), 377–392.

Djabourov, M., Leblond, J., & Papon, P. (1988). Gelation of aqueous gelatin solutions. I. Structural investigation. *Journal de Physique*, *49*(2), 319–332. https://doi.org/10.1051/jphys:01988004902031900

Draget, K. I., Smidsrod, O., & Skjåk-bræk, G. (2005). Alginates from Algae. Chapter 1. *Polysaccharides and Polyamides in the Food Industry: Properties, Production and Patents. Edited by: A. Steinbüchel and S. K. Rhee*, 2–30.

Eghbal, N., & Choudhary, R. (2018). Complex coacervation: Encapsulation and controlled release of active agents in food systems. *LWT - Food Science and Technology*, *90*(October 2017), 254–264. https://doi.org/10.1016/j.lwt.2017.12.036

El-Nawawi, S. A., & Heikel, Y. A. (1997). Factors affecting gelation of high-ester citrus pectin. *Process Biochemistry*, *32*(5), 381–385. https://doi.org/10.1016/S0032-9592(96)00076-3

Fricker, G., Kromp, T., Wendel, A., Blume, A., Zirkel, J., Rebmann, H., … Müller-Goymann, C. (2010). Phospholipids and lipid-based formulations in oral drug delivery. *Pharmaceutical Research*, *27*(8), 1469–1486. https://doi.org/10.1007/s11095-010-0130-x

Garcia, M. A., Martino, M. N., & Zaritzky, N. E. (2000). Lipid Addition to Improve Barrier Properties of Edible Starch-based Films and Coatings. *Journal of Food Science*, *65*(6), 941–944. https://doi.org/10.1111/j.1365-2621.2000.tb09397.x

Gawkowska, D., Cybulska, J., & Zdunek, A. (2018). Structure-related gelling of pectins and linking with other natural compounds: A review. *Polymers*, *10*(7). https://doi.org/10.3390/polym10070762

Gibbs, B. F., & Kermasha, S. (1999). Encapsulation in the food industry - a review.pdf, 213–224.

Gomaa, A. I., Martinent, C., Hammami, R., & Fliss, I. (2017). Dual Coating of Liposomes as Encapsulating Matrix of Antimicrobial Peptides : Development and Characterization, *5*(November). https://doi.org/10.3389/fchem.2017.00103

Gomathi, T., Susi, S., Abirami, D., & Sudha, P. N. (2017). Size Optimization And Thermal Studies On Calcium Alginate Nanoparticles. *IOSR Journal Of Pharmacy*, (December), 1–7.

Gunasekaran, S., Ko, S., & Xiao, L. (2007). Use of whey proteins for encapsulation and controlled delivery applications. *Journal of Food Engineering*, *83*(1), 31–40. https://doi.org/10.1016/j.jfoodeng.2006.11.001

Hermann, J. R. (n.d.). Protein and the Body Protein Is a Nutrient. *Oklahoma Cooperativ Extension Service, Division of Agricultural Sciences and Natural Resources*, 1–4. Retrieved from http://pods.dasnr.okstate.edu/docushare/dsweb/Get/Document-2473/T-3163web.pdf

Hogan, S. A., McNamee, B. F., Dolores O'Riordan, E., & O'Sullivan, M. (2001). Microencapsulating properties of sodium caseinate. *Journal of Agricultural and Food Chemistry*, *49*(4), 1934–1938. https://doi.org/10.1021/jf000276q

Kader, A. (2001). 234-104, (106).

Kakran, M., & Antipina, M. N. (2014). Emulsion-based techniques for encapsulation in biomedicine, food and personal care. *Current Opinion in Pharmacology*, *18*, 47–55. https://doi.org/10.1016/j.coph.2014.09.003

Khanvilkar, A. M., Ranveer, R. C., & Sahoo, A. K. (2016). Carrier materials for encapsulation of bio-active components of food. *International Journal of Pharmaceutical Sciences Review and Research*, *40*(1), 62–73.

Kırtıl, E., Dag, D., Guner, S., Unal, K., & Oztop, M. H. (2017). Dynamics of unloaded and green tea extract loaded lecithin based liposomal dispersions investigated by nuclear magnetic resonance T2 relaxation. *Food Research International*, 807-814.

Korma, S. A., Ali, A. H., Abed, S., Aloys, H., Marie Alice, T., Chantal, N., … Ildephonse, H. (2016). Microencapsulation by Complex Coacervation: Methods, Techniques, Benefits, and Applications. *American Journal of Food Science and Nutrition Research*, *3*(6), 188–192. Retrieved from http://www.openscienceonline.com/journal/fsnr

Kumagai, H., Seto, H., Sakurai, H., Ishii, K., & Kumagai, H. (1997). Analysis of Water Sorption Behavior of Native and Denatured Proteins by Solution Thermodynamics. *Bioscience, Biotechnology and Biochemistry*, *61*(8), 1307–1311. https://doi.org/10.1271/bbb.61.1307

Kurowski, C., Buffler, M., & Labortechnik, B. (2010). Kjeldahl determination of nitrogen and protein Sample preparation, digestion, distillation and titration, 50–70.

Lam, A., Karaca, A. C., Tyler, R., & Nickerson, M. (2018). Pea protein isolates: Structure, extraction, and functionality. *Food Reviews International*, 126-147.

Lebensmittelsicherheit, E. für. (2001). Report of the Scientific Committee on Food on composition and specification of food intended to meet the expenditure of intense

muscular effort, especially. *European Commission*. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Report+of+the+Scientific+Committee+on+Food+on+composition+and+specification+of+food+intended+to+meet+the+expenditure+of+intense+muscular+effort+,+especially+for+sportsmen#0

Lee, S. Y., & Wan, V. C. H. (2005). *Edible films and coatings*. *Handbook of Food Science, Technology, and Engineering - 4 Volume Set*. Elsevier Ltd. https://doi.org/10.1002/9781118229187.ch14

Leick, S., Henning, S., Degen, P., Suter, D., & Rehage, H. (2010). Deformation of liquid-filled calcium alginate capsules in a spinning drop apparatus. *Physical Chemistry Chemical Physics*, *12*(12), 2950–2958. https://doi.org/10.1039/b921116k

Lucy, L., Fries, M., Menezes, C. R. De, Holkem, A. T., Schwan, C. L., & Bastos, J. D. O. (2014). Microencapsulation : concepts , mechanisms , methods and some applications in food technology, 1304–1311.

