Encapsulation of Orange Flavor in Gel Matrix

BY

Ms. Nontawan Boonyapanwanna

ID.5210319

A special project submitted to the Biotechnology of Biotechnology, Assumption University in part of fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology

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Level of study	:	**Bachelor of Science**
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(Advisor)

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Nontawan Boonyapanwanna

ABSTRACT

This study was aimed to optimize the factors for encapsulation of orange flavor and xylitol in gel matrix. The orange oil flavor and xylitol were encapsulated in chitosan coated alginate bead. In order to form a bead, sodium alginate solution mixed with xylitol (1%) and orange flavor was extruded into calcium chloride solution (1%) by a syringe. The beads were also coated with chitosan (1%) which was mixed in calcium chloride solution using one-stage encapsulation procedures. Central composite experimental design and response surface methodology were used to study the effects of two factors, alginate concentration (X_1) and orange flavor concentration (X_2) on encapsulation efficiency (EE) of flavor encapsulation in gel matrix. Each independent variable was studied at three levels as 1, 1.5 and 2% for alginate concentrations; and 1, 2 and 3% for orange flavor concentrations. The result showed that encapsulation efficiency (Y_1) ranged from 4.2% to 64.0%, which was fitted with the polynomial model as $Y_1 = 31.729 - 8.585X_1 - 15.776X_2 - 2.766X_1^2 + 0.530X_1X_2 + 2.563X_2^2$, indicating negatively effects of both factors. The use of 0.6% alginate and 0.2% orange flavor gave the highest encapsulation efficiency as 91.6%. Moreover, the effect of alginate concentrations and orange flavor concentrations on bead size was not found. The diameter of beads varied from 2.97 to 3.40 mm.

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INTRODUCTION

Flavor plays an important role in consumer satisfaction and influences further consumption of foods. People sometimes prefer food because of the flavor to other attributes. Since flavor affects the consumer satisfaction and quality of foods, flavor stability in foods has been of increasing interest. However it is difficult to control as it is delicate and volatile. Manufacturing and storage processes, packaging materials and ingredients in foods often cause modification in overall flavor by reducing aroma compound intensity or producing off-flavor components (Atmane et al., 2005). Flavors form very complex systems because there are many variables. Some are more stable in carbohydrates which are water soluble and some are more stable in lipid-based coating. Many factors linked to aroma affect the overall quality of the food which the examples are physico-chemical properties, concentration and interactions of volatile aroma molecules with food components (Atmane et al., 2005). The method to preserve them is often a top concern of food manufacturers. Therefore, encapsulation of volatile ingredients prior to use in foods and beverages is invented to limit aroma degradation during process and storage. Encapsulation is the technique by coating material or mixture of materials in another material or system to protect against evaporation, reaction, or migration in a food. The retention of flavor is governed by factors related to the chemical nature of the core, including its molecular weight, chemical functionality, polarity and relative volatility, to the wall material properties and to the nature and the parameters of the encapsulation technology (Atmane et al., 2005). Incorporation of small amounts of flavors into foods can greatly influence the finished product, quality, cost, and consumer satisfaction. The food industry is continuously developing ingredients, processing methods, and packaging materials to improve flavor preservation and delivery. Encapsulation technology is now well developed and accepted within the pharmaceutical, chemical, cosmetic, foods and printing industries. In food products, fats and oils, aroma compounds and oleoresins, vitamins, minerals, colorants, and enzymes have been encapsulated (Atmane et al., 2005).

OBJECTIVES

1. To study the effect of orange flavor and alginate concentration in encapsulation efficiency of orange flavor in gel matrix
2. To determine the equation of relationship between encapsulation efficiency and concentration of orange flavor and alginate

LITERATURE REVIEW

1. Encapsulation Technology

1.1 Definition

Encapsulation is a process which a thin coating is formed around solid particles, liquid droplets, or gas cells that are fully contained within the capsule wall. Approximate 80 years ago, encapsulation processes were developed. It involves the coating and entrapment of a pure material or mixture into another material. The coated or entrapped material is usually a liquid but can be a solid or gas (Soottitantawat, n.d.).

The coated material is called active or core material, and the coating material is called shell, wall material, carrier or encapsulant. The core material may be composed of just one or several different types of ingredients and the carrier may be single or multilayered. For encapsulation of the flavor compounds, the carrier material must have no reactivity with the core material; be present in a form that is easy to handle. A good knowledge of the physico-chemical interactions occurring between aroma compounds and the main constituents of foods such as lipids polysaccharides and proteins is required for food flavoring control (Atmane et al., 2005).

1.2 Benefits of encapsulation

1. Reduce the reactivity of the core with regard to the outside environment. Since the encapsulated materials can be protected from oxygen, moisture, heat or other extreme conditions, they enhance the stability and maintaining viability (Gibbs et al., 1999).
2. Decrease the evaporation or transfer rate of the core material with regard to the outside environment.
3. Control the release of the core material so as to achieve the proper delay until the right stimulus.
4. Promote the ease of handling of the core material.
5. Utilized to mask the odor or taste of the core, for example, unpleasant smell from unsaturated fatty acids when they are oxidized. Encapsulation largely overcomes this

problem by taste masking and limiting oxidation. Moreover, it helps to make functional food pleasant to consume by addition of flavor in coating material (Poncelet et al., 2011).

6. Dilute the core material when it is only used in very small amounts; but, achieve uniform dispersion in the host material (Soottitantawat, n.d.).

2. Types of Encapsulated ingredients

Various food ingredients that can be encapsulated

- Flavoring agents
- Acids, akalis, buffers
- Lipids
- Redox agents (bleaching, maturing)
- Antioxidants
- Enzymes and microorganisms
- Artificial sweeteners
- Leavening agents
- Preservatives
- Colorants
- Cross-linking and setting agents
- Agents with undesirable flavor and odors
- Essential oils, amino acids, vitamins, minerals (Soottitantawat, n.d.).

Among others, the use of encapsulation for sweeteners such as aspartame, xylitol and flavors in chewing gum is well known (Gibbs et al., 1999).

3. Wall Material

Many different types of materials can be used. These include proteins, starches, dextrins, lipids, gums and cellulose. For this reason, many coatings are actually composite formulations of any or all the above. The choice of wall materials depends upon a number of factors including: expected product objectives and requirements; nature of the core material;

the process of encapsulation; economics and whether the coating material is approved by the Food and Drug Administration (US) or European Food Safety Authority (Europe) (Gibbs et al., 1999; Atmane et al., 2005).

Alginate

Alginate is a natural polysaccharide in all brown algae as a skeletal component of their cell walls. It is a linear unbranched co-polymer made up from β-(1→4)-linked D-mannuronic acid (**M**) and α-(1→4)-linked L-guluronic acid (**G**) residues. The blocks vary in size and alternating M and G segments as well as random blocks may also be present. The type of structure is influenced by the seaweed source as well as the growing conditions of the weed (CyberColloid Ltd., n.d.).

