CONTROLLING DELIVERY OF CAPSAICIN FROM LAYERED-FLAT NOODLES USING GASTRO-RESISTANT DOUGH LAYERS

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By

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LIST OF SYMBOLS/ ABBREVIATIONS

Symbol/Abbreviation	Caption
CAP	capsaicin
CCD	capsaicin consumption dose
CED	capsaicin-enriched dough
CN	conventional noodle
EE	energy expenditure
HI	hydrolysis index
HPLC	high performance liquid chromatography
LN	layered noodle
MEC	multiple extrusion cell
MTG	microbial transglutaminase
pGI	predicted glycemic index
RP	red pepper
RS	resistant starch
SEM	scanning electron microscope
SHU	Scoville heat units
SPI	soy protein isolate
TPA	texture profile analysis
TRPV1	transient receptor potential vanilloid 1

PENGHANTARAN TERKAWAL KAPSAISIN DARIPADA

MI LEPER-BERLAPIS DENGAN LAPISAN DOH RINTANG-GASTRO

ABSTRAK

Tesis ini membentangkan pembangunan satu sistem pelepas/penghantar-terkawal kapsaisin yang baru dengan menggunakan mi kuning alkali yang direkabentuk semula, dikenali sebagai mi lapisan (LN). LN telah disediakan dengan melapis doh diperkaya dengan kapsaisin (CED) di antara dua lapisan doh rintangan-gastro. Dalam kajian awal, formulasi CED telah direkabentuk dan kemungkinan penggunaan LN sebagai pembawa-kapsaisin telah diterokai. Sebagai perbandingan dengan mi konvensional (CN) dengan jumlah kapsaisin yang setara, LN telah terbukti mengalami kehilangan kapsaisin yang lebih sedikit secara signifikan ($P < 0.05$) semasa memasak dan tempoh kepedasan berlarutan yang lebih singkat di dalam mulut seleps memakan mi tersebut. Untuk menghadapi keadaan di dalam gastro-usus, penggunaan lapisan rintangan-gastro untuk menghasilkan LN diterokai. Lapisan rintangan-gastro dihasilkan daripada (a) tepung gandum (LN-C), (b) tepung gandum dicampur dengan tepung kanji rintangan (Fibersym®) (LN-F) dan (c) tepung gamdum dicampur dengan isolat protein soya (SPI) dan mikrobial transglutaminase (MTG) (LN-MTG). Ciri-ciri fizikokimia, tekstur, pengekalan kapsaisin dan ramalan indeks glisemik (pGI) bagi LN telah dinilai. LN-MTG menunjukkan kekuatan tegangan dan keanjalan yang tertinggi ($P < 0.05$), serta pengekalan kapsaisin di dalam simulasi kondisi mulut dan usus yang tertinggi ($P < 0.05$). Dalam simulasi kondisi gastrik, pengekalan kapsaisin bagi semua jenis mi adalah sama. pGI daripada LN-MTG dan LN-F adalah sama ($P > 0.05$) tetapi lebih rendah secara signifikan ($P < 0.05$) berbanding dengan LN-C. Keputusan ini

mencadangkan berlakunya lebihan hubung-silang antara matrik-matrik protein bagi LN-MTG merupakan alasan untuk integriti struktur mi yang lebih baik dan resapan kapsaisin yang terbantut dari CED. Dalam fasa terakhir kajian ini, kesan paras MTG pada pengekalan kapsaisin, profil tekstur, ciri-ciri pemecahan struktur, mikrostruktur dalaman dan ciri-ciri sensori bagi LN-MTG telah dinilai. LN-MTG dengan 0.5, 1.0 dan 1.5 g MTG/100 g campuran tepung gandum dengan SPI telah dirangka untuk menyediakan LN-MTG0.5, LN-MTG1.0 dan LN-MTG1.5, masing-masing. Secara umumnya, peningkatan paras MTG membawa kepada peningkatan yang signifikan ($P < 0.05$) dalam kekuatan tegangan, pengekalan kapsaisin dan kepadatan struktur, tetapi pengurangan kadar keruntuhan struktur dengan signifikan ($P < 0.05$). Penambahan paras MTG antara 0.5 dan 1.5 g tidak membawa sebarangan perbezaan yang signifikan ($P > 0.05$) dalam penerimaan keseluruhan sensori. Walau bagaimanapun, LN-MTG1.5 mempunyai penerimaan keseluruhan sensori yang lebih rendah secara ketara ($P < 0.05$) berbanding dengan LN-C. Oleh itu, peningkatan paras MTG dalam lapisan rintangan-gastro bagi LN-MTG boleh menghasilkan pengekalan kapsaisin yang lebih baik dan integriti struktur yang lebih kukuh, tetapi pada pampasan penerimaan sensori yang lebih rendah. Kesimpulannya, LN-MTG menunjukkan potensi yang baik untuk menjadi suatu sistem penghantar untuk pelepas/penghantar-terkawal bagi kapsaisin dalam keadaan gastro-usus. Reka bentuk dan pembangunan LN-MTG dalam kajian ini boleh memudahkan penggunaan teknologi LN dalam pelepas/penghantar-terkawal bagi bahan-bahan bioaktif dan berfungsi dalam industri makanan.

