กลไกการสูญเสียสารหอมระเหยหลายชนิดในกระบวนการไมโครเอนแคปซูเลชันด้วยวิธีอบแห้งแบบพ่นฝอย

นางสาวไพลิน เพ็ญบัณฑิตกุล

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรดุษฎีบัณฑิต
สาขาวิชาวิศวกรรมเคมี ภาควิชาวิศวกรรมเคมี
คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

LOSS MECHANISM OF MULTI-FLAVOR COMPOUNDS IN MICROENCAPSULATION PROCESS BY SPRAY DRYING

Ms. Pailin Penbunditkul

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Engineering Program in Chemical Engineering
Department of Chemical Engineering
Faculty of Engineering
Chulalongkorn University
Academic Year 2011

Thesis Title	LOSS MECHANISM OF MULTI-FLAVOR COMPOUNDS IN MICROENCAPSULATION PROCESS BY SPRAY DRYING
By	Miss Pailin Penbunditkul
Field of Study	Chemical Engineering
Thesis Advisor	Associate Professor Tawatchai Charinpanitkul, D.Eng.
Thesis Co-advisor	Professor Hidefumi Yoshii, Ph.D.
Thesis Co-advisor	Assistant Professor Apinan Soottitantawat, Ph.D.

Accepted by the Faculty of Engineering, Chulalongkorn University in Partial Fulfillment of the Requirements for the Doctoral Degree

…………………………………………….… Dean of the Faculty of Engineering
(Associate Professor Boonsom Lerdhirunwong, Dr.Ing.)

THESIS COMMITTEE

……………………………………………….. Chairman
(Professor Suttichai Assabumrungrat, Ph.D.)

……………………………………….……. Thesis Advisor
(Associate Professor Tawatchai Charinpanitkul, D.Eng.)

……………………………………………….. Thesis Co-Advisor
(Professor Hidefumi Yoshii, Ph.D.)

……………………………………………….. Thesis Co-Advisor
(Assistant Professor Apinan Soottitantawat, Ph.D.)

……………………………………………….. Examiner
(Assistant Professor Amornchai Arpronwichanop, D.Eng.)

……………………………………………….. Examiner
(Assistant Professor Varong Pavarajarn, Ph.D.)

……………………………………………….. External Examiner
(Emeritus Professor Wiwut Tanthapanichakoon, Ph.D.)

……………………………………………….. External Examiner
(Uracha Ruktanonchai, Ph.D.)

ไพลิน เพ็ญบัณฑิตกุล : กลไกการสูญเสียสารหอมระเหยหลายชนิดในกระบวนการไมโครเอนแคปซูเลชันด้วยวิธีอบแห้งแบบพ่นฝอย. (LOSS MECHANISM OF MULTI-FLAVOR COMPOUNDS IN MICROENCAPSULATION PROCESS BY SPRAY DRYING) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร.ธวัชชัย ชรินพาณิชกุล, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ.ดร.ฮิเดฟูมิ โยชิอิ, ผศ.ดร.อภินันท์ สุทธิธารธวัช, 127 หน้า.

การกักเก็บกลิ่นจากสารสกัดสมุนไพรไทย มุ่งเน้นสารออกฤทธิ์ที่มีกลิ่นในกลุ่มของน้ำมัน ซึ่งโดยทั่วไปแล้วสารที่มีกลิ่นจะมีคุณสมบัติในการระเหยได้ง่าย และมีความคงตัวที่ต่ำโดยเฉพาะในสภาวะที่มีแสง อากาศ ความชื้น รวมถึงความร้อน ในงานวิจัยนี้ได้พัฒนาผงอนุภาคกักเก็บสารสกัดที่มีกลิ่นในสารกลุ่มกัมอะราบิค และแป้งดัดแปร โดยกระบวนการอบแห้งแบบพ่นฝอย ในงานวิจัยนี้เลือกน้ำมันผิวมะกรูดเป็นน้ำมันหอมระเหยซึ่งน้ำมันดังกล่าวมีสารสำคัญหลายชนิด ได้แก่ ดี-ลิโมนีน, ลินาลูล และ ลินาลิลอะซิเตท เพื่อศึกษาหากลไกการสูญเสียสารหอมระเหยหลายชนิดในกระบวนการกักเก็บระดับไมโครด้วยกระบวนการอบแห้งแบบพ่นฝอย ซึ่งสารสำคัญแต่ละชนิดในน้ำมันผิวมะกรูดส่งผลต่อการกักเก็บที่แตกต่างกันขึ้นกับหลายปัจจัยได้แก่ อัตราส่วนของน้ำมันต่อผงแป้ง, ขนาดอนุภาคของน้ำมันในอิมัลชัน, สมบัติต่างๆของสารสำคัญ, สภาวะที่ใช้ในการอบแห้งแบบพ่นฝอย และค่าสัมปสิทธิ์การแพร่ของน้ำมัน ในงานวิจัยนี้พบว่าการกักเก็บน้ำมันผสม(ผิวมะกรูด)ดีกว่าน้ำมันหอมระเหยชนิดเดียว เนื่องจากในน้ำมันผสมมีแรงกระทำระหว่างน้ำมันแต่ละชนิด ซึ่งส่งผลต่อค่าการกักเก็บ ข้อควรระวังของการกักเก็บน้ำมันผสมโดยวิธีอบแห้งแบบพ่นฝอยคือ การเปลี่ยนรูปของสารสำคัญที่ไวต่อความร้อน ซึ่งทำให้กลิ่นดั้งเดิมของน้ำมันผสมเปลี่ยนไป การปลดปล่อยสารสำคัญในผงแห้งขึ้นอยู่กับความสามารถในการละลายน้ำของน้ำมันหอมระเหย ความชื้นสัมพัทธ์ และขนาดของผงแห้ง เนื่องจากการกระจายตัวของน้ำมันหอมระเหยบนผงแห้งจะกระจายตัวในผงแห้งตามความสามารถในการละลายในน้ำ และจะสามารถแพร่ผ่านตามสามารถในการละลายน้ำเช่นเดียวกัน

ภาควิชา..........วิศวกรรมเคมี.......... ลายมือชื่อนิสิต...
สาขาวิชา.........วิศวกรรมเคมี......... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก..............................
ปีการศึกษา....2554........................ ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม..............................
ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม..............................

5171852521: MAJOR CHEMICAL ENGINEERING
KEYWORDS: MULTI-FLVOR/ MICROENCAPSULATION/ SPRAY DRYING

PAILIN PENBUNDITKUL: LOSS MECHANISM OF MULTI-FLAVOR COMPOUNDS IN MICROENCAPSULATION PROCESS BY SPRAY DRYING, ADVISOR: TAWATCHAI CHARINPANITKUL, D.Eng., CO-ADVISOR: HIDEFUMI YOSHII, Ph.D., APINAN SOOTTITANTAWAT, Ph.D., 127 pp.

Some Thai herbals are volatile and chemically unstable in the presence of air, light, moisture and high temperature. To limit flavor degradation or loss during processing and storage, it is useful to encapsulate volatile compounds prior to its use in foods or beverages. In this research, flavor was encapsulated with carrier solution such as gum arabic or modified starch and transformed to solid by spray drying process. Bergamot oil was used as multi-flavor model. The main compositions of bergamot oil were *d*-limonene, linalool and linalyl acetate. The purpose of this research is to understand the loss mechanism of multi-flavor compound during microencapsulation process by spray drying. The loss of multi-flavor and single flavor were affected by many factors such as ratio of flavor to solid, emulsion droplet size, powder size, flavor properties, spray drying condition and diffusion coefficient of flavor. The results demonstrated that multi-flavor gave higher retention than single flavor because of the interaction between each flavors. The transformation of sensitive flavors was occurred during the spray drying process. The volatile release of spray dried powder depended on the solubility of flavor, powder size, temperature storage and RH. This implied that flavor release was governed by the interaction of flavor and matrices.

Department: ……Chemical Engineering……..Student's Signature……………...…….
Field of Study: ……Chemical Engineering........Advisor's Signature……………..………
Academic Year: ……2011…………………Co-advisor's Signature………..…..…..
Co-advisor's Signature………..…........

ACKNOWLEDGMENTS

First of all, I would like to thank God for his grace, wisdom, favour and faithfulness. I could never have done my dissertation without You. *If any of you lacks wisdom, he should ask God, who gives generously to all without finding fault, and it will be given to him (James 1:5).*

I offer my profound gratitude to my graduate advisor, Assoc. Prof. Tawatchai Charinpanitkul, Prof. Hidefumi Yoshii and Dr. Apinan Soottitantawat for their help and support me. They also allowed me to work in my own way and gave me their good comment and suggestion during the period of my research. My thanks also go to the members of my major committee, Prof. Suttichai Assabumrungrat, Prof. Wiwut Tanthapanichakoon, Dr. Uracha Ruktanonchai, Asst. Prof. Varong Pavarajarn and Asst. Prof. Amornchai Arpornwichanop for reading my first drafts of dissertation and improving the presentation of this dissertation.

I would like to thank my family for their help and encouragement. Thank also to my colleagues both Center of Excellence in Particle Technology, Engineering, Chulalongkorn University (Thailand) and Food Engineering Lab., Department of Applied Biological Science, Kagawa University (Japan) for their help.

Thank also to Prof. Hidefumi Yoshii in Food Engineering Lab., Department of Applied Biological Science, Kagawa University, Japan for their kind helping during my stay and for flavor release experiment. Also thank to Dr. Neoh Tze Loon from Kagawa University for his comment and helping me during my time in Japan.

I also would like to acknowledge to financial support and scholarship of the 90th Anniversary of Chulalongkorn University Fund (Dutsadi Phiphat Scholarship), the Centennial Fund of Chulalongkorn University to the Center of Excellence in Particle Technology and National Nanotechnology Center (NANOTEC).

CONTENTS

Page

Chapter Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

1.1 Motivation

Flavor is the most important of all food characteristics affected by processing conditions. Flavor stability during processing is very important, especially for high-temperature treatments [1]. Many essential oils are known for their antibacterial activity. They are also commonly used in perfumes, cosmetics, bath products, to flavor food and drinks, and in household cleaning products [2].

Microencapsulation is an important process for the food and flavoring industries by which core materials such as oils or flavors are packaged within the wall material. The main advantage of this technique is the protection of core materials from heat, light, and oxygen [1, 3-7]. Microencapsulated oils provide a dried-powder, with reduced volatility and less oxidation, and can be used in many different finished products such as cakes and, beverages. Examples of commonly used encapsulated flavors and oils are citrus oils, artificial or natural flavors, essential oils and spices, tuna oil, fatty acids, soy oil, and sunflower oil [8]. The size of particles from encapsulation process may be classified as: macro (>5000 mm): micro (1.0–5000 mm); and nano (<1.0 mm). Capsules below 1.0 mm in size are frequently referred to as nanocapsules, which are often made by very specialized nanoencapsulation methods [4, 8].

Among many encapsulation techniques, the most common technique is spray drying, which is the transformation of a feed from a fluid state (solution, dispersion, emulsion) to a dried particulate form by spraying the feed into a hot drying medium [4, 6, 9-12]. However, it is quite important to protect the flavor loss during drying, because high temperature air is commonly used in spray drying [4]. Spray drying encapsulation has been used in the food industry since the 1930s to prepare the first encapsulated flavors using gum arabic as the wall material [13]. A successful spray drying encapsulation relies on achieving a high retention of the core materials especially volatiles and minimum amounts of the surface oil on the powder particles for both volatiles and non-volatiles during the process and storage [8]. Spray drying is the most common and cheapest method to produce dry stable food additives and flavors. More than 90% of the encapsulated flavorings are produced by this method [1, 7]. Spray-drying is a widely used technique to facilitate the storage and handling of various fat-containing food systems.

Release mechanism comparison may be carried out using model independent or model dependent methods [14]. In order to investigate the release mechanism, several different models have been employed for outlining the release mechanism from matrices. The release models which are most widely applied and best describe drug release phenomena are, in general, the Higuchi model, the Weibull model, and the Korsmeyer-Peppas model [14-20].

In this study, the encapsulation of flavor (bergamot oil, *d*-limonene, linalool, linalyl acetate) in modified starch was investigated by spray drying. The emulsion (oil in water) was prepared by high speed homogenizer and high pressure homogenizer. The diffusion coefficient of the different flavors and ratios of wall material and oil were evaluated by calculation and DOSY NMR. After the spray drying of powders, size, moisture content, morphology, and flavor retention were analyzed by laser scattering particle size distribution analyzer, vacuum oven, scanning electron microscopy (SEM), and gas chromatograph (GC), respectively. Moreover, the rate of volatile release with rapid analysis was also investigated by humidity ramping and repeating methods.

1.2 Objectives

1.2.1. To determine the effect of different wall materials and various ratio of core and wall material on multi-flavor encapsulation

1.2.2. To obtain the relationship of spray drying (air inlet temperature, feed rate) on total retention and surface oil of encapsulated multi-flavor

1.2.3. To investigate the effect of model flavor on retention and stability

1.2.4. To evaluate the diffusion coefficient of model flavors in different media and study the release mechanism of spray dried powders

1.2.5. To find the factor which affect the chemical transformation of linalyl acetate to linalool in spray dried powder

1.3 Scope of Research

1.3.1. Investigate the emulsifying property and emulsion size of wall material on function group of wall material by varying the following parameters:

- Type of wall material: HI-CAP 100 and gum arabic
- The pressure of high pressure homogenizer (bars): 500-1500
- The number of cycle passes through high pressure homogenizer (bars): 1-5

1.3.2. Determine the optimal ratio of bergamot oil: modified starch (solution) on the total oil retention and surface oil of encapsulated powders by varying the following parameters;

- The mass ratio of bergamot oil: modified starch (solution); 1:2-1:8
- Type of modified starch: HI-CAP 100 and CAPSUL

1.3.3. Determine the influence and find the relation of air inlet temperature and feed rate of spray drying on the total oil retention and surface oil of encapsulated powders by varying the following parameters:

- Air inlet temperature of spray drying (^{o}C): 120 - 180
- Feed rate of emulsion (ml/min): 3-9

1.3.4. Determine the effect of model flavor on surface oil and retention

- Different water solubility: *d*-limonene, linalool, linalyl acetate, citral and benzaldehyde
- Different functional group: linalool (alcohol), *d*-limonene (hydrocarbon), citral (aldehyde)
- The mass ratio of essential oil: modified starch (solution); 1:4-1:8

1.3.5. Determine diffusivity of flavors

- Type of media: air, water, emulsion
- Calculation method: Stokes-Einstein relation’s equation, DOSY NMR

1.3.6. Predict the release mechanism of dried powders from models

- Release mechanism model: zero order kinetics, first order kinetics, Higuchi’s model, Korsmeyer - Peppas’s model, Weibull’s model, Baker–Losdale’s model and Avrami’s equation

- The coefficient of determination: R^2 and $R^2_{adjusted}$

1.3.7. Investigation the release behavior of emulsified flavor powder

- Ramping method (RH=10-50%)

- Repeated method of relative humidity: 0% and 50%RH

- Spray dried powder size: 32, 53 and 75 µm

1.3.8. Investigation chemical transformation of linalyl acetate to linalool in spray-dried powder

- Modified starch (HI-CAP 100) content: 10-40 %w/w

- Air inlet temperature of spray drying: 120 -200 ^oC

- Incubation sample: linalyl acetate(oil), emulsion

1.3.9. Emulsion, reconstituted emulsion and particle size were measured by laser scattering particle size distribution analyzer

1.3.10. The morphology (internal and external) of spray dried powder was characterized by scanning electron microscopy (SEM)

1.3.11. Peak intensity ratio of functional groups of wall materials was measured by using Fourier Transform Infrared (FT-IR) spectroscopy.

1.3.12. Determine the effects of different types of flavors and storage conditions (relative humidity, temperature and time) on the stability.

1.4 Expected Benefits

1.5.1. The relationship between the air inlet temperature and feed rate of spray drying on flavor retention will be obtained.

1.5.2. Understanding the influence of flavor properties such as number of carbon, water solubility of flavor, etc. on the diffusion coefficient and the release mechanism.

CHAPTER II

FUNDAMENTAL THEORY AND LITERATURE REVIEWS

2.1 Microencapsulation

Microencapsulation is an important process for the food and flavoring industries by which core materials such as oils or flavors are packaged within the wall material. The advantages of this technique are the protection of core materials from heat, light, and oxygen [1, 3-7]. Microencapsulated oils provide a dried-powder, with reduced volatility and less oxidation, and can be used in many different finished products such as cakes and, beverages. Examples of commonly used encapsulated flavors and oils are citrus oils, artificial or natural flavors, essential oils and spices, tuna oil, fatty acids, soy oil, and sunflower oil [8]. The size of particles from the encapsulation process may be classified as: macro (>5000 mm): micro (1.0–5000 mm); and nano (<1.0 mm). Capsules below 1.0 mm in size are frequently referred to as nanocapsules, which are often made by very specialized nanoencapsulation methods [4, 8].

The techniques of encapsulation have been described elsewhere and can be classified based on its processes (spraying processes, coating processes, and suspension processes) [21], capsules formation mechanism (physical, chemical, and physicochemical) [4, 7, 22] and encapsulation purposes (glass encapsulation and controlled release system) [1, 4, 7].

The first step in encapsulating a food ingredient is the selection of a suitable wall material, basically a film-forming biopolymer, from a wide variety of natural or synthetic polymers, depending on the core material and the characteristics desired in the final microcapsules. For flavor and oil encapsulation in particular, the ideal wall material should have emulsifying properties; be a good film former; have low viscosity at high solids levels; exhibit low hygroscopicity; release the flavor when reconstituted in a finished food product; be low in cost; bland in taste; stable in supply; and afford good protection to the encapsulated flavor and oil [8]. However, cost considerations in the food industry are much more stringent than in, for instance, the pharmaceutical or cosmetic industries [7]. Therefore, encapsulated flavors have application in the processing of food and also prolong the storage life of the products. Controlled flavor release is another major benefit of encapsulated flavors [1, 3].

The other encapsulation category, controlled release system, comprises encapsulation techniques aimed to enhance flavor impact during consumption. Using these techniques, the release of flavor can be controlled by retaining a core structure in the food matrix. Many different systems of this encapsulation have been established and proposed for food application, such as spray drying, spray chilling or spray cooling, extrusion coating, fluidized-bed coating, air suspension coating, multi-orifice centrifugal extrusion, coacervation/ phase separations, liposome entrapment, coacervation, inclusion complexation, inclusion complexion, co-crystallization, interfacial polymerization and rotational suspension separation [1, 3, 7, 13, 23, 24].

2.2 Spray Drying

Among the many encapsulation techniques, the most common is spray drying, which is the transformation of a feed from a fluid state (solution, dispersion, emulsion) to a dried particulate form by spraying the feed into a hot drying medium [4, 6, 9-12]. However, it is quite important to protect the flavor loss during drying, because high temperature air is commonly used in spray drying [4]. Spray drying encapsulation has been used in the food industry since the 1930s to prepare the first encapsulated flavors using gum arabic as the wall material [13]. A successful spray drying encapsulation relies on achieving a high retention of the core materials especially volatiles and minimum amounts of the surface oil on the powder particles for both volatiles and non-volatiles during the process and storage [8]. Spray drying is the most common and cheapest method to produce dry stable food additives and flavors. More than 90% of the encapsulated flavorings are produced by this method [1, 7]. Spray-drying is a widely used technique to facilitate the storage and handling of various fat-containing food systems. In many cases, it is desirable to keep the fat encapsulated in the powder particle. For example, encapsulation can be used to protect sensitive materials such as flavoring agents, aroma, vitamins, etc. from oxygen in the air, and for the controlled release of the encapsulated substance [25]. Furthermore, spray drying is a commercial process which is widely used in the large-scale production of encapsulated flavors and volatiles [3, 7]. The spray drying technique has been widely used for drying heat-sensitive foods, pharmaceuticals, and other substances, because of the solvent rapid evaporation from the droplets. Although most often considered a dehydration process, spray drying can also be used as an encapsulation method when it entraps 'active' material within a protective matrix [6].

Spray-drying is a unit operation by which a liquid product is atomized in a hot gas current to instantaneously obtain a powder. The gas generally used is air or more rarely an inert gas such as nitrogen. The initial liquid feeding the sprayer can be a solution, an emulsion or a suspension [24]. The carriers commonly used in spray drying are starch, modified starches, dextrins, succinylated gelatin, and gum arabic. Spray-dried encapsulated flavors have moderate stability with a shelf life of 6 months or more. The stability of the encapsulated flavors depends on the type of flavor compounds, carrier used, absence of surface oil (which oxidizes rapidly), moisture content of the powder, powder surface properties, and moisture absorption during storage [1].

2.2.1 Microencapsulation Process Steps

Spray drying is divided into different steps: atomization, mixing of sprayed liquid and air, evaporation of water and separation of product. First, the emulsion is fed into the spray dryer and atomized with a nozzle or spinning wheel, followed by the very rapid evaporation of water in hot air[12]. The microcapsules are then transported to a cyclone separator for recovery [10].

The formation of a stable emulsion, in which the wall material acts as a stabilizer for the flavor, is considerable [3]. Spray-drying is a food manufacturer–friendly technique because it allows the food processor to manipulate the preparation process to improve the quality of the final product [7].

2.2.2 Influence of Drying Operational Conditions

Many investigations have been carried out into the influence of drying operational conditions and the composition of wall materials and core on the retention and shelf life of encapsulated flavors [4, 9].

One limitation of the spray drying technology is the limited number of wall materials available. Since almost all spray drying processes in the food industry are carried out from aqueous feed formulations, the wall material must be soluble in water at an acceptable level, and have effective emulsification and film-forming characteristics and efficient drying properties [3, 13]. Typical shell materials include gum acacia, maltodextrins, hydrophobically modified starch, and mixtures thereof [7].

When core materials of limited water solubility are encapsulated by spray drying, the resulting capsules are of a matrix-type structure. In such, the core is organized into small droplets coated with wall materials that are embedded in the wall matrix. Microstructures of spray-dried capsules have shown to be affected by wall composition and properties, flavor-to-wall ratio, atomization and drying parameters, uneven shrinkage at early stages of drying, the effect of a surface tension-driven viscous flow and storage conditions [3, 10]. Flavor retention is maximized by using a high infeed solids level, high viscosity infeed, optimum inlet (160–210 ^{o}C) and high exit (>100 ^{o}C) air temperatures, and high molecular weight flavor molecules [10].

2.2.3 The Advantages of the Use of Spray Drying

The merits of the spray drying process have ensured its dominance; these include the availability of equipment, low process cost, economical, flexible, wide choice of carrier solids, good retention of volatiles, good stability of the finished product, and large-scale production in continuous model [1, 3, 7, 8, 10, 13, 23].

2.2.4 The Disadvantages of the Use of Spray Drying

One disadvantage of spray drying is that some low-boiling point aromatics can be lost during spray drying and the core material may also be on the surface of the capsule; this would encourage oxidation and possible flavor changes of the encapsulate [3]. Another problem with spray drying is that this technology produces no uniform microcapsules and is not good for heat-sensitive material [3, 23].

2.2.5 The Process of Spray Drying

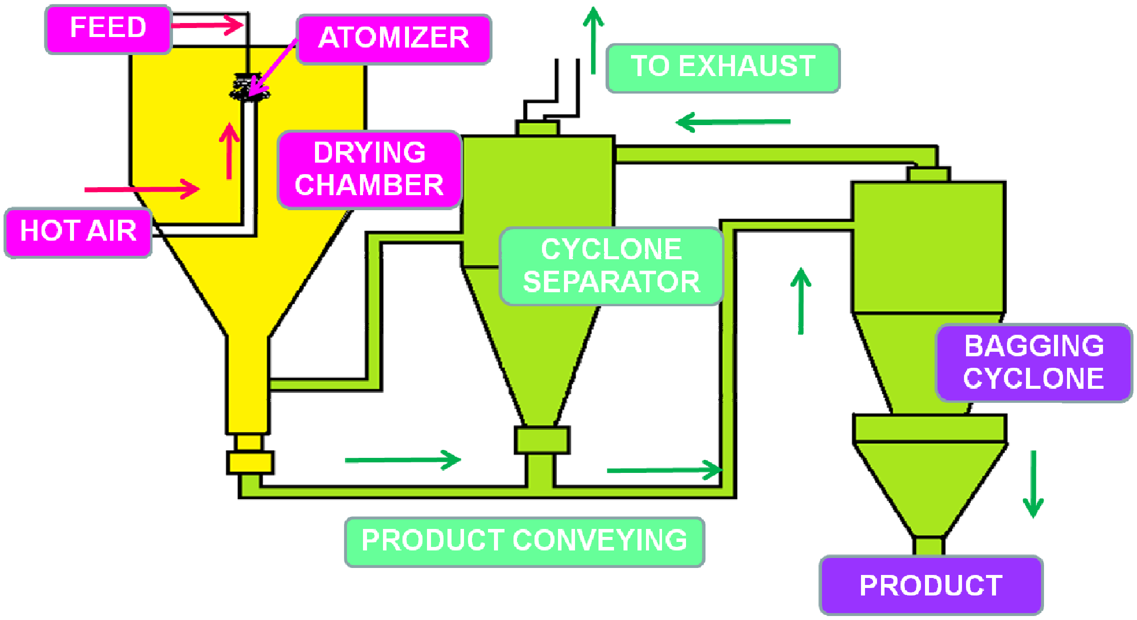

Figure 2-1. Spray drying system

Atomization

Following the preparation of the infeed emulsion, it is pumped into the drying chamber of the spray drier [8]. Liquid atomization in small droplets can be carried out by pressure or centrifugal energy [24]. Selection of the atomizer is one of the most important choices in spray dryer design and has a significant effect upon the size distribution of the final dried paticles. The most important atomizer characteristics from the standpoint of product quality are uniformity of drop-size, control of drop-size distribution, and homogeneity of the spray [6]. Atomizers used include pneumatic (two-fluid) atomizer, pressure nozzle, spinning disk configurations and, recently, two fluid nozzle and sonic nozzle [24]. Two types of atomizers are widely used: the high-pressure nozzle; and the centrifugal (wheel) atomizer [6, 10]. The industry is nearly equally divided between the use of these two types of atomizers. Although each type of atomization has its own advantages and disadvantages, there is no literature suggesting that one type results in a better effect than the other [6, 8].

While a two-fluid nozzle is used in some applications, it is not commonly used in the flavor industry. A two-fluid nozzle has a larger orifice than a single-fluid nozzle and thus, has application for particulate or crystalline materials that would otherwise plug a single fluid nozzle. Centrifugal wheel atomizers have the advantage of handling very viscous, abrasive or particulate infeed materials while the pressure spray systems offer greater flexibility in spray pattern in the dryer and in powder physical properties. An additional advantage of the single-fluid nozzle over the wheel atomizer is that it yields a larger average particle size. The flavor industry is divided nearly equally between these two types of atomization [10].

The goal of this stage is to create a maximum heat-transferring surface between the dry air and the liquid in order to optimize heat and mass transfers. The choice of atomizer configuration depends on the nature and viscosity of the feed and the desired characteristics of dried product [24].

Droplet – hot air contact

This contact takes place during atomization and initiates the drying stage. In comparing the atomizer emplacement to the hot air spreader, one can distinguish the co-current drying and the counter-current one. In the co-current process the liquid is sprayed in the same direction as the flow of hot air through the apparatus with a hot air inlet temperature typically of 150–220 ^{o}C, evaporation occurs instantaneously and dry powders are exposed to moderate temperatures (typically 50–80 ^{o}C) which limit thermal degradations. In contrast, during counter- current drying, the liquid is sprayed in the opposite direction of the flow of hot air and the dry product is exposed to high temperatures which limit the applications of this process to thermo-sensitive products. However, the main advantage of the counter-current process is that it is considered as being more economic in terms of consumed energy [24].

Almost all spray dryers used in the flavor industry are co-current in design, i.e., the product enters the dryer flowing in the same direction as the drying air. This results in very rapid drying and does not subject the flavoring to as much heat as would a counter current system. In the co-current dryer, the spray-dried product never exceeds the exit air temperature of the dryer [8, 10].

Evaporation of water droplets

The following three steps vary in duration depending on both product nature and air inlet temperature. In fact, if the air inlet temperature is high, at dry crust is rapidly formed because of the high water evaporation rate. Due to the large surface-to-volume ratio of the atomized droplets, the drying of formed droplets in the hot atmosphere is a very rapid process [8, 24].

In the first stage, the hot gas causes an increase in the droplet temperature, which promotes liquid evaporation from the droplet surface and a corresponding shrinkage of the droplet. The rapid migration of the water to the droplet surface maintains a constant evaporation rate. At some point the suspended particles form a continuous network. Originally, the use of spray-drying was advocated as a rapid method for drying compounds which showed unacceptable levels of degradation when dried in the slower classic drying processes. In fact, at the moment of the mixing of the air with the atomized liquid, the drying takes place almost instantaneously and intensive evaporation takes place at the surface of each droplet. The evaporation is so rapid that the droplet remains kept cool until the dry state is reached; this is due to the absorption of heat in vaporizing the liquid. After the evaporation has ceased, the temperature of the particle rises to the general temperature of the drying chamber [24].

The rapid evaporation of water from these droplets during surface film solidification keeps the core temperature below 100 ^{o}C in spite of the high temperatures (>150 ^{o}C) used in the process [8].

Dry product-humid air separation

This separation is often done through a cyclone placed outside the dryer which reduces product losses in the atmosphere: the larger particles or agglomerated particles are taken off the bottom of the dryer while the finest ones pass through the cyclone collector to be separated from the humid air. In addition to cyclones, spray-dryers are commonly equipped with filters, called ‘‘bag houses’’ that are used to remove the finest powder, and chemical scrubbers to remove the remaining powder or any volatile pollutants. The obtained powder is made of particles which originate from spherical drops after shrinking. Depending on the composition, the water and gas content of the drop, these particles can be compact or hollow [10, 24].

The exit air from the dryer generally has to be treated to meet local pollution control laws. While some of the older dryers use gas incineration, as energy costs have increased these incineration systems have become quite costly to operate. New dryer installations use scrubbing systems (e.g., aqueous/ chemical sprays) to remove entrained solids and gaseous volatile flavors [10].

2.3 Wall Materials

There are numerous wall materials or encapsulating agents for use in food application [8, 26]. In these techniques, various encapsulating materials, including water-soluble and insoluble materials, are used to encapsulate flavor [1, 8, 26]. Selection of encapsulation technique and material depends on the end use of the microencapsulated product, including the release property of the core material [1]. 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) [3, 24]. The choice of a wall material for microencapsulation by spray-drying is very important for encapsulation efficiency and microcapsule stability [24]. The wall system is designed to protect core material from factors that may cause its deterioration, to prevent a premature interaction between the core material and other ingredients, to limit volatile losses, and also to allow controlled or sustained release under desired conditions [24].

For spray drying microencapsulation, the choice of wall material is critical as it will influence emulsion properties before drying, retention of the volatiles during the process and the shelf-life of the encapsulated powder after drying [8]. Typically, effective wall materials for spray drying should have functional properties (solubility; molecular weight; glass/melting transition; crystallinity; diffusibility), including emulsifying properties, film forming, high solubility, low viscosity at high concentrations and low cost properties [24, 26]. Moreover, the costs should be also considered. Thus, judicious choice of encapsulating material according to the desired application is an important task [24]. 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, i.e. with low viscosity at high concentrations; allow a complete elimination of solvent in any processes requiring a phase of desolvatation; give the maximum protection of the active ingredients against the external factors; ensure good emulsion-stabilization properties and effective redispersion behavior in order to release the flavor at the time and the place desired [3, 6].

