ENCAPSULATION OF PALM OLEIN WITHIN CALCIUM-ALGINATE BEADS

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**SCHOOL OF ENGINEERING AND
INFORMATION TECHNOLOGY
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ABSTRACT

ENCAPSULATION OF PALM OLEIN WITHIN CALCIUM ALGINATE BEADS

Encapsulation of palm oil in Ca-alginate beads using high oil loading has been studied. It was found that the alginate concentration, oil volume fraction and alginate type have significant influence on the encapsulation efficiency and bead properties. The alginate-oil emulsion, comprising oil loading up to 30 vol% (equivalent to oil-to-wall weight ratio up to15 g/g) and 25 g/l of high G alginate solution, was found to be stable and resulted in encapsulation efficiency of 90% before drying. The oil-loaded wet beads were spherical. The sphericity values obtained was less than 2mm. The encapsulation efficiency was dependent on the degree of cross-linking at the surface of the extruded emulsion droplet as well as the emulsion stability. The oil extraction profile can be related to the structural properties of dried beads, which are dependent on the drying method and the oil loading. The overall encapsulation efficiency after freeze-drying and oven-drying were 90% and 79%, respectively, with oil content of over 85 wt% for both types of dried beads. The freeze-dried beads were non-oily and free flowing whereas the oven-dried beads were oily and sticky. The results of this work were compared with existing literature. The challenges and opportunities of the methods were discussed.

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LIST OF ABBREVATIONS

$O_{S,1}$	Surface oil, g
$O_{S,2}$	Oil on Petri dish, g
O_S	Total surface oil, g
O_T	Total oil, g
O_E	Encapsulated oil, g
O_E	Encapsulated oil, g
D	Diameter, mm
D_{max}	Maximum diameter, mm
D_{min}	Minimum diameter, mm
ρ	Density, g/ml
t	Thickness, µm
EE	Encapsulation Efficiency
ES	Emulsion Stability

LIST OF SYMBOLS

>	Greater than
<	Less than
≥	Greater than or equal to
≤	Less than or equal to
=	Equal to
%	Percentage
°C	Degree Celsius

CHAPTER 1

INTRODUCTION

1.1 Research Background

Encapsulation is a well-known technique for protecting the active ingredients, which are susceptible to oxygen, by usage of bio degradable materials, e.g. alginate. These biodegradable materials act as a protective wall material to the sensitive ingredients or core materials. Core materials can thereby be protected from deteriorative reactions or adverse environmental conditions, thus enhancing their stability and range of applications.

Encapsulation is used in the food and beverage industry to control the release of the sensitive ingredients or core materials, lower flavor loss during the product shelf life, extend the flavor perception over a longer period of time and enhance the ingredient bioavailability and efficacy (Klaypradit *et al.,*2008, Shefer and Shefer, 2003; Berry, 2004). Encapsulating agents used in food industry include carbohydrates, gums, lipids and proteins (Jackson and Lee, 1991; Shahidi and Han, 1993; Gibbs *et al.*, 1999).

1.1.1 Alginate as an Encapsulating Material

Alginate has an excellent biocompatibility and biodegradability (Herrero *et al.,* 2005). The most important property of alginates is their ability to form gels by reaction with divalent cations such as calcium or barium by binding between guluronic acid blocks in alginate and the divalent cations. These gels are similar to solids because they retain their shape and resist stress. This process of gelation is an almost instantaneous and irreversible process, which is governed by the relative rate of diffusion of barium ions and polymer molecules into the gelling zone (Herrero *et al.,* 2005).

Alginates, which are natural accruing polymers, have been used for several decades in the food and pharmaceutical industries as emulsifying, thickening, film forming and gelling agent (Dettmar.W *et al.*,2009; Onsoyen, 1996). Within the biomedical field alginates are now well known as immobilization materials for cells, tissue or macromolecules.

However, since alginates are a heterogeneous group of polymers, with a wide range of functional properties, their success will rely on an appropriate choice of materials and methodology for each application. Alginates in general fulfill the requirements as additives in food and pharmaceutical products. (Onsoyen, 1996)

Though there are many other encapsulating materials available, the purpose of using alginate is because, alginate is readily available, it can be easily prepared for lab scale purposes and compared to all the other encapsulating materials, alginate is low in cost.