CONTROLLING DELIVERY OF CAPSAICIN FROM LAYERED-FLAT NOODLES USING GASTRO-RESISTANT DOUGH LAYERS

ABSTRACT

This thesis describes the development of a novel capsaicin controlled-release/delivery using redesigned yellow alkaline noodles (i.e. layered noodles, LN) as the delivery system. The proposed LN was prepared by sandwiching the capsaicin-enriched dough (CED) in between two gastro-resistant layers. In the preliminary study, the formulation of CED was designed and the feasibility of using LN as a capsaicin-carrier was explored. In comparison with conventional noodles (CN) with equal amount of capsaicin, LN was proven to have significantly lower ($P < 0.05$) capsaicin losses during cooking and shorter lingering period of spiciness in the mouth after consuming the noodles. To withstand the gastro-intestinal conditions, application of gastro-resistant layers to produce LN was examined. The gastro-resistant layers were made from (a) wheat flour (LN-C), (b) wheat flour mixed with resistant starch flour (Fibersym®) (LN-F) and (c) wheat flour mixed with soy protein isolate (SPI) and microbial transglutaminase (MTG) (LN-MTG), respectively. Physicochemical properties, texture properties, capsaicin retentions and predicted glycemic index (pGI) of the developed LN were examined. LN-MTG showed the highest ($P < 0.05$) tensile strength and elasticity, as well as the highest ($P < 0.05$) capsaicin retention in stimulated mouth and intestinal conditions. In stimulated gastric conditions, the capsaicin retention of all noodles was similar. The pGI of LN-MTG and LN-F were similar ($P > 0.05$) and were significantly lower ($P < 0.05$) than LN-C. These results suggest the occurrence of additional cross-linking between protein matrices of LN-MTG could be a reason that improved

structural integrity of noodles and retarded the diffusion of capsaicin from CED. In the final phase of this study, the effect of MTG concentration on capsaicin retention, textural profiles, structural breakdown properties, inner microstructures and the sensory properties of LN-MTG were evaluated. LN-MTG with 0.5, 1.0 and 1.5 g MTG/100 g of wheat flour and SPI mixture were formulated to prepare LN-MTG0.5, LN-MTG1.0 and LN-MTG1.5, respectively. Generally, increased in MTG concentration leads to a significant increased ($P < 0.05$) in tensile strength, capsaicin retention and structure density but structure breakdown rate was significantly decreased ($P < 0.05$). Addition of MTG concentration between 0.5 and 1.5 did not caused any significant difference ($P > 0.05$) in sensory overall acceptability. However, LN-MTG1.5 showed significantly lower ($P < 0.05$) overall acceptability compared to LN-C. Hence, increasing the level of MTG within the gastro-resistant layers of LN-MTG could yield better retention of capsaicin and stronger structure integrity but at the compensation of lower sensory acceptability. In conclusion, LN-MTG showed good potential to be a delivery system for controlled-release/delivery of capsaicin in gastro-intestinal conditions. The design and development of LN-MTG in this study could facilitate the application of LN technology in controlled-release/delivery of other bioactive and functional ingredients in the food industry.

CHAPTER 1

INTRODUCTION

1.1 Backgrounds and Rationale

Chili pepper is a common plant found in many parts of the world, which comprise more than 200 varieties (Pruthi *et al.,* 1980). Chili peppers from different regions vary widely in size, shape, flavor and sensory heat (Korel *et al.,* 2002). Capsaicin is the predominate substance responsible for the hot character of chili peppers. The capsaicin is mainly produced by the glands at the junction of placenta and the pod wall of the chili fruit (Rowland *et al.,* 1984; Pandey *et al.,* 2010). The concentrations of capsaicin in strong chili pepper varieties range from 0.1 to 1% of the fresh fruit (Govindarajan *et al.,* 1987).

Capsaicin is known to exert the function of anti-obesity through increasing the energy expenditure and decreasing the appetite. After the ingestion of capsaicin, the energy metabolism is enhanced via the activation of sympathetic nervous system (Kang *et al.,* 2010; Wsterterp-Plantenga *et al.,* 2006). This causes an increased secretion of catecholamine from the adrenal medulla (Yoshioka *et al.,* 1999) and the expression of certain fat degrading proteins (Joo *et al.,* 2010). The feeling of satiety is promoted and the energy intake is reduced (Reinbach *et al.,* 2009; Westerterp-Plantenga *et al.,* 2005). However, the application of capsaicin in the food industry for treating and preventing obesity is limited because the effective dosage of capsaicin required for weight control (10-30 mg) (Reinbach *et al.,* 2010; Yoshioka *et al.,* 2004; Youshioka *et al.,* 1999) is much higher than the limitation of intake for normal people (1-10 mg) (Craft & Porreca, 1992; Yoshioka *et al.,* 1998; Yoshioka *et al.,* 2004). A product that is directly incorporated with capsaicin may be too hot for most

consumers to consume. Some cutting-edge companies introduced delivery systems that are based on the encapsulation technologies using capsules. These products are designed to release the capsaicin in the intestine, not in the stomach, to reduce the irritation and the pain caused by capsaicin. However, these approaches are limited merely for nutraceutical applications. A more innovative technique is desirable in order to achieve similar functions for food products.

"Layered Noodle" (LN) is designed to be a novel product that is prepared by sandwiching a capsaicin-enriched dough (CED) layer in-between of two gastro-resistant layers (Figure 1.1). This might be potentially a good method to convey the functions of capsaicin. The hypothesis of this study is capsaicin could be retained in the LN during mastication and results in less uneasiness in human mouth. The idea is feasible in implementation and practicable in manufacture. Many of the Asian consumers prefer to slurp the noodle rather than chewing them extensively during eating (Kohyama *et al.,* 2010). Therefore, a reduction in irritation is expected by controlled release of capsaicin in the stomach and intestine.

