USE OF IONIC GELATION TO REDUCE PERCEIVED BITTERNESS OF SPIRULINA PROTEIN

By

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PERCEIVED BITTERNESS OF SPIRULINA PROTEIN

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Title of Study: USE OF IONIC GELATION TO REDUCE PERCEIVED BITTERNESS OF SPIRULINA PROTEIN

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Abstract:

Spirulina (blue-green algae) is one of the cheapest sources of protein and essential vitamins. However, bitterness and bad flavor of spirulina protein may limit its use in food products. In this study, ionic gelation was used to facilitate protein delivery and to mask the bitter flavor of the spirulina protein. The objective was to develop a method for encapsulating spirulina protein using sodium alginate, and evaluate its effectiveness in reducing the perceived bitterness of spirulina.

Spirulina protein was encapsulated in alginate using both internal and external gelation methods and varying concentrations of sodium alginate and calcium chloride. A total of six different treatments were evaluated. The crude protein was measured using the Dumas method, and the firmness/hardness was measured using a texture analyzer. The morphology was studied using a scanning electron microscope (SEM). The thickness and width of the beads were measured using a digital caliper. The prepared beads were incorporated into cookies to do a sensory evaluation in comparison with untreated spirulina, a standard bitter blocker flavor, and soy protein.

Results from analysis of the bead characteristics showed that the beads formed by external gelation were superior to those formed with internal gelation. The hardness of the beads prepared by external gelation was significantly higher than the hardness of the beads prepared by internal gelation. External gelation beads show a more smooth and rigid exterior morphology, whereas internal gelation beads show a soft and heterogeneous exterior morphology. External gelation beads also possess higher protein content than the internal gelation beads. Results from sensory evaluation showed that the color of samples with spirulina-alginate beads was significantly better than the samples with untreated spirulina. However, the panelists felt that the cookies with spirulina-alginate beads were more bitter than other the cookie samples.

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CHAPTER I

INTRODUCTION

1.1 Background

An increasing world population and depleting natural resources have created a need to develop a sustainable and cost-effective protein source. Today, protein malnutrition is a problem worldwide, and global annual mortality rates due to protein malnutrition are 7.1 per 100,000 people (Global Health Data Exchange and The World Bank, 2013). Especially in developing countries, malnutrition is the cause of many health problems in young children, including increased risk of mortality, weakened the immune system, and diminished cognitive capacity and school performance (Hug and Weid 2011). Microbial cells have the potential to provide an alternative source of protein around the world. Algae contain very high levels of complete protein, and they are also rich in lipids, minerals, vitamins, soluble fiber and other bioactive compounds (Becker 2007; Chronakis and Madsen 2011).

Among algae proteins, spirulina is considered to be a powerhouse of nutrients. It has high concentrations of beta carotene, vitamin B-12, iron, Gamma Linolenic Acid (GLA) and minerals. It also has a balanced spectrum of amino acids, and pigments like chlorophyll and phycocyanin. Spirulina can also be more sustainably produced than other traditional protein sources. It requires 200 times less land and 50 times less water than beef to produce the same amount of protein (IIMSAM – United Nations, 2015). However, the unpleasant organoleptic properties of spirulina restrict its application in food products. Different physical, chemical and biological methods can be employed to reduce the bitterness of spirulina protein. One method with great potential involves the use of alginate to form a gel matrix and encapsulate the spirulina to mask it's bitter flavor.

1.2 Objectives:

The main objective of this project was to study the use of ionic gelation for reducing the perceived bitterness of spirulina protein.

The specific objectives were to:

A. Compare the effects of different gelation methods on the particle size, texture, morphology and crude protein content of the beads.

B. Evaluate encapsulation efficiency of spirulina with different gelation methods.

C. Compare the sensory perception of spirulina in raw form with spirulina-alginate beads, and in the presence of a commercial bitter blocker flavor.

CHAPTER II

REVIEW OF LITERATURE

2.1 Protein Malnutrition

Protein malnutrition is one of the major global public health concerns, affecting mainly developing countries (Ubesie and Ibeziakor 2012; Colombelli et al. 2016). The World Health Organization reports that protein malnutrition is one of the largest contributors to child mortality. It is an abnormal physiological condition, and it is caused by an inadequate intake of protein (dos Santos et al. 2016). The Reference Daily Intake (RDI) for protein is 50 grams (USDA 2015).

The increasing population growth has also indirectly led to an increase in hungry and malnourished people. This situation has created a demand for an alternative source of protein that can replace the conventional and expensive plant or animal protein. Hence, in recent times, there has been an increased focus on the use of microbes as an alternative and sustainable source of protein (Anupama and Ravindra 2000).

2.2 Single Cell Protein

The protein extracted from different microbial sources is known as "Single Cell Protein" (SCP). Primary sources of single cell protein are Bacteria, Moulds, Yeasts, Green and Blue-Green algae (Adedayo et al. 2011). SCP has many advantages over animal and plant protein in that it's neither seasonal nor climate dependent (Anupama and Ravindra 2000). SCP is gaining popularity because it requires limited land area and water for growth. Waste materials can be used as a substrate for the production of SCP; which helps in reducing the environmental footprint of microbial proteins. These

organisms grow fast and produce large quantities of protein from a relatively small area of land (Adedayo et al. 2011).

The term "microbial protein" or "petro protein" was replaced by the term "Single Cell Protein" at a meeting held at the Massachusetts Institute of Technology (MIT) in1968 (Srividya et al. 2013). Single Cell Protein(SCP) can be produced through fermentation of the substrate –microorganism, as shown in Figure 2.1 (Adedayo et al. 2011).

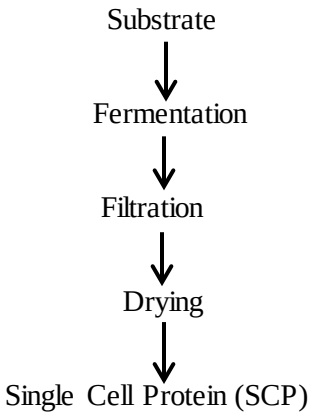

Figure 2.1 Single Cell Protein production. Source: Adedayo et al. (2011).

Besides protein, these microbial cells are rich in carbohydrate, fat, vitamins, fiber, and minerals. Table 2.1 shows the nutritional composition of SCP from algae, fungi, and bacterial sources. It can be seen that protein content ranges from 40 % - 80 % in these sources. On the basis of the amino acid profile, bacterial protein is comparable to that of fish protein, and yeast protein is similar to soy protein (Chronakis and Madsen 2011).

Table 2.1 Nutritional composition of SCP from different sources.

Component	Percentage composition by weight		
	Algae	**Fungi**	**Bacteria**
True Protein	40-60	30-70	50-83
Fats/Lipids	5-10	5-13	8-10
Carbohydrate	9	NA	NA
Bile pigment and Chlorophyll	6	NA	NA
Fiber	3	NA	NA

Source: Anupama and Ravindra (2000).

There are a number of researchers suggesting different organisms as a potential source for SCP, but only a few are suitable for commercial production. Physical and chemical characteristics must be considered to select a potential source for SCP. Table 2.2 lists some of the most important desirable characteristics of SCP, including high growth rates, high yields, stable and inexpensive growth, and high nutritional content.

Table 2.2 Desirable characteristics of microorganisms to be considered as a source of Single Cell Protein (SCP).

Physiologic Characteristics:
High growth rate
Capable of growing on simple media
Generation of high yields on the chosen substrate
Ability to grow at high cell densities
Stable growth in continuous culture
Other Characteristics:
The protein, fat, and carbohydrate content should be of high quality
High digestibility of the product
High nutrient content
Low nucleic acid content
Absence of toxicity
Good taste
Easy recovery
Amenability to further processing (drying)

Source: Kuhad et al. (1997).

Different measures of nutritional value such as protein efficiency ratio (PER), biological value (BV), net protein utilization (NPU) and digestibility also need to be considered to produce SCP. The nutritional benefits of microbial proteins are comparable with that of other plant and animal protein as shown in Table 2.3 (Kuhad et al. 1997).

Table 2.3 Nutritional values of microbial protein.

Organism	Biological Value(BV) %	Digestibility %	Net Protein Utilization(NPU)%
Spirulina sp.	77.6	83.9	65.0
Chlorella sp.	71.6	79.9	57.1
Pichia sp.	51.0	83.0	-
Casein	87.7	95.1	83.4
Egg	94.7	94.2	89.1

Source: Kuhad et al. (1997).

Single cell protein (SCP) nutritional characteristics can cause a few negative impacts to human health. The solid cell wall, high nucleic acid content, and allergies can impart negative health consequences upon consumption (Chronakis and Madsen 2011). The chemical composition of SCP for human consumption should be defined based on percentage protein, amino acid profile, nucleic acid content, lipids, toxins and vitamins (Anupama and Ravindra 2000).

2.3 Spirulina

Spirulina is the most extensively used microorganism to produce Single Cell Protein (Anupama and Ravindra 2000). Algae is considered to be a stable, traditional food for people in Mexico (*Spirulina platensis*) and for people in Chad (Spirulina maxima) (Kuhad et al. 1997). Spirulina is one of the cheapest sources of protein and essential vitamins (Babu and Rajasekaran 1991). It is also rich in ß-carotene and dietary gamma- linolenic acid (GLA) (Chronakis and Madsen 2011). Spirulina has been declared as a GRAS (Generally Recognized as Safe) ingredient by the Food and Drug Administration (FDA 2003). Spirulina is produced extensively around different parts of the world (3000 tons/year) and used in food and animal feed (Gouveia et al. 2008). The comparative values of protein content and cost of different protein sources are given in Table 2.4. Spirulina has the highest protein content per 100 g of food when compared to egg

and milk. The ratio of the costs of different protein sources compared with spirulina clearly shows spirulina protein costs the least (Babu and Rajasekaran 1991).

Table 2.4 Cost and protein comparison of different Sources of Protein.

Sources	Protein Content per 100g (g)	Comparative ratio of cost of Protein with Spirulina
Spirulina	66.00	1: 1
Egg	13.20	1: 8.23
Milk (100 ml)	3.30	1:10.97

Source: Babu and Rajasekaran (1991).

Blue-green micro-algae like spirulina are rich in total amino acids (AAs), essential amino acids (EAAs) and non-essential amino acids (NEAAs) (Table 2.5).Generally, the essential amino acid concentration is less compared to that of non-essential amino acid concentration among major algae proteins (Mišurcová et al. 2014). Spirulina is one of the primary sources of natural phycocyanin, which is used as a natural color in food products like chewing gums, candies, dairy products, jellies, ice creams, soft drinks and used as a biochemical tracer in immunoassays (Gouveia et al. 2008). Spirulina contains natural pigments like carotenoids and phycobiliproteins which have several beneficial biological activities, such as antioxidant, anti- carcinogen, anti-inflammatory, anti- obesity, and neuroprotective activities (Vaz et al. 2016). ß-carotene represents 70% of total carotenoids present in spirulina which is equivalent to 53% more retinol equivalent than the amount present in carrots (Dey and Rathod 2013). The amino acid profile of spirulina is comparable with that of other conventional protein sources such as eggs (Figure 2.2). However, the microalgal protein may have lower biological value, digestibility, net protein utilization and protein efficiency ratio (PER) than conventional protein like egg and casein(Table2.7) (Ejike et al. 2017).

Table 2.5 Amino acid contents (g/ 100 g of protein) of different algae species.

g/ 100 g of protein	Chlorella pyrenoidosa	Spirulina platensis	Palmaria palmata
Amino Acids	84.4	82.1	85.5
Essential Amino Acid	37.2	34.1	32.1
Non-Essential Amino Acid	47.2	47.9	53.4

Source: Mišurcová et al. (2014).

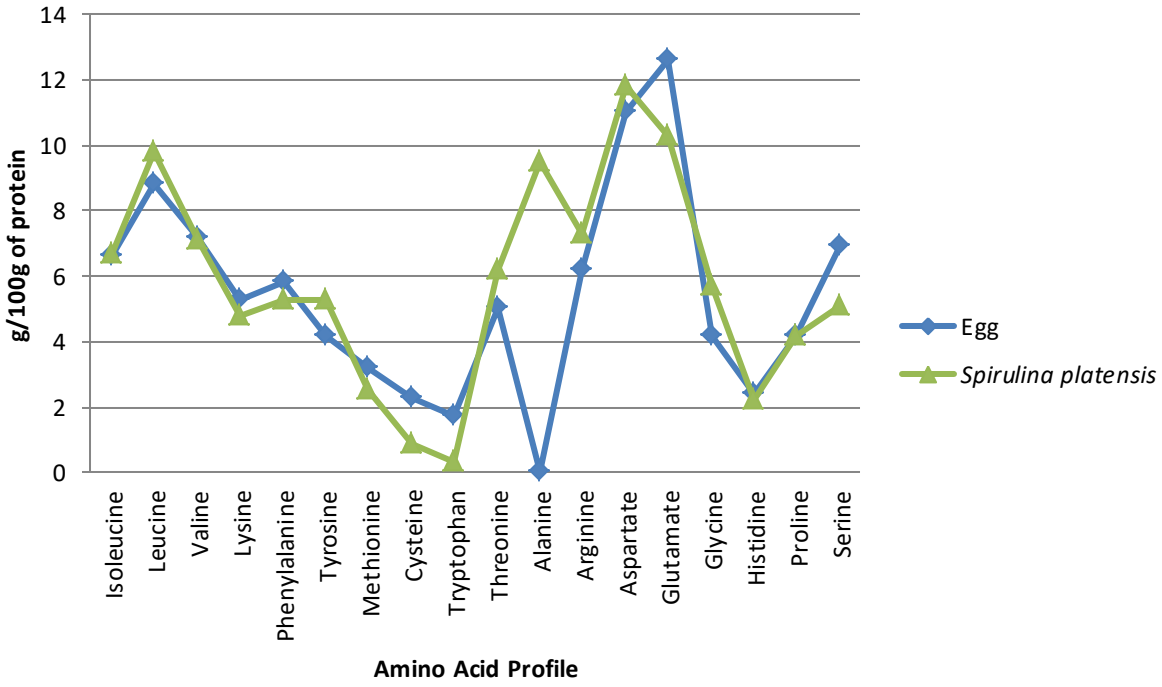

Figure 2.2 Amino acid profile of spirulina compared with egg protein.

Adapted from Chronakis and Madsen (2011).

Table 2.6 Comparative data for protein quality of spirulina with egg and casein protein source.

Protein Source	Biological value(BV) %	Digestibility Coefficient(DC) %	Net Protein Utilization(NPU) %	Protein Efficiency Ratio(PER)
Casein	87.8	95.1	83.4	2.50
Egg	94.7	94.2	89.1	-
Spirulina	77.6	83.9	65.0	1.78

Adapted from Becker (2007).

The alcohol and water extracts of spirulina have a greater antioxidant effect than other chemical antioxidants (α-tocopherol, BHA, and ß-carotene) and natural antioxidants (Gallic acid and chlorogenic acid), respectively (Belay 2002). Spirulina has many potential health benefits like anti-cancer, antiviral and cholesterol-reduction properties (Belay 2002). Incorporation of different levels of spirulina in pasta showed an increase in antioxidant capacity compared to the control (Rodríguez De Marco et al. 2014). Spirulina is also rich in polyunsaturated fatty acids such as ɣ- linolenic acid (GLA), which has been used in the treatment of dermatitis, diabetes and pre-menstrual syndrome (Chaiklahan et al. 2008). Several studies suggest that eating spirulina can increase the Lactobacillus count in the gut and also improves the absorption of vitamin B1(Vaz et al. 2016).

In general, algal proteins like chlorella and spirulina are marketed in the form of tablets and liquids. Different trial experiments were made to add algal proteins to everyday food items like bread, pasta, and noodles. Incorporation of spirulina in food products resulted in a dark-green and a less acceptable "burnt" aftertaste (Becker 2007; Chronakis and Madsen 2011). The unpleasant taste, bad flavor and dark green color of spirulina are the characteristics that limit its application in higher concentration.