Numerous wall materials are commercially available for use as flavor encapsulating agents. Most wall materials used for spray drying applications are selected from the following types [1, 6, 8, 24, 26]:

- Carbohydrates (starch, maltodextrins, corn syrup solids, cyclodextrins, modified starches)
- Cellulose derivatives (carboxy methylcellulose, methylcellulose, ethylcellulose)
- Gums (gum acacia, agar, sodium alginate)
- Lipids (wax, paraffin, fats, oils)
- Proteins (gelatin, soy protein, whey protein, sodium caseinate)

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. Characteristics of the major wall materials used for flavor encapsulation are reported in Table 2-1 [3].

Table 2-1. Characteristics of the wall material used for encapsulating flavors [3]

Wall material	**Characteristics**
Maltodextrin (DE < 20)	Film forming
Corn syrup solid (DE > 20)	Film forming, reductability
Modified starch	Very good emulsifier
Gum Arabic	Emulsifier, film forming
Modified cellulose	Film forming
Gelatin	Emulsifier, film forming
Cyclodextrin	Encapsulant, emulsifier
Lecithin	Emulsifier
Whey protein	Good emulsifier
Hydrogenated fat	Barrier to oxygen and water

Among the available materials, the major encapsulating agents used for spray drying applications are gums and, modified starches [6].

2.3.1 Gum

Gum acacia has been the encapsulating agent of choice for many years [6]. It is the gum that is most often used as a flavor-encapsulating material [3]. Gum acacia, also known as gum arabic, is defined as the gummy exudates flowing naturally or obtained by incision of the trunk and branches of Acacia Senegal or other species [6].

Gum acacia is a polymer consisting of D-glucuronic acid, L-rhamnose, D-galactose and L-arabinose with approximately 2% - 5% protein. This protein fraction is responsible for the emulsification properties of the gum since it acts as an interface between oil and water. The film forming properties come from the arnbinogalactan fraction of the gum. The low viscosity and consequently high solubility of this portion is likely responsible for the barrier film that is formed after the evaporation of water during drying [6, 24].

In particular, due to its specific characteristics like film forming and good emulsifying properties, gum acacia provides high retention of volatiles[6, 24]. In addition, the wall material is ideally suited to the encapsulation of lipid droplets as it fulfills the roles of both surface-active agent and drying matrix, thus preventing the loss of volatiles in contact with the atmosphere. Likewise, expensive cost, limited supply, and quality variations have restricted the use of gum arabic for encapsulation purposes thereby forcing researchers to seek alternative microencapsulating materials [3, 6, 24, 26].

Gum acacia provides very good volatile retention during the drying process as shown in the spray drying microencapsulation of citrus oil, esters, citral and linalyl acetate, among others [6]. Gum arabic was found to be a better wall material for the encapsulation of cardamom oleoresin than maltodextrins and modified starch and the obtained microcapsules exhibited a free flowing character. It was also recently reported that gum arabic is good wall material for the encapsulation of cumin oleoresin by spray-drying. Gum arabic is usually preferred because it produces stable emulsions with most oils over a wide pH range, and it also forms a visible film at the oil interface. Because of this emulsifying efficiency, gum Arabic has been usually used to encapsulate lipids. Typically the ratio of oil/wall material, when gum Arabic is used, is lower than 0.15. However maltodextrin DE 18.5 was considered as the most suitable partial replacer for gum arabic because the obtained wall material presents a good solubility and a rapid reconstitution of the emulsion in water [24].

2.3.2 Starch

Starch and starch-based ingredients (modified starches, maltodextrins, β-cyclodextrins) are extensively used in the food industry to retain and protect volatile compounds due to their aqueous solubility, low viscosity and ease of drying conditions [3, 6, 24]. They can act as carriers for aroma encapsulation, fat replacers and also emulsion stabilizers. Many researchers have created new microporous starch materials with the aim of improving aroma retention. The binding of volatile compounds to starch has been classified into two types. First, the flavor compound surrounded by the amylase helix through hydrophobic bonding is known as an inclusion complex. Second, polar interactions have been determined which involve hydrogen bonds between the hydroxyl groups of starch and aroma compounds. It was demonstrated that amylose is able to form inclusion complexes with a wide spectrum of ligand molecules, for instance with flavor compounds [3].

Chemically modified starches (i.e., incorporating a lipophilic group into their molecules and having emulsifying properties) have been shown in numerous studies, to yield very good retention of volatiles during drying [6].

A novel approach to improve the encapsulating properties of common wall materials consists of chemical modifications of carbohydrates. For example, some modified starches have surface active properties and are widely used in the process of microencapsulation by spray-drying. Hydrolyzed starch products are hydrophilic compounds, thus have little affinity for hydrophobic flavors. Their hydrophilic nature can be modified by linking hydrophobic side chains like 1-octenyl succinate. These wall materials have excellent emulsification properties [6, 24, 26]. In order to give some emulsifying capabilities to starch molecules, side chains of lipophilic succinic acid are inserted into starch to produce modified starches. Various forms of modified starches are used for flavor and oil encapsulation such as CAPSUL, N-lok, HI-CAP and Encapsul [8].

Soybean soluble polysaccharide is considered a superior emulsifier over gum arabic to retain microencapsulated ethyl butyrate during spray drying [9]. In addition, it is known that polysaccharides having gelling properties that could stabilize emulsions towards flocculation and coalescence [24].

Some workers have shown that wall materials based on modified starch leads to very good retention of volatiles and low amounts of unencapsulated oil at the surface of powder particles [8]. For example, Jeon et al.[27] investigated the encapsulation potential of native corn and barley starches and their chemically modified counterparts (Succinylated and Octenyl Succinylated starches) to minimize the evaporative flavor loss and to improve flavor stability. They found that chemically modified (Succinylated) corn and barley starches are more effective than the native starches in the flavor retention. In particular, Succinylated regular starches showed better retention ability than waxy starches [8]. Table 2-2 provides a summary of different wall materials with their properties and applications.

Table 2-2. Different wall materials used in spray-drying microencapsulation of food oils and flavors [8]

Wall materials	Properties	Examples	Flavors and oils
Hydrolyzed starches	Very good oxygen-barrier, cheap, low viscosity at high solids; no/ limited emulsion stabilization	Corn syrup solids, maltodextrins	Citral, linalyl acetate, ethyl caprylate, cheese aroma, linoleic acid, orange peel oil, lemon oil
Modified starches	Very good emulsion stabilization, inexpensive	CAPSUL, N-lok, HI-CAP	Meat flavor, fish oil, orange oil, *d*-limonene, l-mentol, butter oil, cream, black pepper oleoresin, vitamin E
Gums	Good emulsions, very good retention of volatiles	Arabic gum, mesquite gum	Monoterpens, orange peel oil, cardamom oil, vegetable oils, cardamom oleoresin, linoleic acid,

			bixin, lipids, acetyl pyrroline, soy oil, *d*-limonene, ethyl butyrate
Cyclodextrins	Very good inclusion of volatiles, excellent oxygen barrier, relatively expensive	α-, β-, α-Cyclodextrins	Pine flavor, shiitake flavor, *d*-limonene, ethyl hexanote, caraway fruit oil, lemon oil
Milk protein	Very good emulsions, expensive	Whey proteins	Milk fat, linoleic acid, soy oil, ethyl butyrate, ethyl caprylate

2.4 Release Mechanism Model

The release mechanism comparison may be carried out using model independent or model dependent methods. [14] In order to investigate the release mechanism, several different models have been employed for outlining the release mechanism from matrices. The release models most widely applied and which best describe flavor release phenomena are, in general, the Higuchi model, the Weibull model, and the Korsmeyer-Peppas model [14-20].

2.4.1 Zero Order Kinetics [14, 16-20]

Flavor release from dried powders that do not disaggregate but release the flavor slowly (assuming that area does not change and no equilibrium conditions are obtained) can be described by the following equation 1. In this case, the flavor release runs at a constant rate.

$$Q_l = Q_0 + K_0 t \quad (1)$$

where Q_l = the amount of flavor released at time t

Q_0 = the initial amount of flavor in the solution (most time Q_0=0)

K_0 = the zero order release constant

This relation can be used to describe the flavor release of several types of modified release flavor dried powders, as in the case of some transdermal systems, as well as matrix particles with low soluble flavor, coated forms, and osmotic systems, etc. This profile releases the same amount of flavor by unit of time and it is the ideal method of flavor release.

2.4.2 First Order Kinetics [14, 16, 17, 19, 20]

The first application of this model was used to investigate drug dissolution and flavor release [28, 29]. This model has also been used to describe absorption and/ or elimination it is difficult to conceptualize this mechanism on a theoretical basis. The flavor released each time is proportional to the residual flavor inside the dosage form. The relation can be expressed as follows,

$$\log Q_1 = \log Q_0 + \frac{K_l\, t}{2.303} \quad (2)$$

where Q_t = the amount of flavor released in time t

Q_0 = the initial amount of flavor in the solution (most time $Q_0=0$)

K_l = the first order release constant

In this way a graphic of the decimal logarithm of the released amount of flavor versus time will be linear [30].

2.4.3 Higuchi’s Model [14, 16-20, 31-34]

Higuchi’s model has been recognized as one of the most popular models used to describe pure Fickian transport. Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs or flavors incorporated in semi-solids and/or solid matrixes. Mathematical expressions were obtained for flavor particles dispersed in a uniform matrix behaving as the diffusion media. To study the dissolution from a planar system with a homogeneous matrix, the typical relation can be expressed as

$$Q = \frac{\mathrm{M_t}}{\mathrm{M_\infty}} = \sqrt{D(2C - C_s)C_s t} = kt^{0.5} \quad (3)$$

where Q = the amount of flavor released in time t per unit area

M_t = the cumulative amounts of flavor released at time t

M_∞ = the cumulative amounts of flavor released at infinite time

C = the flavor's initial concentration

C_s = the flavor's solubility in the matrix media

D = the diffusivity of the flavor molecules (diffusion constant) in the matrix substance

k = Higuchi release rate

Higuchi describes flavor release as a diffusion process based on Fick's law, square root time dependent. This relation can be used to describe the drug dissolution and flavor release from several types of modified release pharmaceutical dosage forms, as in the case of some transdermal systems [35] and matrix tablets with water soluble drugs [36-39].

2.4.4 Korsmeyer-Peppas's Model [14, 16-20, 31, 32, 40-43]

Another fundamental equation usually applied to fit release data is the power law. Korsmeyer et al. [40] developed a simple, semi-empirical model, relating exponentially the flavor release to the elapsed time (t). This equation is the short time approximation of exact complex relationships to describe general solute release behavior from controlled release matrices and therefore their use is confined for the description of the first 60% of the release curve. Under some experimental situations the release mechanism deviates from the Fick equation, following an anomalous behavior (non-Fickian). In these cases a more generic equation can be used:

$$\frac{\mathrm{M_t}}{\mathrm{M_\infty}} = \mathrm{kt^n} \qquad (4)$$

where M_t = the cumulative amounts of flavor released at time t

M_∞ = the cumulative amounts of flavor released at infinite time

k = kinetic constant

n = an exponent characterizing the diffusional mechanism

This model is generally used to analyze the release of pharmaceutical polymeric dosage forms or dried powder when the release mechanism is not well known or when more than one type of release phenomena could be involved.

From Table 2-3, Peppas [44] used this n value in order to characterize different release mechanisms. In the case of a cylinder, n = 0.45 instead of 0.5, and 0.89 instead of 1.0. To use equation 4, it is also necessary that release occurs in a one-dimensional way.

Table 2-3. Interpretation of diffusional release mechanisms

Release exponent (n)	**Flavor transport mechanism**
0.5	Fickian diffusion
0.5<n<1.0	Anomalous transport
1.0	Case-II transport
Higher than 1.0	Super Case-II transport

Note: To the determination of the exponent n the portion of the release curve where $M_t/M_\infty < 0.6$ should only be used.

2.4.5 Weibull's Model [14, 16-20, 31, 45]

A general empirical equation described by Weibull (1951) could be adapted to the dissolution/ release process. Another alternative for the description of release profiles is based on the empirical use of the Weibull function. This equation can be successfully applied to almost all kinds of dissolution curves.

$$\frac{M_t}{M_\infty} = 1 - \exp(-at^b) \quad (5)$$

where M_t = the cumulative amounts of flavor released at time t

M_∞ = the cumulative amounts of flavor released at infinite time a,b = constants

From this equation a linear relation can be obtained for a log-log plot of –ln (M_t /M_∞) versus time, t. the shape parameter (b) is obtained from the slope of the line and the scale parameter, a is estimated from the ordinate value at time t = 1.

2.4.6 Baker-Lonsdale's Model [14, 17, 20]

This model was developed by Baker and Lonsdale (1974) from the Higuchi model and describes the drug and flavor controlled release from a spherical matrix, being represented by the following expression:

$$\frac{3}{2}\left[1-\left(1-\frac{M_t}{M_\infty}\right)^{2/3}\right]-\frac{M_t}{M_\infty}=kt \quad (6)$$

where M_t = the cumulative amounts of flavor released at time t

M_∞ = the cumulative amounts of flavor released at infinite time

k = release constants

In this way a graphic relating the left side of the equation and time will be linear if the established conditions are fulfilled.

2.4.7 Hixson-Crowell's Model [14, 16, 17, 20]

Hixson-Crowell's model is used for the particle regular area and it is proportional to the cubic root of its volume, deriving an equation that can be described in the following manner.

$$W_0^{1/3}-W_t^{1/3}=K_s t \quad (7)$$

where W_0 = the initial amount of flavor in dried powder

W_t = the remaining amount of flavor in dried powder form at time t

K_s = a constant incorporating the surface-volume relation

This equation applies to pharmaceutical dosage form such as tablets, spherical powder where the release occurs in planes that are parallel to the flavor surface.

2.4.8 Avrami's Equation [7, 9, 11, 46, 47]

Avrami's equation was developed to explain the crystal growth of a polymer. It can be applied to the release time-courses of the encapsulated flavor. In order to evaluate the release rate constant of flavor in the powder, the release data are fitted to Avrami's equation.

$$R = exp[-(kt)^n] \quad (8)$$

where R = the retention of encapsulated flavor(1-α)
t = the storage time
k = the release rate constant
n = a parameter representing the release mechanism

Taking a logarithm of both sides of equation 8, we can get the parameter n as a slope by plotting ln (-ln-R) vs. ln t and release rate constant k from the interception at ln t = 0. For n of value, n = 1 represents the first-order reaction, n = 0.54 represents the diffusion-limiting reaction kinetics. This equation was successfully applied to describe the shelf life failure and later applied to the release time-courses of the encapsulated flavor [9, 11]. Table 2-4 shows the n value for solid reaction by Hancock and Sharp[48]. The model equation can be derived from the rate of product formation decreases proportional to the thickness of the matrix layer[49]. In diffusion controlled mechanisms, the release rate for the diffusion layer model is proportional to the thickness of the barrier layer as depicted in Figure 2-2.

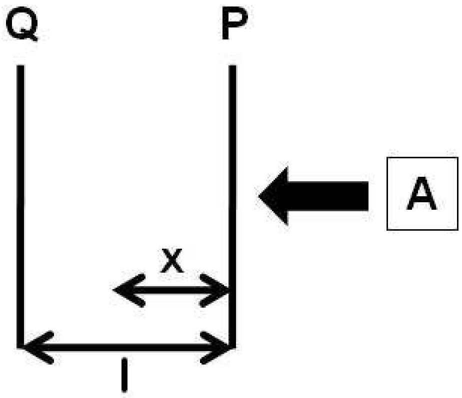

Figure 2-2. One dimensional diffusion through a flat plate

$$\frac{dl}{dt} = -DA\frac{dC}{dx} \qquad (9)$$

where D = diffusion coefficient

l = thickness of the barrier layer

x = the distance diffused through matrix

C = the concentration of volatile compound

A = surface area of product

Assuming a linear concentration gradient of A: $dC/dxl_{x=l}=-(C2-C1)/l$

Equation 9 becomes

$$\frac{dl}{dt} = DA\frac{(C_2 - C_1)}{l} \qquad (10)$$

Separating variables and integrating equation 10 gives

$$l^2 = 2DA(C_2 - C_1)t \qquad (11)$$

If $k=2DA(C_2-C_1)$, equation 11 becomes

$$l^2 = kt \qquad (12)$$

The release rate equation is for a flat plate that does not involve a one-dimensional shape, where the release rate (α=1-R; R=flavor retention) is directly proportional to the product layer thickness of matrix (l). Therefore, equation 12 becomes

$$\alpha^2 = k't \qquad (13)$$

This equation (eq 13) represents the release rate in one dimension.

The three-dimensional diffusional model is based on the assumption of spherical solid particles (Figure 2-3). The release amount involving n spherical particles uses the following equations 14 and 15

$$\alpha = \frac{m_0 - m_t}{m_0} \qquad (14)$$

$$\alpha = \frac{\frac{4}{3}n\rho\pi R^3 - \frac{4}{3}n\rho\pi(R-x)^3}{\frac{4}{3}n\rho\pi R^3} \qquad (15)$$

where x = the thickness of the matrix

Equation 15 can be simplify to

$$\alpha = 1 - \left(\frac{R-x}{R}\right)^3 \qquad (16)$$

Equation 16 can be rearranged to

$$x = R\left(1 - (1-\alpha)^{\frac{1}{3}}\right) \qquad (17)$$

Therefore, substituting equation 17(after squaring x) into equation 12 gives

$$R^2\left(1 - (1-\alpha)^{\frac{1}{3}}\right)^2 = kt \qquad (18)$$

Assuming k'=k/R^2, equation 18 becomes the three-dimensional diffusional model:

$$\left(1 - (1-\alpha)^{\frac{1}{3}}\right)^2 = k't \qquad (19)$$

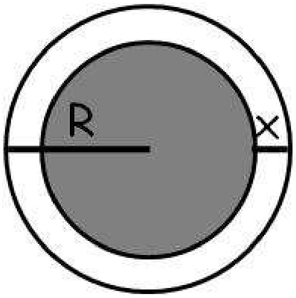

Figure 2-3. Three dimensional diffusion through a sphere

Table 2-4. Comparison of n value (release mechanism) with the release model equation

Value of *n*	Release mechanism	Model equation
0.54	Diffusion controlled (Sphere)	$\left[1-R^{1/3}\right]^2 = kt$
0.57	Diffusion controlled (cylinder)	$R\ln R + 1 - R = kt$
0.62	Diffusion controlled (tablet)	$(1-R)^2 = kt$
1.00	First-order mechanism	$-\ln R = kt$
1.07	Moving interface mechanism (sphere)	$1-R^{1/3} = kt$
1.11	Moving interface mechanism (disk)	$1-R^{1/2} = kt$
1.24	Zero-order mechanism	$1-R = kt$

2.4.9 The Adjusted Coefficient of Determination ($R^2_{adjusted}$) [17, 19]

For the same number of parameters, the coefficient of determination (R^2) can be used to determine the best of the model equations. However, when comparing models with different numbers of parameters, the adjusted coefficient of determination ($R^2_{adjusted}$) is more meaningful:

$$R^2_{\mathrm{adjusted}} = 1 - \frac{(n-1)}{(n-p)}(1-R^2) \qquad (20)$$

where $R^2_{adjusted}$ = the adjusted coefficient of determination

n = the number of dissolution data points (M/t)

p = the number of parameters in the model

R^2 = the coefficient of determination

2.5 Diffusion Coefficient

2.5.1 Diffusion Coefficients in Liquids [50]

2.5.1.1 Liquid Diffusion Coefficients from the Stokes-Einstein Equation [50, 51]

The most common basis for estimating diffusion coefficients in liquids is the Stokes-Einstein equation. Coefficients calculated from this equation are accurate to only about twenty percent. Nonetheless, this equation remains the standard against which alternative correlations are judged. The Stokes-Einstein equation is expressed in the following equations:

$$D = \frac{k_B T}{f} = \frac{k_B T}{6\pi\mu R_0} \qquad (21)$$

where f = the friction coefficient of the solute

k_B = Boltzmann's constant

μ = the solvent viscosity

R_0 = the solute radius

The temperature variation suggested by this equation is apparently correct, but it is much smaller than the effects of solvent viscosity and solute radius. As the standard, the Stokes-Einstein equation has often been extended and adapted.

2.5.1.2 Deriving the Stokes-Einstein Equation [50]

Because the Stokes-Einstein equation is limited to cases in which the solute is larger than the solvent, many investigators have developed correlations for cases in which solute and solvent are similar in size. The impressive aspect of these efforts is their similarly to the Stokes-Einstein equation. Some studies claim better accuracy, but their increased complexity means that their results are rarely used. The exception is the Wilke-Chang correlation (1955), which predicts

$$D = \frac{7.4 \times 10^{-8} (\phi M_2)^{1/2} T}{\mu V_1} \qquad (22)$$

where D = the diffusion coefficient of solute 1 (cm^2/sec)

M_2 = the molecular weight of solvent 2 (g/mol)

μ = the viscosity (centipoises)

V_1 = the molar volume of solute 1 (cm^3/mol)

T = the temperature (K)

ϕ = the association factor of solvent 2

(ϕ=1, for most organic solvents, 1.5 for alcohols, and 2.6 for water)

To estimate the flavor compound diffusion coefficients, the Wilke and Chang estimation method was used to evaluate the diffusion coefficients in the water (flavor) phase. The kinetic release as a function of time from emulsion (oil-in-water) during spray drying can be evaluated from the diffusion coefficient of flavor.

Molar volume in the Wilke-Chang equation can be estimated using various methods such as the Le Bas (1915) and, Schroeder methods (1949), and the Tyn and Calus method (1975) among others.

Le Bas method (1915) [52]

Volume increments from Le Bas are shown in Table 2-5, and the calculated values of V_b (molar volume) are compared with the experimental values. The average error for the compounds tested is 3.9% with 5 substances having errors greater than 10%.

Table 2-5. Group/ atom contributions for Le Bas and Schroeder method

	Increment (cm^3/mol)	
	Schroeder	Le Bas
Carbon	7.0	14.8
Hydrogen	7.0	3.7
Oxygen	7.0	7.4
In methyl esters and ethers		9.1
In ethyl esters and ethers		9.9
In higher esters and ethers		11.0
In acids		12.0
Joined to S, P, and N		8.3
Nitrogen	7.0	
Doubly bonded		15.6
In primary amines		10.5

In secondary amines		12.0
Bromine	31.5	27.0
Chlorine	24.5	24.6
Flourine	10.5	8.7
Iodine	38.5	37.0
Sulfur	21.0	25.6
Ring, three-membered	-7.0	-6.0
Four-membered	-7.0	-8.5
Five-membered	-7.0	-11.5
Six-membered	-7.0	-15.0
Naphthalene	-7.0	-30.0
Anthracene	-7.0	-47.5
Double bond	7.0	
Triplebond	14.0	

<u>Schroeder method (1949) [52]</u>

This method suggests a simple additive method for estimating the molar volume at normal boiling point. His rule is to count the number of atoms of carbon, hydrogen, oxygen, and nitrogen, add one for each double bond, two for each triple bond and then multiply the sum by seven. Schroeder's original rule has been expanded to include halogens, sulfur, and triple bonds. Table 2-5 gives the contributions to be used. The values in the table may be expressed in equation form as:

$$\begin{aligned} V_b = 7(N_C + N_H + N_O + N_N + N_{DB} + 2N_{TB}) + 31.5N_{Br} + 24.5N_{Cl} + 10.5N_F \\ + 38.5N_I + 21N_S \\ - 7^* \qquad (23) \end{aligned}$$

where (subscripts) DB = double bonds

TB = triple bonds

* = counted once if the compound has one or more rings

The average error for the compounds tested is 3.9% with 5 strongly polar and associated substances having errors greater than 10%.

Tyn and Calus method (1975) [52]

In this method, V_b (molar volume) is related to the critical volume by

$$V_b = 0.285 V_c^{1.048} \qquad (24)$$

where V_b = the molar volume (cm^3/mole)

V_c = the critical molar volume (cm^3/mole)

2.5.2 Diffusion Coefficients in Gases [52]

(For binary gas systems at low pressures: empirical correlations)

Several proposed methods for estimating diffusion coefficient (D_{AB}) in low-pressure binary gas systems retain the general from theory. These include the equations proposed by Arnold (1930), Gilliland (1934), Wilke and Lee (1955), Slattery and Bird (1958), Bailey (1975), Chen and Othmer (1962), and Fuller, et al. (1965, 1966, 1969). The values of D_{AB} estimated by these equations generally agree with the experimental values to within 5 to 10%, although discrepancies of more than 20% are possible.

Prediction from the ideal-gas law [52]

The theory describing diffusion in binary gas mixture at low to moderate pressures has been well developed. The theory results from solving the Boltzmann equation, and the results are usually credited to both Chapman and Enskog, who independently derived the working equation as follows:

$$D_{AB} = \frac{0.00266 T^{3/2}}{P M_{AB}^{1/2} \sigma_{AB}^{2} \Omega_{\mathrm{D}}} \qquad (25)$$

where D_{AB} = diffusion coefficient (cm^2/sec)

T = temperature (K)

M_A ,M_B = the molecular weights of A and B (g/mole)

M_{AB} = $2[(1/ M_A) + (1/ M_B)]^{-1}$

P = pressure (bar)
σ_{AB} = characteristic length (Å)
Ω_D = collision coefficient (dimensionless)

Key to the use of this equation is the intermolecular force law and the evaluation of σ_{AB} and Ω_D.

Wilke and Lee (1955) [52]

The Wilke and Lee method is rewritten as:

$$D_{AB} = \frac{\left[3.03 - \left(0.98/M_{AB}^{1/2}\right)\right](10^{-3})T^{3/2}}{PM_{AB}^{1/2}\sigma_{AB}^{2}\Omega_D} \qquad (26)$$

where D_{AB} = binary diffusion coefficient (cm^2/sec)
T = temperature (K)
M_A ,M_B = the molecular weights of A and B (g/mole)
M_{AB} = $2[(1/M_A) + (1/M_B)]^{-1}$
P = pressure (bar)
σ_{AB} = characteristic length (Å)
Ω_D = collision coefficient (dimensionless)

The scale parameter σ_{AB} for each component:

$$\sigma = 1.18V_b^{1/3} \qquad (27)$$

where V_b = the liquid molar volume at normal boiling temperature (cm^3/mole)

Ω_D is determined from this equation with $(\varepsilon/k)_{AB} = 1.15\ T_b$, T_b as the normal boiling point in kelvins:

$$\Omega_D = \frac{A}{(T^*)^B} + \frac{C}{\exp(DT^*)} + \frac{E}{\exp(FT^*)} + \frac{G}{\exp(HT^*)} \qquad (28)$$

where $T^* = kT/\varepsilon_{AB}$

A =1.06036, B = 0.15610, C = 0.19300,

D = 0.47635, E = 1.03587, F = 1.52996,

G = 1.76474, H = 3.89411

Fuller, et al. (1965, 1966, 1969)

These authors modified equation 25 and proposed the following model:

$$\boldsymbol{D_{AB} = \frac{0.00143T^{1.75}}{PM_{AB}^{1/2}\left[(\Sigma_\upsilon)_A^{1/3} + (\Sigma_\upsilon)_B^{1/3}\right]^2}} \qquad (\mathbf{29})$$

where D_{AB} = binary diffusion coefficient (cm^2/sec)

T = temperature (K)

M_A ,M_B = the molecular weights of A and B (g/mole)

$M_{AB} = 2[(1/M_A) + (1/M_B)]^{-1}$

P = pressure (bar)

Σ_υ = summing atomic diffusion volumes in Table 2-6

Table 2-6. Atomic Diffusion Volumes

Atomic and Structural Diffusion Volume Increments			
C	15.9	F	14.7
H	2.31	Cl	21.0
O	6.11	Br	21.0
N	4.54	I	29.8
Aromatic Ring	-18.3	S	22.9
Heterocyclic ring	-18.3		
Diffusion Volumes of Simple Moleculars			
He	2.67	CO	18.0
Ne	5.98	CO_2	26.9
Ar	16.2	N_2O	35.9
Kr	24.5	NH_3	20.7

Xe	32.7	H_2O	13.1
H_2	6.12	SF_6	71.3
D_2	6.84	Cl_2	38.4
N_2	18.5	Br_2	69.0
O_2	16.3	SO_2	41.8
Air	19.7		

2.6 Diffusion-Ordered NMR Spectroscopy (DOSY NMR)

The capability of nuclear magnetic resonance (NMR) spectroscopy to provide valuable information regarding mixture analysis has resulted in its broad applicability to chemistry, biochemistry, biology and medicine. NMR is a good tool for studying such formulations. Unfortunately, NMR has traditionally been sensitivity-limited compared with many other analytical techniques. For a long time it has been preferred, when possible, to isolate each mixture component prior to its study by NMR rather than to analyze the whole mixture. For most scientists, the preferred methods for mixture analysis and/ or trace detection are still the chromatographic methods, generally coupled with spectroscopic methods such as mass spectrometry or NMR. Furthermore, DOSY experiments do not need complicated setups and the method can be easily standardized and automated [53-57].

DOSY NMR was first proposed about 10 years ago. The DOSY method allows measuring the translational self-diffusion of molecules in a solution. Based on the analysis of exponential decay, the spectra of mixture components can be separated depending on the value of their apparent diffusion coefficients. Diffusion measurement by NMR and especially the DOSY-type experiments are thus powerful analytical tools, which have so far been overlooked by most scientists [53, 58].

Diffusion-ordered spectroscopy (DOSY) NMR is a two-dimensional NMR experiment, in which the signal decays exponentially according to the self diffusion behavior of individual molecules. This leads to two dimensions; one dimension accounts for conventional chemical shift and the other for diffusion behavior. Because the diffusion behavior is related to the properties of an individual molecule, such as size, shape, mass and charge as well as its surrounding environment, such as solution, temperature and aggregation state, each component in a mixture can be pseudo-separated, based on its own diffusion coefficient on the diffusion dimension [54-60]. However, the main challenge still lies with the data processing techniques, which limit the wide use of DOSY in practice. There are two classes of techniques: single channel methods and multivariate methods. Single channel methods employ only part of the spectra by considering a limited chemical shift range. The other single channel method is CONTIN, especially used for polydisperse components, where the diffusion coefficient is not a single value but a range with a normal distribution [54, 59].

2.6.1. Principle of DOSY

A diffusion-ordered NMR spectrum is obtained by the use of pulsed magnetic field gradient spin-echo NMR (PFGSE-NMR) according to the different diffusions of the components in the mixture. Diffusion behavior is a measure of the translational motion of a molecule. According to the Stokes–Einstein equation, it is related to the size, shape of individual molecules and specific molecule systems, such as aggregates [54, 58].

A basic presentation of the 2D DOSY NMR experiment can be obtained with reference to Figure 2-4. DOSY NMR is a two-dimensional NMR experiment; one dimension accounts for conventional chemical shifts and the other for diffusion coefficients. Typically, a series of 1D pulsed field gradient stimulated spin-echo experiments is acquired with systematic variations of the gradient pulse amplitude (Figure 2-4A). The rate of signal decay is directly related to the diffusion coefficient of the molecule, which depends on the molecular weight and other hydrodynamic properties (size, shape, charge) as well as on its surrounding environment. The diffusion coefficient can thus be estimated by analysis of the exponential signal decay. Signals from small molecules (large D) decay more rapidly than those from large molecules (small D) as the pulsed field gradient is incremented (Figure 2-4A and 2-4B). The chemical shifts (δ in ppm) lie on the horizontal axis of the Fourier-Laplace spectrum while the diffusion coefficients are on the orthogonal axis (D in $\mu m^2 s^{-1}$) (Figure 2-4D).