The encapsulation of oils involves the emulsification or dispersion of the components in an aqueous alginate solution. This can be performed by external gelation of the aqueous emulsion, which consists of alginate solution & oil, according to the desired concentration, where the hardened wall material (hardens after the drying process) that is contained in the aqueous emulsion entraps the dispersed oil droplets.

1.2 Research Problem

The world food market is currently focusing on foods that provide not only nutritive values but also health benefits to human. Many food active components such as antioxidants, nutraceutics and aromas, exist in oil form which may be easily oxidized. Examples of nutritive oils are palm oil (tocotrienols, carotenoids), fish oils, primrose oil, etc. and they have to be protected against the environment to avoid oil oxidation. Another reason for encapsulating nutritive oils is such oils are not palatable to be consumed directly. Hence, implementation of encapsulation techniques would be necessary, so that it promotes controlled release of nutritive components & also makes it easier for handling purposes (i.e, liquids can be converted to solids).

The most commonly used method to encapsulate oil is by using the spray-drying method. The encapsulation wall materials usually are carbohydrates, proteins and gums. The oil-to-wall weight ratios normally ranged from 0.1 to 1.0, although 0.2 to 0.5 are more common (Tan *et al.,* 2004). The encapsulated oil content is in the range of 20% to 30% of the total weight of the final dried products. In some applications, there is a need of the product containing higher oil content. Peniche *et al.,* (2005) has shown that it is possible to produce dried encapsulated product of high oil content, of 65-70 (wt %), by encapsulating the oil within ca-alginate beads coated with chitosan. However, they did not attempt to further increase the oil content in the ca-alginate beads.

The overall aim of this study was to encapsulate palm oil in ca-alginate beads using high oil loading, in order to produce dried beads containing high oil content. Palm oil was chosen because it has a greater content of nutritive components such as tocotrienols, carotenoids etc. These components require protection against external environmental factors (ex: oxygen). An alginate-oil emulsion was prepared and dropped into a gelling bath to produce the oil loaded ca-alginate beads. The wet beads were then dried by freeze-drying and oven-drying. The influence of process parameters such as alginate type and concentration, oil volume fraction, emulsion stability and drying methods on the encapsulation efficiency and bead properties were evaluated in this study.

1.3 Research Objectives

1.3.1 Overall Objective

To encapsulate palm oil in Ca-Alginate beads using high oil-loading, in order to produce oil-loaded dried beads & attain maximum encapsulation efficiency (EE).

1.3.2 Specific Objectives

i. To study the effect of process parameters on encapsulation efficiency & emulsion stability before drying.
ii. To characterize the size and shape of oil-loaded wet beads.
iii. To develop the oil extraction profile of dried ca-alginate beads.
iv. To study the effect of drying on encapsulation efficiency.

v. To conduct FTIR studies before & after encapsulation of the palm olein within Ca-alginate beads.

vi. To compare the results to previous studies.

1.4 Significance of This Study

There are many studies on the encapsulation of oils or hydrophobic compounds. In order to narrow down the scope of comparison, studies were conducted on encapsulation using alginate or gel-beads system since they are closest to the scope of this study, and the microencapsulation studies using spray-drying because of its popularity.

However, the oil encapsulation by gel-beads system still has its distinct advantages. For example, its high process yield could be an advantage for encapsulating expensive oil. Also, the process could preserve volatile or easily oxidized compounds since the encapsulation process is normally carried out at room temperature without the use of air.

This particular study describes the materials and methods to produce Ca-alginate beads of high oil content, which open up opportunities for development of new products. It also provides a basic understanding on the relationships between process parameters and the product quality or process performance.

1.5 Thesis Organization

Chapter 1 was devoted to the introduction of the thesis topic. The introduction covers the research challenges to implement extrusion dripping method for the encapsulation of the palm oil within Ca-alginate beads.

Chapter 2 presents the literature review on the production of oil encapsulated Ca-alginate particles. The review included an introduction to alginate, oil encapsulated Ca-alginate particles production methods, physical properties of alginate solution, shape and size analysis of oil loaded Ca-alginate particles. In addition, the quantification methods for determination of the encapsulation efficiency, physical properties of alginate solution and the previous studies on the encapsulation of oils by usage of different techniques were also reviewed.

Chapter 3 describes the extrusion dripping system set-up and materials used for the production of the oil loaded Ca-alginate particles. The methods used to determine the emulsion stability of the alginate-oil emulsion, physical properties of Na-alginate solution, encapsulation efficiency were also described in this chapter. Furthermore, the imaging system for the shape and size analysis of the oil encapsulated Ca-alginate beads was introduced.

