Stability and bioactivities of *Mesona chiensis* Benth extract
microbeads under simulated gastrointestinal digestion

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ความคงตัวและฤทธิ์ทางชีวภาพของไมโครบีดของสารสกัดจากเฉาก๊วยภายใต้สภาวะจำลองการย่อยใน
ระบบทางเดินอาหาร

นางสาวชนม์นิภา ว่องวีรวัฒนกุล

Thesis Title   Stability and bioactivities of *Mesona chiensis* Benth extract microbeads under simulated gastrointestinal digestion

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ชนม์นิภา ว่องวีรวัฒนกุล : ความคงตัวและฤทธิ์ทางชีวภาพของไมโครบีดของสารสกัดจาก
เฉาก๊วยภายใต้สภาวะจำลองการย่อยในระบบทางเดินอาหาร (Stability and
bioactivities of *Mesona chiensis* Benth extract microbeads under simulated
gastrointestinal digestion) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.สิริชัย อดิศักดิ์วัฒนา,
หน้า.

ในการศึกษานี้ สารสกัดจากหญ้าเฉาก๊วย (*Mesona chinensis* extract; MCE) ถูกเอน
แคปซูเลชันด้วยแคลเซียมและอัลจิเนต ในขั้นแรก MCE ผ่านการเอนแคปซูเลชันด้วยแคลเซียมคลอ
ไรด์ (3 และ 5% โดยมวลต่อปริมาตร) และโซเดียมอัลจิเนต (1.5 และ 1.8% โดยมวลต่อปริมาตร)
เพื่อทำให้อยู่ในรูปเม็ดไมโครบีดเฉาก๊วย (MC microbeads; MCB) เม็ดไมโครบีดเฉาก๊วยที่ได้จาก
MCE 75% โดยมวลต่อปริมาตร, อัลจิเนต 1.5% โดยมวลต่อปริมาตรและแคลเซียมคลอไรด์ 3% โดย
มวลต่อปริมาตรถูกเลือกไปใช้ในการศึกษาขั้นถัดไปโดยพิจารณาจากประสิทธิภาพในการเอนแคปซูเล
ชันและลักษณะทรงกลมเมื่อเทียบกับเม็ดบีดที่ได้จากสภาวะอื่นๆ ผลการศึกษาด้วยเครื่อง Fourier
transform infrared spectroscopy (FT-IR) รายงานว่าไม่มีปฏิกิริยาทางเคมีเกิดขึ้นระหว่างอัลจิเนต
และสารสกัดเฉาก๊วย เมื่อผ่านระบบจำลองการย่อยสารประกอบฟีโนลิก (TPC) และฤทธิ์การต้าน
ออกซิเดชันที่วัดด้วยวิธี FRAP ของ MCE ลดลง 20.87 ± 3.78% และ 41.95 ± 3.68% อย่างมี
นัยสำคัญทางสถิติ การเอนแคปซูเลชันด้วยอัลจิเนตทำให้เกิดการปลดปล่อย TPC อย่างช้าๆ และทำ
ให้ค่า FRAP ต่ำในระยะกระเพาะอาหาร ในขณะที่เพิ่มการปลดปล่อย TPC และค่า FRAP ในระยะ
ลำไส้ เมื่อนำเจลลี่จากอะการ์และคาราจีแนนที่มีโพลีฟีนอลของเฉาก๊วยทั้งในรูปสารสกัดและไมโครบีด
ไปผ่านระบบการย่อยจำลองพบว่าเจลลี่สามารถปกป้อง TPC ได้ประมาณ 50% ในระยะกระเพาะ
อาหารและทำให้เกิดการปลดปล่อย TPC อย่างช้าๆในระยะลำไส้ นอกจากนี้ค่า FRAP ของโพลีฟีนอล
จากเฉาก๊วยในเจลลี่ทำด้วยอะการ์ยังแสดงถึงการเสริมฤทธิ์กันในฤทธิ์การต้านออกซิเดชัน โดยสรุป
การศึกษานี้พบว่าการเอนแคปซูเลชันด้วยอัลจิเนตหรือการนำไปใส่ในเจลลี่ที่ทำจากอะการ์และคารา
จีแนนสามารถเพิ่มการนำไปใช้ทางชีวภาพ (Bioaccessibility) ของสารสกัดจากหญ้าเฉาก๊วยและฤทธิ์
การต้านออกซิเดชันของสารสกัดดังกล่าวได้

ภาควิชา   โภชนาการและการกำหนดอาหาร   ลายมือชื่อนิสิต ....
สาขาวิชา   อาหารและโภชนาการ   ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
ปีการศึกษา 2560

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KEYWORDS: MESONA CHINENSIS / ALGINATE BEAD / AGAR / CARRAGEENAN

CHONNIPA WONGVERAWATTANAKUL: Stability and bioactivities of *Mesona chiensis* Benth extract microbeads under simulated gastrointestinal digestion. ADVISOR: ASSOC. PROF. SIRICHAI ADISAKWATTANA, Ph.D., pp.

Alginate-calcium microencapsulation of *Mesona chinensis* extract (MCE) was conducted in this study. Firstly, the MCE was encapsulated by using calcium chloride (3 and 5% w/v) and sodium alginate (1.5 and 1.8% w/v) to form MC microbeads (MCB). The MCB with condition of 1.5% w/v sodium alginate, 3% w/v calcium chloride and 75% w/v MCE demonstrated the highest %encapsulation efficiency and suitable spherical shape among other conditions. According to Fourier-transform infrared spectroscopy (FT-IR), there was no strong chemical interaction between alginate and MCE. After simulated digestion, the total phenolic content (TPC) and antioxidant activity measured by ferric reducing antioxidant power (FRAP) of MCE was significantly decreased for $20.87 \pm 3.78\%$ and $41.95 \pm 3.68\%$ respectively. In gastric phase, alginate-encapsulation of MCE slow released the TPC and exhibited low FRAP value whereas it enhanced the release of TPC release and an increase in FRAP value in intestinal phase. The simulated gastric digestion of agar and carrageenan contained MC polyphenols, MCE and MCB, showed partially protection (~50%) and increased the gradual release of polyphenols after entering into the intestinal phase. The FRAP value of MC polyphenols in agar demonstrated the synergistic antioxidant activity. In conclusion, alginate encapsulation or incorporation in agar and carrageenan improve bioassessibility of MC polyphenols and its antioxidant activity.

Department:	Nutrition and Dietetics	Student's Signature ....
Field of Study:	Food and Nutrition	Advisor's Signature ....
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CHULALONGKORN UNIVERSITY

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CHAPTER I

INTRODUCTION

1. Background and significant of the study

Polyphenols are secondary metabolites from plants and can be found abundantly in foods. Scientific evidences have shown that polyphenols exhibit a wide range of bioactivities including antioxidant, anti-inflammation, inhibition of oxidizing LDL, delay of carbohydrate digestion and inhibition of intestinal glucose absorption (Pandey and Rizvi 2009). In addition, their biological effects have been associated with prevention of degenerative diseases such as cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegeneration (Arts and Hollman 2005). Several polyphenols from different dietary sources have been shown health benefits. For example, grape seed flavonoids could inhibit lipid peroxidation and the inflammatory mediators cyclo-oxygenase (COX)-1 and -2 and demonstrated antioxidant and anti-inflammatory effects (Seeram, Cichewicz et al. 2003). Onion quercetin has found to protect the lipid peroxidation in diabetes patient (Rizvi and Mishra 2009). Green tea catechins increased energy expenditure, fat oxidation and promote weight maintenance after weight loss (Kovacs, Lejeune et al. 2004).

According to their beneficial effects to human health, polyphenols have become an interesting alternative option for development of functional food products. Many studies have incorporated these substances into food products to improve

polyphenol stability and its antioxidant properties in order to improve health such as mango polyphenol-added muffin (Ramírez-Maganda, Blancas-Benítez et al. 2015) and peanut skin polyphenol-fortified cookies (De Camargo, Vidal et al. 2014). Even though these polyphenols have beneficial effects to health, they have low bioaccessibility due to the sensitivity to light, heat, pH, temperature (Bell 2001, Arabshahi-D, Devi et al. 2007). Gastrointestinal condition may lead to the unachievable desired concentration (Friedman and Jürgens 2000) due to the drastic change of pH and enzymatic activities. Since the health effects of polyphenols depend on their bioavailability, improving the bioassessibility might increase the benefit the body will gain from polyphenols. There are several methods to improve bioassessibility of polyphenols including thermal and physical treatment to change the polyphenols structure (Wang, He et al. 2014) and microencapsulation to coat polyphenols and promote polyphenols release at the active site (McClements 2014).

Polysaccharides are generally used as the coating agents due to their ability to self-assembly to form gel structure (Imeson 2011). There are several interesting aspects of polysaccharides including alginate, agar and carrageenan on improvement of the polyphenols bioassessibility. Alginate is one of the common coating materials made from brown algae (Smidsrød and Skja 1990). The structure of alginate consists of D-mannuronic acid and L-glucoronic acid residues. Alginate has low toxicity and can easily form to hydrogel by interaction with divalent ion, commonly Ca^{2+} (Lee and Mooney 2012). The cross-linking process between alginate and Ca^{2+} can trap the

active compounds such as drugs or nutraceuticals inside the core structure during microencapsulation process (McClements 2017). This structure can tolerate acidic environment and break down in basic condition (Li, Kim et al. 2016). Therefore, alginate may protect content inside from acidic gastric condition and promote release under intestinal condition. Even though the preparation of alginate beads by cross-linking process is convenient and food-grade available, it can result in rapid and poorly controlled gelation due to high solubility in aqueous solutions (Lee and Mooney 2012). Alginate encapsulation of plant polyphenol extracts have been previously reported such as turmeric's curcumin (Zhang, Zhang et al. 2016) and *Pterospartum tridentatum* (Isailović, Kalušević et al. 2012). They enhance the stability and bioactivities of polyphenols under stimulated digestion. In addition, *Clitoria ternatea* alginate microbeads have shown to promote the release of polyphenols in stimulated intestinal digestion while small release of polyphenols occurs in stimulated gastric digestion (Pasukamonset, Kwon et al. 2016). Moreover, encapsulation of *Hypericum perforatum* increased thermal stability of the plant's flavonoids (Kalogeropoulos, Yannakopoulou et al. 2010). In food industry, alginate microbeads are used for fortification of specific nutrients, improving stability in final product and masking unpleasant sensory, especially for polyphenols bitterness (Nedovic, Kalusevic et al. 2011). Agar is the polysaccharide obtained from red algae and its structure consists of agarose and agaropectin. It can easily form gel structure by the rearrangement from random coil structure to helical structure after heat

treatment. Agar has been used in many applications in both food and research industry including microbial culture, molecular biology and food additive as thickening, gelling and emulsifying agents. In addition, agar-based diet could reduce calorie intake in overweight subjects (Maeda, Yamamoto et al. 2005) and delayed gastric emptying (Sanaka, Yamamoto et al. 2007). Agar is also resistance to gastric digestion (Kozu, Nakata et al. 2015, Kobayashi, Kozu et al. 2017) because it is undigested by pepsin. Carrageenan is another polysaccharide obtained from red algae. The usage of carrageenan is similar to agar as thickening, gelling and emulsifying agents. The carrageenan could form the gel by rearrangement of the structure but the cation such as Na^+, K^+, Ca^{2+} are required. Carrageenan hydrolyzed around 10% in gastric phase (Capron, Yvon et al. 1996). These polysaccharides might be able to prevent polyphenols release in gastric phase which may lead to increased absorption in intestinal phase. Even though several studies were investigated on stability of polyphenols in alginate beads after simulated digestion, there was a very few investigation on the effect of agar and carrageenan on polyphenols release and antioxidant activity. Edible agar film with green tea polyphenols could recover the polyphenols content and antioxidant activity during simulated digestion (López de Lacey, Giménez et al. 2012). In addition, agar and carrageenan might improve the content protection of alginate microbeads. Probiotics alginate microbeads in carrageenan jelly could protect the probiotics against gastric stimulated digestion (Talebzadeh and Sharifan 2017). Therefore, incorporate the alginate microbeads into

jellies such as agar and carrageenan might provide more protection to its polyphenols content.

China grass jelly (*Mesona chinensis* ; MC) is an herb commonly found in East and Southeast Asia. In traditional medicine, MC is used to treat liver diseases, hypertension and diabetes (Shen, Sun et al. 2000). MC extract contains several polyphenols and flavonoids (Hailan, Yingzhen et al. 2011) such as kaempferol and caffeic acid (Yen, Hung et al. 2003). Due to its high polyphenols content, MC exhibited various bioactivities such as antioxidant (Yen, Hung et al. 2003), anti-inflammatory (Huang, Liao et al. 2012) and antihyperlipidemic (Huang, Chuang et al. 2016) activities. Previous studies also reported that MC improved antioxidant status and attenuated postprandial glucose in overweight subjects (Chusak, Thilavech et al. 2014) by intestinal α-glucosidase inhibition and inhibited advanced glycation end products and protein oxidation *in vitro* (Adisakwattana, Thilavech et al. 2014). However, there is no available data on the bioaccessibility of MC extract after gastrointestinal digestion. Moreover, MC is traditionally consumed as herbal drink or jelly-type desserts. There is no investigation on the effects of the different gelling polysaccharides on the release of polyphenols and antioxidant activity of MC. Since these polysaccharides are exist as food matrix in the MC jelly (Kreungngern 2016), they might affect the polyphenol release and antioxidant activity during gastrointestinal digestion.

Therefore, it would be interesting to investigate the effect of digestion process on the stability of the polyphenols in MC extract. The research also investigated the MC extract in different form; extract, beads and jellies, on the release of polyphenols and antioxidant activity. The comparison of different polysaccharides effect on MC polyphenols will also be observed.

2. Objectives of the study

2.1 To develop the microbeads of *Mesona chinensis* and investigate its physical properties

2.2 To investigate the release of polyphenol and antioxidant activity of *Mesona chinensis* extract and its microbeads under stimulated digestion

2.3 To study the effect of different polysaccharides on the release of polyphenol and antioxidant activity of *Mesona chinensis* extract and its microbeads under stimulated digestion

3. Benefits of the study

The main expected outcome of the study is the knowledge on the bioassessibility and bioactivities of *Mesona chinensis* and the effects of different polysaccharides on the bioaccessibility of MC polyphenols. The information of plant extracts microencapsulation techniques from this study might provide more alternative choices to improve the plant health benefits. This study will be beneficial for product development in functional food, nutritional knowledge, raising values of agricultural outcome related to food commerce, business and food industry.

4. Conceptual framework

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┌─────────────────────────────┐
│ Dried Mesona chinensis      │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│ Water extraction            │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐          Simulated digestion    ┌──────────────────────────────┐
│ Mesona chinensis extract    │ ──────────────────────────────▶ │ Polyphenol ↓                 │
│ (MCE)                       │                                 │ Antioxidant activity ↓       │
└─────────────────────────────┘                                 └──────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐
│ Microencapsulation          │
└─────────────────────────────┘
              │
              ▼
┌─────────────────────────────┐          Simulated digestion    ┌──────────────────────────────┐
│ Mesona chinensis microbeads │ ──────────────────────────────▶ │ Polyphenol ↑                 │
│ (MCB)                       │                                 │ Antioxidant activity ↑       │
└─────────────────────────────┘                                 └──────────────────────────────┘
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┌──┐
│ 1. Physical properties   │
│ • Morphology :round, Ø < 2mm   │
│ • No strong chemical interaction   │
│   between alginate and polyphenols   │
│ • Thermal stability ↑   │
│ 2. Release in solvent (water)   │
└──┘