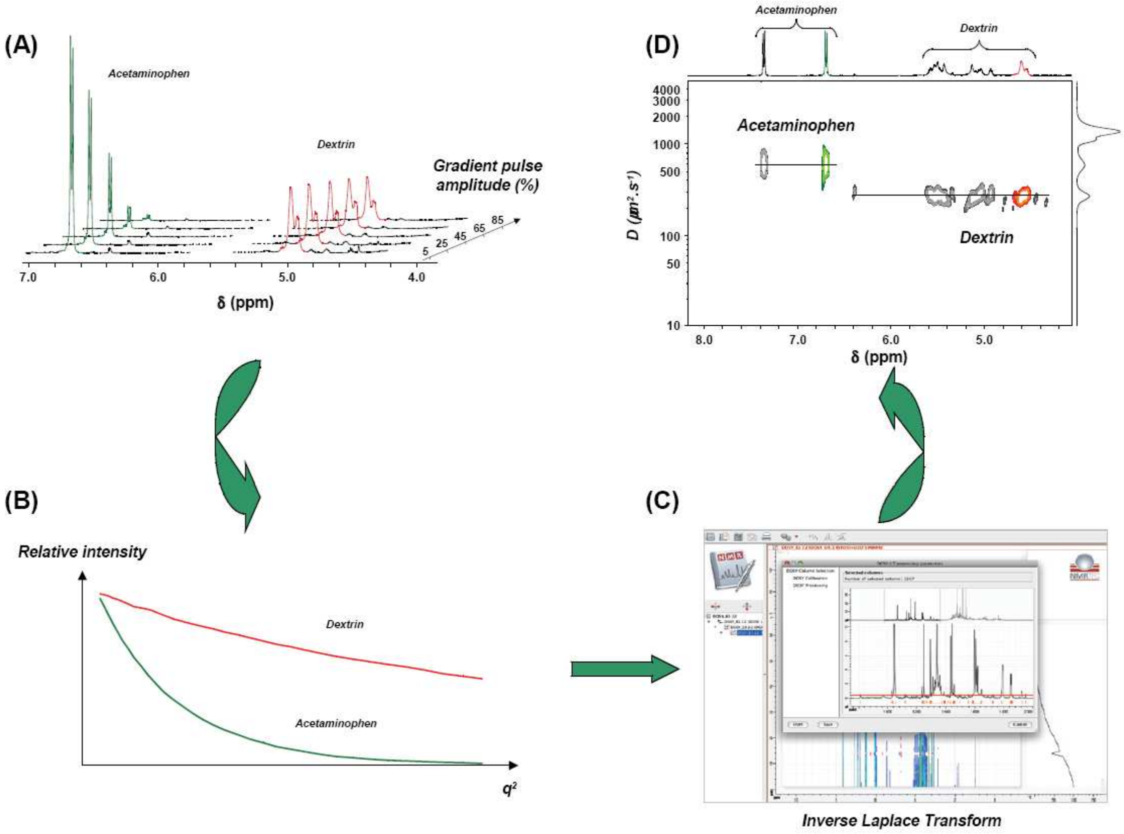

Figure 2-4. Schematic principle of the DOSY NMR experiment. (A) Pulsed field gradient stimulated spin-echo experiments at different gradient pulse amplitudes, (B) this displacement has the effect of reducing the signal intensity with an exponential law (C) data processing using the NMRnotebook package, (D) 2D DOSY ^{1}H NMR spectrum [53, 56]

2.6.2. Single Channel Methods

Method for discrete diffusion coefficient: SPLMOD

SPLMOD is a single channel method which is restricted to analyzing a system with discrete diffusion coefficient. SPLMOD intends to analyze sums of pure exponentials by performing the least square fit of this equation (30):

$$I(\upsilon, s) = \sum_{i=1}^{n} I_0(i) \exp(-\lambda_i s) + E \qquad (30)$$

where I = the in tensity of a specific frequency channel υ and its variation of exponential decay depends on the increase of s

n = the number of components

λ = D(i)(Δ - δ/3)

s = K^2

E = account for noise

Resolution of discrete components using SPLMOD with certain rejection criteria has been presented by Morris and Johnson [61]. However, with the benefit of remedial constraints, SPLMOD still suffers from the overlap problem, i.e. it is difficult to separate more than two components in one single channel. It is also very sensitive to noise and hence SPLMOD often overestimates the number of the components.

Method for continuous diffusion coefficient: CONTIN

Some samples are composed of components with continuous distributions of diffusion coefficients, e.g. polymers and aggregates. For a specific frequency channel of the data of the polydisperse system, the signal can be described by the following equation (31):

$$I(\upsilon, s) = \int_{\min\lambda}^{\max\lambda} g(\lambda)\exp(-\lambda s)d\lambda + E \qquad (31)$$

where g(λ) = the spectrum of diffusion coefficients

λ = D(i)(Δ - δ/3)

s = K^2

E = account for noise

A method called CONTIN, a constrained regularization program, attempts to solve this inverse Laplace transform problem and obtain the Laplace spectrum of the diffusion coefficients. The constraints are based on the non-negativity of the signal and decay constant, statistical prior knowledge and parsimony, which are used to solve the ill-posed problem. If the distribution is narrow, then it can be reanalyzed by SPLMOD if desired.

2.6.3. Multivariate Methods

Direct exponential curve resolution algorithm (DECRA)

DECRA is a multivariate method of calculating the pure spectrum and the corresponding decay profile of each component based on the generalized rank annihilation method (GRAM). GRAM is suitably applied to the two data sets with a correlation like the following:

$$\mathrm{A} = \mathrm{C}\mathrm{S}^{\mathrm{T}}, \qquad \mathrm{B} = \mathrm{C}\alpha\mathrm{S}^{\mathrm{T}} \qquad (32)$$

where α = a diagonal matrix, whose elements contain the constants accounting for the correlation of the two data sets

By using DECRA, however, only one experiment is needed and the data set obtained is split into two sub-matrices, A and B. DECRA is a fast algorithm in obtaining the information of a pure component in a mixture. The limitation of DECRA is that it can only be applied to discrete diffusion components with the range of about two orders of magnitude in the diffusion dimension due to the requirement of equal g^2 steps.

Multivariate curve resolution (MCR)

DOSY data has a bilinear structure and therefore the whole data set can be separated into two matrices, one representing the pure decay profiles and the other the pure spectra of the components. In MCR, DOSY data is first analyzed by principal component analysis (PCA) to obtain the abstract factors or principal components. This iteration with the application of non-negativity constraints continues until the convergence is achieved.

$$\mathrm{C} = \mathrm{I}\mathrm{S}(\mathrm{S}^{\mathrm{T}}\mathrm{S})^{-1} \qquad (33)$$

$$S = I^{T}C(C^{T}C)^{-1} \qquad (34)$$

MCR only depends on the change of intensity with the increase of the square of gradient strength g^2. The key requirement of MCR is a good initial guess of alternating least square (ALS). The main problem of MCR is in the VARIMAX rotation of the abstract factors. Obtaining the largest simplicity is the aim of VARIMAX rotation. However, a rotated factor with maximal simplicity does not necessarily mean that it is a good initial input of ALS.

CHAPTER III

EXPERIMENTAL

3.1 Materials

3.1.1 Model Flavors

3.1.1.1 Bergamot Oil (Thai-China Flavors and Fragrance Industry Co.,Ltd., Bangkok, Thailand and Takasago International Corporation, Tokyo, Japan)

Bergamot or Citrus Bergamia Risso (Figure 3-1) is a hybrid fruit from bitter orange and lemon; it is produced almost solely in the Reggio Calabria province in southern Italy, where the cultivated area is about 1500 hectares with an annual production of 25,000 tonnes. It is mainly used for its essential oil extracted from the peel [62-66] which is an important raw, widely used substance that has great commercial value. It is necessary in the international perfumery industry as it has not only the function of fixing the aromatic bouquets of perfumes, but also that of blending all the other essences contained in them, exalting notes of freshness and fragrance [65]. Bergamot essential oil is used in the pharmaceutical industry because of its antibacterial and antiseptic activity, mainly in dentistry, ophthalmology, gynecology, and dermatology, in the pharmaceutical industries for its antiseptic and antibacterial proprieties, in the cosmetic industries such as in perfumes, body lotions, soaps, and aromatherapy [64, 65, 67]. Bergamot oil is also used in the food and confectionery industries as a flavoring for liquors, teas, toffees, candies, ice creams, and soft drinks [65]. Bergamot peel represents about 60% of the processed fruits and is regarded as primary waste; if not processed further, it may cause environmental problems because of its fermentability. However, bergamot peel contains very useful compounds, such as pectins and flavonoids [67-69].

This essential oil is obtained by pressing the peel of the bergamot fruit. Bergamot oil comprises volatile (93-96% of total) and non-volatile (4-7% of total) compounds[66]. A variable percentage of the essential oil (4-7%) consists of nonvolatile compounds such as pigments,waxes, and above all coumarins (e.g., citropten) and psoralens (e.g., bergapten and bergamottin) [63]. The most important contribution to the flavor of the oil comes from oxygenated compounds, such as linalool, neral, geranial, neryl acetate, geranyl actate and linalyl acetate, whereas monoterpenes, such as *d*-limonene, α- and β-pinene β-myrcene, ɣ-terpinene, terpinolens, sabinene, β-bisabolene, tend to decompose, producing off-flavor compounds upon heating or contact with air [62, 66]. The composition of bergamot oil is determined using GC analysis. The key constituents, *d*-limonene (38.8%), γ-terpinene (9.1%), linalool (16.6%), and linalyl acetate (21.4%), are confirmed by comparison of mass spectra and retention times with those of pure standards [62, 63, 70]. As compared with other citrus oils, bergamot oil as characterized by Verzera et. al showed a lower amount of *d*-limonene (25.6-53.0%) and high qualities of oxygenated compounds, such as linalool (1.7-20%) and linalyl acetate (15.6-40.4%) [62].

Figure 3-1. Bergamot fruit, root and essential oil

3.1.1.2 *d*- Limonene (Purity: 95%, Nacalai Tesque, Kyoto, Japan)

d-Limonene is the major flavor compound present in orange oil. It is a widely used oil in the perfumery industry, as the test active principle. The main component of citrus essential oils is *d*-limonene which is commonly extracted from their matrix by using distillation [71]. *d*-Limonene, which is the solvent used in this study is a major by-product of the citrus fruits industry. The chemical structure is shown in Figure 3-2. This monoterpenic molecule (Figure 3-2) is the major component of essential oils extracted from citrus peels and plays an important role in the field of flavor and fragrances [71, 72].

d-Limonene (Figure 3-2), a well-known non-polar flavoring compound, is encapsulated in different wall material such as gum arabic [73, 74], starch [75], cyclodextrin [1], gellan gum, pectin [76], maltodextrin [10] and combinations of maltodextrins with gum arabic by spray drying [9, 74]. In our research, *d*-limonene was purchased from Nacalai Tesque (Kyoto, Japan)

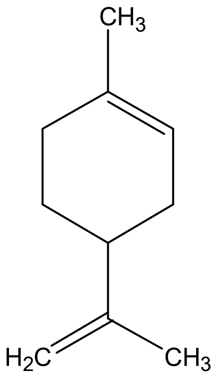

Figure 3-2. Chemical structure of *d*-limonene

3.1.1.3 Linalool (Purity: 98%, Wako Pure Chemical Industries, Osaka, Japan)

Linalool is a monoterpene (Figure 3-3) commonly found as the major volatile component of essential oil from aromatic plant species. These results strengthen the suggestion that inhaling linalool rich essential oils can be useful as a means to attaining a relaxed state and counteracting anxiety [77]. Linalool is a fragrance ingredient used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its worldwide use is in the region of greater than 1000 metric tonnes per annum [78, 79].

Linalool is the major component of the essential oil produced by several well-known species including Lavandulaaugustifolia Mill., Melissaofficinalis L., Rosmarinusofficinals L. and Cymbopogoncitratus DC. Interestingly [80, 81]. Linalool is an essential oil of bergamot, and it is also found in coriander and rosewood [82]. In our research, it was purchased from Wako Pure Chemical Industries (Osaka, Japan).

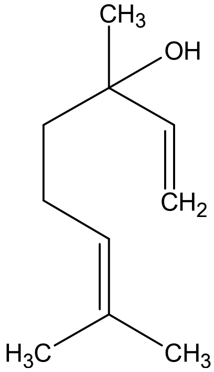

Figure 3-3. Chemical structure of linalool

3.1.1.4 Linalyl acetate (Purity: 95%, Tokyo Chemical Industry, Tokyo, Japan)

Linalyl acetate (Figure 3-4) is a monoterpene compound like linalool and is used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries as well as in non-cosmetic products such as household cleaners and detergents. Its worldwide use is in the region of >1000 metric tons per annum [83]. Linalool is the principal component of many essential oils known to possess several biological activities, attributable to these monoterpene compounds [84].

The industrial uses of linalyl acetate and linalool are different. Linalyl acetate is highly appreciated as a food additive because of its flavor, while linalool is used as a natural insecticide or pesticide as well as in food and fragrance applications. Normally, the market value of the oil increases with the proportion of linalyl acetate [85]. In our research, linalyl acetate was purchased from Tokyo Chemical Industry (Tokyo, Japan)

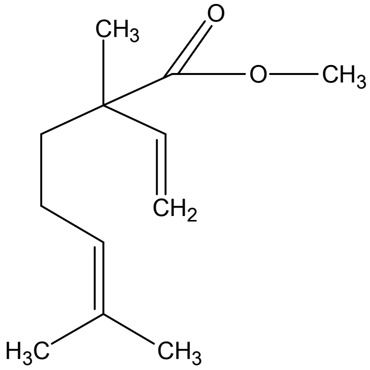

Figure 3-4. Chemical structure of linalyl acetate

Table 3.1 Physicochemical characteristics of flavor compounds

Chemical	Molecular weight (g/mole)	Boiling point (^{o}C)	Solubility (mg/l)	Functional group
Linalool	154.24	198	1589	Alcohol
Linalyl acetate	196.29	220	20	Ester
d-Limonene	136.23	178	Low	Hydrocarbon

3.1.2 Wall Materials

3.1.2.1 HI-CAP 100 (Food and Cosmetic Systems, Bangkok, Thailand)[Ref]

HI-CAP 100 is an n-octenyl succinic anhydride (OSA) modified starch blended with high dextrose (DE= 32-37%) corn syrup solids. HI-CAP 100 is a modified food starch derived from waxy maize and characterized by excellent resistance to oxidation.

HI-CAP 100 is a food starch specifically designed for the encapsulation of high levels of volatile flavor oils. Its low viscosity at high solids, strong oil retention properties, excellent oxidation resistance, even at high oil loads and above all consistency allow for very significant improvements in process efficiency and product quality over traditional encapsulation systems.

[Ref] National Starch & Chemical. 1999 Product data sheet: HI-CAP 100. National Starch & Chemical, A Member of the ICI Group, Manchester, UK

3.1.2.2 CAPSUL (Food and Cosmetic Systems, Bangkok, Thailand) [Ref]

CAPSUL which is a modified food starch derived from waxy maize, is also modified with n-octenyl succinic anhydride (OSA). It is especially suited for the encapsulation of flavor, clouds, vitamins and spices. It is an excellent replacement for expensive gums and proteins commonly used for these applications.

A unique feature of CAPSUL when compared to other encapsulating agents, such as gum arabic, is its ability to form very stable oil-in-water emulsions. The fine particle size of the emulsion results in reduced losses during spray drying.

[Ref] National Starch & Chemical. 1999 Product data sheet: CAPSUL. National Starch & Chemical, A Member of the ICI Group, Manchester, UK

3.1.2.3 Gum arabic (Nacalai Tesque, Kyoto, Japan)

Gum Arabic, also known as acacia gum, is a natural product from two species of acacia tree and it is normally collected by hand when dried. This natural gum is usually free of color, odor and taste.

The main chemical composition of gum arabic is polysaccharide and glycoprotein. Gum arabic is used primarily in the food industry as flavor encapsulating material. Gum arabic has an emulsifier property from glycoprotein, is bland in flavor and provides good retention of volatiles during the drying process.

3.1.2.4 Maltodextrin (Nacalai Tesque Inc., Kyoto, Japan)

Maltodextrin is produced from starch by partial hydrolysis with amylase. It consists of D-glucose units connected in chains of variable length. Maltodextrin can dissolve in cold water and has film forming properties. With these properties, maltodextrin solution is transparent with low viscosity. The degree of hydrolysis is measured by the dextrose equivalent for 100 grams of solids. The higher DE, the more the product is hydrolyzed. In our research, the used maltodextrin was DE 25(Pindex 3).

3.1.3 Analytical chemicals

3.1.3.1 Potassium bromide (KBr)

3.1.3.2 Hexane

3.1.3.3 Isopropanol

3.1.3.4 Distilled water

3.1.3.5 Acetone-D6

3.1.3.6 Dimethyl sulfoxide-D6 (DMSO)

3.2 Apparatus

3.2.1 Spray Dryer

3.2.1.1 Mini Spray Dryer B-290 (BUCHI Labortechnik AG, Switzerland)

Specifications:
Model : B-290
Water evaporation rate: 1 L/hr
Nozzle diameter : 0.7 mm
Dimension (L×W×H) : 60×50×110
Application : Preparation the encapsulated flavor powder

3.2.1.2 Ohkawara L-8 (Ohkawara Kakouki Co., Ltd., Yokohama, Japan)

Specifications:
Model : Ohkawara L-8
Water evaporation rate: 3 kg/hr
Atomizer : Rotating disk
Chamber : 800 mm I.D., 560 mm high and conical chamber of 650 mm high with 60^{o} angle
Application : Preparation the encapsulated flavor powder

3.2.2 Homogenizer

3.2.2.1 High Speed Homogenizer (T-25 ULTRA-TURRAX, IKA, USA)

<u>Specifications:</u>
Model : T-25
Rotating speed: 24000
Display : Digital display
Shaft : Model S25N-18G, Diameter 18 mm, Length 194 mm
Capacity : 1-2000 ml
Application : Preparation the emulsion

3.2.2.2 Polytron Homogenizer (Kinematica GA, Littau, Switzerland)

<u>Specifications:</u>
Model : PT 6100
Rotating speed: 24000
Display : Digital display
Shaft : Model GR, PTA45/6, Diameter 45 mm, Length 270 mm
Capacity : 200-4000 ml
Application : Preparation the emulsion

3.2.2.3 High Pressure Homogenizer (M-110P Laboratory Models, USA)

Specifications:
Model : M-110P
Flow rate : 110-155 ml/min
Pressure range : 5000-10000 psi
Minimum sample size : 50 ml
Application : Preparation the fine droplet emulsion

3.2.3 Particle size analysis

3.2.3.1 Laser Scattering Particle Size Distribution Analyzer (LA-950V2, HORIBA, Kyoto, Japan)

Specifications:
Model : LA-950V2 connected with batch and flow cell sampler
Size range : 0.01-300 µm
Application : Analysis emulsion droplet and particle size

3.2.3.2 Laser Diffraction Particle Size Analyzer (SALD 7100, Shimadzu Corp., Kyoto, Japan)

Specifications:
Model : SALD 7100 connected with batch cell sampler
Size range : 0.01-300 µm
Application : Analysis emulsion droplet and particle size

The average diameter and size distribution of emulsion droplet, reconstituted emulsion and encapsulated powder size were measured by a laser scattering particle size distribution analyzer. Distilled water was used as the dispersant in emulsion and reconstituted emulsion.). In order to determine the change in emulsion droplet size during atomization, the reconstituted emulsion droplet size was also measured by gently mixing 1.0 gram of the spray-dried powder with 9.0 ml of distilled water before the measurement. In order to investigate the size distribution of spray dried powder, the powder was dispersed in isopropanol and analyzed by a laser scattering method with a batch cell unit. Each sample was analyzed in duplicate and the data were reported as an average.

The specific surface area was calculated with the volume surface mean diameter, D_{32} [12]

$$D_{32} = \frac{\sum_i z_i D_i^3}{\sum_i z_i D_i^2}$$

(1)

$$SSA = \frac{6}{D_{32}}$$

(2)

where D_{32} is the volume surface mean diameter, z_i is the number of droplets of diameter D_i and SSA is the specific surface area. Each sample was measured and calculated in duplicate. The data were presented as an average.

3.2.4 Scanning Electron Microscopy (JSM-6060, JEOL Co., Ltd., Tokyo, Japan)

Specifications:

Model	: JSM-6060
Application	: Investigation of the internal and external structure spray-dried powder

3.2.5 Magnetron Sputter (Vacuum Device Inc., Japan)

Specifications:

Model	: MSP-1S
Rotary pump	: 20 L/min
Target size	: diameter 55 mm
Coating material	: Pt-Pd
Application	: Coat the powder sample before observation by SEM

The internal and external morphology of the spray dried powders were determined by scanning electron microscopy (JSM-6060, JEOL Co., Ltd., Tokyo, Japan). The powders were placed on the SEM stub using two-sided adhesive tape (Nisshin EM Co. Ltd., Tokyo, Japan). The samples were coated with Pt-Pd using a Model MSP-1S magnetron sputter coater (Vacuum Device Inc., Tokyo, Japan). The coated samples were analyzed using the SEM operating at 20 kV.

3.2.6 Gas Chromatograph

3.2.6.1 Gas Chromatograph (GC-14B, Shimadzu Corp., Kyoto Japan)

Specifications:

Model	: GC-14B
Detector	: FID
Column	: Packed column with PEG-20M
Carrier gas	: Nitrogen
Application	: Analysis of flavor release content

3.2.6.2 Gas Chromatograph (GC-17A, Shimadzu Corp., Kyoto Japan)

Specifications:
Model : GC-17A
Detector : FID
Column : Capillary column (HR1)
Carrier gas : Nitrogen
Application : Analysis of flavor content in powder

3.2.6.3 Gas Chromatograph (GC MS-QP5050A, Shimadzu Corp., Kyoto Japan)

Specifications:
Model : GC MS-QP5050A
Detector : FID
Column : Capillary column (HR1)
Carrier gas : Nitrogen
Application : Analysis of flavor composition in powder

3.2.7 Fourier Transform Infrared Spectroscopy (FT-IR) (Nicolet 6700, Madison, WI, USA)

Specifications:
Model : Nicolet 6700
Application : Analysis of chemical functional group in modified starch

The chemical functional group of HI-CAP 100 was investigated after passing through the high pressure homogenizer (M-110P Laboratory Models, USA). HI-CAP 100 was dissolved in warm distilled water at 40 wt%. Then, the hydrated solution passed through a high pressure homogenizer at 500, 1000, 1500 bar for 1 and 3 cycles. These solutions were dried using freeze drying. The chemical functional group of high pressurized HI-CAP 100 was investigated by using Fourier transform infrared (FT-IR) spectroscopy (Nicolet 6700, Madison, USA). Samples were prepared by grinding the modified starch with KBr. The spectrum was recorded in the wavenumber range from 400 to 4000 cm^{-1}.

3.2.8 Viscometer (R/S plus, BROOKFIELD, Brookfield Engineering Laboratories, INC., MA, USA)

Specifications:
Model : R/S plus
Speed : 0.01-200 rpm
Temperature range : -20-250^{o}C
Application : Measurement of feed emulsion viscosity

The emulsion viscosity was measured by a rheometer (R/S plus, BROOKFIELD, USA). This instrument is equipped with a temperature-controlled bottom plate. The samples were placed in the temperature controlled measurement vessel at 25^{o}C. Each sample was analyzed in duplicate and the data were reported as an average.

3.2.9 Nuclear Magnetic Resonance (NMR) Spectroscopy (Bruker 600 Ultrashield, USA) and data will be analyzed using Bruker BioSpin XWINNMR software.

Specifications:
Model : Bruker 600 Ultrashield
Type of measurement : 1H, 13 C, 15 N
Application : Estimation of flavor compound diffusion coefficient

3.2.10 Sieve Shaker or Electromagnetic Vibro Sifter (TSUTSUI SCIENTIFIC INSTRUMENTS CO LTD, Tokyo, Japan)

Specifications:
Model : M 100
Application : sieve spray dried powder

3.2.11 Home-Made Dynamic Vapor Sorption System (Food engineering Lab., Department of Applied Biological Science, Kagawa University, Japan)

Specifications:

Application : test stability of spray dried powder

3.3 Methodology

3.3.1 Preparation of Dissolved Solid and Emulsion

All emulsions were oil in water emulsion type. The aqueous phase or hydrated solution of emulsion was prepared by dissolving the wall material in warm distilled water by using hot plate stirrer. The temperature of the hot plate stirrer was adjusted to 50 ^{o}C for 4 hours and the hydrated solution was stirred overnight at room temperature. Then flavor (oil phase) was added to the hydrated solution. The coarse emulsions were produced by a high speed homogenizer at the rotation speed of 8000 rpm for 3-5 minutes. To study the effect of the emulsification process on the size of the emulsion, coarse emulsions were further homogenized by a high pressure homogenizer at 500, 1000, 1500 bar from 1 to 5 cycles.

Table 3-2. Composition of flavor and wall material in emulsion

Part	Flavor	Wall material	Solid content (% w/w)	Ratio of flavor to wall material	Emulsification process
1	Bergamot oil	HI-CAP 100	40	1:4	S, P

	Bergamot oil	GA	10	1:4	S, P
2	Bergamot oil	CAPSUL	40	1:4, 1:8	S
	Bergamot oil	HI-CAP 100	40	1:4, 1:8	S
3	Bergamot oil	HI-CAP 100	10-40	1:4	S
	d-limonene	HI-CAP 100	10-40	1:4	S
	Linalool	HI-CAP 100	10-40	1:4	S
	Linalyl acetate	HI-CAP 100	10-40	1:4	S
4	*d*-limonene	HI-CAP 100	30	1:4	S
	Linalool	HI-CAP 100	30	1:4	S
5	Linalyl acetate	HI-CAP 100	10-30	1:2, 1:4	S
		HI-CAP 100 and MD	10-30, 10-30	1:4	S
		GA and MD	10, 30	1:4	S

Note: S is high speed homogenizer and P is high pressure homogenizer.

3.3.2 Preparation of Encapsulated Powder by Spray Drying

The emulsion was transformed to encapsulated powder using a spray dryer. The spray dryer was integrated with a two-fluid nozzle (Model B-290, BUCHI Labortechnik AG, Switzerland) or a centrifugal atomizer (Okawara-L8, Japan). The compressed air was used to disperse the liquid body into fine droplets which were subsequently dried in the cylinder. Feed emulsion and hot air were in the spray dryer as a co-current. The flavor retention was analyzed by solvent extraction. The spray-dried powder were collected and stored in a sealed bottle at -20 ^{o}C until analysis.

Table 3-3. Spray drying condition for each experiment

Part	Flavor	Wall material	Type of atomization	Air inlet temperature (^{o}C)	Emulsion feed rate (ml/min)
1	Bergamot oil	HI-CAP 100	N	120-180	3-9
2	Bergamot oil	CAPSUL	N	180	9
	Bergamot oil	HI-CAP 100	N		
3	Bergamot oil	HI-CAP 100	C	200	30
	d-limonene	HI-CAP 100	C		

	Linalool	HI-CAP 100	C		
	Linalyl acetate	HI-CAP 100	C		
4	*d*-limonene	HI-CAP 100	C	200	30
	Linalool	HI-CAP 100	C		
5	Linalyl acetate	HI-CAP 100	C	200	30
		HI-CAP 100 and MD	C		
		GA and MD	C	120-200	

Note: N is two fluid nozzles and C is centrifugal atomizer.

3.3.3 The Stability of spray dried powder

3.3.3.1 Vacuum Treatment Powder and Sieved Powder

The vacuum treatment process was used to remove surface oil on the spray dried powder using a vacuum oven. About 0.2 g of the powder was placed into the bottle and covered with aluminum foil (with small holes). These bottles were put into the vacuum oven at room temperature for 2 hours. These samples were used as the vacuum treatment powder.

Sieved powder was prepared using a sieve shaker machine (M 100, Tsutsui Scientific Instruments Co., Ltd., Japan) with 5 aperture sizes (20, 32, 53, 75 and 106 µm). Approximately 2g of non-treatment powder was sieved in the fume hood for 4 min. Then the sieved powder was collected and stored in a sealed bottle until further release experiment. 32 µm and 53 µm particle sizes and no sieved powder were used to investigate the effect of particle size on release behavior.

3.3.3.2 Release Experiment with Humidity Ramping and Repeating Conditions

Both *d*-limonene and linalool powder were subjected to release experiment. Approximately 0.1 g of spray dried powder was filled into a flat bottom aluminum pan (1 mm × 13 mm i.d.). This sample was placed in a glass vial (78 mm × 15 mm i.d.) and the box which kept the vial was maintained at a temperature of 50 ^{o}C. The flow rate of moistened air which flowed through the vial was set at 100 mL/min. In the case of the humidity ramping experiment, the relative humidity (RH) was ramped from 10 to 50% at the rate of 0.17 %RH/min for 4 h. For the humidity repeating method, RH was controlled at 0% and 50%RH. Gas Chromatograph (GC 14B, Shimadzu Corp., Kyoto, Japan) was used to continuously monitor the release of flavor release from the spray dried powder. Flavor release from the vial was collected with a 5.0 mL sampling loop and introduced every 5 min into the gas chromatograph. The gas sample was separated in a glass column (2.1m × 3.2 mm i.d.) packed with PEG-20M (20% on Chromosorb W 80/100 AW mesh, Shinwa Chemical Industries, Ltd, Kyoto, Japan). The conditions used in the gas chromatograph were as follows: flame ionization detector (FID) at 200 °C with N_2 as the carrier gas, inject temperature for d-limonene and linalool: 140 and 180 °C, respectively; column temperature for d-limonene and linalool: 130 and 170 °C, respectively. Avrami's equation was used to evaluate the release rate constant and release mechanism as reported in previous work [12].

$$R = exp[-(kt)^n] \tag{3}$$

where R is the flavor (*d*-limonene and linalool) retention, t is the storage time, k is the release rate constant, and n is a parameter representing the release mechanism. For n = 1 represents the first order release mechanism, n < 1 means diffusion-limiting reaction kinetics and n > 1 indicates rapid release.

3.3.4 Model Transformation of Linalyl Acetate in Emulsion by Incubation

Emulsion and pure flavor of linalyl acetate were used as the models solution to investigate the linalyl acetate transformation. The emulsion described in the following section and pure linalyl acetate were incubated in the water bath at 80 ^{o}C. The samples were placed in the water bath for 4 hours. Every hour, the samples were collected from the water bath in order to extract and measure the amounts of linalyl acetate transformation in emulsion and pure flavor.

3.3.5 Flavor Retention Analysis using GC and GC-MS

Flavor retention is defined as the ratio of the flavors in the powder on a dry basis to the original flavors in the feed emulsion. To determine the amount of each component of the encapsulated powder, 0.1 gram of dried powder was dissolved in 4 mL of water in a glass bottle and then 4 mL of hexane was added, followed by mixing with a vortex mixer for 1 minute. To extract flavor from mixture into the organic solvent, the mixture solution was heated in a heating block with intermittent shaking. The extraction time and temperature were 30 minutes and 90 ^{o}C, respectively. To measure the amount of flavor in the organic phase, two microliters of organic phase were injected twice for each sample into a gas chromatograph equipped with a fused silica capillary column (10 m × 0.53 mm × 2 μm film thickness, DB-WAX) using a flame ionisation detector. The conditions used were as follows: flame ionisation detector (FID): 250 ^{o}C with the N_2 as the carrier gas at 50 kPa, inject temperature: 240 ^{o}C, initial temperature: 50 ^{o}C, initial time: 1 minute, heating rate: 10 ^{o}C/min, final temperature: 200 ^{o}C, final time: 5 minutes. The calibration curve of standard solution (*d*-limonene, linalool and linalyl acetate) was used to calculate the amount of each component. To create the calibration curve of the standard solution, the 2, 4, 6, 8 and 10 microliters of *d*-limonene, linalool and linalyl acetate were added into 10 mL of hexane and measured in the same manner as above. The results were the average of the duplicates and the data were shown as an average value.