Mandal, S., Senthil Kumar, S., Krishnamoorthy, B., & Basu, S. K. (2010). Development and evaluation of calcium alginate beads prepared by sequential and simultaneous methods. *Brazilian Journal of Pharmaceutical Sciences*, *46*(4), 785–793. https://doi.org/10.1590/S1984-82502010000400021

Martins, E., Poncelet, D., Rodrigues, R. C., & Renard, D. (2017). Oil encapsulation techniques using alginate as encapsulating agent: applications and drawbacks. *Journal of Microencapsulation*, *34*(8), 754–771. https://doi.org/10.1080/02652048.2017.1403495

Melgarejo, P., Salazar, D. M., & Artés, F. (2000). Organic acids and sugars composition of harvested pomegranate fruits. *European Food Research and Technology*, *211*(3), 185–190. https://doi.org/10.1007/s002170050021

Mellema, M., Van Benthum, W. A. J., Boer, B., Von Harras, J., & Visser, A. (2006).

Wax encapsulation of water-soluble compounds for application in foods. *Journal of Microencapsulation*, *23*(7), 729–740. https://doi.org/10.1080/02652040600787900

Mession, J. L., Sok, N., Assifaoui, A., & Saurel, R. (2013). Thermal denaturation of pea globulins (Pisum sativum L.) - Molecular interactions leading to heat-induced protein aggregation. *Journal of Agricultural and Food Chemistry*, *61*(6), 1196–1204. https://doi.org/10.1021/jf303739n

Mocanu, G.-D., & Botez, E. (2012). Milk and Dairy Products: Vectors to Create Probiotic Products. *Probiotics*, (October). https://doi.org/10.5772/50044

Moraes, T. B., Monaretto, T., & Colnago, A. L. (2016). Rapid and simple determination of relaxation times in time-domain NMR by Continuous Wave Free Precession sequence. *Journal of Magnetic Resonance*, 1-6.

Mortazavian, A., Razavi, S. H., Ehsani, M. R., & Sohrabvandi, S. (2007). Principles and methods of microencapsulation of probiotic microorganisms, *5*(1), 1–18.

Mustafa, S. (2017). Development of Alginate–Gum Arabic Beads for Targeted Delivery of Protein. *Journal of Biomolecular Research & Therapeutics*, *06*(02). https://doi.org/10.4172/2167-7956.1000155

Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., & Bugarski, B. (2011). An overview of encapsulation technologies for food applications. *Procedia Food Science*, *1*(May), 1806–1815. https://doi.org/10.1016/j.profoo.2011.09.265

Nedovic, V., Kalusevic, A., Manojlovic, V., Petrovic, T., & Bugarski, B. (2013). Chapter 13 Encapsulation Systems in the Food Industry. *Advances in Food Process Engineering Research and Applications*, 687. https://doi.org/10.1007/978-1-4614-7906-2

Nireesha, G. R., Divya, L., Sowmya, C., Venkateshan, N., Babu, M. N., & Lavakumar,

V. (2013). 1.Lyophilization, *3*(4), 87–98.

Norudin, N. S., Mohamed, H. N., & Yahya, N. A. M. (2018). Controlled released alginate-inulin hydrogel: Development and in-vitro characterization. *AIP Conference Proceedings*, *2016*(September). https://doi.org/10.1063/1.5055515

Paulsen, P. V. (2009). *Isolated soy protein usage in beverages*. *Functional and Speciality Beverage Technology*. Woodhead Publishing Limited. https://doi.org/10.1533/9781845695569.3.318

Perez-Gago, M. B., Serra, M., & Río, M. A. D. (2006). Color change of fresh-cut apples coated with whey protein concentrate-based edible coatings. *Postharvest Biology and Technology*, *39*(1), 84–92. https://doi.org/10.1016/j.postharvbio.2005.08.002

Pieczykolan, E., & Kurek, M. A. (2019). Use of guar gum, gum arabic, pectin, beta-glucan and inulin for microencapsulation of anthocyanins from chokeberry. *International Journal of Biological Macromolecules*, *129*, 665–671. https://doi.org/10.1016/j.ijbiomac.2019.02.073

Quirós-Sauceda, A. E., Ayala-Zavala, J. F., Olivas, G. I., & González-Aguilar, G. A. (2014). Edible coatings as encapsulating matrices for bioactive compounds: a review. *Journal of Food Science and Technology*, *51*(9), 1674–1685. https://doi.org/10.1007/s13197-013-1246-x

Quong, D., Neufeld, R. J., Skjåk-Bræk, G., & Poncelet, D. (1998). External versus internal source of calcium during the gelation of alginate beads for DNA encapsulation. *Biotechnology and Bioengineering*, *57*(4), 438–446. https://doi.org/10.1002/(SICI)1097-0290(19980220)57:4<438::AID-BIT7>3.0.CO;2-N

Ramos, P. E., Silva, P., Alario, M. M., Pastrana, L. M., Teixeira, J. A., Cerqueira, M. A., & Vicente, A. A. (2018). Effect of alginate molecular weight and M/G ratio in beads properties foreseeing the protection of probiotics. *Food Hydrocolloids*,

77, 8–16. https://doi.org/10.1016/j.foodhyd.2017.08.031

Ravichandran, K., Palaniraj, R., Saw, N. M. M. T., Gabr, A. M. M., Ahmed, A. R., Knorr, D., & Smetanska, I. (2014). Effects of different encapsulation agents and drying process on stability of betalains extract. *Journal of Food Science and Technology*, *51*(9), 2216–2221. https://doi.org/10.1007/s13197-012-0728-6

Sabaté, J., & Soret, S. (2014). Sustainability of plant-based diets: Back to the future. *American Journal of Clinical Nutrition*, *100*(SUPPL. 1). https://doi.org/10.3945/ajcn.113.071522