┌──────────────────────────────┐
│ Polysaccharides   │
│ • Agar   │
│ • Carrageenan   │
└──────────────────────────────┘

```
┌─────────────────────────────┐          Simulated digestion    ┌──────────────────────────────┐
│ Mesona chinensis jelly       │ ──────────────────────────────▶ │ Polyphenol ↑                 │
│ products                    │                                 │ Antioxidant activity ↑       │
│  • MCE agar jelly           │                                 └──────────────────────────────┘
│  • MCE carrageenan jelly    │
│  • MCB agar jelly           │
│  • MCB carrageenan jelly    │
└─────────────────────────────┘
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CHAPTER II

REVIEW OF LITERATURE

1. Phytochemicals

Phytochemicals are biologically, non-nutrient compounds found in natural products such as fruits and vegetables. These compounds have shown potential health benefits such as anti-inflammatory, anti-bacterial, anti-atherosclerotic, anti-oxidative and anti-tumor (Lagarda, García-Llatas et al. 2006). Based on the chemical structure, phytochemicals can be classified into carotenoids such as lutein and lycopene, polyphenols such as flavonoids and phenolic acids, alkaloids, nitrogen-containing compounds, and organosulfur compounds such as indoles. The most powerful phytochemical class found in plants is polyphenols that are interested as beneficial effects on human health (Scalbert, Johnson et al. 2005).

1.1 Polyphenols

Polyphenols are secondary metabolites from plants which consist of phenol groups in the structure. It can be found in various types of foods such as fruits, vegetables and cereals. According to epidemiological studies, these compounds have been linked to prevention of degenerative diseases such as cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative disease (Arts and Hollman 2005, Graf, Milbury et al. 2005). Polyphenols can be classified to many classes according to the structures such as number of phenol rings and basic structural

elements. The main classes of polyphenols are phenolic acids, flavonoids, stilbenes and lignans (figure 1) (Spencer, El Mohsen et al. 2008).
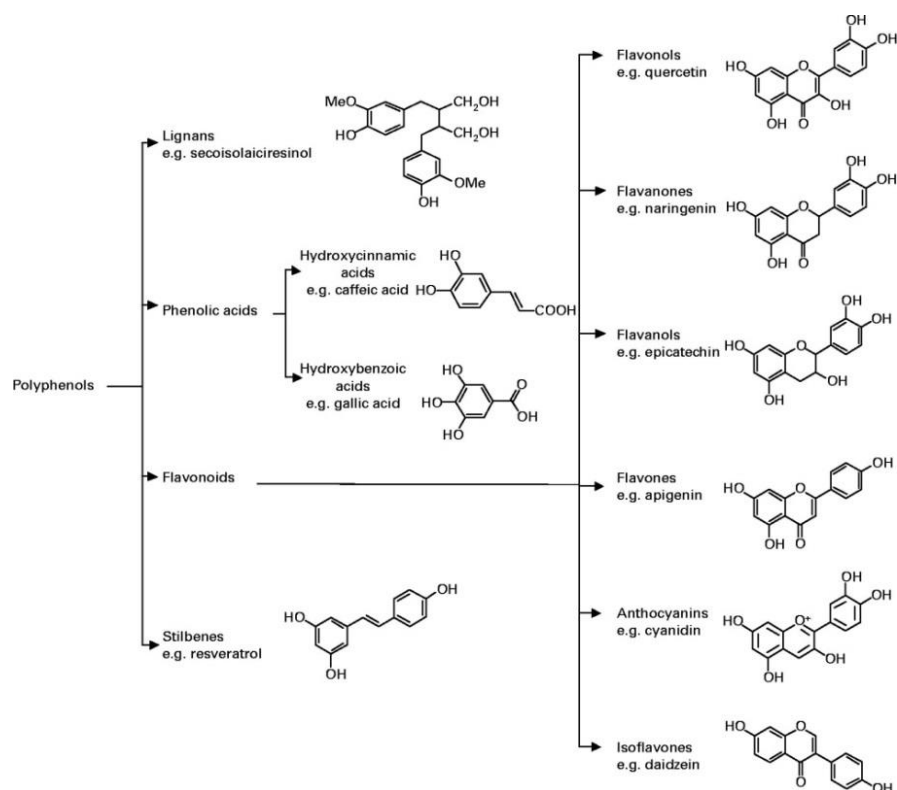
Figure 1. Polyphenol classes
(https://healthjade.com/what-are-polyphenols/)

Phenolic acids are phenol compound with one carboxylic group. They can be further divided to hydroxycinnamic and hydroxybenzoic group. The flavonoids are large group of polyphenolic substances that contain the flavane (2-phenyl-benzo-γ-pyrane) nucleus. This nucleus consists of two benzene rings (A and B rings) linked by pyrane ring (C ring). Flavonoids can be divided into subclasses; flavones, isoflavones, flavanols, flavonols, flavanones, anthocyanins and proanthocyanidins.

1.2 Putative health-promoting effects of polyphenols

1.2.1 Antioxidant effects

Many studies shown that plant extract polyphenols exhibit antioxidant properties (Hung and Yen 2002, Seeram, Cichewicz et al. 2003, Yen, Hung et al. 2003, Scalbert, Johnson et al. 2005, Pandey and Rizvi 2009, Isailović, Kalušević et al. 2012). The mechanism of antioxidant properties of polyphenol had been investigated and shown in figure 2. Polyphenols can inactivate free radical by transfering hydrogen atom (HAT) from O-H bond break or transfering single electron (SET) by donating single electron to free radical molecule (Leopoldini, Russo et al. 2011). Moreover, polyphenol might chelated the transition metals ions forming stable complexed compounds (Van Acker, Tromp et al. 1996, Brown, Khodr et al. 1998). This causes the entrapment of metal ions and prevents them to take part in the reactions generating free radicals.
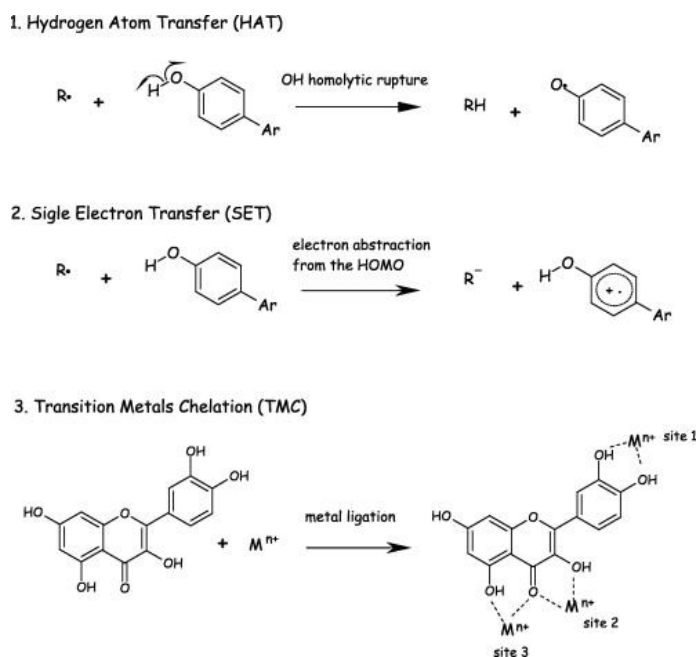
Figure 2. Antioxidant mechanisms of polyphenols

(Leopoldini, Russo et al. 2011)

1.2.2 Anti-inflammation effects

Previous studies had shown that polyphenols exhibited anti-inflammatory effects (Seeram, Cichewicz et al. 2003, Huang, Liao et al. 2012). This is because polyphenols affect the inflammatory pathway.

For example, grape seed extract (Seeram, Cichewicz et al. 2003), cocoa (Lee, Kundu et al. 2006) and green tea (Ahmed, Rahman et al. 2002) had shown inhibition of the prostaglandin formation pathway by inhibiting cyclooxygenase-1 (COX-1) and COX-2 (Yoon and Baek 2005). COX was not only polyphenol target in anti-inflammatory effects, polyphenol such as quercetin (Lee, Matteliano et al. 1982) and curcumin targeted at phospholipase A_2 (PLA_2).

1.2.3 Antidiabetic effects

When carbohydrate is consumed, the digestion started with salivary amylase to breakdown some of the starch into dextrin. Carbohydrate is mainly digested in the small intestine with several enzymes such as α-amylase, α-dextrinase, α-glucosidase. α-amylase hydrolyze the α-1,4 glycosidic linkage of the starch while α-glucosidase is the enzymes located at the brush border and digested starch and disaccharides to monosaccharides then the absorption can occur.

Polyphenols have been found to decrease the postprandial blood glucose level (Chusak, Thilavech et al. 2014, Castro-Acosta, Stone et al. 2017). It is possible due to ability to inhibit α-glucosidase and α-amylase (McDougall, Shpiro et al. 2005, Chusak, Thilavech et al. 2014). This leads to inhibition of starch digestion process. Tea polyphenols have found to inhibit α-amylase, pepsin, trypsin and lipase with the ratio of 61%, 32%, 38% and 54% compare with positive control respectively (He, Lv et al. 2007). *Mesona chinensis* polyphenols has also been reported to inhibit intestinal sucrase and maltase activities with IC50 values of 1.30 ± 0.43 mg/mL and 4.66 ± 0.26 mg/mL respectively (Chusak, Thilavech et al. 2014).

2. Mesona chinensis (MC)

2.1 Origin and distribution

Mesona chinensis (cin-cao or hsian-tsao or china grass tea) is an annual herb belongs to Lamiaceae family. According to Medicinal plant database, School of

Chinese Medicine, China, MC is used as traditional medicine to treat sunstrokes, diabetes, jaundice, diarrhea, dysentery, hypertension, myalgia and arthralgia, acute enteritis, anemogenous toothache, burns and scalding injuries, erysipelas, syphilis, hypersensitivity to pain (Liu 2012). MC is distributed in East and Southeast Asia. Based on plant database, MC is annual herb and can grow to 1 meter with stem upper erect (Liu 2012). MC is consumed as herbal drink (Hsian-tsao tea) or as jelly-type desserts (grass jelly, figure 3B) which made from dried MC. MC can easily form to jelly when adding the gelling agents, which are hydrocolloids such as potato starch, carrageenan, and agar (Kreungngern 2016).
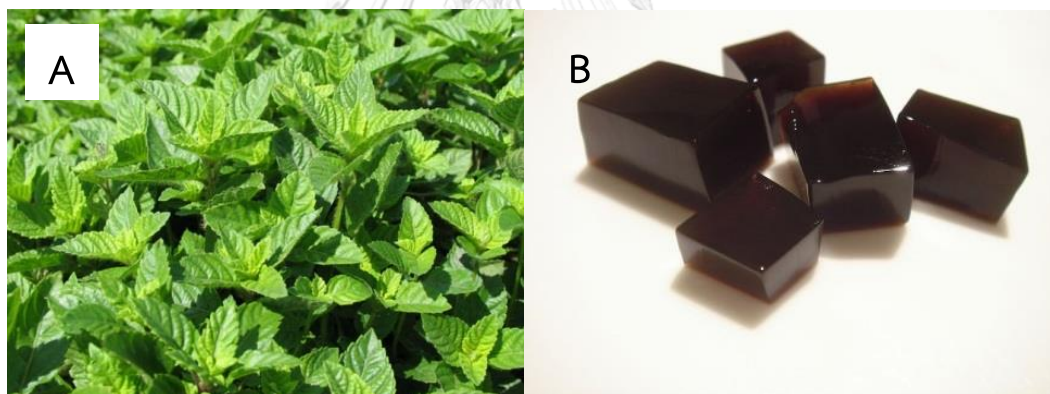
Figure 3. MC plant (A) and jelly made from grass jelly (B)

(https://libproject.hkbu.edu.hk and https://en.wikipedia.org/wiki/Grass_jelly)

2.2 Bioactive compounds

MC water extract contains 17 amino acid (7 essential amino acids), carbohydrates, fat, fiber, polyphenols, and flavonoids (Liu, Xia et al. 2005, Hailan, Yingzhen et al. 2011). The polyphenols found in MC water extract includes

kaempferol, apigenin, caffeic acid, protocatechuic acid, syringic acid, vanillic acid and
p-hydrobenzoic acid (Hailan, Yingzhen et al. 2011).

2.3 Pharmalogical activities

MCE exhibited many bioactivities. Some studies showed that MC exhibited antioxidant activity (Yen, Hung et al. 2003) which may resulted from bioactive compounds found in the extract such as kaempferol and caffeic acid (Yen, Hung et al. 2003). The extract showed hepatoprotective effect in liver fibrosis-induced rat (Shyu, Kao et al. 2008), antihypertensive effect in hypertensive rat (Yeh, Huang et al. 2009) and anti-inflammatory effect in the mouse paw oedema model (Huang, Liao et al. 2012). In addition, MC tea showed hypolipidaemic effects in both *in vivo* and *in vitro* studies by decreasing triacylglycerol(TAG), cholesterol and LDL in atherogenic hamster blood circulation and decreasing TAG and apolipoprotein B release from HepG2 cell (Huang, Chuang et al. 2016).

There are investigations of antidiabetic property of MC both in *in vitro* and clinical study. MC caused the inhibition of intestinal maltase and sucrose *in vitro* (Chusak, Thilavech et al. 2014) which directly affected on postprandial sugar level which proof by attenuate of postprandial sugar level in overweight subjects after consumption of beverage containing MC extract (Chusak, Thilavech et al. 2014). In addition, MC has also been revealed as antiglycation agent by inhibiting the formation of advanced glycation end products (AGEs) formation and the oxidation of

protein mediated by fructose in bovine serum albumin (Adisakwattana, Thilavech et al. 2014).

3. The stability and bioavailability of polyphenol

Bioavailability is the amount of the bioactive compounds ingested that could be use in physiological function. Therefore, it reflects the health benefit body can gain. Bioavailability can be further divided into bioaccessibility and bioactivity (Fernández-García, Carvajal-Lérida et al. 2009). Bioaccessibility is termed as the amount of compounds released from its matrix during digestion and become available for absorption through epithelial tissues, including pre-systemic metabolism (Benito and Miller 1998). The term bioactivity includes the health benefits from the active compounds, which does not depend on the bioaccessibility.

The health benefit of polyphenols is still limited due to the low bioaccessibility (Bell 2001) and the sensitivity to light, heat, pH and temperature (Arabshahi-D, Devi et al. 2007). When human consumed polyphenols: acidic condition in the stomach causes the polyphenol loss before entering the absorption site at small intestine (Friedman and Jürgens 2000). This leads to assume higher effective dose of polyphenols in *in vivo* studies when compared with *in vitro* ones (Bell 2001). In addition to polyphenol loss, antioxidant activities of polyphenol after simulated digestion had been found to decrease as well (Bermúdez-Soto, Tomás-Barberán et al. 2007). However, different types of polyphenols may lead to different results in

the polyphenol contents and antioxidant capacity (Wootton-Beard, Moran et al. 2011). Chemical structure of polyphenol affected the absorption. Some studies found that the polyphenols must be hydrolyzed from glycosylated form to aglycone form to be absorbed (D'Archivio, Filesi et al. 2010). However, glycoside form of anthocyanin group could be absorbed and detected in the circulation (Nurmi, Mursu et al. 2009).

There were several studies aiming to improve the bioavailability of the polyphenols including thermal or mechanical treatment to change its structure (Wang, He et al. 2014) or using encapsulation method to protect the structure and increase absorption (McClements 2014).

4. Microencapsulation

Microencapsulation is defined as a technology of packaging solids, liquids, or gaseous materials in miniature, sealed capsules that can release their contents at controlled rates under specific conditions (Desai and Jin Park 2005, Patil, Chavanke et al. 2012). Microencapsulation can be used in many aspects such as food industry, nutrition and pharmaceutical (McClements 2014). Since encapsulation can design to control release of active ingredients in desired condition, it can lead to release of drugs or nutrients in certain condition such as oral, gastric, small intestine or large intestine (McClements 2014). It can also be used in food industry to add certain active ingredients with specific attributes to food product such as colors, flavors,

antimicrobial, neutraceutials, antioxidants and preservatives which normally could not be added (McClements 2014).

There are varieties of coating substances and techniques to microencapsulated substances such as spray-drying, coacervation, liposomes, inclusion encapsulation, freeze-drying, yeast encapsulation, emulsions, etc (Patil, Chavanke et al. 2012).

4.1 Microencapsulation techniques

There are many types of microencapsulation methods. The suitable method for each microparticle is chosen depends on many factors such as the active compound, desired function and production cost. Some microencapsulation techniques are discussed below.

4.1.1 Ionotropic gelation technique

Ionotropic gelation method principle is based on the ability of polyelectrolytes polymers to cross link in the presence of its counter ions to form hydrogel beads (Patil, Chavanke et al. 2012). There are many types of biopolymers such as anioic polysaccharides (alginate, carrageenan, pectin, etc.), cationic polysaccharides (chitosan) and proteins (whey, casein, etc.). (McClements 2014). The formation can be divided into two process. The first step is to form the mixture of active compounds and biopolymers and the second step is to cross-link these polymers to form hydrogels (McClements 2017). There are several methods to

implement including injection, emulsion templating, electrostatic complexation, antisolvent precipitation and thermodynamic incompatibility (Desai and Jin Park 2005, McClements 2017)

Ionotropic gelation technique is easy to conduct and require simple equipment. However, many neutraceutical had low efficacy when use this method due to low solubility characteristics and low chemical stability (McClements 2017).

4.1.2 Spray-drying

Another encapsulation technique is the spray-drying method. Spray-dry machine can evaporate water from the solution to produce powder form by atomized in hot gas current (I Ré 1998). Encapsulation with spray-drying method contains four steps (Gharsallaoui, Roudaut et al. 2007). The first step is preparation of emulsion or dispersion which compose of core materials inside microcapsule include active compound. The second step is homogenizing the dispersion. The third and fourth step is inside the machine; atomization of the infeed emulsion and dehydration of the particles.

The advantage of spray-drying is the cost-effective and convenience. However, spray-drying machine is required. Another limitation is the limited wall materials available since they must highly soluble in water (Gharsallaoui, Roudaut et al. 2007). This method also produced microcapsules powder so they require further processing such as agglomeration.

4.1.3 Supercritical antisolvent

This technique promotes microencapsulation by using supercritical fluid which has properties as intermediate between liquid and gas. The properties can change by altered the pressure and temperature for example CO_2. In this method the supercritical fluid is use as antisolvent and another solvent that poorly soluble in this supercritical fluid is selected as solvent (Munin and Edwards-Lévy 2011). The difference solubility of solvent and antisolvent can lead to precipitation and form the particle.