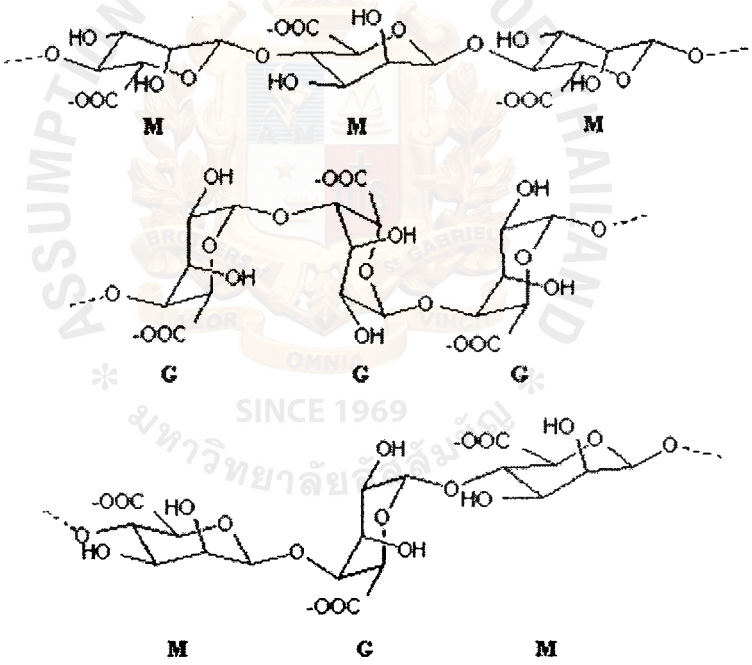

Figure 1: The chemical structure of alginate with β -D-mannuronic (M) acid blocks and α -L-gluronic (G) acid blocks (Brunetti and Martin, 2006)

The block structure within the alginate can vary significantly. The poly guluronic acid blocks bind significantly more effectively with calcium ions than the poly mannuronic acid blocks. The weed with the higher guluronic acid levels normally has a stronger interaction with calcium which gives stronger gel strength. However, the alginate with the strongest calcium

gel not only high guluronic acid level is required but also significant block structures (CyberColloid Ltd., n.d.).

Alginate is used in food because it is a powerful thickening, stabilizing, and gel-forming agent. Most alginate used in foods is in the form of sodium alginate. In order to form a gel, sodium alginate needs to come into contact with divalent ions such as Ca^{2+}, Sr^{2+}, or Ba^{2+}, while monovalent cations and Mg^{2+} do not induce gelation. Ba^{2+} and Sr^{2+} ions produce very strong alginate gels. Numerous other cations including Pb^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, and Mn^{2+} will induce gelation, but due to their toxicity they are rarely used. (Brunetti and Martin, 2006) As soon as sodium alginate (Figure 2) is added to a solution of calcium chloride, a gel forms as the sodium ions (Na^{+}) are exchanged with calcium ions (Ca^{2+}) and the polymers become crosslinked (Figure 3). The structure formed is called 'egg-box' (Figure 4). In the case of Ca2+, the cation binds to the a-L-gluronic acid residues (G-block) forming dimerizing junctions with other chains, producing soluble gellous networks (Brunetti and Martin, 2006).

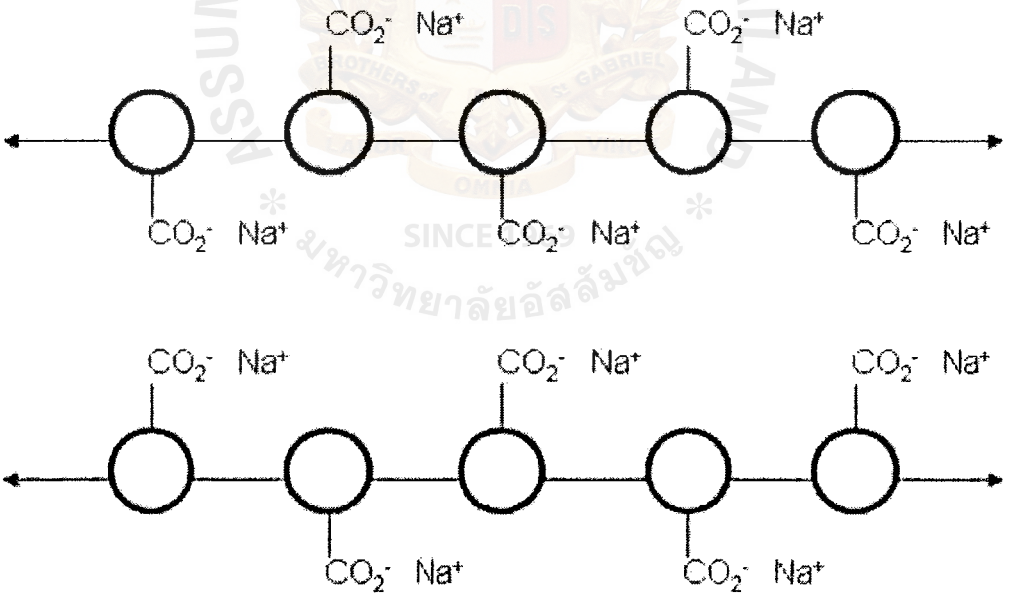

Figure 2: Alginate polymer in NaCl solution (no crosslinking) (Waldman et al., 1998)