Furthermore, the chemical composition of the flavor transformation was confirmed by using gas chromatography-mass spectrometer (GC MS-QP5050A, Kyoto, Japan) equipped with a 30 m × 0.25 mm × 0.5 μm film thickness, fused silica capillary column (ULBAN HR-1, Shinwa Chemical Industries, Ltd., Kyoto, Japan) using a flame ionization detector (FID) at 230 ºC with helium gas as the carrier gas at 70 kPa. The injector temperature was maintained at 210ºC. The GC-MS temperature program was set first at 100 ºC and raised to 180 ºC at a rate of 10 ºC/min which was maintained for 2 minutes. One microliter of the sample and standard solution were injected into the GC-MS.

3.3.6 Surface Oil Analysis using GC

The surface oil content of the encapsulated powder was determined by washing with hexane. 0.5 grams of encapsulated powder was placed on filter paper and washed with 1 mL of hexane for a total of 10 times. The solution which passed through the filter paper was composed of the mixture of flavor on the surface of the dried powder and hexane. The amount of flavor was analyzed by gas chromatograph as described above. The results were the average of the duplicates in each sample.

3.3.7 Statistical Analysis

The analysis of variance (ANOVA) was used to determine the significant differences. Two-way ANOVA was selected to determine the interaction among the effects of pressure on emulsion size and the effect of carrier solution, emulsion feed rate, air inlet temperature on flavor retention and surface oil. The data presented in Table and Figure are at 95% confidence levels of the fitting values.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 The Loss of OSA-Modified Starch Emulsifier Property During the High Pressure Homogenizer for Encapsulating Multi-Flavor Bergamot Oil by Spray Drying

4.1.1 The Effect of Emulsification Method on Emulsion Size Distribution and Chemical Functional Group of Modified Starch

There are various wall materials available to use as hydrophobic flavor and oil encapsulating agents. The type of wall materials showed the influence on the retention of flavor after the spray drying because of their emulsified and film forming properties as well as the water solubility. Soottitantawat *et al.* [12] reported the influence of emulsion size on the retention of model volatile compounds in various types of wall materials. In all wall material systems, high flavor retention was reported at small sizes of emulsion in the case of insoluble flavor. On the other hand, in the case of partly-water solution flavors, the optimal emulsion size shows the highest retention of flavor. However, all results were obtained with only one model flavor in the emulsion. In this study, as shown in Table 4-1, bergamot oil, composed of both insoluble and partly-soluble flavor, was chosen as the model oil. Therefore, the effect of emulsification methods on the emulsion size of flavor was firstly studied.

As shown in Figure 4-1, the effects of the homogenized process using a high speed homogenizer (0 cycles) followed by the high pressure homogenizer (1-5 cycles at 500, 1000 and 1500 bar) on the geometric mean volume emulsion size were reported. The HI-CAP 100 was used as the carrier material compared to the conventional material of GA. When the emulsion was prepared under the same conditions of the high speed homogenizer, the emulsion of the HI-CAP 100 system showed a smaller mean emulsion size compared to the GA system. This could be explained by the different emulsifier properties of each carrier. In case of using high pressure homogenizers at 1000 bar, the mean emulsion size was decreased from about 5.0 µm to about 1.2 µm with the GA carrier solution. The mean emulsion size seemed to decrease with the increasing number cycles which passed through the high pressure homogenizers. However, when the HI-CAP 100 was used as the carrier material, the increasing number of cycles also increased the mean size of the emulsion. Furthermore, at higher operating pressure, larger emulsion size was observed. This might be due to the loss of the chemical functional group which enhanced the emulsifier properties of HI-CAP 100 and the re-coalescence of droplets during the high pressure process.

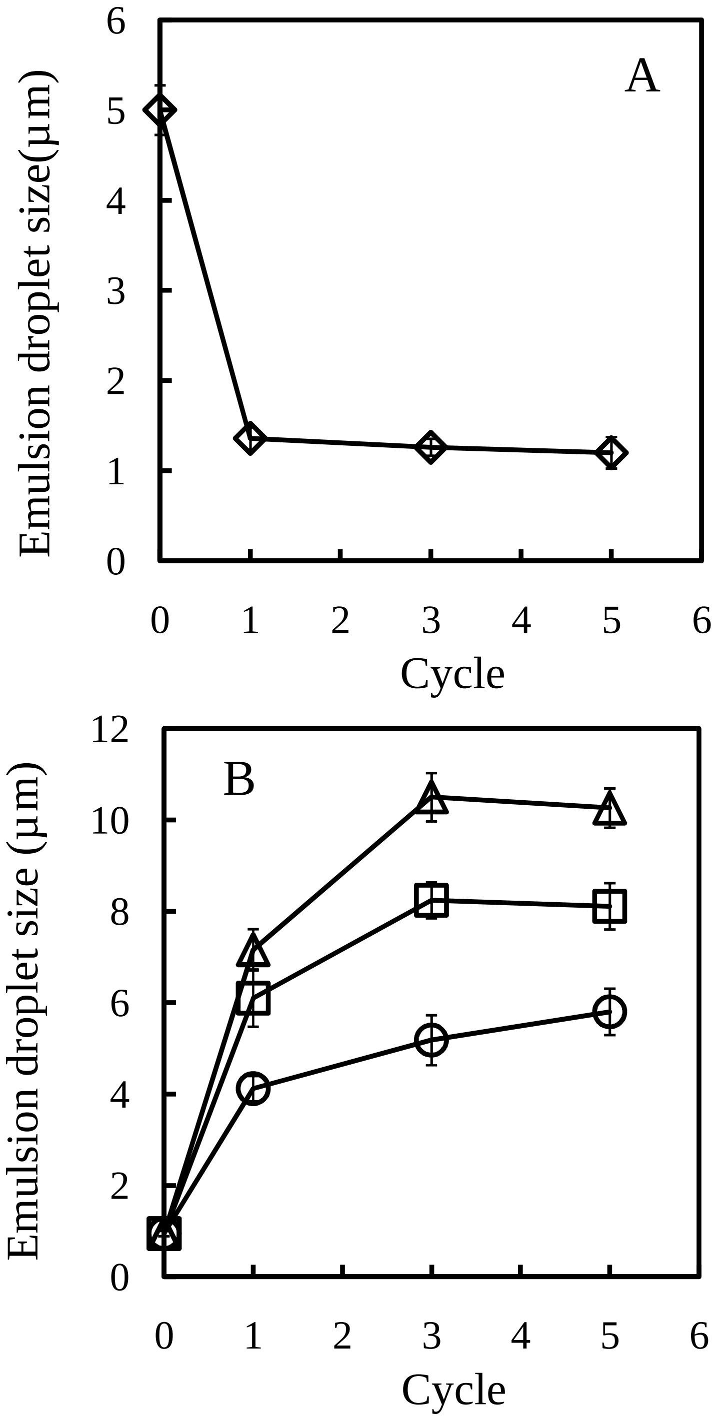

Figure 4-1. Effect of high pressure homogeniser on emulsion size of bergamot oil with different carrier solution (A) gum Arabic solution (10% solids) and (B) HI-CAP 100 solution (40% solids) ○, 500 bars; □, 1000 bars;△, 1500 bars. The error bars indicated 95% confident levels.

4.1.2 The Effect of the High Pressure Homogenizer on the Chemical Functional Groups of Modified Starch

Modified starches give some emulsifying capabilities by hydrolyzing starch with n-octenyl succinic anhydride (OSA) as HI-CAP 100 [8, 86-88]. It is noteworthy that hydrophobically modified polysaccharides, such as octyl-substituted starches, have been used as shell materials in spray drying to encapsulate up to 50% flavor oils, while still maintaining free-flowing properties [89]. From the previous results, the mean emulsion sizes of bergamot oil in the HI-CAP 100 unexpectedly increased with the use of the high pressure homogenizer process. The addition of bifunctional groups were as expected destroyed by pressure and heat from the high pressure homogenizer. Therefore, the chemical functional groups of modified starch after the high pressure homogenizer were investigated. Figure 4-2 shows the chemical structure formula of HI-CAP 100 (octenyl succinylated waxy maize starch) [86, 90] and FT-IR spectroscopy of initial modified starch and high pressure homogenized modified starch at various conditions (Figure 4-2). The FT-IR spectroscopy showed the asymmetric stretching vibration of carboxylate (RCOO-), ester carbonyl and hydroxyl groups (O-H) at 1572 cm^{-1}, 1724 cm^{-1} and 3390 cm^{-1}, respectively [86]. To determine the loss of the emulsifier properties of the modified starch, the amount of OSA groups were measured which were related to the peak intensity of carboxylate and ester carbonyl stretching. However, the FI-IR peak intensity could not be directly converted to the amount of chemical bonds. Therefore, the peak intensity ratio based on the hydroxyl groups was used to calculate the relative content of carboxylate and ester carbonyl group in the modified starch. The peak intensity of hydroxyl stretching was used due to the fact that it was the original chemical functional group in the maize starch/native starch [86, 91]. The peak intensity of carboxylate and ester carbonyl to the hydroxyl stretching of the high pressure homogenized HI-CAP 100 is shown in Table 4-2. A decrease in peak ratios with the number of cycles and pressure of the high pressure homogenization process is statistically significant ($p<0.05$). It implied that the carboxylate and ester carbonyl groups were destroyed by increasing the pressure and cycle. Jafari *et al.* [92] reported that the residence time in the high pressure homogenizer is in the range of milliseconds and the use of higher pressure leads to the collisions of droplets and makes them sensitive to coalesce. To prepare the bergamot oil emulsion from the HI-CAP 100 carrier solution, the high pressure homogenization process was not used due to the loss of the emulsifier functional group.

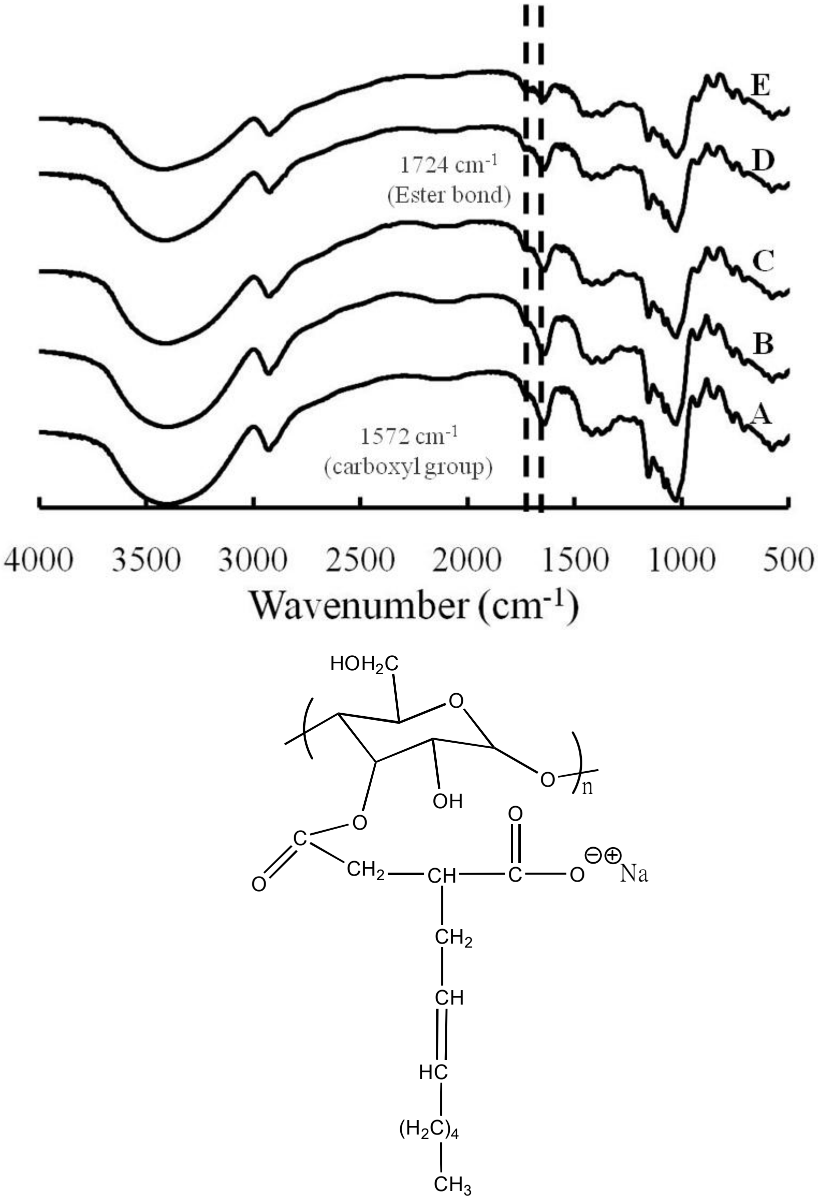

Figure 4-2. FT-IR spectra of octenyl succinic anhydride modified waxy maize starch (HI-CAP 100) and chemical structure of modified starch (octenyl succinic anhydride modified waxy maize starch). (A) native HI-CAP 100, (B-C) 1, 3 cycle, respectively at 500 bar, (D-E) 1, 3cycle, respectively at 1000 bar.

Table 4-1. Properties of main component of bergamot oil

Main component	Chemical functional	Percentage (w/w)	Solubility (mg/l)	Molecular weight (g/mol)	Boiling point (°C)
***d*-Limonene**	Hydrocarbon	40.28	Low	136.23	178
Linalool	Alcohol	17.08	1589	154.3	198
Linalyl acetate	Ester	22.33	30	196	220

Table 4-2. The peak intensity ratio[a] of HI-CAP 100

Functional groups ratio	Wavenumber (cm^{-1})	HI-CAP 100 (no treat)	Pressure (bar)			
			500		1000	
			1 cycle	3 cycles	1 cycle	3 cycles
Carboxylate/ Hydroxyl groups	1572, 3390	4.037±0.17[b]	3.64±0.04[b]	2.63±0.15[b]	2.13±0.03[b]	2.08±0.05[b]
Ester carbonyl groups/ Hydroxyl groups	1724, 3390	3.889±0.14[b]	3.53±0.07[b]	2.60±0.05[b]	2.10±0.09[b]	2.05±0.10[b]

[a] The range indicates 95% confident levels of the fitting values

[b] Mean significant difference between this variety and the other two ($p<0.05$).

Table 4-3. Effect of carrier solution on flavor retention and surfacet oil after spray drying

Type of carrier solution	**Flavor retention[a] (%)**			**Surface oil content[a](%)**		
	***d*-limonene**	**linalool**	**linalyl acetate**	***d*-limonene**	**linalool**	**linalyl acetate**
Non-Satuation	75±2.08[b]	83±1.96[b]	63±5.64[b]	0[b]	10.5±0.94	10.6±1.06
Satuation	89±1.97[b]	114±1.65[b]	91±3.36[b]	0.14±0.06[b]	0.70±0.06	0.64±0.18

[a] The range indicates 95% confident levels of the fitting values

[b] Mean significant difference between this variety and the other two (p<0.05).

Table 4-4. Effect of emulsion feed rate on flavor retention and surfacet oil after spray drying

Emulsion feed rate (ml/min)	**Flavor retention[a] (%)**			**Surface oil content[a](%)**		
	***d*-limonene**	**linalool**	**linalyl acetate**	***d*-limonene**	**linalool**	**linalyl acetate**
3[b]	81±2.36	101±2.90[b]	81±2.65	0.90±0.03[b]	2.56±0.12[b]	2.13±0.22[b]
6	88±2.72	111±6.91[b]	89±2.35	0.37±0.07[b]	1.71±0.15[b]	1.58±0.09[b]
9	89±1.97	114±2.29[b]	91±3.36	0.14±0.06[b]	0.70±0.06[b]	0.64±0.17[b]

[a] The range indicates 95% confident levels of the fitting values

[b] Mean significant difference between this variety and the other two (p<0.05).

Table 4-5. Effect of air inlet temperature on flavor retention and surfacet oil after spray drying

Air inlet temperature (°C)	**Flavor retention[a] (%)**			**Surface oil content[a](%)**		
	***d*-limonene**	**linalool**	**linalyl acetate**	***d*-limonene**	**linalool**	**linalyl acetate**
120	92±2.92	111±1.80[b]	93±3.96	1.40±0.11[b]	1.74±0.11	1.66±0.08
140	95±2.71	112±6.58[b]	93±4.55	0.29±0.07[b]	0.65±0.08	0.58±0.03
160[b]	99±1.75	120±4.07[b]	98±3.82	0[b]	0	0
180	89±1.97	114±2.29[b]	91±3.36	0.14±0.06[b]	0.70±0.06	0.64±0.18

[a] The range indicates 95% confident levels of the fitting values

[b] Mean significant difference between this variety and the other two (p<0.05).

4.1.3 The Effect of the Non-Saturated Carrier Solution of Wall Materials on Retention and Surface Oil

In the literature, saturated carrier solutions were required as prepared by dissolving the wall materials in water and then keeping the solution for at least overnight. [92-95]. However, there was no report on the effect of the saturation of the carrier solution of wall materials on the properties of dried powder as well as the flavor retention. Therefore, in this study, the influence of saturated and non-saturated carrier solution of HI-CAP 100 on flavor retention and surface oil content were investigated. The solid content of HI-CAP 100 and the mass ratio of flavor to solid were 40 %w/w and 1:4, respectively. HI-CAP 100 was dissolved in distilled water at 60^{o}C for both systems. To prepare the saturated and non-saturated carrier solutions, one of them was stirred for 4 hours in order to completely dissolve it to be as the non-saturated carrier solution. The second system was stirred and kept overnight (24 h.) at room temperature as the saturated carrier solution. The feed emulsions were prepared by a high speed homogenizer before spray drying under the same conditions as described above. The emulsion feed rate and the air inlet temperature were kept constant at 9 ml/min and 180 ^{o}C, respectively. Other operational conditions of the spray drying were kept constant.

Table 4-3 shows the effect of the saturated carrier solution on the retention and surface oil of the three components in bergamot oil (*d*-limonene, linalool, and linalyl acetate). In all flavor systems, higher flavor retention was reported when the saturated carrier solution was used to prepare the feed emulsion. The non-saturated carrier solution was affected not only as concerned flavor retention but also surface oil powder. The higher surface oil was obtained when the non-saturated carrier solution was used. The surface oil of linalool and linalyl acetate were dramatically increased by using the non-saturated carrier solution.

These results indicated an important step in preparing the saturated carrier solution. The carrier solution required saturation time even when the clear solution was prepared. This could be explained by the nature of HI-CAP 100, chemical modified starch by octenyl succinic anhydride, which can act as an interface between water and oil. Nilsson et al., studied the adsorption of modified starch at oil/water interface and found that the adsorption was governed by the interfacial area[96]. This means that the dissolving time was an important factor into ensuring that modified starch was extensively solubilized and avoided a residual granule structure [97]. The result implied that the carrier solution should be kept in the stirrer overnight.

Considering the type of flavors, the retention of linalool was unexpectedly higher than 100 % with the saturated carrier solution. This result is explained by the transformation of linalyl acetate to linalool. There were studies that reported the transformation of linalyl acetate to linalool by biotransformation [98] or catalytic reaction [99]. The high temperature during the spray drying can also increase the reaction rate of linalyl acetate. The chemical functional group of flavor was also affected in terms of flavor retention. Le Thanh et al. [100] and Goubet et al. [101] reported higher retention of alcohols than other compounds (ester and aldehydes). The results in this study agreed with the literature that the alcohol of linalool was the most retained compound. Furthermore, the surface oil content of *d*-limonene was the lowest. The water solubility of the flavors was used to explain the surface oil results. The higher water solubility flavor as linalool easily diffused with the water through the crust of the spray dried droplet which acted as a selective membrane. Therefore, the higher surface oil content was obtained. On the other hand, the low solubility flavors as *d*-limonene were more difficult to diffuse through the crust of the droplet resulting in lower surface oil content.

4.1.4 Effect of Emulsion Feed Rate on Encapsulated Powder

In order to evaluate the effect of emulsion feed rate in spray dried powder, the total retention and surface oil of *d*-limonene, linalool and linalyl acetate were investigated. In this study, wall material solutions were prepared and kept overnight to ensure a full saturation of the polymer molecules [100]. For emulsion preparation, the ratio of bergamot oil and wall material solution was 1:4 and mixed by a high speed homogenizer. Table 4-4 shows the flavor retention and surface oil content of *d*-limonene, linalool and linalyl acetate at different feed rates (3, 6 and 9 ml/min) with a constant 180 °C inlet air temperature. The results showed that an increase in emulsion feed rate increased the retention and decreased the surface oil content of each flavor. The higher emulsion feed rate decreased the drying rate of the each atomized droplet which affected the flavor retention and surface content. The high drying rate decreases the flavor retention and surface oil content since the loss of flavor from the initial evaporation is promoted. However, the low drying rate decreased the flavor retention and surface oil because of the slowly crusted formation of droplets which acts as a selective membrane resulting in the increase of flavor loss and surface oil content. These dried crust selective membranes protected against the loss of flavor[102]. That means the optimal drying rate as well as the emulsion feed rate can be expected to give the highest flavor retention and lowest surface oil content.

However, the optimal emulsion feed rate giving the highest retention and lowest surface oil was not observed in this study. It might be that the emulsion feed rate used at a 180 °C inlet air temperature is with in the suitable range to dry the powder. Even at the highest feed rate of 9 ml/min, the drying rate is high enough to immediately form a crust of droplets for preventing loss of flavor.

As for the effect of the type of flavor on the retention and surface oil with different emulsion feed rates, the results followed the same trend as in the previous section. The retention of linalool was the highest with over 100 % value. The surface oil content of *d*-limonene was the lowest. This can be explained by the transformation of linalyl acetate to linalool and the solubility of flavors as mentioned above.

4.1.5 Effect of Air Inlet Temperature on Retention and Surface Oil

The effect of air inlet temperature on the retention of multi-flavor bergamot oil after spray drying is shown in Table 4-5. The feed emulsion was spray dried at various air inlet temperatures (120-180 ºC) while the emulsion feed rate was kept constant at 9 ml/min. The highest flavor retention was found at 160 ^{o}C air inlet temperature. The flavor retention in each air inlet temperature was higher than 90%. The results were explained by the effect of the drying rate on the loss of flavor from the evaporation at the initial state of drying and the rate of semi-membrane crust formation as mentioned in the effect of emulsion feed rate. At the low inlet air temperature (120 ºC), the lower drying rate the slower crust formation resulting in low flavor retention and high surface oil content. At the high inlet air temperature (180 ºC), the higher the drying rate the higher the loss of flavor from evaporation and consequently the decrease in flavor retention and increase in surface oil content. The optimal inlet air temperature at 160 ºC was shown in this study to give the highest flavor retention and lowest surface oil content. Furthermore, the results for the type of flavors followed the same trend as in the previous sections as explained before.

Furthermore, the total retention of linalool was higher than 100% for all inlet air temperature systems. As explained before, these results should be from the transformation of linalyl acetate to linalool. The linalool retention at 180 ºC air inlet temperature was higher than the low range of air inlet temperature of between, 120 and 140 ºC. On the other hand, the linalyl acetate retention was lower at 180 ºC. This can be explained by a higher conversion of linalyl acetate to linalool at a higher temperature.

4.2 Effect of Mass Ratio of Bergamot Oil and Wall Material on Flavor Retention and Surface Oil Content

4.2.1 Effect of Mass Ratio of Bergamot Oil and Wall Material on Retention

Table 4-6 shows the effect of bergamot oil content in feed emulsion on the retention of bergamot oil during spray drying. For both wall materials, the increasing mass ratio of bergamot oil and wall material decreased the retention of bergamot oil. This is similar to previous reports [8, 10-12] and can be explained by the higher wall material content increasing the rate of the formation of the semipermeable membrane resulting in the reduction of flavor loss during spray drying according to the selective diffusion theory [13]. For example, the mass ratio of HI-CAP 100 increased from 0.125 to 0.25, the retention of *d*-limonene, linalool and linalyl acetate, which are the major chemical compounds in bergamot decreased about 10-20%. From the results, at the same oil content and solid feed concentration in the feed emulsion, HI-CAP 100 showed higher flavor retention than CAPSUL. Furthermore, as for the retention of each flavor component, in both types of wall materials, linalool showed higher flavor retention than *d*-limonene and linalyl acetate, respectively. This could be explained by their volatility (boiling point temperature) and solubility as reported by Soottitantawat et al. [6].

Table 4-6. Effect of bergamot oil content on the flavor retention

Mass ratio of bergamot oil and wall materials	**Retention of bergamot oil (%)**					
	HI-CAP 100			**CAPSUL**		
	***d*-limonene**	**Linalool**	**Linalyl acetate**	***d*-limonene**	**Linalool**	**Linalyl acetate**
1:8	95.25	100.00	76.25	87.86	98.50	67.56
1:4	75.14	87.34	62.72	55.05	66.04	47.95

4.2.2 Effect of Wall Material on Emulsion Size and Encapsulated Powder

The type of wall materials (HI-CAP 100 vs. CAPSUL) were investigated in terms of emulsion size, particle size and retention during spray drying. Emulsions were prepared by high speed homogenizer and spray-dried in the same condition. The result (Table 4-7) showed that encapsulated powder consisting of HI-CAP 100 had higher retention than CAPSUL emulsions. For instance, the retention of *d*-limonene, linalool and linalyl acetate for HI-CAP 100 was 95.25%, 100.00% and 76.25%, respectively.This was higher than that of CAPSUL emulsions (87.86%, 98.50% and 67.56% respectively). Also, the emulsion and particle size of HI-CAP 100 as wall material were 0.62 μm and 26.45 μm, which was lower than that of CAPSUL (0.79 μm and 29.62 μm).

HI-CAP 100 emulsions size (0.62μm) demonstrated smaller emulsion size than CAPSUL emulsions size (0.79μm) and could retain more oil droplets inside the microcapsules.

Table 4-7. Effect of the emulsifying agent on encapsulated powder in the ratio of 1:8

Material	**Emulsion size (μm)**	**Particle size (μm)**	**Retention of bergamot oil (%)**		
			***d*-limonene**	**linalool**	**linalyl acetate**
HI-CAP 100	0.62	26.45	95.25	100.00	76.25
CAPSUL	0.79	29.62	87.86	98.50	67.56

4.2.3 Effect of Oil Content on Encapsulated Powder

The content of bergamot oil influenced emulsion size, reconstituted emulsion size, surface oil content and particle size. According to Table 4-8, for the *d*-limonene, represented flavor, retention was decreased with the ratio of bergamot oil and HI-CAP 100 increasing. Therefore, emulsion size is an important factor for flavor retention. According to Soottitantawat et al. [6], the reconstituted emulsion size was in the range of fine emulsion which was stable during atomization and spray drying. In Table 4-6, the retention of *d*-limonene showed that increasing mass ratio of bergamot oil and HI-CAP 100 with decreasing the retention of *d*-limonene of about 20%. Figure 4-3 reveals that indentation, shrinkage and rough surface were frequently found at in the ratio (core: wall material) of 1:4 more than the ratio of 1:8. There were some spherical dried powders in the ratio of 1:4 with smooth surfaces, but there were some holes (Figure 4-3D). The presence of holes on the surface could not prevent any losses of bergamot oil. The comparison of the ratio of (bergamot oil: HI-CAP 100) 1:8 (Figure 4-3A) and the ratio of 1:4 (Figure 4-3C) indicated overall encapsulated powder. Figure 4-3A demonstrates a perfect sphere with little change in particle size while the dried powders in Figure 4-3C were cracked. These results implied that less content HI-CAP 100 was not enough to encapsulate bergamot oil, therefore a ratio of 1:4 of powder with cracks and holes to decreasing retention was found.

Table 4-8. Effect of the ratio (bergamot oil and HI-CAP 100) on encapsulated powder properties

Mass ratio (flavor: solid)	**Emulsion size (μm)**	**Reconstituted emulsion size (μm)**	**Particle size (μm)**	**Retention of *d*-limonene (%)**
1:8	0.62	0.53	26.45	95.25
1:4	1.15	0.81	32.92	75.14

In order to explain the decreasing retention of larger emulsion size, the emulsion size and reconstituted emulsion were investigated. Figure 4-4 shows the distributions size of emulsion before and after atomization when the ratio of bergamot oil and HI-CAP 100 is 1:8 (Figure 4-4A) and 1:4 (Figure 4-4B). In Figure 4-4A, the distribution size of feed emulsion and reconstituted emulsion appear unchanged, but in Figure 4-4B there is little shift in reconstituted size. For this reason, the retention of the ratio of 1:4 is less than 1:8. This shows that the powder at the ratio of 1:4 was not as stable as the ratio of 1:8 during atomization and spray drying. The unstable emulsions will reach lower retention.

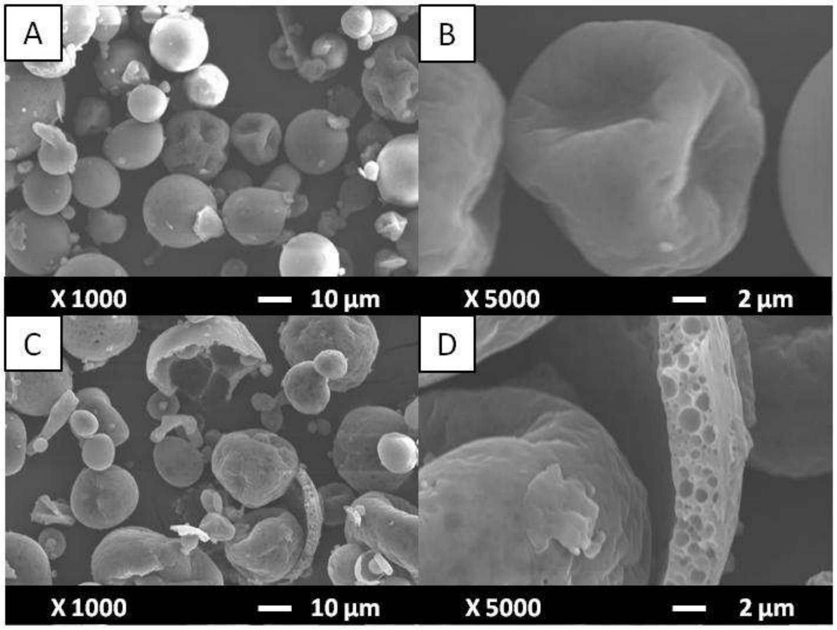

Figure 4-3. The structure of dried powder.