The advantage of this method is the use of lower amount of organic solvent and safety. However, the particle size distribution from this method is heterogeneous (Munin and Edwards-Lévy 2011).

4.2 Coating material types

There are varieties of coating matetrials for encapsulation depends on the active compound properties and the desired function of the final encapsulted particles. According to McClements, there are three major structural components that use in coating food-grade active compounds (McClements 2014). Sometimes encapsulation use more than one types of the coating to produce microparticles.

4.2.1 Biopolymers

Most biopolymers use to encapsulated are proteins and polysaccharides. Common proteins using in encapsulation include whey, casein, soy protein and

gelatin while common polysaccharides include alginate, chitosan, pectin, carrageenan and inulin (McClements 2014). The biopolymers are chosen based on the solubility of active compound and functional requirement of particle.

4.2.2 Surfactants

Surfactants such as tween, span, lecithin are use in the system that contain both polar and non-polar substances since surfactants have amphiphilic structure (McClements 2015). The microparticle and be formed by using the hydrophobic effect. The microparticle form by using surfactants includes micelles and liposomes. It is noted that when using surfactants the addition process might added to ensure the dispersion of the surfactants (McClements 2014).

4.2.3 Lipids

Lipids are use in the emulsion-based techniques which formed the particle by blending of oil, water and surfactants to create the small particles. Lipid sources might came from animal fats, fish oils or plant oils (McClements 2014).

4.3 Formation of alginate beads by extrusion technique

Injection method or extrusion method is conducted by mixing the active compounds with the biopolymer and extrude the fluid through one or more orifices then trapping the particle formed into the stable form such as coating or solidifying (McClements 2014) as shown in figure 4 the biopolymer solution is dropped into the container that have to polyelectrolyte solution to promote gelation. The gelation of

hydrogel beads depends on the types of materials use to encapsulate. In this study, alginate is mixed with active compounds then dropped into calcium chloride solution to cross-link the biopolymers.
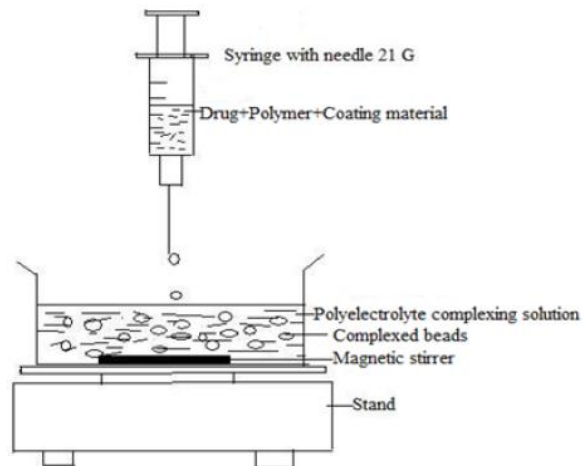
Fig. 3: It shows the diagrammatical presentation of Gelispheres preparation by polyelectrolyte complexation technique"

Figure 4. Hydrobeads preparation from ionotropic gelation technique with extrusion method
(Patil, Chavanke et al. 2012)

4.4 Applications of alginate in food industry

Alginate had been used as food additive as gelling, thickening, stabilizing and emulsifying agents due to its properties to form gel and it can retain food moisture and improve food texture (Rehm 2009). The alginate in the beads form also had been use in food application since many substances could be encapsulated such as neutraceuticals, antioxidants or even probiotics. Alginate had been incorporated into many foods to improve food properties. Alginate beads containing bacteoriocin could decrease the growth of parthogens in ground beef (Millette, Le Tien et al. 2007). Probiotics also had been encapsulated to improve their survival in yoghurt

(Krasaekoopt, Bhandari et al. 2003, Krasaekoopt, Bhandari et al. 2006) and jelly (Talebzadeh and Sharifan 2017).

5. Characterization of microcapsules

5.1 Percentage of Encapsulation efficiency (%EE) of the microcapsules

Encapsulation efficiency is the amounts of polyphenols that can be incorporated into the microbeads compare with initial extract. Higher %EE indicates that more polyphenols are encapsulated. Encapsulation efficiency of microbeads can be affected by many factors. In ionotropic gelation technique, the dropping rate affects the microbeads size range and encapsulation efficiency. Constant dropping rate leads to constant pressure applying to the syringe to drop the alginate-active compound solution. Curing time in calcium chloride solution also affects encapsulation efficiency, prolong curing time (more than 30 minutes) leads to loss of polyphenols and proanthocyanidins content in pomegranates extract (Zam, Bashour et al. 2014).

To form the appropriate calcium alginate microbeads, various concentrations of calcium chloride solution, sodium alginate and plant polyphenol extracts was used (Pasukamonset, Kwon et al. 2016). Higher alginate content increases the viscosity of alginate-polyphenol solution (Zam, Bashour et al. 2014) thus affects the dripping into the calcium chloride solution and may also increase the beads size. However, if the alginate content is too low the microbeads couldn't be formed due

to lack of carboxyl groups to interact with calcium ions. In previous study the range of alginate use is from 1.5-2% (Pasukamonset, Kwon et al. 2016). The concentration of calcium chloride solution provides calcium ion therefore it affects the microbeads formation (Lee, Ravindra et al. 2013). Too low calcium ion leads to lack of interaction to form microbeads. Moreover, excess calcium ion binding at glucuronic acid chain can damage microbeads structure due to lack of calcium ion entrapment in egg-box model (Zam, Bashour et al. 2014). The polyphenol content in plant extract also affects microbeads formation. Low polyphenol concentration filled in the microbeads leads to disheveled and unrounded shape of microbeads while high polyphenol concentration causes oval-like shaped of microbeads. In addition, high polyphenol concentration may exceed the ability of calcium alginate to trap polyphenol inside and loss in calcium solution and decrease %EE.

5.2 Morphology of the microcapsules by Scanning Electron Microscopy (SEM)

SEM is the type of microscope that uses the signal from electron wavelength after the electron beam contact with the surface sample to produce high-resolution image (Padua and Wang 2012).

The characteristics of microbeads are one of the factors that affect its stability and bioactivities. Major crack or rupture on the surface may leads to the content leakage (Pasukamonset, Kwon et al. 2016). The morphology and shape of the microbeads could be observed by using the Scanning Electron Microscopy.

5.3 Particle size analysis of microcapsules

Microbeads are small particulates that range from sub-micron to several millimeters in size (Fang and Bhandari 2010). The particle size can be classified by the diameter (d). The particle is called microcapsules or microbeads when 0.2 ≤ d ≥ 5000 μm while d < 0.2 often termed as nanocapsules or nanobeads (I Ré 1998). The termed capsules or beads are depended on the structure of the particle. The capsule often referred to the particle that has a distinct membrane that enveloped the liquid core inside while the term beads often referred to the particle that can't divided into membrane and core (Leong, Lam et al. 2016). The particle size can be observed by laser diffraction-based Malvern particle size analyzer. The principle is based on the light scattering pattern (intensity and scattering angle) produced after contact with the laser beam. It is noted that refractive index of the substances influences the scattering and absorption of light by the particles. Therefore, the calculate of particle size requires information on the refractive index of both the particle and dispersing medium (McClements 2014).

Sizes of the microbeads affect the performance of microbeads. The larger hydrogel beads lead to higher retention rate of active compound inside and decrease release rate due to the longer diffusion from the hydrogel matrix (Dan 2016). However, the stability age may increase with the diameter especially if the surrounding aqueous phase led to the active compound degradation because the distance between the active compound and the component that may affect the

active compound increases (McClements 2017). Moreover, the beads size affects food product appearance. Very small beads (d < 50 nm) might be optically transparent in the product. Moreover, the beads with d > 50 mm can be perceived as the particle in human mouth (McClements 2017). The size of microbeads differs from many factors such as encapsulation methodology, polymer types, etc (Lee, Ravindra et al. 2013). For example, the minimum diameters of hydrogel beads from injection method (approximately 10 μm) are larger than hydrogel beads from thermodynamic incompatibility (approximately 1 μm) and antisolvent precipitation (approximately 100 nm) (McClements 2017).

5.4 The chemical structure of the microcapsules by Fourier transform infrared (FT-IR) study

FT-IR is chemical analysis method that investigates the bonding presenting in molecules of the substances by detecting the vibration of functional groups in the infrared range (electro-magnetic radiation falling in the region from 0.7 - 1000 cm^{-1}). The information can use to characterize molecular structure and analyze the chemical interaction (Schmitt and Flemming 1998). The mid IR region (4000 to 400 cm^{-1}) are used in chemical analysis because the vibrations of the functional groups of organic molecules are included in this region (Doyle 1992). The example of different wavenumber of each functional group is presented in figure 5.
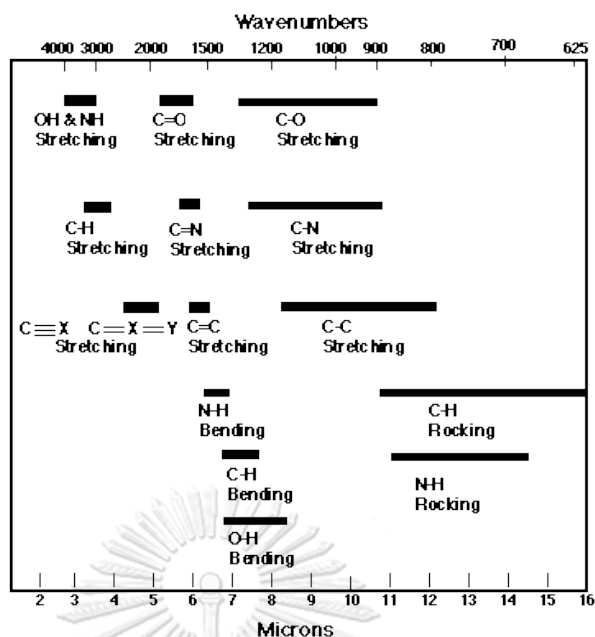
Figure 5. Infrared wavenumbers of different bonds
(http://www.wag.caltech.edu/home/jang/genchem/infrared.htm)

Attenuated Total Reflection (ATR)-FT-IR is the accessories for FT-IR that can use for study the chemical composition of the surface of materials (Schmitt and Flemming 1998). This accessory measures the changes of internally reflected IR beam when the beam comes into contact with a sample (Oelichmann 1989). Briefly, The IR beam is directed onto the crystal with a high refractive index at an angle greater than the critical angle for total reflection (Oelichmann 1989). The internal reflectance then creates a wave that extends beyond the surface of the crystal into the sample held in contact with the crystal then these waves are detected.

Several previous studies have indicated that FTIR shows no interaction between alginate and plant polyphenols such as plant extract from butterfly pea (Pasukamonset, Kwon et al. 2016), lemon (Najafi-Soulari, Shekarchizadeh et al. 2016)

and rose hip (Stoica, Pop et al. 2013). These studies show that there is no additional peak in FT-IR spectra in microbeads comparing with initial extract and alginate. Since there is no interaction between polyphenols and alginate, this may promote polyphenol release from the microbeads.

5.5 Thermal analysis of microcapsules by Differential Scanning Calorimeter (DSC)

Differential scanning calorimetry is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. DSC composed of measurement chamber which have 2 pans inside and the computer. One pan is for placing sample and the other one is left empty as the reference. The heat is applied to the pans at the same time and the computer will plot the difference in the heat output of the two pans against the temperature (Bershteĭn and Egorov 1994). Therefore it can track to changes when the endothermic or exothermic process occurs. The thermal behavior analysis from DSC is present as DSC thermogram. In the thermogram, melting point temperature (Tm), crystallization temperature (Tc) and glass transition temperature (Tg) can be seen.

5.5.1 Tg, Tc and Tm

Glass transition temperature (Tg) is the temperature that the polymers change from the amorphous structure (hard, brittle) to the molten (elastic) structure. When continued to heat the polymer the structures will undergo the rearrangements which

is the crystallization and the temperature at the exothermic peak of crystallization is

Tc. If continue to heat until the structure inside melts and broken down into

amorphous stage again the endothermic curve of melting point will appears on the

thermogram. The Tg, Tc and Tm is shown in the figure 6. Different substances have

different Tm, Tc and Tg. These parameters affect the structure of

polymers.(Bershteǐn and Egorov 1994, Montenegro 2003)
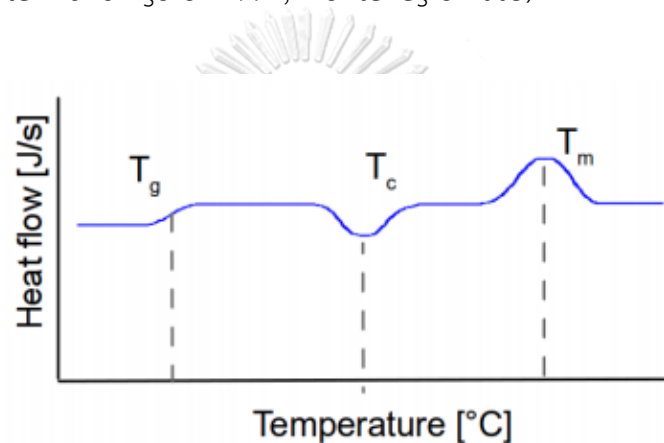
Figure 6. Example plot of thermogram for a polymer that undergoes a glass
transition, crystallization and melting
(Bershteǐn and Egorov 1994)

Encapsulation by alginate could improve thermal stability of active compound by

increasing the melting point temperature. The shift of decomposition peak (melting

point) of the active compound alginate beads to higher temperature compared with

the extract alone could be seen in the previous studies (Santos, Albarelli et al. 2013,

Pasukamonset, Kwon et al. 2016).

6. Advantages of the microencapsulation application

As already mentioned that microencapsulation can be applied to many industries such as pharmaceutical, food, cosmetic and agrochemical (McClements 2014). The advantage of the microencapsulation is the ability to apply with varied types of compounds and living cells. This is because there are many techniques and coating substances to select to match with the properties of active compounds and desired function after encapsulation. For food industry, encapsulation of active compounds can provide more nutritious food, longer shelf life, marked presence of some chemicals that might affect consumer acceptance such as bitterness, separate the two compounds that need to be in the same matrix, etc (Munin and Edwards-Lévy 2011). Moreover, some ingredients could not be directly incorporated in food because different of solubility or the low stability of the compound. These problems might be able to solved by incorporate these substances using microencapsulation techniques (McClements 2014). The microencapsulation of drugs could improve its usage in pharmaceutical industry such as prolonged release or design to release at specific condition to improve the drug performance. Some studies had been proved that microencapsulation could improve bioaccessibility of plant extract (Fernández-García, Rincón et al. 2008, Gómez-Estaca, Gavara et al. 2015, Gómez-Mascaraque, Sipoli et al. 2017) due to increase of polyphenol content after digestion compare with non-encapsulated extract.

7. Application of antioxidant in food products