Based on the acceptability analysis, consumption rate of algal protein (spirulina) will increase if it is incorporated with other food ingredients to enhance the palatability by reducing

the off flavor and odor (Babu and Rajasekaran 1991). Chronakis and Madsen (2011) suggested that mixing algal proteins with conventional plant protein could reduce the bad after taste and improve the consumer acceptability. Cocoa powder, when blended with Spirulina powder, was efficient in masking the bad odor and flavor of spirulina (McCarty et al. 2010). A series of experiments was attempted to modify and combine algal protein in common food products like bread or noodles. However, only small amounts could be added before the appearance and taste of the product became unacceptable. Even though it is clear that all these experiments will not solve the problem, not much research has been done involving the use of other common food processes on spirulina such as emulsification, encapsulation, gelling, bleaching, etc.(Becker 2007).

2.4 Methods to Reduce Bitterness

2.4.1 Encapsulation

Encapsulation may be defined as a process to entrap solid, liquid or gaseous material within different carrier substances. The substance that is encapsulated is called the active/core material, and the substance that is encapsulating is called the carrier/wall material (Mohan et al. 2015). Encapsulation has been used for various applications, including aroma/taste differentiation, stabilize food ingredients or increase their bioavailability. There are a number of different processes possible for achieving encapsulation, including spray drying, spray-bed drying, fluid-bed drying, spray chilling, spray-cooling, and freeze drying (Nedovic et al. 2011).

Novel food products are developed with many physiological benefits by adding bioactive and nutritive compounds to the food products. However, bitterness and off-flavor of these nutritive compounds may limit their use in food products (Favaro-Trindade et al. 2010). Consumers prefer food products that are tasty, healthy and convenient. Encapsulation can mask bad tasting components, stabilize food ingredients and increase their bioavailability (Bainbridge

1994).

Microencapsulation creates a physical barrier/film between the bitter bioactive compounds and the taste buds (Sun-Waterhouse and Wadhwa 2013). Encapsulation using spray-drying with gelatin and soy protein isolates as wall materials masked the bitterness and improved the stability of casein hydrolysates (Favaro-Trindade et al. 2010). Steviol glycosides encapsulated with maltodextrin and insulin using spray drying showed a reduction in the bitter aftertaste with microencapsulation efficiency ranging from 64% to 83% (Chranioti et al. 2015). Encapsulation efficiency is the ratio between the concentration of molecules encapsulated in each encapsulate and the original concentration of the molecules present in the loading solution.

Spirulina was encapsulated using spray-drying with maltodextrin as the wall material and checked for storage stability at different temperatures. The results proved that encapsulation had increased the stability of C-phycocyanin, which has been widely used in commercial applications in the food and cosmetic industry as a natural blue dye (Pruchyathawornkul 2016). However, spray drying is often considered as a "harsh" method, since the bioactive material is subjected to a high temperature, which may affect its nutritional benefits (Yu et al. 2010). As an alternative to this method, water insoluble gelation using sodium alginate can be used to encapsulate bioactive compounds. Since any bioactive material can be easily integrated into alginate-based formulations with mild conditions that minimize any damage to the core material.

2.4.2 Enzymatic Hydrolysis

Bitterness is often associated with the specific composition of amino acids in the peptide sequences. The use of enzymatic hydrolysis to modify the protein structure has been shown to decrease bitterness in some products. Proteases or hydrolases are divided into two groups called exopeptidases and endopeptidases. Endopeptidases hydrolyze specific peptide bonds within the polypeptide chain; exopeptidases catalyze the formation of free amino acids or small peptides

from the N-terminal (aminopeptidase) or C-terminal (carboxypeptidase) end of the polypeptide substrate. Exopeptidases play a major role in the food industry since they can reduce the bitterness and produce flavor-precursors/taste-active compounds. Exopeptidase is not effective on whole protein, thus they are used in combination with endopeptidase (Raksakulthai and Haard 2003).

Studies have shown that numerous factors like pH, temperature, the substrate to enzyme ratio and incubation time can all have a big effect on the overall performance of the enzymatic reaction (Wing and Cheung 2007).

2.4.3 Sodium Alginate and Ionic Gelation

Alginates are unbranched polysaccharides extracted from brown algae and bacteria (Rehm 2009). The two most important compounds of alginate are ß-D- mannuronic acid (M-residues) and α-L-guluronic acid (G-residues). The G and M- blocks are composed of consecutive G- residues and consecutive M-residues respectively (Kuen Yong Lee 2013). Alginates are formed by sequences of M-blocks and G-blocks combined with MG-blocks sequences linked by glycosidic linkages (Pawar and Edgar 2012). The sequence and chemical composition of the G-block and M-block of alginates are dependent on various factors like species, season and growth condition of the algae (Paques 2015).

Alginate can form a gel-like structure when induced by the addition of divalent cations (Lupo et al. 2015). Figure 2.3 shows the chemical reaction between sodium alginate and calcium chloride, where the sodium ions are replaced by the calcium ions to form a gel-like structure. This unique property of the alginate makes it a suitable material for encapsulation of bioactive compounds and protein (Aceval Arriola et al. 2016; Zhang et al. 2016b). The gelation of the alginate is achieved through an ion exchange of the alginate counter ions (sodium or potassium) with the divalent cations (calcium or magnesium) (Paques 2015). The physicochemical properties

of the gel are influenced by the G-to-M block ratio of the alginate. Each cross-linking cation (calcium) binds with two adjacent G- residues and with two G-residues in the opposing chain forming an "egg-box" like structure gel (Rehm 2009). The binding affinity of the alginate differs for various cations and is also dependent on the chemical composition of the alginate. Calcium is the most commonly used cation since it is nontoxic and inexpensive (Paques 2015).

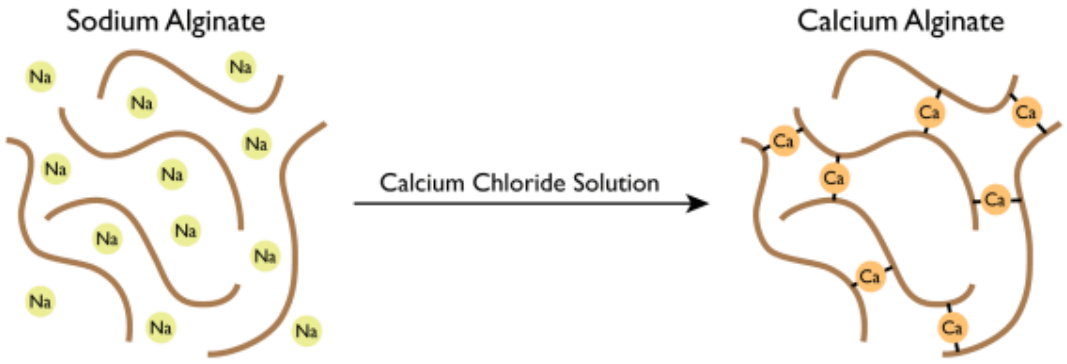

Figure 2.3 Ionic gelation: chemical reaction between sodium alginate and calcium chloride.

Alginates can be cross-linked by external or internal gelation methods. In the external gelation or diffusion method, the cations diffuse from the external medium into the interior of an alginate phase to form the hydrogel beads (Figure 2.4 a). The bioactive compound to be encapsulated is mixed with the alginate solution, and then the solution is extruded dropwise into an aqueous solution with cross-linking cations (calcium chloride solution) to form gelation (Paques 2015). For internal gelation, the cations are released from the interior of the alginate phase to form the hydrogel beads (Figure 2.4 b). The bioactive substance is mixed with the solution of cations and dropped into an alginate solution; the cation is released by acidification of the medium (Lupo et al. 2015).

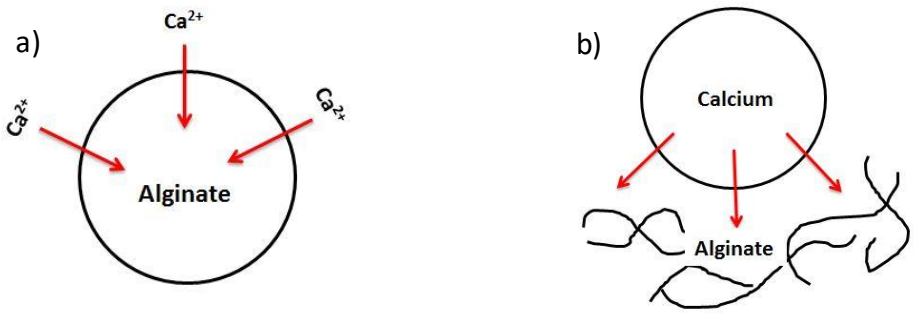

Figure 2.4 Calcium ion diffusion during a) external gelation b) internal gelation.

Belščak-Cvitanović et al. (2015) encapsulated green tea polyphenols using ionic gelation to enhance its stability, bioavailability and sensory properties. Aqueous leaf extract of Stevia rebaudiana Bertoni entrapped in a calcium bead showed high encapsulation efficiency and antioxidant storage stability (Aceval Arriola et al. 2016). Storage studies of hydrogel beads with ß-carotene indicated that the beads partially protected the ß-carotene from chemical degradation (Zhang et al. 2016a). In another study, Belscak-Cvitanovic et al. (2015) evaluated the potential of sodium alginate to encapsulate and mask the bitterness of caffeine. The sensory analysis from the study suggests that the bitterness of formulated alginate beads was lower than that of the caffeine standard (Figure 2.3).

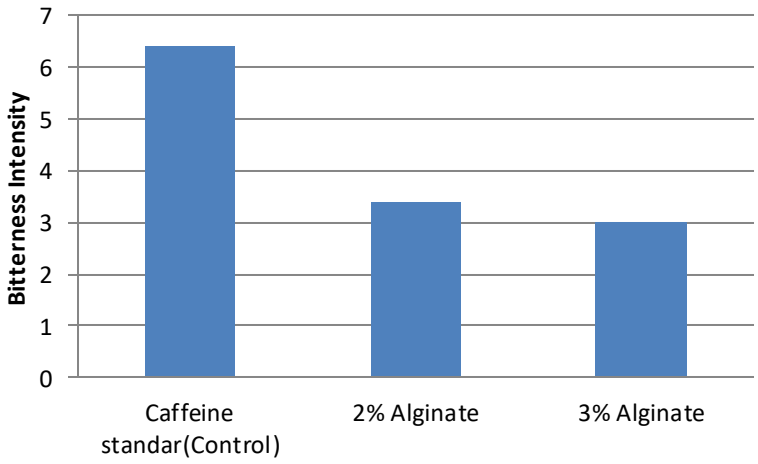

Figure 2.5 Comparison of the bitterness intensity of caffeine (control) and caffeine-alginate microbeads. Adapted from Belscak-Cvitanovic et al. (2015).

A comparative study between internal gelation (IG) and external gelation (EG) of cocoa extract indicated that the beads formed by IG showed lower hardness than beads formed by EG. In external gelation, the calcium ion diffusion from the shell to the core of the alginate makes the beads harder (Lupo et al. 2015). The morphology, texture, and dimensions of the hydrogel beads can be altered by changing the alginate concentration and crosslinking ion concentration.

2.5 Protein Delivery - Ionic Gelation

Incorporation of bioactive proteins and peptides into food products is a challenging task since they are sensitive to chemical or biochemical degradation and susceptible to aggregation. These proteins also possess a potential to cause off-flavors like bitterness or astringency to the food products (Zhang et al. 2016b). Encapsulation using ionic gelation can overcome all these potential challenges. Alginate is also an excellent carrier material for protein delivery since proteins can be easily integrated into alginate-based formulations with mild conditions that minimize protein denaturation. Due to the inherent porosity and hydrophilic nature of the hydrogels, the release rate of protein from these gels is instant (Kuen Yong Lee 2013). Zhang et

al. (2016b) successfully prepared whey protein loaded hydrogel beads using an encapsulation unit with a small vibrating nozzle and studied the effect of pH of alginate/protein solution on hydrogel stability. The results of the study suggest that the protein encapsulation efficiency and retention of the bead reach a maximum at pH 3. However, it is critical to consider the isoelectric point of the protein while deciding the pH of the alginate/protein solution. Encapsulation of bovine serum albumin (BSA) protein using calcium alginate offered high encapsulation efficiency and high particle yield (Yu et al. 2010).

2.6 Cookies

Nowadays, cookies have become one of the most popular and well-accepted snack products worldwide among all age demographics. The low manufacturing cost and stable shell-life with low water activity act as an advantage for both consumers and manufacturers (Cheng and Bhat 2016). Many studies have suggested that fortification of cookies with different sources of bioactive compounds (like high protein sources) can be utilized as a functional food (Tumbas aponjac et al. 2016). Kaur et al. (2016) partially replaced wheat flour with flaxseed flour to make cookies and studied its effect on the sensory, physical, chemical, and antioxidant characteristics of the cookies. The results revealed that incorporation of flaxseed improved the overall acceptability and enhanced the nutritional properties of the cookies. Marques et al. (2016) developed a no sugar added cookie by replacing wheat flour with whey protein and increased the protein levels of the cookies.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

Spirulina powder (*Spirulina platensis*, ID: 7199) was purchased from Nuts.com, NJ, USA. Sodium alginate (W201502) was purchased from Sigma- Aldrich, USA and Calcium chloride was purchased from Modernist Pantry, York, ME, USA. Sodium dodecylbenzene sulfonate (289957) was purchased from Sigma- Aldrich, USA, and Polysorbate 80/ Sorbitan monooleate was purchased from Vantage, Gurnee, IL, USA. Maltodextrin (DE = 18) was purchased from Myprotein, USA.

All other ingredients like brown sugar (C&H Sugar), vegetable oil (Crisco), vegetable shortening (Crisco), molasses (Grandma's Molasses), all-purpose flour (Great Value), baking powder (Great Value), salt (Morton Salt), ground cinnamon (McCormick) and soy protein (Naturade Soy Protein, Natural) were purchased from the local grocery store. A bitter blocking flavor (Natural and Artificial Bitterness Blocker Flavor # 33199) was provided by David Michael Flavors, USA.

3.2 Preparation of Spirulina-Alginate Beads

3.2.1 Preliminary Trials

Preliminary trials were carried out to examine the effect of the concentration of sodium alginate, and calcium chloride on the gel formation. More than 30 different formulations were developed using different concentrations of sodium alginate and calcium chloride.

All preliminary formulations were evaluated for crude protein and hardness of the beads. With external gelation, the beads were not formed when the concentration of calcium chloride was below 10%, and concentration of sodium alginate was below 1%. With internal gelation, the beads were not formed when the concentration of calcium chloride was above 2%. Optimum concentrations of sodium alginate and calcium chloride for internal and external gelation were selected from the preliminary trials.

3.2.2 Beads Prepared by External Gelation (EG)

The plain alginate solution was prepared by dissolving sodium alginate (1% w/w or 7% w/w) and polysorbate-80 surfactant (1%w/w) in distilled water stirring at 65 C for 20 minutes. The spirulina powder was then mixed with the previously prepared alginate solution to obtain 15 % w/w concentration at 65°C for 20 minutes until it formed a homogeneous solution. The cross-linking solution (10% or 15% w/w) was prepared by dissolving calcium chloride powder in distilled water. The spirulina-alginate solution was drawn into a 3ml syringe with 22 G and 26 G needles and dropped manually into the cross-linking solution to form the alginate beads. As shown in Figure 3.1, the spirulina – alginate solution was extruded into calcium chloride solution to form small teardrop shaped spirulina – alginate hydrogel beads. The manual extrusion process was slowed down to form beads of uniform size and shape. The beads were then filtered using a strainer, rinsed with distilled water and stored under refrigeration until further analysis or use.

