บทที่ 7

บรรณานุกรม

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บทที่ 8
ต้นฉบับงานวิจัยเพื่อตีพิมพ์ในวารสารต่างประเทศ

1. การทดสอบฤทธิ์ของซิริซินในการป้องกันมะเร็งลำไส้ใหญ่และทวารหนัก

Sericin Consumption Suppresses Development and Progression of Colon Tumorigenesis in 1,2-Dimethylhydrazine–Treated Rats

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Abstract

Colon cancer is one of the most common cancers in many countries. Dietary factor is a major risk for developing this type of cancer, thus dietary interventions could perhaps prevent the diseases. This study was conducted to examine the chemopreventive effect of sericin against 1,2-dimethylhydrazine (DMH)-induced colon tumorigenesis in rats. Rats were fed with 4% sericin or 4% casein diet for 20 weeks, and given 20 mg/kg DMH subcutaneously injection once a week for the initial ten weeks. Supplementation of sericin did not affect the incidence rate of aberrant crypt foci (ACF) formation, but effectively suppressed tumor development compared to casein supplement. Consumption of sericin prior to carcinogen exposure appeared to reduce the number and multiplicity of ACF indicating its ability to suppress the initiation and promotion stages of tumorigenesis. Sericin supplement after being exposed to DMH could also reduce the development of ACF, while crypt multiplicity was not affected. Although, it's not statistically significance, the level of colonic lipid peroxidation in rats fed sericin diet showed a tendency to be lower than that of casein diet. From Ki67 immunohistochemistry and colonic morphological analysis, sericin seemed to delay tumorigenesis process since colonic crypts of sericin fed rats were mostly in the hyperplastic stage whereas those of casein fed rats showed abnormal and dysplastic morphology indicating more advanced stage of tumorigenesis. This finding suggests that consumption of sericin could reduce the risk of developing colon tumor by suppressing the initiation and progression of tumorigenesis.

Key words: colon cancer, sericin, 1,2-dimethylhydrazine, aberrant crypt foci

1. Introduction

Colon cancer is one of the most common cancers affecting people worldwide. The incidence is high in high income countries, but it is now increasing in middle- and low income countries (Labianca et al. 2010).The incidence rate of colon cancer in Thailand is low when compared with other countries and the highest incidence is seen in Bangkok (Khuhaprema and Srivatanakul 2008). This rate is expected to be rapidly increased in the next decade probably due to the acquisition of Western lifestyle. Western style diet (high levels of fat and red meat, and low dietary fiber) is the major risk factor of colorectal cancer (Rogers et al. 1993; van Breda et al. 2007). Since diet is definitely important for colon cancer development, dietary interventions are received much attention as one of the approaches to prevent this type of cancer (Rogers et al. 1993; DeMarini 1998; Johnson 2004).

Several experimental studies have proposed that the protective effects of diets rich in fruits and vegetables against colon carcinogenesis are thought to be due to their content of anti-oxidant vitamins and fibers (Wogan 1985; Levi et al. 2001; Campos et al. 2005). Many types of dietary proteins such as wheat bran, soy bean, buckwheat, and whey proteins were also found to reduce the development of colon cancer in animals (Compher et al. 1999; Takahashi et al. 1999; Hakkak et al. 2001a; Liu et al. 2001; McIntosh and Le Leu 2001; Belobrajdic et al. 2003; Badger et al. 2005). Most studies have been focused on proteins isolated from regular diets. The present study was aimed to investigate sericin, an eatable protein isolated from silk cocoon.

Sericin is a glue protein that secreted from the middle part of silk gland of silkworm, *Bombyx mori* (Gamo et al. 1977; Grzelak 1995; Kundu et al. 2008). Its molecular weight is ranging from 20 to 400 kDa with high content of serine, glycine and aspartic acid (Tokutake 1980; Zhang 2002). Many pharmacological activities of sericin including anti-oxidation (Kato et al. 1998; Dash et al. 2008), tyrosinase inhibition (Kato et al. 1998), anti-coagulation (Tamada et al. 2004), chemoprevention (Zhaorigetu et al. 2001; Zhaorigetu et al. 2003a; Zhaorigetu et al. 2003b; Zhaorigetu et al. 2007), and liver

and gastric protection (Li et al. 2008) were reported. Regarding to its chemopreventive effect, sericin consumption significantly suppress colon tumorigenesis in 1,2-dimethylhydrazine (DMH)-treated animals (Zhaorigetu et al. 2001; Zhaorigetu et al. 2007). The mechanisms of its chemoprevention are associated with the ability to reduce colonic oxidative stress and cell proliferation (Zhaorigetu et al. 2001; Zhaorigetu et al. 2007). It should be noted that protein preparation of sericin is generally heterogeneous consisting of various sizes of polypeptides depending on separation techniques. Due to sericin's heterogeneity caused by the degumming procedure, different sericin preparations may differently display their pharmacological activities. Small sericin (5-100 kDa) was found to promote mammalian cell proliferation better than large sericin (50-200 kDa) (Terada et al. 2005). Thus, the present study was aimed to test the chemopreventive effect against colon tumorigenesis of sericin isolated by xxxx techniques developing by Institution of Agricultural Technology, Suranaree University of Technology, Thailand. The molecular weight of sericin used in this study was 76-132 kDa.

Ki67, a cellular marker of cell proliferation, was also investigated to determine the potential mechanism of action of sericin.

2. Materials and methods

Animals and Chemicals

Male Spargue-Dawley rats (120-160 g) were purchased from the National Laboratory Animal Centre, Mahidol University, Thailand. All experimental procedures were approved by the Animal Research Ethics Committee, Naresuan University, Thailand. The animals were housed in stainless steel cages at temperature of 24 ± 2 °C with a 12 hours light-dark cycle. All rats were fed the assigned experimental diet and water ad libitum. DMH, methylene blue and EDTA were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade. Sericin was isolated by Institution of Agricultural Technology, Suanaree University of Technology, Nakhon Ratchasima, Thailand.

Experimental protocol

After 1 week acclimatization period, the rats were divided into 5 groups (n = 6/group). The experimental diets were prepared by mixing commercial diet with casein or sericin at the level of 4%. The experimental design is shown in Fig. 1. Group 1 and 3 were fed casein diet whereas group 2 and 4 were fed sericin diet. The last group (post-sericin) was received casein diet for the first 5 weeks and then fed sericin diet until the end of experiment. Group 3, 4 and 5 were subcutaneously injected with DMH (20 mg/kg bodyweight) once a week for 10 weeks. Group 1 and 2 were control groups injected with 1 mM EDTA pH 6.5 used to dissolve DMH for the same period of time. Food consumption and body weight were recorded weekly throughout the experimental period. Ten weeks after the last DMH injection, all rats were sacrificed and colons were removed and flushed with cold phosphate buffer saline (pH 7.4). The colon was divided into three equal parts, proximal, middle and distal. Each part was then cut into three segments for evaluation of aberrant crypt foci (ACF), lipid peroxidation and Ki67 immunohistochemistry.

Determination of aberrant crypt foci (ACF)

The colons were flushed with cold PBS, opened along the longitudinal median axis and fixed flat between two pieces of filter paper in 10% neutral formalin buffer for 6 hours. The colonic tissues were stained with 0.2% methylene blue in PBS for 5 minutes. The colons were placed on microscopic slides and observed ACF formation under a microscope. (Bird and Good 2000). Aberrant crypts were distinguished from the surrounding normal crypts by their increased size and had thicker epithelial lining compare to normal crypts. They usually gathered into a focus, consisting of one or more aberrant crypts (AC). (Bird 1987; McLellan and Bird 1988) The number of ACF in each part of colon was recorded. Crypt multiplicity was analyzed by determining the number of crypts in each focus and was categorized as 1, 2, 3, 4 and 5 or more aberrant crypts per focus or AC/ACF.

Lipid peroxidation of colon tissue

Lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances (TBARs) assay. Briefly, colon was homogenized with ice-cold PBS pH 7.4 containing 1 mM phenylmethanesulfonylfluoride (PMSF), incubated with TBARs reagent (40% TCA: 1.4% TBA: 8% HCl) and heated at 90°C for 1 hour. After, centrifugation, the fluorescent intensity was measured (Ex 535 nm, Em 595 nm). The protein content of colon homogenate was determined using BCA protein assay kit (Pierce).

Immunohistochemical analysis of Ki67

After fixing colon segments in 10% neutral formalin buffer for 48 hours, they were cut into serial strips, processed for paraffin-embedding and cut into 3 μm-thick sections. The expression of Ki67 was detected by immunohistochemistry using avidin-biotin complex method (Vectastain Elite ABC kit, Vector Laboratories, USA). The procedure was performed as the manufacturer recommendation. Colon sections were deparaffinzed with xylene, dehydrated through a graded ethanol series. Hydrogen peroxide at 0.5% was administered to the slides for 10 minutes. Antigen retrieval was accomplished by

heating the sections in 10 mM citrate buffer pH 6.0 for 10 minutes. The slides were then incubated with the rabbit monoclonal antibody Ki67 (Abcam, Cambridge, UK) for 1 hour. Biotinylated secondary antibody was applied for 30 minutes, followed by incubation with DAB peroxidase substrate, counterstained with hematoxylin.

The numbers of Ki67 positive cells in the individual crypt were recorded. In each group, the average number of Ki67 positive cells per crypt was calculated from the total 60 crypts (10 crypts /rat).

Statistical analysis

All data are expressed as means ± standard deviation (SD). The data were analyzed by one-way analysis of variance (ANOVA) and student t-test. Differences were considered to be significance when p value ≤ 0.05.

3. Results

Body weight and food consumption

Initial and final body weights of all groups were not significantly different (Table 2). The initial diet intake of group 4 was unexpectedly lower than the others, probably because rats in this group were slightly smaller than the rest. Food consumption at the end of the experiment significantly increased in DMH-treated groups (group 3-5) compared to control groups (group 1 and 2). Type of protein did not affect body weight and food consumption.

Incidences of ACF and tumor development

Table 3 summarizes the incidence of ACF which is considered as the early sign of colon tumorigenesis and tumor formation. There was no ACF and tumors found in the colon of control rat in group 1 and 2. All DMH-treated rats showed the incidence of ACF development. Two rats in casein fed group appeared to have tumors in their colons, whereas none of rats in sericin diet groups showed sign of tumor. This observation suggests that consumption of sericin containing diet did not completely block the DMH-induced ACF formation, but effectively suppressed tumor development. The density of ACF in casein diet (group 3) was higher than that of sericin diet (group 4 and 5), although it was not statistically different. It should be noted that two of six rats in casein diet group developed tumors in which the number of ACF reported in the result was likely to be underestimated due to the interference from tumorigenesis. Thus, sericin diet appears to suppress or decelerate colon tumorigenesis process whether or not it is intake before or after the exposure of carcinogen.

ACF distribution and multiplicity

ACF found in DMH-treated rats were mostly localized at the distal part of colon (Table 4). The density of ACF in the middle and distal colon of sericin fed rats was lower than that of casein fed rats, although it was not statistically significant. Distribution of ACF in the proximal colon was observed only

in casein diet group. This observation suggests that sericin diet could retard the expanding of ACF formation referring to its ability to delay the progression of colon cancer pathogenesis.

Crypt multiplicity was also analyzed and represent as number of aberrant crypt (AC) per ACF (Table 4). Multiplicity of ACF was analyzed by dividing into five categories depending on the number of AC/ACF (1, 2, 3, 4 and ≥ 5 AC/ACF). Distal colon showed the highest multiplicity, approximately 3 AC/ACF, indicating larger size of ACF compared to ACF in other parts of colon. The multiplicity of the average number of AC/ACF in the distal part of colon was not affected by type of protein supplement. The degree of multiplicity of ACF in distal colon was further analyzed since most ACF were distributed in this segment. The density of ACF containing ≤ 4 AC was not different among three groups (table 4). In both sericin fed groups, the densities of large ACF containing ≥ 5 AC (28.79±15.79 and 21.43±9.03) were lower than that of casein diet group (37.66±10.12). Because of high variation in the actual size among large ACF (≥ 5 AC/ACF), area analysis of individual ACF was performed. Average size of large ACF in casein diet was 0.14±0.06 mm^2 and it was smaller (0.11±0.05 mm^2) in rats fed sericin except, rats in post-sericin group. Taken all data together, sericin consumption prior to carcinogen exposure appears to reduce ACF density and crypt multiplicity suggesting the ability to suppress both ACF development and progression. Sericin could only suppress ACF formation when it was consumed after carcinogen administration.

Lipid peroxidation of distal colon

Lipid peroxidation of the colon was measured by using TBARs assay as showed in table 5. The production of malondialdehyde (MDA) was referred to the intensity of lipid peroxidation reaction. Since sericin diet exhibited beneficial effect in the distal part of rat colon, lipid peroxidation in this segment was firstly determined. Although the lipid peroxidation products in distal colon of sericin diet groups seemed to be lower than those of casein diets (group 1 vs 2 and group 3 vs 4, 5), no significant difference was observed between the two protein types because of high variation among individual rat.

Immunohistochemical and morphological analysis

The marker of cell proliferation, Ki67 was determined in this study. Our finding showed no difference in the average number of Ki67 positive cells presenting in each colonic crypt among groups 1-3 (table 6). Unexpectedly the number of Ki67 positive cells in group 4 and 5 were higher than the first three groups. Compared to DMH-treated rat fed casein diet (group 3), the length of colonic crypt in DMH-treated rat fed sericin diet (group 4 and 5) were higher. In addition, Ki67 staining cells of the last two groups were mostly distributed in the top zone of the crypt. Taken all data together, sericin diet seemed to promote colonic cell proliferation. However, we observed dramatically changes in morphology of colonic crypt of group 3 rats such as abnormal mucosal lining and dysplastic crypts (figure 1C, D). In sericin fed groups, the appearance of colonic crypts was similar to normal but longer in length and hyperplastic morphology was observed (figure 1E, F).

4. Discussions

The incidence of colon cancer is rapidly rising in many areas where Western lifestyle especially type of diets is being immersed into their eating habits. Thus, dietary interventions seem to have a great influence on preventing this kind of cancer. Many animal studies indicated that supplement with dietary proteins including wheat bran, soy bean, buckwheat, and whey proteins could protect against the development of colon cancer (Compher et al. 1999; Takahashi et al. 1999; Hakkak et al. 2001b, 2001a; Liu et al. 2001; McIntosh and Le Leu 2001; Belobrajdic et al. 2003; Badger et al. 2005). Sericin is one of eatable protein that has received of much attention since it was demonstrated to be beneficial for human health in many aspects. The present study was therefore interested to evaluate the chemopreventive effect of sericin against DMH-induced colon cancer in rats.

Sericin is a dietary fiber-like protein with very low digestibility (Sasaki et al. 2000) . Undigested sericin was found to play a role in the mechanism of its chemopreventive effect against colon tumorigenesis (Zhaorigetu et al. 2007). Sericin is a complex mixture of polypeptides differing in size, chemical and structural properties (Gamo et al. 1977). Degumming processes generally affect certain properties of sericin preparations (Freddi et al. 2003; Kurioka et al. 2004), and subsequently could showed to be affect their pharmacological activities, for example 5-100 kDa sericin was superior to 50-200 kDa sericin in accelerating cell proliferation in culture (Terada et al. 2005). Therefore, this study was conducted to test sericin (MW 76-132 kDa) prepared by degumming process developed by Suranaree University of Technology. Our result indicate that sericin diet showed could suppress the development and delay progression of tumorigenesis whether or not sericin is consumed before or after the exposure of DMH carcinogen.

DMH, an alkylating agent, is metabolized in the liver by conjugating with glucuronic acid. This glucuronide is excreted in the bile and then enters to the gut where microbial beta-glucuronidase releases the active metabolite to mediate its carcinogenesis activities on the mucosa (Reddy et al. 1974;

Fiala 1975; Emerich 1977; Chipman 1982). Thus, DMH seems to be highly specific to initiate colon tumorigenesis. In this study, DMH administration effectively induced the development of ACF with 100% incidence rate. Food consumption in DMH-treated rats was higher than control rats, possible due to the reduction in nutrient absorbability. Consistent with previous studies (Zhaorigetu et al. 2001; Zhaorigetu et al. 2007), this study indicated that sericin diet suppressed the development of ACF, an early marker of colon tumorigenesis. Sericin did not provide statistically significant reduction in the density of ACF in the present study, probably because of under estimation of the number of ACF in casein diet group due to tumor development. There was no sign of tumor developed after sericin supplement indicating less tumorigenesis progression than casein supplement.