Santana, A. A., Cano-Higuita, D. M., De Oliveira, R. A., & Telis, V. R. N. (2016). Influence of different combinations of wall materials on the microencapsulation of jussara pulp (Euterpe edulis) by spray drying. *Food Chemistry*, *212*, 1–9. https://doi.org/10.1016/j.foodchem.2016.05.148

Serdaroğlu, M., Öztürk, B., & Kara, A. (2015). An Overview of Food Emulsions : Description , Classification and Recent Turkish Journal of Agriculture - Food Science and Technology An Overview of Food Emulsions : Description , Classification and Recent Potential Applications, (November). https://doi.org/10.24925/turjaf.v3i6.430-438.336

Shaddel, R., Hesari, J., Azadmard-Damirchi, S., Hamishehkar, H., Fathi-Achachlouei, B., & Huang, Q. (2018). Use of gelatin and gum Arabic for encapsulation of black raspberry anthocyanins by complex coacervation. *International Journal of Biological Macromolecules*, *107*(October), 1800–1810. https://doi.org/10.1016/j.ijbiomac.2017.10.044

Silva, C. M., Ribeiro, A. J., Figueiredo, I. V., Gonçalves, A. R., & Veiga, F. (2006). Alginate microspheres prepared by internal gelation: Development and effect on insulin stability. *International Journal of Pharmaceutics*, *311*(1–2), 1–10. https://doi.org/10.1016/j.ijpharm.2005.10.050

Siró, I., Kápolna, E., Kápolna, B., & Lugasi, A. (2008). Functional food. Product

development, marketing and consumer acceptance--a review. *Appetite*, *51*(3), 456–467. https://doi.org/10.1016/j.appet.2008.05.060

Smrdel, P., Bogataj, M., & Mrhar, A. (2008). The influence of selected parameters on the size and shape of alginate beads prepared by ionotropic gelation. *Scientia Pharmaceutica*, *76*(1), 77–89. https://doi.org/10.3797/scipharm.0611-07

Srivastava, Y., Semwal, A. D., & Sharma, G. K. (2013). Application of Various Chemical and Mechanical Microencapsulation techniques in Food Sector-A Review, *3*(10), 1–13.

SUMNER, A. K., NIELSEN, M. A., & YOUNGS, C. G. (1981). Production and Evaluation of Pea Protein Isolate. *Journal of Food Science*, *46*(2), 364–366. https://doi.org/10.1111/j.1365-2621.1981.tb04862.x

Totosaus, A., Ariza-Ortega, T. de J., & Pérez-Chabela, M. de L. (2013). Lactic acid bacteria microencapsulation in sodium alginate and other gelling hydrocolloids mixtures. *Journal of Food and Nutrition Research*, *52*(2), 107–120.

Varankovich, N. V., Khan, N. H., Nickerson, M. T., Kalmokoff, M., & Korber, D. R. (2015). Evaluation of pea protein-polysaccharide matrices for encapsulation of acid-sensitive bacteria. *Food Research International*, *70*, 118–124. https://doi.org/10.1016/j.foodres.2015.01.028

Wandrey, C., Bartkowiak, A., & Harding, S. E. (2010). *Materials for encapsulation*. *Encapsulation Technologies for Active Food Ingredients and Food Processing*. https://doi.org/10.1007/978-1-4419-1008-0_3

Wang, X., Yuan, Y., & Yue, T. (2015). The application of starch-based ingredients in flavor encapsulation. *Starch/Staerke*, *67*(3–4), 225–236. https://doi.org/10.1002/star.201400163

Watson, M. A., Lea, J. M., & Bett-Garber, K. L. (2017). Spray drying of pomegranate juice using maltodextrin/cyclodextrin blends as the wall material. *Food Science and Nutrition*, *5*(3), 820–826. https://doi.org/10.1002/fsn3.467

Zhang, Y., Zheng, W., Gu, J. F., Ni, J., Wang, L., Tang, Z. X., & Shi, L. E. (2015). Soy protein isolate-alginate microspheres for encapsulation of Enterococcus faecalis HZNU P2. *Brazilian Archives of Biology and Technology*, *58*(5), 805–811. https://doi.org/10.1590/S1516-89132015050260

Zhang, Z., Zhang, R., Zou, L., & McClements, D. J. (2016). Protein encapsulation in alginate hydrogel beads: Effect of pH on microgel stability, protein retention and protein release. *Food Hydrocolloids*, *58*(June), 308–315. https://doi.org/10.1016/j.foodhyd.2016.03.015

APPENDICES

A. Statistical Analysis

Table A.1. Rheological properties of Melon Juice

General Linear Model: Viscosity versus Pectin Ratio

Factor	Type	Levels	Values
Pectin Ratio	Fixed	3	0,0; 0,5; 1,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Pectin Ratio	2	0,000617	0,000308	1156,43	0,000
Error	6	0,000002	0,000000		
Total	8	0,000618			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0005164	99,74%	99,66%	99,42%

Coefficient

Terms	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,012944	0,000172	75,20	0,000	
Pectin Ratio					
0,0	-0,008578	0,000243	-35,24	0,000	1,33
0,5	-0,002611	0,000243	-10,73	0,000	1,33

Regression Equation

Viscosity = 0,012944- 0,008578 Pectin Ratio_0,0- 0,002611 Pectin Ratio_0,5
+ 0,011189 Pectin Ratio_1,0

Tukey Pairwise Comparisons: Pectin Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Pectin Ratio	N	Mean	Grouping
0,0	3	0,0241333	A
0,5	3	0,0103333	B
1,0	3	0,0043667	C

Table A.2. Rheological properties of Pomegranate Juice

General Linear Model: Viscosity versus Pectin Ratio

Factor	Type	Levels	Values
Pectin Ratio	Fixed	3	0,0; 0,5; 1,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Pectin Ratio	2	0,000485	0,000243	1436,70	0,000
Error	6	0,000001	0,000000		
Total	8	0,000486			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0004110	99,79%	99,72%	99,53%

Coefficient

Terms	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0,011733	0,000137	85,65	0,000	
Pectin Ratio					
0,0	-0,007033	0,000194	-36,31	0,000	1,33
0,5	-0,003100	0,000194	-16,00	0,000	1,33