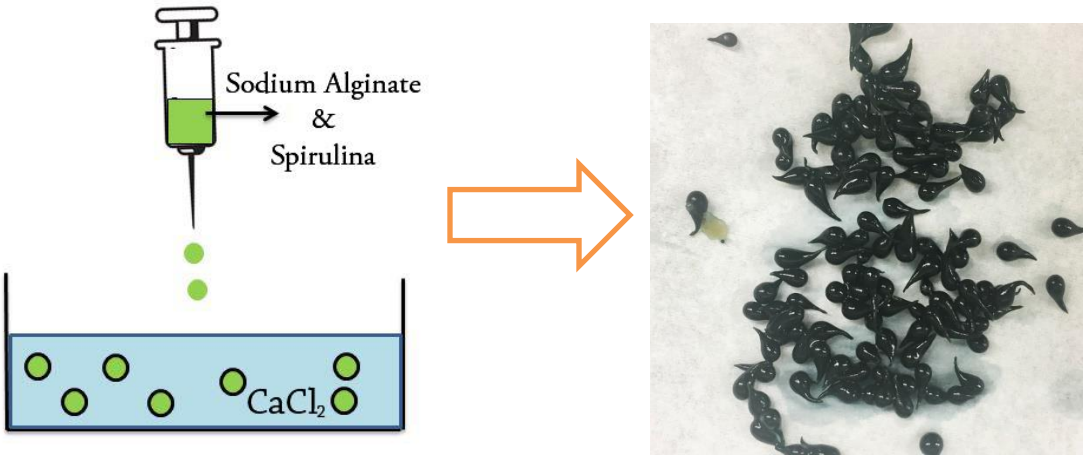

Figure 3.1 Bead formation using the external gelation process.

3.2.3 Beads Prepared by Internal Gelation (IG)

The plain alginate solution was prepared by dissolving sodium alginate (0.5% w/w or 1.5% w/w) and SDS (0.5% w/w) in distilled water stirring at 65°C for 20 minutes. The cross-linking solution was prepared by dissolving calcium chloride (2 % w/w) in distilled water. Maltodextrin (10% w/w) was used to adjust the viscosity of calcium chloride solution and ensure that the alginate beads were in uniform shape. Spirulina powder was mixed with calcium chloride solution to reach 15% w/w concentration. The spirulina- calcium chloride solution was drawn into a 3ml syringe with 26 G needle and dropped manually into the alginate solution to form the beads. Figure 3.2 shows the extrusion of spirulina – calcium chloride solution into sodium alginate solution to form small hydrogel beads. The formed beads were filtered using a strainer, rinsed using distilled water and stored under refrigeration.

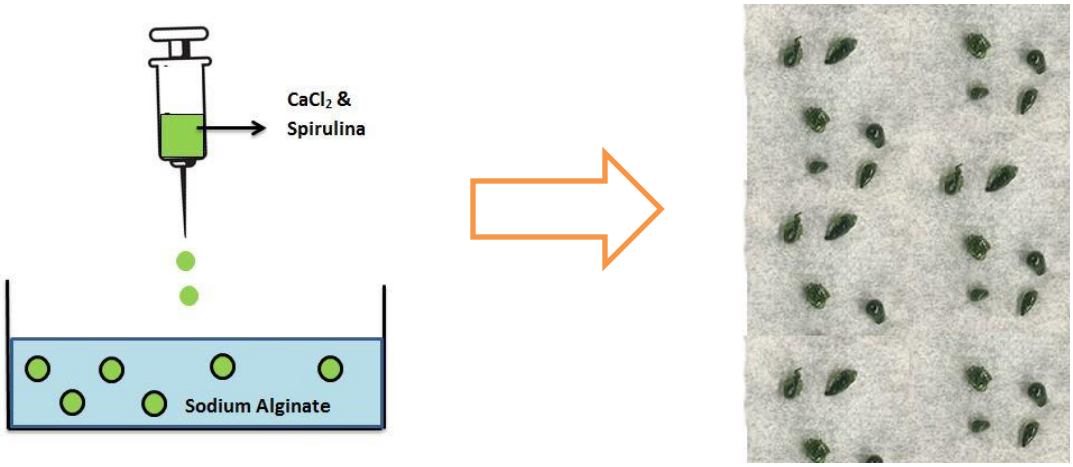

Figure 3.2 Bead formation using the internal gelation process.

3.3 Characterization of Spirulina-Alginate Beads

3.3.1 Determination of Size/Dimension

Samples of 5 spirulina-alginate beads obtained from each formulation and type of gelation were taken at random and measured with a digital caliper (ROHS CE Digital Caliper – SH20, China) to measure their width and length.

3.3.2 Scanning Electron Microscope- Morphological Studies

A pair of beads from each formulation and type of gelation method was viewed under a Scanning Electron Microscope (Joel JSM 6360, Peabody) to determine both external and cross-sectional morphology. The beads were attached to stubs using adhesive tape and coated with gold. Finally, the beads were examined using an acceleration voltage of 10 kV at 25x and 50x magnification.

3.3.3 Determination of Textural/ Mechanical Properties

The texture of the spirulina-alginate bead was analyzed using a texture analyzer (TA-XT 2i), and the compression testing was performed using a cylindrical probe. The samples were

examined at a test speed of 0.5 mm/s, over a varied distance adjusted based on the dimensions of the samples in order to achieve complete compression. The maximum force (N) needed for compression represents the maximum resistance of the bead to compression of the probe, which indirectly gives an indication of the hardness of the samples. In order to obtain representative results of the hardness of the beads, experiments were performed in triplicate (with ten samples per experiment) and expressed as mean ± S.D.

3.3.4 Protein Analysis

The Dumas method (AOCS Official Method Ba 4e-93) for estimation of crude protein is based on combustion of the whole sample in an oxygen- enriched environment at 950 ° C in order to ensure complete combustion. All samples were analyzed for crude protein content using the Dumas method in triplicates. Samples (10 g) from each formulation were dried at 102°C for 18 hours and homogenized. The homogenized samples were analyzed for percent protein using a Leco combustion instrument (TruSpec N -630, St. Joseph, MI).

3.3.5 Encapsulation Efficiency

The encapsulation efficiency (%) of the alginate beads was determined by dividing the amount of spirulina remaining in the beads by the initial amount of spirulina added to each formulation. The amount of spirulina remaining in each formulation was determined based on the protein content of the beads and the total protein content of spirulina.

3.4 Cookie Preparation for Sensory Analysis I

3.4.1 Cookie Samples Prepared with Alginate Beads

A general spice cookie was used for the sensory analysis, comparing different forms of spirulina added to the cookies. Soy protein was used as a control for added protein. Ingredients used in the cookies shown in Table 3.1. All dry ingredients were weighed on a tarred digital

kitchen scale. Once weighed, the spirulina-alginate beads were mixed with other dry ingredients like all-purpose flour, brown sugar, baking powder, salt and ground cinnamon in a large mixing bowl. Then molasses, vegetable oil, and water were added to the dry ingredient mix and mixed into a dough. Mixed dough was rolled into small balls (4.5 grams each) and placed on cookie sheets in a preheated conventional baking oven. The cookies were baked at 325 F for 8 minutes and later cooled at room temperature for 20 minutes. After 20 mins the cookies were placed inside zip-lock bags (Figure 3.3 a).

3.4.2 Cookie Samples with Spirulina and Bitter Blocking Flavors

Spirulina powder was mixed with the bitter blocking flavor and then mixed with all the dry ingredients. Molasses, vegetable oil, and water were mixed with the dry ingredients into a dough consistency. The dough was rolled into small balls (4.5 grams each) and placed on a cookie sheet in a conventional baking oven and baked at 325 F for 8 minutes. The cookies were cooled for 20 minutes and placed inside zip-lock bags (Figure 3.3 b).

3.4.3 Cookie Samples with Untreated Spirulina

Un-treated spirulina protein was mixed with all the dry ingredients. Molasses, vegetable oil, and water were added to the dry ingredient mix and mixed into a dough. The dough was rolled into small balls (4.5 grams each) and placed on a cookie sheet in a conventional baking oven and baked at 325F for 8 minutes. The cookies were cooled for 20 minutes and placed inside zip-lock bags (Figure 3.3 c).

3.4.4 Cookie Samples with Soy Protein

Soy protein was mixed with all the dry ingredients and later mixed with molasses, vegetable oil and water to form the dough. The dough was rolled into small balls (4.5 grams each)

and placed on a cookie sheet in a conventional baking oven and baked at 325 F for 8 minutes. The cookies were cooled for 20 minutes and placed inside zip-lock bags (Figure 3.3 d).

Table 3.1 Ingredient formula for each prepared cookie (Sensory analysis I).

	Spirulina-Alginate Cookie (g)	**Untreated Spirulina Cookie(g)**	**Spirulina with flavor Cookie(g)**	**Soy Protein Cookie (g)**
Brown Sugar	1.5	1.5	1.5	1.5
Oil	0.37	0.37	0.37	0.37
Molasses	0.12	0.12	0.12	0.12
All Purpose Flour	1.46	1.83	1.83	1.86
Baking Powder	0.031	0.031	0.031	0.031
Salt	0.01	0.01	0.01	0.01
Ground Cinnamon	0.05	0.05	0.05	0.05
Water	0.02	0.43	0.43	0.43
Spirulina Beads (20%)	0.88	0	0	0
Spirulina	0	0.10	0	0
Spirulina + Flavor	0	0	0.10	0
Soy Protein	0	0	0	0.07
Total	4.5	4.5	4.5	4.5

3.5 Cookie Preparation for Sensory Analysis II

During the first sensory analysis, the panelists sensed a strong bitter aftertaste from the cookies with the spirulina-alginate beads. One of the hypothesized reasons for the bitterness was leaching of spirulina in the presence of oil in the cookies, which might accentuate the bitterness. Therefore, for the second sensory analysis, the vegetable oil was replaced with vegetable shortening to achieve better sensory attributes. Ingredients used in the cookies are shown in Table 3.2.

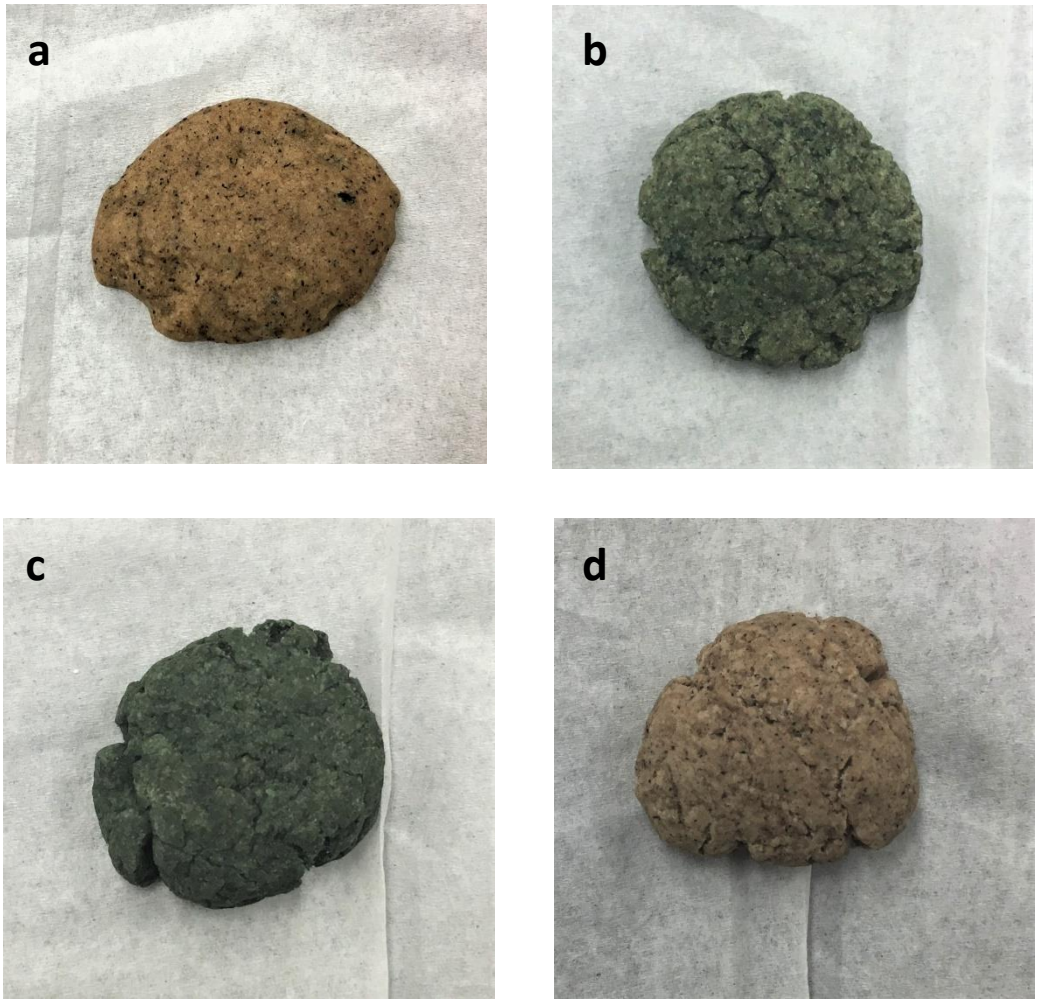

Figure 3.3 Cookies with a) spirulina – alginate beads b) spirulina with bitter blocker flavor c) untreated spirulina d) soy protein.

Table 3.2 Ingredient formula for each prepared cookie (Sensory analysis II).

	Spirulina-Alginate Cookie (g)	Untreated Spirulina Cookie(g)	Spirulina with flavor Cookie(g)	Soy Protein Cookie (g)
Brown Sugar	1	1	1	1
Shortening	0.37	0.37	0.37	0.37
Molasses	0.05	0.05	0.05	0.05
All Purpose Flour	1.25	1.79	1.79	1.81
Baking Powder	0.05	0.05	0.05	0.05
Salt	0.05	0.05	0.05	0.05
Ground Cinnamon	0.1	0.1	0.1	0.1
Water	0.5	0.69	0.69	0.69
Spirulina Beads (20%)	0.83	-	-	-
Spirulina	-	0.1	-	-
Spirulina + Flavor	-	-	0.1	-
Soy Protein	-	-	-	0.08
Total	4.2	4.2	4.2	4.2

3.6 Sensory Analysis

A consumer acceptance test was carried out with two different population groups to cover a wide range of demographics. The first sensory analysis was conducted with a population of 22 untrained panelists of age 18- 60 years, most of whom were students, staff and faculty members from the Food and Agricultural Products Center (FAPC), Oklahoma State University. For the second sensory analysis, a consumer acceptance test was conducted with 87 untrained panelists

ranging in age from 18 to 24 years. All the panelists evaluated four different cookies for seven different sensory attributes (sweetness, bitterness, aroma, mouth feel, aftertaste, color, overall palatability). The consumer acceptance test used a 9-point hedonic scale (pleasantness dimension). The sensory evaluation was approved by the Institutional Review Board (IRB) at Oklahoma State University (Appendix).

3.7 Statistical Analysis

The research study was designed as a completely randomized design. The ANOVA procedure was used to evaluate any significant differences between the gelation methods in terms of bead dimensions, protein content, and hardness of the beads. In the case of sensory analysis, AVONA was used to find any differences between different treatments in terms of seven sensory attributes (sweetness, bitterness, aroma/ flavor, aftertaste, mouthfeel, color, overall palatability) with a 9- point hedonic scale. A generalized linear model was used with different factors being the dependent variables and treatments being the independent variables. Tukey's Studentized Range Test was used to detect the significantly different treatments using $\alpha = 0.05$. Table 3.2 shows the sample size for each dependent variable.

Table 3.3 Dependent variables and number of observations for statistical analysis.