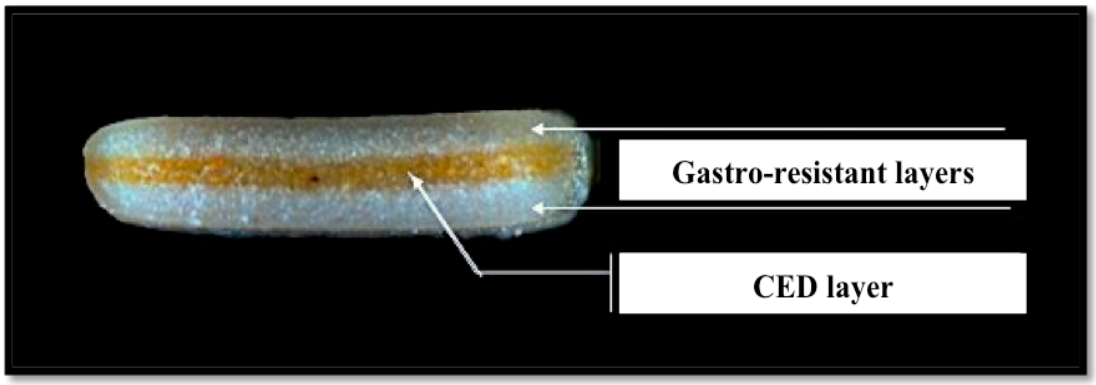


Figure 1.1 Cross-section image of LN

The gastro-resistant materials used for the preparation of the sandwiching noodle layers should have controlled-delivery properties and lower digestion capabilities so that the noodles could retain capsaicin for a longer period of time. A modified resistant wheat starch product (Fibersym®) that contains a high content (76%) of dietary fiber is one of the options for producing the gastro-resistant dough. The intensive studies in high fiber products exert positive effects on the human health, by reducing the glycemic response, the levels of serum cholesterol and the risk of coronary diseases while enhancing weight management (Appleby *et al.,* 1998; Rigaud *et al.,* 1990). Some studies demonstrated that the organoleptic qualities of the products enriched with resistant starch (RS) were better than those with traditional dietary fiber. This could be owed to a few properties of the RS namely the low water-holding properties, the white and smooth appearance as well as the bland flavor (Sanz *et al.,* 2008).

Another alternative in producing gastro-resistant dough is to use a protein substance (e.g. soy protein isolate, SPI) cross-linked via a cross-linking agent (e.g. microbial transglutaminase, MTG). The cross-linking reaction catalyzed by the MTG enzyme (Nonaka *et al.,* 1996) occurred between the γ-carboxyamidegroup of peptide-bound glutamine residues (acyl donors) with a variety of primary amines (acyl acceptors) (Motoki & Seguro, 1998). Incorporation of MTG into the mixture of SPI and wheat flour has been reported to reduce the predicted glycemic index (pGI), strengthen the dough network and enhance the gastro-resistant properties (Gan *et al.,* 2008; Gan *et al.,* 2009). The reduction in the pGI value means that the noodles are more resistant to the human digestion.

It is rational to incorporate the RS and the cross-linked protein into the outer layers of the newly designed LN, in order to increase the retentions of capsaicin, i.e. improving the gastro-resistant properties of the LN outer layers.

From the literature search, no study was carried out regarding LN, not to mention the evaluation of the ingredients used in LN. This project can be considered as a pioneering research to develop the capsaicin enriched LN with controlled-release properties.

1.2 Objectives

The main objective of this research is to innovate a capsaicin delivery system by using a redesigned yellow alkaline noodle (i.e. LN) as a food matrix. The LN is designed to release capsaicin in a controlled manner in order to deliver its weight controlling function without causing excessive uneasiness and suffering to the consumers. In order to achieve the main goal, the study is subdivided into the following objectives:

a. To explore the feasibility in using the LN as a capsaicin-carrier to convey the high amount of capsaicin.

b. To estimate the physicochemical properties and the capsaicin retention of the LN prepared from different selected gastro-resistant materials.

c. To improve the capsaicin retention and evaluate the sensory characteristics of the LN enriched with capsaicin.

1.3 Research Protocol

The experimental works of this study are divided into three main phases as listed below:

a. First phase describes the preparation method and the formulation of the LN enriched with capsaicin. During an exploration study the knowledge on the lingering of spiciness sensation in human mouth after the consumption of the capsaicin-enriched LN will be acquired.

b. Second phase covers the characterization of the physical properties (i.e. cooking yield, pH, color, tensile strength and elasticity), in-vitro starch digestibility and the capsaicin retentions of the LN. These samples will be prepared by sandwiching the CED sheet with two gastro-resistant sheets that will be prepared from the RS and SPI-MTG respectively.

c. The attempts to improve the capsaicin retention of LN in the simulated mouth, gastric and intestinal conditions are described in phase three. The sensory characteristics of the LN will also be carried out to appraise the acceptance of LN by consumers.

CHAPTER 2

LITERATURE REVIEWS

2.1 Capsaicin

2.1.1 Backgrounds

Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is the pungent compounds of chili pepper (plants belonging to the genus *Capsicum*), which may cause the burning sensation in any human tissue that it comes into contact with. Pure capsaicin is a crystalline to waxy compound that is hydrophobic, colorless and odorless. It possesses the physical properties of the melting point at 62-65°C and the molecular mass at 305.41 g/mol. It is a synthesized alkaloid and accumulated in the intermolecular septum of the chili pepper (Iwai *et al.,* 1979; Suzuki *et al.,* 1980).

The structure of capsaicin is shown in Figure 2.1, which has a benzene ring and a long hydrophobic carbon tail with the polar amide group. The vanillyl (4-hydroxy-3-methoxybenzyl) moiety confers the biological activities of capsaicin (Suzuki *et al.,* 1980).