According to previous reports, the increased number of ACF seemed to reflect the initiation step of colon carcinogenesis, whereas the increase in number of aberrant crypts (AC) per ACF or crypt multiplicity might correspond to the progression or promotion step of the disease (Zhang et al. 1992; Magnuson et al. 1993; Fenoglio-Preiser and Noffsinger 1999) Consumption of sericin prior to the exposure of carcinogen appeared to suppress both initiation and promotion stages. Even thought sericin diet was consumed after receiving carcinogen, it could still reduce the number of ACF formation. Post-sericin might somehow affect the progression of colon tumorigenesis despite crypt multiplicity is not obviously reduced but tumor development was not occurred in this group of rat.

Consumption of sericin could reduce cell proliferation possibly by suppressing *c-myc* and *c-fos* proliferation-related genes (Zhaorigetu et al. 2001). Sericin diet also suppressed oxidative stress in the colon mucosa that might at least in part lead to the reduction of cell proliferation (Zhaorigetu et al. 2001). The reduction of colon oxidative stress by sericin supplement in the present study was not clearly demonstrated as the previous report. The Ki67, a nuclear protein associated with cell proliferation (Pollack et al. 2004), was determined in this study. In normal colonic mucosa, the proliferating cells is

localized to the basal and lower one third of the crypt, then the cells migrate from the base of the crypts upwards towards the luminal surface, where they are sloughed off (Ishida et al. 2004).

Ki67 is a biomarker of proliferation presenting in all phases of cell cycle except for G0 phase. Ki67 positive cells were stained along whole crypts up to the mucosal surface. Unexpectedly, sericin diet in DMH-treated rats appeared to increase the number of Ki67 positive cells and the length of colonic crypts indicating its cell proliferation promoting properties. Colonic crypts from these rats were also appeared in hyperplastic morphology. In tumorigenesis process, ACF show variable histological features, ranging from hyperplasia to dysplasia (Cheng and Lai 2003). Colonic crypts of casein diet DMH-treated rats were histologically heterogeneous, hyperplastic and dysplastic crypts were observed together with abnormal mucosal lining indicating more advanced stage of tumorgenesis than sericin diet groups. Therefore, sericin consumption might not directly promote colonic cell proliferation but instead delay the progression of colon tumorigenesis.

High content of arginine and glycine in buckweath protein was suggested to be responsible for its chemopreventive effect against colon tumorigenesis (Liu et al. 2001). Supplementation of glycine was also found to inhibit the growth of B16 melanoma tumors in mice (Rose et al. 1999). Sericin protein contains higher concentration of glycine (13.5% w/w) compared to casein (1.6% w/w) (Morita et al. 1997). It is possible that glycine component is involved in the protective effect of sericin against colon tumorigenesis but further study is necessary before coming to this conclusion.

In conclusion, the chemopreventive effect of sericin consumption on ACF development occurred in both initiation and promotion stages. Sericin diet could suppress or decelerate colon tumorigenesis process whether or not it is intake before or after the exposure of carcinogen. Thus, sericin is one of the interesting food supplements exhibiting various beneficial effects for human health, particularly its preventive effect to colon tumorigenesis.

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Table 1

Amino acid composition of the sericin

Amino acid	% w/w
Serine	33.40
Aspartate	16.70
Glutamate	4.40
Glycine	13.50
Threonine	9.70
Lysine	3.30
Tyrosine	2.60
Arginine	3.10
Alanine	6.00
Valine	2.80
Histidine	1.30
Luecine	1.10
Isoluecine	0.70
Phenylalanine	0.50
Trytophan	0.20
Proline	0.70
Cystine	0.20
Methionine	0.04

Table 2

Body weight and food consumption

Group	Treatment groups	Initial body weight (g)	Final body weight (g)	Body-weight gain (g)	Initial food consumption (g/rat/day)	Final food consumption (g/rat/day)
1	Casein	237.38 ± 11.61	502.75 ± 28.25	265.37 ± 16.64	10.73 ± 2.76	18.21 ± 0.90
2	Sericin	234.67 ± 30.53	488.83 ± 40.81	254.16 ± 10.28	12.52 ± 2.40	19.37 ± 2.70
3	Casein + DMH	228.54 ± 37.24	498.08 ± 29.73	269.54 ± 7.54	11.84 ± 2.70	22.38 ± 2.70*
4	Sericin + DMH	206.04 ± 25.24	483.38 ± 24.84	277.34 ± 0.40	8.41 ± 3.00#	21.89 ± 2.70*#
5	Post-sericin + DMH	229.00 ± 35.06	486.83 ± 48.42	257.83 ± 13.36	12.37 ± 2.80	21.21 ± 1.00*

Values are mean ± SD of 6 rats

* Significantly different from casein diet by Student's *t*-test ($p < 0.05$)

Significantly different from sericin diet by Student's *t*-test ($p < 0.05$)

Table 3

Incidences of aberrant crypt foci (ACF) and tumor formation in rat colon

Group	Treatment groups	No. of rats	ACF and tumor formation		
			Tumor Incidence (%)	ACF Incidence (%)	Number of ACF/cm^2/rat
1	Casein	6	0	0	0
2	Sericin	6	0	0	0
3	Casein + DMH	6	2 (33)	6 (100)	116.01 ± 48.52
4	Sericin + DMH	6	0	6 (100)	79.96 ± 29.04
5	Post-sericin + DMH	6	0	6 (100)	82.35 ± 39.83

Values are mean ± SD

Table 4

Distribution and multiplicity of aberrant crypt foci (ACF) in rat colon

	Treatment groups		
	DMH	Sericin + DMH	Post-sericin + DMH
ACF distribution (No. ACF/cm^2)			
Proximal colon	3.90 ± 5.30	0	0
Middle colon	37.99 ± 27.50	21.00 ± 18.47	28.35 ± 29.78
Distal colon	73.38 ± 27.02	57.79 ± 26.38	53.46 ± 22.78
Average ACF multiplicity (AC/ACF)			
Proximal colon	0.44 ± 0.44	0	0
Middle colon	0.78 ± 0.17	0.67 ± 0.16	0.60 ± 0.13
Distal colon	3.57 ± 0.17	3.53 ± 0.57	3.25 ± 0.28
Type of ACF in distal colon (No. ACF/cm^2)			
1 AC/ACF	6.49 ± 6.27	6.06 ± 7.69	5.84 ± 6.96
2 AC/ACF	12.01 ± 8.70	12.34 ± 10.48	11.26 ± 7.19
3 AC/ACF	9.74 ± 6.49	7.58 ± 6.49	9.52 ± 7.47
4 AC/ACF	7.47 ± 7.14	3.68 ± 1.73	5.41 ± 4.89
≥ 5 AC/ACF	37.66 ± 10.12	28.79 ± 15.79	21.43 ± 9.03*
(Area of individual ACF, mm^2)	(0.14 ± 0.06)	(0.11 ± 0.05*)	(0.14 ± 0.06)

Values are mean ± SD

*Significantly different from casein diet by Student's *t*-test ($p < 0.05$)

Table 5

Lipid peroxidation of distal part of colon

Group	Treatment groups	MDA concentration (μM)
1	Casein	1.26 ± 0.51
2	Sericin	0.81 ± 0.21
3	Casein + DMH	1.77 ± 1.26
4	Sericin + DMH	1.08 ± 0.71
5	Post-sericin + DMH	1.15 ± 0.64

Values are mean ± SD

Table 6

Immunohistochemical analysis of Ki67 proliferation marker

	Treatment groups				
	Casein	Sericin	Casein +DMH	Sericin + DMH	Post-sericin + DMH
i67 positive cells					
Total number (cells/crypts)	15.78 ± 3.00	18.25 ± 6.97	17.08 ± 4.42	28.55 ± 10.32*†	29.82 ± 9.55$^{*\dagger\#}$
ength of crypt (μm)					
Total length (μm)	220.62 ± 34.90	228.64 ± 34.90	224.42 ± 19.55	237.30 ± 46.67	270.08 ± 13.83$^{*\dagger\#}$
Ki67 length (μm)	102.93 ± 18.08	107.61 ± 29.74	101.66 ± 16.14	147.83 ± 46.58	174.12 ± 27.45$^{*\dagger\#}$
Relative distance of Ki67	0.46 ± 0.05	0.47 ± 0.08	0.45 ± 0.05	0.62 ± 0.10$^{*\dagger\#}$	0.64 ± 0.10$^{*\dagger\#}$
Relative total number of Ki67	0.08 ± 0.02	0.08 ± 0.04	0.08 ± 0.02	0.12 ± 0.03$^{*\#}$	0.11 ± 0.04
)istribution of Ki67 positive cells					
Top zone	11.63 ± 2.30	14.05 ± 5.36	12.33 ± 3.39*	17.63 ± 3.22$^{*\dagger\#}$	17.67 ± 5.23*†
Middle zone	5.02 ± 2.17	4.52 ± 2.17	4.73 ± 2.30	9.70 ± 5.89	10.85 ± 4.45$^{*\dagger\#}$
Basal zone	0.07 ± 0.10	0.05 ± 0.12	0.03 ± 0.05	1.17 ± 1.79	1.30 ± 1.35†

Values are mean ± SD

Data were analyzed from 60 crypts (10 crypts/rat)

* Significantly different from casein diet by Student's *t*-test ($p < 0.05$)

† Significantly different from sericin diet by Student's *t*-test ($p < 0.05$)

$^{\#}$ Significantly different from casein+ DMH by Student's *t*-test ($p < 0.05$)

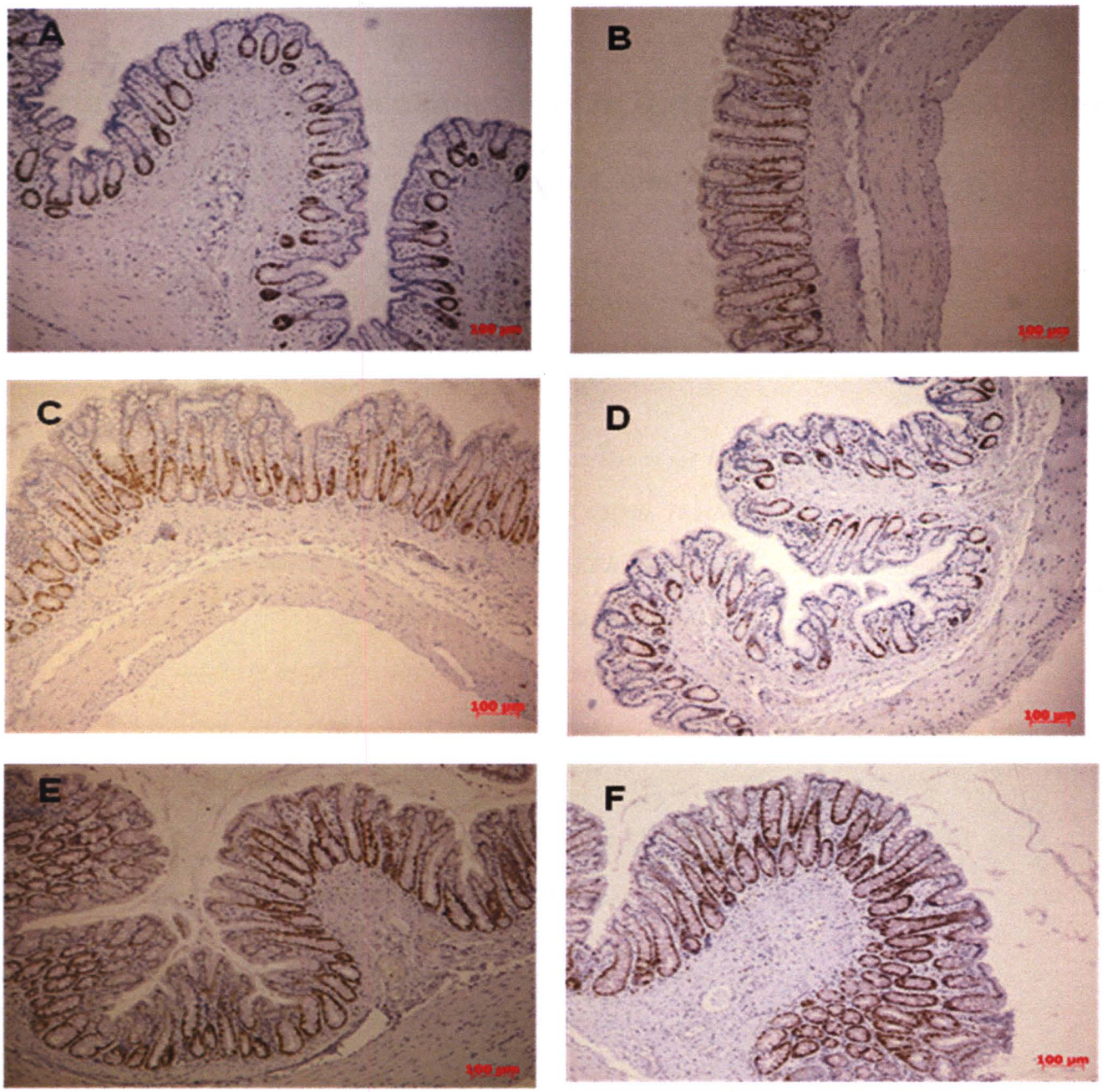

Figure 1. Morphology of colonic crypt from normal rats fed casein diet (A) and sericin diet (B) as well as DMH-treated rats fed with casein diet (C, D), sericin diet (E) and post sericin diet (F)

2. การทดสอบฤทธิ์ลดการดูดซึมโคเลสเตอรอลในเซลล์ลำไส้เพาะเลี้ยงและในสัตว์ทดลอง

Sericin Reduces Plasma Cholesterol in Rats and Cholesterol Uptake in Caco-2 Cells

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Keywords: hypocholesterolemic effect, sericin, cholesterol uptake, cholesterol, protein

ABSTRACT

Sericin is an eatable protein isolated from silk cocoon and its consumption has been suggested to be beneficial for health. Many dietary proteins have been shown to reduce serum lipids, but this lipid lowering effect of sericin has not been studied. The purpose of this study was to evaluate the hypocholesterolemic effect of sericin in rats and its potential mechanism of actions *in vitro* models. The hypocholesterolemic effect was tested in rats fed a high cholesterol diet for 14 days. Total cholesterol and non-high density lipoprotein (non-HDL) cholesterol were significantly reduced in rats fed high-cholesterol diet together with all three tested doses of sericin (10, 100 and 1000 mg/kg/day). HDL cholesterol and triglyceride levels were not affected by sericin supplement. The effect of sericin on cholesterol absorption was determined by measuring the uptake of radio-labeled cholesterol into differentiated Caco-2 cells. Low concentrations of sericin (25 and 50 μg/ml) inhibited cholesterol uptake about 30%, whereas higher concentrations showed no effect. Cholesterol micellar solubility was also reduced in the presence of sericin. This study is the first evidence suggesting the hypocholesterolemic of sericin is partially the result of its inhibitory activity on cholesterol absorption in intestinal cells.

Keywords: hypocholesterolemic effect, sericin, cholesterol uptake, cholesterol, protein

1. Introduction

There is growing interest not only in functional foods but also dietary supplements and nutraceutical products to improve human health, especially those that promote prevention of certain diseases. Since high cholesterol level increases risk of many cardiovascular diseases, the cholesterol lowering agents have become one of the major targets for prevention of those diseases. Several reports have indicated that various types of dietary proteins could affect serum cholesterol levels [1-5]. The most extensively studied proteins are soybean and milk proteins. Some other sources of proteins such as rice, buckwheat, potato and sunflower appeared to have cholesterol lowering effect [1,2,6,7]. It should be noted that most studies have been focused on proteins isolated from regular diets. The present study was aimed to investigate cholesterol lowering effect of sericin, an eatable protein found in silk cocoon.