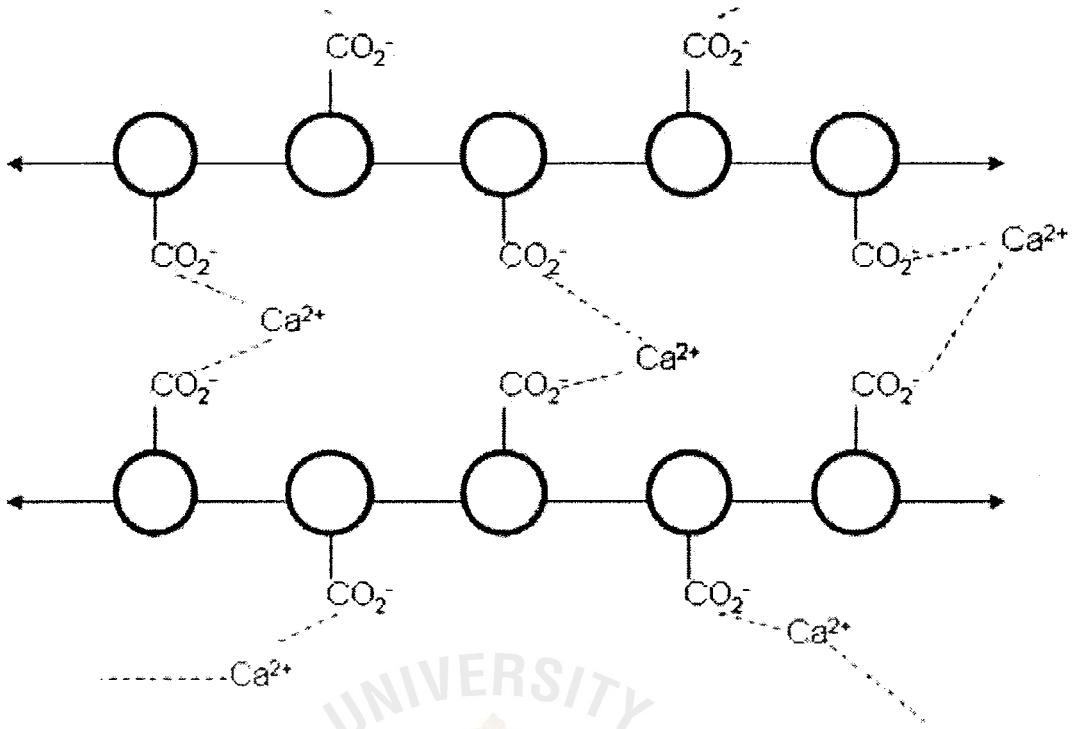

Figure 3: Alginate polymer in $CaCl_2$ solution (crosslinking) (Waldman et al., 1998)

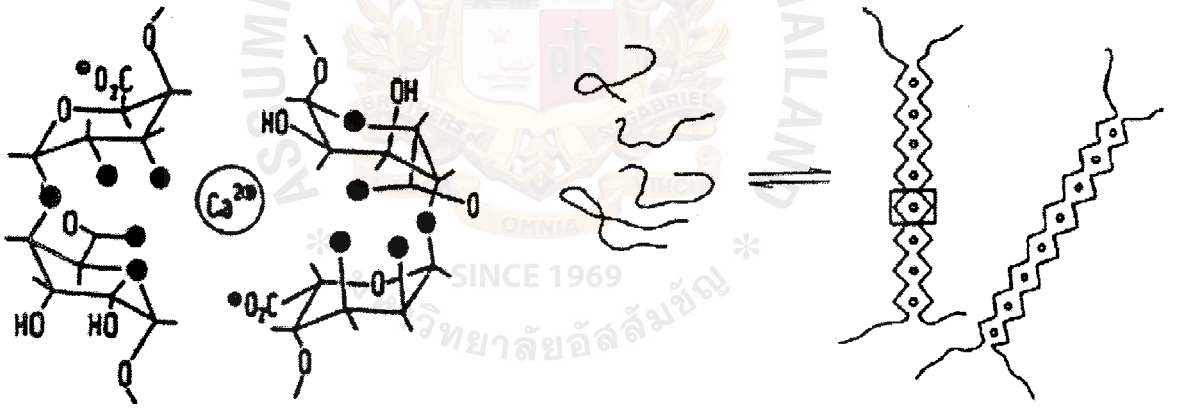

Figure 4: Egg-Box association of poly-L-guluronate sequences of alginate and conversion of random coils to ribbon structures when cross-linked with calcium ions (Brunetti and Martin, 2006)

The calcium ions are able to crosslink the alginate polymers because they can form two bonds, as opposed to monovalent ions such as sodium, which can only form one bond. If a fine jet of sodium alginate solution is forced into a bath of a calcium chloride solution, calcium alginate is formed as fibers. If low viscosity alginates are used, a strong solution can

be used without any viscosity problems and the calcium bath is not diluted as rapidly (Fisheries and Aquaculture Department, FAO, n.d.). The longer the alginate is in contact with the calcium chloride solution, the more rigid the gel will become, as more crosslinks are formed. During the longer soak, more calcium ions were able to move further into the mesh of the gel bead, resulting in more cross-linking and a firmer texture (Anon., n.d.). Also, depending on the concentration of calcium ions, the gels are either thermoreversible (low concentrations) or not (high concentrations) (Waldman et al., 1998; Belitz and Grosch, 1999).

Alginate matrices contain aqueous internal environments ideal for the encapsulation of proteins and small molecules. The encapsulations can be done at room temperature. The size of the needle and the viscosity of the alginate solution will determine the diameter of bead formed. Larger needle and more viscous solutions produce larger diameter beads. Moreover, as concentration of the sodium alginate is increased, the beads produced become more spherical. The beads have a high rate of macromolecular diffusion due to their porous gel state that may be controlled through specific coating procedures. In addition, alginate matrices are very biodegradable and can be broken down under normal physiological conditions (Brunetti and Martin, 2006).

4. Encapsulation Technique

Various techniques are used for encapsulation. The main process consists of two steps. The first is often emulsification of a core material. The second is drying or cooling of the emulsions, ensuring that leakage does not occur, and ensuring that undesired materials are kept out. The two major industrial processes for flavor encapsulation are spray drying and extrusion. The following are encapsulation techniques (Atmane et al., 2005).

- Spray drying
- Spray chilling
- Liposome entrapment
- Coacervation
- Spray cooling
- Extrusion coating
- Inclusion complexation
- Centrifugal extrusion
- Fluidized bed coating
- Co-crystallization
- Rotational suspension separation
- Interfacial polymerization