Core material and HI-CAP100 in the ratio (A,B) 1:8 and (C,D) 1:4

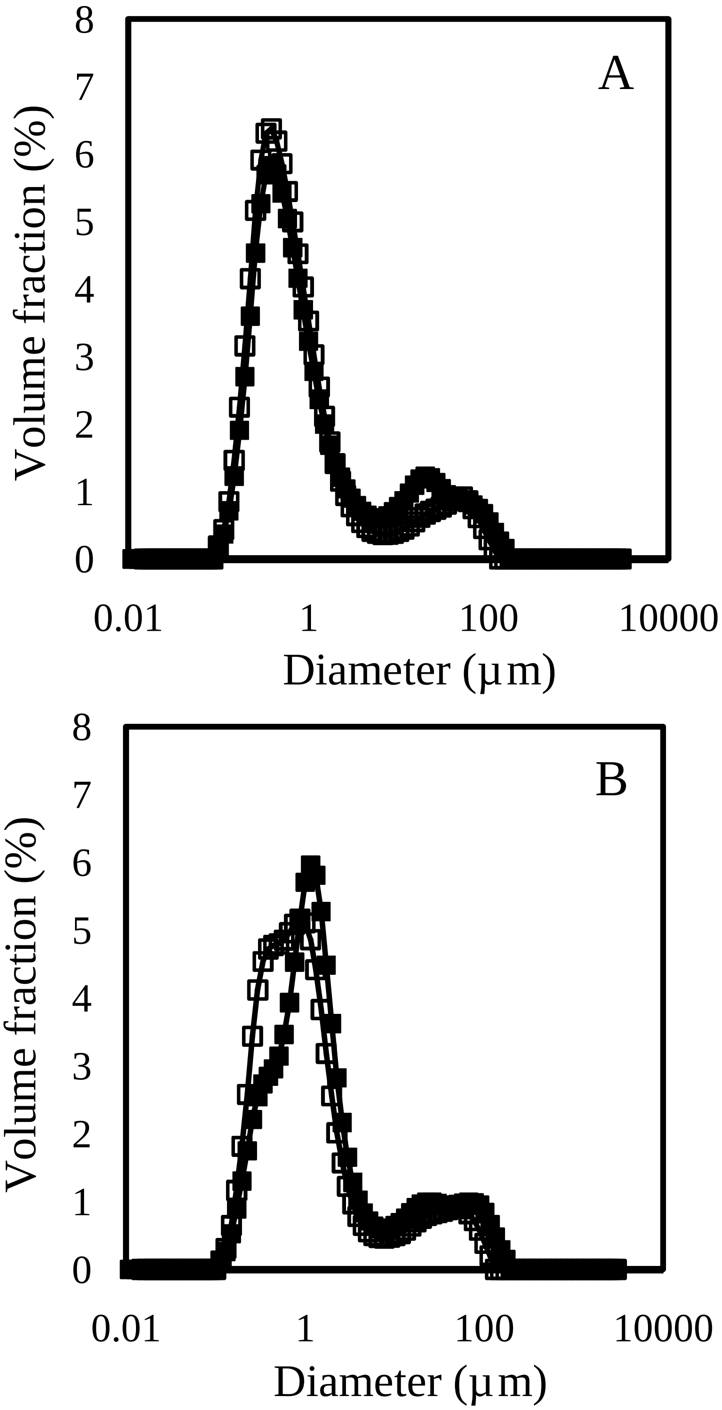

Figure 4-4. Emulsion size distribution before spray drying (closed symbols) and reconstituted emulsion size (opened symbols) in the ratio (bergamot oil and HI-CAP 100) of 1:8 (A) and 1:4 (B)

4.3 Encapsulated in Spray-Dried Modified Starch Powder

4.3.1 Effect of Feed Solution Viscosity on Emulsion Droplet Size and Spray-Dried Powder Size

The viscosity of feed solution could be controlled by varying the solid concentration of modified starch. It affects the circulation movement in the drops resulting in a rapid skin formation. Moreover, the emulsion viscosity also had influence on emulsion droplet size, reconstituted emulsion droplet size and particle size. The reconstituted emulsion droplet size was expressed through the stability of emulsion during atomization. In these experiments, the solid contents varied from 10 %w/w to 40 %w/w. Bergamot oil, which is used as multi-flavors was compared with 3 single flavors (*d*-limonene, linalool and linalyl acetate). Figure 4-5 shows the correlation of mean emulsion droplet size versus emulsion viscosity and mean emulsion droplet size versus solid content of wall material. In the case of the bergamot oil and linalyl acetate, the emulsion droplet size of the initial emulsified solution and the reconstituted solution were almost the same. However, for *d*-limonene, the reconstituted emulsion droplet size was smaller than the initial feed *d*-limonene emulsion. The results also showed that the multi flavor of bergamot oil emulsion was more stable and gave smaller emulsion droplet size than pure flavors.

Furthermore, the viscosity of the feed emulsified flavor liquid was related to the spray-dried powder size. The correlation between emulsion viscosity versus particle size and solid content versus particle size are shown in Figure 4-6. The empirical relationship between feed emulsion viscosity and mass mean powder size was $d_p = 36.5\mu^{0.24}$ with $R^2 = 0.7052$when d_p is the mass mean diameter of emulsion and μ is the emulsion viscosity.

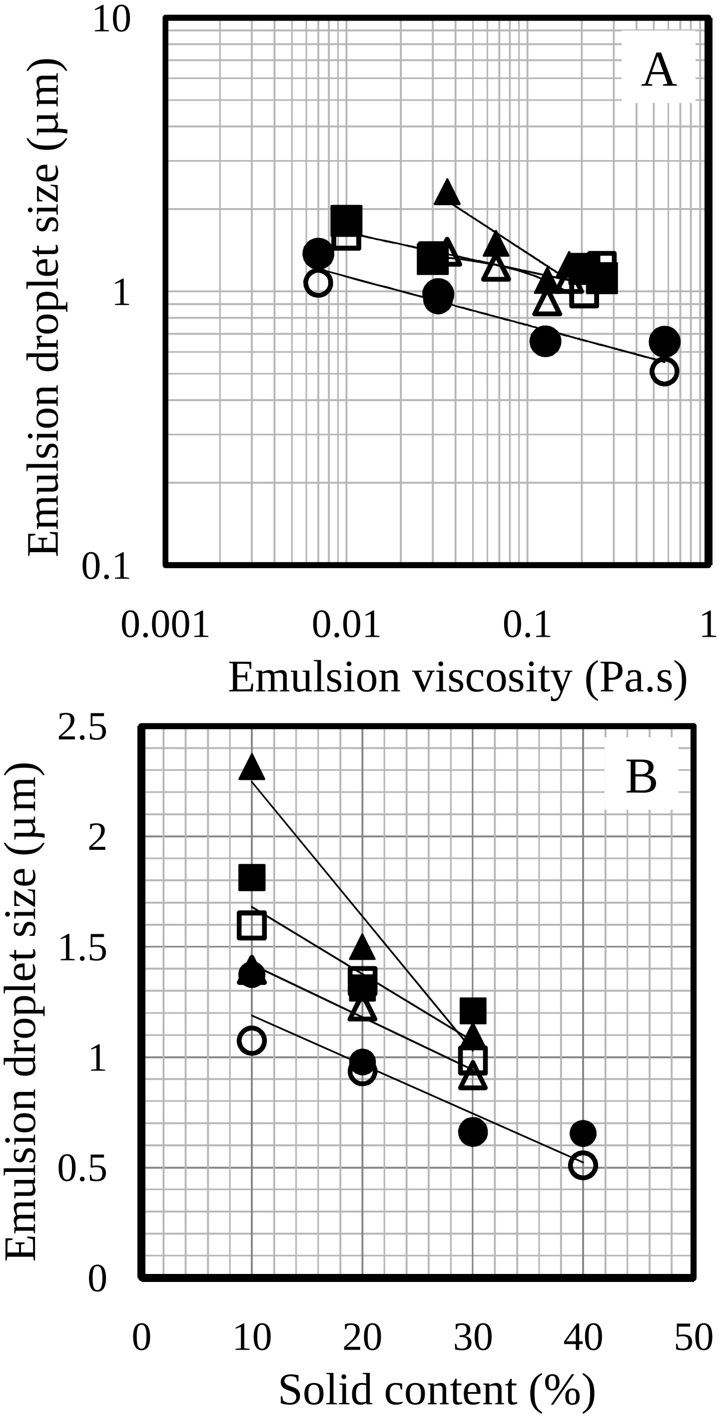

Figure 4-5. The correlation between emulsion droplet size and emulsion viscosity (A) and solid content (B). The mass ratio of flavor to solid is 1:4.

○●, bergamot oil; △▲, *d*-limonene; □■, linalyl acetate.

Closed symbols represent flavors in bergamot oil and opened symbols for pure flavor.

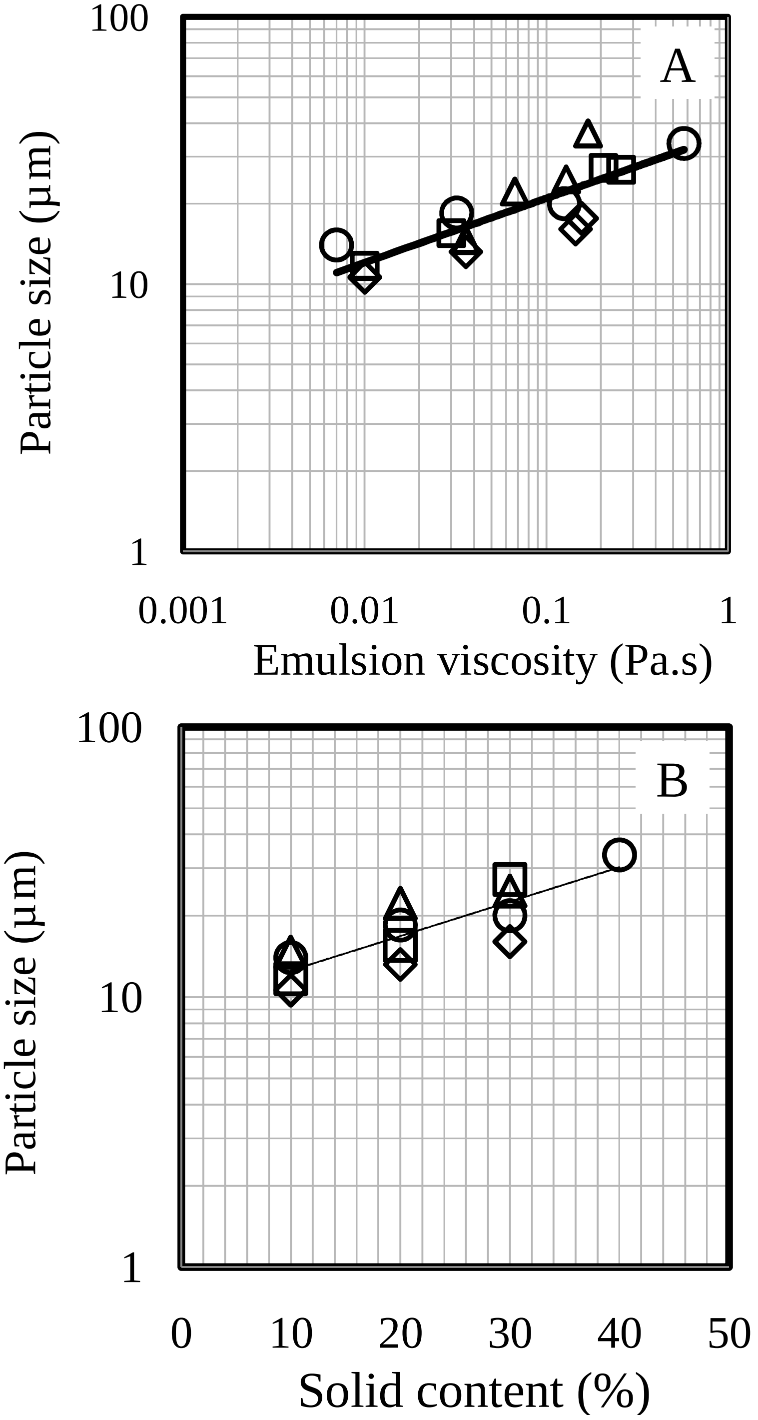

Figure 4-6. The correlation between particle size of spray-dried powder and emulsion viscosity (A) and solid content (B)

□, bergamot oil; □, *d*-limonene; □, linalool; □, linalyl acetate.

4.3.2 Effect of Emulsion Viscosity and Emulsion Droplet Size on Flavor Retention

The flavour retention of the spray-dried powders was determined at various emulsion viscosities and emulsion droplet sizes. Besides the effect of the solid content in the feed emulsion, the emulsion viscosity should also affect the flavor retention as shown in Figures 4-7, 4-8 and 4-9. For all flavor systems, the higher the viscosity the higher retention could be obtained especially when the viscosity was higher than 0.03 Pa.s. As concerns to the retention of the multi-flavors in the bergamot oil, nearly 100% flavor retention could be obtained with viscosity above 0.03 Pa.s. However, the flavor retention of the single flavor system was lower than the multi-flavor retention in the bergamot oil, especially for *d*-limonene and linalyl acetate. The interaction between each flavor was expected to have higher retention in the case of the multi-flavors in bergamot oil. Furthermore, the unexpected results could be observed in the lowest retention of linalool at the low viscosity emulsion in both the single and multi-flavor system.

Some researchers [12, 24, 103] reported that the emulsion droplet size affected flavor retention during spray drying. The flavor retention increased with decreased emulsion droplet size especially for the high hydrophobic flavor [103]. In Figure 4-9, the same results could also be observed even for the different type of flavors. Furthermore, the retention of multi-flavor in the bergamot oil was higher than the single flavor retention. These results could be explained by the smaller emulsion in the multi-flavor system resulting in higher retention. The smaller size emulsion of multi-flavor in the bergamot oil might be from the change of mixture properties. Even at the same emulsification condition, the size of emulsion from the single flavor was larger than the multi-flavor emulsion. These results also suggested that the emulsion droplet size should be less than 1 μm to obtain almost 100% flavor retention.

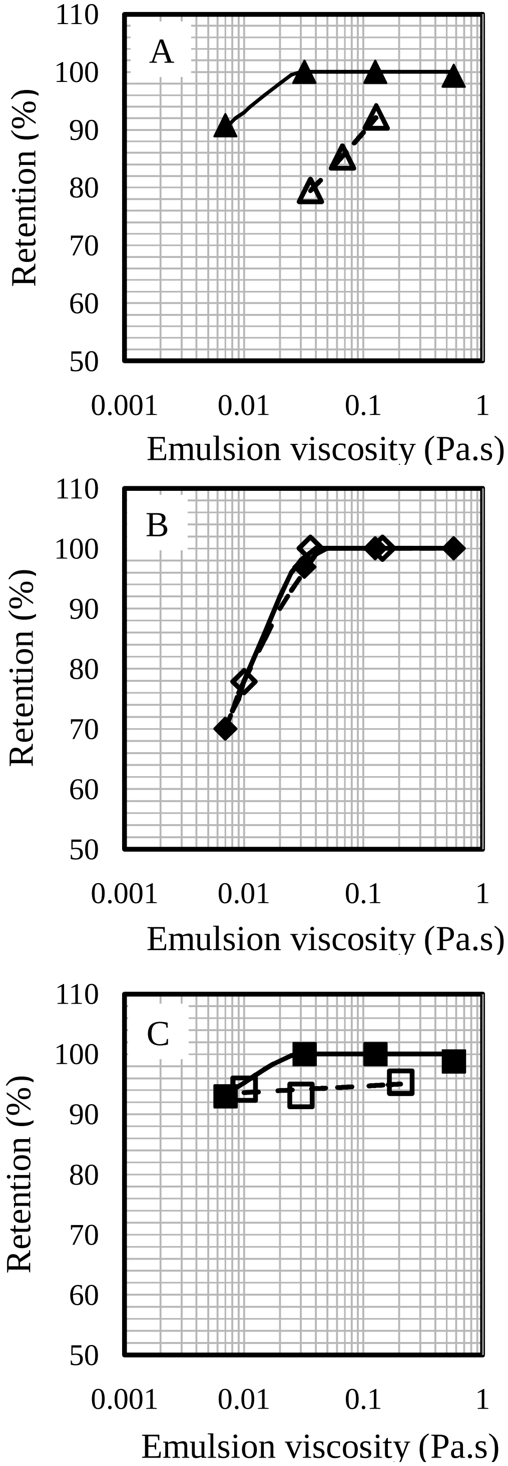

Figure 4-7. The correlation between flavor retention and emulsion viscosity (A) *d*-limonene (B) linalool (C) linalyl acetate.

Closed symbols represent flavors in bergamot oil and opened symbols for pure flavor.

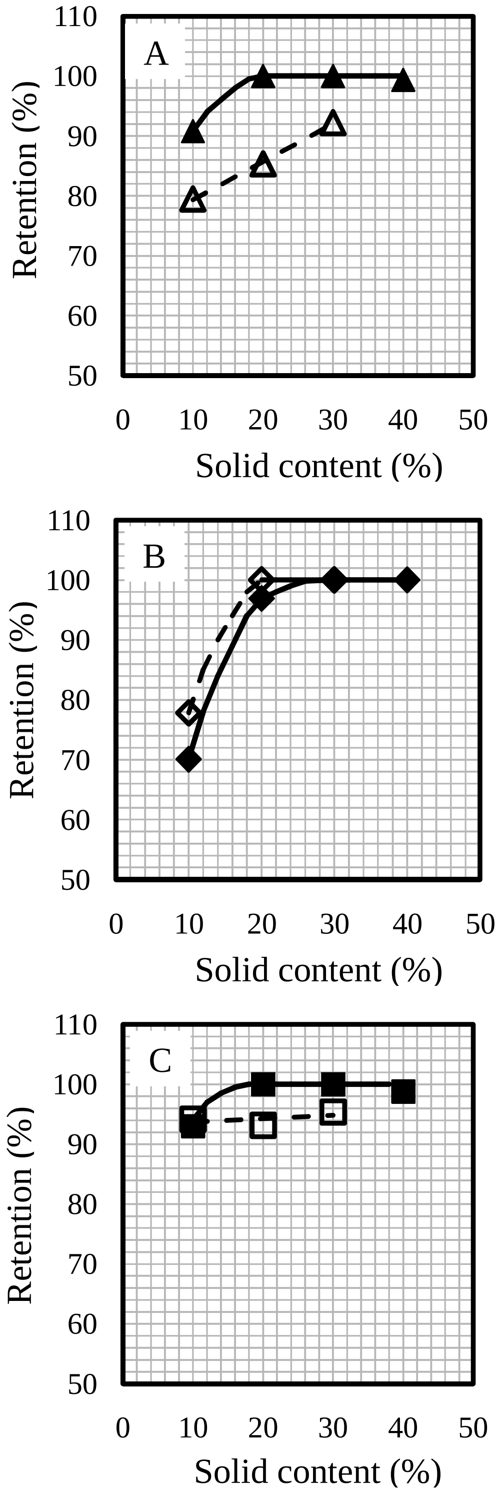

Figure 4-8. The correlation between flavor retention and solid content (A) *d*-limonene (B) linalool (C) linalyl acetate.

Closed symbols represent flavors in bergamot oil and opened symbols for pure flavor.

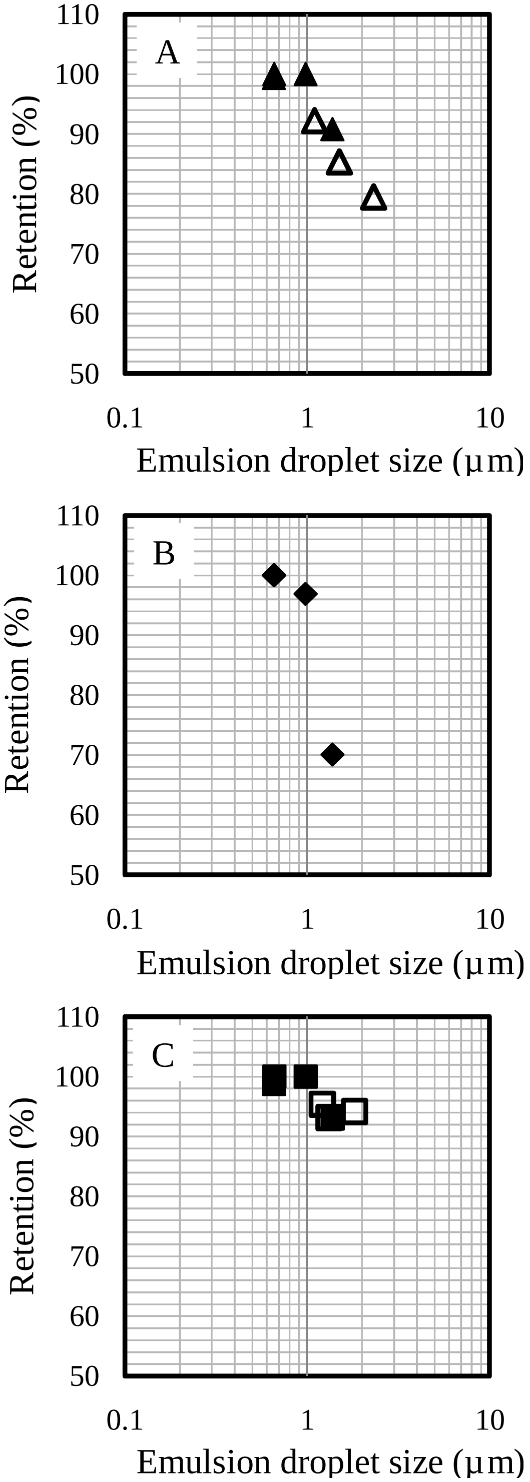

Figure 4-9. The correlation between flavor retention and emulsion droplet size (A) *d*-limonene (B) linalool (C) linalyl acetate.

Closed symbols represent flavors in bergamot oil and opened symbols for pure flavor.

4.3.3 The Morphology of Spray-Dried Powder with Different Solid Concentration

For the structure of encapsulated powder, the scanning electron microscopy was used to observe morphology and surface integrity. The morphology of spray-dried powder was related to the atomized droplet during drying. Spray-dried powders were obtained from bergamot oil emulsion at 10 %w/w- 40 %w/w of solid concentration. The external morphology of spray-dried powder is shown in Figure 4-10. The particle sizes increased with increasing the solid content. At lower solid content and low emulsion viscosity as in Figure 4-10A, the oil droplets could be observed at the outer surface. On the other hand, higher solid content was not noticed from the external morphology and the powder had a smooth surface. However, the smooth powder surface was observed more at 30%w/w and 40%w/w than with the others.

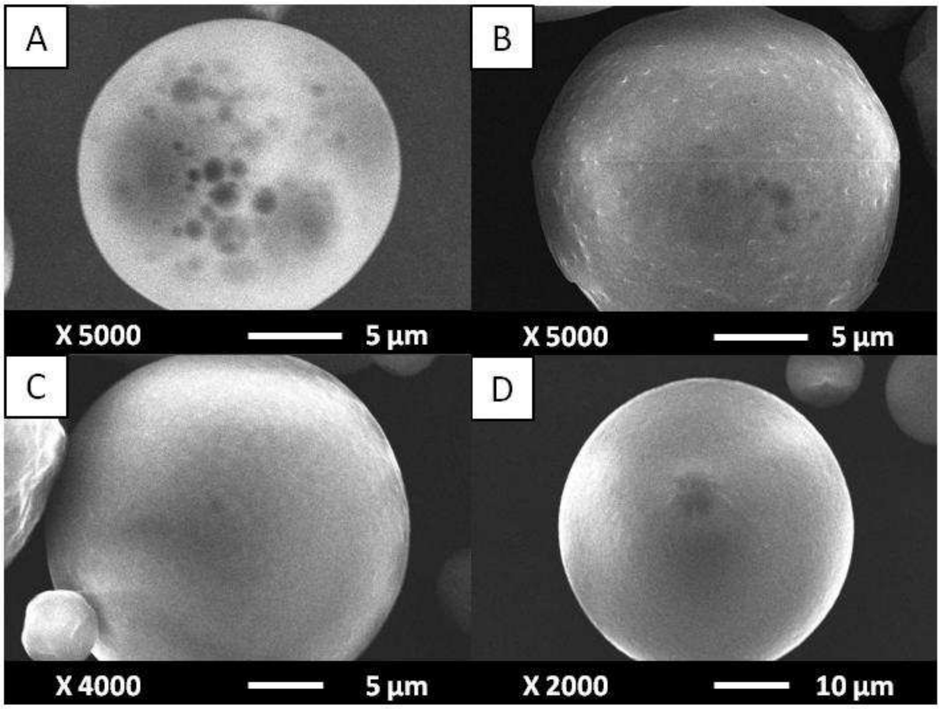

Figure 4-10. External morphology of spray-dried powders.

The mass ratio of flavor (bergamot oil) to solid is 1:4.

Solid concentration: (A) 10%w/w, (B) 20%w/w, (C) 30%w/w, (D) 40%w/w

Moreover, the internal structure of spray-dried powders was presented in Figure 4-11. Oil droplet was observed and embedded in the shell of wall matrix. These SEM photographs were also confirmed the emulsion droplet size in spray-dried powder.

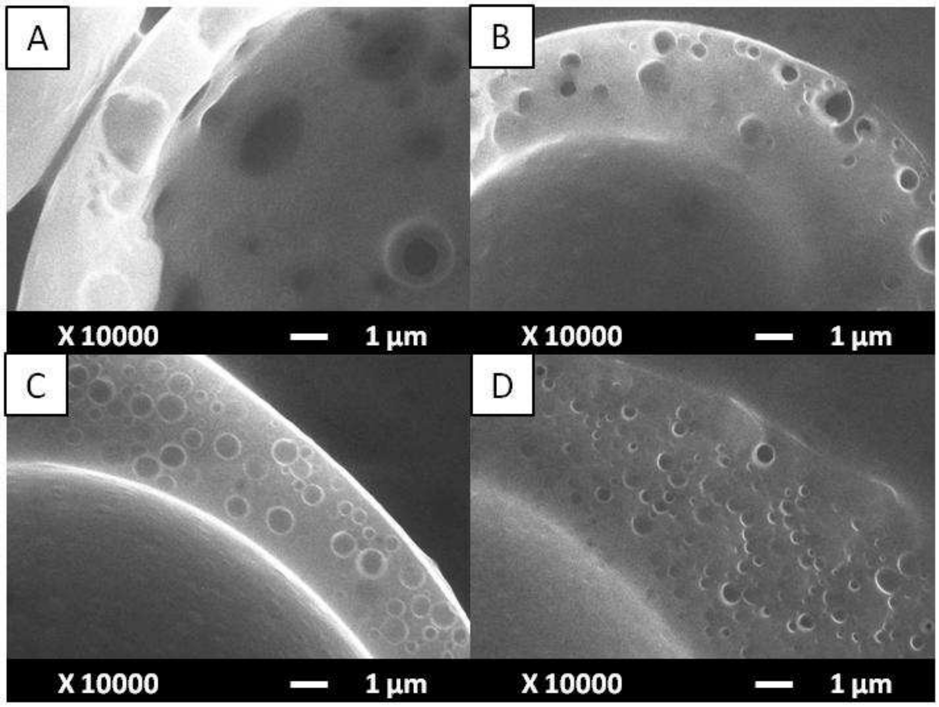

Figure 4-11. Internal morphology of dried powders.
The mass ratio of flavor (bergamot oil) to solid is 1:4.
Solid concentration: (A) 10%w/w, (B) 20%w/w, (C) 30%w/w, (D) 40%w/w

4.3.4 The Diffusivity of Flavor in Emulsion using DOSY-NMR and Wilke-Chang equation[104]

Diffusion is controlled by the water solubility of the volatile compound in emulsion and the permeability of the volatile compound through the shell of the matrix. Diffusion is important in food because it is a strong mechanism to evaluate flavor loss from the diffusion of the volatile compound through encapsulation matrix during atomization in the spray drying process and flavor release from spray dried powder. Therefore, the diffusion coefficient should be estimated to find the correlation of flavor diffusion and retention. Diffusion coefficient was estimated from DOSY-NMR and the Wilke-Chang equation (as shown in Chapter sections 2.5 and 2.6). Table 4-9 shows the diffusion coefficient of each flavor in water and emulsion using DOSY-NMR. The diffusion coefficient of pure flavor in water was higher than in the emulsion system because the emulsion system has a barrier (wall material) to prevent the flavor from diffusing into the surroundings. In the same way, the trend of the diffusion coefficient from the Wilke-Change equation was similar to DOSY-NMR data. The results implied that the emulsion system could protect flavor loss during atomization in the spray drying process. In the case of *d*-limonene, flavor retention was lower than linalool and linalyl acetate because it had a high diffusion coefficient.

Table 4-9. Diffusion coefficient of single flavor in pure flavor and emulsion system by using DOSY-NMR

Flavor	**Diffusion coefficient (m^2/s)**	
	Pure flavor	**Emulsion**
d-limonene	6.6E-10	3.03E-10
linalool	3.85E-10	2.29E-10
linalyl acetate	3.35E-10	2.24E-10

The Wilke-Chang equation was used to calculate the diffusion coefficient in different emulsion viscosity as shown in Table 4-10. At low solid content of wall material, the diffusion of linalool was higher than *d*-limonene and linalyl acetate with linalool retention being lower than any another flavors (Figures 4-7 and 4-8). With increasing emulsion viscosity, the diffusion coefficient of *d*-limonene was highest and this influenced low flavor retention (Figures 4-7 and 4-8). The result implied that flavor retention not only affected the volatile compound but also the emulsion viscosity in each flavor.

Table 4-10. Diffusion coefficient of single flavor in pure flavor and emulsion system by using Wilke-Change equation

Flavor	Solid content (w/w)	Viscosity (Pa.s)	Diffusion (m2/s)
d-limonene	10	36	1.51E-09
	20	67	8.12E-10
	30	128.5	4.23E-10
linalool	10	10	4.99E-09
	20	36	1.39E-09
	30	144.5	3.45E-10
linalyl acetate	10	10	4.53E-09
	20	20	2.27E-09
	30	206	2.2E-10

4.4 Effect of Flavor Volatility on the Release Behavior of Emulsified Flavor Powder

4.4.1 The Stability of Spray-dried Powder in 20 Days using Multi-flavor and Single Flavor Powder

The flavor releases of multi-flavor (bergamot oil) and single flavor (*d*-limonene, linalool and linalyl acetate) were measured at 50%RH and 50^{o}C. The effects of the flavor release behavior during storage are shown in Figure 4-12. It can be seen that the type of flavors influenced the flavor loss from the spray-dried powder. As concerns only multi-flavor (Figure 4-12A), linalool and linalyl acetae retention was not simple over time because the hydrolysis reaction of linalyl acetate to linalool and acetic acid occurred. This reaction is reversible, so linalool and acetic acid can produce linalyl acetate again. Therefore, the retention of linalool and linalyl acetate in multi-flavor was not stable over time. However, *d*-limonene release did function with time.