Regression Equation

Viscosity = 0,011733- 0,007033 Pectin Ratio_0,0- 0,003100 Pectin Ratio_0,5
+ 0,010133 Pectin Ratio_1,0

Tukey Pairwise Comparisons: Pectin Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Pectin Ratio	N	Mean	Grouping
0,0	3	0,0218667	A
0,5	3	0,0086333	B
1,0	3	0,0047000	C

Table A.3. Comparison of rheological properties of melon juice versus pomegranate juice

Tukey Pairwise Comparisons: Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Juice Type	N	Mean	Grouping
Melon	9	0,0129444	A
Pomegranate	9	0,0117333	B

Tukey Pairwise Comparisons: Pectin Ratio*Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Pectin Ratio*

Juice Type	N	Mean	Grouping
1,0 Melon	3	0,0241333	A
1,0 Pomegranate	3	0,0218667	B
0,5 Melon	3	0,0103333	C
0,5 Pomegranate	3	0,0086333	D
0,0 Pomegranate	3	0,0047000	E
0,0 Melon	3	0,0043667	E

Table A.4. Protein content of non-heated beads

General Linear Model: Protein Content versus Alginate Ratio

Factor	Type	Levels	Values
Alginate Ratio	Fixed	3	1,0; 1,5; 2,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Alginate Ratio	2	148,71	74,353	10,24	0,012
Error	6	43,58	7,263		
Total	8	192,28			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2,69501	77,34%	69,78%	49,01%

Coefficient

Terms	Coef	SE Coef	T-Value	P-Value	VIF
Constant	25,266	0,898	28,12	0,000	
Alginate Ratio					
1,0	4,99	1,27	3,93	0,008	1,33
1,5	-0,03	0,000243	-0,02	0,985	1,33

Regression Equation

Protein Content = 25,266+ 4,99 Alginate Ratio_1,0- 0,03 Alginate Ratio_1,5 - 4,97 Alginate Ratio_2,0

Tukey Pairwise Comparisons: Alginate Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Alginate Ratio	N	Mean	Grouping
1,0	3	30,2567	A
1,5	3	25,2400	AB
2,0	3	20,3000	B

Table A.5. Protein content of heated beads

General Linear Model: Protein Content versus Alginate Ratio

Factor	Type	Levels	Values
Alginate Ratio	Fixed	3	1,0; 1,5; 2,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Alginate Ratio	2	117,56	58,781	21,48	0,002
Error	6	16,42	2,737		
Total	8	133,98			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1,65432	87,74%	83,66%	72,42%

Coefficient

Terms	Coef	SE Coef	T-Value	P-Value	VIF
Constant	23,530	0,551	42,67	0,000	
Alginate Ratio					
1,0	4,587	0,780	5,88	0,001	1,33
1,5	-0,340	0,780	-0,44	0,678	1,33

Regression Equation

Protein Content = 23,530+ 4,587 Alginate Ratio_1,0- 0,340 Alginate Ratio_1,5 - 4,247 Alginate Ratio_2,0

Tukey Pairwise Comparisons: Alginate Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Alginate Ratio	N	Mean	Grouping
1,0	3	28,1167	A
1,5	3	23,1900	B
2,0	3	19,2833	B

Table A.6. Comparison of protein content of heated beads versus non-heated beads

Tukey Pairwise Comparisons: Heat Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment	N	Mean	Grouping
Non-Heated	9	25,2656	A
Heated	9	23,5300	A

Tukey Pairwise Comparisons: Alginate Ratio*Heat Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Alginate Ratio*

Heat Treatment	N	Mean	Grouping
1,0 Non-Heated	3	30,2567	A
1,0 Heated	3	28,1167	AB
1,5 Non-Heated	3	25,2400	ABC
1,5 Heated	3	23,1900	BC
2,0 Non-Heated	3	20,3000	C
2,0 Heated	3	19,2833	C

Table A.7. Protein recovery of non-heated beads

General Linear Model: Protein Recovery versus Alginate Ratio

Factor	Type	Levels	Values
Alginate Ratio	Fixed	3	1,0; 1,5; 2,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Alginate Ratio	2	45,28	22,64	0,61	0,575
Error	6	223,29	37,21		
Total	8	268,56			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
6,10036	16,86%	0,00%	0,00%

Coefficient

Terms	Coef	SE Coef	T-Value	P-Value	VIF
Constant	47,29	2,03	23,25	0,000	
Alginate Ratio					
1,0	2,07	2,88	0,72	0,499	1,33
1,5	-3,12	2,88	-1,08	0,320	1,33

Regression Equation

Protein Recovery = 47,29+ 2,07 Alginate Ratio_1,0- 3,12 Alginate Ratio_1,5 + 1,05 Alginate Ratio_2,0

Tukey Pairwise Comparisons: Alginate Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Alginate Ratio	N	Mean	Grouping
1,0	3	49,3583	A
1,5	3	48,3333	A
2,0	3	44,1714	A

Table A.8. Protein recovery of heated beads

Factor	Type	Levels	Values
Alginate Ratio	Fixed	3	1,0; 1,5; 2,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Alginate Ratio	2	5,910	2,955	0,26	0,779
Error	6	68,129	11,355		
Total	8	74,039			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3,36070	7,98 %	0,00%	0,00%

Coefficient

Terms	Coef	SE Coef	T-Value	P-Value	VIF
Constant	45,32	1,12	40,35	0,000	
Alginate Ratio					
1,0	0,55	1,59	0,35	0,741	1,33
1,5	-1,15	1,59	-0,72	0,498	1,33

Regression Equation

Protein Recovery = 45,32+ 0,55 Alginate Ratio_1,0- 1,15 Alginate Ratio_1,5 + 0,60 Alginate Ratio_2,0

Tukey Pairwise Comparisons: Alginate Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Alginate Ratio	N	Mean	Grouping
1,0	3	45,9127	A
1,5	3	45,8673	A
2,0	3	44,1714	A

Table A.9. Comparison of protein recovery of heated beads versus non-heated beads

Tukey Pairwise Comparisons: Heat Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment	N	Mean	Grouping
Non-Heated	9	47,2877	A
Heated	9	45,3171	A