Dependent Variable	Number of Observations (n)
Thickness (mm),	60 (6 treatments * 10 reps)
Length (mm)	60 (6 treatments * 10 reps)
Hardness (g)	180 (6 treatments * 30 reps)
Protein Content (%)	18 (6 treatments * 3 reps)
Sensory Analysis 1	616 (4 samples * 22 participants * 7 attributes)
Sensory Analysis 2	2436 (4 samples * 87 participants * 7 attributes)

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Size and Dimension of Spirulina- Alginate Beads

Spirulina beads were prepared using both external and internal gelation methods, and varying levels of sodium alginate and calcium chloride. Differences in the formation methods resulted in different size beads. Due to the formation method, the bead shapes were not truly spherical but were more teardrop shaped, so two different dimensions were measured, termed thickness and length. Table 4.1 shows the mean thickness (mm) and length (mm) of the beads for each formulation and gelation mechanism. The thickness and length measurements were analyzed by ANOVA, and the results are shown in the Appendix. The external gelation (EG) beads had a mean thickness around 2 mm, whereas internal gelation (IG) beads had a mean thickness around 1.5 mm. Mean length of the external gelation beads ranged between 2.11 mm and 4.5 mm, and the mean length of the beads formed with internal gelation was approximately 3 mm. Irrespective of the gelation method, an increase in the concentration of sodium alginate significantly increased the thickness of the beads. This finding can be attributed to a less cross-linked gel, which consequently decreases syneresis (Ren 2009). Syneresis is defined as a release of water from the gel with a consequent decrease in its dimensions(Rehm 2009). However, an increase in calcium chloride concentration, while keeping the alginate concentration constant, did not significantly affect either thickness (mm) or length (mm) of the beads.

For incorporation into food products, the smallest possible beads would be ideal, because they are easier to 'hide' in existing food products (Belščak-Cvitanović et al. 2015). Bead size in

these experiments was controlled by the diameter of the syringe needle used to prepare the beads(Ren 2009). Obviously, a smaller diameter needle will create smaller beads. However, the limiting factor in this case was the pressure required to dispense the droplets, which was conducted by hand. In a commercial setting, it is likely that an extruder would be used to generate the beads, and therefore, much higher pressures and smaller outlet diameters would be possible.

Table 4.1 Mean length and thickness of spirulina – alginate beads.

Gelation	Sodium Alginate %	Calcium Chloride %	Thickness (mm)	Length (mm)
External	1%	10%	1.40 ± 0.09^{c}	2.11 ± 0.25^{c}
	1%	15%	1.58 ± 0.09^{cb}	2.08 ± 0.16^{c}
	7%	10%	2.48 ± 0.09^{a}	4.77 ± 0.30^{a}
	7%	15%	2.39 ± 0.017^{a}	4.48 ± 0.25^{a}
Internal	0.50%	2%	1.42 ± 0.12^{c}	3.03 ± 0.23^{b}
	1.50%	2%	1.68 ± 0.23^{b}	2.98 ± 0.28^{b}

Data reported is mean ± standard deviation (n=10), values for each treatment with different letters are significantly different ($\alpha = 0.05$).

4.2 Scanning Electron Microscope (SEM) – Morphological Studies

A scanning electron microscope was used to evaluate the structural differences among the spirulina beads prepared in different ways. The internal structures of the beads prepared by the two different gelling mechanisms are shown in Figures 4.1 to 4.6. Figures 4.1 and 4.2 show external gelation beads with alginate concentration 1%, Figures 4.3 and 4.4 show external gelation with alginate concentration 7%, and Figure 4.5 and 4.6 show internal gelation beads with alginate concentrations of 0.5% and 1.5%, respectively. The SEM micrograph reveal differences in the cross-sectional morphology of external gelation beads and internal gelation beads. Beads obtained by external gelation show a more smooth and rigid exterior (Figures 4.1, 4.2, 4.3, 4.4)

(Khosravi Zanjani et al. 2014; Belscak-Cvitanovic et al. 2015), whereas beads formulated by internal gelation show a soft and heterogeneous exterior (Figure 4.5, 4.6). The structure obtained by external gelation can be attributed to the formation of the gel layer on the surface of the droplet which yields a rigid exterior (Chan et al. 2006; Lupo et al. 2015). The calcium ions would first cross-link with the bead surface which would draw the polymer chains closer to form a less permeable surface to the diffusion of calcium ions into the interior. This phenomenon results in a highly cross-linked surface and less cross-linked core (Chan et al. 2006). This behavior is in accordance with the results reported by Aceval Arriola et al. (2016) for the encapsulation of aqueous leaf extract of stevia rebaudiana. The external gelation beads appeared to have a more porous interior than the internal gelation beads due to the inward movement of Ca^{2+} ions from the shell to the core (Figure 4.1, 4.2, 4.3, 4.4). In contrast, with internal gelation, diffusion of calcium ions from the core to the surface leads to a more homogeneous internal structure. A similar structure was observed by Lupo et al. (2015) for encapsulation of cocoa extract by both internal and external gelation methods.

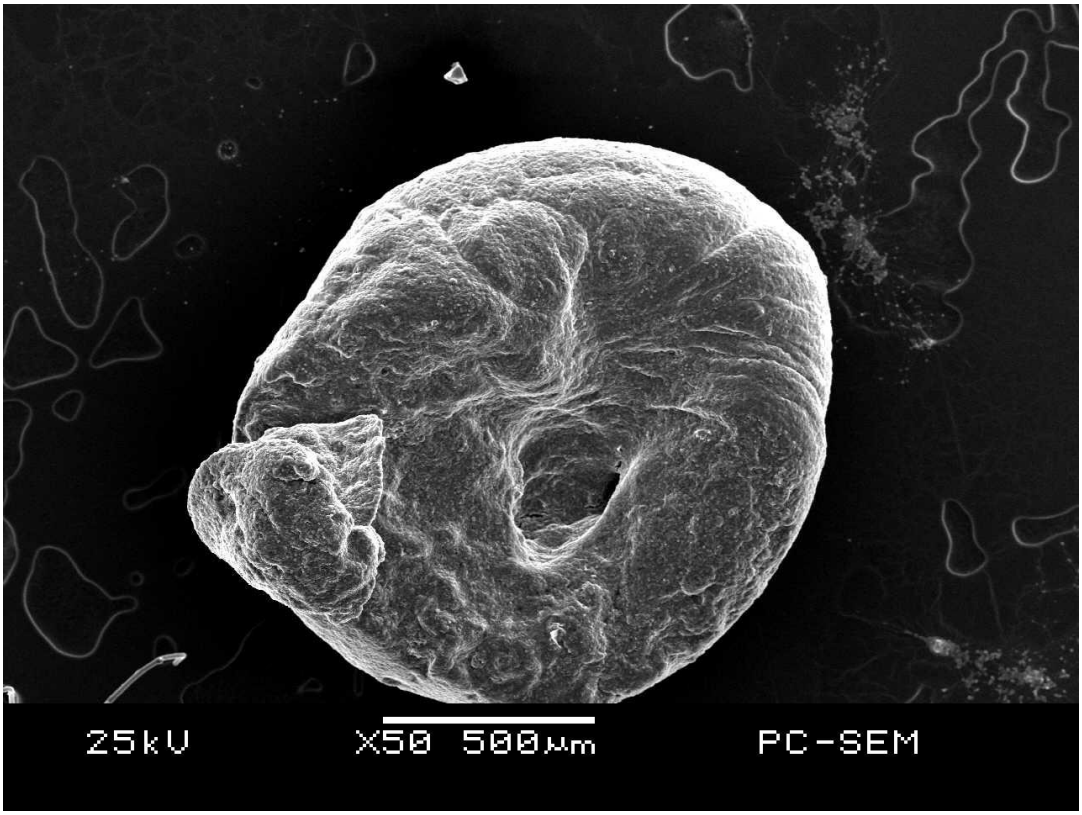

Figure 4.1 SEM micrograph showing the cross-sectional morphology of spirulina–alginate beads made with external gelation process with alginate 1% and $CaCl_2$ 10%.

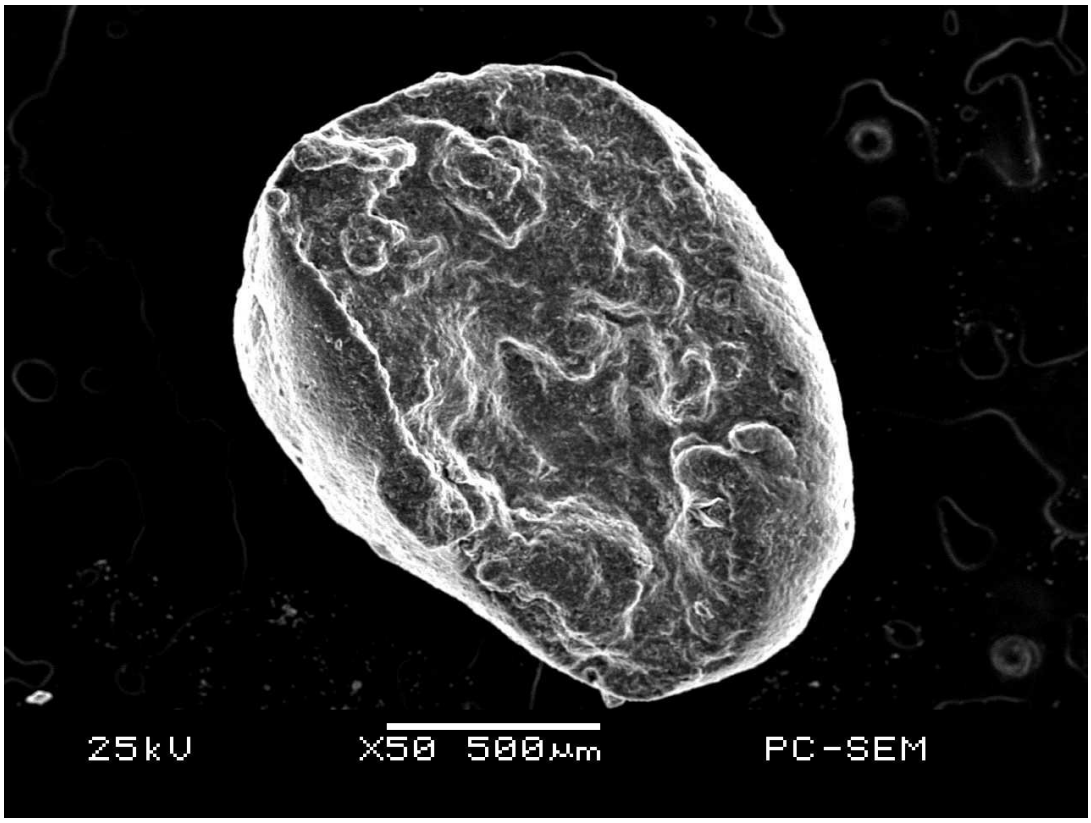

Figure 4.2 SEM micrograph showing the cross-sectional morphology of spirulina–alginate beads made with external gelation process with alginate 1% and $CaCl_2$ 15%.

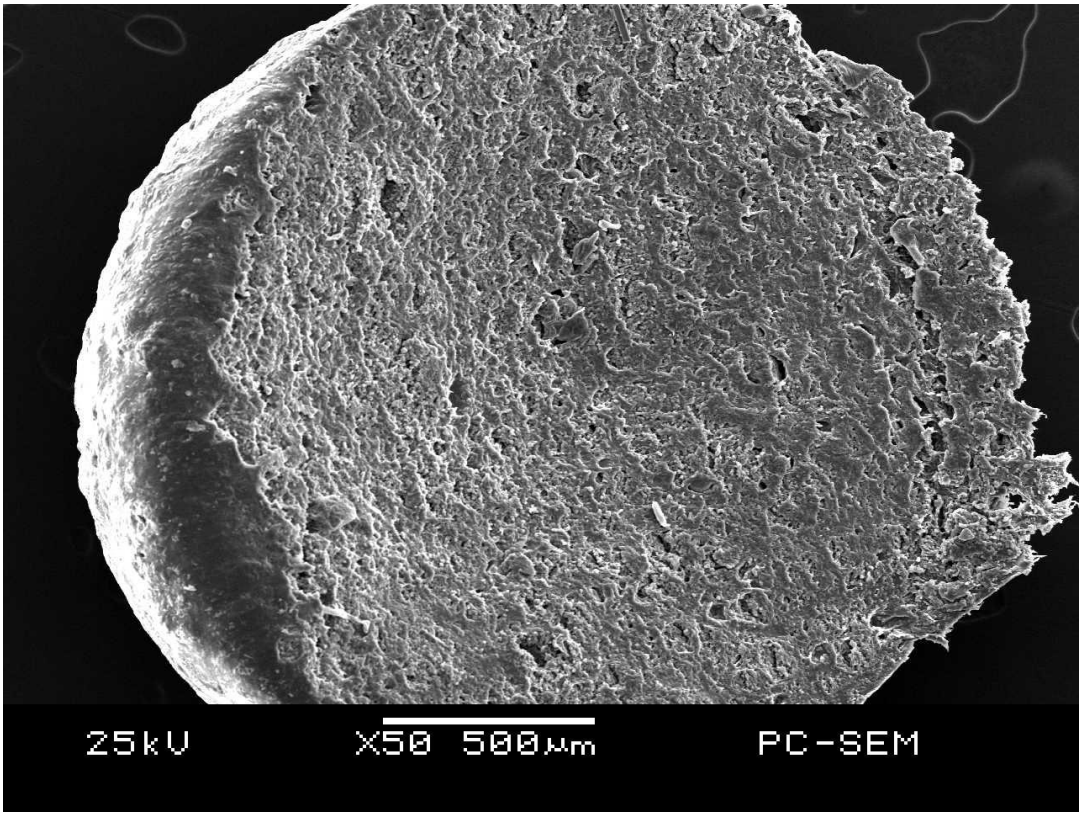

Figure 4.3 SEM micrograph showing the cross-sectional morphology of spirulina–alginate beads made with external gelation process with alginate 7% and $CaCl_2$ 10%.

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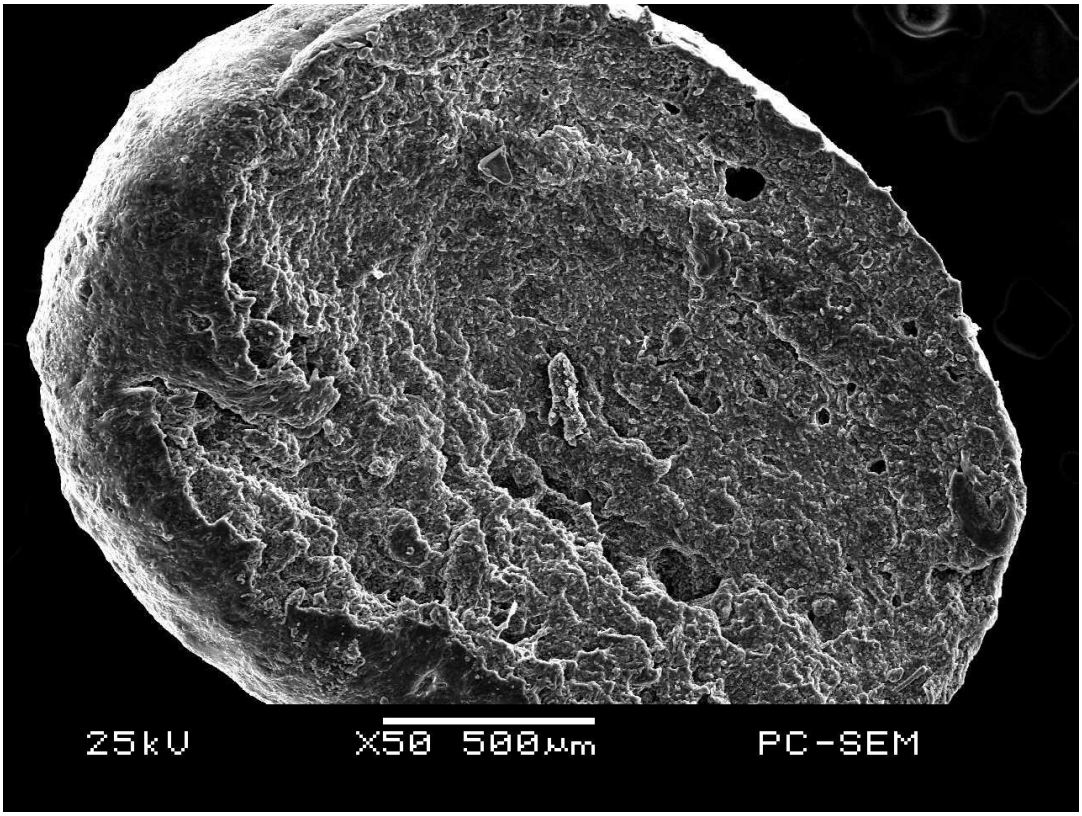

Figure 4.4 SEM micrograph showing the cross-sectional morphology of spirulina–alginate beads made with external gelation process with alginate 7% and $CaCl_2$ 15%.