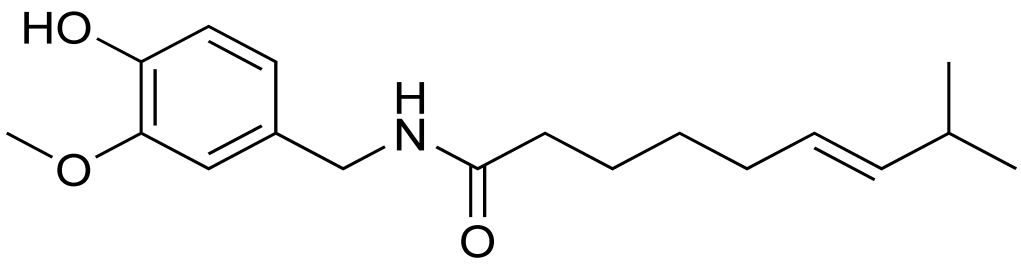


Figure 2.1 Chemical structure of capsaicin (citation from Suzuki *et al.,* 1980)

The biosynthetic pathway of capsaicin is well characterized. The vanillyl moiety of capsaicin is derived from phenylalanine, while the branched fatty acid moiety is originating from L-valine or L-leucine (Bennett & Kirby, 1968; Diaz *et al.,* 2004). The quantities of capsaicin vary with genotype and the maturity of the chili plant (Zewdie & Bosland, 2001). The accumulation of capsaicin increases with the maturity until the maximum is reached. However, due to the presence of photooxidation or oxidizing enzymes, the capsaicin content then decreases up to 60% (Contreras-Padilla & Yahia, 1998; Iwai *et al.,* 1979).

Capsaicin produces a large number of physiological and pharmacological effects on human body. These effects demonstrated on the taste, the digestive tract, the thermoregulatory system, cardiovascular system and respiratory system. It is also widely used as a neuropharmacological tool. A general review of the capsaicin applications on human body are listed in Table 2.1.

Table 2.1: The overview applications of capsaicin on human body

Effect	Application	Reference
Effects of sensory	Flavoring substance	Precott & Stevenson, 1995.
Effects on gastrointestinal tract	Increase the secretion of salivary and gastric fluid	Platel & Srinivasan, 2004.
	Against gastric ulcer	Adbel-Salam *et al.,* 1997.
Effects on thermoregulation and metabolism	Weight management	Yoshioka *et al,* 1995; Lim *et al.,* 1997; Yoshioka *et al.,* 1998; Matsumoto *et al.,* 2000; Ludy & Mattes, 2011.
	Lipid peroxidation in various organs	Kogure *et al.,* 2002.
Pain management	Rheumatic diseases	Tsuchiya, 2001.
	Cluster headache	Fusco *et al.,* 1994.
	Painful diabetic neuropathy	Bernstein & Phillips, 2006.
	Post herpetic neuralgia	Hempenstall *et al.,* 2005.
	Arthritis pain	Deal, 1994.
	Post mastectomy pain	Watson & Evans, 1992.
	Cystitis	Peter & Watson, 1994.
	Bladder dysfunction	Fowler *et al.,* 1992.
	Chronic musculoskeletal pain	Mason *et al.,* 2004.
	Human immunodeficiency virus induced pain	Newshan, 1997.
Effects on anti-inflammatory	Treatment of gastric infections.	Lee *et al.,* 2007; Choi *et al.,* 2011.
Effects on anti cancer and mutagen		Laviada, 2006; Huynh & Teel, 2005; Yang *et al.,* 2010.
Effects on antioxidant		Henning *et al.,* 2011.
Reduce post-operative nausea and vomiting		Agarwal *et al.,* 2005.

2.1.2 Scoville Heat Units (SHU)

The sensation of spiciness triggered by capsaicin is historically measured in Scoville heat units (SHU). The SHU system is based on an organoleptic method. This method is created by Wilbur Scoville, a renowned American pharmacist, in 1912. It measures the amount of sugar syrup diluted in the chili extract until its heat becomes undetectable by a panel of tasters (usually five). The more sugar added, the greater degree of pungency and the higher measurement in SHU (Topuz & Ozdemir, 2007). Pure capsaicin ranked at 16,000,000 SHU which means the extract of the pure capsaicin must be diluted over 16,000,000 times before the sensation of hotness is undetectable.

Generally, anything less than 5,000 SHU is considered as 'mild' while that over 20,000 SHU is 'hot'. Moderated pungency is between 5,000 and 20,000 SHU. The tolerance dose of capsaicin for most people is around 30,000 SHU (Topuz & Ozdemir, 2007; Krajewska & Powers, 1988; Othman *et al.,* 2011; Caldecott, 2002).

Nowadays, due to the side effects caused by the sensory methods, e.g. buildup of heat, fatigue and gastric inconsistencies (Korel *et al.,* 2002), the hotness is more frequently determined by the high performance liquid chromatography (HPLC) (Sanatombi & Sharma, 2008). This measurement by modern equipment is considered to be more reliable, accurate and modern.

2.1.3 Capsaicin Daily Consumption and Side Effects

Capsaicin is widely consumed as a food additive throughout the world, particularly in the South East Asian and Latin American countries (Surh & Lee, 1995). The average consumptions of capsaicin in India, Thailand and Mexico, where chili products are heavily consumed, are reported to be 25-200 mg/person/day

(Mathew *et al.,* 2000; Othman *et al.,* 2011; López-Carrillo *et al.,* 2003). However, in the low chili consumption regions, like US and Europe, the average daily intake of capsaicin is roughly estimated to be 1.5 mg/person/day (Othman *et al.,* 2011; Govindarajan & Sathyanarayana, 1991). Some studies suggest that people whose daily intake of capsaicin in a quantity range from 90 to 250 mg may have a higher possibility of developing gastric cancer than those whose daily capsaicin consumption less than 30 mg (López-Carrillo *et al.,* 2003; López-Carrillo *et al.,* 1994). Other than that, occasional and lower intake of chili has little impact on the increasing cancer cases (Govindarajan *et al.,* 1991). Some studies even suggest that consumption of capsaicin at decent level has possible cancer prevention feature, e.g. inducing the autophagy in HTC116 human colon cancer cells (Arora *et al.,* 2011; Chueh *et al.,* 1997).