Silk proteins produced by silkworm are composed of fibroin and sericin. Sericin contributes 25-30% of the total cocoon weight. It has high serine content (30-33%) and its role is to help the formation of a cocoon by gluing the fibroin together. Sericin is a mixture of macromolecule polypeptides, its molecular weight ranges widely from about 10 to over 300 kDa [8]. In general silk processing, sericin is removed and mostly discarded in the wastewater. In the past decade, there are attempts to discover its biological activities which then make it a valuable natural ingredient particularly in food and cosmetic purposes. Sericin's biological functions include anti-oxidation [9], tyrosinase inhibition [9], chemoprevention [10-12], and protection against alcohol-induced liver and gastric injuries [13], UV-induced keratinocyte apoptosis [14].

Since sericin is protease-resistant with low digestibility property [15], it was received much attention to be beneficial for the health of intestines. Consumption of sericin suppressed colon tumorigenesis in animals treated with 1,2-dimethylhydrazine-treated (DMH), a potent colon carcinogen [10,11]. Consumption of sericin could have more interesting undiscovered advantages, such as cholesterol lowering effect as above mentioned dietary proteins. Thus, the present study was carried out to obtain the hypocholesterolemic activity of sericin in rats fed high cholesterol diet and its potential mechanism of actions to inhibit cholesterol absorption in differentiated Caco-2 cells and cholesterol micellar solubility.

2. Materials and Methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM)/F12 and all the materials used in cell culture and cholesterol, phosphatidylcholine and sodium taurocholate were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum (FBS) was purchased from Gibco. [1α,2α(n)-^{3}H]cholesterol was purchased from PerkinElmer. Sericin was provided by the Institution of Agricultural Technology, Suanaree University of Technology, Nakhon Ratchasima, Thailand. The analysis of amino acid composition is showed in Table 1.

2.2. Animal experiment

Twenty five male Sprague-Dawley rats (200–250 g) were obtained from the National Laboratory Animal Centre, Mahidol University, Thailand. All animal experimental procedures were approved by the Animal Research Ethics Committee, Naresuan University, Thailand. Animals were housed in stainless steel cages at 22 ± 2 ^{o}C and 12 h light/dark cycle and had free access to water and food for 1 week of acclimatization before the beginning experiment. Rats were randomly divided into four groups consisting of 5 animals each. The animals were fed a standard diet with high-cholesterol supplement (cholesterol, bile extract, and coconut oil at 1.5, 0.75 and 0.75 g/kg body weight/day, respectively). The amount of cholesterol each animal received was approximately 2% of daily diet. The cholesterol mixtures were daily administered with and without sericin solutions by gastric tube for 14 days. Fasting blood was collected at day 0 (baseline), 4, 8, 11 and 14 from the tail vein to measure plasma lipid levels. During the experimental period, body weight and food intake were regularly recorded.

2.3. Measurement of plasma lipid levels

Plasma total cholesterol, triglyceride and HDL-cholesterol levels were measured with commercial enzymatic assay kits (HUMAN GmbH, Germany). The procedures were conducted as manufacturer recommendation. The levels of non-HDL cholesterol were calculated by subtracting HDL-cholesterol from total cholesterol.

2.4. Cell culture preparation

Caco-2 cells were obtained from the American Type Culture Collection (ATCC). Cells were grown in Dulbecco's modified Eagle's medium (DMEM)/F12 containing 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. Cells were maintained at 37 °C in CO_2 incubator in a saturated humidity atmosphere containing 95% air and 5% CO_2. All cells were propagated in culture flasks and subsequently plated in 24-well plates for cholesterol uptake experiment.

2.5. Cholesterol micelle preparation

Briefly, stock solutions of [1α,2α(n)-^{3}H]cholesterol, cholesterol, phosphatidylcholine were dissolved in chloroform. A stock solution of sodium taurocholate was prepared in methanol. The lipid and bile salt solutions were mixed and evaporated under a stream of N_2. The lipid film was stored under N_2 at -20 °C until use. The micelle solutions were freshly prepared by hydrating the lipid film in serum-free DMEM/F12 so that the final concentrations of the micelle were 1 μM cholesterol, 2 mM sodium taurocholate, 50 μM phosphatidylcholine, and 1 μCi/ml [1α,2α(n)-^{3}H]cholesterol. The micelle solutions were sonicated and passed through 0.2 μm syringe filters and kept at 37 °C before adding to the cells.

2.6. Cholesterol uptake assay

Caco-2 cells were seeded on 24-well plate at a cell density 50,000 cells/well and cultured for 14 days to allow them to differentiate. During this period cells were fed with fresh medium every 2 days. After 14 days, cells were incubated with serum-free medium overnight. Cells were treated with sericin or ezetimibe (positive control) for 1 h before adding [1α,2α(n)-^{3}H]cholesterol-micelles. After 3 h incubation, medium were removed and cells were washed twice with ice-cold PBS. The cells were disrupted with 0.2 N NaOH and 0.1 % Triton-X 100. One part of the aliquot was added to scintillation cocktail (MicroScint™-20; PerkinElmer), the other part was taken for protein determination by BCA protein assay kit (Pierce). The radioactivities of cell lysates were measured in a Packard β-counter.

2.7. Micellar cholesterol solubility assay

Cholesterol micelles were prepared with ultrasonication of a 7ml micellar dispersion containing 10 mM sodium taurocholate, 2 mM cholesterol, 5 mM oleic acid, 132 mM NaCl and 15 mM sodium phosphate (pH 7.4). Various concentration of sericin or BSA (500-2000μg/ml) were added to the mixed micelle solution before and after micelle preparation and incubated for 24 h at 37°C. The precipitated cholesterol was separated from the intermicellar cholesterol by filtering through a 0.22 μm. The intermicellar cholesterol in the filtrates were determined by cholesterol assay kit (Human).

2.8. Statistical analysis

All data from animal experiments are expressed as means ± standard deviation (SD). Means ± standard error of means (SEM) were used for the *in vitro* experiments. The data were analyzed by one-way analysis of variance (ANOVA). Differences were considered to be significance when p value ≤ 0.05.

3. Results

3.1. Lipid levels in rat plasma

The mean of total cholesterol levels of four groups are showed in Fig. 1. Before feeding high-cholesterol mixture, the baseline levels of plasma lipids were measured. Since the entire experimental period is 14 days in which the increasing age was expected not to influence the lipid profiles, therefore plasma lipid levels at day 0 (baseline) were used as control levels. Rats fed normal diet were not included in this study in order to minimize the number of animals. After cholesterol administration, plasma cholesterol level was continuously increased and a significant increase was observed after days 4 of feeding. Plasma cholesterol levels were significantly reduced in rats fed high-cholesterol mixtures with sericin. All tested doses of sericin, 10, 100 and 1000 μg/kg body weight/day, effectively reduced cholesterol levels after days 8 of feeding but dose-dependent pattern is not clearly established. This data suggest that sericin supplement could suppress plasma cholesterol level in rats given high-cholesterol diet.

The plasma levels of high density lipoprotein cholesterol (HDL-C), non-HDL cholesterols and triglyceride (TG) were showed in Fig. 2. The levels of plasma HDL cholesterol in all groups were not much different across 14 days of administrations, although there was slightly increase at days 4 in sericin fed groups. Non-HDL cholesterol (VLDL and LDL cholesterols) levels were appeared in the similar pattern to total cholesterol levels, but the dose-dependent effect of sericin to reduce these types of lipoproteins was apparently demonstrated at days 11 and 14. Plasma TG levels were also slightly increased from baseline in all groups over treatment period, and sericin showed no TG lowering effect (Fig. 2). Taken all data together, sericin supplement effectively reduce plasma levels of total cholesterol and non-HDL cholesterol in rats fed high-cholesterol diet.

3.2. Cholesterol uptake in differentiated Caco-2 cells

To examine the effect of the sericin on cholesterol absorption in the intestine, differentiated Caco-2 cells were used as an *in vitro* model. The level of [1α2α(n)-^{3}H] cholesterol captured in Caco-2 cells was measured and calculated as the amount of tritium cholesterol per mg

protein of cell lysates. In this study, ezetimibe was used as a positive control since it is a cholesterol absorption inhibitor by blocking cholesterol transporter protein. Bovine serum albumin (BSA) was used as a control protein. The results showed the significant decreases in uptake of cholesterol micelles into differentiated Caco-2 cells pre-treated with sericin at 25 and 50 μg/ml (Fig. 3). Unexpectedly, cholesterol uptake inhibitory activity was decreasing with increasing concentration of sericin. Sericin at 1000 μg/ml somehow increased the uptake of cholesterol. This pattern of cholesterol uptake was also observed with BSA, but BSA at 25 and 50 μg/ml did not significantly reduced cholesterol uptake whereas its higher concentrations (100-1000 μg/ml) slightly increased the uptake of cholesterol. This data suggest that sericin could either reduce or promote cholesterol uptake into intestinal cells depending on its concentrations.

3.3. Lipid micellar cholesterol solubility

The effect of sericin on the micellar solubility of cholesterol is shown in Table 3. The cholesterol solubility in mixed lipid micelles was significantly decreased in the presence of sericin particularly before and after micelle preparation. The inhibitory activity of sericin on cholesterol solubility was dose-dependent. BSA as control protein showed no reduction of cholesterol micellar solubility.

4. Discussion

To our knowledge, this is the first study showing that silk protein, sericin, lowers cholesterol levels in rats. Our data provide evidence that sericin significantly suppresses the plasma levels of total cholesterol and non-HDL cholesterol in high-cholesterol diet-induced hypercholesterolemia in rats. This cholesterol lowering effect is dose-dependent. Although its effect is significant, sericin could not bring cholesterol levels back to the baseline.

The present study choose to reports non-HDL cholesterol since calculated LDL-cholesterol is still controversial especially in rat serum. In human, serum LDL cholesterol can be calculated by Friedewald equation, [LDL-Cholesterol] = [Total-Cholesterol] - [HDL-Cholesterol] - [Triglyceride]/5 [16]. In rat, this equation however seemed not to be suitable for certain circumstances, particularly in hypercholesterolaemic rats [17]. Non-HDL cholesterol in the present study mainly represents LDL and partially VLDL cholesterols.

Because a strong positive correlation was observed between serum cholesterol concentration and dietary methionine concentration or methionine:glycine ratio [6], the low methionine content in dietary proteins was though to be involved in their cholesterol lowering effects [6,18]. Sericin is one of the protein that contains low methionine concentration (<0.05 mol %) [11,13]. The glycine content in sericin is about 200 times higher than methionine, which makes methionine:glycine ratio to 0.005 [11,13]. The amino acid composition of the sericin protein used in the present study is similar to previous reports [11,13]. Our sericin contains 0.04% w/w methionine and 13.5% w/w glycine which makes its ratio about 0.003. The decreases in apoprotein A-I and HDL secretion from rat livers were suggested to partially responsible for cholesterol lowering effect of low methionine protein diet [19,20]. In this study, the increases in HDL cholesterol were observed at days 4 of sericin administration, but this effect was not clear at longer periods of treatment. Although the exact mechanism to lower serum cholesterol level is not clearly identified, low-methionine content seems be involved in the hypocholesterolemic effect of some dietary proteins such as soybean, potato and rice [6]. It should be noted that the amount of methionine and

methionine:glycine ratio in sericin protein are far lower than those dietary proteins. Methionine and glycine contents in these dietary proteins are 1-2 and 3-4.5 % w/w, respectively [6,21].

Sericin is a dietary fiber-like protein with very low digestibility [15]. Undigested sericin was found to play a role in the mechanism of its chemopreventive effect against colon tumorigenesis [10]. According to its protease resistance property, undigested sericin might affect the absorption of some substances in the intestinal lumen. Sericin consumption was showed to enhance intestinal absorption of zinc, iron, magnesium and calcium in rats [22]. Disruption of cholesterol absorption of undigested sericin might responsible for its cholesterol lowering effect. However, the *in vivo* experiment did not allow determination the effect of sericin on cholesterol absorption. To investigate this mechanism of action, differentiated Caco-2 cell cultures were therefore used. Caco-2 cells at 2-3 weeks in culture were proliferated and differentiated to become the monolayer intestinal epithelium [23]. The present study demonstrated that sericin reduced cholesterol uptake into differentiated Caco-2 but surprisingly showed inverse dose-dependent activity. In cellular experiment, sericin protein was tested in the comparison with BSA. High concentration of both proteins seem to promote cholesterol uptake. The explanation for this observation have not been thoroughly investigated at this time, it at least give us the direction for further investigation.

There are evidences indicated that dietary proteins decreased cholesterol absorption in Caco-2 cell model [21,24]. Disruption of cholesterol solubility referring to incorporation of cholesterol into micelles is one of the mechanisms of plant phytosterols (Jesch 2006) and some proteins [2,24] to reduce cholesterol absorption in animal models. Alteration of cholesterol solubility in lipid micelles may affect the efficacy to micelles to be taken up by Caco-2 cells. Micellar cholesterol solubility *in vitro* was significantly lower in the presence of soy protein derivatives and possibly lead to cholesterol absorption inhibition in rats and in caco-2 cells [21]. Buckwheat protein was shown to reduce cholesterol solubility in lipid micelles only when it was added prior to micelle formation but this effect was not observed if buckwheat protein was

added after lipid micelle formation [24]. In the current study, cholesterol solubility in mixed lipid micelles was significantly reduced in the presence of increasing concentration of sericin either before and after micelle formation.

We have found for the first time that protein from silk cocoon, sericin, has cholesterol lowering effect which partially due to its inhibitory activity against cholesterol absorption silk cocoon. Although higher concentration of sericin unexpectedly exhibits lesser inhibitory activity against cholesterol absorption in cell culture models, this inverse correlation between dose and activity is not observed in animal experiment.

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Table 1
Amino acid composition of the sericin

Amino acid	% w/w
Serine	33.40
Aspartate	16.70
Glutamate	4.40
Glycine	13.50
Threonine	9.70
Lysine	3.30
Tyrosine	2.60
Arginine	3.10
Alanine	6.00
Valine	2.80
Histidine	1.30
Luecine	1.10
Isoluecine	0.70
Phenylalanine	0.50
Trytophan	0.20
Proline	0.70
Cystine	0.20
Methionine	0.04

Table 2

Changes of plasma lipid levels and amount of food intake after 14 days of treatment

Groups	Changes from day 0 (mg/dl)				Food intake (g/day/100g BW)
	Total cholesterol	HDL-C	Non-HDL-C	Triglyceride	
High cholesterol	97.45±31.52	-0.38±1.49	97.83±31.01	56.05±40.70	6.56±1.34
High cholesterol+ Sericin 10 mg/kg/day	41.06±7.59**	1.80±3.87	39.25±4.26**	30.71±29.00	6.18±0.29
High cholesterol+ Sericin 100 mg/kg/day	38.61±12.45**	2.64±4.80	35.97±16.84**	72.79±43.02	8.43±1.86
High cholesterol+ Sericin 1000 mg/kg/day	49.05±13.02*	-2.70±9.54	51.75±9.84*	97.87±16.61	7.52±0.51

Values are expressed as mean ± SD (n = 5)

Significantly different from high cholesterol group (* $p \leq 0.05$, ** $p \leq 0.01$)

Table 3.
Inhibition of cholesterol micellar solubility of sericin

Protein concentration (μg/ml)	Before micelle preparation Cholesterol solubility (%)		After micelle preparation Cholesterol solubility (%)	
	Sericin	BSA	Sericin	BSA
Control	94.46 ± 3.34	94.46 ± 3.34	83.24 ±12.02	83.24 ±12.02
0	92.11 ± 7.80	92.11 ± 7.80	85.01 ± 8.86	85.01 ± 8.86
500	76.72 ± 7.98*	87.96 ± 4.37	77.84 ± 4.14	85.46 ± 5.39
1000	71.78 ± 9.76*	85.92 ± 1.62	66.67 ± 3.45	84.65 ± 7.52
2000	71.30 ± 3.39*	87.36 ± 6.14	64.52 ± 1.70	86.31 ± 6.20

Values are expressed as mean ± SD (n = 3)
Significantly different from control (* $p \leq 0.05$)