Since antioxidants are able to inhibit oxidation of other molecules it could protect food from oxidation process. Oxidation of food can lead to changes in nutrients structure, organoleptic properties and decrease shelf-life. Antioxidants had been used to slow down the lipid oxidation in foods (Thorat, Jagtap et al. 2013). Some antioxidant substances also exhibit other biological function such as antibacterial effects so it could extend the shelf-life of food products(Pina-Pérez, Rodrigo et al. 2011). Moreover, antioxidants had shown potential to improve health since they can scavenge the free radicals. Oxidative stress in the body had been found to relate with diseases such as inflammatory diseases, ischemic diseases, and smoking-related disease. They also postulated with many health condition such as atherosclerosis, certain cancers, and aging process. This is because the excess free radicals could damage the structure and function of the DNA, lipids and proteins (Lobo, Patil et al. 2010).

8. Natural polysaccharides in food products

Polysaccharides are polymer consists of long chains of monosaccharides units linked together by glycosidic bond (Lovegrove, Edwards et al. 2017). There are many types of polysaccharides including starch, glycogen, cellulose, alginate, agar, carrageenan, etc. Since polysaccharides are one of the building block of the organism, it could be found in many diet especially plant diet (Lovegrove, Edwards et al. 2017). Polysaccharides from plant could be divided into two groups; storage

polysaccharides (ex. starch) and dietary fibers (ex. pectin, cellulose and hemicellulose) (Lovegrove, Edwards et al. 2017). However, some polysaccharides also obtained from other organism such as algae (ex. agar, alginate and carrageenan) (Imeson 2011). Many polysaccharides are use as stabilizer in foods including agar, alginate, carrageenan, gallen gum and etc. (Imeson 2011)

8.1 Alginate

Alginate is the linear polymer obtained from brown algae (Phaeophyceae) (Smidsrød and Skja 1990). Major components of alginate are L-guluronate (G) residue and D-mannuronate (M) (Lee and Mooney 2012) residue and these residues linked by β-1,4 to form the blocks. The blocks may consist of linear G residues (G block), M residues (M block) or both (GM block) as shown in figure 7. M and G content of alginate is different depends on the source of alginate (Tønnesen and Karlsen 2002).
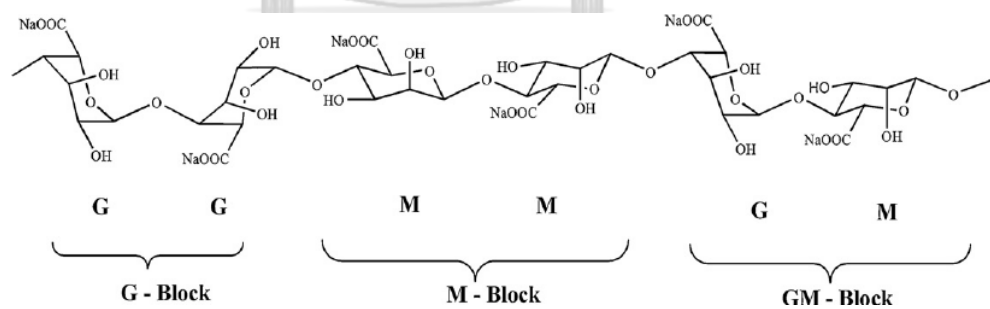
Figure 7. Sodium alginate chemical structure

(Daemi and Barikani 2012)

8.1.1 Application of alginate

Alginate is use in many biomedical approaches because of its biocompatibility, low toxicity and ability to form gelation by crosslinking method such as divalent ion (ex. Ca^{2+}, Ba^{2+}, Fe^{2+}, Sr^{2+}, etc.) (Lee and Mooney 2012). When divalent ion is added, it is bind to G block of alginate to form junctions with another G block of adjacent polymers and form the "egg-box" structure (Figure 8).
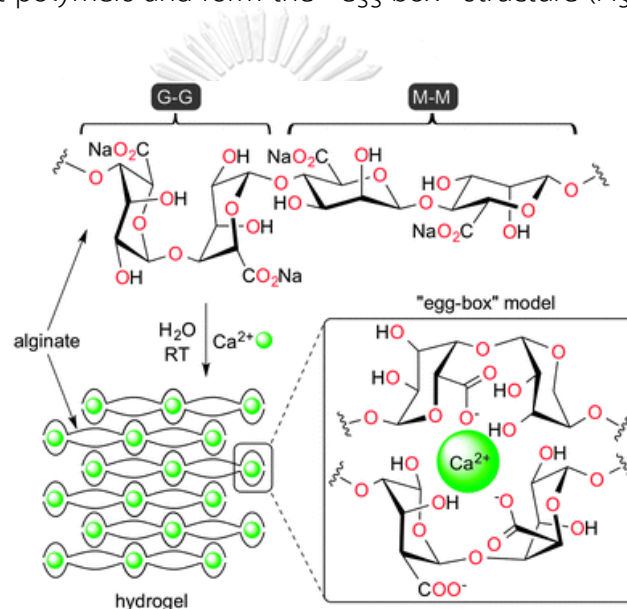
Figure 8. Egg-box model

(Kühbeck, Mayr et al. 2015)

Another feature of alginate that leads to application in controlled release is the pH (Tønnesen and Karlsen 2002). The release mechanism occurs due to the change of pore size of the biopolymers structure by the change of surrounding pH. When pH drop below pKa of the alginate (3.38 for mannuronic and 3.65 for glucuronic), the hydrogen bonding is stable (Chuang, Huang et al. 2017) but when the pH is raised higher than alginate pKa the hydrogen bond network are broken causing the

microbeads to swell. The release occurs when the size of the pore is larger than the active compound inside (figure 9).
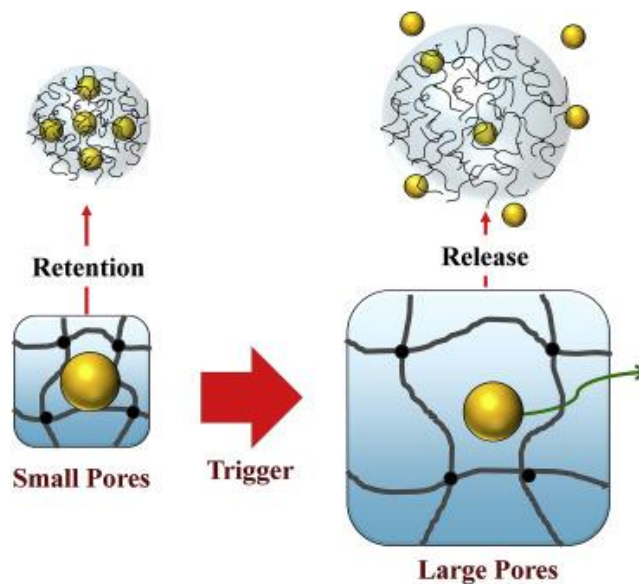
Figure 9. Release mechanism from hydrogel particle

(McClements 2017)

In physiological condition, these beads may protect its content from acidic condition in stomach and may promote the content release in basic condition in the small intestine condition (Tønnesen and Karlsen 2002). Therefore, the use of alginate in polyphenol microencapsulation may increase the amount of polyphenol at the absorption site and increase the absorption of polyphenol into the body. From the properties of alginate, it may protect content from gastric condition and promote release under intestinal condition.

Even though the preparation of alginate beads by cross-linking with Ca^{2+} is convenient, regard as safe and easy to conduct, it can result in rapid and poorly

controlled gelation due to high solubility in aqueous solutions (Lee and Mooney 2012). Therefore, several studies tried to overcome this by coating the alginate microbeads (Jayant, McShane et al. 2009) or combine with other substances such as chitosan (Santagapita, Mazzobre et al. 2012), gelatin (Song, Yang et al. 2014).

Many studies have been encapsulated polyphenols using sodium alginate and calcium. For example, encapsulation of *Clitoria ternatea* petal flower extract (Pasukamonset, Kwon et al. 2016) and *Pterospartum tridentatum* extract (Isailović, Kalušević et al. 2012) enhanced the polyphenol stability and bioactivities under stimulated digestion. Encapsulation of *Hypericum perforatum* improved thermal stability of flavonoids (Kalogeropoulos, Yannakopoulou et al. 2010).

8.2 Agar

Agar is a gelling agent extracted from red algae, consisted of at least two monomers; agarose and agaropectin (Figure 10). Agarose is a linear polymer structure consisting of alternating D-galactose and 3,6-anhydrous-L-galactose.
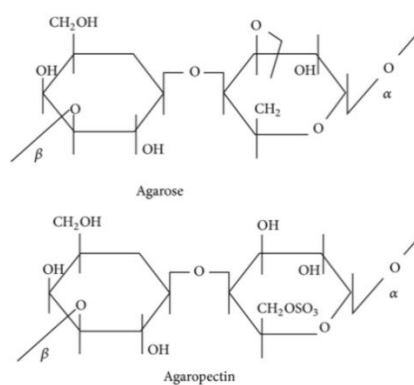
Figure 10. Agarose and agaropectin structure

(Varshosaz, Zaki et al. 2015)

8.2.1 Application of agar

Agar is widely use in food industry, it is ingredients for varieties of foods including jelly desserts, confectionaries, soups, dairy products (ex. Ice cream and custards) and canned meat products (Imeson 2011). Moreover, agar is use in many researches such as culture media in microbiology (Imeson 2011), agarose gel electrophoresis (Johansson 1972), or even use as model for mechanical movement digestion (Kozu, Nakata et al. 2015). Agar can form cold-set gel (Saha and Bhattacharya 2010) . Short-term study on agar diet had found that intake of agar diet in obese patients resulted in marked weight loss due to the maintenance of reduced calorie intake and to an improvement in metabolic parameters (Maeda, Yamamoto et al. 2005).

Using of agar as coating substance may protect the polyphenol digestion. Previous studies had incorporated green tea polyphenols into agar film. The film slow down the polyphenol release into the water surrounding (Giménez, López de Lacey et al. 2013) and the percentage of polyphenol recovery after simulated gastric phase approximate 50-80% and almost no addition of the percentage of polyphenol recover in simulated intestinal phase (López de Lacey, Giménez et al. 2012). The antioxidant activity (FRAP and ABTS) recovered after simulated digestion was approximately 40%.

8.3 Carrageenan

Carrageenans are polysaccharides extracted from the red algae. There are three basic types of carrageenan: iota carrageenan, kappa carrageenan and lambda carrageenan (Figure 8) (Chauhan and Saxena 2016).
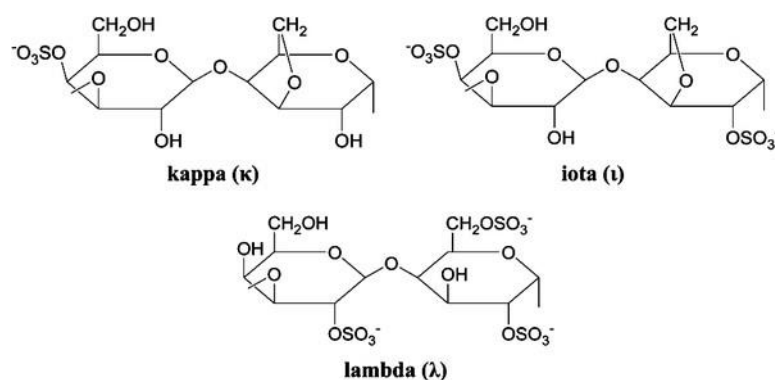
Figure 11. kappa, iota and lambda carrageenan structure
(Chauhan and Saxena 2016)

8.3.1 Application of carrageenan

Carrageenan have many applications in food industry such as cooking ingredients in many foods including milk-based pudding, dessert jelly, ice cream mix, etc. (Imeson 2011), thickening agent and stabilizing agents in foods (Saha and Bhattacharya 2010). Carrageenan gel could form the cold-set gel same as agar (McClements 2014) by rearrangement of structure from random to helix coil with cross-link to cation (Saha and Bhattacharya 2010). Each types of carrageenan lead to different gel type (Imeson 2011). Kappa carrageenan cross-link with K^+ to form brittle gel, while iota carrageenan cross-link with Ca^{2+} to form elastic and soft gel. Lambda carrageenan can form gel in very high salt concentration (Saha and Bhattacharya

2010, Imeson 2011). In research, lambda carrageenan is use as paw edema inducer in rodents to study inflammation (Morris 2003). The carrageenan was used as gelling agent to develop fruit jelly product with probiotic alginate microbeads (Talebzadeh and Sharifan 2017) the result show improvement of microbeads content protection from low pH and high temperature. Previous study shown that carrageenan hydrolysis occurs 10% in the simulated artificial stomach digestion (Capron, Yvon et al. 1996). Therefore it might protect the content inside during gastric phase.

9. Jelly product

Jelly is one of the common food products in Thailand and available in many forms such as jelly gummy and jelly drinks. Even though some products have claimed on antioxidant, information of the antioxidant capacity of jelly with polyphenols is still missing.

9.1 Polyphenol jellies

Several studies had investigated on the polyphenol content and antioxidant properties of jelly with polyphenols from various sources such as tea polyphenols (Gramza-Michalowska and Regula 2007), purple sweet potatoes (Choi and Lee 2013) and wild carrots (Kang, Kim et al. 2017). These studies found that the jelly product exhibited polyphenol content and antioxidant properties. However, these studies focused on the development of jelly product and the sensory acceptance of the product. There is one study on agar product with green tea polyphenols (López de

Lacey, Giménez et al. 2012) that investigated the bioaccessibility of polyphenols and found that agar film could decreased polyphenol release and antioxidant activity in gastric phase and provided maximum release in intestinal phase.

9.2 Microbeads jelly

Application of microbeads into jelly in food industry may provide extra protection layer for microbeads. It is possible that jelly will affect releasing pattern of polyphenols from microbeads and may delay the digestion on microbeads (López de Lacey, Giménez et al. 2012). Previous studies had incorporated probiotics alginate microbeads into carrageenan jelly product and found that jelly can protect the probiotics against high temperature and stimulated digestion (Talebzadeh and Sharifan 2017). However there are many gelling agents that can be added to form jelly such as agar and carrageenan and the study on these polysaccharides as the matrix for microbeads is still limited.

CHAPTER III

MATERIALS AND METHODS

1. Materials and equipment

Plant Materials	Company
Dried *Mesona chinensis* whole plant	a specific herbal drugstore (Ran-Khay-Ya-Chao-Krom-Poe)

Chemicals	Company
Sodium alginate (food grade)	Nerdy gummy (Bangkok, Thailand)
Calcium chloride (food grade; $CaCl_2$)	Nerdy gummy (Bangkok, Thailand)
Tri-Sodium citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$)	Ajax Finechem (Taren Point, Australia)
Sodium carbonate anhydrous (Na_2CO_3)	Ajax Finechem (Taren Point, Australia)
Folin-Ciocalteau's phenol reagent	Sigma-Aldrich CO. (St. Loius, MO, USA)
Gallic acid	Fluka™ (Seelze, Germany)
2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ)	Sigma-Aldrich CO. (St. Loius, MO, USA)
Iron (II) sulfate ($FeSO_4 \cdot 7H_2O$)	Ajax Finechem (Taren Point, Australia)
Iron (III) chloride anhydrous ($FeCl_3$)	Ajax Finechem (Taren Point, Australia)
Rat intestinal acetone powder	Sigma-Aldrich CO. (St. Loius, MO, USA)

Chemicals	Company
D-Maltose	Ajax Finechem (Taren Point, Australia)
D-Sucrose	Ajax Finechem (Taren Point, Australia)
Glucose liquicolor	HUMAN (GmbH, Germany)
Pepsin from porcine extract mucosa	Sigma-Aldrich CO. (St. Loius, MO, USA)
Amyloglucosidase	Roche (Basel, Switzerland)
Pancreatin	Sigma-Aldrich CO. (St. Loius, MO, USA)
Bile extract porcine	Sigma-Aldrich CO. (St. Loius, MO, USA)
Glacial acetic acid (CH_3COOH)	Merck (Darmstadt, Germany)
Sodium acetate hydrate ($CH_3COONa \cdot 3H_2O$)	Ajax Finechem (Taren Point, Australia)
Hydrochloric acid (HCl)	Merck (Darmstadt, Germany)
Sodium hydroxide (NaOH)	Ajax Finechem (Taren Point, Australia)
Sodium bicarbonate ($NaHCO_3$)	Amresco (Solon, OH, USA)
Sodium chloride (NaCl)	Ajax Finechem (Taren Point, Australia)
Potassium dihydrogen orthophosphate (KH_2PO_4)	Ajax Finechem (Taren Point, Australia)

Chemicals	Company
Di-potassium hydrogen orthophosphate (K_2HPO_4)	QreC chemical co, Ltd. (New Zealand)
Sodium dihydrogen phosphate (NaH_2PO_4)	Ajax Finechem (Taren Point, Australia)
Disodium hydrogen phosphate (Na_2HPO_4)	Ajax Finechem (Taren Point, Australia)
Agar	Pearl Mermaid (Thailand)
Carrageenan	Chemipan (Thailand)

Laboratory Equipment	Company
Syringe pump	Aitecs (Lithuania)
Incubator	Labnet (USA)
Spectrophotometer	Biotac, (USA)
Centrifuge	Hettich zentrifugen (USA)
pH meter	Thermo Fisher Scientific Inc., USA)
Hot plate	IKA-works (Germany)
Vortex mixer	Gemmy industrial (Taiwan)
Scanning electron microscopy	JEOL (Japan)
Ion sputter instrument	Bal-Tec (Liechtenstein)
Laser diffraction-based particle size analyzer	Malvern (UK)

Fourier-transform infrared spectroscopy	Perkin Elmer (USA)
Differential scanning calorimetry	Netzsch (Germany)
Miscellaneous	**Company**
Syringe	Nipro (Japan)
Hypodermic needle	Nipro (Japan)
Nylon filter 0.22 μm	

2. Methods

2.1 Preparation of *Mesona chinensis* extraction (MCE)

Dried stems and leafs of *Mesona chinensis* were purchased from the local market in Thailand. The *Mesona chinensis* water extract (MCE) was conducted based on the previous study (Chusak, Thilavech et al. 2014) with some modifications. Six hundred grams of dried MC was boiled in approximately 4 liters of water at 90°C for 4 hours and then leave at 60°C for another 4 hour to evaporate the solvent. The extract then sieved through cheesecloth and filtered through Whatman paper no.4 and no.1. The extract was stored in -20°C in dark place prior use.

2.2 Preparation of alginate beads

The microencapsulation process was conducted based on previous study (Pasukamonset, Kwon et al. 2016). Sodium alginate was dissolved in warm water, approximately 70°C, until dissolved then the MCE were added and mixed until became homogenous solution. For the control microbeads, the alginate was fully

dissolved in distilled water without adding the plant extract. Then the alginate mixture either with or without MC extract was loaded into the syringe then sonicated for 15 minutes to get rid of the air bubbles. After sonication, the loaded syringe was put into the syringe pump as shown in the figure. The needle no. 25G x 1" (0.5x25 mm) was used in the microbeads formation.
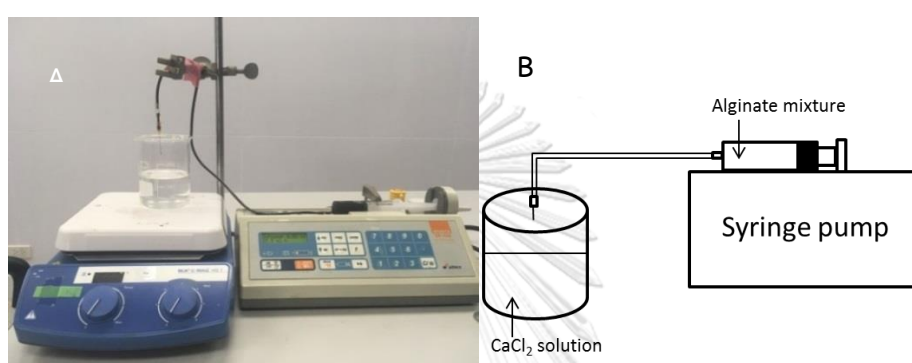
Figure 12. (A) Ionotropic gelation technique (B) experiment model