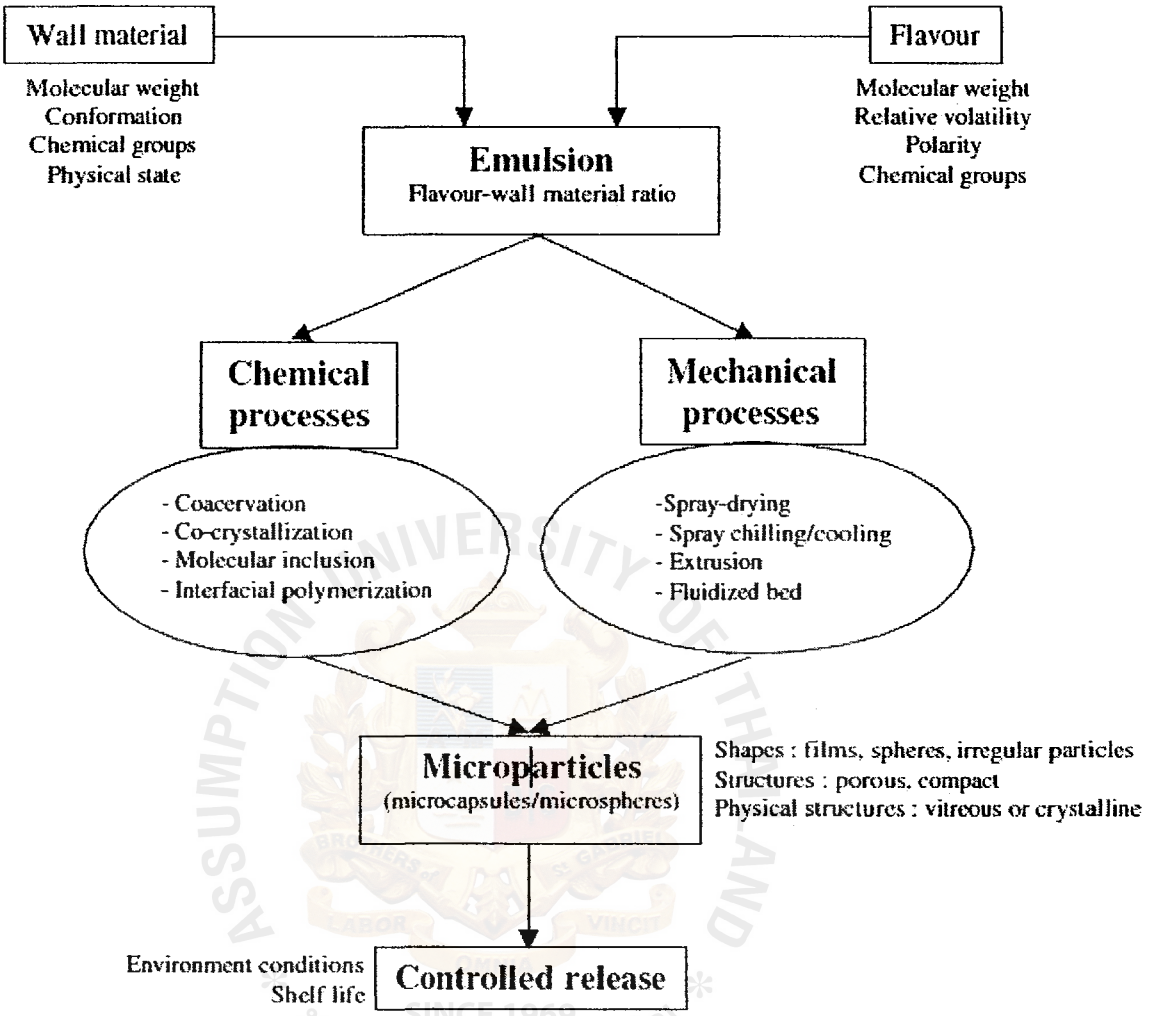

Figure 5: A schematic illustration of different processes of encapsulation of flavor compounds (Atmane et al., 2005)

Extrusion is the encapsulation technique used in this research. Encapsulation of flavors via extrusion has been used for volatile and unstable flavors in glassy carbohydrate matrices. Advantage of this method is the stability of flavors against oxidation. Carbohydrate matrices in the glassy state have very good barrier properties and extrusion is a convenient process enabling the encapsulation of flavors in such matrices. However, if there are structural defects such as cracks, thin wall, or pores formed during or after processing process, the flavor can diffuse from the extruded carbohydrate. Extrusion of polymer solutions through nozzles to produce either beads or capsules is mainly used on a laboratory scale (Atmane et al., 2005).

5. Special Treatment

Chitosan

Chitin is classified as non-starch polysaccharide and often cellulose derivatives due to its identical structure to cellulose. Chitosan is a cationic, non-toxic, biocompatible, and biodegradable polymer chitin derivative derived by *N*-deacetylation of chitin and becomes a copolymer of *N*-acetylglucosamine and glucosamine. It is found to be very useful than chitin and also can be formed many more useful derivatives which their applications are useful in diversified areas (Dutta et al., 2004).

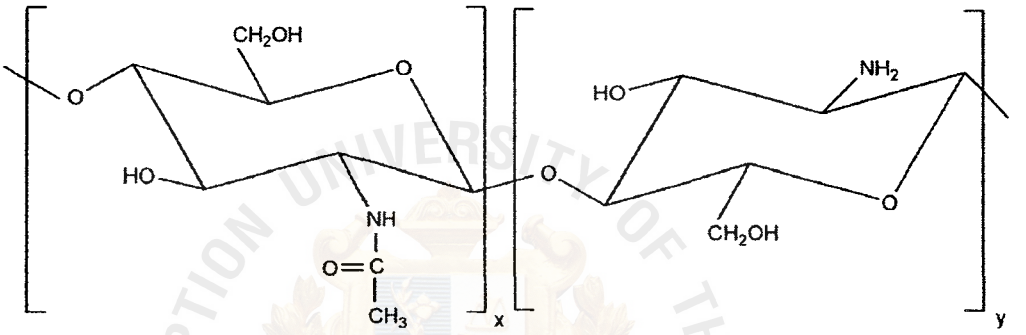

Figure 6: Chemical structure of chitosan (Alves and Manoa, 2008)

Chitosan films are extensively used in biomedical applications such as drug and gene delivery and wound dressing. In all these applications of chitosan, some of the most important factors to consider include the physicochemical and mechanical properties of chitosan. The amino- and hydroxyl groups on chitosan chains allow for relatively easy derivatization and immobilization of chitosan as compared to gelatin. Chitosan chains tend to have inter- and intra- molecular hydrogen bonding. Currently, research groups are looking into the possibility of improving the mechanical properties of chitosan by addition of cross linkers such as glutaraldehyde and genipin (Diop, 2009).

The permeability of sodium alginate capsules can be modified by placing the capsules into a solution of a polycation, such as chitosan. Chitosan is soluble in acidic conditions due to the free protonable amino groups present in the D-glucosamine units. It reacts with alginate to form a polyelectrolyte complex.

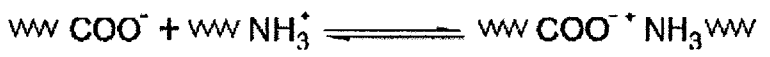

Figure 7: A polyelectrolyte complex of alginate and chitosan (Peniche et al., 2004)

As a result of this reaction, the alginate beads become covered by a chitosan shell. The thickness of this layer depends on the molecular weight of chitosan as well as on the pH and chitosan concentration of the solution (Peniche et al., 2004).

MATERIALS AND METHODS

1. Preparation of chitosan solution

Low-molecular-weight chitosan (0.8g; specification: low viscosity 14 mPa in 1% w/v solution; Fluka, Australia) was dissolved in 90 mL distilled water, acidified with 0.8 mL of glacial acetic acid to achieve a final concentration of 0.8% (w/v). The pH was then adjusted to between 5.7 and 6 by adding 1 M NaOH. The mixture was filtered through Whatman #4 filter paper and the volume adjusted to 100 mL.