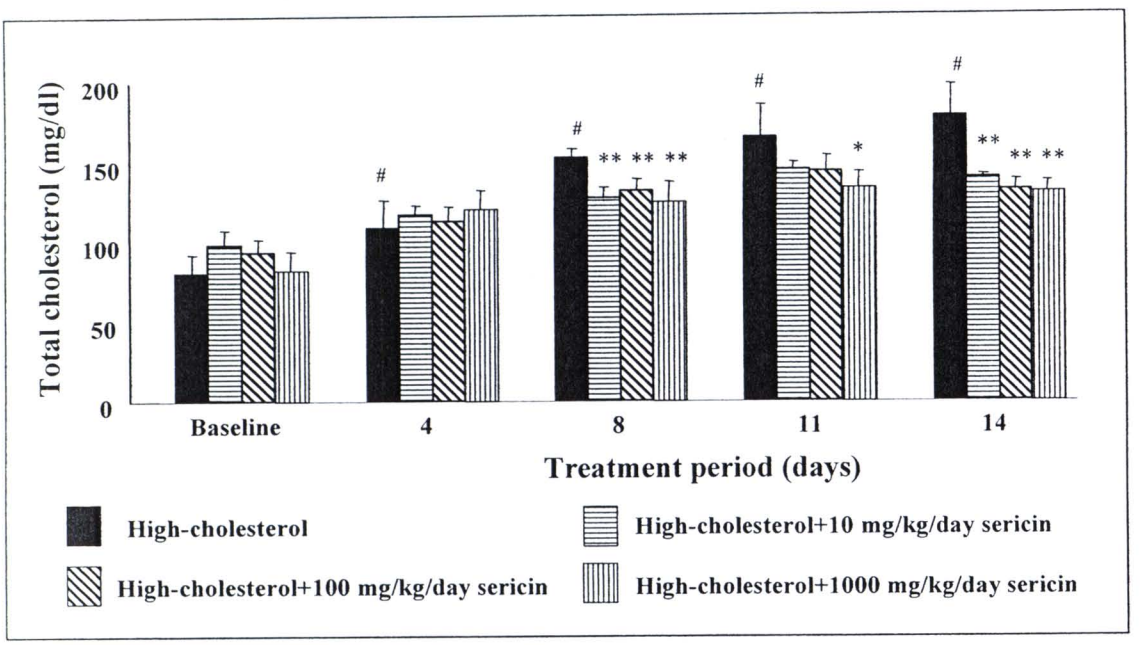

Fig. 1. Plasma total cholesterol in rats fed high-cholesterol diet. Rats were fed cholesterol (2% cholesterol of total daily diet) with and without sericin at 10, 100 and 1000 mg/kg for 14 days. The data represent mean ± SD. (# $p < 0.05$ compared to baseline; * $p < 0.05$, ** $p < 0.01$ compared to high-cholesterol, n =5)

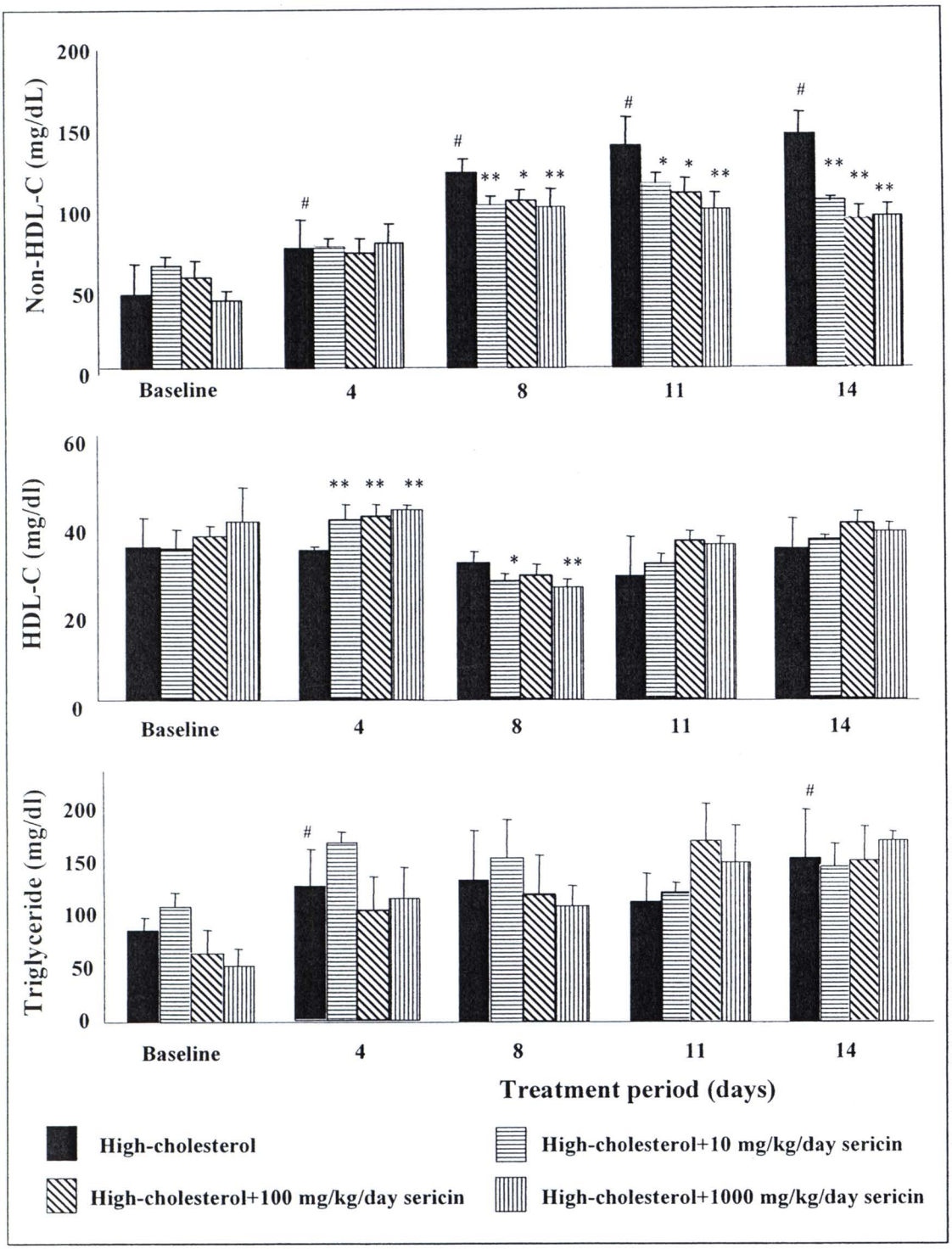

Fig. 2. Plasma HDL cholesterol (HDL-C), non-HDL cholesterol (non-HDL-C) and triglyceride in rats fed high-cholesterol diet. Rats were fed cholesterol (2% cholesterol of total daily diet) with and without sericin at 10, 100 and 1000 mg/kg for 14 days. The data represent mean ± SD. (# $p < 0.05$ compared to baseline; * $p < 0.05$, ** $p < 0.01$ compared to high-cholesterol, n =5)

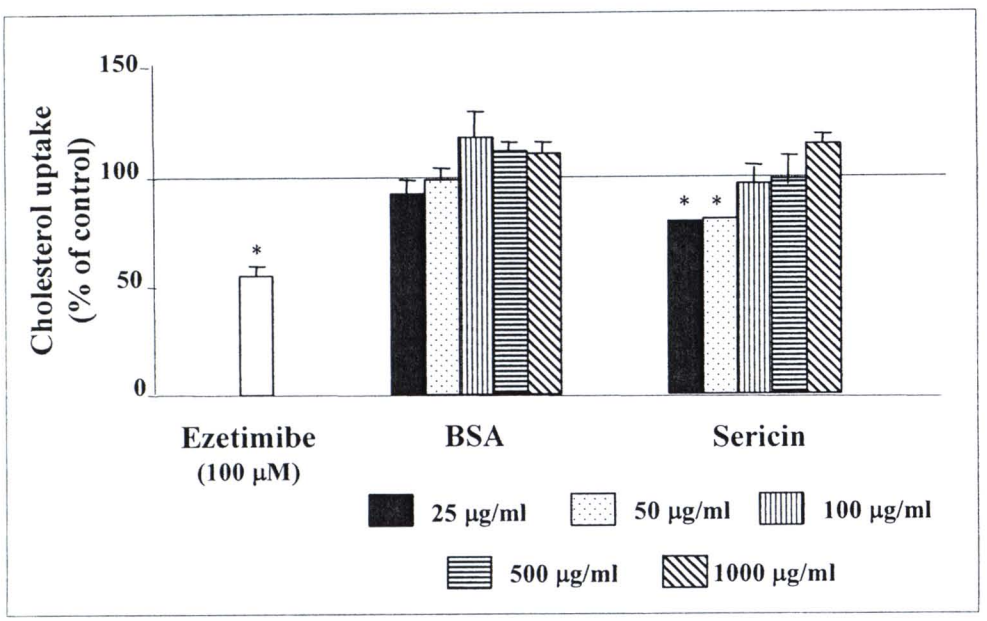

Fig. 3. Cholesterol uptake in differentiated Caco-2 cells. Diffrentiated Caco-2 cells were pretreated with various concentrations of either sericin or BSA (25-1000 μg/ml) for 1 h before incubating with [1α,2α(n)-^{3}H]cholesterol-micelles for 3 h. Ezetimibe was used as positive control. The data represent mean ± SEM from 5-6 experiments. * indicates significant different between treatment and control or PBS-treated groups with $p < 0.05$.

บทที่ 9

ภาคผนวก

1. การผลิตอาหารสัตว์ทดลองเสริมซิริซิน

1.1. ข้อมูลผลการวิเคราะห์องค์ประกอบทางเคมี

1.) ความชื้น (Moisture content)

ตารางที่ 43 ปริมาณความชื้นที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน

ตัวอย่าง	Rep.	น้ำหนักถ้วย (g)	น้ำหนักตัวอย่างเริ่มต้น (g)	น้ำหนักตัวอย่างและถ้วยหลังอบ (g)	น้ำหนักตัวอย่างหลังอบ (g)	ความชื้น (moisture content) (%)	Ave.	SD
COM	1	12.4725	2.0205	14.3536	1.8811	6.90	**6.74**	0.136
	2	13.8974	2.0170	15.7796	1.8822	6.68		
	3	12.4820	2.0062	14.3548	1.8728	6.65		
CN5	1	14.0073	2.0090	15.9079	1.9006	5.40	**5.44**	0.106
	2	12.7835	2.0103	14.6821	1.8986	5.56		
	3	13.8369	2.0236	15.7521	1.9152	5.36		
CN6	1	13.7775	2.0205	15.6549	1.8774	7.08	**7.13**	0.137
	2	13.6533	2.0066	15.5191	1.8658	7.02		
	3	13.8920	2.0194	15.7644	1.8724	7.28		
CN7	1	12.4225	2.0138	14.3674	1.9449	3.42	**3.94**	0.875
	2	14.4931	2.0052	16.4293	1.9362	3.44		
	3	14.7884	2.0196	16.7081	1.9197	4.95		
CC7	1	15.3824	2.0219	17.3220	1.9396	4.07	**4.20**	0.115
	2	13.5662	2.0199	15.5002	1.9340	4.25		
	3	13.5102	2.0150	15.4389	1.9287	4.28		
CS7	1	13.3306	2.0188	15.2990	1.9684	2.50	**2.52**	0.068
	2	13.6843	2.0181	15.6526	1.9683	2.47		
	3	13.2191	2.0254	15.1919	1.9728	2.60		
ST5	1	13.2949	2.0080	15.1904	1.8955	5.60	**5.61**	0.079
	2	13.1869	2.0113	15.0838	1.8969	5.69		
	3	13.9811	2.0252	15.8943	1.9132	5.53		
ST6	1	11.8656	2.0276	13.7754	1.9098	5.81	**5.88**	0.068
	2	12.5311	2.0185	14.4309	1.8998	5.88		
	3	11.5725	2.0219	13.4742	1.9017	5.94		
ST7	1	12.6640	2.0256	14.5779	1.9139	5.51	**5.56**	0.071
	2	11.4922	2.0118	13.3905	1.8983	5.64		
	3	11.8648	2.0066	13.7606	1.8958	5.52		
SS7	1	12.3935	2.0233	14.3627	1.9692	2.67	**2.64**	0.041
	2	11.8586	2.0221	13.8282	1.9696	2.60		
	3	12.6481	2.0200	14.6144	1.9663	2.66		
SA7	1	12.0472	2.0106	13.9768	1.9296	4.03	**4.06**	0.028
	2	11.5541	2.0251	13.4965	1.9424	4.08		
	3	12.6988	2.0243	14.6410	1.9422	4.06		

ตารางที่ 44 ปริมาณน้ำอิสระและความชื้นที่เป็นองค์ประกอบในสูตรอาหารหนูเสริมซิริซินและสูตรที่มีปริมาณโคเลสเตอรอลสูง

ตัวอย่าง	Rep.	น้ำหนักถ้วย	น้ำหนักตัวอย่างเริ่มต้น	น้ำหนักตัวอย่างและถ้วยหลังอบ	น้ำหนักตัวอย่างหลังอบ	ความชื้น (moisture content)			อุณหภูมิขณะวัด	น้ำอิสระ (water activity)		
		(g)	(g)	(g)	(g)	(%)	Ave	SD	(°C)	(%)	Ave	SD
CC	1	11.5725	2.0698	13.5294	1.9569	5.45	**5.32**	0.120	30.1	0.428	**0.36**	0.058
	2	13.3318	2.0658	15.2896	1.9578	5.23			30.4	0.314		
	3	13.5119	2.0333	15.4380	1.9261	5.27			30.8	0.350		
SA	1	12.4219	2.0728	14.4154	1.9935	3.83	**3.82**	0.070	29.6	0.230	**0.25**	0.024
	2	13.7797	2.0040	15.7059	1.9262	3.88			30.0	0.243		
	3	13.9826	2.0328	15.9393	1.9567	3.74			30.5	0.277		
SB	1	13.1884	2.1590	15.1988	2.0104	6.88	**6.86**	0.024	30.9	0.499	**0.50**	0.005
	2	12.1018	2.0399	14.0021	1.9003	6.84			31.0	0.496		
	3	14.0087	2.1141	15.9782	1.9695	6.84			31.0	0.505		
SC	1	13.6550	2.0443	15.5879	1.9329	5.45	**5.43**	0.025	31.1	0.351	**0.35**	0.003
	2	11.6550	2.0822	-	-	-			31.2	0.345		
	3	12.6424	2.0338	14.5661	1.9237	5.41			31.3	0.347		
HC	1	13.2122	2.1082	15.2397	2.0275	3.83	**4.78**	1.831	29.8	0.265	**0.27**	0.020
	2	13.2958	2.0707	15.2238	1.9280	6.89			30.2	0.289		
	3	12.6437	2.0707	14.6394	1.9957	3.62			30.9	0.250		
HB	1	12.7842	2.0712	14.7343	1.9501	5.85	**5.69**	0.168	31.4	0.401	**0.39**	0.008
	2	12.5321	2.0940	14.5107	1.9786	5.51			31.5	0.386		
	3	11.5129	2.0674	13.4625	1.9496	5.70			31.6	0.392		

2.) ปริมาณเถ้า (Ash content)