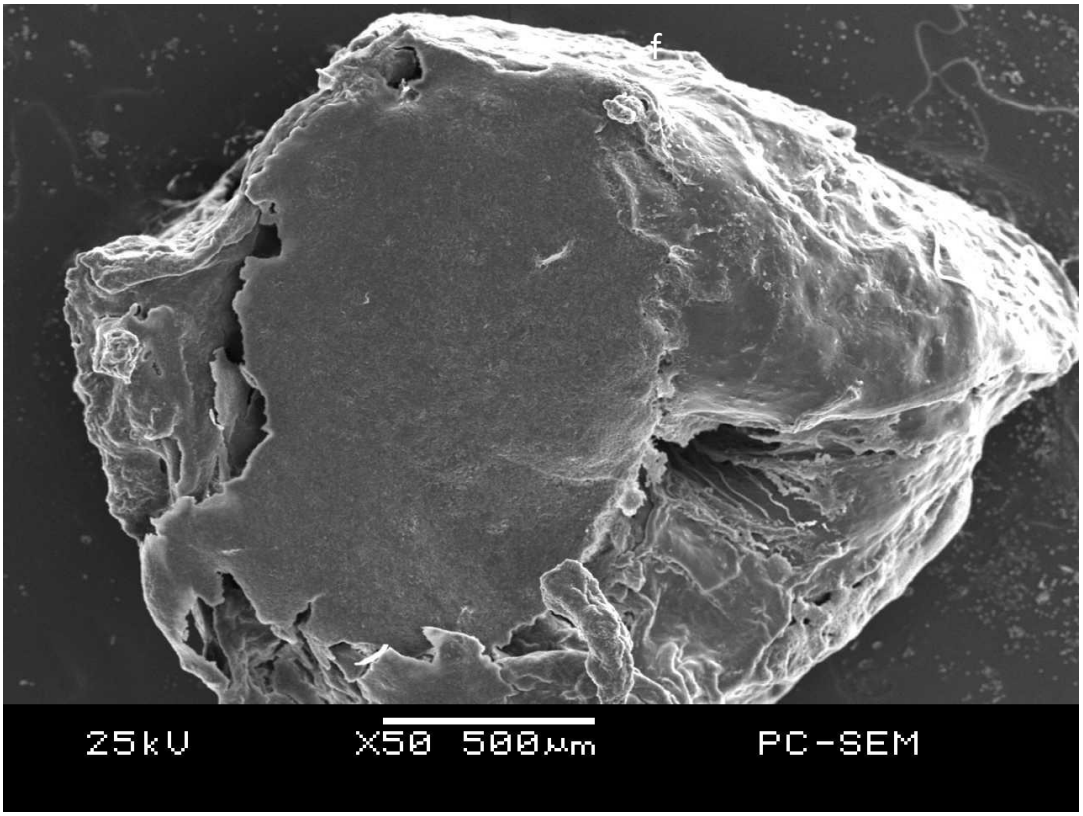

Figure 4.5 SEM micrograph showing the cross-sectional morphology of spirulina–alginate beads made with internal gelation process with alginate 0.5% and $CaCl_2$ 2%.

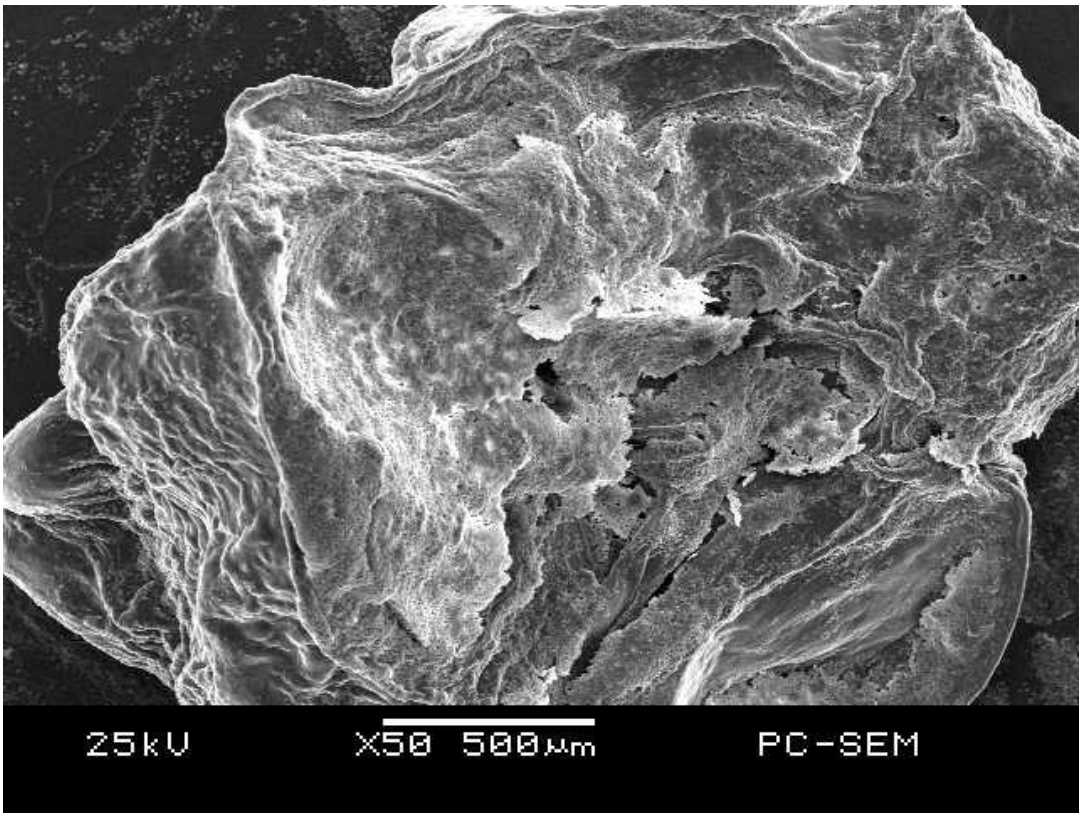

Figure 4.6 SEM micrograph showing the cross-sectional morphology of spirulina–alginate beads made with internal gelation process with alginate 1.5% and $CaCl_2$ 2%.

Figures 4.7 to 4.12 show the external morphology of the spirulina–alginate beads formulated by the two different gelling mechanisms. Figures 4.7 and 4.8 show the exterior morphology of external gelation beads with alginate concentration 1%, Figures 4.9 and 4.10 show external gelation with alginate concentration 7%, and Figures 4.11 and 4.12 show internal gelation beads with alginate concentration of 0.5% and 1.5%, respectively. The SEM photographs of the alginate beads prepared by external gelation compared with the internal gelation beads show a difference in the surface morphology. Detailed examination of the surface structure of external gelation beads (Figures 4.9 & 4.10) displays a sponge-like or porous structure, which is due to the inward movement of calcium ions from the exterior (Pasparakis and Bouropoulos 2006; Belščak-Cvitanovic et al. 2016). Figures 4.11 & 4.12 show that the internal

gelation beads possess a heterogeneous structure, internal gelation beads have a strong cross-linked gel structure core and a weakly cross-linked gel at the surface.

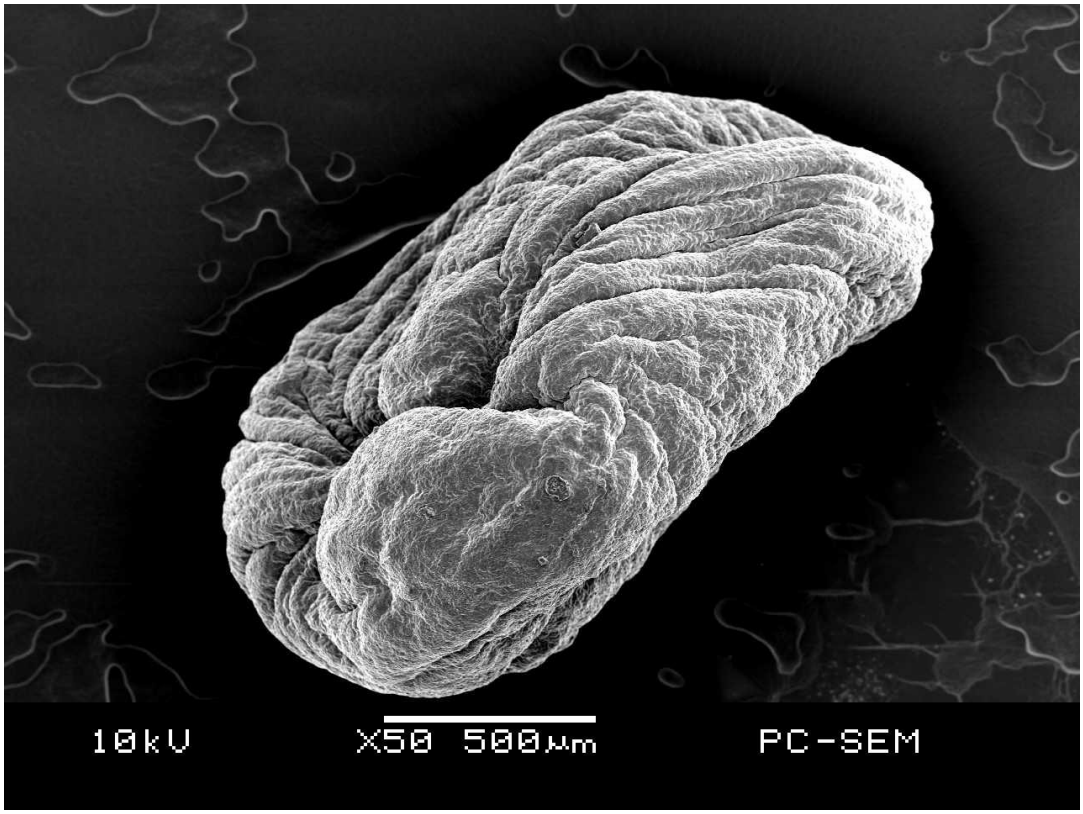

Figure 4.7 SEM micrograph showing the exterior morphology of spirulina–alginate beads made with external gelation process with alginate 1% and $CaCl_2$ 10%.

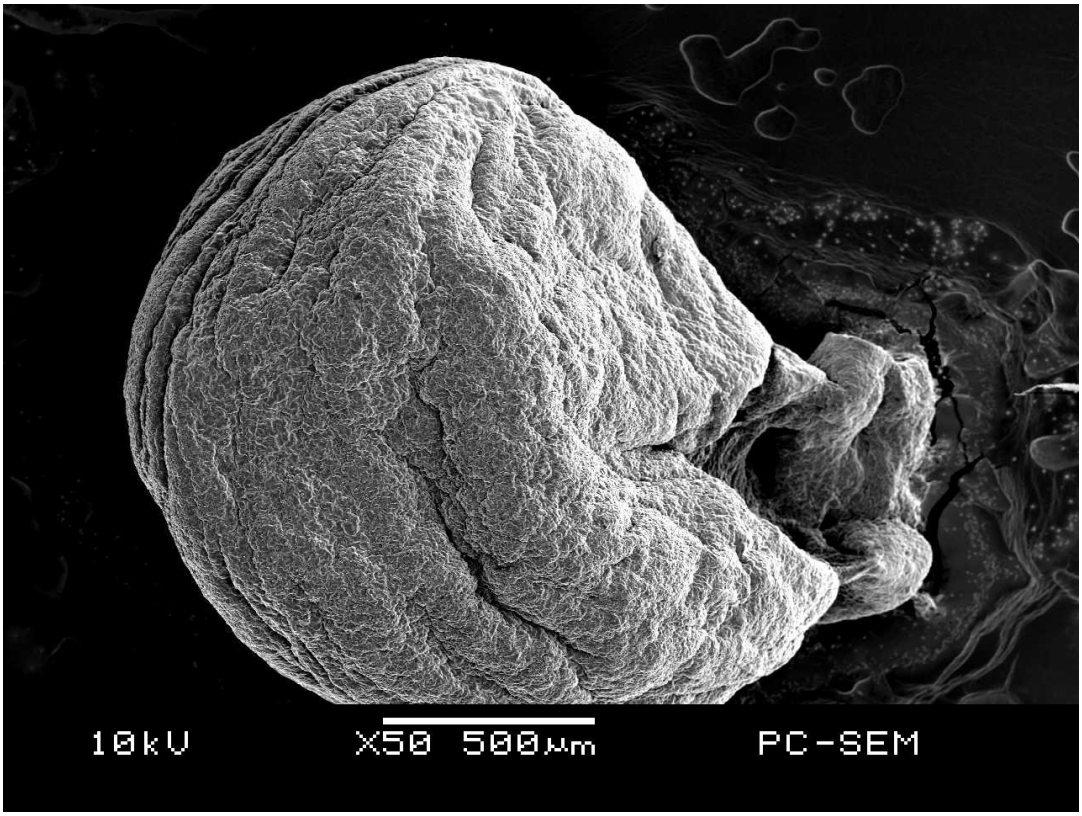

Figure 4.8 SEM micrograph showing the exterior morphology of spirulina–alginate beads made with external gelation process with alginate 1% and $CaCl_2$ 15%.

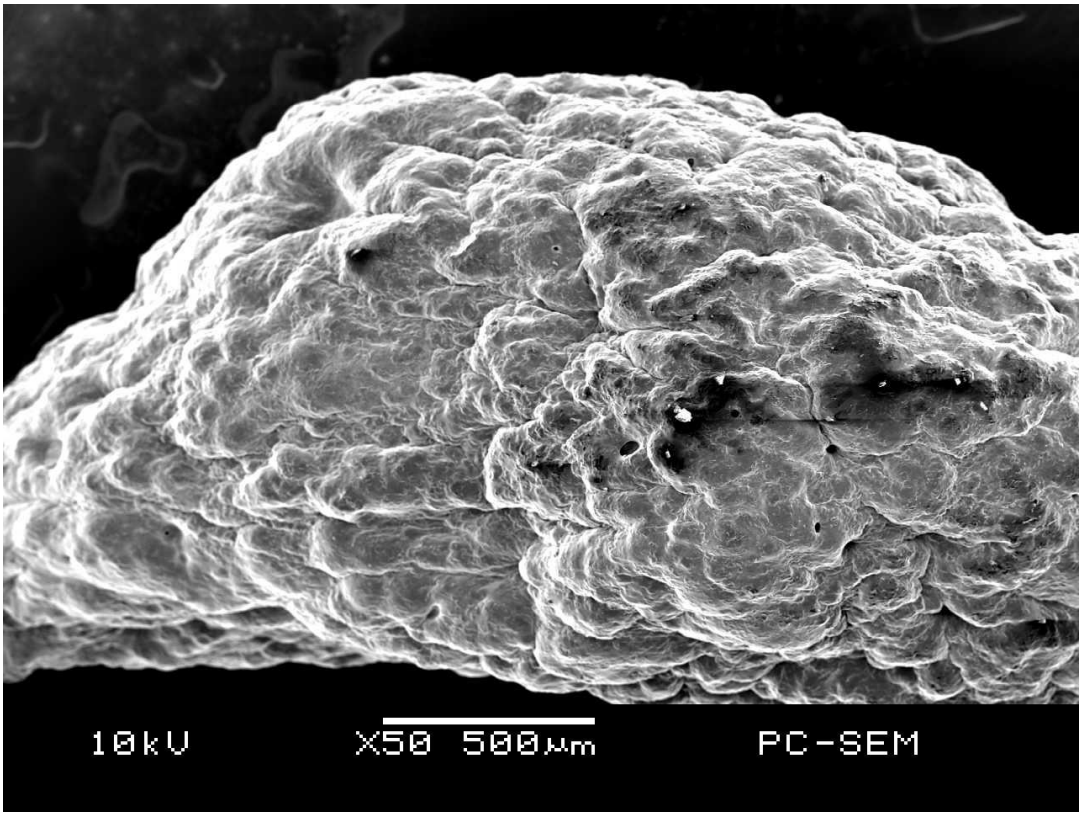

Figure 4.9 SEM micrograph showing the exterior morphology of spirulina–alginate beads made with external gelation process with alginate 7% and $CaCl_2$ 10%.