2. Encapsulation of orange flavor and coating with chitosan

Orange flavor (specification: Dos. Approx. 0.4-0.6: 1000; Silesia, Singapore) was mixed with 20 mL alginate (CTi & SCIENCE, Thailand) solution containing 1% (w/w) xylitol (CTi & SCIENCE, Thailand) and 0.1% (w/w) Tween 80 and stirred using a mechanical stirrer at room temperature to form an oil-in-water emulsion. The concentration of orange flavor and alginate used in this experiment is shown in Table 2. The emulsion was dropped into 0.05M $CaCl_2$ mixed with chitosan solution through a syringe under gentle stirring. The beads were stand for 30 minutes for gelification. The chitosan-coated beads were washed with 10 mL of 1% (w/v) $CaCl_2$ to remove unbound chitosan, and kept in $CaCl_2$ solution at 4°C to avoid the loss of calcium.

3. Total oil extraction

The total oil content of the bead samples was analyzed by using the Rose-Gottlieb method. Ten grams of beads were blended with 10 mL 1% sodium citrate. The mixture was then transferred into a separatory funnel. Ammonia (1.25 mL), specific gravity 0.8974, was added. The mixture was mixed and shaken thoroughly. Ethyl alcohol (10 mL) was added and mixed. Diethyl ether (peroxide free) (25 mL) was added and shaken vigorously for 1 minute. The petroleum ether (boiling range 40-60 °C) (25 mL) was then added and shaken for 30 seconds. The mixture was left to stand until the clear upper ethereal layer had separated completely. The clear ethereal layer was transferred into a flask and the remaining solution was repeatedly extracted twice using 15 mL of each solvent every time. The ethereal extract was collected into the same flask. The ethereal extract was dried in an air oven at 100 °C for 2

hours, cooled in a desicator and weighted. The total oil was calculated based on the difference weight between the initial flask and the flask containing extracted oil.

4. Encapsulation efficiency

To monitor the encapsulation process, encapsulation efficiency was determined. It was defined as the percentage of total oil load to the amount of oil used at the beginning.

$$\textbf{Encapsulation efficiency (EE) (\%)} = \frac{\textbf{Weight of total oil load}}{\textbf{Amount of oil used at initial}} \times \mathbf{100}$$

5. Bead size measurement

The diameters of 120 randomly selected beads of each treatment were measured with a vernier caliper.

6. Experimental Design and Statistical analysis

A central composite experimental design and response surface methodology were used to study the effects of two factors on encapsulation efficiency (EE) of flavor encapsulation in gel matrices. The two investigated factors (independent variables) were alginate concentration (X_1, 1, 1.5 and 2% w/v) and orange flavor concentration (X_2, 1, 2 and 3% w/v). The analyzed dependent variables were the encapsulation efficiency (EE) and the mean bead size. Each independent variable was studied at three levels. Coded working levels were: minimum level (-1), maximum level (+1), and central level (0) (Table 1), where the central point was repeated six times, obtaining 14 experimental runs (Table 2).

The use of a response surface method experimental design permits the construction of second-order poly-nomial models that can describe quantitatively the linear, quadratic and interaction effects of the selected factors on the studied response variables. For two factors, the general model corresponds to the following equation:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{11}X_1^2 + b_{22}X_2^2 + b_3X_1X_2 \qquad (1)$$

In this equation, X_1 and X_2 are the independent variables (factors) and Y is the investigated dependent variable (response). Also, b_0 represents the arithmetic average of all quantitative outcomes of all the runs, b_1 and b_2 are related with the independent variables effect on the

response, b_{11} and b_{22} are two quadratic relationships and b_{12} represents the interaction effect between the two variables. A coefficient with a positive sign signifies a synergistic effect, whereas a negative sign stands for an antagonistic effect.

Table 1: Factors and their concentration in CCD

Variables	Code	Code Level			ΔX
		-1	0	1	
Alginate (%)	X_1	1	1.5	2	0.5
Flavor (%)	X_2	1	2	3	1

Table 2: Experimental design for concentration of alginate and flavor in CCD (2 factors)

Trial	Alginate	Flavor
1	-1 (1%)	-1 (1%)
2	1 (2%)	-1 (1%)
3	-1 (1%)	1 (3%)
4	1 (2%)	1 (3%)
5	0 (1.5%)	0 (2%)
6	0 (1.5%)	0 (2%)
7	-1.414 (0.79%)	0 (2%)
8	1.414 (2.21%)	0 (2%)
9	0 (1.5%)	-1.414 (0.59%)
10	0 (1.5%)	1.414 (3.41%)
11	0 (1.5%)	0 (2%)
12	0 (1.5%)	0 (2%)
13	0 (1.5%)	0 (2%)
14	0 (1.5%)	0 (2%)

RESULTS AND DISCUSSIONS

Encapsulation of orange flavor in alginate bead in this experiment aimed to optimize the retention of orange oil flavor. In order to form a bead, sodium alginate solution mixed with xylitol and orange flavor was extruded into calcium chloride solution by a syringe. A gel forms as the sodium ions (Na^+) were exchanged with calcium ions (Ca^{2+}) and the polymers become crosslinked, attaching them to each other at many points. This crosslinking creates a soft and flexible gel bead. The bead was also coated with chitosan which mixed in calcium chloride solution using one-stage encapsulation procedures. Coating with chitosan prevents diffusion of substance (Peniche et al., 2004). After the beads were left hardening for 30 minutes, they were kept in $CaCl_2$ solution at 4°C to avoid the loss of calcium. Then the total oil extraction was performed to determine the encapsulation efficiencies.

As referred, the independent variables studied in this work were alginate concentration (X_1) and orange flavor concentration (X_2), while the analyzed response variables were the encapsulation efficiency (Y_1) and the mean bead size (Y_2). The results of 14 trials have shown in Table 4. The polynomial models equations of each response variables were generated from multiple regression analysis. The obtained models to describe variables were selected at the 95% confidential level.

Table 3: Encapsulation efficiency and bead size of orange flavor and xylitol encapsulated in alginate beads coated with chitosan

Trial	Encapsulation efficiency (%)	Average bead diameter (mm)
1 (Alginate 1%, flavor 1%)	55.0	3.11
2 (Alginate 2%, flavor 1%)	34.3	3.25
3 (Alginate 1%, flavor 3%)	33.1	2.97
4 (Alginate 2%, flavor 3%)	14.6	3.24
5 (Alginate 1.5%, flavor 2%)	41.6	3.40
6 (Alginate 1.5%, flavor 2%)	34.7	3.32
7 (Alginate 0.79%, flavor 2%)	33.9	3.24
8 (Alginate 2.21%, flavor 2%)	13.0	3.09
9 (Alginate 1.5%, flavor 0.59%)	64.0	3.25

10 (Alginate 1.5%, flavor 3.41%)	4.2	3.23
11 (Alginate 1.5%, flavor 2%)	20.0	3.23
12 (Alginate 1.5%, flavor 2%)	35.3	3.32
13 (Alginate 1.5%, flavor 2%)	21.8	3.10
14 (Alginate 1.5%, flavor 2%)	37.0	3.18

1. Encapsulation efficiency (EE)

From 14 trials, the encapsulation efficiency (EE) ranged from 4.2% (Alginate 1.5%, orange flavor 3.41%) to 64.0% (Alginate 1.5%, flavor 0.59%). After analysis of variance (ANOVA), regression equations were used as a model to predict encapsulation efficiency obtained. Encapsulation efficiency can be predicted from the model:

$$Y_1 = 31.729 - 8.585X_1 - 15.776X_2 - 2.766X_1^2 + 0.530X_1X_2 + 2.563X_2^2 \quad (2)$$

When X_1 = Concentration of alginate (%)

X_2 = Concentration of flavor (%)

Y_1 = Encapsulation efficiency (%)

This obtained result for EE were fitted by a quadratic model from multiple regression using enters method. Regression coefficient (R^2) was calculated as 0.798, indicating that 79.8 % of data was compatible with experimental data in the model predictions.