Tukey Pairwise Comparisons: Alginate Ratio*Heat Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment	N	Mean	Grouping
1,0 Non-Heated	3	49,3583	A
1,0 Heated	3	48,3333	A
1,5 Non-Heated	3	45,9127	A
1,5 Heated	3	45,8673	A
2,0 Non-Heated	3	44,1714	A
2,0 Heated	3	44,1714	A

Table A.10. Particle size of non-heated beads

General Linear Model: Particle size versus Alginate Ratio

Factor	Type	Levels	Values
Alginate Ratio	Fixed	3	1,0; 1,5; 2,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Alginate Ratio	2	3,177	1,5883	9,86	0,000
Error	297	47,847	0,1611		
Total	299	51,024			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,401375	6,23 %	5,59%	4,32%

Coefficient

Terms	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1,7123	0,0232	73,89	0,000	
Alginate Ratio					
1,0	-0,1047	0,0328	-3,20	0,002	1,33
1,5	0,1399	0,0328	4,27	0,000	1,33

Regression Equation

Particle size = 1,7123- 0,1047 Alginate Ratio_1,0+ 0,1399 Alginate Ratio_1,5 - 0,0351 Alginate Ratio_2,0

Tukey Pairwise Comparisons: Alginate Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Alginate Ratio	N	Mean	Grouping
1,5	100	1,85220	A
2,0	100	1,67721	B
1,0	100	1,60760	B

Table A.11. Particle size of heated beads

General Linear Model: Particle size versus Alginate Ratio

Factor	Type	Levels	Values
Alginate Ratio	Fixed	3	1,0; 1,5; 2,0

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Alginate Ratio	2	0,9045	0,4523	3,01	0,051
Error	297	44,6932	0,1505		
Total	299	45,5977			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,387920	1,98%	1,32%	0,00%

Coefficient

Terms	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1,7866	0,0224	79,77	0,000	
Alginate Ratio					
1,0	-0,0673	0,0317	-2,12	0,034	1,33
1,5	0,0672	0,0317	2,12	0,035	1,33

Regression Equation

Particle size = 1,7866- 0,0673 Alginate Ratio_1,0+ 0,0672 Alginate Ratio_1,5 + 0,0001 Alginate Ratio_2,0

Tukey Pairwise Comparisons: Alginate Ratio

Grouping Information Using the Tukey Method and 95% Confidence

Alginate Ratio	N	Mean	Grouping
1,5	100	1,8538	A
2,0	100	1,7867	AB
1,0	100	1,7193	B

Table A.12. Comparison of particle size of non-heated and heated beads

Tukey Pairwise Comparisons: Heat Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment	N	Mean	Grouping
Heated	300	1,78660	A
Non-Heated	300	1,71234	B

Tukey Pairwise Comparisons: Alginate Ratio*Heat Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment	N	Mean	Grouping
1,5 Heated	100	1,85380	A
1,5 Non-Heated	100	1,85220	A
2,0 Heated	100	1,78670	AB
1,0 Heated	100	1,71930	ABC
2,0 Non-Heated	100	1,67721	BC
1,0 Non-Heated	100	1,60760	C

Table A.13 Release rate of pea protein from beads

General Linear Model: Release Rate versus Alginate concentration; Heat Treatment; Pectin Concentration; Juice Type

Factor Information

Factor	Type	Levels	Values
Alginate concentration	Fixed	3	1; 1,5; 2
Heat Treatment	Fixed	2	N; Y
Pectin Concentration	Fixed	3	0,0; 0,5; 1,0
Juice Type	Fixed	2	m; p

Analysis of Variance

Source	DF	Adj SS	Adj MS
Alginate concentration	2	1393,01	696,50
Heat Treatment	1	825,35	825,35
Pectin Concentration	2	632,11	316,06
Juice Type	1	2848,23	2848,23
Alginate concentration*Heat Treatment	2	22,86	11,43
Alginate concentration*Pectin Concentration	4	11,64	2,91
Alginate concentration*Juice Type	2	92,42	46,21
Heat Treatment*Pectin Concentration	2	52,93	26,47
Heat Treatment*Juice Type	1	0,12	0,12
Pectin Concentration*Juice Type	2	484,40	242,20
Alginate concentration*Heat Treatment*Pectin Concentration	4	5,72	1,43
Alginate concentration*Heat Treatment*Juice Type	2	9,89	4,94
Alginate concentration*Pectin Concentration*Juice Type	4	3,57	0,89
Heat Treatment*Pectin Concentration*Juice Type	2	7,56	3,78
Alginate concentration*Heat Treatment*Pectin Concentration*Juice Type	4	6,04	1,51
Error	36	0,18	0,00
Total	71	6396,02	

	F-Value	P-Value

Source		
Heat Treatment	169194,19	0,000
Pectin Concentration	64790,95	0,000
Juice Type	583880,81	0,000
Alginate concentration*Heat Treatment	2343,61	0,000
Alginate concentration*Pectin Concentration	596,50	0,000
Alginate concentration*Juice Type	9473,09	0,000
Heat Treatment*Pectin Concentration	5425,47	0,000
Heat Treatment*Juice Type	23,74	0,000
Pectin Concentration*Juice Type	49650,61	0,000
Alginate concentration*Heat Treatment*Pectin Concentration	293,20	0,000
Alginate concentration*Heat Treatment*Juice Type	1013,51	0,000
Alginate concentration*Pectin Concentration*Juice Type	182,94	0,000
Heat Treatment*Pectin Concentration*Juice Type	774,91	0,000
Alginate concentration*Heat Treatment*Pectin Concentration*Juice Type	309,40	0,000
Error		
Total		

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0,0698434	100,00%	99,99%	99,99%

Coefficients

Term	Coef	SE Coef
Constant	18,4506	0,0082
Alginate concentration		
1	-5,0435	0,0116
1,5	-0,6315	0,0116
Heat Treatment		
N	-3,38573	0,00823
Pectin Concentration		
0,0	2,2248	0,0116
0,5	1,9627	0,0116
Juice Type		
m	-6,28957	0,00823
Alginate concentration*Heat Treatment		
1 N	0,7955	0,0116
1,5 N	-0,3565	0,0116
Alginate concentration*Pectin Concentration		
1 0,0	-0,7199	0,0165
1 0,5	0,1773	0,0165
1,5 0,0	0,4233	0,0165