The solution containing alginate and MCE was added dropwise into the calcium chloride solution by the syringe pump. The dropping rate was fixed at 15 ml/h. The distance between needle and solution surface was fixed at 3 cm. After curation for 15 minutes, the microbeads were washed with distilled water and let air-dried overnight at room temperature. The percentage of sodium alginate, *Mesona chinensis* extract (MCE) and calcium chloride was varied as follow: sodium alginate: 1.2, 1.5 and 1.8 % w/v; MCE: 25, 50 and 75 % w/v; calcium chloride: 3 and 5 % w/v.

2.3 Determination of total polyphenol content and encapsulation efficiency

TPC of the MCE and MC microbeads (MCB) were measured by Folin-ciocalteau method (Chusak, Thilavech et al. 2014). The Folin-Ciocalteau method principle is based on the color change from yellow color of the reagent to blue color product. The absorbance of the product then measured.

Folin reagent was diluted 1:10 using distilled water. 50 µl of diluted Folin reagent was added into 50 µl of sample and incubated for 5 minutes then 50 µl of 10% sodium carbonate was added and incubated for another 30 minutes. The absorbance was measured at 760 nm. Gallic acid 10, 20, 40, 60, 80 and 100 µg/ml was used as the standard curve. The content of total phenolics was expressed as mg gallic acid equivalents/ml (mgGAE/ml).

For MCB, all microbeads made from 2 ml of MC extract in each condition were dissolved in 5% sodium acetate 4 ml with vortex mixing until fully dissolved then the solution was centrifuged at 3,000 rpm for 5 minutes to separate alginate. The supernatant was used to measure the total polyphenol content by Folin-ciocalteau assay. The MC extract and digesta samples (samples from *in vitro* digestion) were directly used in total phenolic content measurement without further preparation.

The encapsulation efficiency was calculated to select the forming condition. The % encapsulation efficiency was calculated based on the equation (Pasukamonset, Kwon et al. 2016):

$$\text{Encapsulation efficiency (\%)} = (TPCe \div TPCm) \times 100$$

TPCe = Total polyphenol content in the extract

TPCm = Total polyphenol content in microbeads

The encapsulation efficiency calculation was conducted in triplicate and the condition with highest encapsulation efficiency was selected for further studies.

2.4 Physical properties of microbeads

2.4.1 Determination of microbeads morphology

The morphology of the surface of MCB and control beads (microbeads without MCE) and the overall shape of the microbeads of the selected condition was observed under Scanning Electron Microscope (SEM). The microbeads sample were coated with gold layer (40nm) by an ion sputter instrument prior observation under SEM at 15 kV.

2.4.2 Determination of particle size analysis

The particle diameter of the MCB and control beads (microbeads without MCE) of the selected condition was observed by using laser diffraction-based particle size analyzer. The instrument measured the diameter of microbeads based on the light diffraction. A refractive index for the median of the particle size distribution calculation was chosen based on previous study (Pasukamonset, Kwon et al. 2016); 1.52 for calcium chloride, 1.37 for sodium alginate, and 1.38 for plant extract.

2.4.3 Determination of chemical interaction

Chemical interactions of the MCE, MCB, control beads and sodium alginate were observed by using the FT-IR with ATR accessory. The FT-IR condition was conducted follow the previous study (Pasukamonset, Kwon et al. 2016); 32 scans in the transmission mode with resolution of ± 4 cm^{-1}. The spectra wavelength was 400 - 4000 cm^{-1}.

The wavelength of each bonding in each sample types was compare to examine the interaction between polyphenols and alginate.

2.4.4 Determination of thermal bahavior

The thermal behavior of sodium alginate, MCE, control microbeads and MCB was scanned by differential scanning calorimetry (DSC) to examine the thermal stability of each substance. Dry samples were weight then sealed in the pan and hold at 25°C for 1 min then scanned from 25°C - 350°C. The heating rate was 10°C/min under 50 ml/min dry N_2 purge. Melting temperature (Tm) and crystallization temperature (Tc) present in the thermograms were compared between each sample.

2.5 Release study of microbeads in water

One milligram of MCB was soaked in distilled water 1 ml and total phenolic content is measured in the solution at 0.5, 1, 2, 3 and 4 hour according to method

mentioned previously. Then the graph between total phenolic content and time was plotted.

2.6 Preparation of jelly product

The grass jelly will be made in similar manner to previous research (Kreungngern 2016). In total, 3 types of jelly were made: control jelly, MCE jelly and MCB. The jellies were made by using agar or carrageenan powder. The content of each jelly types are lists in table 1. The percentage of agar and carrageenan were chosen based on sensory test from previous study (Kreungngern 2016).

The agar or carrageenan powder was added into boiling water with continuous stirring until everything dissolved. After that MCE or MCB was added into the jelly solution. The jellies were poured in silicone mold (figure 13), each mold contain jelly with TPC 1 mgGAE/ml. After pouring jelly mixture into the mold the jellies were left in 4°C to harden for 30 minutes before used in the simulated digestion study.

Table 1. Jellies product content

Jellies	Agar (% w/v)	Carrageenan (% w/v)	Polyphenol sources
Con A3	3	-	None
Con A5	5	-	None
MCE A3	3	-	MC extract
MCE A5	5	-	MC extract
MCB A3	3	-	MC microbeads
MCB A5	5	-	MC microbeads
Con C3	-	3	None
Con C5	-	5	None
MCE C3	-	3	MC extract
MCE C5	-	5	MC extract
MCB C3	-	3	MC microbeads
MCB C5	-	5	MC microbeads

Figure 13. Silicone mold containing 1 ml jelly blocks

2.7 Simulated gastrointestinal digestion

The in vitro digestion of was MC extract, MC microbeads, control jelly, MC extract jelly and MC microbeads jelly conducted based on a previous study (Pasukamonset, Kwon et al. 2016). The amount of total polyphenol contents before simulated digestion was controlled at 2 mgGAE/ml by calculating the TPC from MCE, MCB and jelly products.

The simulated digestion was separated into 2 parts: gastric phase and intestinal phase.

Sample Preparation:

- MCE and MCB 2 mgGAE were directly use in simulated digestion

- MCB and MCE jellies contained 2 mgGAE were chopped into small pieces prior the gastric phase

Gastric phase:

- Samples were loaded in the 20 ml tube.

- Porcine pepsin solution (40 mg/ml in 0.1 N HCl) was added into each sample tube and adjusted pH to 2.0 ± 0.1 using 0.1 N HCl and 1 N NaOH.

- The solution was incubated at 37°C for 1 hour in shaking water bath 100 rpm.

- pH was adjusted to 4.5 ± 0.1

Intestinal phase

- Amyloglucosidase solution (0.125 mg/ml) 0.15 ml was added and incubated for 30 mins.

- pH was adjusted to 5.3 ± 0.1 with 0.1 N HCl and mixture of 1N NaOH and 0.1N NaHCO$_3$ and small intestinal enzyme solution containing pancreatin (3 mg/mL) and bile acid (12 mg/mL) in 0.1N NaHCO$_3$ were added.

- Adjusted the final pH to 7.2 ± 0.1 and volume to 20 mL with phosphate buffer saline pH 7.2.

- Incubated the samples for 0, 0.5, 1, 1.5 and 2 hours.

The samples were collected after gastric phase and at 0, 0.5, 1, 1.5 and 2 hours after intestinal phase. The simulated digestion was stopped by centrifugation at 12,000 rpm, 4°C and filtered through a 0.22μm nylon filter. The digesta samples were either immediately use for analysis which are total phenolic content measurement, FRAP activity assay and α-glucosidase inhibitory assay or stored frozen at -20°C.

2.8 Determination of antioxidant activity by ferric reducing antioxidant power (FRAP) assay

FRAP assay were conducted based on previous study (Jorjong, Butkhup et al. 2015) to investigate the MCE, MCB and digesta samples antioxidant activities. The

principle is based on ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex reduced to ferrous-tripyridyltriazine (Fe^{2+}-TPTZ).

Sample 20 µl was incubated with 180 µl of FRAP reagent (0.3 M Sodium Acetate buffer pH 3.6 12 ml, 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine or TPTZ 1 ml and 20 mM $FeCl_3$ 1 ml) for 30 minutes. The FRAP reagent were incubated at 37°C for 15 minutes prior use. Then the absorbance was measured at 593 nm. Fe^{2+} from $FeSO_4$ 0.1, 0.2, 0.3, 0.4 and 0.5 mM were used as the standard curve and the result was expressed as $mMFe^{2+}$ equivalents/ml extract.

The MCE was used directly in the assay while the digesta samples reaction was conducted in the 1.5 ml microtube with the same ratio of sample and reagents due to the precipitation issue. After adding the FRAP reagent for 20 minutes the reaction was centrifuged at 3000 rpm, 4°C for 1 minutes then 200 µl of supernatant was aliquot and measured at 593 nm at 30 minutes.

2.9 Determination of pancreatic α-glucosidase activity

The α-glucosidase inhibitory activity of MCE, MCB and digesta samples were measured based on intestinal α-glucosidase inhibition assay with some modifications (Adisakwattana, Lerdsuwankij et al. 2011). The enzyme extraction was conducted by dissolved 100 mg of rat intestinal acetone powder in 3 ml of 0.9% NaCl along with vortex for 15 minutes then centrifuged at 12,000 g for 30 minutes at 4°C. The supernatant was used in maltase and sucrase inhibitory assay. For maltase assay, 10

µl of sample was incubated with 75 µl of 86 mM maltose and 15 µl of enzyme for 30 minutes while in sucrase assay the 10 µl sample was incubated with 50 µl of 400 mM sucrose and 40 µl enzyme solutions for 60 minutes. Then samples were incubated at 100°C for 10 minutes and the glucose concentration was measured by glucose oxidase method using the glucose oxidase assay kit (Glucose liquicolor kit, Human). Briefly, 5 µl of each sample was incubated with the working reagent 500 µl for 10 minutes at room temperature. After that, the absorbance was measured at 500nm. The %inhibition was calculated based on the equation:

$$\%\text{inhibition} = \left(\frac{\text{negative control absorbance} - \text{sample absorbance}}{\text{negative control absorbance}} \times 100\% \right)$$

2.10 Statistical analysis

All experiments were performed independent at least triplicately. The results are expressed as mean ± SEM. One-way ANOVA followed by Tukey's post hoc test was evaluated for the significant differences among groups (P<0.05) for comparing mean between %Encapsulation efficiency in each microencapsulation condition, the polyphenol content, FRAP value and % α-glucosidase inhibition after simulated digestion. Two-way ANOVA followed by Tukey's post hoc test was evaluated for the significant differences among groups (P<0.05) and investigated the interaction between polyphenol content or FRAP activity and time points during simulated digestion. All statistical analysis was conducted using SPSS version 16.0.

CHAPTER IV

RESULTS

1. Phytochemical and functional properties of *Mesona chinensis* extract (MCE)

1.1 The phytochemical composition of *Mesona chinensis* extract *(MCE)*

In this study, the polyphenol content of the MCE was 24.20 ± 1.35 mgGAE/ml extract whereas flavonoids and anthocyanin were undetectable (data not shown). The calibration curve of gallic acid on Folin-Ciocalteau's assay is shown in figure 14.

$Absorbance_{760nm}$
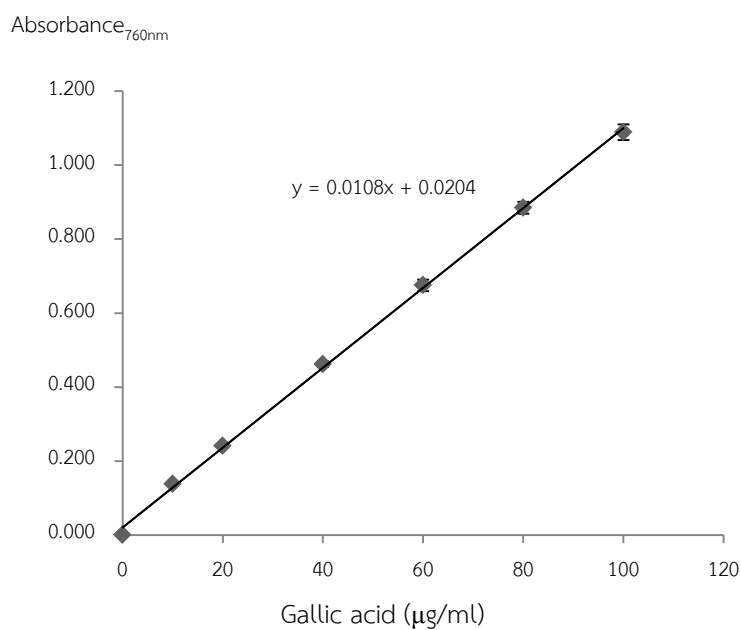
Figure 14. The calibration curve of gallic acid (0 - 100 μg/ml).

Data are expressed as mean ± S.E.M (n=3)

1.2 Antioxidant activity of MCE

The antioxidant activity of MCE was measured by FRAP assay. In this study, FRAP value of MCE was 18.80 ± 1.24 mM Fe^{2+}. The calibration curve of $FeSO_4$ on FRAP assay is shown in figure 15.
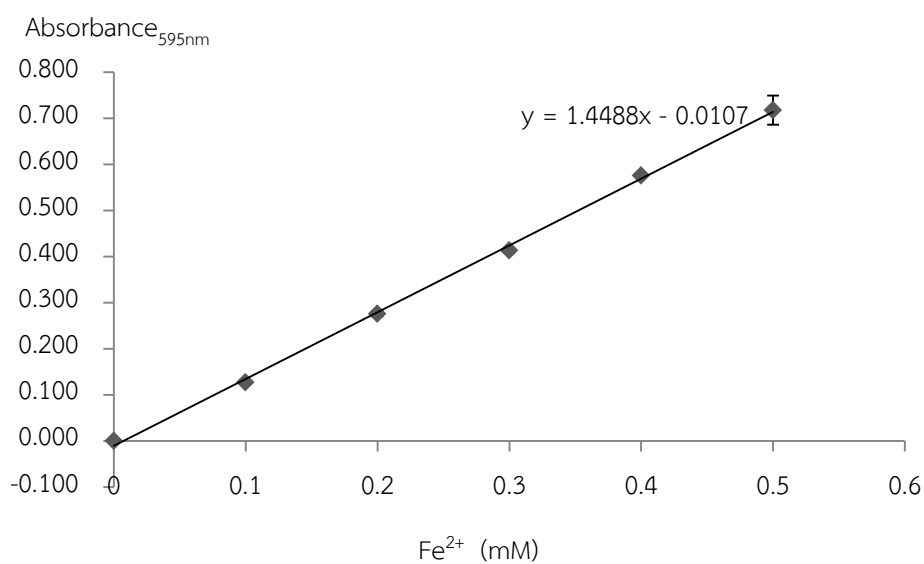
Figure 15. The calibration curve of $FeSO_4$ (0.0 - 0.5 mM).

Data are expressed as mean $\pm$ S.E.M (n=3).

1.3 α-glucosidase inhibitory activity of MCE

The maltase and sucrase inhibitory activity of MCE at various concentrations are shown in figure 16. The results showed that the MCE markedly inhibited the intestinal maltase and sucrase in a concentration-dependent manner. The IC_{50} of maltase and sucrase inhibition was 11.24 ± 3.80 mgGAE/ml and 16.81 ± 2.04 mgGAE/ml respectively.
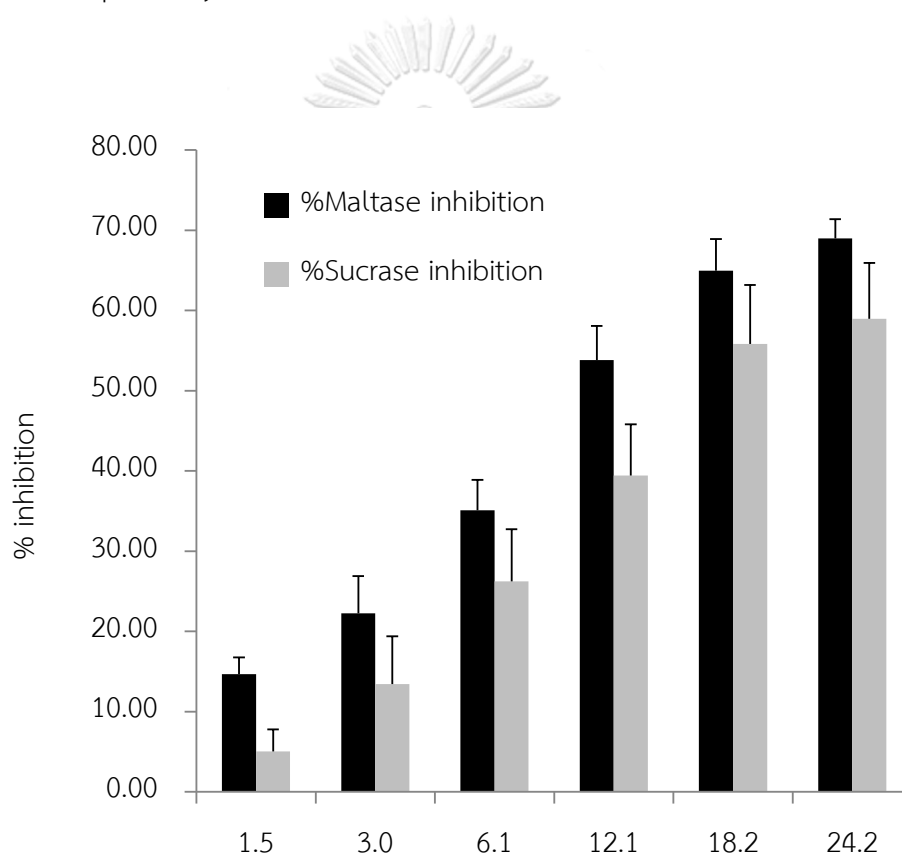
Figure 16. The percentage **α**-glucosidase inhibition (maltase and sucrase) of MCE. Data are expressed as mean ± S.E.M (n=3).

2. The alginate-based encapsulation of polyphenols from *Mesona chinensis*

2.1 Morphology and percentage of encapsulation efficiency (%EE) of MCE in calcium alginate beads in different formulations

The MC beads were formed by varied concentration of MC extract and sodium alginate. The %EE ranged from 41.1% - 56.7% which are shown in the table 2. The MC beads could not hold the bead shape under 1.5% w/v alginate or 3% w/v $CaCl_2$ and 50% w/v MCE. There was no significant difference of %EE at various concentration of $CaCl_2$ ($p<0.05$). The results found that the concentration of alginate affected the shape of MC beads. The spherical beads could form when the solution contained 1.5% w/v alginate, conversely, 1.8% w/v alginate caused the formation of teardrop-shaped beads resulting in the presence of tail structure in the beads (data not shown). Therefore, 75% w/v MCE, 1.5% w/v alginate, 3% w/v Ca was selected for the formation of microbeads. The morphology of control beads with and without MCE were observed under Scanning Electron Microscope (SEM). The control microbeads showed the non-spherical shape with the presence of surface gaps (Figure 17A) while the MCB showed the spherical shape with less visible cracks (Figure 17B).

Table 2. The percentage of encapsulation efficiency (%EE) of microbeads with different concentration of MC extract, $CaCl_2$ and sodium alginate.

MCE (%)	TPC (mgGAE/ml)	$CaCl_2$ (% w/v)	Sodium alginate (% w/v)	%EE
50	12.15	3	1.5	41.1 ± 4.7^{a}
50	12.15	5	1.5	42.2 ± 5.0^{a}
50	12.15	3	1.8	42.0 ± 6.1^{a}
50	12.15	5	1.8	48.7 ± 4.3^{ab}
75	18.15	3	1.2	52.6 ± 1.0^{bc}
75	18.15	5	1.2	52.0 ± 2.4^{bc}
75	18.15	3	1.5	56.7 ± 3.4^{c}
75	18.15	5	1.5	55.7 ± 2.4^{c}
75	18.15	3	1.8	$53.4\pm4.9b^{c}$
75	18.15	5	1.8	55.0 ± 1.8^{c}

MCE = *Mesona chinensis* extract, TPC = total phenolic content

The results are expressed as mean ± SEM (n=3). Means with the same letter are not significantly different ($p < 0.05$).
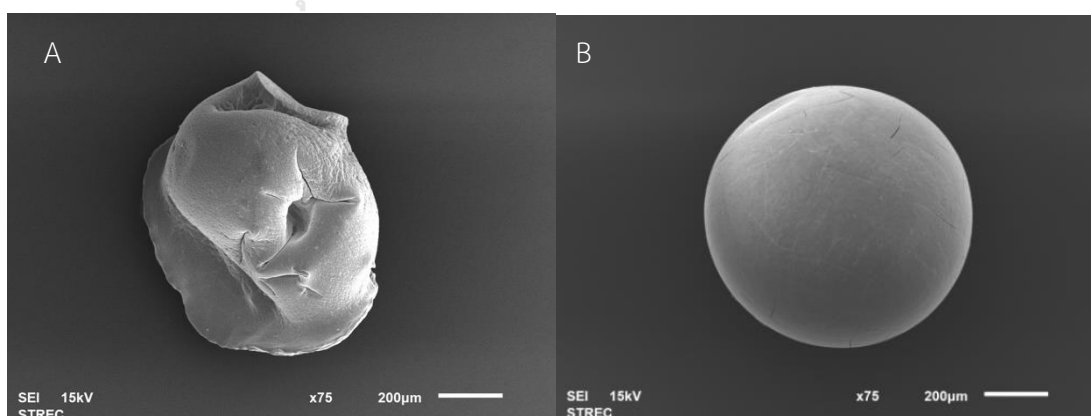
Figure 17. Morphology of control microbeads (A) and MC microbeads (B) under Scanning Electron Microscope.

2.2 Particle size

The microbeads size was analyzed by laser particle size distribution analyzer. The average diameter of control beads was 792.33 ± 26.12 μm while the presence of MC in the microbeads resulted in an increase in the diameter to 1,516.67 ± 40.96 μm.