Chapter 4 reports and discusses the process parameters (emulsion stability, alginate concentration, oil loading, alginate type, drying techniques, size & shape of the beads) that influence the encapsulation efficiency of the palm oil within Ca-alginate beads. The encapsulation efficiency was determined before & after drying as well by usage of the mathematical formulae. The oil extraction profile was developed for the dried oil loaded Ca-alginate beads at different time intervals.

Chapter 5 concludes the findings of this thesis. The outcome of this thesis was highlighted. The future works to extend the application of the encapsulation of oils were also mentioned.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The encapsulation of oils within Ca-alginate beads is currently being applied in bio-encapsulation industries. In this chapter, the details about the production of oil loaded Ca-Alginate beads will be thoroughly reviewed. The review included introduction to alginate, oil loaded Ca-Alginate beads production methods & various techniques on encapsulation of oils. In addition, the methods for the determination of the encapsulation efficiency and the factors that would influence the encapsulation process and also the past studies on the encapsulation will also be reviewed.

2.2 Definition of Encapsulation

Encapsulation is a process in which sensitive ingredients are encapsulated in encapsulating agents or wall materials such as bio polymers, to protect them against oxygen, moisture, light & also allows a controlled release of the encapsulated ingredients. The simplest microcapsule consists of a core surrounded by a wall or barrier. The wall materials protect the sensitive ingredients or core materials from environmental conditions and deteriorative reactions to enhance their stability.

Basically, there are two types of immobilization materials, which are the natural polymers and synthetic polymers. The natural polymers are attractive due to their abundance and apparent biocompatibility. They provide a wide range of physical properties that offer unique characteristics for encapsulation technologies. Examples of natural polymers consist of alginate, collagen, chitosan, agarose, pectin, k-carageenan and etc. Synthetic polymers are widely used due to the high degree of control over the structure, and resultant properties that is possible with this class of materials. The synthetic polymers are poly(glycolide) (PGA), poly(lactide) (PLA), poly (lactide-co-glycolide) (PLGA), poly anhydrides, poly(ethylene glycol)(PEG) and etc (Riddle and Mooney, 2004)

2.2.1 Purpose of Encapsulation

Encapsulation offers distinct advantages as the core containing the biological materials is completely surrounded by a semi-permeable membrane, acting as a barrier (Marison et.al., 2004). The purpose of encapsulation can be categorized into four main categories:

- Protection
- Controlled release
- Easier handling and scale-up
- Higher yield

a) Protection

Since the materials are completely surrounded by a layer of coating, it completely protects them from environment and allows selective transfer of compounds in either direction. (Marison *et al.,* 2004).

For bioprocesses that involve agitation, the biological materials of the immobilization matrix are protected from the shear forces in the medium necessary for agitation and sparging (Muralidhar *et al.,* 2001). Therefore, there is no danger of the core substances being washed out during operation. In addition, encapsulated materials can be protected from chemical and physical degradation resulting in prolonged cell stability and shelf life. Due to their protection, the cells can tolerate higher level of mechanical stress, which will extend the range of reactor suitable for fermentation. The deliverance of encapsulated nutraceutics in foods are necessary since they are fragile and need protection during storage, processing and often during gastro-transit (Poncelet and Teunou, 2002).

b) Controlled release

The demand for nutritional and value added food have been on the rise. Nutraceutics include a larger range of molecules types such as inorganic and organic salts, active molecules (vitamins, fatty acid), polymers (fibers, prebiotic, proteins), enzymes, plants and yeast extracts and biological cells (probiotics) (Poncelet and Teunou, 2002).

Nutraceutics components may interact with other food ingredients, so these components have the necessity to be protected from other substances. They are added at a very low concentration in food and acquire a form easily and reproducibly dispersible in a larger system. They have to be released at the right place in body and even performed to increase their absorption in the intestine.

c) Easier handling and process scale-up

The process of encapsulation makes it easy for handling purposes and to do scale up for large scale production. Since the stability and mechanical strength of the matrix were significantly enhanced, reusability of the immobilized biocatalyst was possible (Chen *et al.,* 2005). This process (encapsulation) made it easier to retrieve the capsules or beads for further usage especially in fermentation processes.