ตารางที่ 45 ปริมาณเถ้าที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน

ตัวอย่าง	Rep.	Crucible	ตัวอย่างเสริม		ตัวอย่างและ crucible หลังเผา	ตัวอย่างหลังเผา	ปริมาณเถ้าที่เป็นองค์ประกอบ (ash content)					
		(g)	(g_{wet})	(g_{dry})	(g)	(g)	($\%_{wet}$)	Ave.	SD	($\%_{dry}$)	Ave.	SD
COM	1	29.3183	2.1762	2.0294	29.4520	0.1337	6.14	**6.16**	0.015	6.59	**6.60**	0.017
	2	27.7448	2.0198	1.8836	27.8695	0.1247	6.17			6.62		
	3	23.4940	2.1763	2.0295	23.6279	0.1339	6.15			6.60		
CN5	1	35.7927	2.0685	1.9561	35.9246	0.1319	6.38	**6.36**	0.027	6.74	**6.72**	0.028
	2	28.6663	2.1066	1.9921	28.7996	0.1333	6.33			6.69		
	3	24.9811	2.1663	2.0485	25.1191	0.1380	6.37			6.74		
CN6	1	26.2434	2.2347	2.0755	26.3742	0.1308	5.85	**5.91**	0.071	6.30	**6.36**	0.076
	2	30.1829	2.2401	2.0805	30.3170	0.1341	5.99			6.45		
	3	23.4667	2.0776	1.9295	23.5888	0.1221	5.88			6.33		
CN7	1	36.4905	2.0365	1.9563	36.6192	0.1287	6.32	**6.32**	0.019	6.58	**6.58**	0.019
	2	25.9935	2.0495	1.9688	26.1233	0.1298	6.33			6.59		
	3	28.6180	2.1853	2.0993	28.7556	0.1376	6.30			6.55		
CC7	1	29.7542	2.0470	1.9610	29.8819	0.1277	6.24	**6.19**	0.050	6.51	**6.47**	0.053
	2	26.4047	2.0637	1.9770	26.5314	0.1267	6.14			6.41		
	3	26.8830	2.1818	2.0901	27.0184	0.1354	6.21			6.48		
CS7	1	29.7981	2.0184	1.9675	29.9525	0.1544	7.65	**7.64**	0.005	7.85	**7.84**	0.005
	2	32.2167	2.0982	2.0453	32.3771	0.1604	7.64			7.84		
	3	26.6211	2.0379	1.9865	26.7768	0.1557	7.64			7.84		
ST5	1	34.4002	2.1079	1.9897	34.5256	0.1254	5.95	**5.95**	0.009	6.30	**6.31**	0.009
	2	28.8864	2.1434	2.0232	29.0139	0.1275	5.95			6.30		
	3	29.6803	2.0139	1.9010	29.8004	0.1201	5.96			6.32		
ST6	1	36.7154	2.1850	2.0566	36.8514	0.1360	6.22	**6.20**	0.041	6.61	**6.58**	0.044
	2	28.5276	2.1042	1.9805	28.6570	0.1294	6.15			6.53		
	3	28.5858	2.1925	2.0636	28.7221	0.1363	6.22			6.60		
ST7	1	23.8227	2.0362	1.9230	23.9470	0.1243	6.10	**6.05**	0.062	6.46	**6.41**	0.065
	2	25.8902	2.1488	2.0293	26.0188	0.1286	5.98			6.34		
	3	29.8387	2.1319	2.0134	29.9681	0.1294	6.07			6.43		
SS7	1	26.3892	2.0144	1.9613	26.5357	0.1465	7.27	**7.31**	0.036	7.47	**7.50**	0.037
	2	28.0776	2.1215	2.0656	28.2334	0.1558	7.34			7.54		
	3	30.6504	2.1824	2.1249	30.8097	0.1593	7.30			7.50		
SA7	1	28.7596	2.0679	1.9840	28.8836	0.1240	6.00	**6.15**	0.231	6.25	**6.41**	0.241
	2	32.0228	2.0615	1.9779	32.1472	0.1244	6.03			6.29		
	3	23.0386	2.0157	1.9339	23.1679	0.1293	6.41			6.69		

ตารางที่ 46 ปริมาณเถ้าที่เป็นองค์ประกอบในสูตรอาหารหนูเสริมซิริซินและสูตรที่มีปริมาณโคเลสเตอรอลสูง

ตัวอย่าง	Rep.	Crucible	ตัวอย่างเริ่มต้น		ตัวอย่างและ crucible หลังเผา	ตัวอย่างหลังเผา	ปริมาณเถ้าที่เป็นองค์ประกอบ (ash content)					
		(g)	(g_{wet})	(g_{dry})	(g)	(g)	($\%_{wet}$)	Ave.	SD	($\%_{dry}$)	Ave.	SD
CC	1	19.8737	2.0563	1.9469	20.0092	0.1355	6.59	**6.57**	0.036	6.96	**6.94**	**0.038**
	2	21.4807	2.0118	1.9048	31.6121	0.1314	6.53			6.90		
	3	20.6791	2.0875	1.9765	20.8168	0.1377	6.60			6.97		
SA	1	19.3323	2.0222	1.9147	19.4685	0.1269	6.28	**6.37**	0.152	6.63	**6.73**	**0.161**
	2	21.5631	2.0533	1.9441	21.6933	0.1344	6.55			6.91		
	3	19.9278	2.0272	1.9194	20.0594	0.1275	6.29			6.64		
SB	1	22.1589	2.0555	1.9462	22.2845	0.1256	6.11	**6.08**	0.023	6.45	**6.43**	**0.025**
	2	22.8482	2.0408	1.9323	22.9721	0.1239	6.07			6.41		
	3	21.1522	2.0943	1.9829	21.2793	0.1271	6.07			6.41		
SC	1	20.1308	2.0774	1.9669	20.2580	0.1272	6.12	**6.08**	0.043	6.47	**6.42**	**0.046**
	2	20.3058	2.0286	1.9207	20.4283	0.1225	6.04			6.38		
	3	21.9837	2.0529	1.9437	22.1082	0.1245	6.06			6.41		
HC	1	20.3969	2.0916	1.9804	20.5186	0.1362	6.51	**6.47**	0.040	6.88	**6.83**	**0.042**
	2	20.4659	2.0185	1.9112	20.5912	0.1302	6.45			6.81		
	3	22.4845	2.0445	1.9358	22.6118	0.1316	6.44			6.80		
HB	1	21.3829	2.0051	1.8985	21.5098	0.1217	6.07	**6.07**	0.020	6.41	**6.41**	**0.021**
	2	20.4134	2.0696	1.9595	20.5478	0.1253	6.05			6.39		
	3	20.7008	2.0890	1.9779	20.8283	0.1273	6.09			6.44		

3.) ปริมาณทราย (Sand content)

ตารางที่ 47 ปริมาณทรายที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน

ตัวอย่าง	Rep.	Crucible	ตัวอย่างเริ่มต้น		ตัวอย่างและ crucible หลังเผา	ตัวอย่างหลังเผา	ปริมาณทรายที่เป็นองค์ประกอบ (sand content)					
		(g)	(g_{wet})	(g_{dry})	(g)	(g)	($\%_{wet}$)	Ave.	SD	($\%_{dry}$)	Ave.	SD
COM	1	29.3183	2.1762	2.0294	29.3273	0.0090	0.41	**0.29**	0.109	0.44	**0.31**	0.117
	2	27.7448	2.0198	1.8836	27.7494	0.0049	0.24			0.26		
	3	23.4940	2.1763	2.0295	23.4986	0.0046	0.21			0.23		
CN5	1	35.7927	2.0685	1.9561	35.8031	0.0104	0.50	**0.53**	0.048	0.53	**0.56**	0.051
	2	28.6663	2.1066	1.9921	28.6767	0.0104	0.49			0.52		
	3	24.9811	2.1663	2.0485	24.9937	0.0126	0.58			0.62		
CN6	1	26.2434	2.2347	2.0755	26.2508	0.0074	0.33	**0.35**	0.012	0.36	**0.37**	0.013
	2	30.1829	2.2401	2.0805	30.1908	0.0079	0.35			0.38		
	3	23.4667	2.0776	1.9295	23.4740	0.0073	0.35			0.38		
CN7	1	36.4905	2.0365	1.9563	36.4996	0.0091	0.45	**0.42**	0.033	0.47	**0.43**	0.035
	2	25.9935	2.0495	1.9688	26.0013	0.0078	0.38			0.40		
	3	28.6180	2.1853	2.0993	28.6272	0.0092	0.42			0.44		
CC7	1	29.7542	2.0470	1.9610	29.7615	0.0073	0.36	**0.34**	0.040	0.37	**0.35**	0.042
	2	26.4047	2.0637	1.9770	26.4123	0.0076	0.37			0.38		
	3	26.8830	2.1818	2.0901	26.8894	0.0064	0.29			0.31		
CS7	1	29.7981	2.0184	1.9675	29.8072	0.0091	0.45	**0.45**	0.043	0.46	**0.46**	0.044
	2	32.2167	2.0982	2.0453	32.2253	0.0086	0.41			0.42		
	3	26.6211	2.0379	1.9865	26.6312	0.0101	0.50			0.51		
ST5	1	34.4002	2.1079	1.9897	34.4106	0.0104	0.49	**0.48**	0.032	0.52	**0.51**	0.033
	2	28.8864	2.1434	2.0232	28.8971	0.0107	0.50			0.53		
	3	29.6803	2.0139	1.9010	29.6892	0.0089	0.44			0.47		
ST6	1	36.7154	2.1850	2.0566	36.7253	0.0099	0.45	**0.40**	0.045	0.48	**0.43**	0.048
	2	28.5276	2.1042	1.9805	28.5355	0.0079	0.38			0.40		
	3	23.8227	2.1925	2.0636	28.5940	0.0082	0.37			0.40		
ST7	1	23.8227	2.0362	1.9230	23.8303	0.0076	0.37	**0.34**	0.030	0.40	**0.36**	0.031
	2	25.8902	2.1488	2.0293	25.8972	0.0070	0.33			0.34		
	3	29.8387	2.1319	2.0134	29.8455	0.0068	0.32			0.34		
SS7	1	26.3892	2.0144	1.9613	26.3978	0.0086	0.43	**0.39**	0.043	0.44	**0.40**	0.044
	2	28.0776	2.1215	2.0656	28.0849	0.0073	0.34			0.35		
	3	30.6504	2.1824	2.1249	30.6592	0.0088	0.40			0.41		
SA7	1	28.7596	2.0679	1.9840	28.7689	0.0093	0.45	**0.30**	0.128	0.47	**0.31**	0.133
	2	32.0228	2.0615	1.9779	32.0274	0.0046	0.22			0.23		
	3	23.0386	2.0157	1.9339	23.0433	0.0047	0.23			0.24		

4.) ปริมาณโปรตีนทั้งหมด (Total nitrogen and protein content)

ตารางที่ **48** ปริมาณโปรตีนทั้งหมดที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน

ตัวอย่าง	Rep.	ตัวอย่างเริ่มต้น		0.1 N HCl	N HCl	ปริมาณโปรตีนที่เป็นองค์ประกอบ (as total nitrogen content)					
		($g\text{-}_{wet}$)	($g\text{-}_{wet}$)	(ml)	Actual	($\%_{wet}$)	Ave.	SD	($\%_{dry}$)	Ave.	SD
COM	1	2.0211	1.8848	53.30	0.1061	23.96	**24.62**	1.198	25.69	**26.40**	1.285
	2	2.0222	1.8858	57.80	0.1061	26.01			27.89		
	3	2.0288	1.8920	53.40	0.1061	23.91			25.64		
CN5	1	2.0218	1.9119	57.25	0.1061	25.76	**26.36**	0.868	27.24	**27.88**	0.918
	2	2.0245	1.9144	58.05	0.1056	25.97			27.47		
	3	2.0013	1.8925	60.40	0.1056	27.36			28.93		
CN6	1	2.0269	1.8825	60.55	0.1061	27.21	**27.70**	0.861	29.29	**29.83**	0.927
	2	2.0120	1.8686	60.10	0.1061	27.20			29.29		
	3	2.0144	1.8708	63.70	0.1506	28.70			30.90		
CN7	1	2.0279	1.9481	55.25	0.1061	24.77	**25.49**	0.727	25.78	**26.54**	0.757
	2	2.0283	1.9485	58.70	0.1056	26.22			27.30		
	3	2.0138	1.9345	56.70	0.1056	25.49			26.54		
CC7	1	2.0222	1.9372	60.80	0.1061	27.39	**25.36**	1.818	28.59	**26.47**	1.900
	2	2.0131	1.9285	54.95	0.1061	24.81			25.90		
	3	2.0187	1.9339	53.30	0.1056	23.87			24.92		
CS7	1	2.0064	1.9558	62.45	0.1061	28.36	**29.36**	1.000	29.10	**30.12**	1.026
	2	2.0148	1.9640	67.35	0.1056	30.36			31.15		
	3	2.0095	1.9589	65.00	0.1056	29.36			30.12		
ST5	1	2.0173	1.9042	56.50	0.1061	25.47	**28.31**	2.572	26.99	**29.99**	2.720
	2	2.0125	1.8997	67.25	0.1061	30.49			32.30		
	3	2.0029	1.8906	63.90	0.1056	28.95			30.67		
ST6	1	2.0167	1.8981	67.90	0.1061	30.59	**28.61**	2.613	32.50	**30.39**	2.776
	2	2.0100	1.8918	65.20	0.1061	29.58			31.43		
	3	2.0035	1.8857	56.75	0.1056	25.65			27.25		
ST7	1	2.0060	1.8945	65.50	0.1061	29.78	**29.81**	0.176	31.53	**31.56**	0.186
	2	2.0307	1.9178	66.00	0.1061	29.65			31.39		
	3	2.0195	1.9072	66.70	0.1056	30.00			31.76		
SS7	1	2.0027	1.9499	65.80	0.1056	29.83	**29.90**	0.097	30.64	**30.71**	0.100
	2	2.0278	1.9744	66.70	0.1061	30.01			30.82		
	3	2.0098	1.9561	66.05	0.1056	29.85			30.66		
SA7	1	2.0277	1.9455	69.40	0.1061	31.25	**30.34**	0.979	32.57	**31.62**	1.021
	2	2.0279	1.9456	67.70	0.1061	30.47			31.76		
	3	2.0153	1.9336	65.05	0.1056	29.30			30.54		

ตารางที่ 49 ปริมาณโปรตีนที่เป็นองค์ประกอบในสูตรอาหารหนูเสริมซิริซินและสูตรที่มีปริมาณโคเลสเตอรอลสูง

ตัวอย่าง	Rep.	ตัวอย่างเริ่มต้น		0.1N HCl	ปริมาณโปรตีนที่เป็นองค์ประกอบ (as total nitrogen content)					
		(g_{wet})	(g_{dry})	(ml)	($\%_{wet}$)	Ave.	SD	($\%_{dry}$)	Ave.	SD
CC	1	0.5392	0.5105	17.05	25.88	**26.14**	0.289	27.34	**27.60**	0.305
	2	0.5118	0.4846	16.35	26.07			27.54		
	3	0.5111	0.4839	16.55	26.45			27.94		
SA	1	0.5217	0.5018	16.80	26.33	**27.19**	1.216	27.38	**28.27**	1.264
	2	-	-	-	-			-		
	3	0.5521	0.5310	18.80	28.05			29.17		
SB	1	0.5168	0.4814	-	-	**29.70**	0.278	-	**31.89**	0.299
	2	0.5709	0.5318	20.35	29.50			31.68		
	3	0.5546	0.5166	20.05	29.90			32.10		
SC	1	0.5517	0.5217	20.30	30.45	**29.14**	1.859	32.20	**30.81**	1.965
	2	0.5252	0.4967	17.80	27.82			29.42		
	3	-	-	-	-			-		
HC	1	0.5316	0.5062	18.30	28.31	**28.05**	0.704	29.73	**29.46**	0.739
	2	0.5395	0.5137	18.73	28.59			30.03		
	3	0.5104	0.4860	17.00	27.26			28.63		
HB	1	0.5418	0.5110	18.30	27.78	**26.95**	1.164	29.45	**28.58**	1.234
	2	0.5221	0.4924	-	-			-		
	3	0.5324	0.5021	17.00	26.13			27.51		

5.) ปริมาณไขมันทั้งหมด (Fat content)