1,5 0,5	-0,3307	0,0165
Alginate concentration*Juice Type		
1 m	1,3402	0,0116
1,5 m	0,0904	0,0116
Heat Treatment*Pectin Concentration		
N 0,0	-0,3659	0,0116
N 0,5	-0,8182	0,0116
Heat Treatment*Juice Type		
N m	-0,04011	0,00823
Pectin Concentration*Juice Type		
0,0 m	3,6549	0,0116
0,5 m	-2,0976	0,0116
Alginate concentration*Heat Treatment*Pectin Concentration		
1 N 0,0	0,1276	0,0165
1 N 0,5	-0,0276	0,0165
1,5 N 0,0	0,1450	0,0165
1,5 N 0,5	0,2781	0,0165
Alginate concentration*Heat Treatment*Juice Type		
1 N m	0,4120	0,0116
1,5 N m	0,0744	0,0116
Alginate concentration*Pectin Concentration*Juice Type		
1 0,0 m	-0,4111	0,0165
1 0,5 m	0,1927	0,0165
1,5 0,0 m	0,1116	0,0165

1,5 0,5 m	0,0493	0,0165
Heat Treatment*Pectin Concentration*Juice Type		
N 0,0 m	0,2852	0,0116
N 0,5 m	-0,4532	0,0116
Alginate concentration*Heat Treatment*Pectin Concentration*Juice Type		
1 N 0,0 m	-0,0273	0,0165
1 N 0,5 m	0,3333	0,0165
1,5 N 0,0 m	0,1227	0,0165
1,5 N 0,5 m	0,0993	0,0165

Term	T-Value	P-Value	VIF
Constant	2241,56	0,000	
Alginate concentration			
1	-433,27	0,000	1,33
1,5	-54,25	0,000	1,33
Heat Treatment			
N	-411,33	0,000	1,00
Pectin Concentration			
0,0	191,13	0,000	1,33
0,5	168,61	0,000	1,33
Juice Type			
m	-764,12	0,000	1,00

Alginate concentration*Heat Treatment			
1 N	68,34	0,000	1,33
1,5 N	-30,63	0,000	1,33
Alginate concentration*Pectin Concentration			
1 0,0	-43,73	0,000	1,78
1 0,5	10,77	0,000	1,78
1,5 0,0	25,72	0,000	1,78
1,5 0,5	-20,09	0,000	1,78
Alginate concentration*Juice Type			
1 m	115,13	0,000	1,33
1,5 m	7,76	0,000	1,33
Heat Treatment*Pectin Concentration			
N 0,0	-31,43	0,000	1,33
N 0,5	-70,29	0,000	1,33
Heat Treatment*Juice Type			
N m	-4,87	0,000	1,00
Pectin Concentration*Juice Type			
0,0 m	313,98	0,000	1,33
0,5 m	-180,19	0,000	1,33
Alginate concentration*Heat Treatment*Pectin Concentration			
1 N 0,0	7,75	0,000	1,78
1 N 0,5	-1,68	0,102	1,78
1,5 N 0,0	8,81	0,000	1,78
1,5 N 0,5	16,89	0,000	1,78

Alginate concentration*Heat Treatment*Juice Type			
1 N m	35,40	0,000	1,33
1,5 N m	6,40	0,000	1,33
Alginate concentration*Pectin Concentration*Juice Type			
1 0,0 m	-24,97	0,000	1,78
1 0,5 m	11,71	0,000	1,78
1,5 0,0 m	6,78	0,000	1,78
1,5 0,5 m	2,99	0,005	1,78
Heat Treatment*Pectin Concentration*Juice Type			
N 0,0 m	24,50	0,000	1,33
N 0,5 m	-38,94	0,000	1,33
Alginate concentration*Heat Treatment*Pectin Concentration*Juice Type			
1 N 0,0 m	-1,66	0,106	1,78
1 N 0,5 m	20,25	0,000	1,78
1,5 N 0,0 m	7,45	0,000	1,78
1,5 N 0,5 m	6,03	0,000	1,78

Table A.14. Comparisons of release rate in with respect to alginate ratio, pectin ratio, heat treatment and juice type

Tukey Pairwise Comparisons: Alginate concentration

Grouping Information Using the Tukey Method and 95% Confidence

Alginate concentration	N	Mean	Grouping
2	24	24,1256	A
1,5	24	17,8191	B
1	24	13,4071	C

Tukey Pairwise Comparisons: Heat Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment	N	Mean	Grouping
Y	36	21,8363	A
N	36	15,0649	B

Tukey Pairwise Comparisons: Pectin Concentration

Grouping Information Using the Tukey Method and 95% Confidence

Pectin Concentration	N	Mean	Grouping
0,0	24	20,6754	A
0,5	24	20,4133	B
1,0	24	14,2630	C

Tukey Pairwise Comparisons: Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Juice Type	N	Mean	Grouping
p	36	24,7402	A
m	36	12,1610	B

Tukey Pairwise Comparisons: Alginate concentration*Heat Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Alginate concentration*Heat Treatment	N	Mean	Grouping
2 Y	12	27,9503	A
1,5 Y	12	21,5613	B
2 N	12	20,3009	C
1 Y	12	15,9973	D
1,5 N	12	14,0768	E
1 N	12	10,8169	F

Tukey Pairwise Comparisons: Alginate concentration*Pectin Concentration

Grouping Information Using the Tukey Method and 95% Confidence

Alginate concentration*Pectin Concentration	N	Mean	Grouping
2 0,0	8	26,6470	A
2 0,5	8	26,2417	B
1,5 0,0	8	20,4673	C
2 1,0	8	19,4882	D
1,5 0,5	8	19,4512	D
1 0,5	8	15,5471	E
1 0,0	8	14,9120	F
1,5 1,0	8	13,5388	G
1 1,0	8	9,7620	H