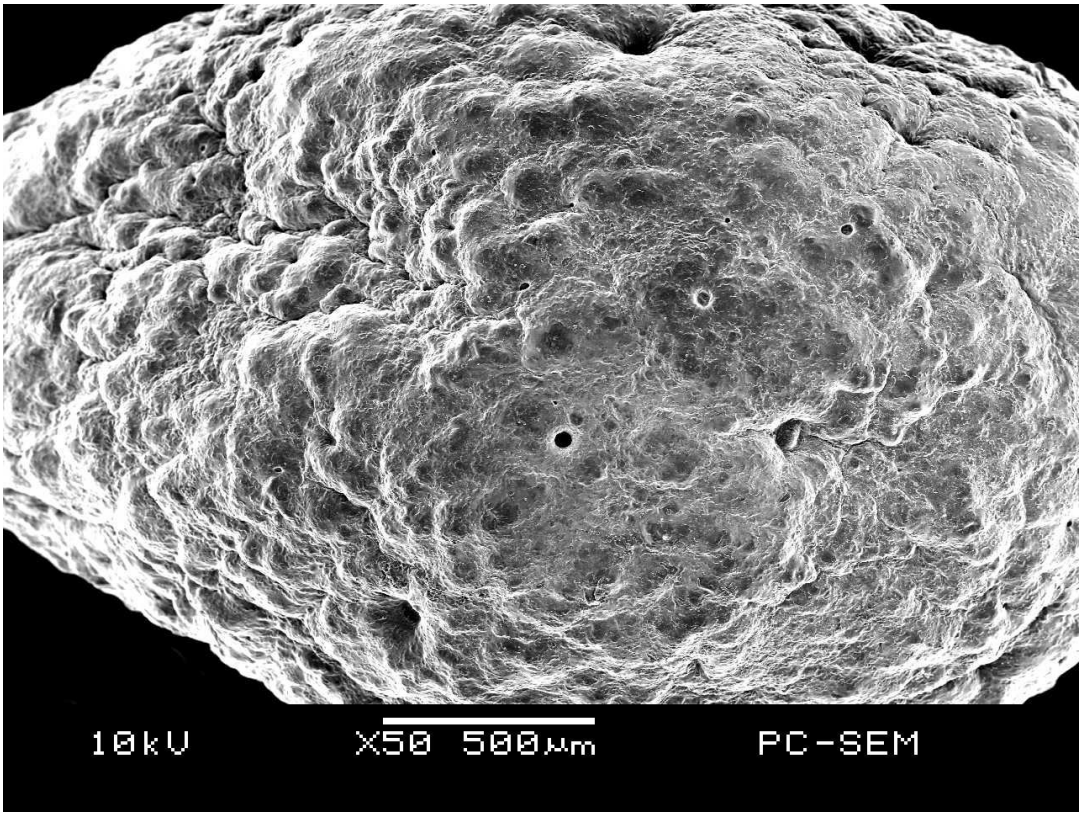

Figure 4.10 SEM micrograph showing the exterior morphology of spirulina–alginate beads made with external gelation process with alginate 7% and $CaCl_2$ 15%.

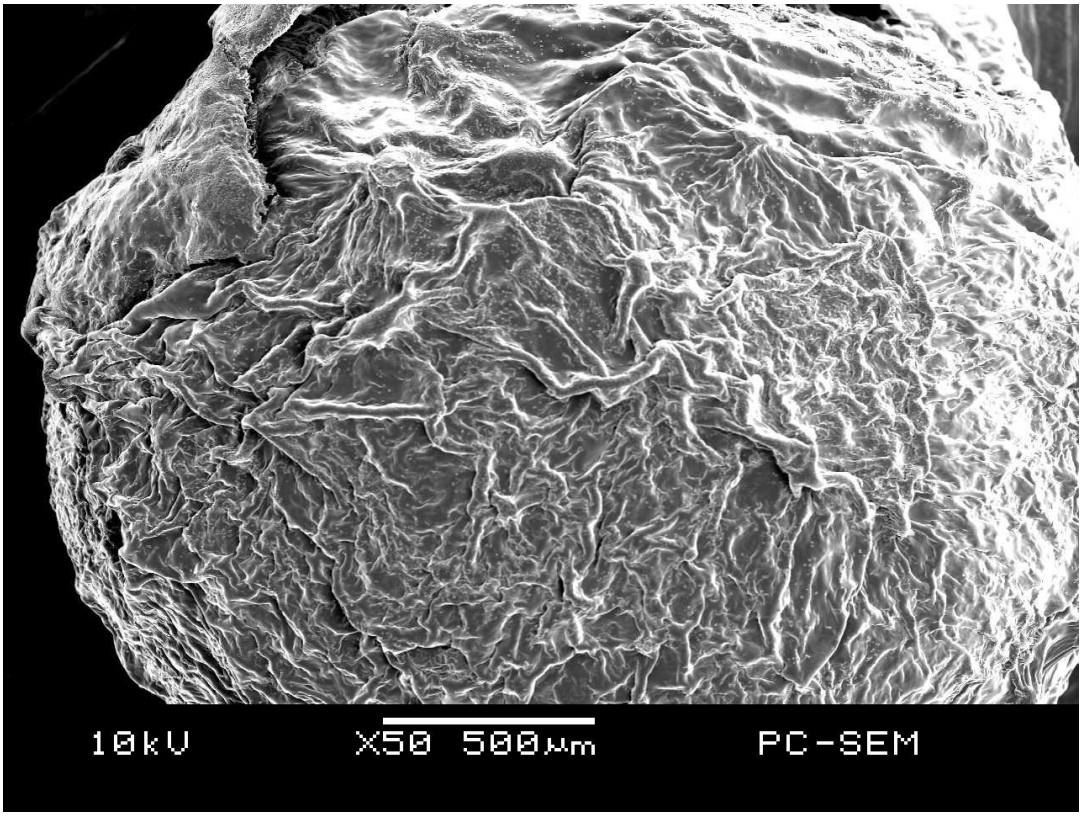

Figure 4.11 SEM micrograph showing the exterior morphology of spirulina–alginate beads made with internal gelation process with alginate 0.5% and $CaCl_2$ 2%.

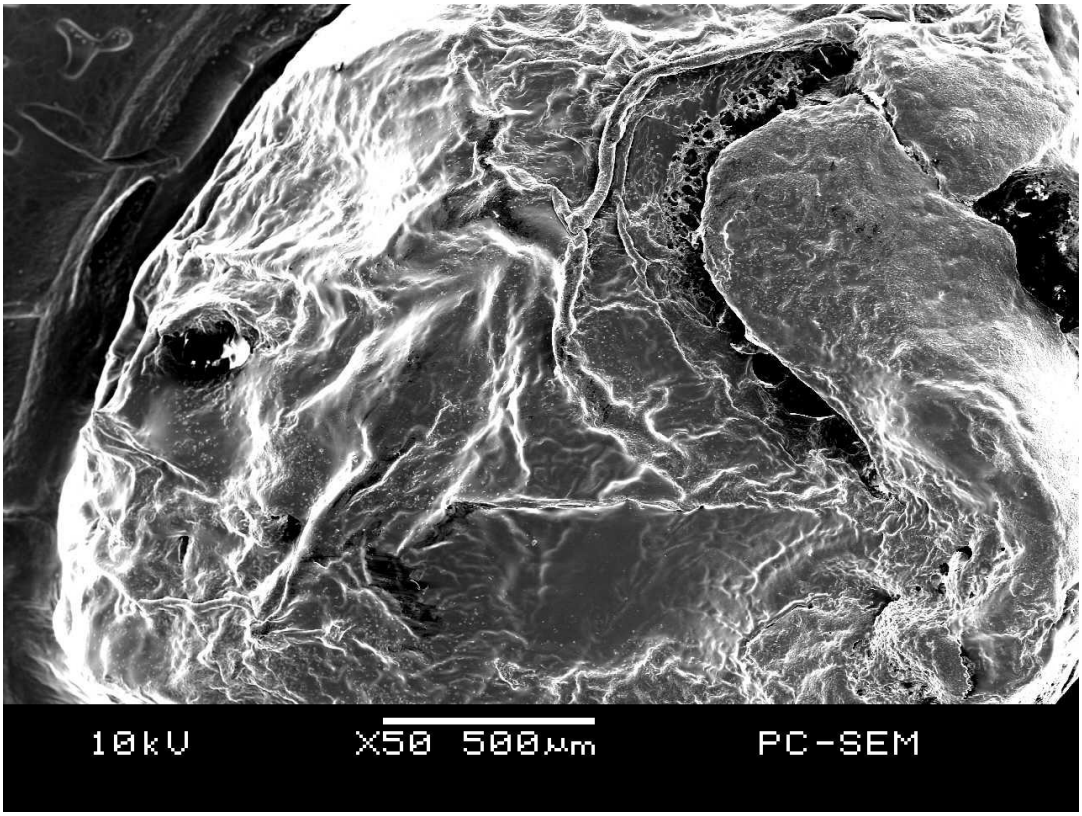

Figure 4.12 SEM micrograph showing the exterior morphology of spirulina–alginate beads made with internal gelation process with alginate 1.5% and $CaCl_2$ 2%.

4.3 Textural/ Mechanical Properties

A texture analyzer was used to evaluate the hardness of the beads. A compression test was used to determine the maximum force required for complete compression of the spirulina – alginate beads, which indicates the hardness of the beads. The hardness data was analyzed by ANOVA, and the results are presented in the Appendix. Figure 4.13 shows the average hardness for each of the six different bead preparation treatments. The external gelation beads with 7% alginate had a maximum force of around 5600 g, but the external gelation beads with 1 % alginate had a maximum force of around 3500g. In the case of internal gelation, beads had a maximum force of around 1300g.

The hardness of the beads prepared by external gelation was significantly higher than the hardness of the beads prepared by internal gelation. This behavior is in accordance with the results reported by Lupo et al. (2015) for the encapsulation of cocoa extract. The concentration of calcium chloride did not significantly influence the hardness of the beads formulated by internal gelation. However, the increase in calcium chloride concentration increased the hardness of the beads formed by external gelation with 1 % alginate from an average of 3186 g to 3744 g. It can also be seen that irrespective of the gelation methodology beads with higher alginate concentration were harder than the beads with lower alginate concentration. This behavior is in accordance with the results reported by Ren (2009) for the encapsulation of sucrose.

Overall, the spirulina beads produced using external gelation with alginate 7% and calcium chloride 15 % had the maximum resistance against compression and exhibited the greatest hardness. Alginate beads are largely used for food applications, and therefore they should possess suitable mechanical properties to withstand the stresses exerted during food processing (Rehm 2009).

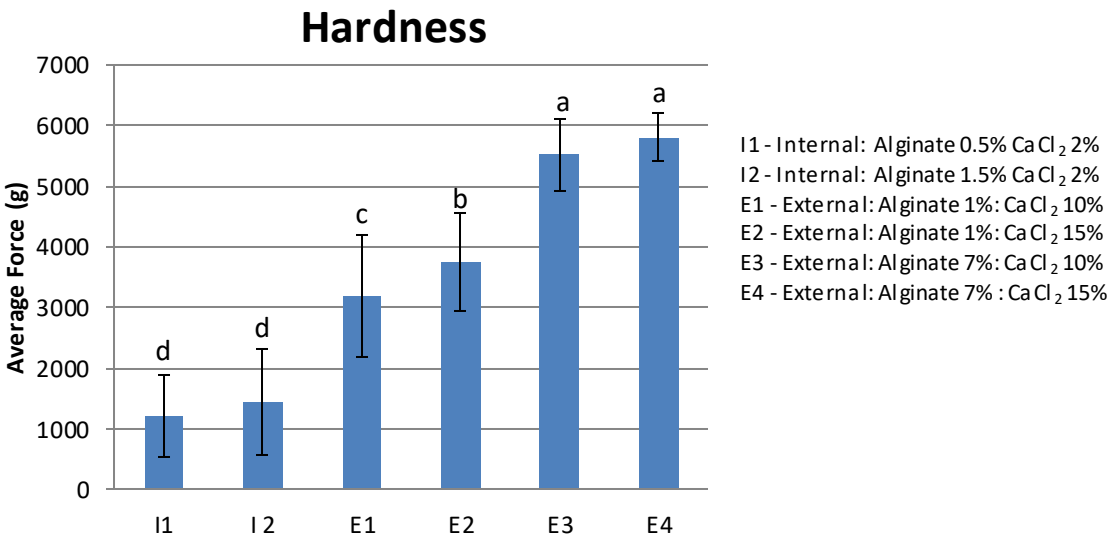

Figure 4.13 Hardness of spirulina–alginate beads for different formulations. Error bars represent ±S.D (n = 30). The bars with different letters are significantly different (α =0.05).

4.4 Protein Analysis

The protein content of the beads produced using each of the six different treatment methods was evaluated using the Dumas method. The data showing crude protein of the spirulina- alginate beads was analyzed by ANOVA, and the results are presented in the Appendix. Figure 4.14 shows the crude protein content of spirulina–alginate beads prepared by external gelation and internal gelation. From the figure, it can be seen that external gelation beads possess higher protein content than the internal gelation beads. The external gelation beads had protein content ranging between 7.29% and 7.59%, while internal gelation beads had protein content around 2.2 %. The method of gelation had a significant impact on the protein content of the beads. However, in both external and internal gelation, the concentration of sodium alginate or calcium chloride did not significantly influence the protein content of the formulated beads.

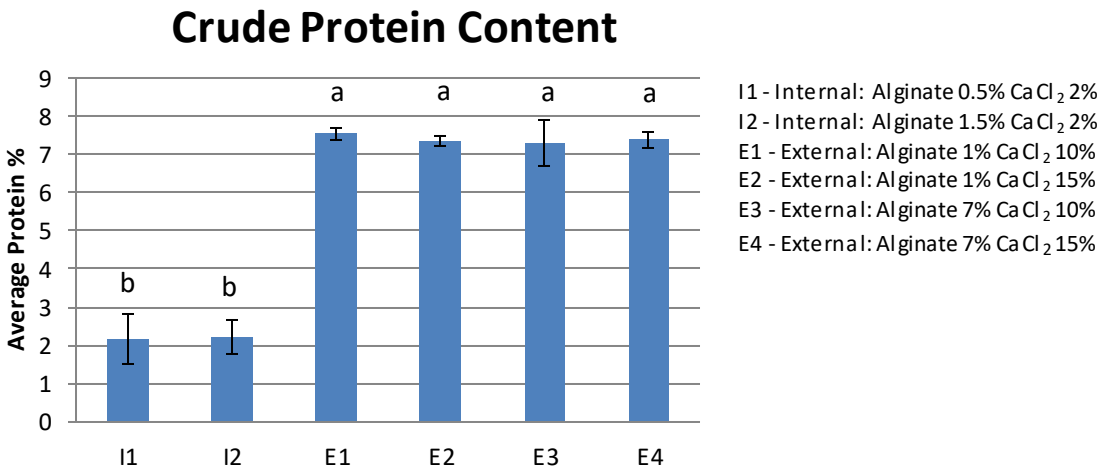

Figure 4.14 Crude protein content of spirulina–alginate beads for different formulations. Error bars represent ±S.D (n= 3). The bars with different letters are significantly different (α =0.05).