ตารางที่ 50 ปริมาณไขมันที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน

ตัวอย่าง	Rep.	กระดาษกรอง (g)	ตัวอย่างเริ่มต้น ($g-_{wet}$)	($g-_{wet}$)	ตัวอย่างและกระดาษกรองหลังสกัด (g)	ไขมัน (g)	($\%_{wet}$)	ปริมาณไขมันที่เป็นองค์ประกอบ (fat content) Ave.	SD	($\%_{dry}$)	Ave.	SD
COM	1	2.2192	3.0270	2.8229	5.1092	0.1370	4.53	**4.78**	0.323	4.85	**5.12**	0.346
	2	2.2073	3.0702	2.8631	5.1343	0.1432	4.66			5.00		
	3	2.0170	3.0281	2.8239	4.8894	0.1557	5.14			5.51		
CN5	1	2.2139	3.0225	2.8582	5.1206	0.1157	3.83	**4.28**	0.449	4.05	**4.53**	0.475
	2	2.2066	3.0241	2.8597	5.1010	0.1297	4.29			4.53		
	3	2.0125	3.0404	2.8751	4.9091	0.1438	4.73			5.00		
CN6	1	2.1663	3.0067	2.7924	5.0613	0.1117	3.72	**4.46**	0.665	4.00	**4.80**	0.716
	2	2.1680	3.0147	2.7999	5.0422	0.1405	4.66			5.02		
	3	1.9869	3.0351	2.8188	4.8702	0.1518	5.00			5.38		
CN7	1	2.1611	3.0184	2.8996	5.0311	0.1484	4.92	**5.12**	0.173	5.12	**5.33**	0.180
	2	2.1452	3.0195	2.9006	5.0069	0.1578	5.22			5.44		
	3	2.1704	3.0212	2.9023	5.0342	0.1574	5.21			5.42		
CC7	1	2.1764	3.0297	2.9024	5.0711	0.1350	4.46	**4.54**	0.379	4.65	**4.74**	0.396
	2	2.1944	3.0089	2.8825	5.0767	0.1266	4.21			4.39		
	3	1.9574	3.0429	2.9150	4.4896	0.1507	4.95			5.17		
CS7	1	2.2384	3.0250	2.9488	5.1784	0.0850	2.81	**2.72**	0.120	2.88	**2.79**	0.123
	2	2.1764	3.0174	2.9413	5.1159	0.0779	2.58			2.65		
	3	1.6965	3.0515	2.9746	4.6638	0.0842	2.76			2.83		
ST5	1	2.1631	3.0047	2.8362	5.0665	0.1013	3.37	**3.61**	0.364	3.57	**3.82**	0.386
	2	2.2032	3.0180	2.8488	5.1180	0.1032	3.42			3.62		
	3	1.7049	3.0468	2.8760	4.6290	0.1227	4.03			4.26		
ST6	1	2.2600	3.0039	2.8273	5.1342	0.1297	4.32	**4.51**	0.197	4.59	**4.97**	0.210
	2	2.2748	3.0082	2.8314	5.1480	0.1350	4.49			4.77		
	3	1.7183	3.0477	2.8685	4.6224	0.1436	4.71			5.01		
ST7	1	2.1647	3.0204	2.8525	5.0782	0.1069	3.54	**3.69**	0.131	3.75	**3.91**	0.139
	2	2.2074	3.0045	2.8375	5.0982	0.1137	3.78			4.01		
	3	1.7066	3.0323	2.8637	4.6252	0.1137	3.75			3.97		
SS7	1	2.2090	3.0095	2.9302	5.1333	0.0852	2.83	**2.98**	0.275	2.91	**3.06**	0.283
	2	2.1755	3.0168	2.9373	5.1076	0.0847	2.81			2.88		
	3	1.6958	3.0253	2.9456	4.6214	0.0997	3.30			3.38		
SA7	1	2.1574	3.0135	2.8913	5.0993	0.0716	2.38	**2.63**	0.216	2.48	**2.74**	0.225
	2	1.7060	3.0099	2.8878	4.6340	0.0819	2.72			2.84		
	3	1.6884	3.0358	2.9127	4.6399	0.0843	2.78			2.89		

ตารางที่ 51 ปริมาณไขมันที่เป็นองค์ประกอบในสูตรอาหารหนูเสริมซิริซินและสูตรที่มีปริมาณโคเลสเตอรอลสูง

ตัวอย่าง	Rep.	กระดาษกรอง (g)	ตัวอย่างเริ่มต้น ($g\text{-}_{wet}$)	($g\text{-}_{wet}$)	ตัวอย่างและกระดาษกรองหลังสกัด (g)	ไขมัน (g)	($\%_{wet}$)	ปริมาณไขมันที่เป็นองค์ประกอบ (fat content) Ave.	SD	($\%_{dry}$)	Ave.	SD
CC	1	2.2037	3.0639	3.0639	2.9010	5.1350	4.33	**4.30**	0.124	4.57	**4.54**	0.130
	2	2.1929	3.0060	3.0606	2.8978	5.1188	4.40			4.65		
	3	2.2511	3.0064	3.1369	2.9701	5.2575	4.16			4.39		
SA	1	2.2454	3.0062	3.0062	2.8914	5.1439	3.58	**3.45**	0.084	3.72	**3.68**	0.087
	2	2.2323	3.0081	3.0081	2.8933	5.1368	3.44			3.58		
	3	2.2538	3.0704	3.0704	2.9532	5.2138	3.60			3.74		
SB	1	2.2310	3.0150	3.0152	2.8085	5.0962	4.97	**4.78**	0.281	5.34	**5.13**	0.301
	2	2.1728	3.0364	3.0364	2.8282	5.0702	4.58			4.91		
	3	2.1949	3.0900	3.0062	2.8001	-	-			-		
SC	1	2.1870	3.0792	3.0792	2.9120	5.1375	4.18	**4.54**	0.503	4.42	**4.80**	0.532
	2	2.8590	3.0590	2.3859	2.2563	-	-			-		
	3	2.7630	3.0032	2.4165	2.2853	5.0613	4.89			5.17		
HC	1	2.2257	3.0215	3.0215	2.8771	5.0956	5.02	**5.15**	0.253	5.27	**5.41**	0.266
	2	2.1884	3.0889	3.0889	2.9412	5.1230	5.00			5.25		
	3	2.1710	3.0621	3.0621	2.9157	5.0664	5.44			5.72		
HB	1	2.2016	3.0881	3.0881	2.9125	5.1180	5.56	**5.43**	0.361	5.90	**5.75**	0.383
	2	2.1911	3.0802	3.0502	2.8768	5.0674	5.70			6.04		
	3	2.1689	3.0571	3.0571	2.8833	5.0726	5.02			5.32		

6.) ปริมาณเส้นใยหยาบ (Crude fiber)

ตารางที่ 52 ปริมาณเส้นใยหยาบที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน

ตัวอย่าง	Rep.	Crucible	กระดาษกรอง	ตัวอย่างเริ่มต้น		กาก กระดาษกรองและcrucible หลังอบ (g)	กาก และcrucible หลังเผา (g)	กาก หลังอบ (g)	เถ้า หลังเผา (g)	ปริมาณเส้นใยหยาบที่เป็นองค์ประกอบ (crude fiber)					
		(g)		(g-wet)	(g-wet)					(%wet)	Ave.	SD	(%dry)	Ave.	SD
COM	1	24.8197	1.1880	3.0270	2.8229	26.0668	24.8253	0.0591	0.0056	1.77	**1.66**	0.347	1.90	**1.78**	0.372
	2	28.4581	1.1702	3.0702	2.8631	29.6765	28.4673	0.0482	0.0092	1.27			1.36		
	3	26.4052	1.1468	3.0281	2.8239	27.6161	26.4106	0.0641	0.0054	1.94			2.08		
CN5	1	26.1081	1.1501	3.0225	2.8582	27.3024	25.1353	0.0442	.	.	**2.32**	0.670	.	**2.45**	0.709
	2	23.9395	1.2001	3.0241	2.8597	25.2310	23.9465	0.0914	0.0070	2.79			2.95		
	3	26.2449	1.1888	3.0404	2.8751	27.4977	26.2529	0.0640	0.0080	1.84			1.95		
CN6	1	29.3192	1.1835	3.0067	2.7924	30.5657	29.3273	0.0630	0.0081	1.82	**1.91**	0.391	1.96	**2.06**	0.421
	2	28.7598	1.1723	3.0147	2.7999	29.9876	28.7680	0.0555	0.0082	1.57			1.69		
	3	29.7539	1.1687	3.0351	2.8188	31.0009	29.7613	0.0783	0.0074	2.34			2.52		
CN7	1	28.8887	1.1967	3.0184	2.8996	30.1415	28.9009	0.0561	0.0122	1.45	**2.08**	0.642	1.51	**2.16**	0.668
	2	27.7293	1.1865	3.0195	2.9006	28.9887	27.7407	0.0729	0.0114	2.04			2.12		
	3	31.5155	1.1566	3.0212	2.9023	32.7631	31.5238	0.0910	0.0083	2.74			2.85		
CC7	1	23.8237	1.1692	3.0297	2.9024	25.0036	23.8507	0.0107	.	.	**0.99**	0.084	.	**1.04**	0.088
	2	32.2176	1.1630	3.0089	2.8825	33.4128	32.2217	0.0322	0.0041	0.94			0.98		
	3	29.6811	1.1787	3.0429	2.9150	30.8963	29.6855	0.0365	0.0044	1.05			1.10		
CS7	1	28.3172	1.1630	3.0250	2.9488	29.5397	28.3240	0.0595	0.0068	1.74	**1.46**	0.399	1.79	**1.50**	0.410
	2	.	.	3.0174	2.9413	.	.	.	.	.			1.21		
	3	28.5280	1.1754	3.0515	2.9746	29.7500	28.5391	0.0466	0.0111	1.18			.		
ST5	1	29.7994	1.1907	3.0047	2.8362	31.0651	29.8140	0.0750	0.0146	2.01	**1.64**	0.611	2.13	**1.74**	0.648
	2	28.6186	1.1922	3.0180	2.8488	29.8805	28.6288	0.0697	0.0102	1.97			2.09		
	3	25.8908	1.1469	3.0468	2.8760	27.0751	25.8998	0.0374	0.0090	0.93			0.99		
ST6	1	30.1825	1.1804	3.0039	2.8273	31.4593	30.2220	0.0964	0.0395	1.90	**1.75**	0.211	2.01	**1.86**	0.224
	2	27.2032	1.1568	3.0082	2.8314	28.0480	27.2120	.	.	.			.		
	3	28.5865	1.1708	3.0047	2.8685	29.8145	28.5950	0.0572	0.0085	1.60			1.70		
ST7	1	27.6020	1.1408	3.0204	2.8525	28.8104	27.6116	0.0676	0.0096	1.92	**1.90**	0.032	2.03	**2.01**	0.034
	2	32.0383	1.1403	3.0045	2.8375	33.2400	32.0434	0.0614	0.0051	1.87			1.98		
	3	.	.	3.0323	2.8637	30.3346	29.1015	.	.	.			.		
SS7	1	29.0874	1.1781	3.0095	2.9302	30.3346	29.1015	0.0691	0.0141	1.83	**1.47**	0.510	1.88	**1.51**	0.523
	2	25.9949	1.1918	3.0168	2.9373	27.2296	26.0044	0.0429	0.0095	1.11			1.14		
	3	.	.	3.0253	2.9456	29.9071	.	.	.	.			.		
SA7	1	24.9841	1.1515	3.0135	2.8913	26.1974	24.9954	0.0618	0.0113	1.68	**1.58**	0.256	1.75	**1.65**	0.267
	2	25.6836	1.1725	3.0099	2.8878	26.1988	25.6930	0.0627	0.0094	1.77			1.85		
	3	28.7879	1.1726	3.0358	2.9127	30.0068	28.7951	0.0463	0.0072	1.29			1.34		

7.) ปริมาณสตาร์ช (Starch content)

ตารางที่ 53 ปริมาณสตาร์ชที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน

ตัวอย่าง	Rep.		Absorbance (620m)						Carbohydrate conc. (ug/ml)						Total carbohydrate (g)						Total carbohydrate (%)					
			1	2	3	Ave	Ave	SD	1	2	Ave	Ave	SD		1	2	3	Ave	Ave	SD	1	2	3	Ave	Ave	SD
COM	1	1	0.156	0.156	0.158	0.157	**0.147**	0.012	297.94	297.94	301.76	299.21	**280.04**	22.98	0.745	0.745	0.745	0.748	**0.700**	0.057	37.24	37.24	37.72	37.40	**35.01**	2.87
		2	0.150	0.153	0.149	0.151			286.48	292.21	284.57	287.75			0.716	0.731	0.711	0.719			35.81	36.53	35.57	35.97		
		3	0.157	0.150	0.157	0.155			299.85	286.48	299.85	295.39			0.750	0.716	0.750	0.738			37.48	35.81	37.48	36.92		
	2	1	0.130	0.137	0.139	0.135			248.28	261.65	265.47	258.47			0.621	0.654	0.664	0.646			31.04	32.71	33.18	32.31		
		2	0.134	0.133	0.129	0.132			255.92	254.01	246.37	252.10			0.640	0.635	0.616	0.630			31.99	31.75	30.80	31.51		
		3	0.125	0.125	0.125	0.125			238.73	238.73	238.73	238.73			0.597	0.597	0.597	0.597			29.84	29.84	29.84	29.84		
	3	1	0.156	0.158	0.156	0.157			297.94	301.76	297.94	299.21			0.745	0.754	0.745	0.748			37.24	37.72	37.72	37.40		
		2	0.154	0.150	0.154	0.153			294.12	285.48	294.12	291.57			0.735	0.716	0.735	0.729			36.76	35.81	35.81	36.45		
		3	0.156	0.156	0.156	0.156			297.94	297.94	297.94	297.94			0.745	0.745	0.745	0.745			37.24	37.24	37.24	37.42		
CN5	1	1	0.192	0.193	0.194	0.193	**0.196**	0.004	366.69	368.60	370.51	368.60	**374.61**	7.63	0.917	0.922	0.926	0.922	**0.937**	0.019	45.84	46.08	46.31	46.08	**46.83**	0.95
		2	0.192	0.197	0.191	0.193			366.69	376.24	364.78	369.24			0.917	0.941	0.912	0.923			45.84	47.03	45.60	46.15		
		3	0.196	0.193	0.197	0.195			374.33	368.60	376.24	373.06			0.936	0.922	0.941	0.933			46.79	46.08	47.03	46.63		
	2	1	0.202	0.202	0.203	0.202			385.79	385.79	387.70	386.43			0.964	0.964	0.969	0.966			48.22	48.22	48.46	48.30		
		2	0.198	0.196	0.196	0.197			378.15	374.33	374.33	375.60			0.945	0.936	0.936	0.939			47.27	46.79	46.79	46.95		
		3	0.202	0.202	0.202	0.202			385.79	385.79	385.79	385.79			0.964	0.964	0.964	0.964			48.22	48.22	48.22	48.22		
	3	1	0.197	0.197	0.197	0.197			376.24	376.24	376.24	376.24			0.941	0.941	0.941	0.941			47.03	47.03	47.03	47.03		
		2	0.191	0.190	0.190	0.190			364.78	362.87	362.87	363.51			0.912	0.907	0.907	0.909			45.60	45.36	45.36	45.44		
		3	0.196	0.195	0.195	0.195			374.33	372.42	372.42	373.06			0.936	0.931	0.931	0.933			46.79	46.55	46.55	46.63		
CN6	1	1	0.177	0.176	0.176	0.176	**0.223**	0.029	338.04	336.13	336.13	336.77	**426.46**	54.68	0.845	0.840	0.840	0.842	**1.066**	0.137	42.26	42.02	42.02	42.10	**53.31**	6.84
		2	0.175	0.176	0.178	0.176			334.22	336.13	339.95	336.77			0.836	0.840	0.850	0.842			41.78	42.02	42.49	42.10		
		3	0.207	0.243	0.245	0.232			395.34	464.09	467.91	442.45			0.988	1.160	1.170	1.106			49.42	58.01	58.49	55.31		
	2	1	0.225	0.224	0.223	0.224			429.72	427.81	425.90	427.81			1.074	1.070	1.065	1.070			53.71	53.48	53.24	53.48		
		2	0.225	0.224	0.223	0.224			429.72	427.81	425.90	427.81			1.074	1.070	1.065	1.070			53.71	53.48	53.24	53.48		
		3	0.223	0.227	0.223	0.224			425.90	433.54	425.90	428.44			1.065	1.084	1.065	1.071			53.24	54.19	53.24	53.56		
	3	1	0.250	0.252	0.252	0.251			477.46	481.28	481.28	480.01			1.194	1.203	1.203	1.200			59.68	60.16	60.16	60.00		
		2	0.249	0.248	0.252	0.250			475.55	473.64	481.28	476.83			1.189	1.184	1.203	1.192			59.44	59.21	60.16	59.60		
		3	0.251	0.252	0.253	0.252			479.37	481.28	483.19	481.28			1.198	1.203	1.208	1.203			59.92	60.16	60.40	60.16		
CN7	1	1	0.238	0.231	0.232	0.234	**0.237**	0.004	454.55	441.18	441.18	446.27	**452.85**	7.25	1.136	1.103	1.108	1.116	**1.132**	0.018	56.82	55.15	55.39	55.78	**56.61**	0.91
		2	0.236	0.236	0.234	0.235			450.73	450.73	450.73	449.45			1.127	1.127	1.117	1.124			56.34	56.34	55.86	56.18		
		3	0.231	0.234	0.232	0.232			441.18	446.91	446.91	443.72			1.103	1.117	1.108	1.109			55.15	55.86	55.39	55.47		
	2	1	0.236	0.237	0.235	0.236			450.73	452.64	452.64	450.73			1.127	1.132	1.122	1.127			56.34	56.58	56.10	56.34		
		2	0.237	0.239	0.240	0.239			452.64	456.46	456.46	455.82			1.132	1.141	1.146	1.140			56.58	57.06	57.30	56.98		
		3	0.236	0.236	0.236	0.236			450.73	450.73	450.73	450.73			1.127	1.127	1.127	1.127			56.34	56.34	56.34	56.34		
	3	1	0.240	0.239	0.239	0.239			458.37	456.46	456.46	457.09			1.146	1.141	1.141	1.143			57.30	57.06	57.06	57.14		
		2	0.241	0.237	0.236	0.238			460.28	452.64	452.64	454.55			1.151	1.132	1.127	1.136			57.53	56.58	56.34	56.82		
		3	0.245	0.244	0.245	0.245			467.91	466.00	467.91	467.28			1.170	1.165	1.170	1.168			58.25	58.49	58.49	58.41		