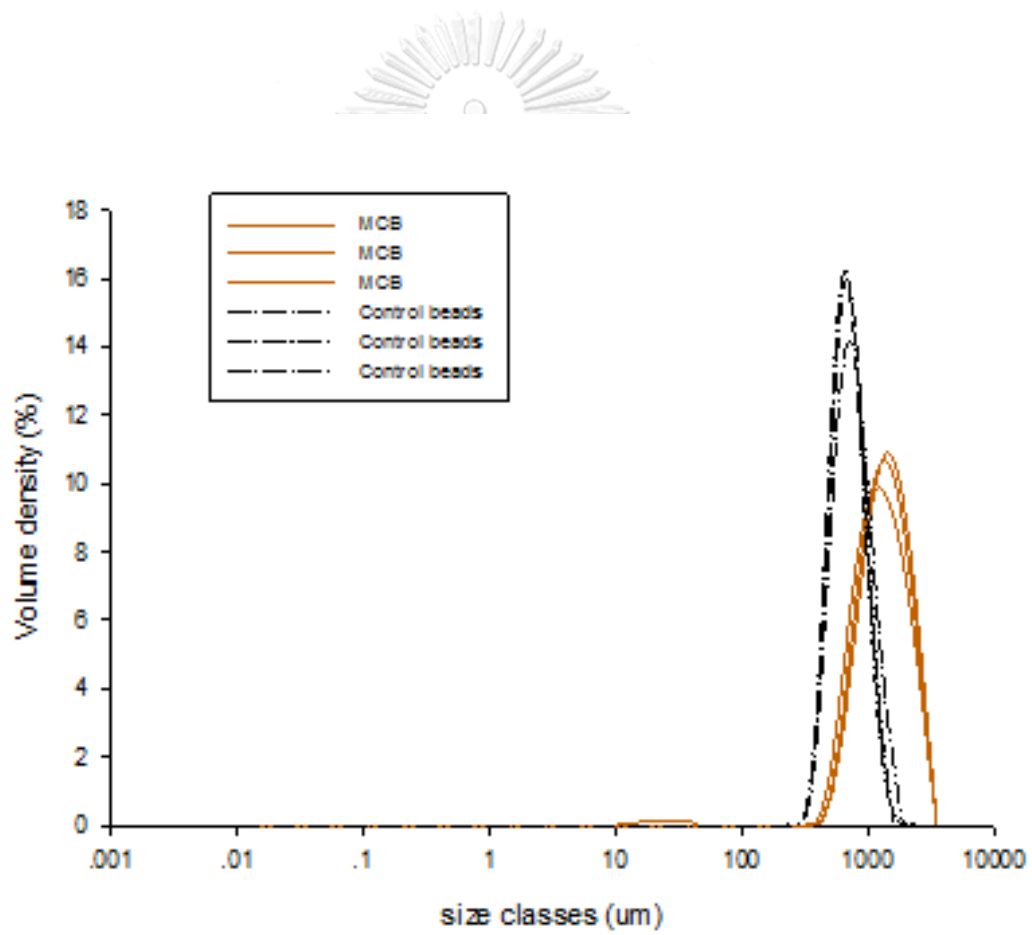
Figure 18. Particle size distribution obtained from light-diffraction particle size analyzer of microbeads with and without MC (n=3).

2.3 The thermograms by Differential Scanning Calorimeter (DSC) of MCE and MCB

The thermal profiles of sodium alginate, lyophilized MCE, MCB and control beads were analyzed by a Differential Scanning Calorimeter (figure19). All samples showed broad endothermic transition temperature of glass transition temperature (T_g) approximately at 100ºC. Sodium alginate presented exothermic decomposition peak (T_c) at 243.6ºC. The endothermic peak of alginate beads with and without MCE (T_m) was 190.2ºC and 202.4ºC respectively. In addition, the control beads and MCE demonstrated broad exothermic peak (T_c) at 295.4ºC and 310.2ºC respectively.
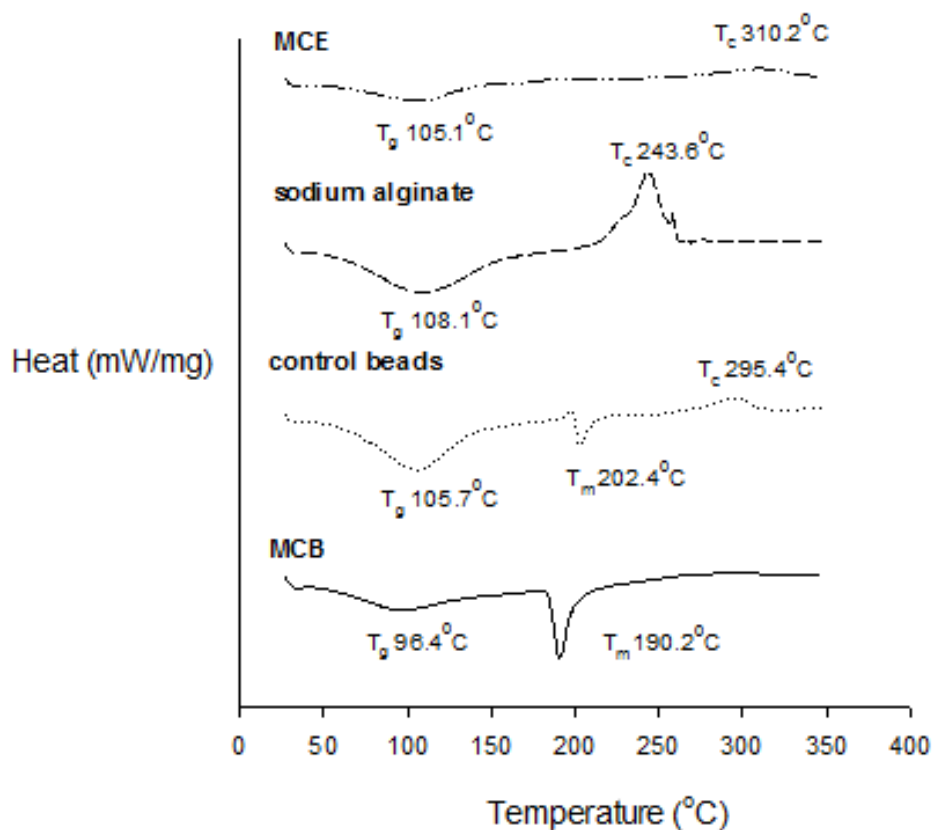
Figure 19. Thermogram obtained from DSC for MC extract (MCE), sodium alginate, control beads and MC microbeads (MCB).

2.4 Fourier transform infrared spectroscopy (FT-IR)

The chemical interactions in MCE, sodium alginate, the control beads and MCB were investigated using FTI-IR with attenuated total reflection accessory (ATR). The main stretching bands of the alginate were O-H band stretching at 3253.50 cm^{-1}, asymmetrical COO$^-$ band at 1592.48 cm^{-1}, symmetrical COO$^-$ band at 1407.45 cm^{-1} and C-O-C band at 1026.15 cm^{-1}. For the control beads, O-H peak was present at 3336.75 cm^{-1}, weak symmetric peak at 1418.58 cm^{-1}, strong asymmetric COO$^-$ peak at 1594.91 cm^{-1} and C-O-C peak at 1023.23 cm^{-1}. According to the MCB spectra, O-H stretching was present at 3333.79 cm^{-1} while asymmetrical COO$^-$ band, symmetrical COO$^-$ band and C-O-C band were present at 1591.72, 1415.97 and 1023.38 cm^{-1} respectively.
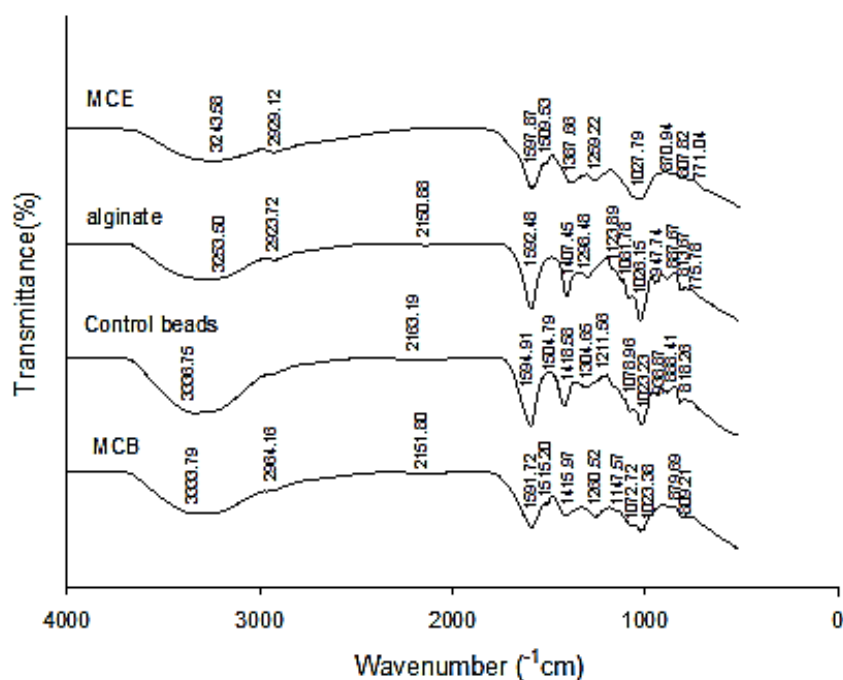
Figure 20. FT-IR spectra of MCE, sodium alginate, control beads and MCB

3. The release of microbeads in water

The total phenolic content of MCB released in distilled water are shown in figure 21. At 1 mg of MCB containing 169.31 µgGAE, the release of polyphenol from MCB was 2.29%, 3.06%, 5.78%, 7.21% and 8.93% after 0.5, 1, 2, 3 and 4 hours incubation, respectively.
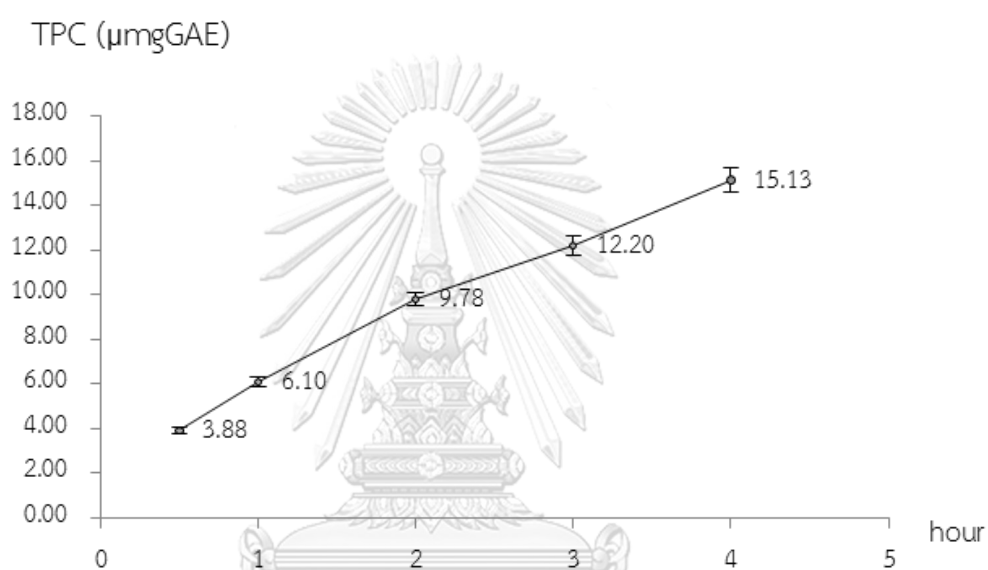
Figure 21. The release of total phenolics in distilled water.. The results are expressed as mean ± SEM (n=3).

4. The appearance of jelly products containing MCE and MCB

The jelly products containing MC and its microbeads made from 3% agar or carrageenan are present in figure 22. Each jelly volume was 1 ml.
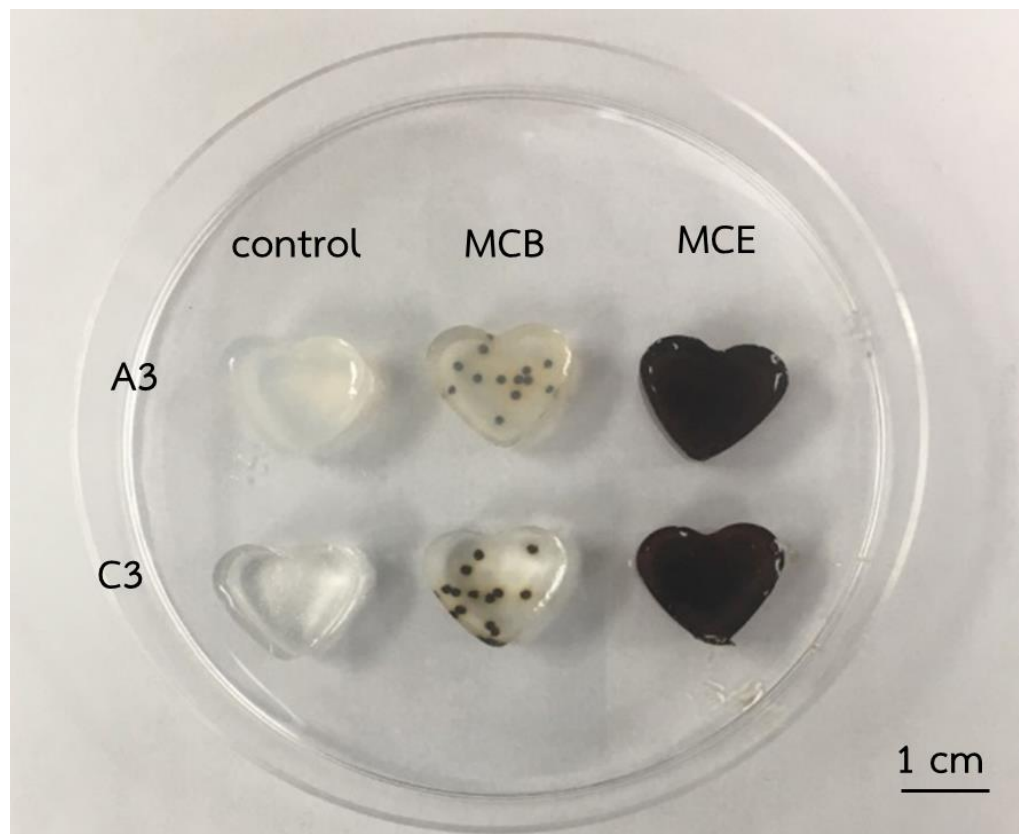
Figure 22. Control jelly, MCB jelly and MCE jelly (from left to right) made from 3% agar (A3) or 3% carrageenan (C3).

5. *In vitro* gastrointestinal digestion

5.1 The change of appearance of jelly products during *in vitro* gastrointestinal digestion

Figure 23 demonstrates 3% agar jellies and 3% carrageenan jellies after gastric phase (figure 23A), before intestinal phase (figure 23B), after intestinal phase for 0.5 h (figure 23C), 1 h (figure 23D), 1.5 h (figure 23E) and 2 h (figure 23F). After simulated gastric phase, the MCB in carrageenan was more swollen than MCB in agar (23A). The swelling MCB in agar could be observed at the beginning of simulated intestinal phase (figure 23B). The diameter of swollen MCB in carrageenan was larger than MCB in agar when compared to the beads size at the same phase. In simulated intestinal phase, MCB in jelly became fragile and some microbeads could not be held in spherical shape (figure 23 D-F). The release of polyphenol from MCB into the polysaccharide jellies could be noticed by the spread of brown color into the jelly.
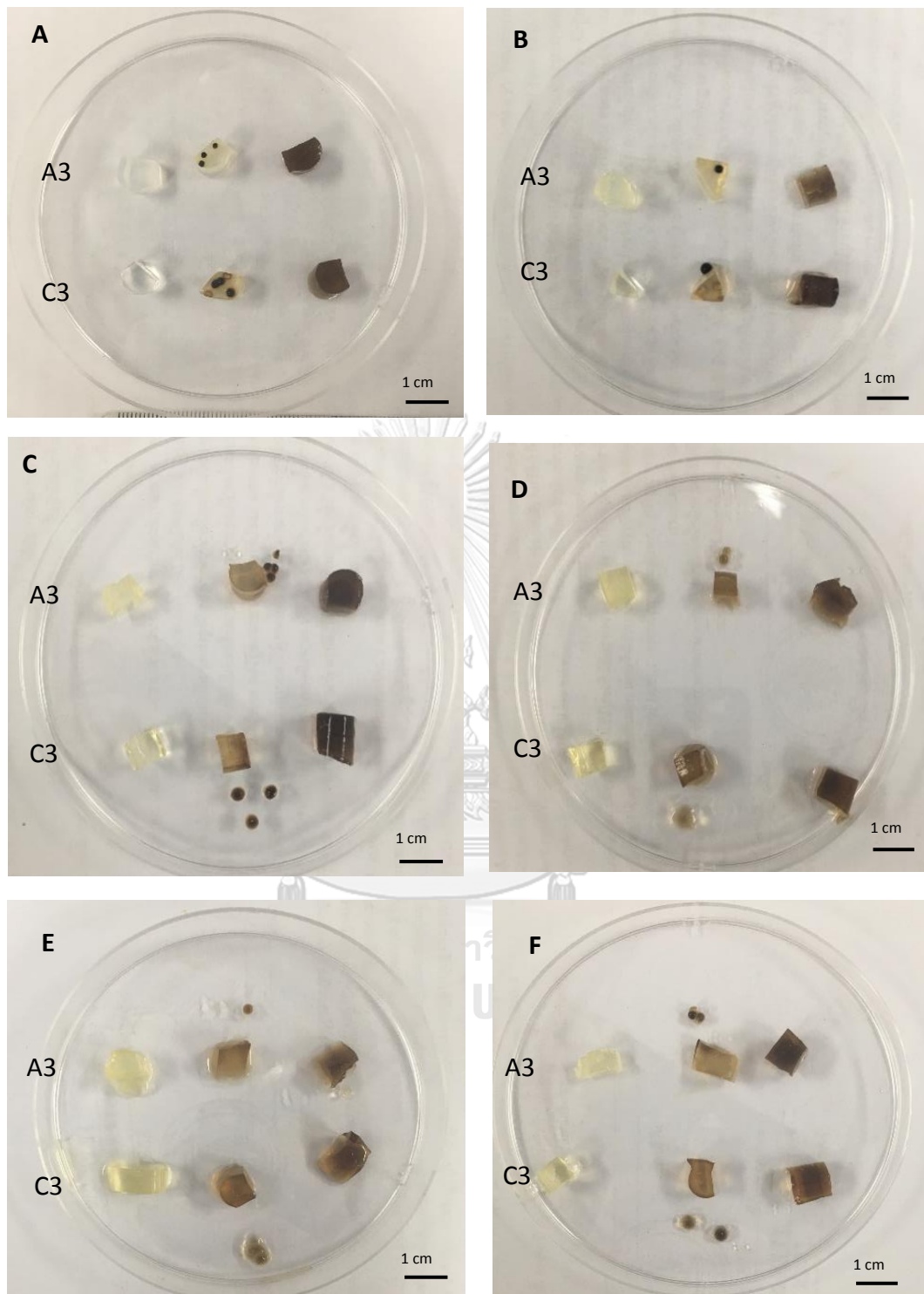
Figure 23. The shapes of 3% agar jelly (A3) and 3% carrageenan jelly (C3) after simulated gastric phase (A), before simulated intestinal digestion (B) and after simulated digestion for 0.5 (C), 1 (D), 1.5 (E) and 2 hours (F).

5.2 Phenolic content after *in vitro* gastrointestinal digestion

Total phenolic content of the MCE was measured during the simulated digestion at after gastric phase (G), before intestinal phase (I0), after intestinal phase for 0.5 (I0.5), 1 (I1), 1.5 (I1.5) and 2 (I2) hours.

The result showed that the polyphenol content of MCE was decreased 20.87% after simulated digestion (table 3). In the gastric phase, total phenolic content released from MCE in 3% and 5% agar was 39.52% and 45.52% respectively (figure 24) while total phenolic content released from MCE in 3% and 5% carrageenan was 45.72% and 37.45% respectively (figure 25). There was no significant difference in the polyphenol release between 4 types of MCE jellies (table 3). Moreover, the polyphenol released from these MCE jellies were significant lower than that of MCE. In the intestinal phase, an increase in the total phenolic content of MCE jellies in digesta was detected after passing gastric digestion (figure 24-25). There was no significant difference in polyphenol released from MCE and MCE jellies during 0.5 – 2 hours of the intestinal digestion.

Encapsulation of MCE affected the release of polyphenol. In the gastric phase, the total phenolic content was released from MCB at 10.36%. The total phenolic content released from MCB in 3% and 5% agar were 9.42% and 7.07% (figure 26), and the total phenolic content released from 3% and 5% carrageenan jellies were 11.92% and 9.11% respectively (figure 27). There were no significant differences in the total phenolic content released from MCB, MCB agar jellies and

MCB carrageenan jellies during the gastric phase (table 3). In intestinal phase, the release of polyphenols from MCB and MCB in jellies increased (figure 26-27) and there was no significant difference in the polyphenol release between MCB and 4 types of MCB jellies (table 3). However, the polyphenol content detected in digesta from MCE and MCE jellies were significantly higher than those of MCB and MCB jellies (table 3).
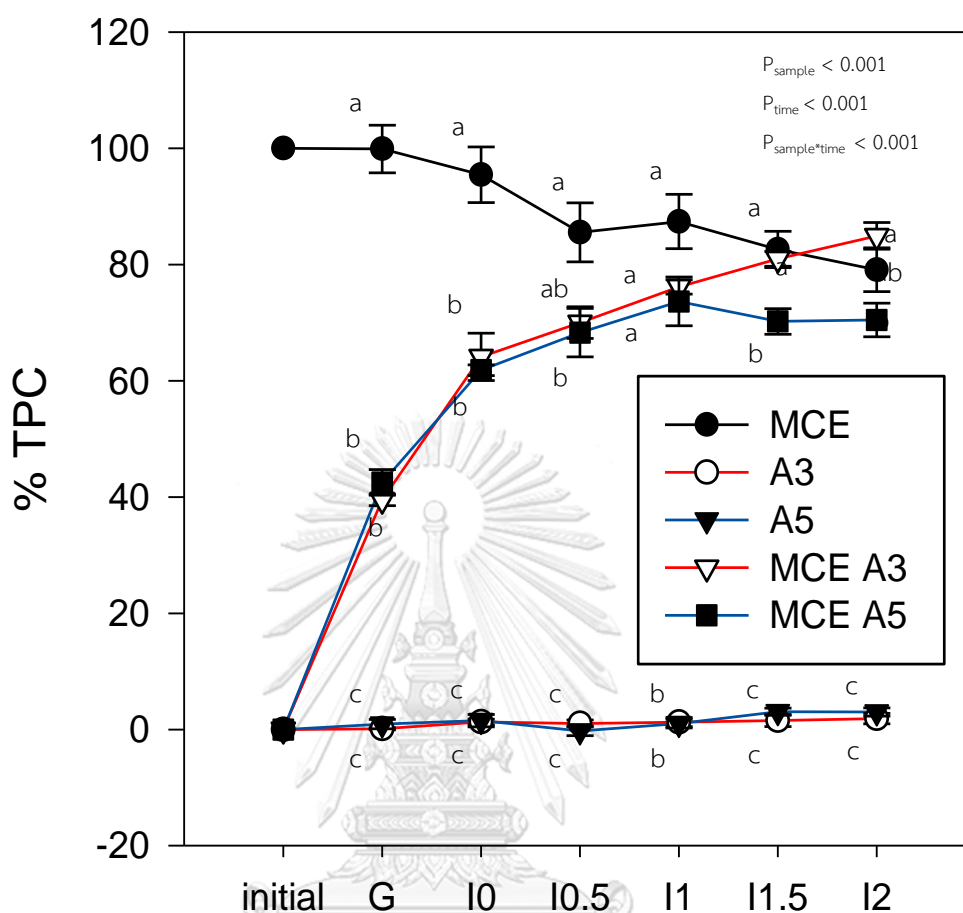
Figure 24. The percentage of polyphenol release of *Mesona chinensis* extract (MCE)
and its encapsulation in agar after simulated gastrointestinal digestion.
(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after
intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after
intestinal phase and I2 = 2 hours after intestinal phase) The results are
expressed as mean ± SEM (n=3). Means with the different letter are
significant differences. Analysis was performed with one-way analysis of
variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).
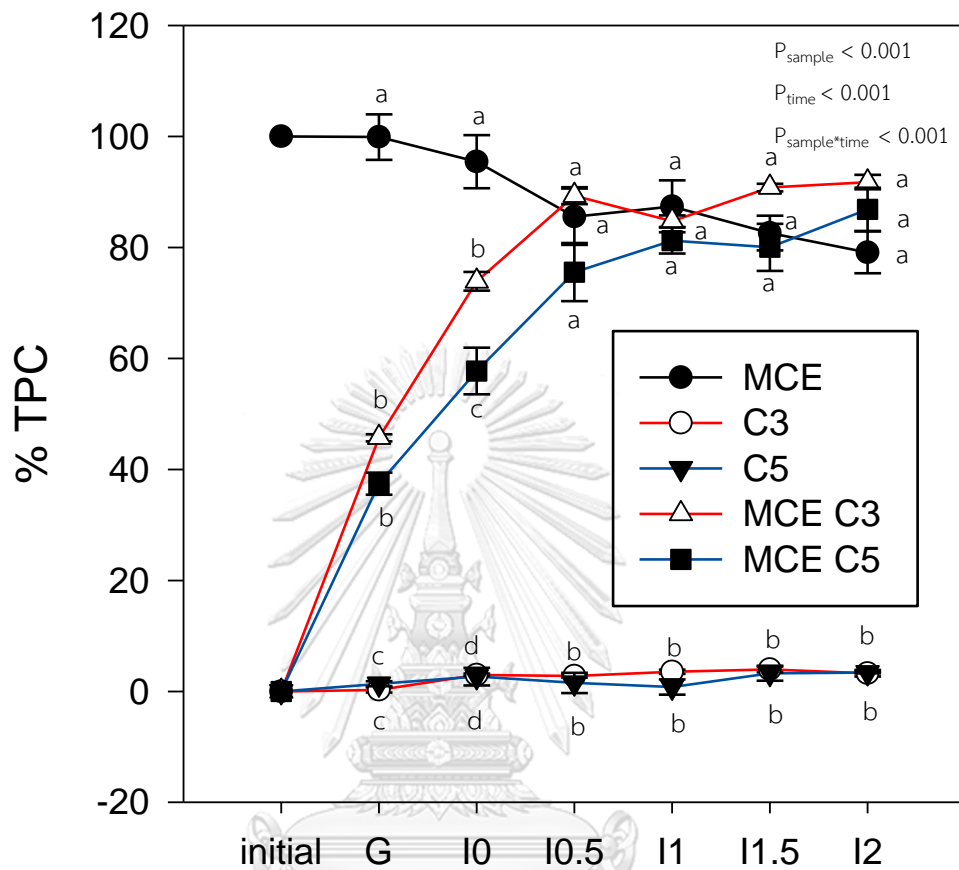
Figure 25. The percentage of polyphenol release of Mesona chinensis extract (MCE) and its encapsulation in carrageenan after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after intestinal phase and I2 = 2 hours after intestinal phase) The results are expressed as mean ± SEM (n=3). Means with the different letter are significant differences. Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).
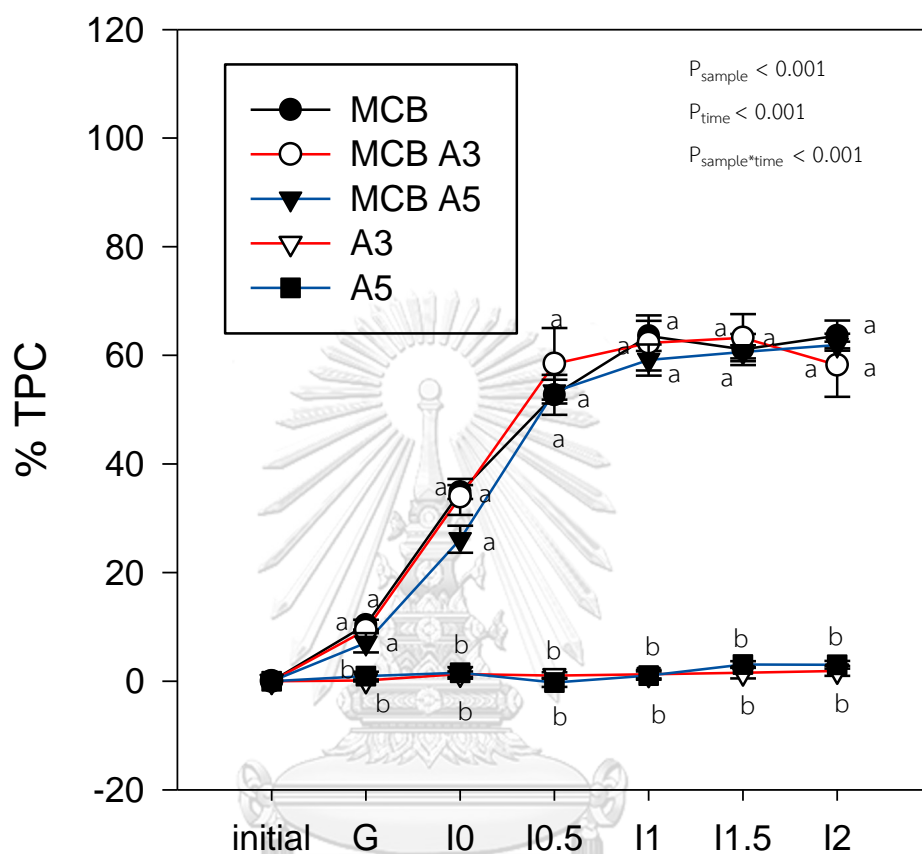
Figure 26. The percentage of polyphenol release of *Mesona chinensis* microbead (MCB) and its encapsulation in agar after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after intestinal phase and I2 = 2 hours after intestinal phase) The results are expressed as mean ± SEM (n=3). Means with the different letter are significant differences. Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).

Figure 27. The percentage of polyphenol release of *Mesona chinensis* microbead (MCB) and its encapsulation in carrageenan after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after intestinal phase and I2 = 2 hours after intestinal phase) The results are expressed as mean ± SEM (n=3). Means with the different letter are significant differences. Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).

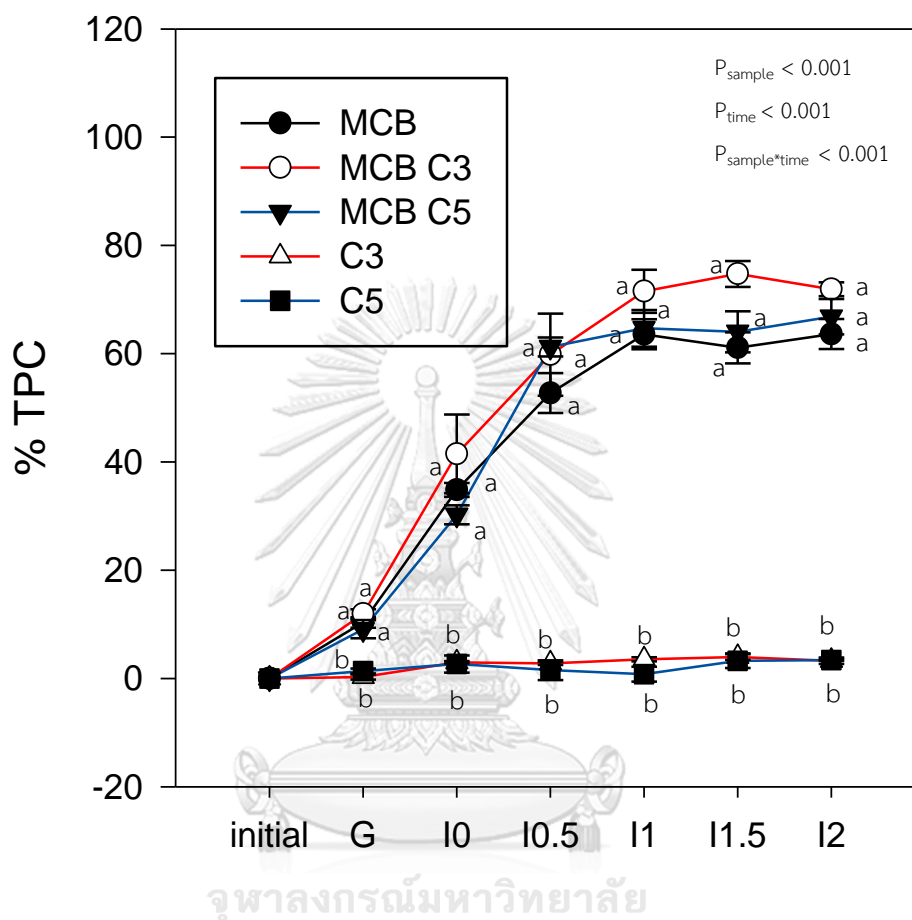Table 3. The total phenolic content of *Mesona chinensis* extract (MCE), MC microbeads, MC agar jellies and MC carrageenan jellies after simulated gastrointestinal digestion.

	Total phenolic content (µg/ml)					
	Gastric	Intestinal 0h	Intestinal 0.5h	Intestinal 1h	Intestinal 1.5h	Intestinal 2h
MCE	99.91 ± 4.10[a]	95.47 ± 4.80[a]	85.55 ± 5.09a[a]	87.42 ± 4.67[a]	82.60 ± 3.13[ab]	79.13 ± 3.78[abcd]
MCE A3	39.52 ± 1.01[b]	64.14 ± 4.08[b]	70.02 ± 2.73[abc]	76.18 ± 1.23[ab]	80.99 ± 1.28[ab]	84.96 ± 2.31[abc]
MCE A5	42.52 ± 2.21[b]	61.83 ± 0.91[b]	68.30 ± 4.16[abc]	73.67 ± 4.20[abc]	70.23 ± 2.20[bcd]	70.46 ± 2.89[cde]
MCE C3	45.72 ± 0.65[b]	73.91 ± 1.69[b]	89.37 ± 1.52[a]	84.77 ± 1.04[a]	90.81 ± 0.69[a]	91.79 ± 1.31[a]
MCE C5	37.45 ± 1.99[b]	57.74 ± 4.20[bc]	75.57 ± 5.23[ab]	81.26 ± 2.34[a]	80.04 ± 4.24[ab]	86.89 ±3.90[ab]
MCB	10.36 ± 0.97[c]	34.84 ± 1.29[d]	52.72 ± 3.68[c]	63.58 ± 2.79[bc]	61.08 ±2.88[cd]	63.63 ±2.78[e]
MCB A3	9.42 ± 0.10[c]	33.95 ± 3.32[d]	58.45 ± 6.58[bc]	62.28 ± 5.06[bc]	63.24 ± 4.33[cd]	58.19 ± 5.80[e]
MCB A5	7.07 ± 1.80[c]	26.11± 2.49[d]	53.32 ± 2.17[c]	59.14 ± 2.87[c]	60.64 ± 1.22[cd]	61.90 ± 0.62[e]
MCB C3	11.92 ± 0.86[c]	41.49 ± 7.26[cd]	59.83 ± 7.59[bc]	71.52 ± 3.99[abc]	74.74 ± 2.39[bc]	71.89 ± 1.27[bcde]
MCB C5	9.11 ± 1.67[c]	30.24 ± 1.76[d]	61.22 ± 1.75[bc]	64.69 ± 3.39[bc]	64.03 ± 3.78[cd]	66.83 ± 3.27[cde]
A3	0.13 ±0.20[d]	1.29 ± 0.49[e]	1.04 ± 0.58[d]	1.27 ± 0.78[d]	1.56 ± 1.05[e]	1.87 ± 0.91[f]
A5	0.95 ± 0.81[d]	1.54 ± 1.06[e]	0.24 ± 0.85[d]	1.04 ± 0.76[d]	3.06 ± 0.63[e]	3.02 ± 0.72[f]
C3	0.25 ± 0.47[d]	2.98 ± 0.21[e]	2.78 ± 0.29[d]	3.54 ± 0.36[d]	3.98 ± 0.53[e]	3.27 ± 0.59[f]
C5	1.38 ± 0.44[d]	2.67 ± 1.60[e]	1.53 ± 1.82[d]	0.79 ± 1.37[d]	3.28 ± 1.35[e]	3.42 ± 0.16[f]

(MCE = *Mesona chiensis* extract, MCB = *Mesona chinensis* microbeads, A3 = 3% agar jelly, A5 = 5% agar jelly, C3 = 3% carrageenan and C5 = 5% carrageenan)

The results are expressed as mean ± SEM (n=3). Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).

5.3 Antioxidant activity after in vitro gastrointestinal digestion

This study showed that the FRAP value of MCE significantly declined during simulated digestion; 19.3% decreased after gastric phase digestion and 42.0% reduced after intestinal phase digestion (figure 28). In the gastric phase, the percentage of FRAP value from MCE in 3% and 5% agar were 52.73% and 41.16% (figure 28) while the percentage of FRAP value released from MCE in 3% and 5% carrageenan were 48.57% and 40% accordingly (figure 29). There was no significant difference in the FRAP value of 4 types of MCE jellies and they were significantly lower than MCE (table 4). In the intestinal phase, the increased FRAP value from MCE jellies was detected in digesta. The percentage of FRAP value from MCE in 3% and 5% agar was significantly higher than that of MCE at 1.5 – 2 hours of intestinal phase (figure 28). There was no significant difference between the percentage of FRAP value of MCE and those 2 types of MCE carrageenan jellies (figure 29).

After encapsulated MCE into MCB, the FRAP value significantly decreased after simulated digestion (table 4). In the gastric phase, the percentage of FRAP from MCB was 8.97%. The FRAP value of MCB in 3% agar, 5% agar, 3% carrageenan and 5% carrageenan were 8.76%, 9.55%, 8.88% and 9.27%, respectively. However, these values did not show significant differences when compared to the control (figure 30-31). At the beginning to 2 hours of intestinal phase, the percentage of FRAP value from MCB in agar and carrageenan were significantly higher than MCB alone, but

there was no significant difference in the FRAP value of all MCB jelly types (table 4).

However, the observed FRAP value of MCE and MCE jellies in digesta were

significantly higher than those of MCB and MCB jellies (table 3).
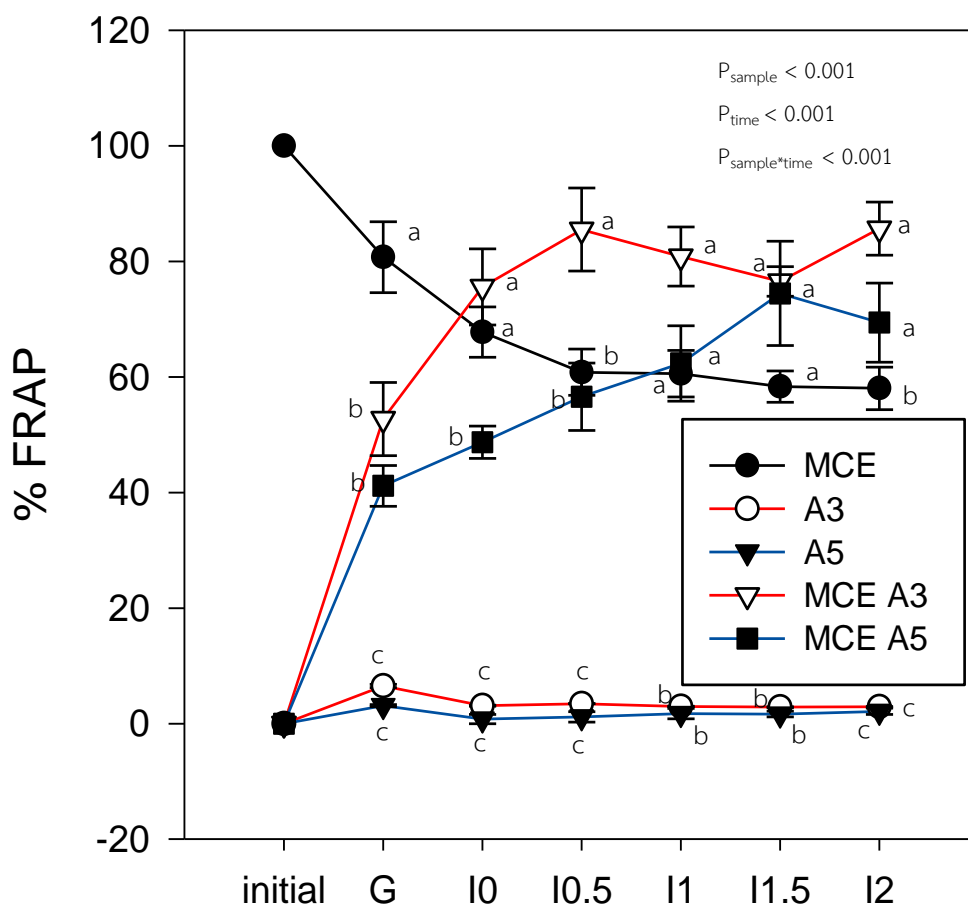
Figure 28. The percentage of FRAP value of *Mesona chinensis* extract (MCE) and its encapsulation in agar after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after intestinal phase and I2 = 2 hours after intestinal phase) The results are expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis ($P < 0.05$).

Figure 29. The percentage of FRAP value of *Mesona chinensis* microbeads (MCB) and

its encapsulation in carrageenan after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after

intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after

intestinal phase and I2 = 2 hours after intestinal phase) The results are

expressed as mean ± SEM (n=3). (a-c) Means with the different letter are

significant differences. Analysis was performed with one-way analysis of

variance (ANOVA) followed by Tukey post hoc analysis ($P < 0.05$).
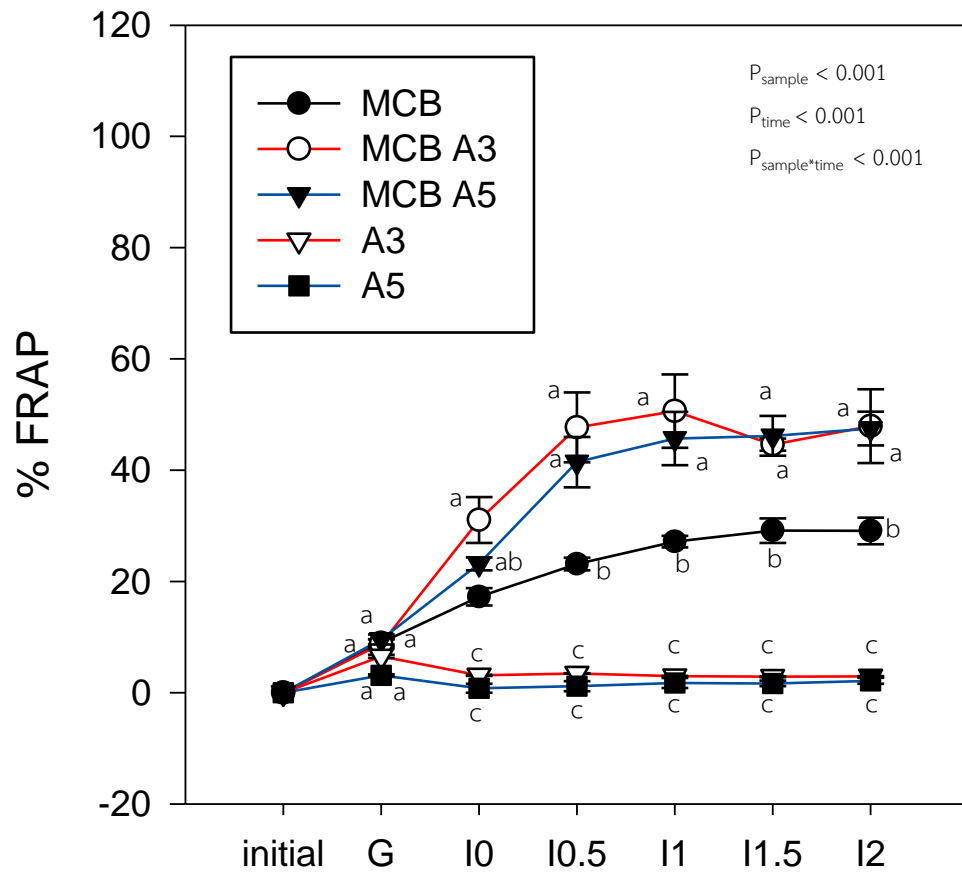
Figure 30. The percentage of FRAP value of *Mesona chinensis* microbead (MCB) and
its encapsulation in agar after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after
intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after
intestinal phase and I2 = 2 hours after intestinal phase) The results are
expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was
performed with one-way analysis of variance (ANOVA) followed
by Tukey post hoc analysis (P < 0.05).

Figure 31. The percentage of FRAP value of *Mesona chinensis* microbead (MCB) and
its encapsulation in carrageenan after simulated gastrointestinal digestion.
(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after
intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after
intestinal phase and I2 = 2 hours after intestinal phase) The results are
expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was
performed with one-way analysis of variance (ANOVA) followed
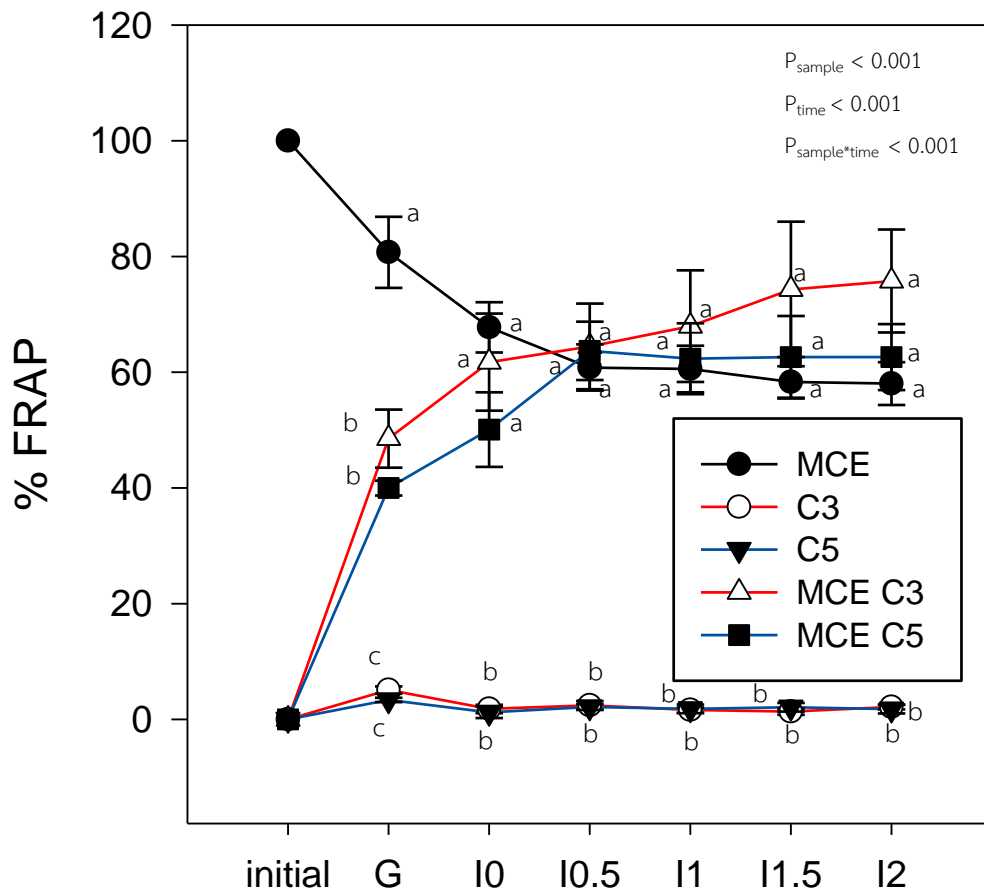by Tukey post hoc analysis (P < 0.05).

Table 4. The FRAP value of *Mesona chinensis* extract, MC microbeads, MC agar jellies and MC carrageenan jellies after simulated gastrointestinal digestion.

	FRAP value (μM FeSO₄)					
	Gastric	Intestinal 0h	Intestinal 0.5h	Intestinal 1h	Intestinal 1.5h	Intestinal 2h
MCE	630.68 ± 48.04[a]	529.42 ± 34.01[ab]	475.03 ± 31.26[abcd]	473.10 ± 31.54[abcd]	455.60 ± 21.21[ab]	453.35 ± 28.75[bcd]
MCE A3	411.81 ± 49.33[b]	590.57 ± 51.53[a]	667.96 ± 56.05[a]	631.47 ± 40.07[a]	597.85 ± 19.89[a]	669.38 ± 35.83[a]
MCE A5	321.49 ± 27.50[b]	380.48 ± 22.00[bc]	442.05 ± 45.54[abc]	486.96 ± 51.00[abc]	581.69 ± 70.49[a]	542.15 ± 53.52[abc]
MCE C3	379.33 ± 39.23[b]	482.28 ± 65.52[ab]	503.50 ± 57.84[ab]	530.93 ± 75.33[ab]	580.61 ± 91.32[a]	591.85 ± 69.50[ab]
MCE C5	312.43 ± 10.08[b]	391.39 ± 50.30[bc]	497.49 ± 39.38[abc]	487.16 ± 47.83[abc]	489.05 ± 55.79[ab]	489.13 ± 44.61[abcd]