Whereas the ability of the matrixes to degrade ensured that the rate of tissues regeneration and the rate of biodegradation were comparable so that the scaffold is completely replaced and integrated into the host tissue. (Riddle and Mooney, 2004).

d) Higher yield

In bioprocess, many ways have been developed and implemented to improve the productivity or yield of the process. With encapsulation, continuous operation of the system at high dilution rates can be carried out with no danger of the core substances being washed out, resulting in high localized biocatalyst densities. As the number of viable biocatalyst per unit volume increased, a faster reaction rate at high density can be achieved. Therefore, higher yield of the product can be obtained (Muralidhar *et al.,* 2001).

2.2.2 Applications of Encapsulation

Microcapsules, which consist of a polymeric shell and an encapsulated component, are utilized in a diverse range of fields, including the chemical, pharmaceutical, cosmetics and food industries. In many of these applications, the microcapsules interact with or bind to a surface in order to carry out their desired function, such as release of the encapsulated components to a targeted site. In addition to interacting with a surface the microcapsules can interact with other surface localized capsules. In the case of the pharmaceutical or chemical applications, the microcapsules typically also interact with a flowing fluid, i.e., the surrounding blood or the imposed processing conditions. In order to optimize the dispersion or control the aggregation of the microcapsules on the substrates, it is vital to understand how the properties of the substrates, the capsules and the fluid flow contribute to the interactions within this complex system (Alexander *et al.,* 2006).

In addition to the applications cited above, microcapsules could also be harnessed in creating an array of maneuverable micro reaction vessels. Microcapsule could be potentially utilized as a reaction flask and two or more of these capsules could be driven to move to a specified site on a substrate, where they could merge and exchange contents in order to carry out micro scale analyses or fabrication (Alexander *et al.,* 2006).

The encapsulation of human, animal, plant cells, microbes, enzymes and drugs or other into microbes may be performed for different reasons, the encapsulated items have to be protected from environmental influences, e.g. immune system of a patient, or has to resist shear forces in a bioreactor or has to be protected against oxidation (Heizelmann *et al.,* 2000).

Encapsulation is considered as a powerful method of immobilization. There are many examples of application of this technique in different fields (Gouin, 2004):

- Soil inoculation is often unsuccessful because cells are washed out by rain. Cell encapsulation allows the maintenance of continuous inoculation and reaches ten times higher cell concentrations
- Plant cell cultures allow production of different metabolites used for medical, pharmacological and cosmetic purposes. Cell immobilization improves the efficiency of the cultures by imitating cell natural environment.
- Immobilization seems to be the technique of choice in many industrial processes in food and especially in beverage production. The technologies such as beer, wine and vinegar already experienced some immobilization approaches traditionally with adhesion culture (i.e. acetobacter in vinegar

production) and a modern approach with entrapment of yeast biomass (i.e. sparkling wine)

- To avoid wash-out of the biological catalyst from the reactor that applied in ethanol and solvent production and sugar conversion.
- Encapsulation is also applied in waste water treatment.

2.2.3 Methods of Encapsulation

Numerous methods have been developed for the manufacture of microcapsules, and there are many techniques that can be used to encapsulate food ingredients, enzymes, cells, or other materials (Gibbs *et al.*, 1999). Over the years, oils have been encapsulated by techniques like complex-coacervation, extrusion, interfacial polymerization, emulsification/internal gelation and many others. The simplest of all is the extrusion method.

a) Simple extrusion

Extrusion microencapsulation has been used almost exclusively for the encapsulation of volatile and unstable flavors in glassy carbohydrate matrices. The main advantage of this process is the very long shelf life imparted to normally oxidation-prone flavour compounds, such as citrus oils, because atmosphere gases diffuse very slowly through the hydrophilic glassy matrix, thus providing an almost impermeable barrier against oxygen (Gouin, 2004). The basic idea behind encapsulation using extrusion is to create a molten mass in which the active agents, *i.e.* either liquids or solids, are dispersed. Upon cooling, this mass will solidify, thereby entrapping the active components.

Most extrusion-encapsulation processes are dealing with carbohydrates as matrix material. The success of the particular matrix depends on its functionalities (*e.g.* emulsifying properties), on its ability to create a plastic flow, and on interactions that take place between the matrix and the ingredients (Vingerhoeds and Harmsen, 2004).

b) Complex coacervation

Complex coacervation occurs through the interaction of two oppositely charged