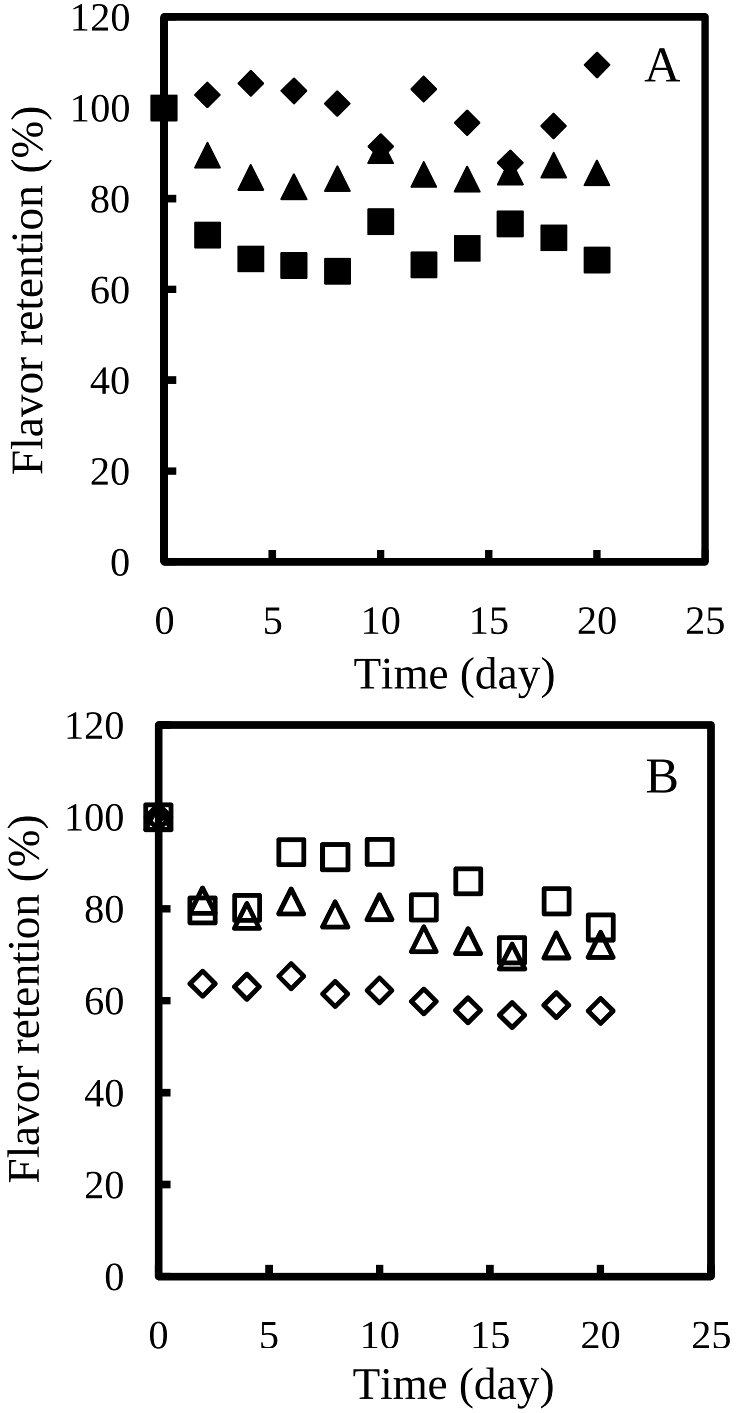

Figure 4-12. Flavor release from spray-dried powder in 20 days at 50%RH and 50^{o}C

△▲, *d*-limonene; ◇◆ , linalool; □■, linalyl acetate.

Closed symbols represent flavors in bergamot oil and opened symbols for pure flavor.

give the same result as multi-flavor due to linalool cannot produce linalyl acetate because linalool does not have acetic acid in flavor.

As can be seen in Figure 4-12B, linalyl acetate retention was not simply the same as multi-flavor because the hydrolysis reaction occurred during storage. However, linalool in single flavor did not give the same result as multi-flavor due to linalool not being able to produce linalyl acetate as it does not have acetic acid in the flavor. The release behavior of *d*-limonene and linalool was governed by the water solubility of flavor. In the case of the insoluble flavor as *d*-limonene, the flavor released less than 25% of total flavor in powder over 20 days. On the other hand, linalool could release more flavor than *d*-limonene and the amount of linalool release was about 40%. The resulted implied that the release behavior of flavor was governed by the water solubility of flavor and the reaction of sensitive flavors.

4.4.2 Effect of RH Dependent Phenomena on Volatile Release Profile

d-Limonene and linalool were used as model flavors to investigate the effect of volatile properties on release behaviors. Emulsion was transformed to powder by the spray drying process and it was used in release experiments. Spray dried powder properties (particle size, surface oil and flavor in powder) of *d*-limonene and linalool are shown in Table 4-11. The flavor release behavior was investigated using the humidity ramping and repeating method. The initial flavor release with humidity ramping measured the flavor distribution in the emulsified flavor of the spray dried powder. The humidity ramping experiment was studied at 50 ^{o}C and 10-50 %RH. High surface oil content as linalool was released at low relative humidity (10-15 %RH) as shown in Figures 4-13 and 4-14. Therefore, *d*-limonene and linalool powders removed the surface oil by vacuum treatment. The released amount of linalool was influenced by the vacuum treatment of spray-dried powder, but the *d*-limonene amount was not affected by this treatment. The release profile of *d*-limonene and linalool (Figure 4-13A and Figure 4-14A) did not change before and after treatment from 10 %RH to 50 %RH. On the other hand, the total amount of linalool release from the vacuum treatment powder decreased from non-treatment powder (Figures 4-13B and 4-14B). The results implied that the vacuum treatment could remove some surface oil of linalool. Because of flavor properties, the release behavior of *d*-limonene and linalool powders had different release profiles.

Table 4-11. Spray dried powder properties

Flavor	**Particle size (μm)**	**SSA (m^2/m^3)**	**Surface oil (mg-flavor/g-powder)**	**Flavor in powder (g-flavor/g-powder)**
d-limonene	24.70	0.13	0.013	0.125
linalool	16.06	0.24	0.069	0.166

At low RH, the release profile depended on surface oil content and vacuum treatment. With the increase of the relative humidity in a glassy state, the loss mechanism of volatile flavors was governed by flavor diffusion in the powder. The spray dried powders were not agglomerated at 10-20 %RH (Figure 4-15). At 25-40 %RH (before collapse point), the amount of *d*-limonene release was greater than linalool release. Since the molecular weight of *d*-limonene is lower than linalool, *d*-limonene is easy to diffuse through the matrix. From Figure 4-15A, the collapse point of emulsified *d*-limonene and linalool powder was about 45 %RH and the morphologies of the powders were confirmed by SEM photograph (Figure 4-16). At high relative humidity, the powders were collapsed and the carrier was changed from a glassy state to a rubbery state. At this point, *d*-limonene and linalool releases decreased near 0 mg/s.g-powder over time and started to re-encapsulate the flavor. After collapse point, the release behavior could be related with the water solubility of flavor. The amount of linalool release from spray-dried powders increased dramatically but the amount of *d*-limonene release decreased.

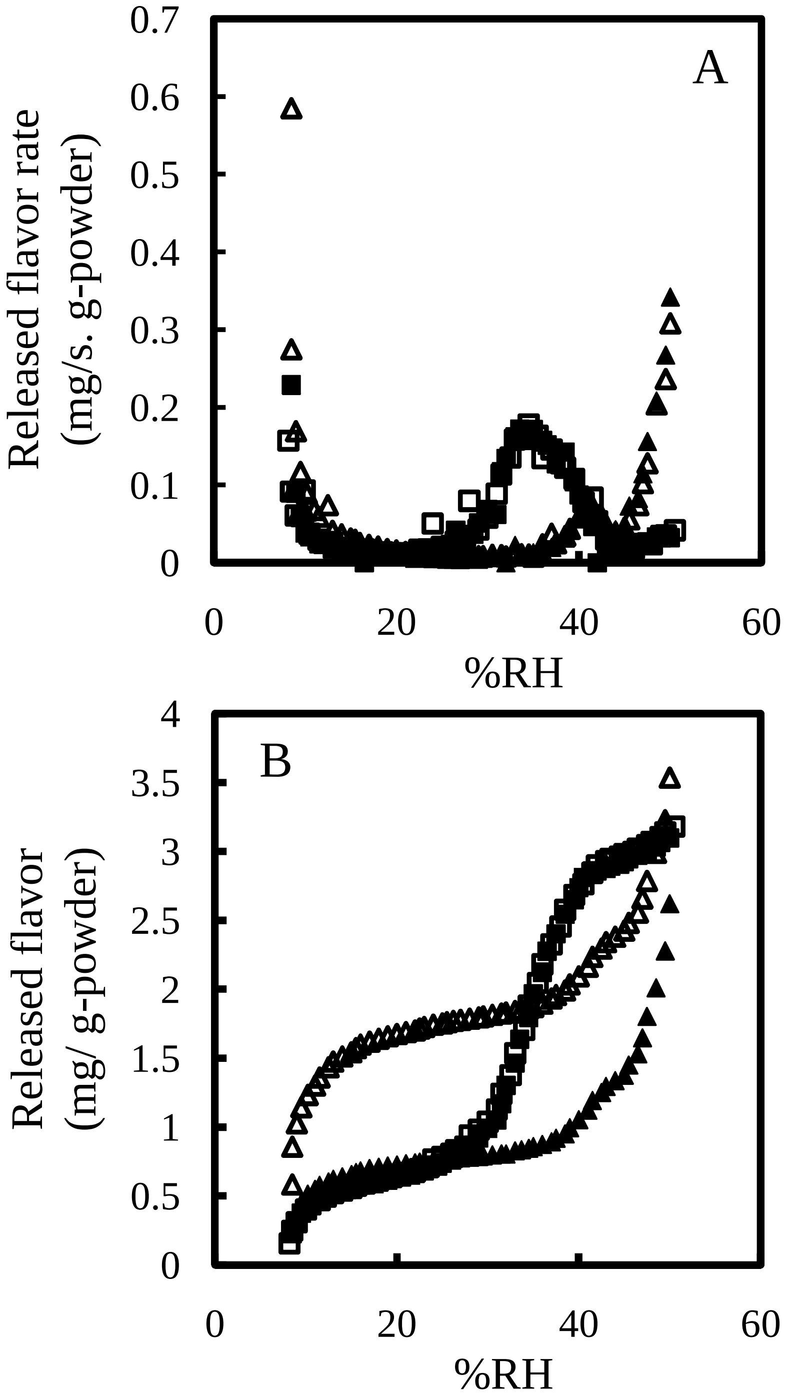

Figure 4-13. Flavor release from spray dried powder with ramping RH (10%-50%RH) at 50 ^{o}C. (A) flavor release profile with RH and (B) flavor release rate with RH. ■, *d*-limonene; ▲, linalool. Closed symbols represent vacuum treatment powder and opened symbols for original powder original powder.

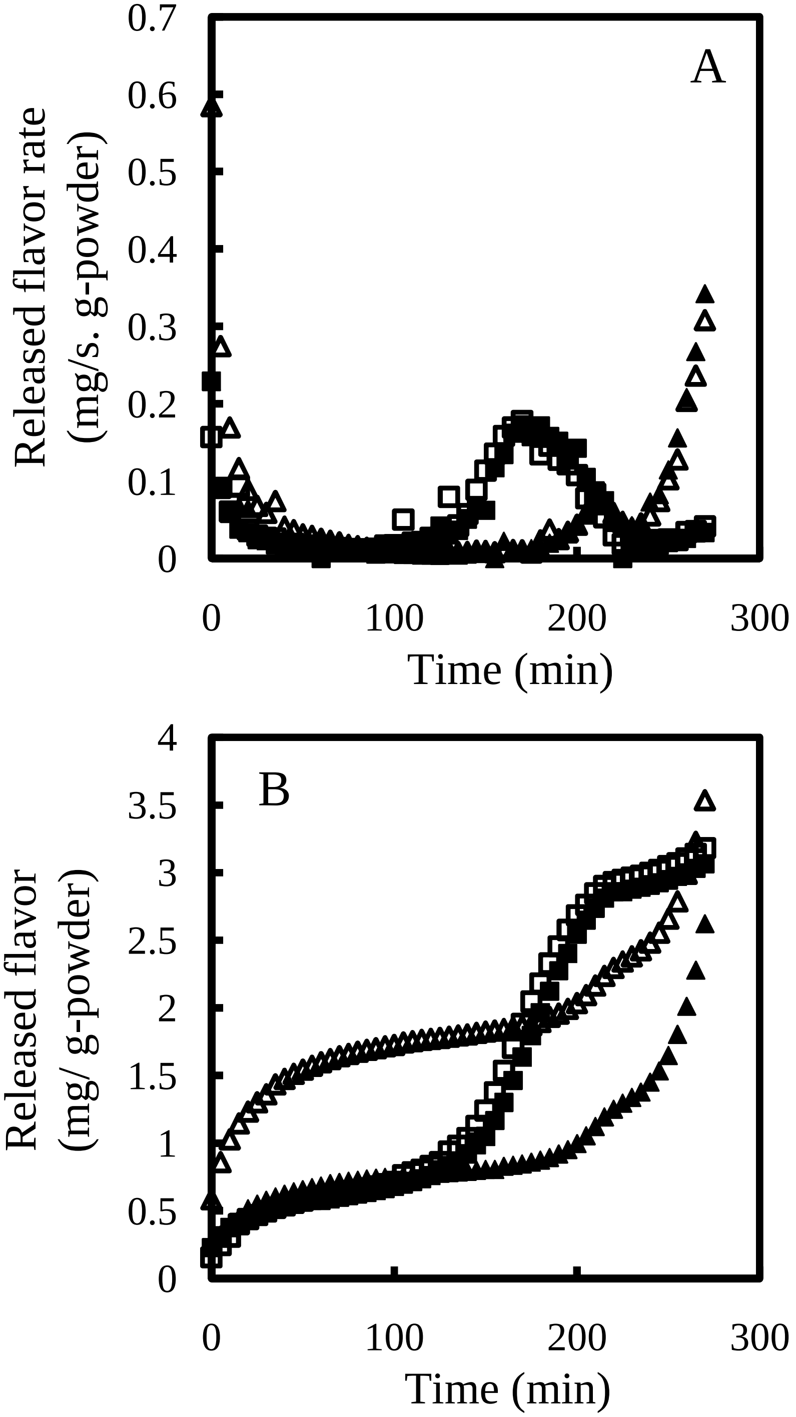

Figure 4-14. Flavor release from spray dried powder with ramping RH (10%-50%RH) at 50 ^{o}C. (A) flavor release profile with time and (B) flavor release rate with time. ■, *d*-limonene; ▲, linalool. Closed symbols represent vacuum treatment powder and opened symbols for original powder

microscopy (SEM). At 0% (data did not show) and 20 %RH (Figure 4-15), *d*-limonene and linalool powders were spherical as initial powders. Average *d*-limonene powder size was bigger than linalool powder size (Figures 4-15A, 4-15C). As shown in Figures 4-15B and 4-15D, the spray dried powders began to contact each particle but did not show the structure agglomerate or collapse. In both flavor powders, the structural agglomerate occurred at 45 %RH (Figure 4-16). The powders clumped and adhered together, so the flavor was entrapped and difficult to diffuse through the surface powder. The initial collapse directly affected release behavior as shown in Figures 4-13 and 4-14. The spherical changes were related with relative humidity or water content. The outer surface of powder started to melt and adhere to each other (Figure 4-16) until collapse (Figure 4-17). At the final point (50 %RH), the sample photographs are shown in Figure 4-17. The powders were melted and collapsed. Moreover, the spherical particle was lost and stressed on the surface (Figure 4-17). The results suggested that the physical change of microcapsules is related to the with release of active and water content in the system.

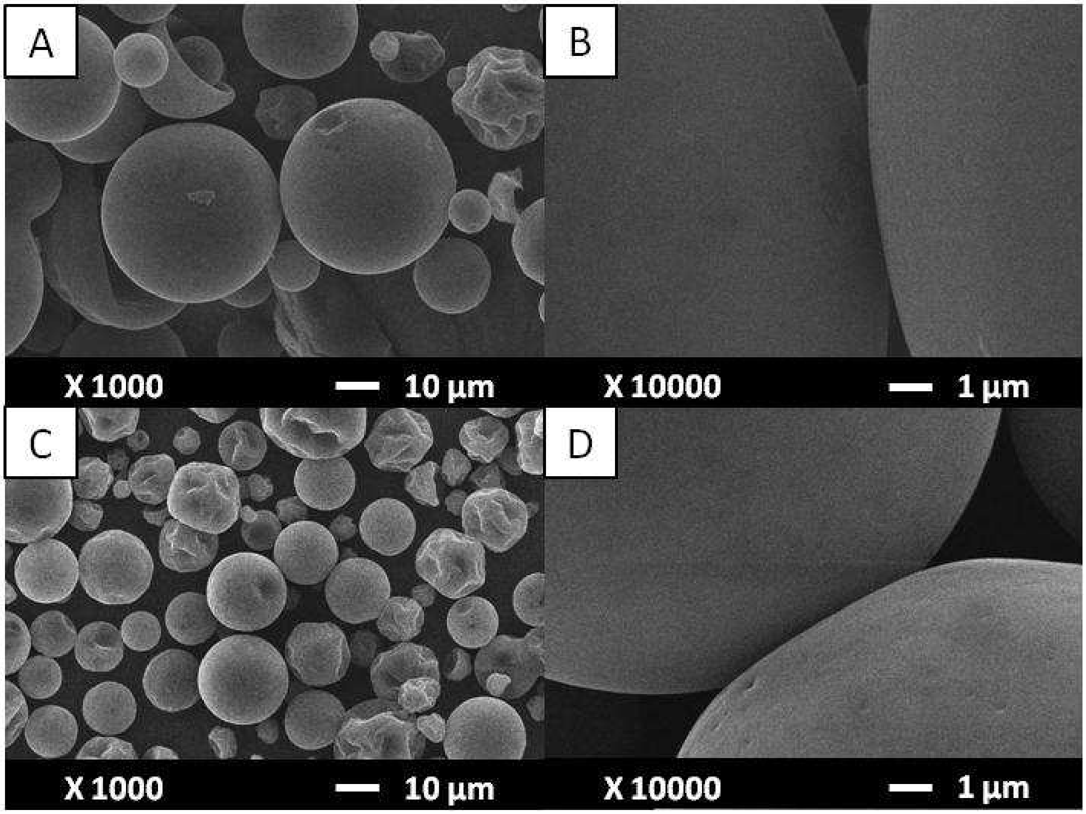

Figure 4-15. External morphology changes in spray-dried powder with ramping RH from 10% to 20% RH at 50 °C. (A, B) *d*-limonene, (C, D) linalool

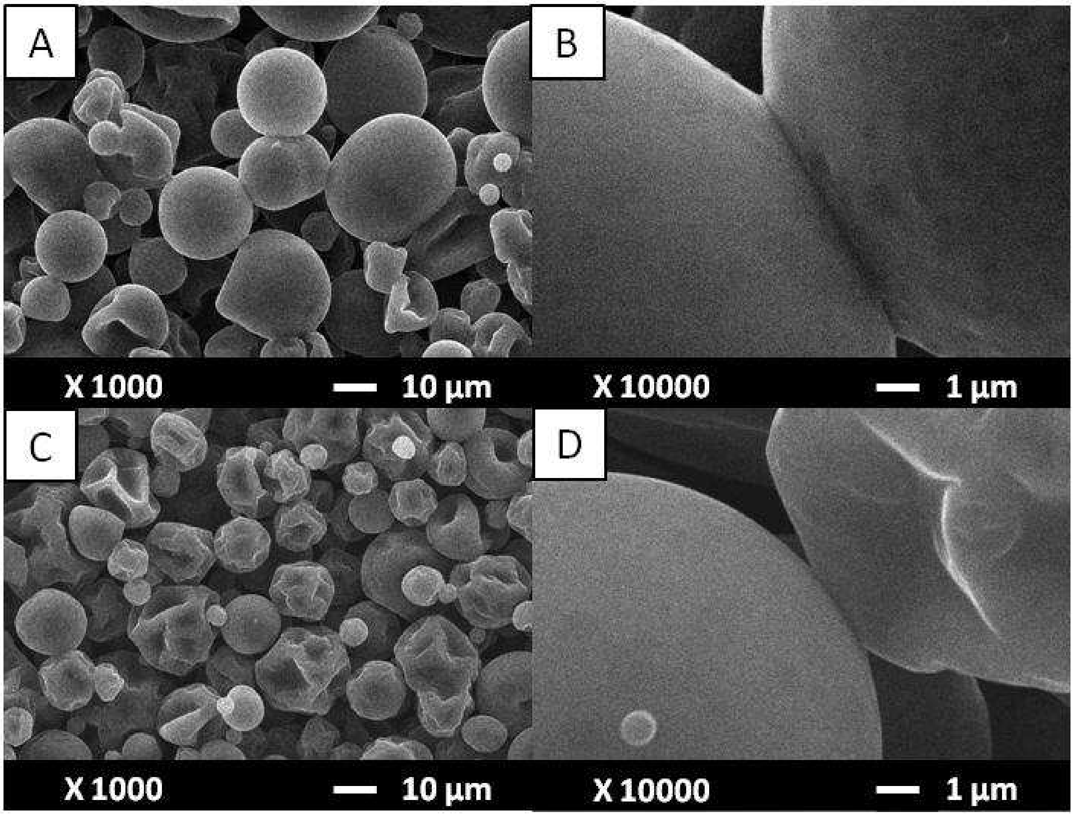

Figure 4-16. External morphology changes in spray-dried powder with ramping RH from 10% to 45% RH at 50 °C. (A, B) *d*-limonene, (C, D) linalool

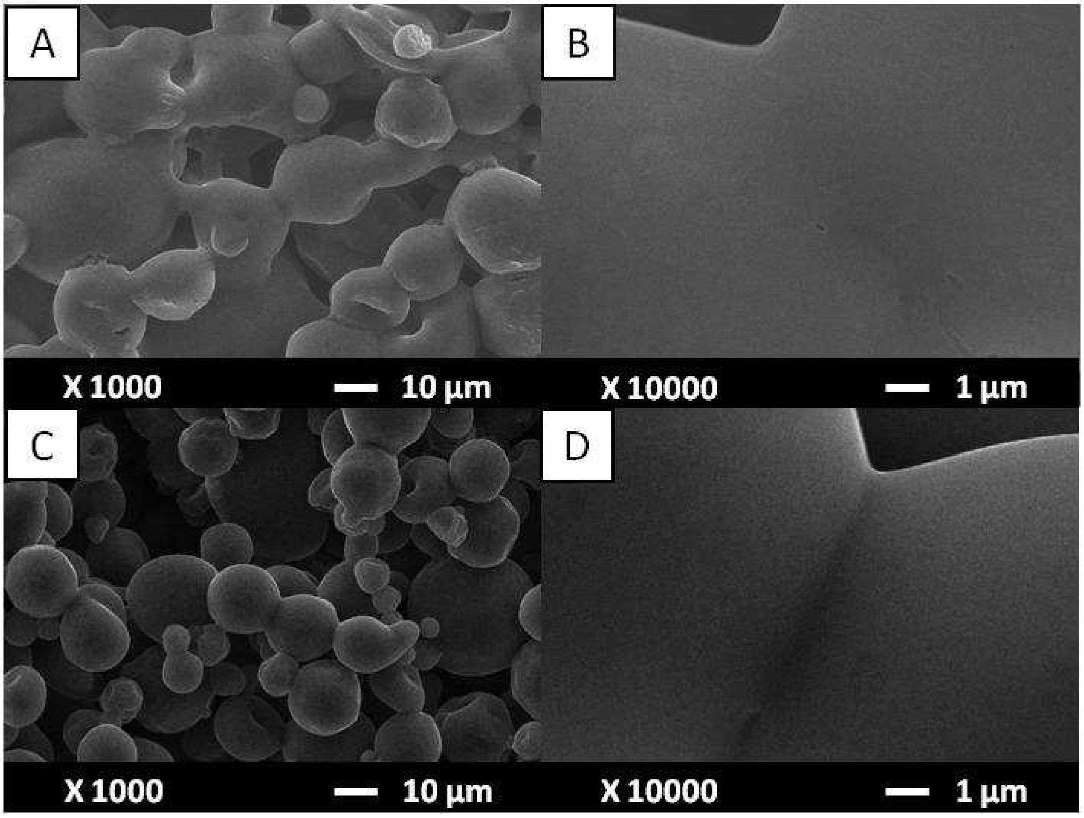

Figure 4-17. External morphology changes in spray-dried powder with ramping RH from 10% to 50% RH at 50 ^{o}C. (A, B) *d*- limonene, (C, D) linalool

4.4.3 Comparison of *d*-Limonene and Linalool Release Using Repeated Method of Relative Humidity

The effect of flavor properties on RH was studied using the humidity repeating method. This method fixed the RH at 0% and 50% for 4 hours and desiccator temperature was kept constant at 50 ^{o}C. The flavor release was studied using a dynamic vapor sorption system. The released data was collected every 5 minutes and analyzed using GC. The result of the *d*-limonene and linalool release is shown in Figures 4-18 and 4-19. The effect of vacuum treatment was also investigated in terms of the surface oil content. At 0 %RH (Figure 4-18), *d*-limonene release behavior did not differ between treatment and non-treatment powders but the release amount of linalool from the vacuum treatment powder was lower than non-treatment powders because the surface oil content of linalool was high. The morphology of spray dried powders in both flavor powders did not change at 0 %RH (Figure 4-20A, 4-20C). At low RH, the release behavior of flavor from the dried powder was controlled by the surface oil content.

Moreover, release behavior and physical change in the capsule wall was also investigated for high relative humidity (50 %RH). Figure 4-18 shows release behavior over time; the release amount of *d*-limonene was almost same as low relative humidity but linalool was released rapidly from the spray-dried powders. At high RH spray-dried powder became higher in water content and it affected the release behavior of flavor. The surface of the capsule absorbed water from the system and then the spherical powder was hydrated and began to adhere and agglomerate until complete collapse together. Therefore, hydrophobic as *d*-limonene was re-encapsulated and could not diffuse through wall material but high water solubility as linalool easily diffused through the capsule even when the wall closed. As we know from the dynamic release results (Figures 4-13 and 4-14), the amount of *d*-limonene release dropped after collapse point but linalool release content increased rapidly over time because the solubility of linalool was higher than *d*-limonene. From the SEM photograph (Figures 4-20B, 4-20D), both flavors of spray dried powder agglomerated and collapsed. The morphology of spray dried powder changed from spherical (Figures 4-20A, 4-20C) to partially collapsed (Figures 4-20B, 4-20D). The results implied that the release amount of *d*-limonene from the spray-dried powder was lower than linalool because the amount of surface oil and water solubility of linalool were higher than *d*-limonene. These results suggested that linalool might distribute in the surface shell of spray dried powder and *d*-limonene might distribute homogeneously in the shell.

Table 4-12. Avrami's equation parameters of *d*-limonene and linalool as a function of treatment condition

%RH	**Treatment condition**	***d*-limonene**		**linalool**	
		n	**k (1/min)**	**n**	**k (1/min)**
0%	Vacuum treatment	0.40	7.05E-07	0.31	1.29E-07
	Non treatment	0.30	9.24E-09	0.22	2.2E-09
50%	Vacuum treatment	0.16	0	0.78	1.37E-02
	Non treatment	0.14	0	0.68	6.4E-03

Avrami's Equation was used to evaluate the release rate constant of *d*-limonene and linalool in spray-dried powder. This equation was applied to the release time-course and correlated well as shown in Tables 4-12 and 4-13. The release rate constant and n-value were obtained by linear plot of ln(-lnR) versus ln t. At 0%RH, the release rate constant of both flavors was similar but these values were quite different with increasing RH. The release rate constant of *d*-limonene slightly decreased with increasing RH in both treatment and non-treatment powder. These results could be explained by re-encapsulation of carrier and collapsed powder from SEM photograph (Figure 4-20B). This implied that *d*-limonene in powder could release more slowly in a glassy state (low RH) but it was trapped inside the matrix in a rubbery state (high RH). On the other hand, linalool release rate constant was not similar to *d*-limonene at high RH. Although the morphology of linalool powder changed as *d*-limonene from a glassy state to a rubbery state, it did not affect linalool release. The release rate constant of linalool increased significantly with the increase in RH. Due to the water solubility of linalool being higher than *d*-limonene, linalool could diffuse through the matrix. As can be seen in Table 4-12, the n-values were in the range of 0.1 to 0.8 which implies that *d*-limonene and linalool release should be controlled by the diffusion mechanism.