MCB	70.09 ± 05.05[c]	134.68 ± 12.20[d]	180.51 ± 08.77[d]	211.93 ± 08.15[e]	227.49 ± 17.13[d]	227.11 ± 18.74[e]
MCB A3	68.44 ± 06.46[c]	242.48 ± 32.29[cd]	372.48 ± 49.01[bcd]	395.32 ± 51.51[bcde]	348.27 ± 08.56[cde]	374.36 ± 51.85[cde]
MCB A5	74.57 ± 06.99[c]	180.83 ± 0 9.12[d]	323.77 ± 35.28[bcd]	356.83 ± 37.49[bcde]	360.67 ± 27.99[cde]	370.82 ± 23.78[cde]
MCB C3	69.35 ± 05.45[c]	174.37 ± 23.78[d]	240.32 ± 40.43[cd]	293.91 ± 20.25[cde]	311.83 ± 27.52[cde]	334.83 ± 12.37[cde]
MCB C5	72.37 ± 12.92[c]	160.64 ± 19.72[d]	278.95 ± 07.05[de]	272.24 ± 08.58[de]	297.91 ± 22.59[cde]	307.59 ± 12.62[cde]
A3	25.47 ± 2.15[d]	24.32 ± 0.86[e]	27.06 ± 0.25[f]	23.19 ± 0.27[f]	22.30 ± 0.62[e]	22.72 ± 0.59[f]
A5	18.74 ± 1.40[d]	6.17 ± 6.45[e]	9.10 ± 6.91[f]	13.58 ± 7.15[f]	12.94 ± 3.82[e]	16.71 ± 4.26[f]
C3	34.26 ± 4.96[d]	14.23 ± 5.64[e]	19.06 ± 5.93[f]	12.80 ± 3.56[f]	10.67 ± 4.19[e]	16.73 ± 3.21[f]
C5	20.94 ± 2.96[d]	9.68 ± 7.79[e]	16.88 ± 4.10[f]	14.28 ± 5.89[f]	16.42 ± 5.82[e]	14.08 ± 6.11[f]

(MCE = *Mesona chiensis* extract, MCB = *Mesona chinensis* microbeads, A3 = 3% agar jelly, A5 = 5% agar jelly, C3 = 3% carrageenan and C5 = 5% carrageenan)

The results are expressed as mean ± SEM (n=3). Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).

5.4 α-glucosidase inhibitory activity after *in vitro* gastrointestinal digestion

The digesta from MCE, MCB, MCE jellies and MCB jellies were measured for α-glucosidase inhibitory activity including intestinal maltase and sucrase. There was no significant difference in the inhibition of maltase and sucrase between all samples after simulated digestion (figure 32-39) and (table 5-6).

Figure 32. The percentage inhibition of intestinal maltase of *Mesona chinensis* extract
(MCE) and its encapsulation in agar after simulated gastrointestinal digestion.
(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after
intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after
intestinal phase and I2 = 2 hours after intestinal phase) The results are
expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was
performed with one-way analysis of variance (ANOVA) followed
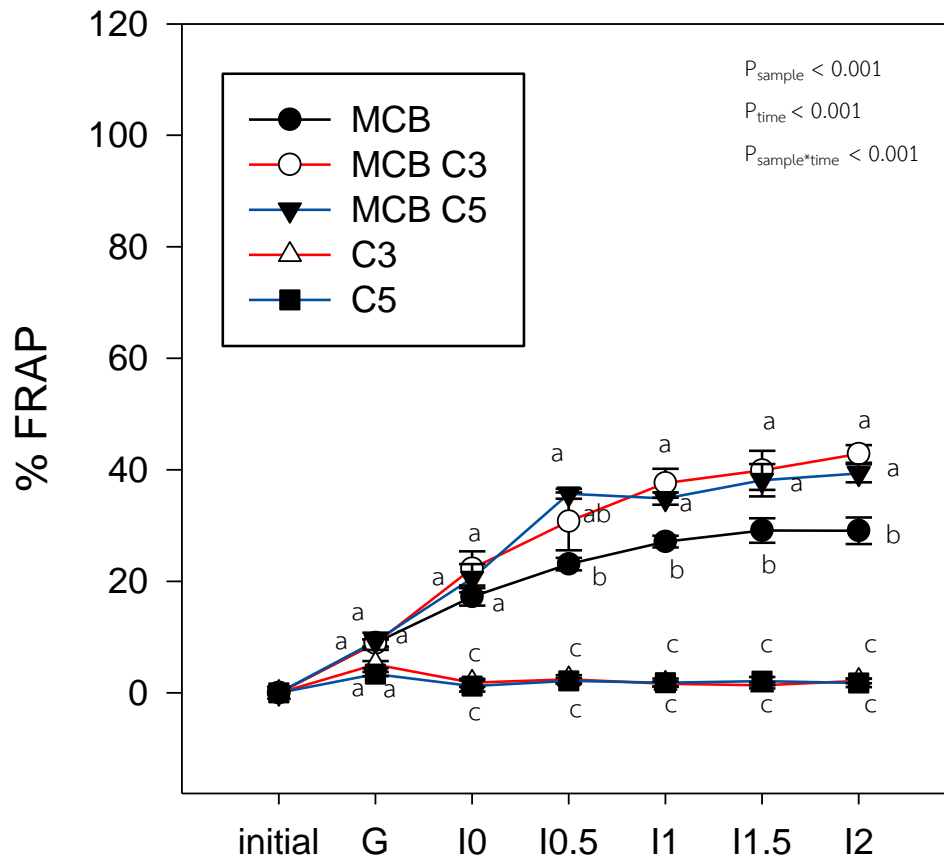by Tukey post hoc analysis (P < 0.05).

Figure 33. The percentage inhibition of intestinal maltase of *Mesona chinensis* extract
(MCE) and its encapsulation in carrageenan after simulated gastrointestinal
digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after
intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after
intestinal phase and I2 = 2 hours after intestinal phase) The results are
expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was
performed with one-way analysis of variance (ANOVA) followed
by Tukey post hoc analysis (P < 0.05).

Figure 34. The percentage inhibition of intestinal maltase of *Mesona chinensis* microbead (MCB) and its encapsulation in agar after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after intestinal phase and I2 = 2 hours after intestinal phase) The results are expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).

Figure 35. The percentage inhibition of intestinal maltase of *Mesona chinensis* microbead (MCB) and its encapsulation in carrageenan after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after intestinal phase and I2 = 2 hours after intestinal phase) The results are expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).

Table 5. The percentage inhibition of intestinal maltase of *Mesona chinensis* extract, MC microbeads, MC agar jellies and MC carrageenan jellies after simulated gastrointestinal digestion.

	Gastric	Intestinal 0h	Intestinal 0.5h	Intestinal 1h	Intestinal 1.5h	Intestinal 2h
MCE	7.50 ± 2.39^{bc}	5.04 ± 3.55^{a}	6.02 ± 4.43^{a}	5.77 ± 3.52^{a}	7.68 ± 3.57^{a}	2.86 ± 4.90^{a}
MCE A3	2.44 ± 0.33^{abc}	-1.18 ± 0.26^{a}	-1.48 ± 2.68^{a}	-4.56 ± 5.67^{a}	-1.72 ± 2.02^{a}	1.75 ± 1.64^{a}
MCE A5	-6.44 ± 1.75^{a}	-5.05 ± 2.05^{a}	-1.91 ± 2.30^{a}	-2.49 ± 3.02^{a}	-0.85 ± 1.09^{a}	1.91 ± 3.08^{a}
MCE C3	3.02 ± 2.06^{abc}	3.84 ± 5.43^{a}	-2.13 ± 1.65^{a}	0.90 ± 1.29^{a}	1.18 ± 3.11^{a}	-3.64 ± 2.47^{a}
MCE C5	2.04 ± 2.00^{abc}	0.07 ± 6.40^{a}	3.24 ± 2.22^{a}	4.88 ± 1.14^{a}	3.78 ± 2.10^{a}	6.44 ± 0.85^{a}
MCB	4.40 ± 2.47^{abc}	7.22 ± 3.00^{a}	2.02 ± 2.65^{a}	7.16 ± 0.65^{a}	6.66 ± 2.25^{a}	4.90 ± 2.24^{a}
MCB A3	2.34 ± 0.74^{a}	2.24 ± 1.76^{a}	7.58 ± 2.40^{a}	-0.58 ± 5.92^{a}	5.00 ± 5.08^{a}	3.75 ± 2.91^{a}
MCB A5	-4.90 ± 3.09^{a}	-6.98 ± 1.37^{a}	-2.67 ± 3.40^{a}	0.78 ± 1.64^{a}	0.05 ± 1.11^{a}	-2.85 ± 0.56^{a}
MCB C3	10.89 ± 2.06^{c}	6.95 ± 5.49^{a}	10.57 ± 0.66^{a}	4.24 ± 0.19^{a}	6.15 ± 3.30^{a}	3.95 ± 4.59^{a}
MCB C5	-3.69 ± 4.40^{ab}	1.50 ± 1.52^{a}	4.93 ± 4.59^{a}	3.29 ± 5.14^{a}	1.42 ± 5.45^{a}	5.33 ± 5.47^{a}

(MCE = *Mesona chiensis* extract, MCB = *Mesona chinensis* microbeads, A3 = 3% agar jelly, A5 = 5% agar jelly, C3 = 3% carrageenan and C5 = 5% carrageenan)

The results are expressed as mean ± SEM (n=3). Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).
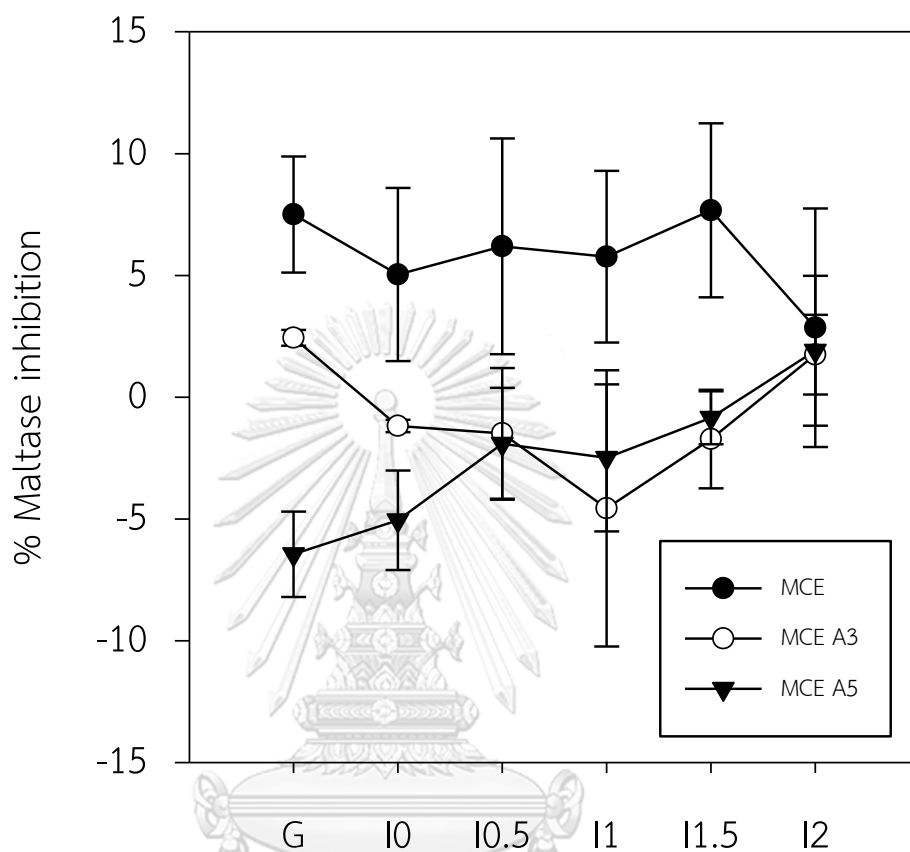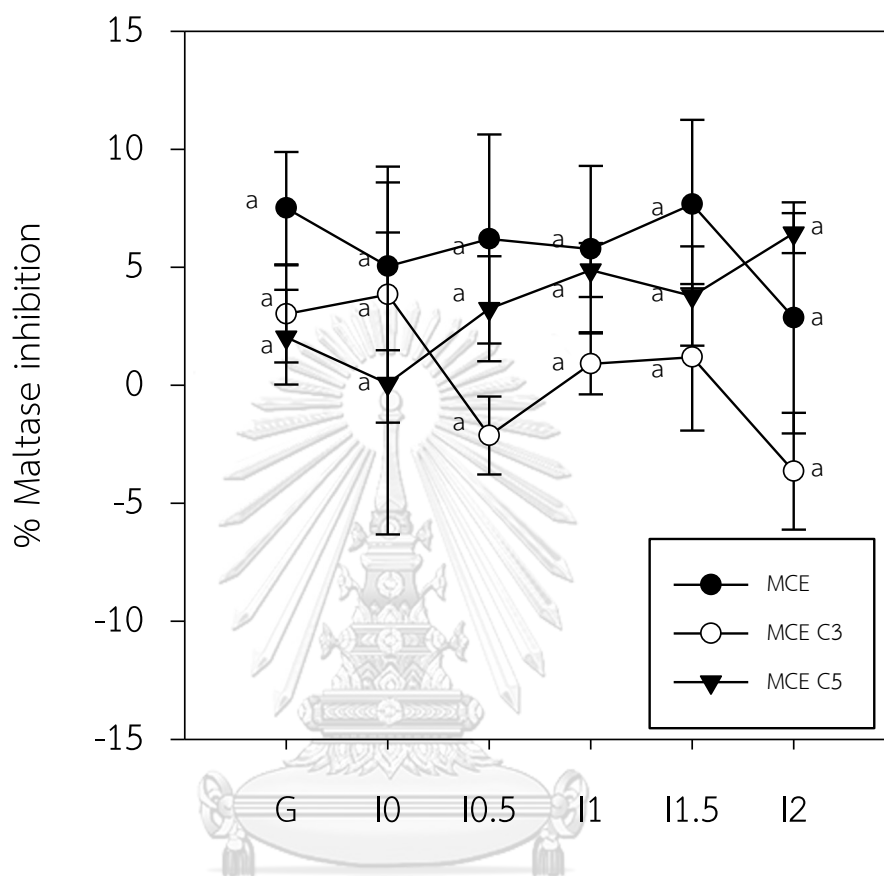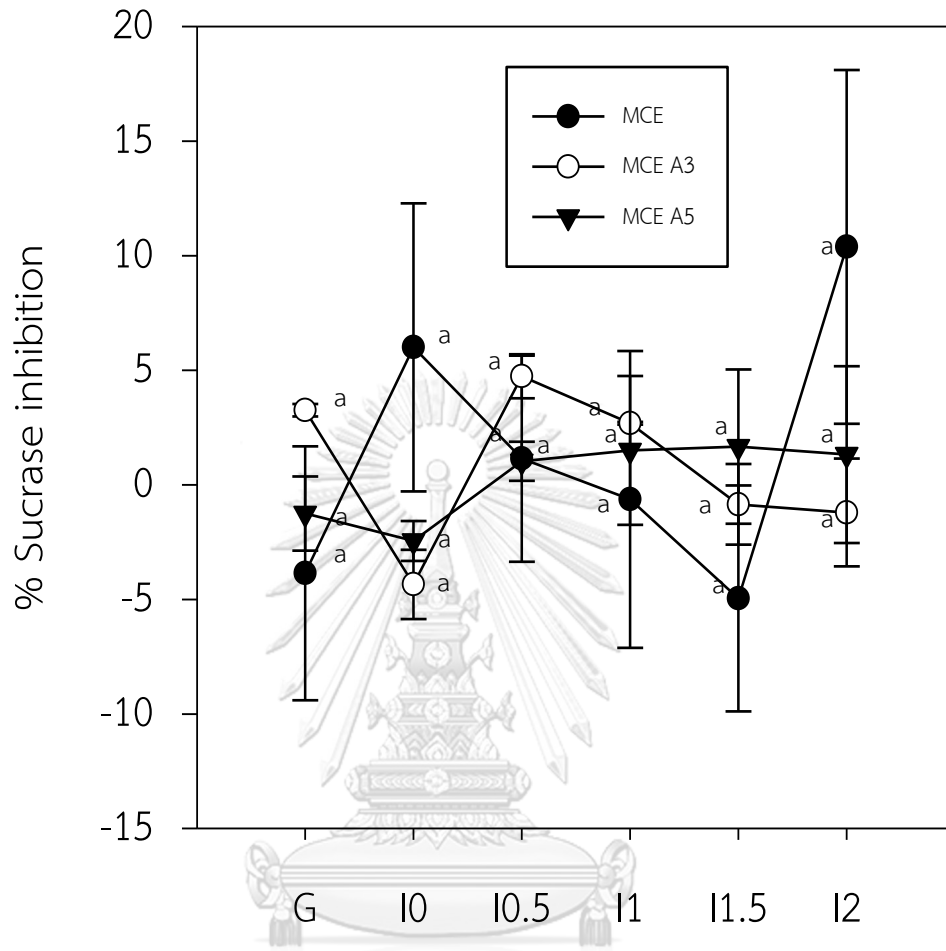
Figure 36. The percentage inhibition of intestinal sucrase of *Mesona chinensis* extract

(MCE) and its encapsulation in agar after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after

intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after

intestinal phase and I2 = 2 hours after intestinal phase) The results are

expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was

performed with one-way analysis of variance (ANOVA) followed
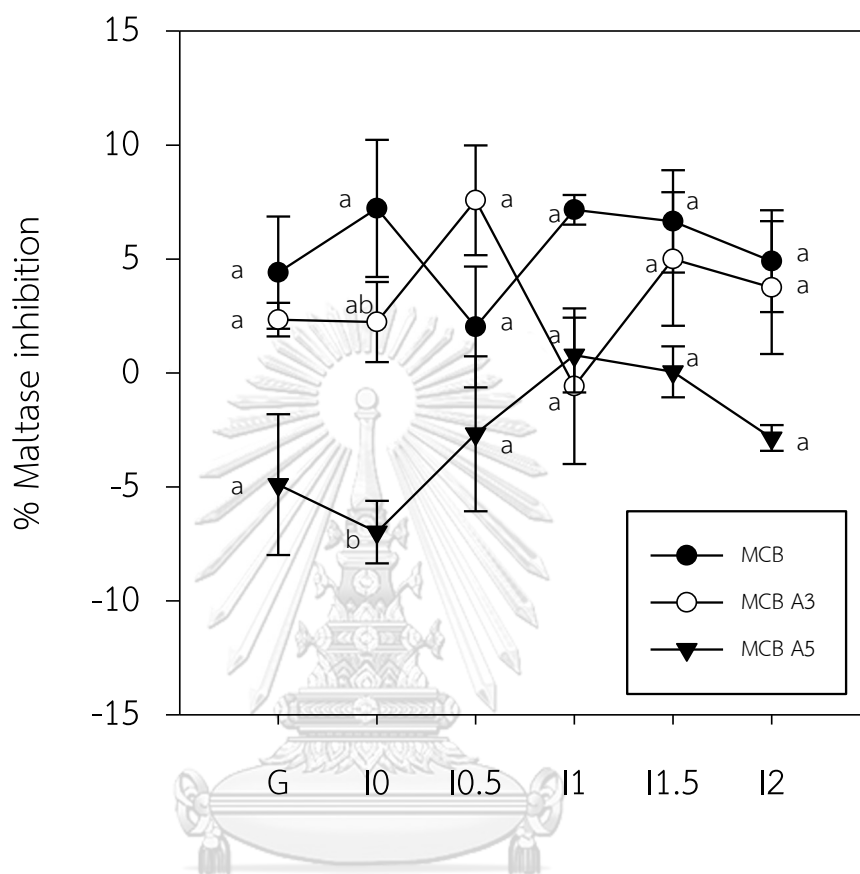
by Tukey post hoc analysis (P < 0.05).
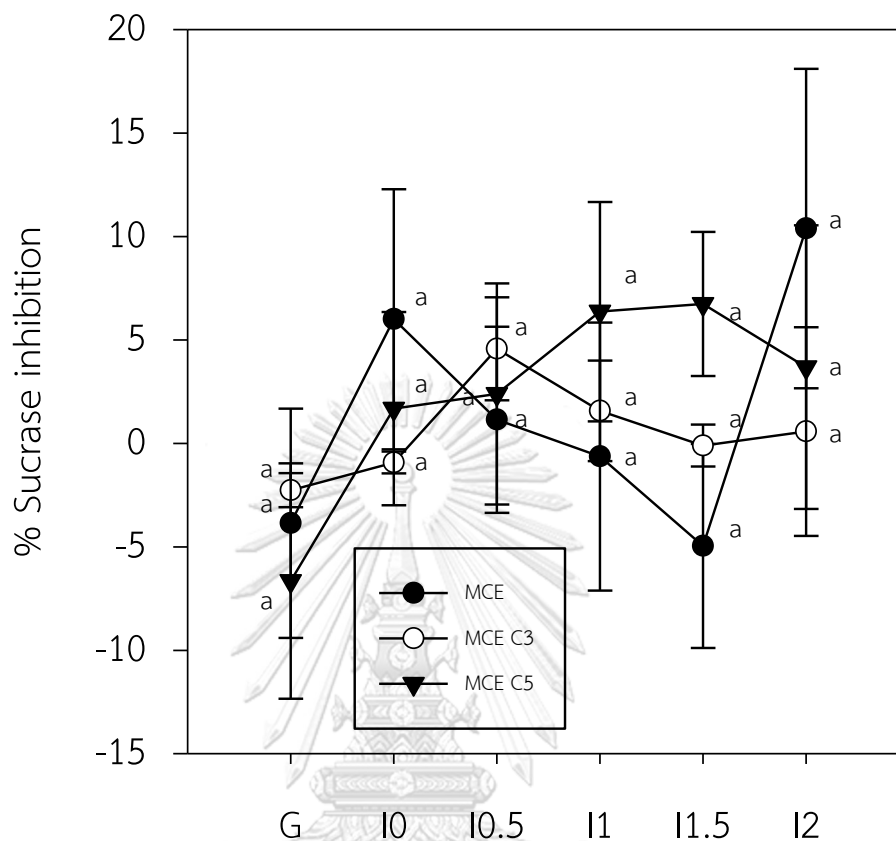
Figure 37. The percentage inhibition of intestinal sucrase of *Mesona chinensis* extract
(MCE) and its encapsulation in carrageenan after simulated gastrointestinal
digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after
intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after
intestinal phase and I2 = 2 hours after intestinal phase) The results are
expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was
performed with one-way analysis of variance (ANOVA) followed
by Tukey post hoc analysis (P < 0.05).
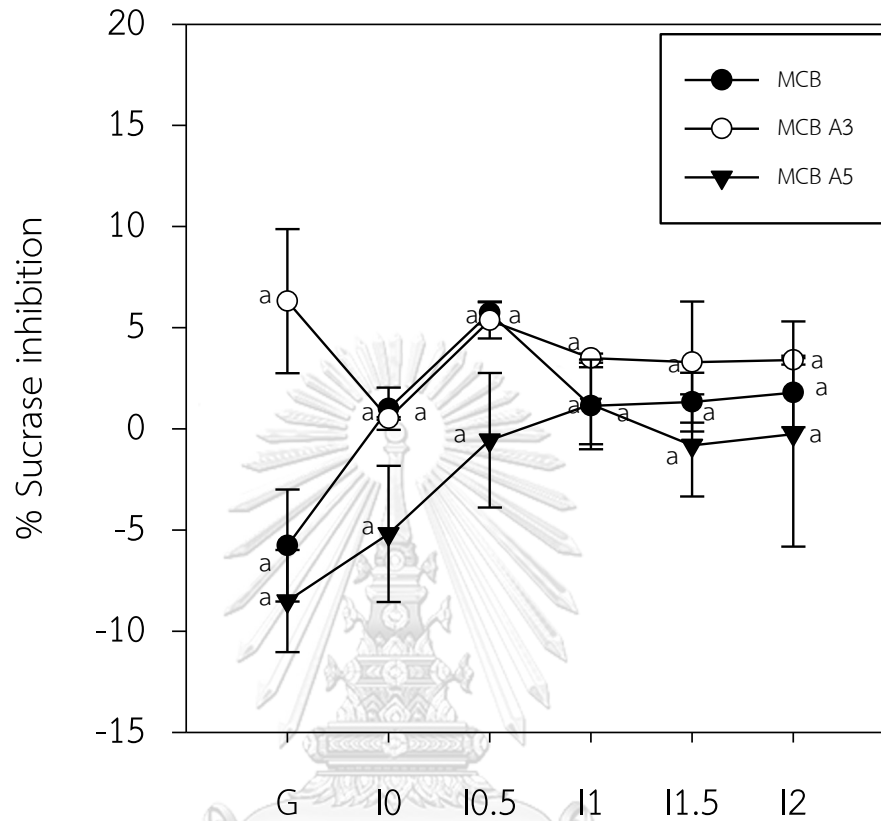
Figure 38. The percentage inhibition of intestinal sucrase of *Mesona chinensis* microbead (MCB) and its encapsulation in agar after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after intestinal phase and I2 = 2 hours after intestinal phase) The results are expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).
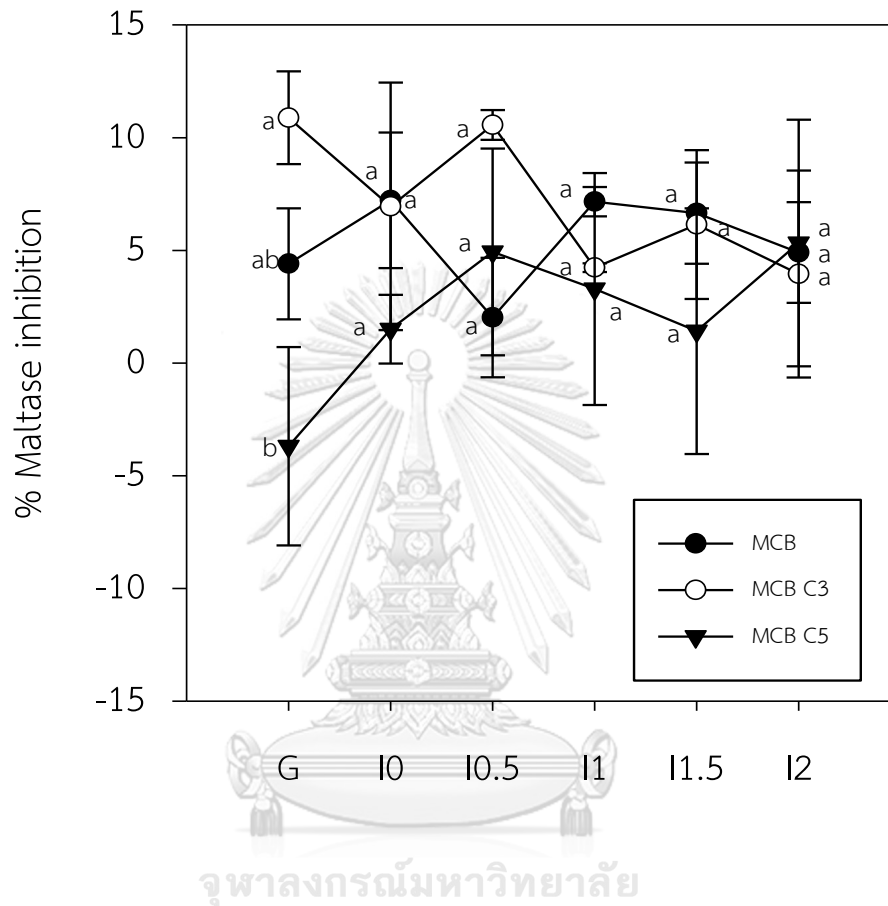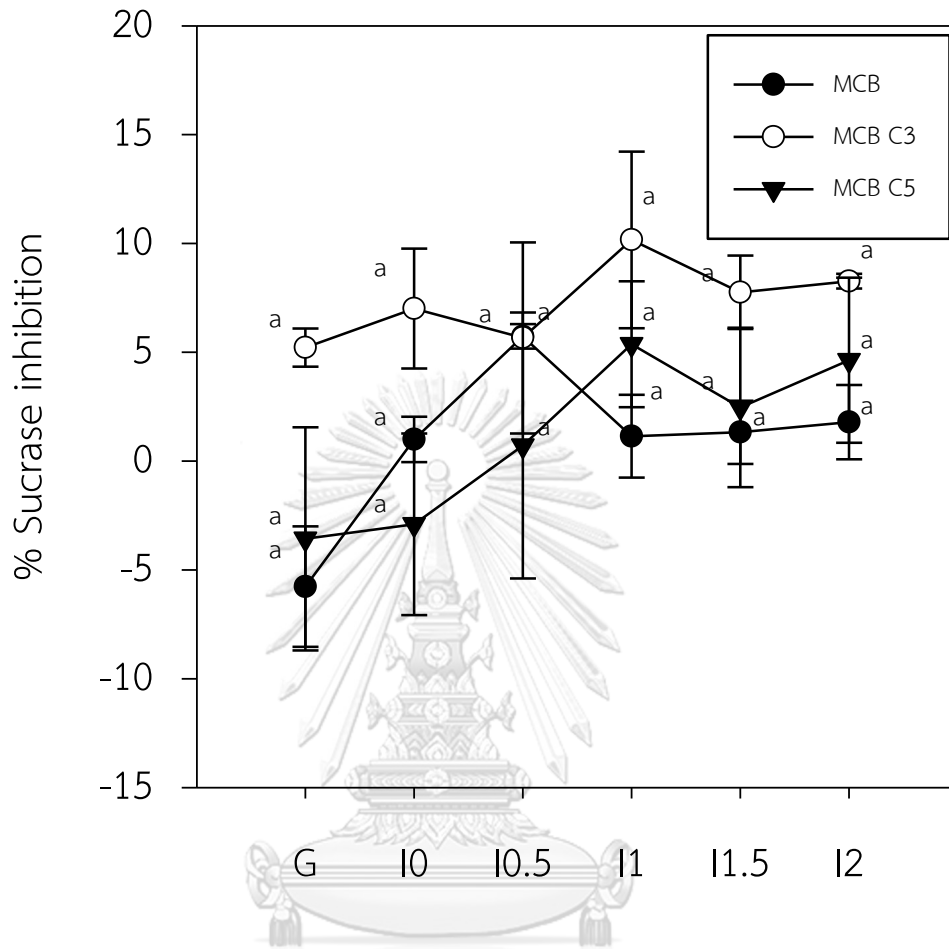
Figure 39. The percentage inhibitions of intestinal sucrase of *Mesona chinensis* microbead (MCB) and its encapsulation in carrageenan after simulated gastrointestinal digestion.

(G = gastric phase, I0 = before intestinal phase, I0.5 = 0.5 hour after intestinal phase, I1 = 1 hour after intestinal phase, I1.5 = 1.5 hours after intestinal phase and I2 = 2 hours after intestinal phase) The results are expressed as mean ± SEM (n=3).

Means with the different letter are significant differences. Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).

Table 6. The percentage inhibition of intestinal sucrase of *Mesona chinensis* extract, MC microbeads, MC agar jellies and MC carrageenan jellies after simulated gastrointestinal digestion.

	Gastric	Intestinal 0h	Intestinal 0.5h	Intestinal 1h	Intestinal 1.5h	Intestinal 2h
MCE	-3.86 ± 5.54[a]	6.00 ± 6.29[a]	1.15 ± 4.50[a]	-0.63 ± 6.48[a]	-4.96 ± 4.93[a]	10.39 ± 7.72[a]
MCE A3	3.26 ± 0.27[a]	-4.34 ± 1.51[a]	4.74 ± 0.96[a]	2.69 ± 0.06[a]	-0.85 ± 1.21[a]	-1.21 ± 2.35[a]
MCE A5	-1.25 ± 1.62[a]	-2.45 ± 0.87[a]	1.03 ± 0.86[a]	1.50 ± 3.25[a]	1.67 ± 3.37[a]	1.32 ± 3.86[a]
MCE C3	-2.26 ± 0.83[a]	-0.92 ± 0.53[a]	4.58 ± 2.49[a]	1.57 ± 2.43[a]	-0.10 ± 1.01[a]	0.58 ± 5.04[a]
MCE C5	-6.65 ± 5.69[a]	1.68 ± 4.67[a]	2.39 ± 5.35[a]	6.37 ± 5.31[a]	6.74 ± 3.49[a]	3.69 ± 6.86[a]
MCB	-5.77 ± 2.77[a]	0.99 ± 1.05[a]	5.73 ± 0.56[a]	1.14 ± 1.91[a]	1.33 ± 1.45[a]	1.79 ± 1.71[a]
MCB A3	6.31 ± 3.56[a]	0.51 ± 0.07[a]	5.36 ± 0.89[a]	3.49 ± 0.23[a]	3.30 ± 2.99[a]	3.40 ± 0.22[a]
MCB A5	-8.51 ± 2.53[a]	-5.20 ± 3.36[a]	-0.56 ± 3.33[a]	1.21 ± 2.22[a]	-0.82 ± 2.52[a]	-0.26 ± 5.56[a]
MCB C3	5.21 ± 0.88[a]	7.01 ± 2.76[a]	5.66 ± 4.39[a]	10.17 ± 4.06[a]	7.75 ± 1.69[a]	8.26 ± 0.34[a]
MCB C5	-3.57 ± 5.13[a]	-2.90 ± 4.18[a]	0.71 ± 6.11[a]	5.37 ± 2.89[a]	2.47 ± 3.66[a]	4.63 ± 3.80[a]

(MCE = *Mesona chiensis* extract, MCB = *Mesona chinensis* microbeads, A3 = 3% agar jelly, A5 = 5% agar jelly, C3 = 3% carrageenan and C5 = 5% carrageenan)

The results are expressed as mean ± SEM (n=3). Analysis was performed with one-way analysis of variance (ANOVA) followed by Tukey post hoc analysis (P < 0.05).

CHAPTER V

DISCUSSIONS

In this study, the polyphenols of *Mesona chinensis* extract (MCE) exhibited the antioxidant and anti-diabetic properties. The ferric reducing antioxidant power (FRAP) assay showed the antioxidant activity of MC extract which consistent with previous studies which observed the antioxidant activities by ferric reducing antioxidant power, DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, trolox equivalent antioxidant capacity, oxygen radical absorbance capacity and ferrous ion cheating activity (Hung and Yen 2002, Chusak, Thilavech et al. 2014). In current study, MC polyphenols might suppress carbohydrate digestion by inhibiting intestinal α-glucosidase enzymes including maltase and sucrase. These results are consistent with a previous study indicating that MC extract acts as α-glucosidase inhibitors (Chusak, Thilavech et al. 2014).

In this study, varied concentration of $CaCl_2$ (3-5%) and alginate (1.2-1.8%) did not alter the encapsulation efficiency of microbeads. In the preliminary studies, the microbeads could not be formed by using sodium alginate at less than 1%. According to the formation of alginate cross-link with the Ca^{2+}, low alginate concentration might cause lacking of COO^- group to interact with Ca^{2+} (Liu, Bao et al. 2003,

Pasukamonset, Kwon et al. 2016). When increased the percent of sodium alginate to 1.8% or above, the MC microbeads (MCB) occurred the tailed structure due to high viscosity of MCE-alginate solution dropped into the calcium chloride solution. The reason for explanation is that high viscosity of alginate solution can increase the resistance to form spherical shape within the time the droplet fell (Voo, Lee et al. 2015). When sodium alginate concentration was more than 2%, it caused the occlusion during the encapsulation process, leading to irregular dropping rate and varied in size.

The morphology of microbeads when using 75% MCE, 1.5% alginate and 3% calcium was in spherical shape. In contrast, the control beads could not form the shape without any extract (a shriveled shape). The spherical shape of microbeads is formed due to the linkage of polyphenol and alginate (Trifković, Milašinović et al. 2014). Spherical shape of microbeads was reported to be more gel bead strength when compared with non-spherical shaped (Al-Hajry, Al-Maskry et al. 1999). The diameter of the alginate microbeads without the extract in this study was similar to a previous study (Belščak-Cvitanović, Jurić et al. 2017) although the percent sodium alginate solution in this study was different about 0.1%. After incorporated MCE into the beads, its diameter was increased because the plant extract was trapped inside the beads. The simple injection method used in this study is commonly produced the beads with diameter which was greater than 1,000 μm (Lee, Ravindra et al. 2013).

However, the alginate beads size greatly varied due to many factors for instance alginate density and viscosity, concentration of alginate, calcium and active compounds, dripping tip diameter, dripping tip types, curing time, etc. Even though injection method produced larger diameter as compared with other methods such as mulsion templating and antisolvent precipitation, this method exhibited a trend to produce highly uniform size beads (Lee, Ravindra et al. 2013, Leong, Lam et al. 2016, Pasukamonset, Kwon et al. 2016, Zhang, Zhang et al. 2016).

The interaction between alginate and MCE was investigated by ATR-FT-IR. In this study, alginate powder represented O–H, COO- (asymmetric), COO-(symmetric), and C–O–C stretching at 3253.50, 1592.48, 1407.45 and 1026.15 cm^{-1} respectively. These stretching bands were similar to the previous studies (Santagapita, Mazzobre et al. 2012, Pasukamonset, Kwon et al. 2016). Theoretically, when cross-linking occurred between G-block alginate and Ca^{2+} and the COO^- groups of alginate polymers, these phenomena affect the intensity of the asymmetry and symmetry COO^- stretching bands (Pongjanyakul and Puttipipatkhachorn 2007). After encapsulation, strong asymmetry COO^- stretching could be seen at 1591.72 and 1594.91 cm^{-1} on spectra of MCB and the control beads, respectively while weak symmetry COO^- stretching presented at 1415.97 and 1418.58 cm^{-1} for MCB and the control beads. The wavenumber shifting of C-O-C and intensity was decreased in the control beads and MCB as compared with the alginate. These changes occur due to the presence of an

ionic bond between COO⁻ of alginates and Ca^{2+} (Kalogeropoulos, Yannakopoulou et al. 2010, Pongjanyakul and Rongthong 2010, Pasukamonset, Kwon et al. 2016). The O-H stretching of alginate microbeads (both the control and MCB) shifted to higher wavenumber and increased intensity as indicating in the previous studies (Pongjanyakul and Puttipipatkhachorn 2007, Pasukamonset, Kwon et al. 2016). This is possibly due to intermolecular hydrogen bonds between the plant extract and alginate. In addition, small peak at 2151.80 and 2163.19 cm^{-1} in the microbeads with and without polyphenols might occur due to crosslinking between calcium and alginate as suggested in the previous study (Pasukamonset, Kwon et al. 2016). The peak presented around 1260 cm^{-1} in MCE and MCB which were the aromatic rings stretching in plant polyphenols (Chupin, Motillon et al. 2013, Ricci, Olejar et al. 2015). These results suggested that were no chemical interactions between MCE and alginate. Therefore, the encapsulation by using alginate as biopolymer is appropriate for development of encapsulated MCE.

Thermal bahavior by DSC also shown the changes of decomposition peaks before and after encapsulation. In this study, alginate powder showed exothermic decomposition peak at 243.6°C which was the similarity to previous studies (Santagapita, Mazzobre et al. 2012, Pasukamonset, Kwon et al. 2016). The incorporation of MCE into alginate beads caused the shift of melting point from 202.4°C in control beads to 190.2°C in MCB. The similar shift was confirmed by