2.1.4 Mechanisms of Capsaicin on Weight Management Action

Over weight and obesity is mainly caused by the energy intake is over than the expenditure. Capsaicin may help to fight obesity by enhancing the energy output and reducing the amount of calories absorbed.

(a) Mechanisms of Capsaicin on Energy Expenditure Enhancement

The basic principle of capsaicin on the energy expenditure enhancement is the formation of thermogenesis. It is likely due to the involvement of the transient receptor potential vanilloid 1 (TRPV1), which distributes among the digestive tract surface (Eldershaw *et al.,* 1994). The TRPV1 (capsaicin receptor) channel is expressed in the sensory neurons, brain and various of the non-neuronal tissues (Szallasi, 2005; Caterina *et al.,* 2000; Westerterp-Plantenga *et al.,* 2006). The

regulation of TRPV1 is complex. Multiple mechanisms act jointly to keep the TRPV1 in a closed and inactive state. Some parameters like heat, protons and vanilloids can activate this receptor (Szallasi, 2005). However, the TRPV1 agonists have different responses with the activation kinetic (Liu & Simon, 2003).

After ingestion of capsaicin, the TRPV1 on the digestive tract surface is activated without being absorbed into the circulation. Once being activated, the signal of capsaicin is transmitted and then activates the sympathetic nervous system (Kang *et al.,* 2010; Wsterterp-Plantenga *et al.,* 2006). The sympathetic nervous system is involved in the regulation of the energy balance as an important regulator of energy expenditure. The activation of the sympathetic nervous system enhances the thermogenesis through the stimulation of catecholamine (i.e. norepinephrine or epinephrine). The catecholamine is secreted from the adrenal medulla (Kawada *et al.,* 1988; Kawada *et al.,* 1986; Watanabe *et al.,* 1988; Watanabe *et al.,* 1987). Some studies demonstrate that the β-adrenergic receptors in the body are stimulated during the infusion of norepinephrine and epinephrine (Diepvens *et al.,* 2007). These receptors control the biochemical mechanisms that lead to an increased activity of the muscular tissues. These muscular tissues are responsible for the increasing of heart rate, oxygen consumption, blood vessels contraction, and the dilating of airway passages (Simonsen *et al.,* 1992). All of these actions increase the total energy output and the fat degradation, therefore help in the prevention of the body weight gain (Kurpad *et al.,* 1994). This pathway is evidenced by the abolishment of the increasing in thermogenesis after the administration of β-adrenergic blockers (Yoshioka *et al.,* 1995).

Another route of amplifying the thermogenesis after the ingestion of capsaicin is the stimulating expression of the certain fat degradation proteins, e.g. uncoupling proteins. These proteins are formed after the stimulation of β-adrenergic receptors. They are located in the inner membrane of mitochondria (Kopeckÿ *et al.,* 1990). The uncoupling proteins are primarily responsible for the increasing of thermogenesis through 'uncoupling' of the respiratory chain and the oxidative phosphorylation. The 'uncoupling' means that during the process of the protons transit down the electrochemical gradient across the inner mitochondrial membrane, the energy produced is released as heat rather than being used to generate ATP. Other than the inhibition of oxidative phosphorylation, the influences on mitochondrial functions included the retention of calcium ions and the stimulation of ATP activity (Reanmongkol *et al.,* 1988; Westerterp-Plantenga *et al.,* 2006). In this case, the size and mass of the fat cells can be shrink since they are partially burned in the thermogenesis process. Thus the ingestion of capsaicin may lead to the weight reduction of the consumers.

(b) Mechanisms of Capsaicin on Food Intake Reduction

The effects of capsaicin on the food intake reduction are achieved via the stimulation of the sympathetic nervous system (Watanabe *et al.,* 1987). A reciprocal relation between the activity of the sympathetic nervous system and the food intake has been described in several different experiments (Yoshioka *et al.,* 1999; Westerterp-Plantenga *et al.,* 2005; Yoshioka *et al.,* 2004; Reinbach *et al.,* 2009). A large reduction in the energy intake is observed in both the experimental animals and the human beings. It may be due to the anorectic effect of the catecholamine secreted

from the adrenal medulla after the stimulation of the sympathetic nervous system activity (Yoshioka *et al.,* 1999).

The overall mechanisms of capsaicin on the weight management action are shown in Figure 2.2.