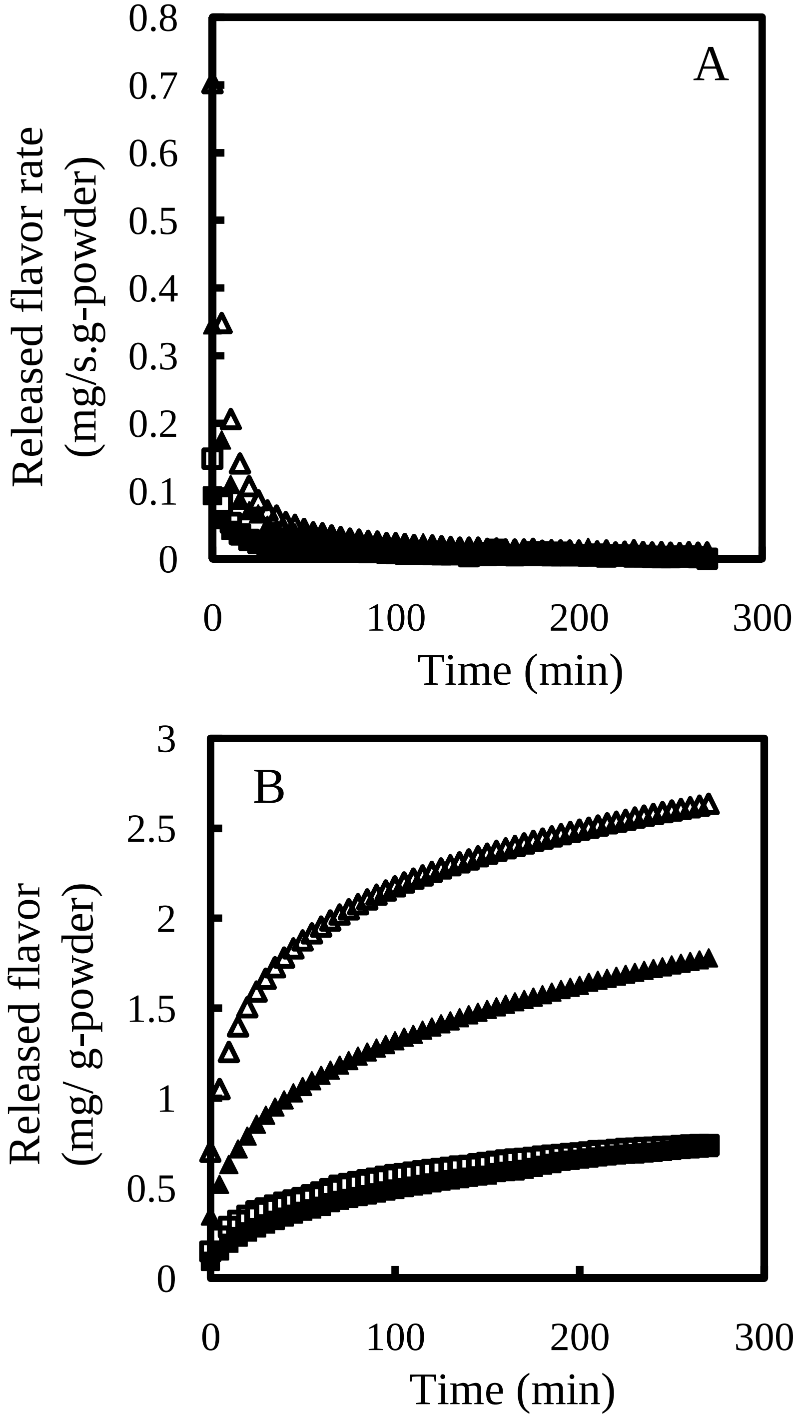

Figure 4-18. Flavor release from spray dried powder with constant RH at 50 ^{o}C and 0% RH. (A) flavor release profile with time and (B) flavor release rate with time. ■, *d*-limonene; ▲, linalool. Closed symbols represent vacuum treatment powder and opened symbols for original powder

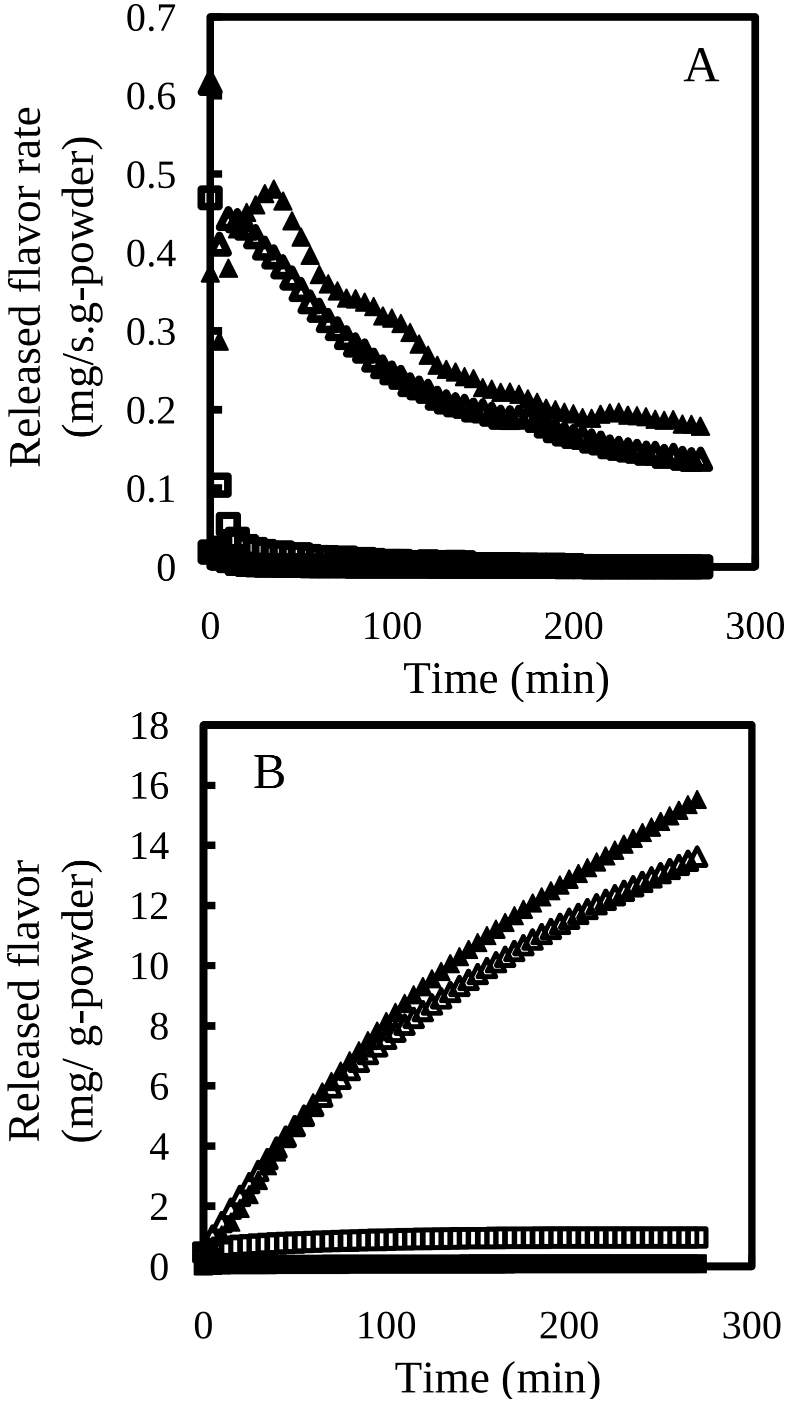

Figure 4-19. Flavor release from spray dried powder with constant RH at 50 °C and 50% RH. (A) flavor release profile with time and (B) flavor release rate with time. ■, *d*-limonene; ▲, linalool. Closed symbols represent vacuum treatment powder and opened symbols for original powder

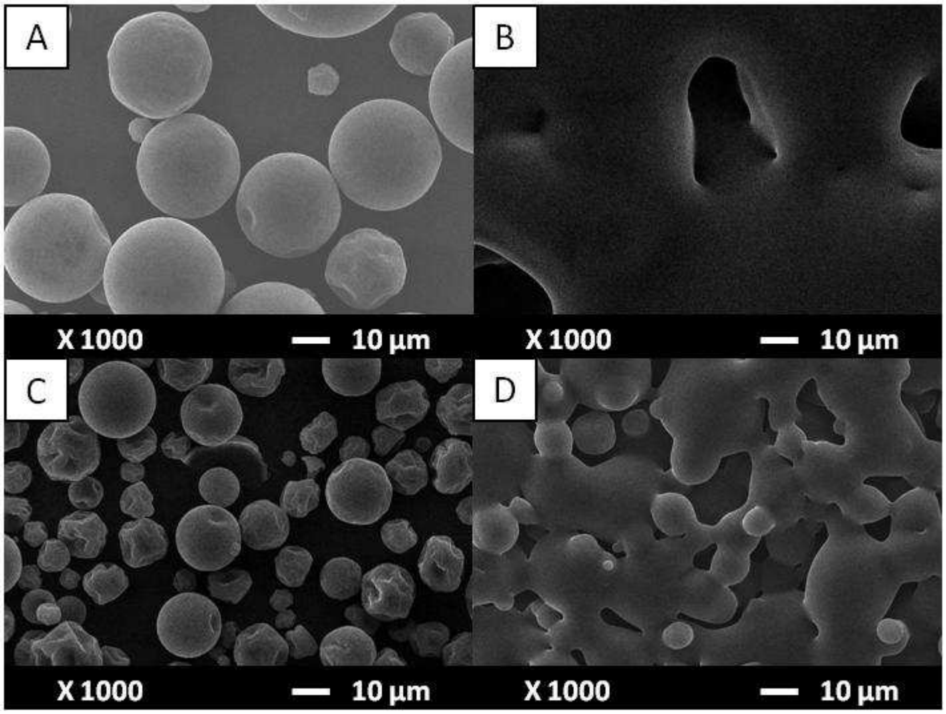

Figure 4-20. External morphology changes in spray-dried powder by using constant RH at 50 ^{o}C. (A) 0%RH, *d*-limonene, (B) 50%RH, *d*-limonene, (C) 0%RH, linalool, (D) 50%RH, linalool

4.4.4 Effect of Spray Dried Particle Size on the Flavor Release Behavior using Repeated Method of Relative Humidity

In this section, the release time course of *d*-limonene and linalool from the spray dried powder using non treatment powders were also investigated in the experiment as described above at 50 ^{o}C and at 0 and 50 %RH. The relationship between the release behavior of powder size and RH during short time storage (4h) is shown in Figures 4-21 and 4-22. Non-treatment powders in both flavor powders were sieved and 3 sizes were used (32, 53, 75 µm). Due to the 75 µm powder being the agglomerate powder and giving the same release profile as 53 µm, 32 and 53 µm powders were used for comparison with no sieve powders. The release profile of both flavors in different powder sizes was similar as there was no sieve powder from 10 %RH to 50 %RH (data did not show). From Figures 4-21 and 4-22, RH also greatly affected the release rate of linalool but *d*-limonene release content was similar in both RH. At low RH (Figure 4-21), no sieve powder was slightly affected in terms of the amount of flavor release due to it having surface oil and irregular powder (from SEM photograph). When water activity or high RH was involved in the release system, the amount of flavor release in small particles increased dramatically. However, the influence of flavor release on the particle size effect occurred only for the water solubility flavor (in case of re-encapsulation matrix). At 50 %RH, *d*-limonene can be released through the matrix only 40 min after being re-encapsulated by modified starch. However, linalool can diffuse through the matrix even after re-encapsulated matrix appeal. At 32µm powder of linalool had higher flavor content than other sizes. Due to the small powder size, there was high specific surface area and effective surface area. This result implied that small particle size could have a higher release rate than large particle size for high RH. The large powder size was chosen for long shelf life. SEM photographs show the external morphology of spray dried powder at 0 and 50 %RH. Encapsulated *d*-limonene and linalool powder did not change at 0%RH (Figure 4-23A, 4-23C). When water concentration or %RH increased, the state of modified starch was transformed from a solid or glassy state to a rubbery state. Samples in the glassy state were originally spherical (Figures 4-23A, 4-23C) but powders began to agglomerate until collapse with increasing RH (Figures 4-23B, 4-23D). Spherical powder became flat with smooth surface area (full collapse) when the temperature and RH passed the glass transition point. Because *d*-limonene spray dried powder has low surface oil, it collapses more easily than linalool powder, which has high surface oil and high water solubility.

Table 4-13. Avrami's equation parameters of *d*-limonene and linalool as a function of powder size

%RH	Powder size (µm)	***d*-limonene**		**linalool**	
		n	**k (1/min)**	**n**	**k (1/min)**
0%	32	0.22	5.05E-13	0.31	1.02E-07
	53	0.19	5.13E-14	0.31	3.91E-09
50%	32	0.01	0	0.73	1.24E-02
	53	0.14	0	0.66	6.00E-03

The release rate constant as calculated from Avrami's equation was similar in different powder sizes but it different in each flavor and RH. For *d*-limonene, the release rate constant of sieved powder did not change from the original powder. In the case of linalool, powder size affected release rate constant. Small powder had higher release rate than large powder because small powder gave a high specific surface area. The values of n with different powder sizes and flavor were in the range of 0.01 to 0.7. These values indicate that flavor release is controlled by the diffusion mechanism.

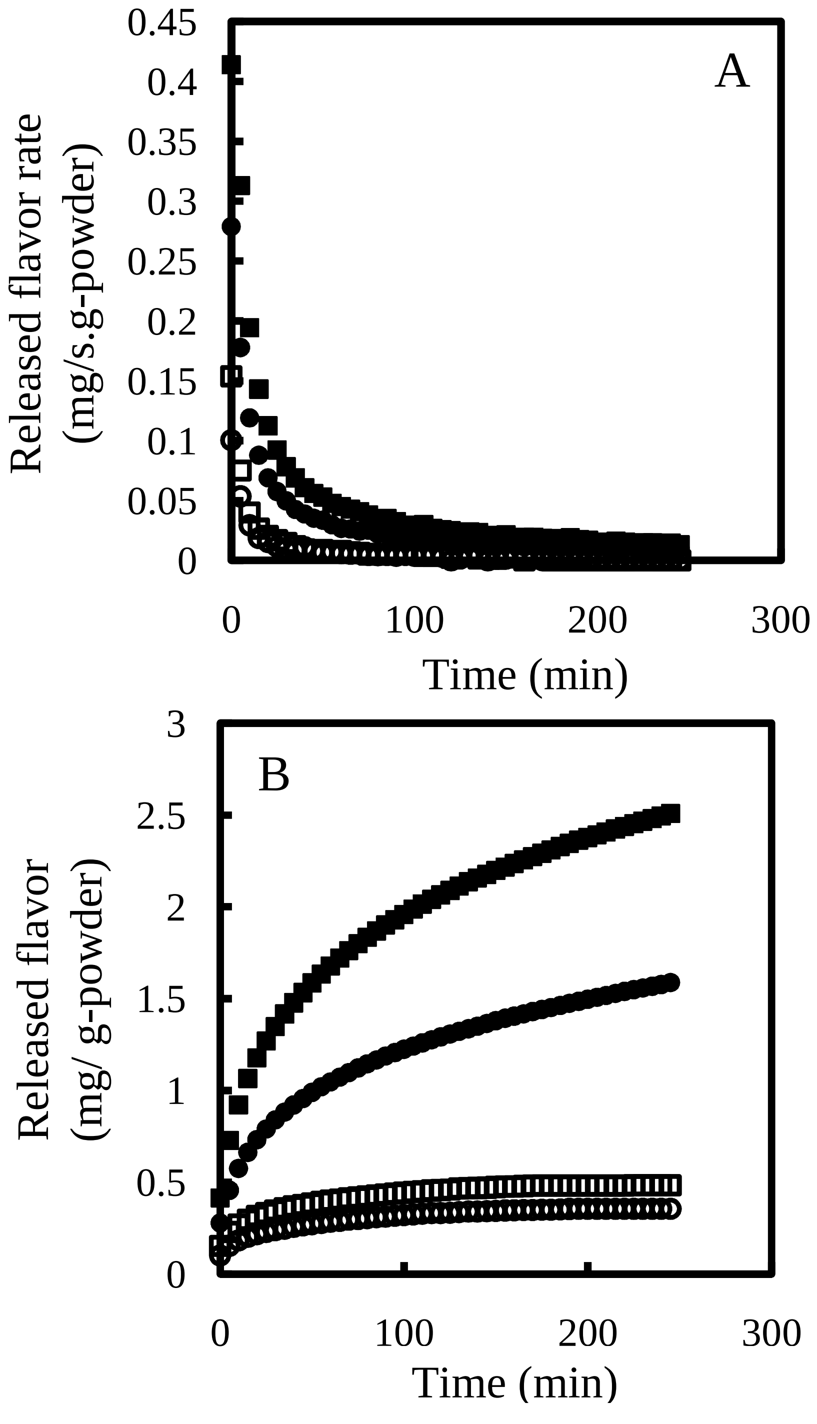

Figure 4-21. Flavor release from spray dried powder with constant RH at 50 °C and 0% RH. (A) flavor release profile with time and (B) flavor release rate with time. ●, 32 μm; ■, 53 μm. Closed symbols represent linalool and opened symbols for *d*-limonene.

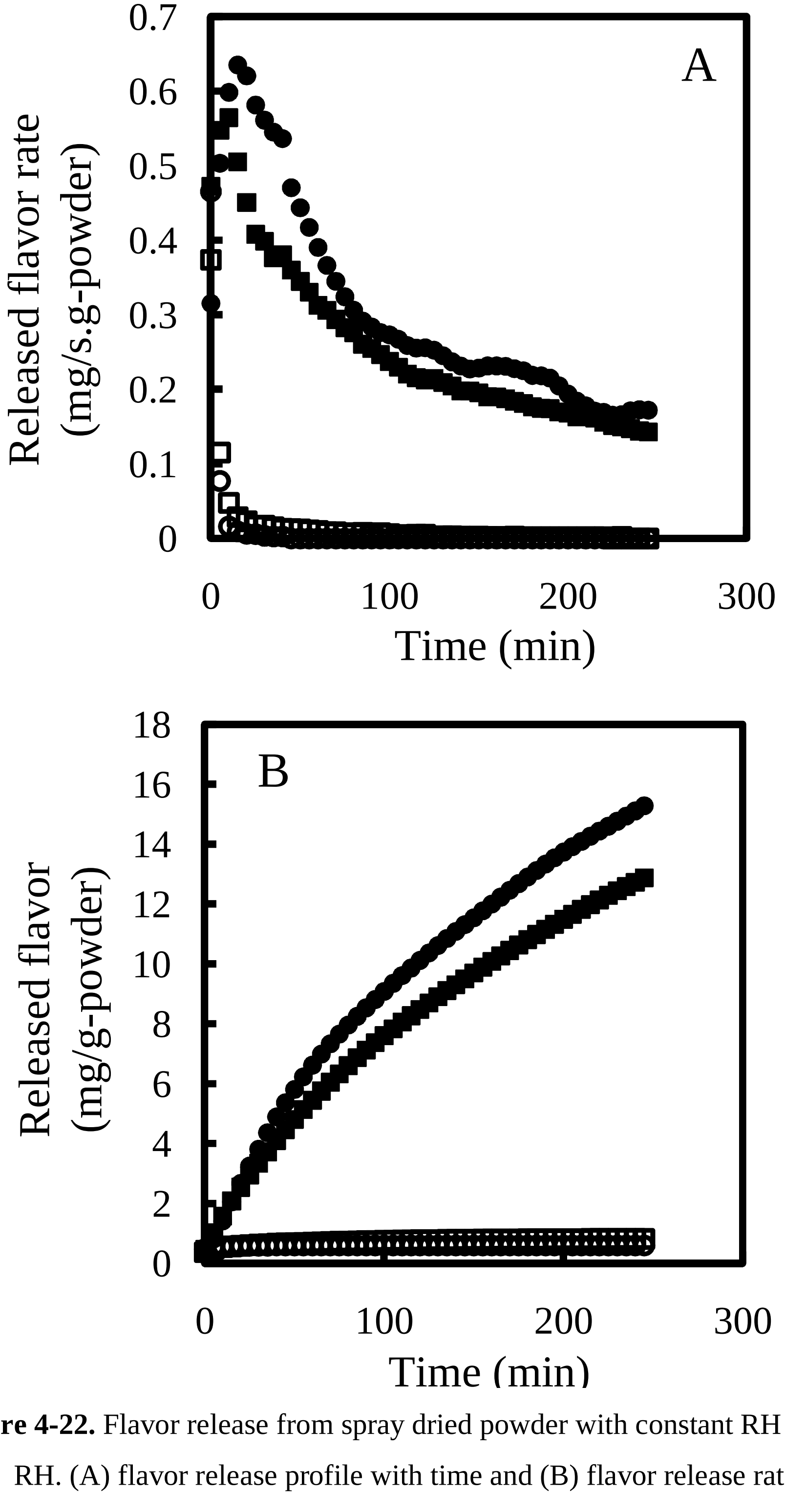

Figure 4-22. Flavor release from spray dried powder with constant RH at 50 ^{o}C and 50% RH. (A) flavor release profile with time and (B) flavor release rate with time. ●, 32 µm; ■, 53 µm. Closed symbols represent linalool and opened symbols for *d*-limonene.

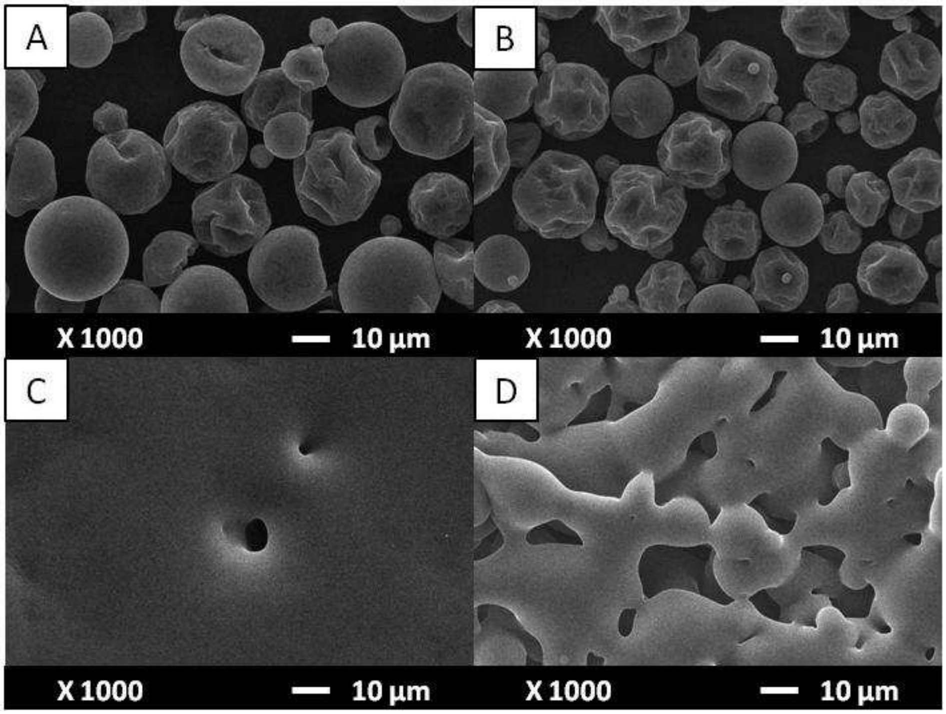

Figure 4-23. External morphology changes in spray-dried powder (32 µm) by using constant RH at 50 °C.

(A) 0%RH, *d*-limonene, (B) 50%RH, *d*-limonene, (C) 0%RH, linalool, (D) 50%RH, linalool

4.5 Chemical Transformation of Linalyl Acetate to Linalool in Spray-Dried Powder

Emulsion (oil in water) was prepared from linalyl acetate as flavor, gum arabic as carrier material and MD as additive wall material using a high speed homogenizer. Original emulsion droplet size and reconstituted droplet size of the emulsion were in the range of 1.8 to 2.2 μm as shown in Table 4-14. The slight difference between the original emulsion droplet size and the reconstituted emulsion droplet size reveals that the emulsion was stable during the spray drying process. Only water was evaporated from emulsion, resulting in the transformation of gum arabic to a solid state by the spray drying process. Spray dried powder of emulsified linalyl acetate and gum arabic was formed by the spray dryer with air inlet temperature between 120 - 200 ºC. The average particle size of the dried powder was in the range of 12 to 22 μm as shown in Table 4-14. Interestingly, the transformation of linalyl acetate to linalool occurred during spray drying. The amount of linalool conversion from spray dried powder was almost similar at 120-180 ^{o}C of air inlet temperature; it was about 7 - 8 %. However the conversion dramatically increased at 200 ^{o}C of air inlet temperature. Percentages of flavor determined before and after the spray drying process are shown in Figure 4-24. Before the spray drying process, pure linalyl acetate used as core in the emulsion was analyzed and it was found that almost 100% of linalyl acetate still remained without existence of linalool. After the spray drying process, both linalyl acetate and linalool were detected within the spray-dried powders. It was found that 22% of linalool was produced and 37% of linalyl acetate was retained in the spray-dried powder as shown in Figure 4-24. The retention of linalyl acetate was only 37% because of flavor loss and chemical transformation during spray drying. Linalool formation was ascribed to hydrolysis reaction (Figure 4-25) which was stimulated during the spray drying process. Many researchers have reported that the flavor retention could be governed by many factors related to the chemical nature of the core or wall material, weight ratio of core to wall materials, inlet gas temperature and drying rate (Wang, Lu, Lv & Bie. 2009; Yoshii et al., 2001). However, no one has reported on the effect of chemical transformation on flavor retention. For the hydrolysis reaction, the water is added to split linalyl acetate into linalool and acetic acid as shown in Figure 4-25. The key factors which induced the hydrolysis reaction of linalyl acetate in the spray drying process were heat transfer from hot air to droplets and the evaporation of water droplet. Due to hot air contracting the droplets and heat from the hot air being transferred to droplets, the droplet temperature increased. Then the water in emulsion evaporated and a dry powder was formed. For a few seconds the hydrolysis reaction occurred and stopped after the dry powder was formed. The rate of hydrolysis occurring in the spray drying process depends on the reaction temperature. At 200^{o}C of air inlet temperature, linalyl acetate can transform to about 22 % linalool without catalyst.

Table 4-14. Properties of feed emulsion and the spray-dried powder for encapsulated linalyl acetate

Air inlet temp (^{o}C)	**Emulsion droplet size (µm)**	**Reconstituted emulsion size (µm)**	**Powder size (µm)**	**Conversion (%)**
120	2.01	2.17	12.10	7.11
140	2.05	2.08	11.65	7.52
160	1.99	2.09	12.43	7.72
180	1.95	2.14	15.47	8.49
200	1.80	1.97	22.21	21.18

For confirmation, formation of emerging linalool within the spray dried powders was again examined by GC-MS in comparison with the linalyl acetate standard solution. As can be observed in Figure 4-26a, linalyl acetate and linalool peaks were found in the dried powder but there was no linalool in the linalyl acetate standard solution (Figure4-26b). The result confirms that linalool formation occurs when the emulsion is subject to the spray drying process. The transformation of linalyl acetate to linalool is attributed to the hydrolysis reaction of linalyl acetate with the presence of water content [99] or its biotransformation [98, 105-107]. Madysta et al. [105] reported transformation of linalyl acetate when incubated with *Aspergillus niger*. Pure linalyl acetate (100%) was transformed to 25% of linalool. Similarly, Ramishvili et al. [99] showed that some catalysts could be used to give a high conversion of linalyl acetate. These previous results support the transformation of linalyl acetate in the prepared emulsion of gum arabic reasonably taking place when subject to the spray drying process. The results implied that the chemical transformation of linalyl acetate to linalool was affected not only by the hot air in the spray drying process but also by small oil droplets in the emulsion system.

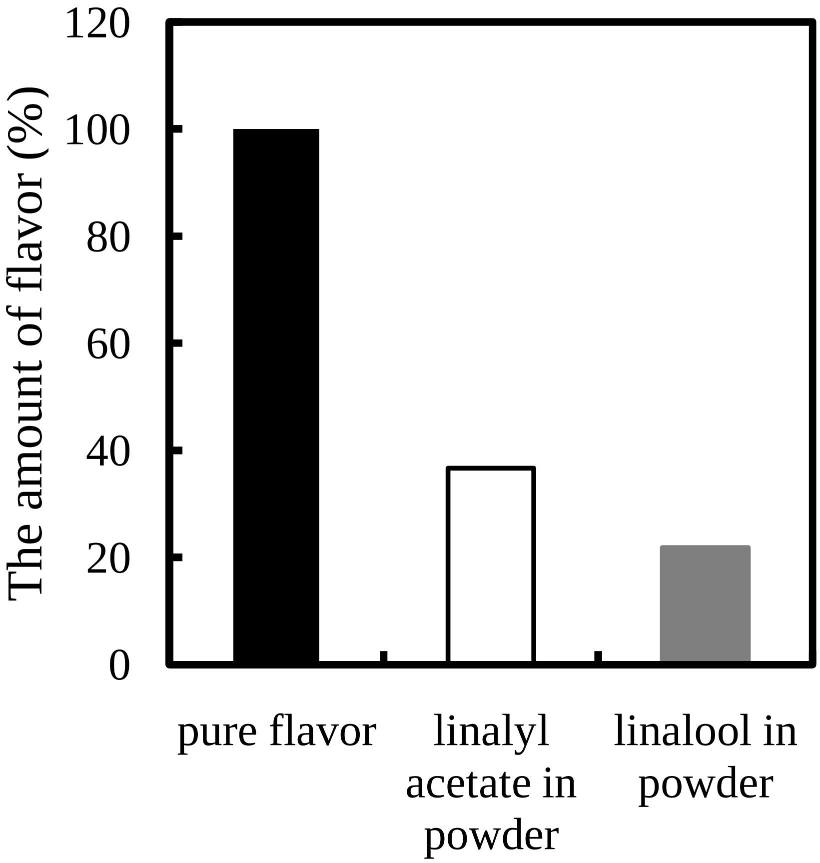

Figure 4-24. The amount of linalyl acetate and linalyl acetate transformation before and after spray drying.

and pure linalyl acetate were incubated at 80 ℃ for some designated incubation times. Every hour a sample was collected and analyzed by GC. After emulsion preparation

(0 hour), only 3% of linalool was found in the emulsion but in the incubated pure linalyl acetate there was no linalool formation found. With the longer incubation time applied to the emulsion of linalyl acetate and gum arabic, a higher content of linalool was detected as shown in Figure 4-27. Incubation of linalyl acetate emulsion for 4 hours resulted in the transformation to linalool from 3% to 6% by weight. These results suggested that the key factor which affected linalyl acetate transformation or hydrolysis reaction was the presence of water content in the emulsion. The emulsion with smaller oil droplets which occupy higher surface area would enhance the hydrolysis reaction of linalyl acetate. Lee et al.[102] reported that only small oil droplets could undergo hydrolysis reaction by lipase. Based on all the above mentioned results, it would be reasonable to summarize that not only the spray drying process but also the surface area of droplets in emulsion play an important role in the chemical transformation of linalyl acetate to linalool. Therefore, encapsulation of sensitive flavor components like linalyl acetate must be carefully carried out to avoid the chemical transformation of linalyl acetate to linaool otherwise the odor of encapsulated oil will be different from the original extracted one.

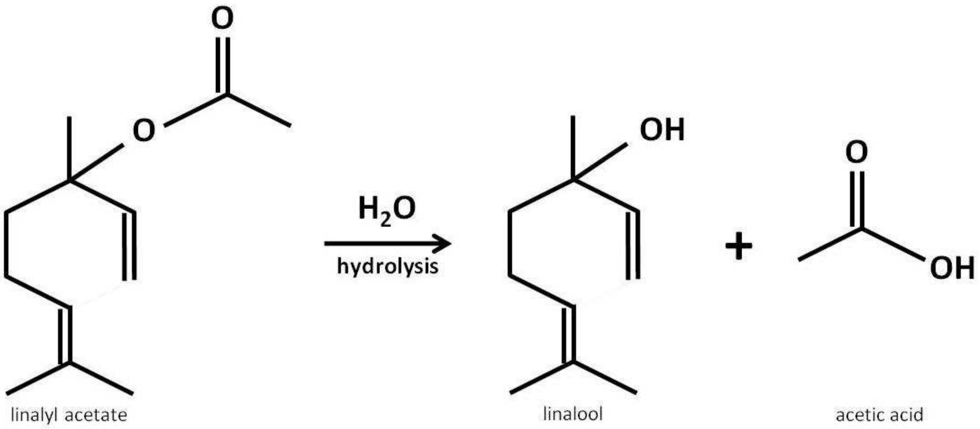

Figure 4-25. Transformation of linalyl acetate to linalool and acetic acid by hydrolysis reaction.

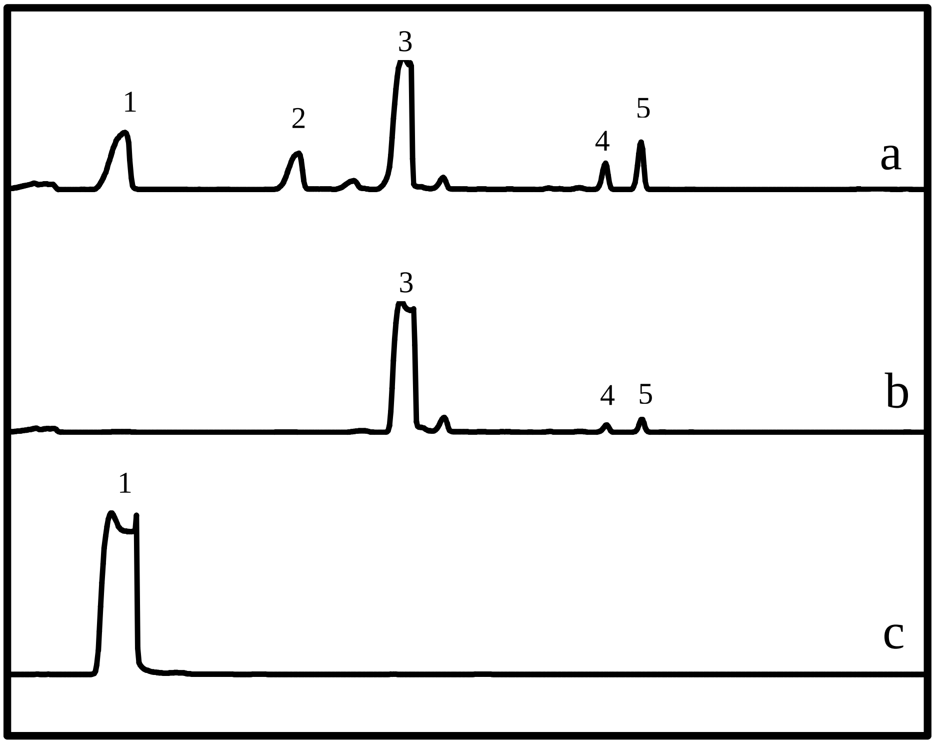

Figure 4-26. Chromatogram of flavors which analyzed by using GC-MS**.** (a) flavor compounds in linalyl acetate spray-dried powder (spray drying condition: 30ml/min of feed flow rate, 200 ºC of air inlet temperature, 30,000 rev/min of speed atomizer and 110 kg/h of air flow rate), (b) linalyl acetate standard solution, (c) linalool standard solution.