previous studies (Santagapita, Mazzobre et al. 2012, Pasukamonset, Kwon et al. 2016). In previous studies the shifted the melting point of plant extract to higher temperature occurred, indicating that encapsulation by alginate caused an increase in thermal stability of plant extract (Santagapita, Mazzobre et al. 2012, Pasukamonset, Kwon et al. 2016). However, in this study, the observed sharp melting point of plant extract in previous study (Pasukamonset, Kwon et al. 2016) disappeared. The similar occurrence was also found in lyophilized yerba mate extract DSC thermogram (Córdoba, Deladino et al. 2013). It suggests that the absence of melting point was due to diversity of compounds in the extract.

When MCB was added into agar jelly, the alginate beads retained its jelly structure. In contrast, the swelling jelly was observed in the carrageenan jelly. The similar observation was found in mixture of alginate and carrageenan film, increasing of carrageenan content led to more swelling of the film (Roh and Shin 2006). The gel formation of carrageenan needs K^+ and Ca^{2+} in the solution (McClements 2014). When MCB was added during the processing formation of carrageenan gel, carrageenan might interrupt the link of Ca^{2+} and alginate in egg-box structure which led to the swelling of alginate bead. Therefore, agar might be more suitable for produce the food product with alginate beads.

The polyphenol content of MCE significantly decreased after simulated digestion. Previous studies showed that polyphenols are sensitive to many factors

such as light, heat, pH, temperature (Bell 2001, Arabshahi-D, Devi et al. 2007). This might cause the instability of some polyphenols such as catechin at near neutral or alkaline pH and led to oxidation and epimerization of polyphenols (Roginsky and Alegria 2005, Neilson, Hopf et al. 2007, Ananingsih, Sharma et al. 2013). However, the different stability of various subclasses of polyphenols were reported when they were in simulated digestion (Arabshahi-D, Devi et al. 2007, Wootton-Beard, Moran et al. 2011). Polyphenols in the aqueous extract of *Mesona chinensis* are mostly flavonones such as kaempferol (a major compound) and apigenin. Phenolic acids such as caffeic acid (major compound), protocatechuic acid, syringic acid, vanillic acid and p-hydrobenzoic acid (Hailan, Yingzhen et al. 2011, Huang, Liao et al. 2012) are also found in the aqueous extract of *Mesona chinensis*. Previous studies reported that the bioassessibility of flavones such as apigenin and kaempferol-3-rutinoside increased after simulate digestion while bioaccessibility of phenolic acids such as caffeic acid and syringic acid decreased (Ortega, Reguant et al. 2009, Wong, Tan et al. 2014). Interestingly, the stability of kaempferol decreased while the kaempferol-3-rutinoside form was increased after simulated digestion (Gil-Izquierdo, Zafrilla et al. 2002, Goh and Barlow 2004, Jiao, Li et al. 2018). Previous study reported the low conversion of kaempferol-3-rutinoside to aglycone form in in vitro digestion (Goh and Barlow 2004). This leads to increase amount of kaempferol-3-rutinoside in the small intestine. The conversion of kaempferol to quercetin and 3′-O-methylated quercetin had also been observed in rat in the intestine (Barve, Chen et al. 2009). This might be

the reason to explain why the decrease of kaempferol occurs during simulated digestion.

The loss of polyphenol content in MCE after digestion might be due to instability of its major polyphenols such as caffeic acid and kaempferol. It found that high pH exposure (>7) cause the irreversible change to OH-group (Friedman and Jürgens 2000) and changed the reactive structure to metabolite structure of phenolic compounds.

Previous studies showed the potential of agar and carrageenan as the coating substance to protect its content during simulated digestion. These two polysaccharides could not be digested by pepsin (Kozu, Nakata et al. 2015, Kobayashi, Kozu et al. 2017). Moreover, agar and carrageenan structure consist of α-1,3-glycosidic bond and β-1,4-glycosidic bonds (Hehemann, Smyth et al. 2012, Kariduraganavar, Kittur et al. 2014). These glycosidic linkages were not digested by mammal α-amylase which hydrolyses at α-1,4-glycosidic bond (Butterworth, Warren et al. 2011). Previous studies revealed that the indigestibility of agar and carrageenan was observed in simulated digestion (Capron, Yvon et al. 1996, Kozu, Nakata et al. 2015, Kobayashi, Kozu et al. 2017). It found that agar remains in intact form while carrageenan is broken down less than 10% in the simulated gastric phase. The characteristic properties of agar enhanced the stability of polyphenols in the extract. For example, green tea polyphenols were incorporated into agar film leading to slow

release of the polyphenol into gastric solution but it provided maximum the release of polyphenol during intestinal phase (López de Lacey, Giménez et al. 2012). In this study, the amounts of polyphenols in gastric phase were detected from all types of MCE jellies approximately 50%, indicating that agar and carrageenan could partially protect the release of the *Mesona chinensis* phenolic compounds into the gastric solution. However, the release of polyphenols in the intestinal phase from MCE jellies slightly increased but it was not significant differences when compared to the digested MCE. In human digestive tract, foods are digested and absorbed in small intestine approximately for 3-4 hours (DeSesso and Jacobson 2001). In this study, the content of phenolics from MCE jellies continuously released during intestinal digestion, suggesting that it might increase amount of polyphenol and enhance its absorption in small intestine. However, the MC polyphenols still retain inside agar and carrageenan jellies approximately 40% after simulated digestion. A previous study has shown the retained polyphenols in agar (López de Lacey, Giménez et al. 2012).

The results demonstrated that the structure of MCB was able to retain the *Mesona chinensis* polyphenol content in the beads after gastric phase and it promoted the content release in intestinal phase. It might cause the calcium alginate response to the environmental pH. In the simulated gastric phase (pH 2), the pH solution was lower than pKa value of mannuronic and glucuronic acid (3.38 and 3.65

respectively). This leads to stabilization of hydrogen bonding network between Ca^{2+} and alginate (Chuang, Huang et al. 2017) and the structure of beads remained intact. In the intestinal phase, the polyphenols released from MCB to digesta was very fast which indicates by 6.1-fold increase phenolic content. The release of the alginate beads content in intestinal phase was consistence with previous studies (Córdoba, Deladino et al. 2013, Pasukamonset, Kwon et al. 2016). The reason for explanation is when the pH in intestinal phase (7.2) was higher than the pKa of alginate monomers, therefore the hydrogen bonds between Ca^{2+} and alginate were broken and it caused alginate beads swelling and consequently release of polyphenols (Chuang, Huang et al. 2017, McClements 2017). However, the incorporation of MCB into agar and carrageenan did not demonstrate the similarity of release of phenolic compounds as MCB when they were digested in gastrointestinal phase. It is suggested that these two polysaccharides protect the releasing polyphenol mechanisms of the alginate microbeads.

The antioxidant activity was measured by FRAP method. The antioxidant activity of *Mesona chiensis* extract significantly decreased after simulated digestion especially in intestinal phase. Since polyphenols of the MC extract have antioxidant activity (Hung and Yen 2002, Yen, Hung et al. 2003), the reduction of polyphenol content might led to decrease antioxidant activity of the extract. The current study showed that the percentage of FRAP value after gastric phase of MCB was only

8.76% and it significantly 3.3-fold rose to 29.08% after intestinal phase. The increasing of FRAP value might be attributed from phenolic content released from MCB into the digesta. The FRAP method is based on the ability of antioxidant compound to transfer the single electron to the reagent complex. This mechanism is dependent on the ionization potential of the complex (Leopoldini, Russo et al. 2011). The compound with low ionization potential is prone to donate electron. Therefore, each polyphenol could react to FRAP method with different potentials. The phenolic compounds that have low ionization potentials are likely to exhibit high antioxidant activity through the single electron transfer mechanism. There are a report mentioned about MCE polyphenols such as kaempferol, caffeic acid, apigenin had low ionization potential values (<180) compared with other compounds such as cyanidins (246) and phenol (192) (Leopoldini, Russo et al. 2011). The previous study also support that flavonoids with the 3-OH group at C ring, such as kaempferol, was the most reactive substituent in concomitant with FRAP value. (Csepregi, Neugart et al. 2016). In addition, the study by Csepregi and colleagues also found that the phenolic acid compounds containing 3-OH group such as caffeic acid and protocatechuic acid demonstrated a good reaction with FRAP method (Csepregi, Neugart et al. 2016). After simulated digestion, the increase in polyphenol and antioxidant activity might be attributed to the remaining high FRAP-reacted phenolic compounds such as kaempferol, caffeic acid and protocatechuic acids in the MC.

The incorporation of MC polyphenols and its alginate beads with the polysaccharides jellies resulted in the alteration of FRAP value during simulated digestion. In the intestinal phase, the MC agar jellies (MCE and MCB) showed synergistic effects on antioxidant activity (the FRAP value) when compared to agar and MC polyphenols. Even though there was no direct investigation on the interaction between agar and polyphenols, some studies also reported synergistic effects on antioxidant activity of other polysaccharides and polyphenols. It revealed that the interactions of polysaccharides and polyphenols could occur in the conjugated form and the physical mixture of polysaccharides and polyphenols (Wang, Hu et al. 2016). The synergistic antioxidant effects between tea polyphenols and low concentration of β-glucan (<0.05 mg/ml) mixture were observed in DPPH assay. However, the conjugated form of tea polyphenols and β-glucan demonstrated additive effects in DPPH assay (Wu, Ming et al. 2011). Moreover, the mixture of dextran and EGCG (Epigallocatechin gallate) from tea polyphenols also showed the synergistic antioxidant activity (DPPH free radical scavenging activity) (Wang, Zhao et al. 2013). The researchers revealed that the hydrogen bonds were formed between OH group of polyphenols and oxygen atoms of the cross-linking ether bonds in β-glucan or EGCG (Wu, Ming et al. 2011, Wang, Zhao et al. 2013). Moreover, the formation of hydrogen bond between OH groups of polyphenols and OH groups of β-glucan was also observed (Wu, Ming et al. 2011). The results from these two previous studies suggest that the hydrogen bonds might be formed between OH

group of MC polyphenols and oxygen atom in glycosidic bond of agar. However, the chemical reactions regarding synergistic antioxidant effects in single electron transfer mechanism of antioxidant are needed to further investigation.

The antioxidant effects resulted from these polyphenols and polysaccharides during the intestinal phase might benefit for the enterocytes in the small intestine. Previous study had shown that reactive oxygen species (ROS) could be generated from break down of some foods such as high fat diets and alcohol in the intestinal tract (Bhattacharyya, Chattopadhyay et al. 2014, Park, Kim et al. 2016). High ROS production can disrupt intestinal cell membrane and led to intestinal turmogenesis (Park, Kim et al. 2016) or cause the gastrointestinal diseases including peptic ulcers and inflammatory-related diseases (Bhattacharyya, Chattopadhyay et al. 2014). Moreover, ROS could directly induce apoptosis in intestinal Caco-2 cells (Prosperini, Juan-García et al. 2013). Since polyphenols exhibited antioxidants properties and neutralize these ROS (Scalbert, Johnson et al. 2005), they might protect the enterocytes from ROS damage. The synergistic antioxidant effects from agar and MC polyphenols might provide more potent on protective ability to the enterocytes.

The α-glucose inhibitory activity (both maltase and sucrase) of the MCE extract and its microbeads as well as jellies showed no significant differences during simulated digestion. This might occur from insufficient polyphenols from initial concentration of MC polyphenols before simulated digestion. In this study, the IC_{50} of

maltase and sucrase inhibition was 11.24 ± 3.80 mgGAE/ml and 16.81 ± 2.04 mgGAE/ml while the initial total phenolic content of simulated digestion were 2 mgGAE (0.5 mgGAE/ml).

CHAPTER VI

CONCLUSION

The bioassessibility of polyphenol in *Mesona chinensis* extract (MCE) decreased after simulated digestion. The encapsulation of MCE using the alginate showed the ability to retain the polyphenols in gastric phase and promote polyphenol release in intestinal phase. The physical and chemical characteristics of MC microbeads supported that the MCB can be an alternative method to improve plant polyphenol bioaccessibility. The incorporation of MC polyphenols into agar and carrageenan jellies were partially protect the MC polyphenol during gastric phase. In intestinal phase, agar showed synergistic antioxidant effect when mixed with MC polyphenols regardless of polyphenol sources. Therefore, products containing MC extract in agar jelly might enhance the prolonged release of polyphenols with concomitant increase antioxidant activity in the intestinal phase.

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APPENDIX

VITA

Chonnipa Wongverawattanakul was born on November 8th, 1991 in Bangkok, Thailand. She graduated with Bachelor's degree of Science in Biology (first class honor) in 2012 from Department of Biology, Faculty of Science, Chulalongkorn University. After that she entered the Graduate School of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University and majored in Applied Food and Nutrition.

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