Since X_1 and X_2 were significant ($p<0.05$) model terms, the obtained regression coefficients showed that EE was significantly ($p<0.05$) negative. EE was reduced when the concentrations either of alginate or orange flavor increased. As the increase in the sodium alginate concentration resulted in more dense structure, higher alginate concentration decreases the pore size in the bead (B. and L., 2011). Then the oil entrapped in these pores also decrease. $X_1^2$and X_2^2 were also kept in the model as well as the interaction effect between variables (X_1X_2), which were not statistically different ($p>0.05$), only to support the hierarchy of the polynomial equation. The relationship between EE and the two independent variables is illustrated using a response surface plot in Figure 8. The optimal value of each factor, leading to reach maximal response levels could be determined.

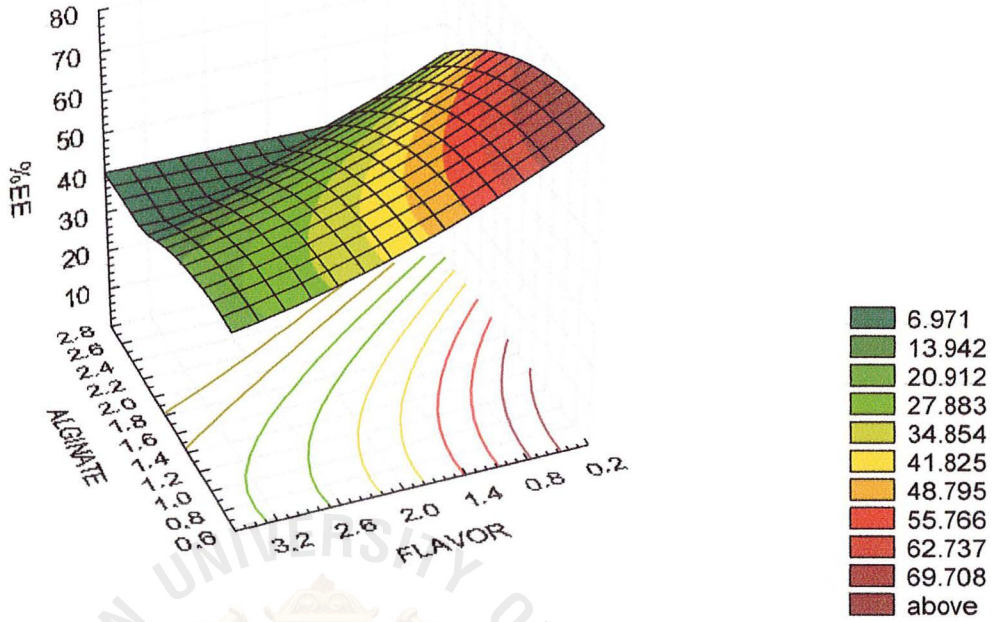

Figure 8: Contour graph and response surface plotted from concentration of alginate and flavor (%) and encapsulation efficiency (%)

To fit the model with the experimental data, five alginate concentrations and orange flavor concentrations in the area that provided the highest EE were chosen and the results are shown in Table 4 and Figure 9.

Table 4: Encapsulation efficiency from chosen five alginate concentrations and orange flavor concentrations

Trial	Average encapsulation efficiency (%)	Theoretical encapsulation efficiency (%)
1 (Alginate 0.6%, Flavor 0.2%)	91.6^{a}	22.6
2 (Alginate 1 %, flavor 0.2%)	62.4^{b}	17.4
3 (Alginate 0.6%, flavor 0.45%)	54.8^{bc}	19.1
4 (Alginate 1%, flavor 0.45%)	12.2^{d}	15.1
5 (Alginate 0.8%, flavor 0.35%)	37.6^{c}	18.0

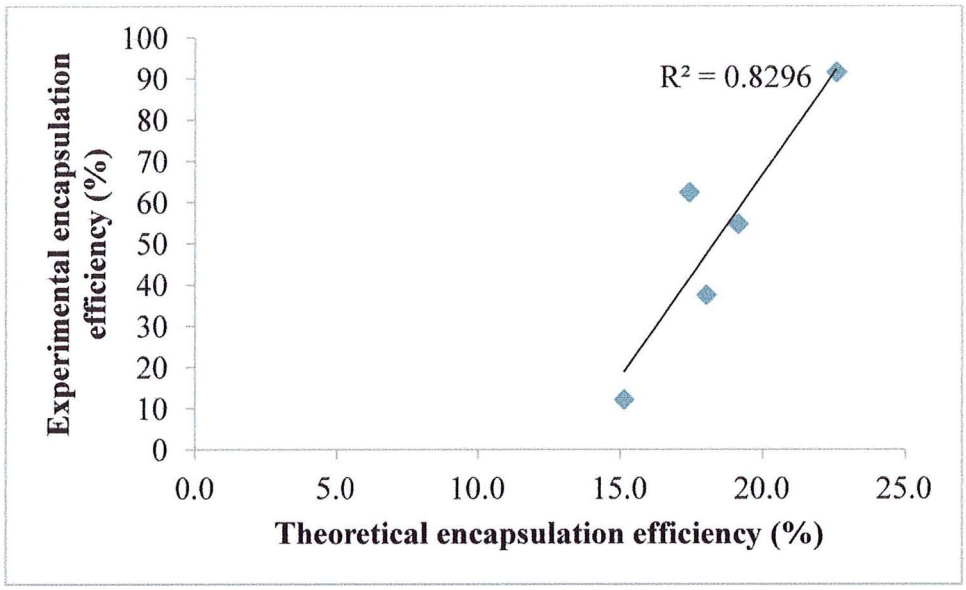

Figure 9: Correlation between theoretical and experimental data of encapsulation efficiency (EE)

From t-test of experimental encapsulation efficiency and theoretical encapsulation efficiency, there was a significant different between the data in trials 1, 2 and 3. Since the equation used to predict the theoretical encapsulation efficiency was obtained from the first 14 trials, the result that using different concentration of alginate and orange flavor from those 14 trials might be different. It was recognized that square of correlation coefficient (R^2) between theoretical and experimental data of encapsulation efficiency (EE) was as high as 0.8296, indicating that this model was fitted with this experiment. The fitted model showed that the alginate concentration and orange flavor concentration were the factors that had a higher impact in the EE, with a negative effect. It was also recognized that encapsulation of orange oil flavor and xylitol using 0.6% alginate and 0.2% orange oil flavor provided the highest encapsulation efficiency (91.6%).