(1) linalool; (2) α-terpineol; (3) linalyl acetate; (4) (Z)-geranyl acetate; (5) (E)-geranyl acetate.

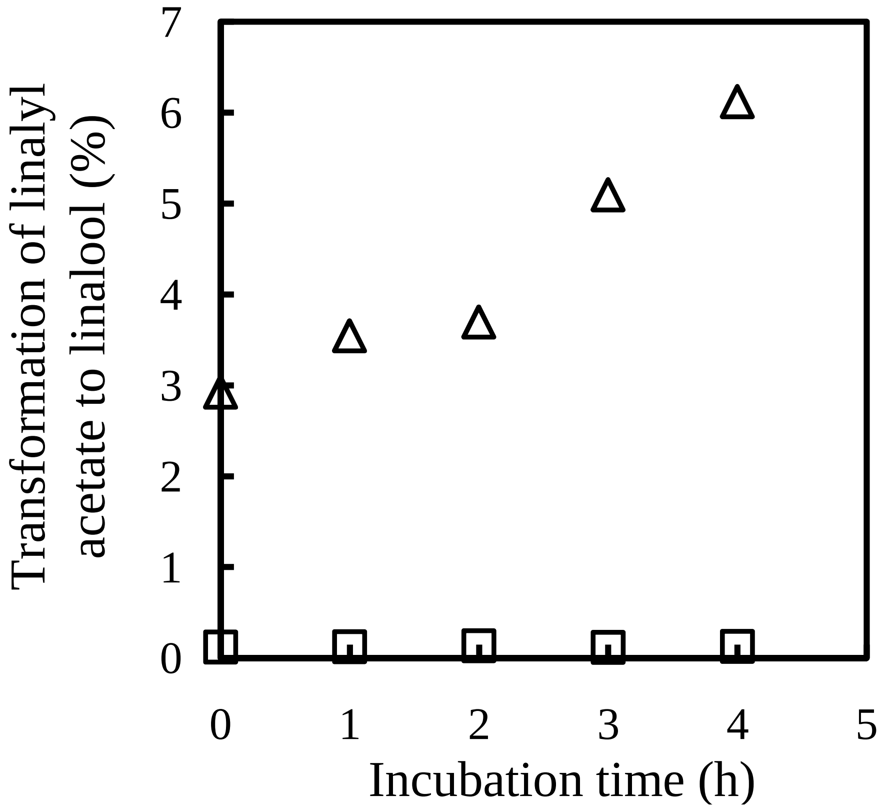

Figure 4-27. Chemical transformation in pure linalyl acetate and emulsion and samples were incubated at 80ºC for 4 hours. (☐) pure linalyl acetate; (△) emulsion.

CHAPTER V

SUMMARY

5.1 The loss of OSA-modified starch emulsifier property during the high pressure homogenizer for encapsulating multi-flavor bergamot oil by spray drying

Encapsulation of multi-flavor was studied using modified starch as the wall material and bergamot oil as flavor. The mean volume emulsion size was governed by the emulsification process type of the wall material. For gum arabic, the larger emulsion size was decreased using a high pressure homogenizer but modified starch could not reduce the emulsion size by the same process as shown in Figure 5-1. This is due to the chemical functional group of modified starch being destroyed by the pressure from high pressure homogenizer process. In the preparation of emulsion from the modified starch carrier solution, the high pressure homogenizer process was not suitable.

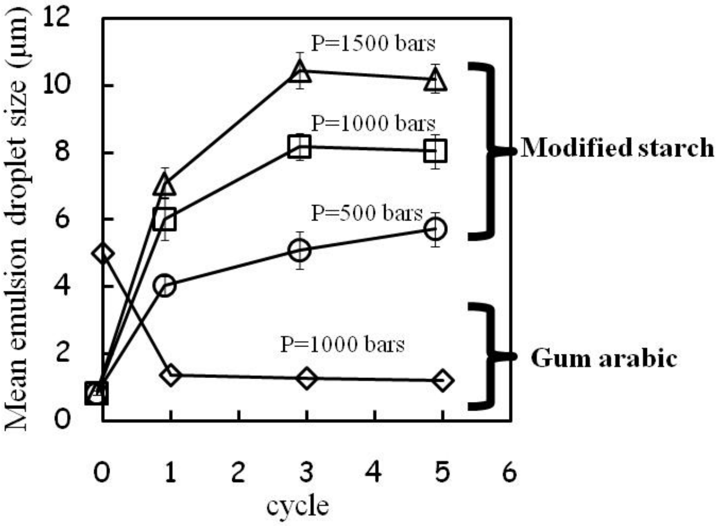

Figure 5-1. Effect of pressure and cycle in high pressure homogenizer on emulsion droplet size

5.2 Effect of mass ratio of bergamot oil and wall material on flavor retention and surface oil content

The mass ratio of bergamot oil and wall materials can influence the properties of encapsulated powder in a number of different ways, including emulsion size, reconstituted emulsion size, particle size, moisture content and retention. Low ratio of core to wall materials is more efficient in both smaller emulsion droplet sizes and retention as shown in Figure 5-2. This is due to the fact that these emulsions had more stability. The smaller emulsion size had better retention because emulsion droplets were retained and encapsulated more efficiently in larger particles.

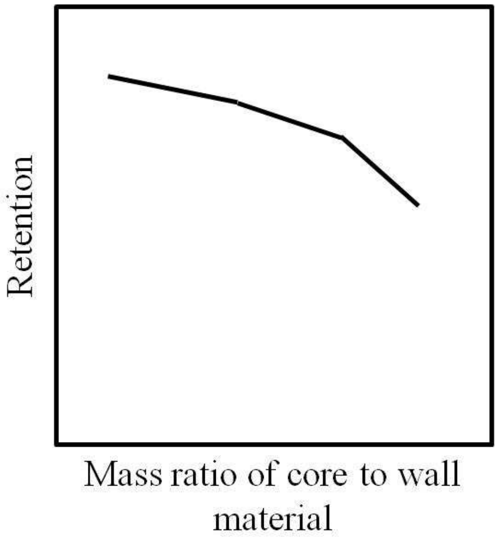

Figure 5-2. Effect of mass ratio of core to wall material on flavor retention

5.3 Effect of feed liquid viscosity on flavor retention of bergamot oil encapsulated in spray-dried modified starch powder

The viscosity of feed solution influenced the physical properties of spray-dried power such as flavor retention, reconstituted emulsion droplet size and powder size. High emulsion viscosity indicates higher retention as shown in Figure 5.3. The viscosity of feed solution significantly affected flavor retention. The powder size also could be effectively controlled by changing the emulsion viscosity. The flavor was optimal in emulsion viscosity for the retention of volatiles. Furthermore, the retention of multi-flavor was higher than the single flavor retention. The interaction between each flavor was expected for higher retention in the case of multi-flavor.

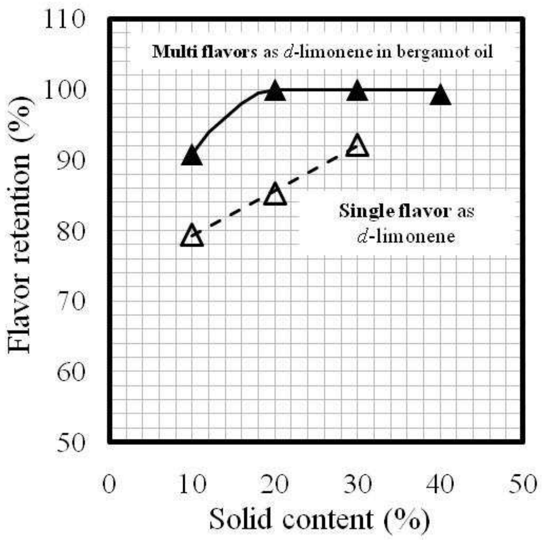

Figure 5-3. Effect of emulsion viscosity on flavor retention

5.4 Effect of flavor volatility on the release behavior of emulsified flavor powder

From Figure 5-2, the release amount of insoluble flavor from the spray-dried powder was lower than the soluble flavor because the solubility and surface oil content of soluble flavor was higher than the insoluble flavor. Therefore, the soluble flavor might easily diffuse through the wall material. The flavor solubility in water might affect the distribution of flavor in the spray dried powder and surface oil content. The release behavior of dried powders was closely related to the humidity and the flavor distribution in the emulsified flavor of spray-dried powder. Therefore, the volatile release of spray dried powder depended on the solubility of flavor, powder size, temperature storage and RH. This implied that flavor release was governed by the interaction of flavor and matrices.

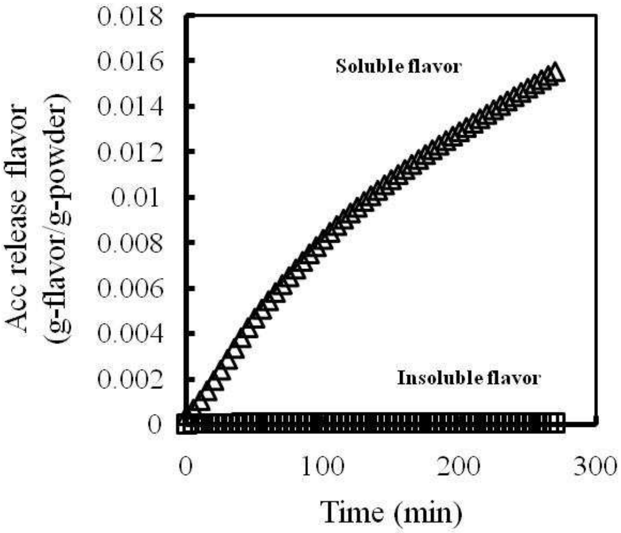

Figure 5-4. Effect of flavor volatile on release behavior

5.5 Chemical transformation of linalyl acetate to linalool in spray-dried powder

Chemical transformation of linalyl acetate to linalool was affected by not only the characteristics of oil droplets in the emulsion process but also the spray drying process. The encapsulation of sensitive flavors like linalyl acetate should be carefully handled to prevent chemical transformation otherwise the odor of encapsulated oil would be different from the original one.

REFERENCES

[1] Yuliani, S., et al., Application of Microencapsulated Flavor to Extrusion Product. Food Reviews International 20,2(2004): 163 - 185.

[2] Comai, S., et al., Essential oil of Lindera neesiana fruit: Chemical analysis and its potential use in topical applications. Fitoterapia 81,1(2010): 11-16.

[3] Atmane, M., et al., Flavour encapsulation and controlled release - a review. International Journal of Food Science & Technology 41,1(2006): 1-21.

[4] Liu, X.-D., et al., Microencapsulation of emulsified hydrophobic flavors by spray drying. Drying Technology: An International Journal 19,7(2001): 1361 - 1374.

[5] Given Jr, P.S., Encapsulation of Flavors in Emulsions for Beverages. Current Opinion in Colloid & Interface Science 14,1(2009): 43-47.

[6] I., R.M., MICROENCAPSULATION BY SPRAY DRYING. Drying Technology: An International Journal 16,6(1998): 1195 - 1236.

[7] Desai, K.G.H. and H.J. Park, Recent Developments in Microencapsulation of Food Ingredients. Drying Technology: An International Journal 23,7(2005): 1361 - 1394.

[8] Jafari, S.M., et al., Encapsulation Efficiency of Food Flavours and Oils during Spray Drying. Drying Technology: An International Journal 26,7(2008): 816 - 835.

[9] Yoshii, H., et al., Flavor release from spray-dried maltodextrin/gum arabic or soy matrices as a function of storage relative humidity. Innovative Food Science & Emerging Technologies 2,1(2001): 55-61.

[10] Reineccius, G.A., The Spray Drying of Food Flavors. Drying Technology: An International Journal 22,6(2004): 1289 - 1324.

[11] Soottitantawat, A., et al., Effect of Water Activity on the Release Characteristics and Oxidative Stability of d-Limonene Encapsulated by Spray Drying. Journal of Agricultural and Food Chemistry 52,5(2004): 1269-1276.

[12] Soottitantawat, A., et al., Influence of emulsion and powder size on the

stability of encapsulated d-limonene by spray drying. Innovative Food Science & Emerging Technologies 6,1(2005): 107-114.

[13] Gouin, S., Microencapsulation: industrial appraisal of existing technologies and trends. Trends in Food Science & Technology 15,7-8(2004): 330-347.

[14] Costa, P. and J.M. Sousa Lobo, Divisability of Diltiazem Matrix Sustained-Release Tablets. Pharmaceutical Development and Technology 6,3(2001): 343-351.

[15] Costa, P., An alternative method to the evaluation of similarity factor in dissolution testing. International Journal of Pharmaceutics 220,1-2(2001): 77-83.

[16] Nagarwal, R., D. Ridhurkar, and J. Pandit, *In Vitro* Release Kinetics and Bioavailability of Gastroretentive Cinnarizine Hydrochloride Tablet. AAPS PharmSciTech 11,1(2010): 294-303.

[17] Costa, P. and J.M. Sousa Lobo, Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences 13,2(2001): 123-133.

[18] Giovagnoli, S., et al., Physicochemical characterization and release mechanism of a novel prednisone biodegradable microsphere formulation. Journal of Pharmaceutical Sciences 97,1(2008): 303-317.

[19] Costa, P. and J.M. Sousa Lobo, Evaluation of Mathematical Models Describing Drug Release from Estradiol Transdermal Systems. Drug Development and Industrial Pharmacy 29,1(2003): 89-97.

[20] Costa, P. and J.M.S. Lobo, Influence of Dissolution Medium Agitation on Release Profiles of Sustained-Release Tablets. Drug Development and Industrial Pharmacy 27,8(2001): 811-817.

[21] Shahidi, F. and X.-Q. Han, Encapsulation of food ingredients. Critical Reviews in Food Science and Nutrition 33,6(1993): 501 - 547.

[22] Arshady, R., Microcapsules for food. Journal of Microencapsulation 10,4(1993): 413-435.

[23] Bylaitë, E., P. Rimantas Venskutonis, and R. Maþdþierienë, Properties of caraway (Carum carvi L.) essential oil encapsulated into milk protein-

based matrices. European Food Research and Technology 212,6(2001): 661-670.

[24] Gharsallaoui, A., et al., Applications of spray-drying in microencapsulation of food ingredients: An overview. Food Research International 40,9(2007): 1107-1121.

[25] Faldt, P. and B. Bergenstahl, Spray-dried whey protein/lactose/soybean oil emulsions. 1. Surface composition and particle structure. Food Hydrocolloids 10,4(1996): 421-429.

[26] Loksuwan, J., Characteristics of microencapsulated [beta]-carotene formed by spray drying with modified tapioca starch, native tapioca starch and maltodextrin. Food Hydrocolloids 21,5-6(2007): 928-935.

[27] Jeon, Y.-J., et al., The suitability of barley and corn starches in their native and chemically modified forms for volatile meat flavor encapsulation. Food Research International 36,4(2003): 349-355.

[28] Gibaldi, M. and S. Feldman, Establishment of sink conditions in dissolution rate determinations. Theoretical considerations and application to nondisintegrating dosage forms. Journal of Pharmaceutical Sciences 56,10(1967): 1238-1242.

[29] Wagner, J.G., Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules. Journal of Pharmaceutical Sciences 58,10(1969): 1253-1257.

[30] Mulye, N.V. and S.J. Turco, A Simple Model Based on First Order Kinetics to Explain Release of Highly Water Soluble Drugs from Porous Dicalcium Phosphate Dihydrate Matrices. Drug Development and Industrial Pharmacy 21,8(1995): 943-953.

[31] Papadopoulou, V., et al., On the use of the Weibull function for the discernment of drug release mechanisms. International Journal of Pharmaceutics 309,1-2(2006): 44-50.

[32] Ford, J.L., et al., Mathematical modelling of drug release from hydroxypropylmethylcellulose matrices: Effect of temperature. International Journal of Pharmaceutics 71,1-2(1991): 95-104.

[33] Brophy, M.R. and P.B. Deasy, Application of the Higuchi model for drug

release from dispersed matrices to particles of general shape. International Journal of Pharmaceutics 37,1-2(1987): 41-47.

[34] Ginebra, M.P., T. Traykova, and J.A. Planell, Calcium phosphate cements as bone drug delivery systems: A review. Journal of Controlled Release 113,2(2006): 102-110.

[35] Costa, P., Ferreira, D.C., Sousa Lobo, J.M. , Determination of drug release profiles of nitroglycerin transdermal therapeutic systems [NITROGLICERINA EM SISTEMAS DE LIBERTACAO TRANSDERMICA - DETERMINACAO DA VELOCIDADE DE LIBERTACAO]. Revista Portuguesa de Farmacia 46,1(1996): 4-8.

[36] Desai, S.J., et al., Investigation of factors influencing release of solid drug dispersed in inert matrices III. Quantitative studies involving the polyethylene plastic matrix. Journal of Pharmaceutical Sciences 55,11(1966): 1230-1234.

[37] Desai, S.J., et al., Investigation of factors influencing release of solid drug dispersed in inert matrices IV. Some studies involving the polyvinyl chloride matrix. Journal of Pharmaceutical Sciences 55,11(1966): 1235-1239.

[38] Schwartz, J.B., A.P. Simonelli, and W.I. Higuchi, Drug release from wax matrices I. Analysis of data with first-order kinetics and with the diffusion-controlled model. Journal of Pharmaceutical Sciences 57,2(1968): 274-277.

[39] Schwartz, J.B., A.P. Simonelli, and W.I. Higuchi, Drug release from wax matrices II. Application of a mixture theory to the sulfanilamide—wax system. Journal of Pharmaceutical Sciences 57,2(1968): 278-282.

[40] Korsmeyer, R.W., et al., Mechanisms of solute release from porous hydrophilic polymers. International Journal of Pharmaceutics 15,1(1983): 25-35.

[41] Ritger, P.L. and N.A. Peppas, A simple equation for description of solute release II. Fickian and anomalous release from swellable devices. Journal of Controlled Release 5,1(1987): 37-42.

[42] Lee, P.I., Kinetics of drug release from hydrogel matrices. Journal of

Controlled Release 2(1985): 277-288.

[43] Gbureck, U., E. Vorndran, and J.E. Barralet, Modeling vancomycin release kinetics from microporous calcium phosphate ceramics comparing static and dynamic immersion conditions. Acta Biomaterialia 4,5(2008): 1480-1486.

[44] Peppas, N.A., Analysis of Fickian ans non-Fickian drug release from polymers. Pharmaceutica Acta Helvetiae 60,4(1985): 110-111.

[45] Jorgensen, K. and L. Jacobsen, Factorial design used for ruggedness testing of flow through cell dissolution method by means of Weibull transformed drug release profiles. International Journal of Pharmaceutics 88,1-3(1992): 23-29.

[46] Yoshii, H., et al., Release kinetics of (−)-menthol from chewing gum. Journal of Inclusion Phenomena and Macrocyclic Chemistry 57,1(2007): 591-596.

[47] Shiga, H., et al., FLAVOR ENCAPSULATION AND RELEASE CHARACTERISTICS OF SPRAY-DRIED POWDER BY THE BLENDED ENCAPSULANT OF CYCLODEXTRIN AND GUM ARABIC. Drying Technology: An International Journal 19,7(2001): 1385 - 1395.

[48] Hancock, J.D. and J.H. Sharp, Method of Comparing Solid-State Kinetic Data and Its Application to the Decomposition of Kaolinite, Brucite, and BaCO3. Journal of the American Ceramic Society 55,2(1972): 74-77.

[49] Khawam, A. and D.R. Flanagan, Solid-State Kinetic Models: Basics and Mathematical Fundamentals. The Journal of Physical Chemistry B 110,35(2006): 17315-17328.

[50] Cussler, E.L., Diffusion : Mass Transfer In Fluid Systems. 3rd ed. 2009, United Kingdom: Cambridge University. 629.

[51] Cayot, N., et al., Measurement of transport phenomena of volatile compounds: A review. Food Research International 41,4(2008): 349-362.

[52] Poling, B.E., J.M. Prausnitz, and J.P. O'Connell, Properties of Gases and Liquids (5th Edition), McGraw-Hill.

[53] Balayssac, S., Gilard, V. , Delsuc, M.A. , Malet-Martino, M., DOSY NMR, a

new tool for fake drug analyses. Spectroscopy Europe 21,3(2009): 10-14.

[54] Huo, R., et al., Assessment of techniques for DOSY NMR data processing. Analytica Chimica Acta 490,1-2(2003): 231-251.

[55] Kapur, G.S., M. Findeisen, and S. Berger, Analysis of hydrocarbon mixtures by diffusion-ordered NMR spectroscopy. Fuel 79,11(2000): 1347-1351.

[56] Morris, K.F., P. Stilbs, and C.S. Johnson, Analysis of mixtures based on molecular size and hydrophobicity by means of diffusion-ordered 2D NMR. Analytical Chemistry 66,2(1994): 211-215.

[57] Gil, A.M., et al., Exploratory applications of diffusion ordered spectroscopy to liquid foods: an aid towards spectral assignment. Analytica Chimica Acta 506,2(2004): 215-223.

[58] Macchioni, A., et al., Determining accurate molecular sizes in solution through NMR diffusion spectroscopy. Chemical Society Reviews 37,3(2008): 479-489.

[59] Morris, K.F. and C.S. Johnson, Diffusion-ordered two-dimensional nuclear magnetic resonance spectroscopy. Journal of the American Chemical Society 114,8(1992): 3139-3141.

[60] Savary, G., et al., Influence of Ingredients on the Self-Diffusion of Aroma Compounds in a Model Fruit Preparation:â€‰ An Nuclear Magnetic Resonanceâˆ’Diffusion-Ordered Spectroscopy Investigation. Journal of Agricultural and Food Chemistry 54,3(2006): 665-671.

[61] Morris, K.F. and C.S. Johnson, Resolution of discrete and continuous molecular size distributions by means of diffusion-ordered 2D NMR spectroscopy. Journal of the American Chemical Society 115,10(1993): 4291-4299.

[62] Fantin, G., et al., Selective Removal of Monoterpenes from Bergamot Oil by Inclusion in Deoxycholic Acid. Journal of Agricultural and Food Chemistry 58,9(2010): 5438-5443.

[63] Guerrini, A., et al., Bergamot (Citrus bergamia Risso) Fruit Extracts as alfa-Globin Gene Expression Inducers: Phytochemical and Functional

Perspectives. Journal of Agricultural and Food Chemistry 57,10(2009): 4103-4111.

[64] Pernice, R., et al., Bergamot: A source of natural antioxidants for functionalized fruit juices. Food Chemistry 112,3(2009): 545-550.

[65] Verzera, A., et al., Effects of Rootstock on the Composition of Bergamot (Citrus bergamia Risso et Poiteau) Essential Oil. Journal of Agricultural and Food Chemistry 51,1(2002): 206-210.

[66] Sakurada, T., et al., Chapter 18 Intraplantar Injection Of Bergamot Essential Oil Into The Mouse Hindpaw: Effects On Capsaicin[hyphen (true graphic)]Induced Nociceptive Behaviors, in International Review of Neurobiology.237-248. Academic Press.2009

[67] Mandalari, G., et al., Antimicrobial activity of flavonoids extracted from bergamot (Citrus bergamia Risso) peel, a byproduct of the essential oil industry. Journal of Applied Microbiology 103,6(2007): 2056-2064.

[68] Mandalari, G., et al., Enzymatic Hydrolysis of Flavonoids and Pectic Oligosaccharides from Bergamot (Citrus bergamia Risso) Peel. Journal of Agricultural and Food Chemistry 54,21(2006): 8307-8313.

[69] Mandalari, G., et al., Characterization of Flavonoids and Pectins from Bergamot (Citrus bergamia Risso) Peel, a Major Byproduct of Essential Oil Extraction. Journal of Agricultural and Food Chemistry 54,1(2005): 197-203.

[70] Verzera. A.; Lamonica. G.;Mondello. L., T.A., Dugo. G., The composition of bergamot oil. Perfum. Flavor. 21,6(1996): 19-28, 30-32, 34.

[71] Virot, M., et al., Green procedure with a green solvent for fats and oils' determination: Microwave-integrated Soxhlet using limonene followed by microwave Clevenger distillation. Journal of Chromatography A 1196-1197(2008): 147-152.

[72] Selli, S., T. Cabaroglu, and A. Canbas, Volatile flavour components of orange juice obtained from the cv. Kozan of Turkey. Journal of Food Composition and Analysis 17,6(2004): 789-796.

[73] Bertolini, A.C., A.C. Siani, and C.R.F. Grosso, Stability of Monoterpenes Encapsulated in Gum Arabic by Spray-Drying. Journal of Agricultural

and Food Chemistry 49,2(2001): 780-785.

[74] Kaushik, V. and Y.H. Roos, Limonene encapsulation in freeze-drying of gum Arabic-sucrose-gelatin systems. LWT - Food Science and Technology 40,8(2007): 1381-1391.

[75] Boutboul, A., et al., Influence of the nature and treatment of starch on aroma retention. Carbohydrate Polymers 47,1(2002): 73-82.

[76] Eugenia Monge, M., et al., Correlation between rheological properties and limonene release in pectin gels using an electronic nose. Food Hydrocolloids 22,5(2008): 916-924.

[77] Linck, V.M., et al., Effects of inhaled Linalool in anxiety, social interaction and aggressive behavior in mice. Phytomedicine 17,8-9(2010): 679-683.

[78] Lapczynski, A., C.S. Letizia, and A.M. Api, Addendum to Fragrance material review on linalool. Food and Chemical Toxicology 46,11, Supplement 1(2008): S190-S192.

[79] Lapczynski, A., et al., Fragrance material review on l-linalool. Food and Chemical Toxicology 46,11, Supplement 1(2008): S195-S196.

[80] Linck, V.d.M., et al., Inhaled linalool-induced sedation in mice. Phytomedicine 16,4(2009): 303-307.

[81] Elisabetsky, E., Coelho de Souza, G.P., Dos Santos, M.A.C., Siqueira, I.R., Amador, T.A., Sedative properties of linalool. Fitoterapia 66,5(1995): 407-414.

[82] Hardin, A., P.G. Crandall, and T. Stankus, Essential Oils and Antioxidants Derived From Citrus By-Products in Food Protection and Medicine: An Introduction and Review of Recent Literature. Journal of Agricultural & Food Information 11,2: 99 - 122.

[83] Letizia, C.S., et al., Fragrance material review on linalyl acetate. Food and Chemical Toxicology 41,7(2003): 965-976.

[84] Peana, A.T., et al., Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. Phytomedicine 9,8(2002): 721-726.

[85] Varona, S., et al., Supercritical carbon dioxide fractionation of Lavandin essential oil: Experiments and modeling. The Journal of Supercritical

Fluids 45,2(2008): 181-188.

[86] Xiaoyan, S., et al., Preparation and Properties of Octenyl Succinic Anhydride Modified Early Indica Rice Starch. Starch - Starke 58,2(2006): 109-117.

[87] Bao, J., et al., Physical Properties of Octenyl Succinic Anhydride Modified Rice, Wheat, and Potato Starches. Journal of Agricultural and Food Chemistry 51,8(2003): 2283-2287.

[88] Liu, Z., et al., Production of Octenyl Succinic Anhydride-Modified Waxy Corn Starch and Its Characterization. Journal of Agricultural and Food Chemistry 56,23(2008): 11499-11506.

[89] Baranauskiene, R., et al., Flavor Retention of Peppermint (Mentha piperita L.) Essential Oil Spray-Dried in Modified Starches during Encapsulation and Storage. Journal of Agricultural and Food Chemistry 55,8(2007): 3027-3036.

[90] Bai, Y., Y.-C. Shi, and D.L. Wetzel, Fourier Transform Infrared (FT-IR) Microspectroscopic Census of Single Starch Granules for Octenyl Succinate Ester Modification. Journal of Agricultural and Food Chemistry 57,14(2009): 6443-6448.

[91] Randal, L.S., et al., Distribution of Octenyl Succinate Groups in Octenyl Succinic Anhydride Modified Waxy Maize Starch. Starch - Stirke 52,6-7(2000): 196-204.

[92] Jafari, S.M., Y. He, and B. Bhandari, Effectiveness of encapsulating biopolymers to produce sub-micron emulsions by high energy emulsification techniques. Food Research International 40,7(2007): 862-873.

[93] Jafari, S.M., et al., Nano-particle encapsulation of fish oil by spray drying. Food Research International 41,2(2008): 172-183.

[94] Jafari, S.M., Y. He, and B. Bhandari, Production of sub-micron emulsions by ultrasound and microfluidization techniques. Journal of Food Engineering 82,4(2007): 478-488.

[95] Jafari, S., Y. He, and B. Bhandari, Optimization of nano-emulsions production by microfluidization. European Food Research and Technology

225,5(2007): 733-741.

[96] Nilsson, L. and B. Bergenstahl, Adsorption of Hydrophobically Modified Starch at Oil/Water Interfaces during Emulsification. Langmuir 22,21(2006): 8770-8776.

[97] Yusoff, A. and B.S. Murray, Modified starch granules as particle-stabilizers of oil-in-water emulsions. Food Hydrocolloids 25,1(2011): 42-55.

[98] Zhu, W. and G.B. Lockwood, Enhanced biotransformation of terpenes in plant cell suspensions using controlled release polymer. Biotechnology Letters 22,8(2000): 659-662.

[99] Ramishvili, T., V. Yushchenko, and M. Charkviani, Catalytic conversions of linalool and linalyl acetate over large-pore zeolites and mesoporous MCM-41. Moscow University Chemistry Bulletin 62,4(2007): 180-186.

[100] Le Thanh, M., et al., Interactions between volatile and non-volatile compounds in the presence of water. Food Chemistry 43,2(1992): 129-135.

[101] Goubet, I., J.L. Le Quere, and A.J. Voilley, Retention of Aroma Compounds by Carbohydrates: Influence of Their Physicochemical Characteristics and of Their Physical State. A Review. Journal of Agricultural and Food Chemistry 46,5(1998): 1981-1990.

[102] Lee, S., et al., Microscopic analysis of ester hydrolysis reaction catalyzed by Candida rugosa lipase. Colloids and Surfaces B: Biointerfaces 47,1(2006): 78-84.

[103] Soottitantawat, A., et al., Microencapsulation by Spray Drying: Influence of Emulsion Size on the Retention of Volatile Compounds. Journal of Food Science 68,7(2003): 2256-2262.

[104] Wilke, C.R. and P. Chang, Correlation of diffusion coefficients in dilute solutions. AIChE Journal 1,2(1955): 264-270.

[105] Gunning, Y.M., et al., Factors Affecting the Release of Flavor Encapsulated in Carbohydrate Matrixes. Journal of Agricultural and Food Chemistry 47,12(1999): 5198-5205.

[106] Renganathan, V. and K.M. Madyastha, Linalyl Acetate Is Metabolized by

Pseudomonas incognita with the Acetoxy Group Intact. Appl. Environ. Microbiol. 45,1(1983): 6-15.

[107] Whorton, C. and A. Reineccius Gary, Evaluation of the Mechanisms Associated with the Release of Encapsulated Flavor Materials from Maltodextrin Matrices, in Encapsulation and Controlled Release of Food Ingredients.143-160. American Chemical Society.1995

BIOGRAPHY

Pailin Penbunditkul was born on October 20, 1985, in Chainat, Thailand but grew up in Nakhonsawan, Thailand. She graduated from high school from Takhliprachason School (Nakhonsawan, Thailand). In 2007, she earned a bachelor's degree in Chemical Engineering from Thammasat University (Bangkok, Thailand) with second class honors and a 1st ranking in the Chemical Engineering class. She then studied in Chemical Engineering at Chulalongkorn University from 2008 to 2012 graduating on a Master and Doctor of Engineering course.