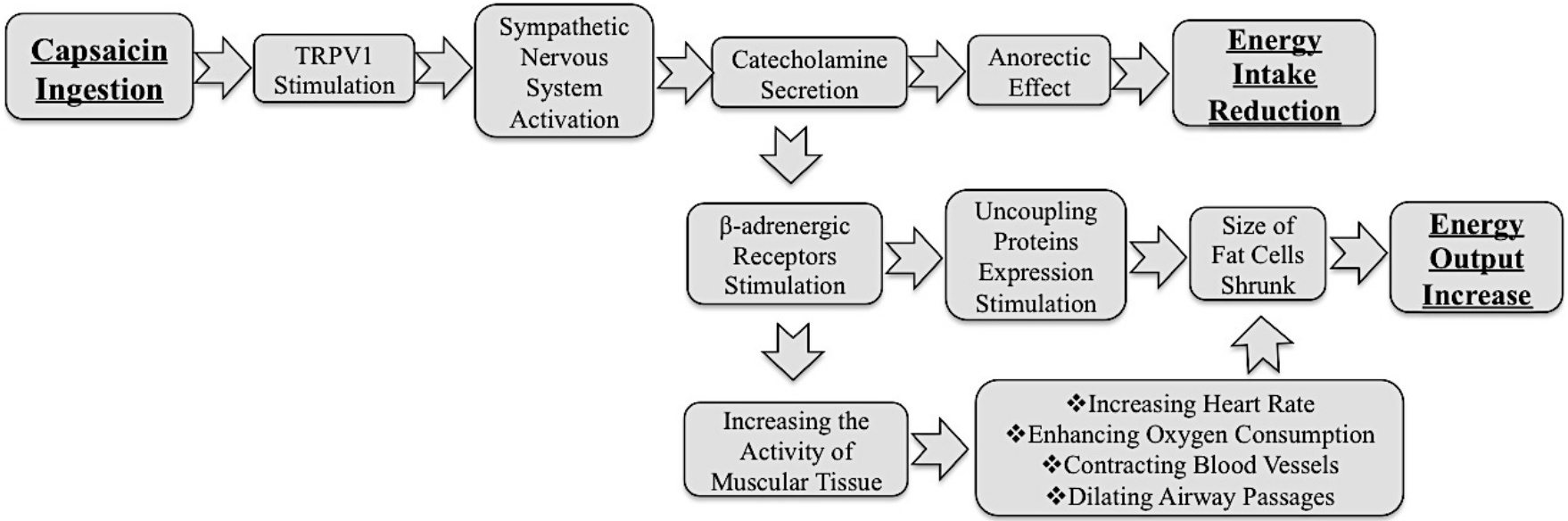


Figure 2.2 Mechanisms of capsaicin on weight management action

2.1.5 Clinical Trials of Capsaicin on Body Weight Regulation

It is important to review the clinical trials on the use of capsaicin to regulate body weight. The evidence that a dietary source of capsaicin ingestion can have a potential role in the weight management strategies can be exciting. The effects of capsaicin on the energy expenditure and the food intake are reviewed and summarized in Table 2.2. These studies are performed through the randomized and controlled experiments.

Table 2.2: Studies on the effects of capsaicin on thermogenesis and appetite

No	Reference	Sample Size (n)	Ethnicity	Gender	Age (years)	BMI (kg/m^2)	Dosage (mg)	Duration (hours/ weeks)	Thermogenesis	Appetite
1	Ludy *et al.*, 2011	25	Caucasian	F=14 M=11	23 ± 0.05	22.6 ± 0.3	RP = 1 g; CAP = 2 mg		Increase in EE, core body temperature; Increase in fat oxidation.	No difference on desire to eat in general, fullness, prospective food intake, thirst, or hunger.
2	Reinbach *et al.,* 2010	40	Caucasian	F=17 M=23	24.6 ± 2.5	22.5 ± 7.0	CAP = 0.375 mg	1 h	-	Decrease the desire to eat; No difference on energy intake.
3	Smeet & Westerterp-Plantenga, 2009	30	Caucasian	F=11 M=19	31 ± 14	23.8 ± 2.8	RP =1.03 g; CAP =5 mg	4 h	No difference in EE between CAP and Control.	No difference on satiety, but tended to decrease ghrelin (P=0.07) between CAP and Control.
4	Snitker *et al.,* 2009	80	Caucasian	F/M	30 - 60	25 - 35	CAP =6 mg	12 wk	No difference in EE between CAP and Control; Increase fat oxidation in CAP.	-
5	Westerterp-Plantenga *et al.,* 2005	24	Caucasian	F=12 M=12	35 ± 10	25.0 ± 2.4	CAP = 0.225 mg (ingested 30 min before meal)	16 h	-	Decrease in energy (0.9-1.6 MJ) and fat intake; Increase in satiety.
6	Yoshioka *et al.,* 2004	16	Japanese	M	22.4 ± 3.2	Weight = 79.4 ± 19.4 kg; Height = 176.1 ± 6.7 cm	CAP = 2.769 ± 4.131 mg	-	-	Decrease in energy and fat (13.3%) intake.

Table 2.2 Continued

No	Reference	Sample Size (n)	Ethnicity	Gender	Age (years)	BMI (kg/m^2)	Dosage (mg)	Duration (hours/ weeks)	Thermogenesis	Appetite
7	Chaiyata *et al.,* 2003	22	Thai	F	-	-	RP = 5 g CAP = 25 mg	0.5 h	Increase in EE (20%).	Decrease in the raising of plasma glucose (20.6%).
8	Yoshioka, *et al.,* 2001	8	Caucasian	M	25	20.7 - 26.8	CAP = 21.8 mg/day	25 h	Increase in EE (3.2%).	Decrease in energy intake (17.3%).
9	Matsumoto *et al.,* 2000	16	Japanese	F	Lean = 19.6 ± 0.26; Overweight =20.1 ± 0.4	Lean = 21.0 ± 0.57; Overweight = 28.8 ± 1.01	CAP =3 mg	0.5 h	Increase EE in lean, but not in overweight.	
10	Yoshioka *et al*., 1999, Study 1	13	Japanese	F	25.8 ± 2.8	Weight = 54.2 ± 6.4 kg; Height = 157 ± 4 cm	RP = 10 g; CAP = 30 mg	3 h	-	Decrease appetite and subsequent protein (6-20%) and fat (11-17%) intake.
11	Yoshioka *et al.*, 1999, Study 2	10	Caucasian	M	32.9 ± 7.8	Weight = 72.5 ± 10.1 kg; Height = 175 ± 6 cm	RP = 6 g; CAP = 18 mg	3 h	-	Decrease energy (11%) intake.
12	Yoshioka *et al*., 1998	13	Japanese	F	25.8 ± 2.8	Weight = 54.2 ± 6.4 kg; Height = 157.2 ± 4.5 cm	RP = 10 g; CAP = 30 mg	3.5 h	Increase in EE.	-