2. Bead size measurement

From 14 trials, bead size ranged from 2.97 mm (Alginate 1%, flavor 3%) to 3.40 mm (Alginate 1.5%, flavor 2%) After analysis of variance (ANOVA), regression equations were used as a model to predict bead size obtained from the model:

$$Y_2 = 3.258 + 0.025X_1 - 0.022X_2 - 0.062X_1^2 + 0.032\,X_1X_2 - 0.024\,X_2^2 \qquad (3)$$

When X_1 = Concentration of alginate (%)

X_2 = Concentration of flavor (%)

Y_2 = Bead diameter (mm)

This obtained result for bead size showed that it did not fitted with a quadratic model from multiple regression using enters method. Regression coefficient (R^2) was calculated as 0.273, indicating that only 27.3% of data was compatible with experimental data in the model predictions. Moreover, all the variables were not significant ($p>0.05$). Therefore, this model may not suitable to predict the bead size.

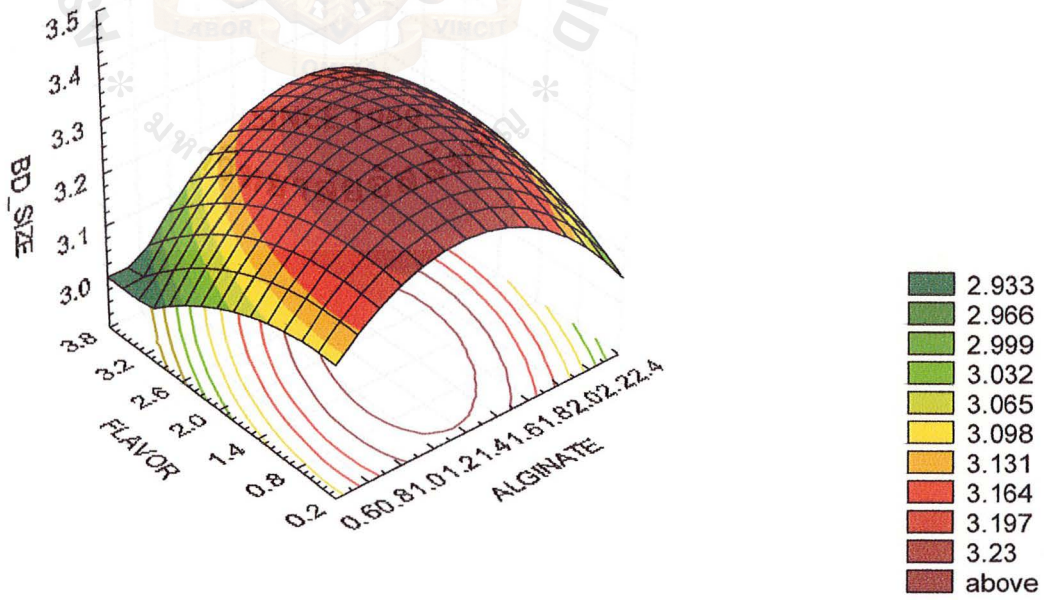

Figure 10: Contour graph and response surface plotted from concentration of alginate and flavor (%) and bead diameter (mm)

Table 5: Average bead diameter from chosen five alginate concentrations and orange flavor concentrations

Trial	Average bead diameter (mm)	Theoretical bead diameter (mm)
1 (Alginate 0.6%, Flavor 0.2%)	3.06^{b}	3.25
2 (Alginate 1 %, flavor 0.2%)	3.21^{a}	3.22
3 (Alginate 0.6%, flavor 0.45%)	3.07^{ab}	3.24
4 (Alginate 1%, flavor 0.45%)	3.15^{ab}	3.22
5 (Alginate 0.8%, flavor 0.35%)	3.19^{ab}	3.24

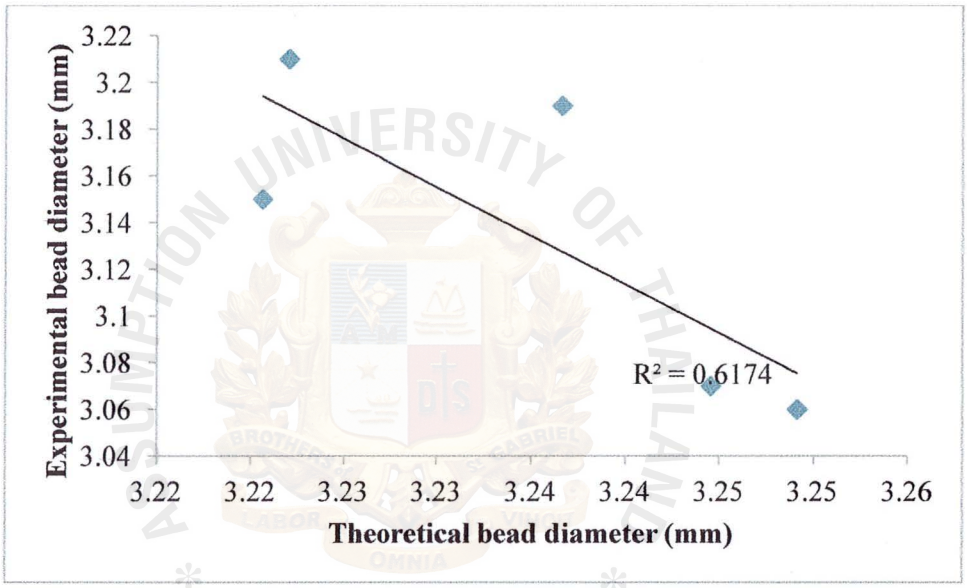

Figure 11: Correlation between theoretical and experimental data of bead size

From the chosen five alginate concentrations and orange flavor concentrations that provided the highest EE, the average bead diameters ranged from 3.06 mm to 3.21 mm which was shown in Table 5. The bead size data was plotted to determine R^2 between theoretical and experimental data. R^2 obtained was 0.6174, indicating that the model was not much fitted with this experiment. Therefore, this model may not suitable to predict the bead size.

Moreover, alginate concentrations and orange flavor concentrations did not affect to the size of beads in this experiment.