4.5 Encapsulation Efficiency

The encapsulation efficiency was determined based on the fraction of protein in the initial mixtures before forming beads compared to the amount of protein in the final beads. Figure 4.15 shows the encapsulation efficiency for each of the six different treatments. From the figure, it is clear that gelation method has a huge influence on the encapsulation efficiency. Irrespective of the sodium alginate and calcium chloride concentration, external gelation beads had an encapsulation efficiency around 78 %, and internal gelation beads had an encapsulation efficiency around 23 %. Overall, the encapsulation efficiency of external gelation beads was significantly higher than the encapsulation efficiency of internal gelation beads.

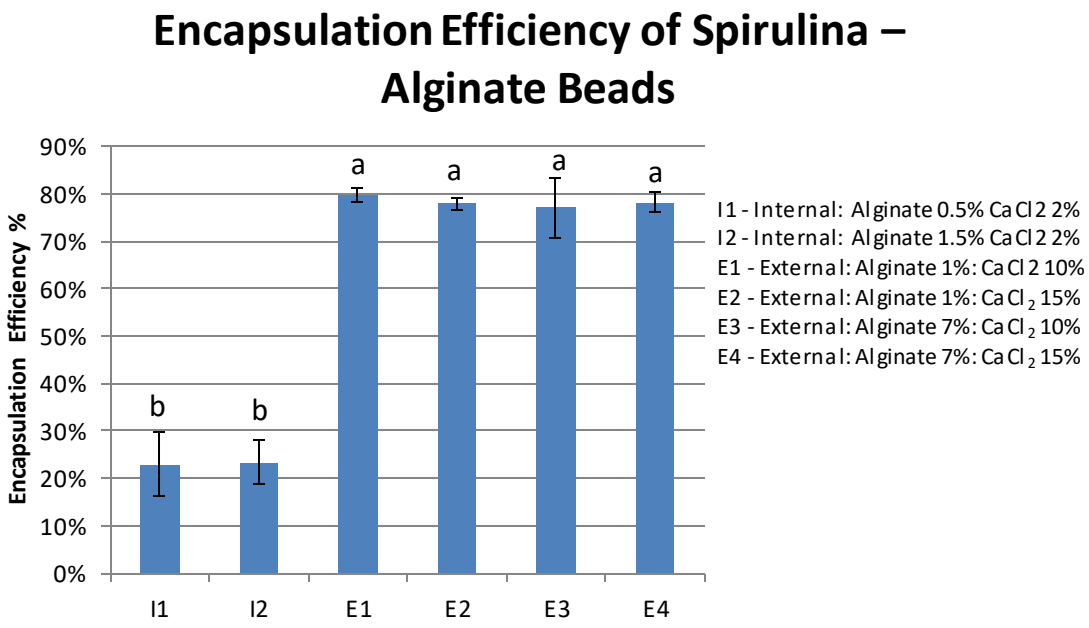

Figure 4.15 Encapsulation efficiency of spirulina–alginate beads for different formulations. Error bars represent ±S.D (n= 3). The bars with different letters are significantly different (α =0.05).

4.6 Sensory Analysis

A sensory analysis was conducted to compare the perceived flavors of cookies made with spirulina beads, plain spirulina, spirulina in combination with a bitter blocker flavor, and soy protein. Table 4.2 shows the sensory score results for seven different attributes as evaluated by panelists (n=22) during the first sensory analysis. In terms of sweetness, aroma/ flavor, and mouth feel, none of the treatments were significantly different. Most of the panelists did not detect any difference in the sweetness, mouth feel and aroma levels among different samples. They also liked the golden color that the cookies with spirulina–alginate and soy protein had, but did not like the green color of the other two cookies with spirulina. However, with respect to bitterness

and aftertaste, the samples with spirulina–alginate beads were significantly different from the other treatments.

Table 4.3 shows the sensory score results for seven different attributes as evaluated by panelists (n=87) during the second sensory analysis. In terms of color, the samples with spirulina-alginate beads and soy protein were significantly better than the samples with untreated spirulina and samples with spirulina & bitter blocker flavor. With respect to bitterness, aftertaste and overall palatability, the scores from the second sensory analysis were similar to that of the first sensory analysis. The panelists felt that the cookies with spirulina-alginate beads were more bitter than other cookie samples. However, the mean bitterness scores of the sample with spirulina-alginate beads from the second sensory analysis were better than those from the first sensory analysis. This indicates that the change from vegetable oil to vegetable shortening in the cookie recipe did have an impact on the perceived bitterness of the beads.

During an informal sensory analysis, five untrained panelists compared the spirulina-alginate beads as a whole with raw spirulina protein. The panelists did not detect any bitterness in the formulated beads when tasted as individual beads. However, when the beads were incorporated into a cookie, it imparted a bitter aftertaste. There might be several reasons for the bitterness such as the cooking of alginate, the interaction between alginate and other ingredients within the food matrix, or the diffusion of spirulina protein in the presence of fat and moisture, etc. The protein release rate from alginate gel depends on the porosity of the gel, and there are different factors like gel strength, gelation mechanism which can affect the porosity of the beads (van den Berg et al. 2007; Kuen Yong Lee 2013). Future studies need to be carried out to study the effect of various factors like the concentration of sodium alginate, etc on the rate of release of spirulina protein from the alginate gel. Also, the size of the spirulina-alginate beads was larger compared to the other dry ingredients in the cookie, which might impact the mouthfeel thereby affecting the sensory perception. A commercial extruder can be used to form smaller sized beads,

which might improve the mouth feel. However, future studies have to be carried out to find the reason behind this behavior.

Table 4.2 Sensory scores for seven different attributes for prepared cookies (sensory analysis I).

	Spirulina – Alginate	Spirulina and Bitter Blocker Flavor	Untreated Spirulina	Soy Protein
Sweetness	5.8 ± 2.2^{a}	6.9 ± 1.6^{a}	6.7 ± 1.6^{a}	7.1 ± 1.5^{a}
Bitterness	4.5 ± 2.3^{b}	6.1 ± 1.7^{a}	6.1 ± 1.7^{a}	6.8 ± 1.7^{a}
Aroma/ Flavor	6.3 ± 1.8^{a}	6.8 ± 1.7^{a}	6.5 ± 1.5^{a}	6.9 ± 1.5^{a}
Mouth Feel	6.3 ± 2.4^{a}	7.0 ± 1.7^{a}	6.6 ± 1.5^{a}	6.7 ± 1.7^{a}
Aftertaste	4.4 ± 2.2^{b}	6.1 ± 1.9^{a}	6.2 ± 1.8^{a}	6.5 ± 1.8^{a}
Color	5.9 ± 1.7^{ab}	4.2 ± 2.4^{c}	4.5 ± 2.7^{bc}	7.2 ± 1.2^{a}
Overall Palatability	4.9 ± 2.1^{b}	5.9 ± 2.2^{ab}	6.2 ± 1.9^{ab}	6.9 ± 1.4^{a}

Data reported in mean ± S.D (n = 22). Values with different letters for each treatment are significantly different from each other ($\alpha = 0.05$). Numbers correspond to a 9- point hedonic scale which goes as follows: 9–Like extremely, 8–Like very much, 7–Like moderately, 6–Like slightly, 5–Neither like nor dislike, 4–Dislike slightly, 3–Dislike moderately, 2–Dislike very much, 1–Dislike extremely.

Table 4.3 Sensory scores for seven different attributes for prepared cookies (Sensory analysis II).

	Spirulina – Alginate	Spirulina and Bitter Blocker Flavor	Untreated Spirulina	Soy Protein
Sweetness	4.9 ± 2.2^{b}	5.5 ± 1.9^{b}	5.5 ± 2.1^{b}	6.6 ± 1.5^{a}
Bitterness	4.4 ± 2.1^{b}	$5.1 \pm 2.2a^{b}$	5.1 ± 2.1^{ab}	5.7 ± 1.7^{a}
Aroma/ Flavor	5.7 ± 2.4^{b}	5.5 ± 2.3^{b}	5.4 ± 2.2^{b}	6.9 ± 1.6^{a}
Mouth Feel	4.5 ± 2.3^{b}	6.1 ± 2.1^{a}	6.3 ± 1.9^{a}	6.8 ± 1.9^{a}
Aftertaste	4.2 ± 2.2^{c}	5.1 ± 2.3^{b}	4.9 ± 2.3^{bc}	6.3 ± 2.0^{a}
Color	6.8 ± 1.8^{a}	3.5 ± 2.1^{b}	3.4 ± 2.1^{b}	7.4 ± 1.3^{a}
Overall Palatability	4.8 ± 2.2^{b}	5.5 ± 2.2^{b}	5.4 ± 2.2^{b}	6.9 ± 1.7^{a}

Data reported in mean ± S.D (n = 87). Values with different letters for each treatment are significantly different from each other ($\alpha = 0.05$). Numbers correspond to a 9- point hedonic scale which goes as follows: 9–Like extremely, 8–Like very much, 7–Like moderately, 6–Like slightly, 5–Neither like nor dislike, 4–Dislike slightly, 3–Dislike moderately, 2–Dislike very much, 1–Dislike extremely.

CHAPTER V

CONCLUSIONS AND FUTURE RECOMMENDATIONS

5.1 Conclusions

This study showed that it is possible to encapsulate spirulina protein using ionic gelation.

Some specific conclusions are as follows:

- Irrespective of the gelation method, an increase in the concentration of sodium alginate significantly increased the thickness of the beads. However, an increase in calcium chloride concentration, did not significantly affect either thickness (mm) or length (mm) of the beads.
- External gelation beads exhibited a more uniform, homogeneous morphology compared to internal gelation beads. Beads obtained by external gelation showed a more smooth and rigid exterior, whereas beads formulated by internal gelation showed a soft and heterogeneous exterior.
- The hardness of the beads prepared by external gelation was significantly higher than the hardness of the beads prepared by internal gelation.
- The external gelation bead with alginate 7% had the maximum resistance against compression and is likely the most suitable for food processing.
- The external gelation beads possessed significantly higher protein content than the internal gelation beads.

- External gelation beads possessed higher encapsulation efficiency than the internal gelation beads.
- Encapsulation of spirulina protein by external gelation and internal gelation resulted in an encapsulation efficiency of 78% and 23% respectively.
- In terms of color of the cookie, the samples with spirulina-alginate beads and soy protein were significantly better than the samples with untreated spirulina and samples with spirulina & bitter blocker flavor. However, the panelists felt that the cookies with spirulina-alginate beads were more bitter than other cookie samples.

5.2 Future Recommendations

- Future studies could involve the use of a mechanical injector to form beads with better characteristics. The concentrations of sodium alginate and spirulina could be increased since a mechanical injector has the potential to create high pressure to extrude highly viscous liquid. A mechanical injector/ extruder would also have the capability to formulate smaller size beads.
- Different methods to reduce bitterness like physical encapsulation and enzymatic hydrolysis could be used to reduce the perceived bitterness.
- Future studies should be performed to understand the reaction between spirulina-alginate beads with other ingredients within the food matrix.
- Apart from cookies, spirulina – alginate beads could be incorporated into other products such as non – heat treated products to understand the consumer acceptance.

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APPENDICES

Appendix 1: Sensory Evaluation Score Sheet

Sample No: **Date_____**

Instructions:

1. FOOD ALLERGEN WARNING: contains Gluten and Spirulina protein**.**
2. Mark **with an "X" or** ✓ beside the answer that best describes your response to the sensory attribute at the top of the column. E.g. If you do not find any bitterness in the product, decide if you like it and how much.

Responses	9 Like extremely	8 Like very much	7 Like moderately	6 Like slightly	5 Neither like, nor dislike	4 Dislike slightly	3 Dislike moderately	2 Dislike very much	1 Dislike extremely
Sweetness									
Bitterness									
Aroma / Flavor									
Mouth Feel/ Bite									
After taste									
Color									
Overall palatability									

Appendix 2 : SAS Outputs for Chapter 3

Key:

Iga	Internal: Alginate 0.5%
Igb	Internal: Alginate 1.5%
Ega	External: Alginate 1%: CaCl 10%
Egb	External: Alginate 1%: CaCl 15%
Egc	External: Alginate 7%: CaCl 10%
Egd	External: Alginate 7% : CaCl 15%

2.1 Length Measurement

```
data lengthcc;
input trt $ length;
cards;
iga 2.9
….
egd 4.78
proc anova data=lengthcc;
class trt;
model length=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information

Class	Levels	Values
trt	6	ega egb egc egd iga igb

Number of Observations Read	60
Number of Observations Used	60

The ANOVA Procedure

Dependent Variable: length

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	66.06679333	13.21335867	210.97	<.0001
Error	54	3.38214000	0.06263222		
Corrected Total	59	69.44893333			

R-Square	Coeff Var	Root MSE	length Mean
0.951300	7.716268	0.250264	3.243333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	5	66.06679333	13.21335867	210.97	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for length

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	54
Error Mean Square	0.062632
Critical Value of Studentized Range	4.17818
Minimum Significant Difference	0.3307

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt
A	4.766	10	egc
A			
A	4.485	10	egd
B	3.034	10	iga
B			
B	2.984	10	igb
C	2.108	10	ega
C			
C	2.083	10	egb

2.2 Thickness Measurement

```
data thickcc;
input  trt $ thick;
cards;
iga  1.38
…….
egd 2.65
proc anova data=thickcc;
class trt;
model thick=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values

trt	6ega egb egc egd iga igb

Number of Observations Read	60
Number of Observations Used	60

The ANOVA Procedure

Dependent Variable: thick

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	11.80249500	2.36049900	112.46	<.0001
Error	54	1.13347000	0.02099019		
Corrected Total	59	12.93596500			

R-Square	Coeff Var	Root MSE	thick Mean
0.912378	7.923429	0.144880	1.828500

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	5	11.80249500	2.36049900	112.46	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for thick

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	54
Error Mean Square	0.02099
Critical Value of Studentized Range	4.17818
Minimum Significant Difference	0.1914

Means with the same letter are not significantly different.				
Tukey Grouping		Mean	N	trt
	A	2.48600	10	egc
	A			
	A	2.39400	10	egd
	B	1.68000	10	igb
	B			
C	B	1.58900	10	egb
C				
C		1.41900	10	iga
C				
C		1.40300	10	ega

2.3 Texture Analysis

```
data texturecc;
input trt $ texture;
cards;
iga 117.818
……..
egd 5955.74
proc anova data=texturecc;
class trt;
model texture=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	6	ega egb egc egd iga igb

Number of Observations Read	180
Number of Observations Used	180

The ANOVA Procedure

Dependent Variable: texture

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	569560137.3	113912027.5	207.88	<.0001
Error	174	95347901.4	547976.4		
Corrected Total	179	664908038.7			

R-Square	Coeff Var	Root MSE	texture Mean
0.856600	21.24401	740.2543	3484.532

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	5	569560137.3	113912027.5	207.88	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for texture

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	174
Error Mean Square	547976.4
Critical Value of Studentized Range	4.07540
Minimum Significant Difference	550.8

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt
A	5805.7	30	egd
A			
A	5513.2	30	egc
B	3744.2	30	egb
C	3186.4	30	ega
D	1454.9	30	igb
D			
D	1202.8	30	iga

2.4 Protein Analysis