Table 2.2 Continued

No	Reference	Sample Size (n)	Ethnicity	Gender	Age (years)	BMI (kg/m^2)	Dosage (mg)	Duration (hours/ weeks)	Thermogenesis	Appetite
13	Lim *et al.,* 1997	-	Japanese	M	18 - 23	-	RP = 10 g; CAP = 30mg	2.5 h	Slightly increase but no significant difference in oxygen consumption in the initial 30 min; Increase respiratory quotient, blood lactate levels, plasma epinephrine and norepinephrine levels.	-
14	Yoshioka *et al.,* 1995	-	Japanese	M	-	-	RP = 10 g; CAP = 30 mg	2.5 h	Increase in EE (30%) in initial 30 min, no difference for the remaining 120 min.	-

Abbreviations:
F=female, M=male, RP = red pepper, CAP = capsaicin, EE = energy expenditure

The doses of capsaicin used in the previous studies (Table 2.2) carried widely. They ranged from 0.3 to 30 mg. Some of the studies reported that capsaicin stimulates the thermogenesis (Ludy *et al.,* 2011; Chaiyata *et al*., 2003; Yoshioka *et al.,* 2011; Matsumoto *et al.,* 2000; Yoshioka *et al.,* 1998; Lim *et al*., 1997; Yoshioka *et al.,* 1995) and prevents the hunger sensation (Reinbach *et al.,* 2010; Westerterp-Plantenga *et al.,* 2005; Yoshioka *et al.,* 2004; Yoshioka *et al*., 2001; Yoshioka *et al.,* 1999). However, there have been conflicting reports on other outcomes (Smeet *et al.,* 2009; Snitker *et al.,* 2009). The rational reason might be the level of the maximum capsaicin consumption dose (CCD), which could be necessary to have a suppressive effect on the energy balance (Yoshioka *et al.,* 2004).

Although capsaicin has anti-obesity affects through the regulating of the energy metabolism, the potential side effects can limit its application. During the process of the previous reviewing experiments, parts of the test subjects dropped the test during the study due to the intolerance of capsaicin irritation (~ 20 mg capsaicin/meal) (Ludy *et al.,* 2011). A decrease in the liking of food as well as the heat and burning sensations in the mouth cavity could be felt at a capsaicin dose of 1-10 mg (Yoshioka *et al.,* 2004). However, it is a higher dose of capsaicin (10-30 mg) that is effective for the prevention and treatment of obesity (Chaiyata *et al.,* 2003; Yoshioka *et al.,* 2001; Yoshioka *et al*., 1998; Lim *et al.,* 1997; Yoshioka *et al.,* 1995; Yoshioka *et al.,* 2004; Youshioka *et al.,* 1999; Youshioka *et al*., 1998; Lim *et al*, 1997; Yoshioka *et al.,* 1995). It suggests that the incorporation of an ample amount of capsaicin to achieve the anti-obesity functions may yield a product that is too spicy for most of the consumers. Thus the products that can deliver capsaicin in a controlled manner are expected.

2.2 Food Controlled-delivery System

The edible delivery system is the food matrices that possess the capabilities of encapsulating, protecting and releasing the sensitive bioactive components (McClement *et al.,* 2007). After food ingestion, some of the sensitive functional food ingredients are easy to degrade (e.g. oil-soluble vitamins) while some others will irritate the digestive tract (e.g. capsaicin) (Matalanis *et al.,* 2011). An edible controlled-delivery system is required to protect these materials from being digested before exerting their functions and release them in the specific locations. A well-designed controlled-delivery system in food field should have several attributes. For example, the food controlled-delivery system should be compatible with the food matrix. The process of food controlled-delivery system needs to be easy to implement with reasonable cost (Uz & Altinkaya, 2011; Gemili *et al.,* 2010; Karim, 1986; Jipa *et al.,* 2012; Mastromatteo *et al*., 2009; McClements & Li, 2010).

The controlled-delivery systems have been studied and widely used in pharmaceutical and food applications (Göning, 1997; Mulbacher *et al.,* 2001; Waterman *et al.,* 2011). The different strategies for controlled-release of the drugs and the food materials have been reported (Ferrero *et al.,* 2010; Chang & Himmelstein, 1990; Huang & Brazel, 2001; Atyabi *et al.,* 1996; Yang & Fassihi, 1997).

A controlled-delivery system fabricated by creating a physical barrier on the surface of the products is reported in food packaging industries (Han & Floros, 1998; Foros *et al.,* 2000). The primary function of this controlled-delivery system is to prevent the contaminations from microorganisms. One of the most efficient systems is built using multilayer film. The film is comprised of three layers and it structure is presented in Figure 2.3. The outer barrier layer of the film is created to prevent the

loss of active ingredient. The middle matrix layer contains a high concentration of the active substance that can be diffused rapidly. The inner layer is the most important one because it is directly in contact with the product and should possess the controlled-release characteristic (Han *et al.,* 1998).