ตารางที่ 53 ปริมาณสตาร์ชที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน (ต่อ)

ตัวอย่าง	Rep		Absorbance (620m)						Carbohydrate conc. (μm/ml)						Total carbohydrate (g)						Total carbohydrate (%)					
			1	2	3	Ave	Ave	SD	1	2	3	Ave	Ave	SD	1	2	3	Ave	Ave	SD	1	2	3	Ave	Ave	SD
CC7	**1**	**1**	0.242	0.243	0.243	0.243	**0.238**	0.009	462.18	464.09	464.09	463.46	**453.84**	17.61	1.155	1.160	1.160	1.159	**1.135**	0.044	57.77	58.01	58.01	57.93	**56.73**	2.20
		2	0.245	0.245	0.241	0.244			467.91	467.91	460.82	465.37			1.170	1.170	1.151	1.163			58.49	58.49	57.53	58.17		
		3	0.247	0.243	0.246	0.245			471.73	464.09	469.82	468.55			1.179	1.160	1.175	1.171			58.97	58.01	58.73	58.57		
	2	**1**	0.223	0.224	0.224	0.224			425.90	427.81	427.81	427.17			1.065	1.070	1.070	1.068			53.24	53.48	53.48	53.40		
		2	0.227	0.223	0.230	0.227			433.54	425.90	439.27	432.90			1.084	1.065	1.980	1.082			54.19	53.24	54.91	54.11		
		3	0.227	0.224	0.224	0.225			433.54	427.81	427.81	429.72			1.084	1.070	1.070	1.074			54.19	53.48	53.48	53.71		
	3	**1**	0.244	0.246	0.244	0.245			466.00	469.82	466.00	467.28			1.165	1.175	1.165	1.168			58.25	58.73	58.25	58.41		
		2	0.243	0.243	0.241	0.242			464.09	464.09	460.28	462.82			1.160	1.160	1.151	1.157			58.01	58.01	57.53	57.85		
		3	0.244	0.246	0.244	0.245			466.00	469.82	466.00	467.28			1.165	1.175	1.165	1.168			58.25	58.73	58.25	58.41		
CS7	**1**	**1**	0.258	0.257	0.255	0.257	**0.254**	0.006	492.74	490.83	487.01	490.20	**485.24**	12.40	1.232	1.227	1.218	1.225	**1.213**	0.031	61.59	61.35	60.88	61.27	**60.66**	1.55
		2	0.265	0.266	0.267	0.266			506.11	508.02	509.93	508.02			1.265	1.270	1.275	1.270			63.26	63.50	63.74	63.50		
		3	0.259	0.259	0.253	0.257			494.56	494.65	483.19	490.83			1.237	1.237	1.208	1.227			61.83	61.83	60.40	61.35		
	2	**1**	0.251	0.266	0.267	0.261			479.37	475.55	483.19	479.37			1.198	1.189	1.208	1.198			59.92	59.44	60.40	59.92		
		2	0.251	0.250	0.254	0.252			479.37	477.46	485.10	480.65			1.198	1.194	1.213	1.202			59.92	59.68	60.64	60.08		
		3	0.254	0.245	0.261	0.253			485.10	467.91	498.47	483.83			1.213	1.170	1.246	1.210			60.64	58.49	62.31	60.48		
	3	**1**	0.241	0.244	0.244	0.243			460.28	466.00	466.00	464.09			1.151	1.165	1.165	1.160			57.53	58.25	58.25	58.01		
		2	0.254	0.251	0.252	0.252			485.10	479.37	481.28	481.92			1.213	1.198	1.203	1.205			60.64	59.92	60.16	60.24		
		3	0.256	0.254	0.257	0.256			488.92	485.10	490.83	488.29			1.222	1.213	1.227	1.221			61.12	60.64	61.35	61.04		
ST5	**1**	**1**	0.248	0.246	0.246	0.247	**0.236**	0.007	473.64	469.82	469.82	471.10	**451.01**	13.22	1.184	1.175	1.175	1.178	**1.128**	0.033	59.21	58.73	58.73	58.89	**56.38**	1.65
		2	0.242	0.244	0.242	0.243			462.18	466.00	462.18	463.46			1.155	1.165	1.155	1.159			57.77	58.25	57.77	57.93		
		3	0.245	0.244	0.244	0.244			467.91	466.00	466.00	466.64			1.170	1.165	1.165	1.167			58.49	58.25	58.25	58.33		
	2	**1**	0.231	0.231	0.230	0.231			441.18	441.18	439.27	440.54			1.103	1.103	1.098	1.101			55.15	55.15	54.91	55.07		
		2	0.235	0.235	0.236	0.235			448.82	448.82	450.73	449.45			1.122	1.122	1.127	1.124			56.10	56.10	56.34	56.18		
		3	0.234	0.238	0.234	0.235			446.91	454.55	446.91	449.45			1.117	1.136	1.117	1.124			55.86	56.82	55.86	56.18		
	3	**1**	0.227	0.227	0.231	0.228			433.54	433.54	441.18	436.08			1.084	1.084	1.103	1.090			54.19	54.19	55.15	54.51		
		2	0.233	0.233	0.239	0.235			445.00	445.00	456.46	448.82			1.112	1.112	1.141	1.122			55.62	55.62	57.06	56.10		
		3	0.227	0.227	0.227	0.227			433.54	433.54	433.54	433.54			1.084	1.084	0.012	1.084			54.19	54.19	54.19	54.19		
ST6	**1**	**1**	0.210	0.207	0.205	0.207	**0.204**	0.006	401.07	395.34	391.52	395.98	**389.19**	10.74	1.003	0.988	0.979	0.990	**0.973**	0.027	50.13	49.42	48.94	49.50	**48.65**	1.34
		2	0.211	0.204	0.209	0.208			402.98	398.61	399.16	397.25			1.007	0.974	0.998	0.993			50.37	48.70	49.89	49.65		
		3	0.205	0.205	0.203	0.204			391.52	391.52	387.70	390.25			0.979	0.979	0.969	0.976			48.94	48.94	48.46	48.78		
	2	**1**	0.188	0.191	0.192	0.190			359.05	364.78	366.69	363.51			0.898	0.912	0.917	0.909			44.88	45.60	45.84	45.44		
		2	0.201	0.201	0.203	0.202			383.88	383.88	387.70	385.15			0.960	0.960	0.969	0.963			47.99	47.99	48.86	48.14		
		3	0.205	0.206	0.203	0.205			391.52	393.43	387.70	390.88			0.979	0.984	0.969	0.977			48.94	49.18	48.86	48.86		
	3	**1**	0.200	0.203	0.203	0.202			381.97	387.70	387.70	385.79			0.955	0.969	0.969	0.964			47.75	48.46	48.46	48.22		
		2	0.208	0.208	0.208	0.208			397.25	397.25	397.25	397.35			0.993	0.993	0.993	0.993			49.66	49.66	49.66	49.66		
		3	0.208	0.208	0.207	0.208			397.25	397.25	395.34	396.61			0.993	0.993	0.988	0.992			49.66	49.66	49.42	49.58		

ตารางที่ 53 ปริมาณสตาร์ทที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน (ต่อ)

ัวอย่าง	Rep.		Absorbance (620 nm)						Carbohydrate conc.(µg/ml)						Total carbohydrate (g)						Total carbohydrate (%)					
			1	2	3	Ave	Ave	SD	1	2	3	Ave	Ave	SD	1	2	3	Ave	Ave	SD	1	2	3	Ave	Ave	SD
;T7	1	1	0.194	0.200	0.199	0.198	0.191	0.023	370.51	381.97	380.06	377.51	365.31	43.87	0.926	0.955	0.950	0.944	0.913	0.110	46.31	47.75	47.51	47.19	45.66	5.48
		2	0.149	0.149	0.151	0.150			284.57	284.57	288.39	285.84			0.711	0.711	0.721	0.715			35.57	35.57	36.05	35.73		
		3	0.148	0.152	0.148	0.149			282.66	290.30	282.66	285.20			0.707	0.726	0.707	0.713			35.33	36.29	35.33	35.65		
	2	1	0.208	0.208	0.207	0.208			397.25	397.25	395.34	396.61			0.993	0.993	0.988	0.992			49.66	49.66	49.42	49.58		
		2	0.206	0.207	0.207	0.207			393.43	395.34	395.34	394.70			0.984	0.988	0.988	0.987			49.18	49.42	49.42	49.34		
		3	0.203	0.202	0.202	0.202			387.70	385.79	386.79	386.76			0.969	0.964	0.964	0.966			48.46	48.22	48.22	48.30		
	3	1	0.202	0.203	0.202	0.202			385.79	387.70	385.79	386.43			0.964	0.969	0.964	0.966			48.22	48.46	48.22	48.30		
		2	0.205	0.203	0.207	0.205			391.52	387.70	395.34	391.52			0.979	0.969	0.988	0.979			48.94	48.46	49.42	48.94		
		3	0.201	0.201	0.200	0.201			383.88	383.88	381.97	383.24			0.960	0.960	0.955	0.958			47.99	47.99	47.75	47.91		
;C7	1	1	0.214	0.216	0.216	0.215	0.178	0.020	408.71	412.53	412.53	411.26	340.87	37.58	1.022	1.031	1.031	1.028	0.852	0.094	51.09	51.57	51.57	51.41	42.61	4.70
		2	0.194	0.191	0.195	0.193			370.51	364.78	372.42	369.24			0.926	0.912	0.931	0.923			46.31	45.60	46.55	46.15		
		3	0.200	0.201	0.200	0.200			381.97	383.88	381.97	382.61			0.955	0.960	0.955	0.957			47.75	47.99	47.75	47.83		
	2	1	0.180	0.184	0.181	0.182			343.77	351.41	345.68	346.96			0.859	0.879	0.864	0.867			42.97	43.93	43.21	43.37		
		2	0.170	0.168	0.169	0.169			324.68	320.86	322.77	322.77			0.812	0.802	0.807	0.807			40.58	40.11	40.35	40.35		
		3	0.165	0.169	0.165	0.166			315.13	322.77	315.13	317.68			0.788	0.807	0.788	0.794			39.39	40.35	39.39	39.71		
	3	1	0.159	0.161	0.166	0.162			303.67	307.49	317.04	309.40			0.759	0.769	0.793	0.774			37.96	38.44	39.63	38.67		
		2	0.159	0.158	0.157	0.158			303.67	301.76	299.85	301.76			0.759	0.754	0.750	0.754			37.96	37.72	37.48	37.72		
		3	0.156	0.161	0.164	0.160			297.94	307.49	313.22	306.21			0.745	0.769	0.783	0.766			37.24	38.44	39.15	38.28		
;A7	1	1	0.204	0.206	0.204	0.205	0.182	0.029	389.61	393.43	389.61	390.88	348.02	52.75	0.974	0.984	0.974	0.977	0.870	0.132	48.70	49.18	48.70	48.86	43.50	6.59
		2	0.208	0.207	0.208	0.208			397.25	395.34	397.25	396.61			0.993	0.988	0.993	0.992			49.66	49.42	49.66	49.58		
		3	0.208	0.207	0.208	0.208			397.25	395.34	397.25	396.61			0.993	0.988	0.993	0.992			49.66	49.42	49.66	49.58		
	2	1	0.195	0.194	0.195	0.195			372.42	370.51	372.42	371.79			0.931	0.926	0.931	0.929			46.55	46.31	46.55	46.47		
		2	0.194	0.194	0.195	0.194			370.51	370.51	374.33	371.79			0.926	0.926	0.936	0.929			46.31	46.31	46.79	46.47		
		3	0.196	0.197	0.196	0.196			374.33	376.24	374.33	374.97			0.936	0.941	0.936	0.937			46.79	47.03	46.79	46.87		
	3	1	0.140	0.136	0.140	0.139			267.38	259.74	267.38	264.83			0.668	0.649	0.668	0.662			33.42	32.47	33.42	33.10		
		2	0.156	0.149	0.149	0.151			297.94	284.57	284.57	289.02			0.745	0.711	0.711	0.723			37.24	35.57	35.57	36.13		
		3	0.144	0.145	0.144	0.144			275.02	276.93	275.02	275.66			0.688	0.692	0.688	0.689			34.38	34.62	34.38	34.46		
;TD		1	0.289	0.288	0.288	0.288	0.262	0.026																		
Glycogen		2	0.269	0.270	0.270	0.270																				
		3	0.270	0.271	0.271	0.271																				
		4	0.215	0.215	0.212	0.214																				
		5	0.287	0.289	0.288	0.288																				

8.) ปริมาณเกลือ **(Salt content)**

ตารางที่ 54 ปริมาณเกลือที่เป็นองค์ประกอบในตัวอย่างสูตรอาหารหนูเสริมซิริซิน

ตัวอย่าง	ปริมาตร KSCN (ml)			ปริมาณเกลือที่เป็นองค์ประกอบ (salt content)				
	R1	R2	R3	R1	R2	R3	Ave.	SD
COM	24.30	24.23	24.15	0.14	0.15	0.17	**0.15**	0.015
CN5	23.60	23.70	23.60	0.27	0.25	0.27	**0.27**	0.011
CN6	23.55	23.57	23.60	0.28	0.28	0.27	**0.28**	0.005
CN7	23.40	23.43	23.65	0.31	0.31	0.26	**0.29**	0.027
CC7	23.90	23.80	23.80	0.21	0.23	0.23	**0.23**	0.011
CS7	23.55	23.60	23.65	0.28	0.27	0.26	**0.27**	0.010
ST5	23.95	23.70	23.67	0.20	0.25	0.26	**0.24**	0.030
ST6	24.00	23.60	23.70	0.19	0.27	0.25	**0.24**	0.041
ST7	24.05	24.00	23.80	0.19	0.19	0.23	**0.20**	0.026
SS7	23.75	23.70	23.75	0.24	0.25	0.24	**0.25**	0.006
SA7	24.10	24.00	24.05	0.18	0.19	0.19	**0.19**	0.010

1.2. ข้อมูลผลการวิเคราะห์ทางจุลชีววิทยา

1.) ค่า Log จำนวนจุลินทรีย์ทั้งหมดในตัวอย่างอาหารหนูที่เก็บรักษาไว้ที่อุณหภูมิและระยะเวลาต่าง ๆ

ตารางที่ 55 ค่า Log ของจำนวนจุลินทรีย์ทั้งหมดในตัวอย่างอาหารหนูเมื่อเก็บรักษาไว้ที่อุณหภูมิและระยะเวลาต่าง ๆ