```
data proteincc;
input trt $ protein;
cards;
iga 2.791
…….
egd 7.6343
proc anova data=proteincc;
class trt;
model protein=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	6	ega egb egc egd iga igb

Number of Observations Read	18
Number of Observations Used	18

The ANOVA Procedure

Dependent Variable: protein

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	108.7516710	21.7503342	122.26	<.0001
Error	12	2.1347529	0.1778961		
Corrected Total	17	110.8864239			

R-Square	Coeff Var	Root MSE	protein Mean

0.980748	7.446348	0.421777	5.664217

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	5	108.7516710	21.7503342	122.26	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for protein

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	0.177896
Critical Value of Studentized Range	4.75020
Minimum Significant Difference	1.1567

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	7.5478	3	ega
A			
A	7.3979	3	egd
A			
A	7.3695	3	egb
A			
A	7.2905	3	egc
B	2.2091	3	igb
B			
B	2.1705	3	iga

2.5 Encapsulation Efficiency

```
data encapsulationcc;
input  trt $ encapsulation;
cards;
iga  29.53
……..
egd 80.79
proc anova data= encapsulationcc;
class trt;
model encapsulation=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	6	ega egb egc egd iga igb

Number of Observations Read	18
Number of Observations Used	18

The ANOVA Procedure

Dependent Variable: encapsulation

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	12178.36731	2435.67346	122.33	<.0001
Error	12	238.93247	19.91104		
Corrected Total	17	12417.29978			

R-Square	Coeff Var	Root MSE	encapsulation Mean
0.980758	7.444547	4.462179	59.93889

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	5	12178.36731	2435.67346	122.33	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for encapsulation

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	12
Error Mean Square	19.91104
Critical Value of Studentized Range	4.75020
Minimum Significant Difference	12.238

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	79.870	3	ega
A			
A	78.287	3	egd
A			
A	77.983	3	egb
A			
A	77.150	3	egc
B	23.377	3	igb
B			
B	22.967	3	iga

Key:

Sa	Cookies with spirulina – alginate beads
Sf	Cookies with spirulina and bitter blocker flavor
Su	Cookies with untreated spirulina
So	Cookies with soy protein

2.6 Sensory Analysis 1 – Sweetness

```
data sweet;
input trt $ sweetcc;
cards;
Sa 2
……..
So 5
proc anova data=sweet;
class trt;
model sweetcc=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Dependent Variable: sweetcc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	21.5454545	7.1818182	2.31	0.0819
Error	84	260.8181818	3.1049784		
Corrected Total	87	282.3636364			

R-Square	Coeff Var	Root MSE	sweetcc Mean
0.076304	26.55211	1.762095	6.636364

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	21.54545455	7.18181818	2.31	0.0819

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for sweetcc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	84
Error Mean Square	3.104978
Critical Value of Studentized Range	3.70696

Minimum Significant Difference	1.3926

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	7.1364	22	So
A			
A	6.8636	22	Sf
A			
A	6.7273	22	Su
A			
A	5.8182	22	Sa

2.7 Sensory Analysis 1 – Bitterness

```
data bitter;
input  trt $ bittercc;
cards;
Sa 1
…..
So 5
proc anova data=bitter;
class trt;
model bittercc=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	88
Number of Observations Used	88

The ANOVA Procedure

Dependent Variable: bittercc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	59.1363636	19.7121212	5.68	0.0014
Error	84	291.7272727	3.4729437		
Corrected Total	87	350.8636364			

R-Square	Coeff Var	Root MSE	bittercc Mean
0.168545	31.65933	1.863584	5.886364

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt		359.1363636	419.71212121	5.68	0.0014

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for bittercc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	84
Error Mean Square	3.472944
Critical Value of Studentized Range	3.70696
Minimum Significant Difference	1.4728

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	6.7727	22	So
A			
A	6.1364	22	Su
A			
A	6.0909	22	Sf
B	4.5455	22	Sa

2.8 Sensory Analysis 1 – Aroma/ Flavor

```
data aroma;
input trt $ aromacc;
cards;
Sa 2
……..
So 4
proc anova data=aroma;
class trt;
model aromacc=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	88

Number of Observations Used	88

The ANOVA Procedure

Dependent Variable: aromacc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4.4886364	1.4962121	0.57	0.6391
Error	84	222.1363636	2.6444805		
Corrected Total	87	226.6250000			

R-Square	Coeff Var	Root MSE	aromacc Mean
0.019806	24.54620	1.626186	6.625000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	4.48863636	1.49621212	0.57	0.6391

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for aromacc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	84
Error Mean Square	2.644481
Critical Value of Studentized Range	3.70696
Minimum Significant Difference	1.2852

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt
A	6.8636	22	So
A			
A	6.8182	22	Sf
A			
A	6.5000	22	Su
A			
A	6.3182	22	Sa

2.9 Sensory Analysis 1 – Color

```
data color;
input trt $ colorcc;
cards;
Sa 7
……..
So 8
proc anova data= color;
class trt;
```

```
model colorcc=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	88
Number of Observations Used	88

The ANOVA Procedure

Dependent Variable: colorcc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	123.1250000	41.0416667	9.30	<.0001
Error	84	370.5909091	4.4117965		
Corrected Total	87	493.7159091			

R-Square	Coeff Var	Root MSE	colorcc Mean
0.249384	38.58823	2.100428	5.443182

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	123.1250000	41.0416667	9.30	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for colorcc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	84
Error Mean Square	4.411797
Critical Value of Studentized Range	3.70696
Minimum Significant Difference	1.66

Means with the same letter are not significantly different.				
Tukey Grouping		Mean	N	trt
	A	7.1818	22	So
	A			
B	A	5.8636	22	Sa
B				
B	C	4.5455	22	Su
	C			

	C	4.1818	22	Sf

2.10 Sensory Analysis 1 – Mouth Feel

```
data bite;
input trt $ bitecc;
cards;
Sa 2
…….
So 4
proc anova data=bite;
class trt;
model bitecc=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	88
Number of Observations Used	88

The ANOVA Procedure

Dependent Variable: bitecc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	5.9090909	1.9696970	0.57	0.6339
Error	84	288.4545455	3.4339827		
Corrected Total	87	294.3636364			

R-Square	Coeff Var	Root MSE	bitecc Mean
0.020074	27.92344	1.853101	6.636364

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	5.90909091	1.96969697	0.57	0.6339

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for bitecc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	84
Error Mean Square	3.433983

Critical Value of Studentized Range	3.70696
Minimum Significant Difference	1.4646

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	7.0000	22	Sf
A			
A	6.6818	22	So
A			
A	6.5909	22	Su
A			
A	6.2727	22	Sa

2.11 Sensory Analysis 1 – Aftertaste

```
data aftertaste;
input  trt $ aftertastecc;
cards;
Sa 1
……
So 5
proc anova data= aftertaste;
class trt;
model aftertastecc=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	88
Number of Observations Used	88

The ANOVA Procedure

Dependent Variable: aftertastecc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	62.9545455	20.9848485	5.55	0.0016
Error	84	317.3636364	3.7781385		
Corrected Total	87	380.3181818			

R-Square	Coeff Var	Root MSE	aftertastecc Mean
0.165531	33.53910	1.943743	5.795455

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt		362.9545454	520.98484848	5.55	0.0016

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for aftertastecc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	84
Error Mean Square	3.778139
Critical Value of Studentized Range	3.70696
Minimum Significant Difference	1.5362

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	6.5455	22	So
A			
A	6.2273	22	Su
A			
A	6.0455	22	Sf
B	4.3636	22	Sa

2.21 Sensory Analysis 1 – Overall Palatability

```
data overall;
input  trt $ overallcc;
cards;
Sa 2
……
So 3
 proc anova data= overall;
class trt;
model overallcc=trt;
means trt/tukey lines;
run;
```

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	88

Number of Observations Used	88

The ANOVA Procedure

Dependent Variable: overallcc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	43.1250000	14.3750000	3.92	0.0113
Error	84	307.8636364	3.6650433		
Corrected Total	87	350.9886364			

R-Square	Coeff Var	Root MSE	overallcc Mean
0.122867	31.96772	1.914430	5.988636

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	43.12500000	14.37500000	3.92	0.0113

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for overallcc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	84
Error Mean Square	3.665043
Critical Value of Studentized Range	3.70696
Minimum Significant Difference	1.513

Means with the same letter are not significantly different.

Tukey Grouping		Mean	N	trt
	A	6.9091	22	So
	A			
B	A	6.1818	22	Su
B	A			
B	A	5.9091	22	Sf
B				
B		4.9545	22	Sa

2.22 Sensory Analysis 2 – Sweetness

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	348
Number of Observations Used	348

The ANOVA Procedure

Dependent Variable: sweetcc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	128.330460	42.776820	11.30	<.0001
Error	344	1302.597701	3.786621		
Corrected Total	347	1430.928161			

R-Square	Coeff Var	Root MSE	sweetcc Mean
0.089683	34.42713	1.945924	5.652299

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	128.3304598	42.7768199	11.30	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for sweetcc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	344
Error Mean Square	3.786621
Critical Value of Studentized Range	3.65098
Minimum Significant Difference	0.7617

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	6.6322	87	So
B	5.5172	87	Su
B			
B	5.4943	87	Sf
B			
B	4.9655	87	Sa

2.23 Sensory Analysis 2 – Bitterness

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	348
Number of Observations Used	348

The ANOVA Procedure

Dependent Variable: bittercc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	79.525862	26.508621	6.38	0.0003
Error	344	1429.954023	4.156843		
Corrected Total	347	1509.479885			

R-Square	Coeff Var	Root MSE	bittercc Mean
0.052684	40.24470	2.038834	5.066092

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	79.52586207	26.50862069	6.38	0.0003

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for bittercc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	344
Error Mean Square	4.156843
Critical Value of Studentized Range	3.65098
Minimum Significant Difference	0.7981

Means with the same letter are not significantly different.				
Tukey Grouping		Mean	N	trt
	A	5.6897	87	So
	A			
B	A	5.1264	87	Su
B	A			
B	A	5.1034	87	Sf
B				
B		4.3448	87	Sa

2.24 Sensory Analysis 2 – Aroma/ Flavor

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	348
Number of Observations Used	348

The ANOVA Procedure

Dependent Variable: aromacc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	139.011494	46.337165	10.06	<.0001
Error	344	1585.264368	4.608327		
Corrected Total	347	1724.275862			

R-Square	Coeff Var	Root MSE	aromacc Mean
0.080620	36.40605	2.146701	5.896552

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	139.0114943	46.3371648	10.06	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for aromacc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	344
Error Mean Square	4.608327
Critical Value of Studentized Range	3.65098
Minimum Significant Difference	0.8403

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	6.9770	87	So
B	5.7011	87	Sa
B			
B	5.4713	87	Sf
B			
B	5.4368	87	Su

2.25 Sensory Analysis 2 – Mouth Feel

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	348
Number of Observations Used	348

The ANOVA Procedure

Dependent Variable: mouthfeelcc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	257.617816	85.872605	19.43	<.0001
Error	344	1520.620690	4.420409		
Corrected Total	347	1778.238506			

R-Square	Coeff Var	Root MSE	mouthfeelcc Mean
0.144872	35.56937	2.102477	5.910920

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	257.6178161	85.8726054	19.43	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for mouthfeelcc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	344
Error Mean Square	4.420409
Critical Value of Studentized Range	3.65098
Minimum Significant Difference	0.823

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	trt
A	6.7816	87	So
A			
A	6.2529	87	Su
A			
A	6.1264	87	Sf
B	4.4828	87	Sa

2.26 Sensory Analysis 2 – Color

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	348
Number of Observations Used	348

The ANOVA Procedure

Dependent Variable: colorcc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	1181.824713	393.941571	117.69	<.0001
Error	344	1151.425287	3.347167		
Corrected Total	347	2333.250000			

R-Square	Coeff Var	Root MSE	colorcc Mean
0.506514	34.84812	1.829526	5.250000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	1181.824713	393.941571	117.69	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for colorcc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	344
Error Mean Square	3.347167
Critical Value of Studentized Range	3.65098
Minimum Significant Difference	0.7161

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	N	trt
A	7.3793	87	So
A			
A	6.7816	87	Sa
B	3.4598	87	Sf
B			
B	3.3793	87	Su

2.27 Sensory Analysis 2 – Aftertaste

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	348
Number of Observations Used	348

The ANOVA Procedure

Dependent Variable: aftertastecc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	196.491379	65.497126	13.46	<.0001
Error	344	1674.137931	4.866680		
Corrected Total	347	1870.629310			

R-Square	Coeff Var	Root MSE	aftertastecc Mean
0.105040	43.15386	2.206055	5.112069

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	196.4913793	65.4971264	13.46	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for aftertastecc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	344
Error Mean Square	4.86668
Critical Value of Studentized Range	3.65098
Minimum Significant Difference	0.8635

Means with the same letter are not significantly different.				
Tukey Grouping		Mean	N	trt
	A	6.2759	87	So
	B	5.0805	87	Sf
	B			
C	B	4.9080	87	Su
C				
C		4.1839	87	Sa

2.28 Sensory Analysis 2 – Overall Palatabillity

The ANOVA Procedure

Class Level Information		
Class	Levels	Values
trt	4	Sa Sf So Su

Number of Observations Read	348
Number of Observations Used	348

The ANOVA Procedure

Dependent Variable: overallcc

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F

Model	3	227.801724	75.933908	17.60<.0001
Error	344	1483.862069	4.313553	
Corrected Total	347	1711.663793		

R-Square	Coeff Var	Root MSE	overallcc Mean
0.133088	36.66994	2.076909	5.663793

Source	DF	Anova SS	Mean Square	F Value	Pr > F
trt	3	227.8017241	75.9339080	17.60	<.0001

The ANOVA Procedure

Tukey's Studentized Range (HSD) Test for overallcc

Note: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	344
Error Mean Square	4.313553
Critical Value of Studentized Range	3.65098
Minimum Significant Difference	0.813

Means with the same letter are not significantly different.			
Tukey Grouping	Mean	Ntrt	
A	6.9885	87	So
B	5.4713	87	Sf
B			
B	5.4023	87	Su
B			
B	4.7931	87	Sa

Appendix 3 : Institutional Review Board Approval

Oklahoma State University Institutional Review Board

Date: Tuesday, February 28, 2017 **Protocol Expires: 2/8/2020**

IRB Application No: AG175

Proposal Title: Incorporation of Spirulina Protein into Cookies

Processed as: **Modification**

Status Recommended by Reviewer(s) **Approved**

Principal Investigator(s):

Danielle Bellmer
108 FAPC
Stillwater, OK 74078

Deepak Kumar Rajmohan
Stillwater, OK 74078

The requested modification to this IRB protocol has been approved. Please note that the original expiration date of the protocol has not changed. The IRB office MUST be notified in writing when a project is complete. All approved projects are subject to monitoring by the IRB.

The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

The reviewer(s) had these comments:

Mod to add recruitment from FDSC 1133

Signature :

Hugh Crethar, Chair, Institutional Review Board

Tuesday, February 28, 2017
Date

VITA

Deepak Kumar Duraivelu Rajmohan

Candidate for the Degree of

Master of Science

Thesis: USE OF IONIC GELATION TO REDUCE BITTERNESS OF SPIRULINA PROTEIN

Major Field: Food Science

Biographical:

Education:

Completed the requirements for the Master of Science in Food Science at Oklahoma State University, Stillwater, Oklahoma in May, 2017

Completed the requirements for the Bachelor of Engineering in Agricultural Engineering at Anna University, Chennai, India in June, 2013
.

Professional Memberships: Institute of Food Technologists (IFT)