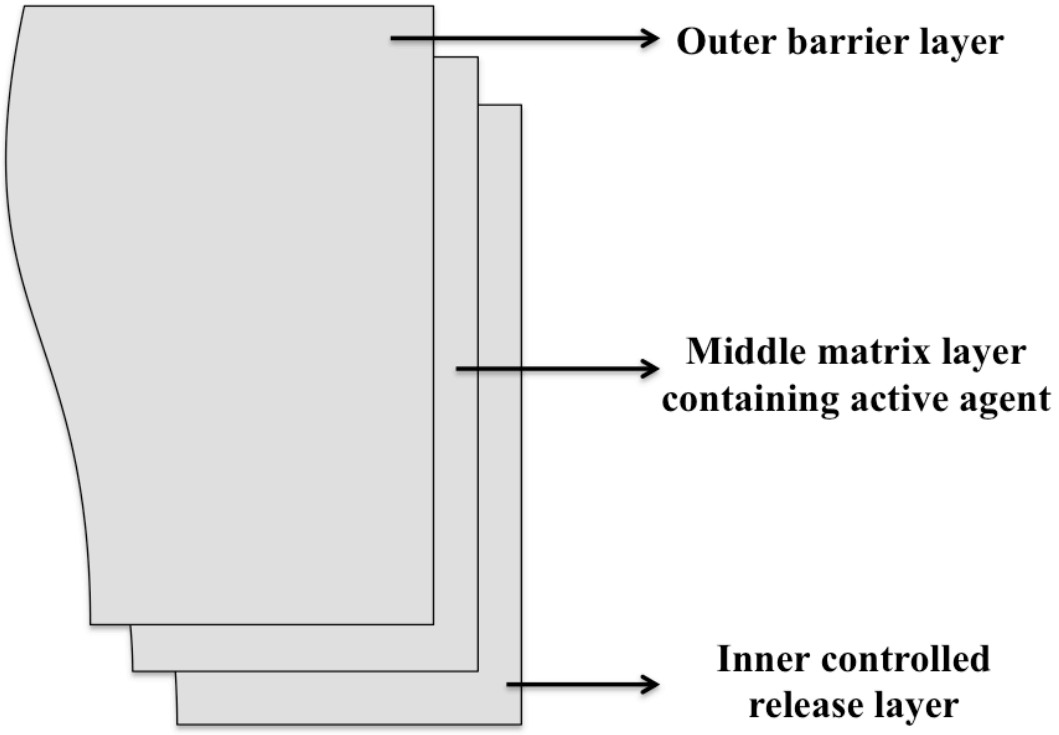


Figure 2.3 Structure of multilayer film

Base on the previous studies, some researches propose an alternative structure of the multilayer film. The alternative structure is comprised of two external control layers and an inner layer containing the active substance (Buonocore *et al.,* 2005; Stroescu *et al.,* 2013; Uz & Altinkaya, 2011; Mastromatteo *et al.,* 2009). The results in both delivery models exhibit that the multilayer film can be used as the food coating materials to achieve the controlled-release properties of the functional materials. Furthermore, the release profile of active compounds is influenced by the films composition and can be restrained by modifying the structure of the outer

barrier layers (Buonocore *et al.,* 2005; Mastromatteo *et al.,* 2009). From this perspective, the multilayer delivery system may be improved and utilized in the controlled-delivery of the functional ingredients (e.g. capsaicin) within digestive tract. The structure of the multilayer film can be employed in the design of the LN. The bioactive compound, i.e. capsaicin, is incorporated in the middle layers of the LN. Two sandwiching layers are made to protect the capsaicin, ergo, to achieve the controlled-release function. The structures of the sandwiching layers are enhanced by using the selected gastro-resistant materials, in order to avoid capsaicin from being released early.

2.3 Gastro-resistant Materials

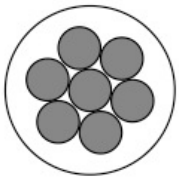
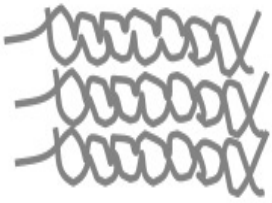
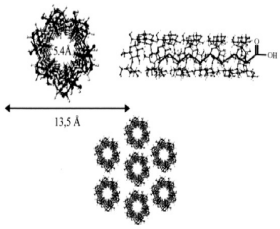
The gastro-resistant materials used for the preparation of the noodle sandwiching layers should possess the strong network that enable the noodles to retain capsaicin for longer period of time. It should also have the slow digestion capability in order to exert its controlled-delivery properties.

2.3.1 Resistant Starch (RS)

(a) Backgrounds

Resistant starch is a type of starch that is not absorbed in the small intestine but partially or completely fermented in the large intestine (Champ *et al*., 2003; Higgins, 2004). As RS delivers the digestive healthy benefits similar like dietary fiber, some of the recent literatures claim that RS can be considered as the third type of dietary fiber (Euentes-Zaragoze, *et al.,* 2010). RS is categorized into five types (RS1, RS2, RS3, RS4 and RS5) and listed in Table 2.3.

Table 2.3: The classification of resistant starch (RS)

Types of RS	Definition	e.g
RS1 	Physically inaccessible starch	coarsely milled grains, seeds and legumes, like cracked wheat, farina, semolina, red beans, pinto beans, and white beans.
RS2 	Granular starch, particularly resistant to digestion	starch granules from raw potato, unripe or green banana, and high-amylose corn, wheat, barley or rice
RS3 	Nongranular, retrograded amylose	exists in cooked and cooled potato, products based on tapioca starch, normal corn starch, and high-amylose corn starch are commercially available
RS4 	Chemically modified starch, may or may not be fermented in the colon	cross-linked starch and hydroxyproylated starch
RS5 	Exhibits resistance to amylolysis, proposed by Brown *et al.* (2006)	exists as amylose-lipid complex

In this research, a type of RS4 named Fibersym® is introduced. Due to the cross-linking modified nature of Fibersym®, the products incorporated with Fibersym® possess the properties of resistance to enzyme digestion and low glucose response (Al-Tamimi, 2007).

(b) Fibersym®

Fibersym® is one of the RS4 industry products, which is produced by cross-linking the wheat starch with phosphating agents. The manufacture process of Fibersym® is illustrated in Figure 2.4. Firstly the slurry of wheat starch is treated with sodium trimetaphosphate and sodium tripolyphosphate under the alkaline conditions (pH=11) at mildly elevated temperatures (44−47°C). The slurry is then adjusted to approximately pH 6.0 and washed. The washed slurry is dried to produce Fibersym® (Seib & Woo, 1999). This process leads to the phosphorylation of wheat starch, thus formed the cross-linking bonds within and between individual starch molecules.