Tukey Pairwise Comparisons: Alginate concentration*Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Alginate concentration*Juice Type	N	Mean	Grouping
2 p	12	31,8458	A
1,5 p	12	24,0183	B
1 p	12	18,3564	C
2 m	12	16,4055	D
1,5 m	12	11,6199	E
1 m	12	8,4577	F

Tukey Pairwise Comparisons: Heat Treatment*Pectin Concentration

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment*Pectin Concentration	N	Mean	Grouping
Y 0,5	12	24,6173	A
Y 0,0	12	24,4270	B
N 0,0	12	16,9238	C
Y 1,0	12	16,4646	D
N 0,5	12	16,2094	E
N 1,0	12	12,0614	F

Tukey Pairwise Comparisons: Heat Treatment*Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment*Juice Type	N	Mean	Grouping
Y p	18	28,0858	A
N p	18	21,3945	B
Y m	18	15,5868	C
N m	18	8,7352	D

Tukey Pairwise Comparisons: Pectin Concentration*Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Pectin Concentration*Juice Type	N	Mean	Grouping
0,5 p	12	28,8005	A
0,0 p	12	23,3101	B
1,0 p	12	22,1099	C
0,0 m	12	18,0407	D
0,5 m	12	12,0262	E
1,0 m	12	6,4161	F

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Tukey Pairwise Comparisons: Alginate concentration*Heat Treatment*Pectin Concentration

Grouping Information Using the Tukey Method and 95% Confidence

Alginate concentration*Heat Treatment*Pectin Concentration	N	Mean	Grouping
2 Y 0,5	4	31,1352	A
2 Y 0,0	4	31,1102	A
1,5 Y 0,0	4	24,4304	B
1,5 Y 0,5	4	23,7336	C
2 N 0,0	4	22,1837	D
2 Y 1,0	4	21,6057	E
2 N 0,5	4	21,3482	F
1 Y 0,5	4	18,9832	G
1 Y 0,0	4	17,7405	H
2 N 1,0	4	17,3707	I
1,5 Y 1,0	4	16,5201	J
1,5 N 0,0	4	16,5041	J
1,5 N 0,5	4	15,1688	K
1 N 0,5	4	12,1111	L
1 N 0,0	4	12,0836	L
1 Y 1,0	4	11,2681	M
1,5 N 1,0	4	10,5576	N
1 N 1,0	4	8,2559	O

Tukey Pairwise Comparisons: Alginate concentration*Heat Treatment*Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Alginate concentration*Heat Treatment*Juice Type	N	Mean	Grouping
2 Y p	6	35,1439	A
2 N p	6	28,5476	B
1,5 Y p	6	27,7949	C
1 Y p	6	21,3186	D
2 Y m	6	20,7568	E
1,5 N p	6	20,2417	F
1 N p	6	15,3943	G
1,5 Y m	6	15,3278	G
2 N m	6	12,0541	H
1 Y m	6	10,6759	I
1,5 N m	6	7,9120	J
1 N m	6	6,2394	K

Tukey Pairwise Comparisons: Alginate concentration*Pectin Concentration*Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Alginate concentration*Pectin Concentration*Juice Type	N	Mean	Grouping
2 0,5 p	4	36,3014	A
2 0,0 p	4	30,4127	B
2 1,0 p	4	28,8232	C
1,5 0,5 p	4	27,6987	D
1,5 0,0 p	4	22,8999	E
2 0,0 m	4	22,8812	E
1 0,5 p	4	22,4013	F
1,5 1,0 p	4	21,4562	G
1,5 0,0 m	4	18,0346	H
1 0,0 p	4	16,6176	I
2 0,5 m	4	16,1820	J
1 1,0 p	4	16,0503	J
1 0,0 m	4	13,2064	K
1,5 0,5 m	4	11,2037	L
2 1,0 m	4	10,1531	M
1 0,5 m	4	8,6929	N
1,5 1,0 m	4	5,6214	O
1 1,0 m	4	3,4737	P

Tukey Pairwise Comparisons: Heat Treatment*Pectin Concentration*Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Heat Treatment*Pectin Concentration*Juice Type	N	Mean	Grouping
Y 0,5 p	6	32,5111	A
Y 0,0 p	6	27,3067	B
N 0,5 p	6	25,0899	C
Y 1,0 p	6	24,4395	D
Y 0,0 m	6	21,5473	E
N 1,0 p	6	19,7803	F
N 0,0 p	6	19,3134	G
Y 0,5 m	6	16,7235	H
N 0,0 m	6	14,5342	I
Y 1,0 m	6	8,4897	J
N 0,5 m	6	7,3289	K
N 1,0 m	6	4,3424	L

Tukey Pairwise Comparisons: Alginate concentration*Heat Treatment*Pectin Concentration*Juice Type

Grouping Information Using the Tukey Method and 95% Confidence

Alginate concentration*Heat Treatment*Pectin Concentration*Juice Type	N	Mean	Grouping
2 Y 0,5 p	2	39,7824	A
2 Y 0,0 p	2	34,5391	B
2 N 0,5 p	2	32,8204	C
1,5 Y 0,5 p	2	31,6614	D
2 Y 1,0 p	2	31,1102	E
2 Y 0,0 m	2	27,6813	F
1,5 Y 0,0 p	2	27,3053	G
2 N 1,0 p	2	26,5363	H
2 N 0,0 p	2	26,2863	H I
1 Y 0,5 p	2	26,0894	]
1,5 Y 1,0 p	2	24,4179	J
1,5 N 0,5 p	2	23,7359	K
2 Y 0,5 m	2	22,4879	L
1,5 Y 0,0 m	2	21,5555	M
1 Y 0,0 p	2	20,0759	N
1 N 0,5 p	2	18,7133	O
1,5 N 0,0 p	2	18,4946	O
1,5 N 1,0 p	2	18,4946	O