CONCLUSION

Encapsulation of 0.2% orange flavor and 1% xylitol using 0.6% alginate and 1% chitosan provided the highest encapsulation efficiency as 91.6%. The changes in alginate and orange flavor concentrations negatively influenced to the encapsulation efficiency (EE), which could be predicted by using this model equation:

$Y_1 = 31.729 - 8.585X_1 - 15.776X_2 - 2.766X_1^2 + 0.530X_1 X_2 + 2.563 X_2^2$,whereas the diameter of beads was not affected and varied from 2.97 mm to 3.4 mm.

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APPENDIXES

A. 14 trials in CCD

Encapsulation efficiency

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	AF, FF, Flavor, Alginate, AA[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: EE

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.893[a]	.798	.671	9.240333

a. Predictors: (Constant), AF, FF, Flavor, Alginate, AA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	2695.123	5	539.025	6.313	.012[a]
	Residual	683.070	8	85.384		
	Total	3378.193	13			

a. Predictors: (Constant), AF, FF, Flavor, Alginate, AA

b. Dependent Variable: EE

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	31.729	3.772		8.411	.000
	Alginate	-8.585	3.267	-.418	-2.628	.030
	Flavor	-15.776	3.267	-.768	-4.829	.001
	AA	-2.766	3.401	-.130	-.813	.440
	FF	2.563	3.401	.120	.754	.473
	AF	.530	4.620	.018	.115	.911

a. Dependent Variable: EE

Bead size

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	AF, FF, Flavor, Alginate, AA[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: SIZE

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.523[a]	.273	-.181	.120989

a. Predictors: (Constant), AF, FF, Flavor, Alginate, AA

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.044	5	.009	.601	.702[a]
	Residual	.117	8	.015		
	Total	.161	13			

a. Predictors: (Constant), AF, FF, Flavor, Alginate, AA

b. Dependent Variable: SIZE

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.258	.049		65.967	.000
	Alginate	.025	.043	.174	.578	.579
	Flavor	-.022	.043	-.157	-.521	.616
	AA	-.062	.045	-.419	-1.385	.203
	FF	-.024	.045	-.164	-.543	.602
	AF	.032	.060	.162	.537	.606

a. Dependent Variable: SIZE

B. 5 alginate concentration and orange flavor concentration chosen from the contour graph in the area provide highest encapsulation efficiency

% Encapsulation efficiency

Randomized complete block with one factors

The GLM Procedure

Class Level Information

Class	Levels	Values
Trial	5	1 2 3 4 5
Rep	3	1 2 3

Number of Observations Read	15
Number of Observations Used	15

The GLM Procedure

Dependent Variable: percentEE

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	10587.46629	1764.57771	20.01	0.0002
Error	8	705.59072	88.19884		
Corrected Total	14	11293.05701			

R-Square	Coeff Var	Root MSE	percentEE Mean
0.937520	18.15542	9.391424	51.72793

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trial	4	10429.72038	2607.43010	29.56	<.0001
Rep	2	157.74591	78.87295	0.89	0.4462

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trial	4	10429.72038	2607.43010	29.56	<.0001
Rep	2	157.74591	78.87295	0.89	0.4462

LSD test

The GLM Procedure

t Tests (LSD) for percentEE

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	88.19884
Critical Value of t	2.30600
Least Significant Difference	17.683

Means with the same letter are not significantly different.

t Grouping	Mean	N	Trial
A	91.637	3	1
B	62.371	3	2
B			
C B	54.808	3	3
C			
C	37.619	3	5
D	12.205	3	4

The GLM Procedure

t Tests (LSD) for percentEE

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	88.19884
Critical Value of t	2.30600
Least Significant Difference	13.697

Means with the same letter are not significantly different.

t Grouping	Mean	N	Rep
A	56.261	5	1
A			
A	50.064	5	2
A			
A	48.859	5	3

t-test for theoretical and experimental data

Trial 1

The TTEST Procedure

Variable: EET1

N	Mean	Std Dev	Std Err	Minimum	Maximum
3	91.6366	0.8656	0.4998	90.6713	92.3439

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
91.6366	89.4862	93.7870	0.8656	0.4507	5.4404

DF	t Value	Pr > |t|
2	138.13	<.0001

Trial 2

The TTEST Procedure

Variable: EET2

N	Mean	Std Dev	Std Err	Minimum	Maximum
3	62.3706	8.8397	5.1036	54.2265	71.7714

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
62.3706	40.4116	84.3296	8.8397	4.6025	55.5552

DF	t Value	Pr > |t|
2	8.81	0.0126

Trial 3

The TTEST Procedure

Variable: EET3

N	Mean	Std Dev	Std Err	Minimum	Maximum
3	54.8083	12.6952	7.3296	41.4614	66.7318

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
54.8083	23.2717	86.3449	12.6952	6.6099	79.7859

DF	t Value	Pr > |t|
2	4.87	0.0396

Trial 4

The TTEST Procedure

Variable: EET4

N	Mean	Std Dev	Std Err	Minimum	Maximum
3	12.2052	9.2622	5.3475	2.8991	21.4228

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
12.2052	-10.8033	35.2137	9.2622	4.8224	58.2103

DF	t Value	Pr > |t|
2	-0.54	0.6425

Trial 5

The TTEST Procedure

Variable: EET5

N	Mean	Std Dev	Std Err	Minimum	Maximum
3	37.6189	10.2870	5.9392	29.6488	49.2316

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
37.6189	12.0645	63.1733	10.2870	5.3560	64.6512

DF	t Value	Pr > |t|
2	3.30	0.080

Bead size

Randomized complete block with one factors

The GLM Procedure

Class Level Information

Class	Levels	Values
Trial	5	1 2 3 4 5
Rep	3	1 2 3

Number of Observations Read	15
Number of Observations Used	15

The GLM Procedure

Dependent Variable: beadsize

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	0.06776000	0.01129333	1.88	0.2002
Error	8	0.04801333	0.00600167		
Corrected Total	14	0.11577333			

R-Square	Coeff Var	Root MSE	beadsize Mean
0.585282	2.471409	0.077470	3.134667

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Trial	4	0.05870667	0.01467667	2.45	0.1311
Rep	2	0.00905333	0.00452667	0.75	0.5011

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trial	4	0.05870667	0.01467667	2.45	0.1311
Rep	2	0.00905333	0.00452667	0.75	0.5011

LSD test

The GLM Procedure

t Tests (LSD) for beadsize

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.006002
Critical Value of t	2.30600
Least Significant Difference	0.1459

Means with the same letter are not significantly different.

t Grouping	Mean	N	Trial
A	3.20667	3	2
A			
B A	3.19333	3	5
B A			
B A	3.15000	3	4
B A			
B A	3.06667	3	3
B			
B	3.05667	3	1

The GLM Procedure

t Tests (LSD) for beadsize

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	8
Error Mean Square	0.006002
Critical Value of t	2.30600
Least Significant Difference	0.113

Means with the same letter are not significantly different.

t Grouping	Mean	N	Rep
A	3.15400	5	2
A			
A	3.15000	5	1
A			
A	3.10000	5	3