2 N 0,0 m	2	18,0812	P
1 Y 1,0 p	2	17,7905	P
1,5 Y 0,5 m	2	15,8057	Q
1 Y 0,0 m	2	15,4051	R
1,5 N 0,0 m	2	14,5136	S
1 N 1,0 p	2	14,3102	S
1 N 0,0 p	2	13,1594	T
2 Y 1,0 m	2	12,1012	U
1 Y 0,5 m	2	11,8770	U
1 N 0,0 m	2	11,0078	V
2 N 0,5 m	2	9,8761	W
1,5 Y 1,0 m	2	8,6222	X
2 N 1,0 m	2	8,2051	Y
1,5 N 0,5 m	2	6,6016	Z
1 N 0,5 m	2	5,5089	AA
1 Y 1,0 m	2	4,7458	AB
1,5 N 1,0 m	2	2,6207	AC
1 N 1,0 m	2	2,2016	AD

Table A.15. T2 values of the beads

Factor	Type	Levels	Values
Alginate ratio	Fixed	3	1.0; 1.5; 2.0
Type of solution	Fixed	3	M; P; W
Heat treatment	Fixed	2	N; Y

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Concentration	2	76.48	38.239	5.35	0.009
Heat treatment	1	282.23	282.230	39.48	0.000
Type of solution	2	302.26	151.131	21.14	0.000
Concentration*					
Type of solution	4	141.52	35.381	4.95	0.003
Concentration*					
Heat treatment	2	5.85	2.925	0.41	0.667
Type of solution*					
Heat treatment	2	60.21	30.106	4.21	0.023
Concentration*					
Type of solution*					
Heat treatment	4	325.38	81.346	11.38	0.000
Error	36	257.37	7.149		

Total	53	1451.31

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.67380	82.27%	73.89%	60.10%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	32.624	0.364	89.66	0.000	
Concentration					
1.0	1.531	0.515	2.98	0.005	1.33
1.5	-0.161	0.515	-0.31	0.757	1.33
Heat treatment					
N	2.286	0.364	6.28	0.000	1.00
Type of solution					
M	-2.054	0.515	-3.99	0.000	1.33
P	-1.260	0.515	-2.45	0.019	1.33
Concentration*					
Type of solution					
1.0 M	-0.085	0.728	-0.12	0.908	1.78
1.0 P	-0.258	0.728	-0.35	0.725	1.78

1.5	M	2.826	0.728	3.88	0.000	1.78
1.5	P	-1.382	0.728	-1.90	0.066	1.78
Concentration*						
Heat treatment						
1.0	N	0.408	0.515	0.79	0.433	1.33
1.5	N	-0.398	0.515	-0.77	0.445	1.33
Type of solution*						
Heat treatment						
M	N	-1.455	0.515	-2.83	0.008	1.33
P	N	0.435	0.515	0.84	0.404	1.33
Concentration*						
Type of solution*						
Heat treatment						
1.0	M	N	-3.994	0.728	-5.49	0.000	1.78
1.0	P	N	1.071	0.728	1.47	0.150	1.78
1.5	M	N	-0.245	0.728	-0.34	0.738	1.78
1.5	P	N	0.178	0.728	0.24	0.809	1.78

Table A.16. Comparison of T2 values

Tukey Pairwise Comparisons: Concentration

Grouping Information Using the Tukey Method and 95% Confidence

Concentration	N	Mean	Grouping	
1.0	18	34.1552	A	
1.5	18	32.4633	A	B
2.0	18	31.2534	B	

Tukey Pairwise Comparisons: Heat treatment

Grouping Information Using the Tukey Method and 95% Confidence

Heat treatment	N	Mean	Grouping
N	27	34.9101	A
Y	27	30.3378	B

Tukey Pairwise Comparisons: Type of solution

Grouping Information Using the Tukey Method and 95% Confidence

Typeof solution	N	Mean	Grouping
W	18	35.9383	A
P	18	31.3641	B
M	18	30.5696	B

Tukey Pairwise Comparisons: Concentration*Type of solution

Grouping Information Using the Tukey Method and 95% Confidence

Concentration*Type

of solution	N	Mean	Grouping		
1.0 W	6	37.8125	A		
2.0 W	6	35.6692	A	B	
1.5 W	6	34.3331	A	B	C
1.5 M	6	33.2349	A	B	C
1.0 P	6	32.6374	B	C	
1.0 M	6	32.0157	B	C	
2.0 P	6	31.6330	B	C	
1.5 P	6	29.8217	C	D	
2.0 M	6	26.4581	D		

Tukey Pairwise Comparisons: Concentration*Heat treatment

Grouping Information Using the Tukey Method and 95% Confidence

Concentration*Heat

treatment	N	Mean	Grouping	
1.0 N	9	36.8496	A	
1.5 N	9	34.3517	A	B
2.0 N	9	33.5291	A	B
1.0 Y	9	31.4608	B	C

1.5 Y	9	30.5748	B	C	
2.0 Y	9	28.9778	C		

Tukey Pairwise Comparisons: Type of solution*Heat treatment

Grouping Information Using the Tukey Method and 95% Confidence

Typeof solution*Heat treatment	N	Mean	Grouping		
W N	9	39.2443	A		
P N	9	34.0850	B		
W Y	9	32.6323	B	C	
M N	9	31.4011	B	C	D
M Y	9	29.7381	C	D	
P Y	9	28.6431		D	

Tukey Pairwise Comparisons: Concentration*Type of solution*Heat treatment

Grouping Information Using the Tukey Method and 95% Confidence

Concentration*Type ofsolution*Heat treatment	N	Mean	Grouping	
1.0 W N	3	44.4505	A	
1.5 W N	3	37.3093	A	B

1.0 P N	3	36.8372	A	B		
2.0 W N	3	35.9730	B	C		
2.0 W Y	3	35.3654	B	C	D	
1.0 M Y	3	34.7703	B	C	D	
1.5 M N	3	33.4232	B	C	D	
2.0 P N	3	33.0953	B	C	D	
1.5 M Y	3	33.0467	B	C	D	
1.5 P N	3	32.3225	B	C	D	
2.0 M N	3	31.5190	B	C	D	
1.5 W Y	3	31.3569	B	C	D	
1.0 W Y	3	31.1744	B	C	D	
2.0 P Y	3	30.1707	B	C	D	
1.0 M N	3	29.2611	B	C	D	E
1.0 P Y	3	28.4377	C	D	E	
1.5 P Y	3	27.3210	D	E		
2.0 M Y	3	21.3973	E			