Temp. (°C)	Time (weeks)	Aerobic Plate Count (CFU/g)						Log TCP (Log A)					
		M	C	S	CNC	SNC	A	M	C	S	CNC	SNC	A
25	0	3200	1300	18550	650	221000	ND	3.505	3.114	4.268	2.813	5.344	ND
	1	ND	3850	ND	1000	185000	250	ND	3.585	ND	3.000	5.267	2.398
	2	54500	5500	58500	2000	204000	5000	4.736	3.740	4.767	3.301	5.310	3.699
	3	185000	7000	30500	ND	ND	ND	5.267	3.845	5.484	ND	ND	ND
	4	41500	ND	19850	1750	109500	2000	4.618	ND	4.298	3.243	5.491	3.301
	5	10000	3000	16000	2650	ND	200	4.000	3.477	4.204	3.423	ND	2.301
	6	6150	4500	10000	1250	575000	250	3.789	3.653	4.000	3.097	5.760	2.398
	8	ND	1000	16450	1050	ND	1850	ND	3.000	4.216	3.021	ND	3.267
	10	3000	ND	25000	1000	410000	ND	3.477	ND	4.398	3.000	5.613	ND
	12	ND	ND	30000	ND	ND	2300	ND	ND	4.477	ND	ND	3.362
35	0	3200	1300	18550	650	221000	ND	3.505	3.114	4.268	2.813	5.344	ND
	1	ND	2000	ND	ND	169000	ND	ND	3.301	ND	ND	5.228	ND
	2	10500	4500	14500	3000	248500	3000	4.021	3.653	4.161	3.477	5.395	3.477
	3	2000	4000	24750	ND	ND	1800	3.301	3.602	4.394	ND	ND	3.255
	4	2000	5500	32550	2050	418000	5000	3.301	3.740	4.513	3.312	5.621	3.699
	5	1000	5000	ND	ND	ND	ND	3.000	3.699	ND	ND	ND	ND
	6	8000	30000	124000	10650	4225000	3950	3.903	4.477	5.093	4.027	6.626	3.597
	8	100	30000	14500	14000	1155000	1500	2.000	4.477	4.161	4.146	6.063	3.176
	10	ND	13500	16500	12850	70000	50	ND	4.130	4.217	4.109	4.845	1.699
	12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
55	0	3200	1300	18550	650	221000	ND	3.505	3.114	4.268	2.813	5.344	ND
	1	ND	1000	20650	800	ND	ND	ND	3.000	4.315	2.903	ND	ND
	2	4000	1500	18500	2500	352500	5500	3.602	3.176	4.267	3.398	5.547	3.740
	3	1000	3500	25500	ND	ND	ND	3.000	3.544	4.407	ND	ND	ND
	4	1000	9500	22050	9100	216000	2050	3.000	3.978	4.343	3.959	5.334	3.312
	5	ND	ND	8500	ND	ND	ND	ND	ND	3.929	ND	ND	ND
	6	40000	85000	5000	16000	235000	2450	4.602	4.929	3.699	4.204	5.371	3.389
	8	10000	29500	4000	17500	26500	2500	4.000	4.470	3.602	4.243	4.423	3.398
	10	ND	ND	7150	7050	10000	300	ND	ND	3.854	3.848	4.000	2.477
	12	ND	ND	15000	ND	ND	ND	ND	ND	4.176	ND	ND	ND

ตารางที่ 56 การเปลี่ยนแปลงจำนวนจุลินทรีย์ทั้งหมด และราและยีสต์ในตัวอย่างอาหารหนูเมื่อเก็บรักษาที่อุณหภูมิ 25°C ที่ระยะต่างๆ

Sample	Time	(weeks)	0		1		2		3		4		5		6		8		10		12	
M		bacteria	2900	3500	ND	ND	49000	60000	ND	260000	36000	34000	ND	10000	6500	5600	ND	ND	2000	3000	16000	14000
	PCA	yeast	ND	ND	ND	ND	ND	ND	10000	100000	7000	6000	ND	ND	ND	200	ND	ND	1000	ND	ND	ND
		yeast	100	ND	1000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1300	900
	PDA	mold	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	2900	3500	ND	ND	49000	60000	10000	360000	43000	40000	ND	10000	6500	5800	ND	ND	3000	3000	16000	14000
	Y&M	(colonies)	200	ND	1000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1300	900
C		bacteria	1300	ND	ND	2000	6000	5000	9000	2000	ND	ND	ND	ND	2000	ND	1000	ND	ND	ND	ND	ND
	PCA	yeast	ND	ND	2500	3200	ND	ND	1000	2000	ND	1000	3000	ND	5000	2000	ND	ND	ND	TNTC	20000	10000
		yeast	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	20	30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	1300	ND	2500	5200	6000	5000	10000	4000	ND	1000	3000	ND	7000	2000	1000	ND	23000	ND	20000	10000
	Y&M	(colonies)	20	30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S		bacteria	17700	19400	9100	8800	42000	75000	320000	270000	19800	19200	16000	16000	11000	9000	18200	14000	22000	28000	10000	50000
	PCA	yeast	ND	ND	ND	ND	ND	ND	10000	10000	400	300	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		yeast	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	10	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	17700	19400	9100	8800	42000	75000	330000	280000	20200	19500	16000	16000	11000	9000	18200	14700	22000	28000	10000	50000
	Y&M	(colonies)	10	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CNC		bacteria	ND	650	700	ND	0	3000	273000	22000	1300	1100	100	700	400	ND	ND	100	ND	ND	6000	4000
	PCA	yeast	ND	ND	900	400	1000	ND	1000	1000	600	500	2300	2200	800	1300	900	1100	1000	1000	5000	5000
		yeast	70	100	ND	700	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	ND	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	ND	650	1600	400	1000	3000	274000	223000	1900	1600	2400	2900	1200	1300	900	1200	1000	1000	11000	9000
	Y&M	(colonies)	70	110	ND	700	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SNC		bacteria	TNTC	TNTC	180000	190000	ND	407000	ND	ND	292000	327000	242000	209000	590000	560000	171000	178000	320000	500000	1080000	890000
	PCA	yeast	ND	ND	ND	ND	ND	1000	30000	20000	ND	ND	ND	ND	ND	ND	ND	1000	ND	ND	ND	ND
		yeast	10	10	ND	ND	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	ND	ND	180000	190000	ND	408000	30000	20000	292000	327000	242000	209000	590000	560000	171000	179000	320000	500000	1080000	890000
	Y&M	(colonies)	10	10	ND	ND	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A		bacteria	ND	ND	500	ND	4000	4000	ND	ND	2500	1200	300	100	300	100	1500	2000	ND	ND	2600	2000
	PCA	yeast	ND	ND	ND	ND	1000	1000	400	100	ND	300	ND	ND	ND	100	200	ND	ND	ND	ND	ND
		yeast	ND	ND	ND	300	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	ND	ND	500	ND	5000	5000	400	100	2500	1500	300	100	300	200	1700	2000	ND	ND	2600	2000
	Y&M	(colonies)	ND	ND	ND	300	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ตารางที่ 57 การเปลี่ยนแปลงจำนวนจุลินทรีย์ทั้งหมดและรา และยีสต์ในตัวอย่างอาหารหนูเมื่อเก็บรักษาที่อุณหภูมิ 35 ° C ที่ระยะเวลาต่างๆ

Sample	Time	(week)	0		1		2		3		4		5		6		8		10		12	
M		bacteria	2900	3500	ND	ND	7000	14000	ND	ND	1000	2000	1000	ND	5000	11000	ND	100	ND	1000	23000	11000
	PCA	yeast	ND	ND	ND	ND	ND	ND	ND	2000	ND	1000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		yeast	100	ND	1000	ND	1000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	100	ND	2000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	2900	3500	ND	ND	7000	14000	ND	2000	1000	3000	1000	ND	5000	11000	ND	100	ND	1000	23000	11000
	Y&M	(colonies)	200	ND	3000	ND	1000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C		bacteria	1300	ND	ND	ND	4000	3000	6000	2000	2000	3000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PCA	yeast	ND	ND	1000	3000	1000	1000	ND	ND	1000	5000	5000	5000	50000	10000	40000	20000	17000	10000	80000	40000
		yeast	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	20	30	ND	100	5000	4000	6000	2000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	1300	ND	1000	3000	ND	ND	ND	ND	3000	8000	5000	5000	50000	10000	40000	20000	17000	10000	80000	40000
	Y&M	(colonies)	20	30	ND	100	14000	15000	24200	24900	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S		bacteria	17700	19400	3000	3000	ND	ND	200	200	32700	31600	7000	11000	112000	136000	15200	12400	19000	11000	300000	210000
	PCA	yeast	ND	ND	1000	ND	ND	ND	ND	ND	200	600	ND	6000	ND	ND	800	600	1000	2000	ND	ND
		yeast	10	20	ND	ND	14000	15000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	17700	19400	4000	3000	ND	ND	24400	25100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	10	20	ND	ND	0	3000	ND	ND	32900	32200	7000	17000	112000	136000	16000	13000	20000	13000	300000	210000
	Y&M	(colonies)	ND	650	700	ND	1000	ND	273000	222000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CNC		bacteria	ND	ND	900	400	ND	ND	1000	1000	1300	1100	100	700	400	ND	ND	100	ND	ND	6000	4000
	PCA	yeast	70	100	ND	700	ND	ND	ND	ND	600	500	2300	2200	800	1300	900	1100	1000	1000	5000	5000
		yeast	ND	10	ND	ND	1000	3000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	ND	650	1600	400	ND	ND	274000	223000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	70	110	ND	700	2E+05	251000	ND	ND	1900	1600	2400	2900	1200	1300	900	1200	1000	1000	11000	9000
	Y&M	(colonies)	2E+05	TNTC	175000	163000	1000	1000	192000	185000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SNC		bacteria	ND	ND	ND	ND	ND	ND	ND	1000	485000	350000	396000	344000	4E+06	4E+06	1E+06	1E+06	60000	60000	2080000	2E+06
	PCA	yeast	10	10	ND	ND	ND	ND	ND	ND	ND	1000	4000	2000	30000	40000	ND	10000	10000	10000	60000	30000
		yeast	ND	ND	100	ND	2E+05	252000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	2E+05	ND	175000	163000	ND	ND	192000	186000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	10	10	100	ND	4000	2000	ND	ND	485000	351000	400000	346000	4E+06	4E+06	1E+06	1E+06	70000	70000	2140000	2E+06
	Y&M	(colonies)	ND	ND	ND	ND	ND	ND	100	3500	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A		bacteria	ND	ND	ND	ND	100	ND	ND	ND	4000	5200	200	400	4300	3500	1000	2000	ND	100	6700	7000
	PCA	yeast	ND	ND	ND	ND	ND	ND	ND	ND	500	300	ND	100	100	ND	ND	ND	ND	ND	100	ND
		yeast	ND	ND	ND	ND	4000	2000	100	3500	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	ND	ND	ND	ND	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	ND	ND	ND	ND	ND	ND	ND	ND	4500	5500	200	500	4400	3500	1000	2000	ND	100	6800	7000
	Y&M	(colonies)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ตารางที่ 58 การเปลี่ยนแปลงจำนวนจุลินทรีย์ทั้งหมด และราและยีสต์ในตัวอย่างอาหารหนูเมื่อเก็บรักษาที่อุณหภูมิ 55°C ที่ระยะเวลาต่างๆ

Sample	Times	(weeks)	0		1		2		3		4		5		6		8		10		12	
M		bacteria	2900	3500	ND	ND	2000	5000	1000	1000	1000	1000	ND	ND	40000	30000	ND	10000	ND	ND	28000	41000
	PCA	yeast	ND	ND	ND	ND	ND	1000	ND	ND	ND	ND	ND	100	ND	10000	ND	ND	ND	ND	ND	ND
		yeast	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	100	ND	2000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	2900	3500	ND	ND	2000	6000	1000	1000	1000	1000	ND	100	40000	40000	ND	10000	ND	ND	28000	41000
	Y&M	(colonies)	200	ND	2000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C		bacteria	1300	ND	ND	ND	2000	1000	1000	3000	2000	2000	ND	ND	ND	ND	ND	ND	ND	ND	40000	80000
	PCA	yeast	ND	ND	1000	ND	ND	ND	3000	ND	8000	7000	10000	ND	7000	10000	30000	29000	ND	ND	40000	70000
		yeast	ND	ND	1100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	20	30	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	1300	ND	1000	ND	2000	1000	4000	3000	10000	9000	10000	ND	7000	10000	30000	29000	ND	ND	80000	15000
	Y&M	(colonies)	20	30	1100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
S		bacteria	17700	19400	21200	20100	17000	20000	23000	28000	22600	18700	7000	10000	7000	1000	4000	4000	105000	100	10000	20000
	PCA	yeast	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1000	1000	ND	ND	1900	1800	ND	ND
		yeast	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	10	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	17700	19400	21200	20100	17000	20000	23000	28000	24100	20000	7000	10000	8000	2000	4000	4000	124000	1900	10000	20000
	Y&M	(colonies)	10	20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CNC		bacteria	ND	250	ND	ND	ND	ND	18000	ND	9500	7500	ND	ND	5000	4400	ND	ND	ND	ND	10000	ND
	PCA	yeast	ND	ND	1000	600	3000	2000	ND	ND	700	500	1800	1900	8700	13900	19000	16000	6700	7400	10000	10000
		yeast	70	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	ND	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	ND	650	1000	600	3000	2000	18000	ND	10200	8000	1800	1900	13700	18300	19000	16000	6700	7400	20000	10000
	Y&M	(colonies)	70	110	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SNC		bacteria	NE+05	TNTC	22500	18700	NE+05	335000	ND	ND	201000	211000	26000	39000	210000	140000	12000	22000	ND	ND	90000	40000
	PCA	yeast	ND	ND	800	800	ND	ND	20000	80000	10000	10000	ND	ND	40000	80000	13000	6000	10000	ND	ND	ND
		yeast	10	10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	ND	ND	100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	NE+05	ND	23300	19500	NE+05	335000	20000	80000	211000	221000	26000	39000	250000	220000	25000	28000	10000	ND	90000	40000
	Y&M	(colonies)	70	110	100	ND	70	110	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
A		bacteria	ND	ND	ND	ND	6000	5000	13000	14000	19000	17000	100	300	TNTC	TNTC	3000	2000	300	200	50000	20000
	PCA	yeast	ND	ND	ND	ND	ND	ND	ND	ND	200	300	200	ND	2000	2900	ND	ND	ND	ND	ND	ND
		yeast	ND	ND	ND	ND	200	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	PDA	mold	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	APC	(CFU/g)	ND	ND	ND	ND	6000	5000	13000	14000	2100	2000	300	300	2000	2900	3000	2000	300	200	50000	20000
	Y&M	(colonies)	ND	ND	ND	ND	200	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

2. การทดสอบฤทธิ์ของซิริซินในการยับยั้งการเกิดมะเร็งลำไส้ใหญ่

ตารางที่ 59 น้ำหนักเฉลี่ยของหนูทดลองทั้ง 5 กลุ่มในระยะเวลา 5 เดือน

เดือน	น้ำหนัก (กรัม)				
	กลุ่ม 1	กลุ่ม 2	กลุ่ม 3	กลุ่ม 4	กลุ่ม 5
1	237.38 ± 11.61	234.67 ± 30.53	228.54 ± 37.24	206.04 ± 25.24	229.00 ± 35.06
2	352.96 ± 18.86	336.92 ± 53.01	342.83 ± 42.14	339.54 ± 28.61	343.67 ± 45.30
3	425.88 ± 27.40	411.17 ± 35.07	423.04 ± 31.32	413.17 ± 26.64	405.83 ± 55.70
4	477.75 ± 29.36	455.83 ± 37.84	473.29 ± 29.85	458.85 ± 21.44	446.65 ± 50.64
5	502.75 ± 28.25	488.83 ± 40.81	498.08 ± 29.73	483.375 ± 24.84	486.83 ± 48.42

หมายเหตุ แสดงค่าเป็น Mean±SD

ตารางที่ 60 ปริมาณการกินอาหารของหนูทดลองทั้ง 5 กลุ่มในระยะเวลา 5 เดือน

เดือน	น้ำหนัก (กรัม)				
	กลุ่ม 1	กลุ่ม 2	กลุ่ม 3	กลุ่ม 4	กลุ่ม 5
1	10.73 ± 2.76	12.52 ± 2.40	11.84 ± 2.70	8.41 ± 3.00	12.37 ± 2.80
2	17.90 ± 2.00	18.88 ± 2.30	19.07 ± 1.90	17.51 ± 3.40	17.78 ± 4.40
3	19.37 ± 0.90	20.10 ± 1.60	19.51 ± 0.90	21.97 ± 3.10	19.90 ± 1.90
4	21.59 ± 2.70	22.21 ± 1.60	24.62 ± 4.00	22.11 ± 1.20	21.89 ± 0.50
5	18.21 ± 0.90	19.37 ± 2.70	22.38 ± 2.70	21.89 ± 2.70	21.21 ± 1.00

หมายเหตุ แสดงค่าเป็น Mean±SD

ตารางที่ 61 น้ำหนักเฉลี่ยของอวัยวะต่างๆ หนูทดลองทั้ง 5 กลุ่มในระยะเวลา 5 เดือน

อวัยวะ	น้ำหนัก (กรัม)				
	กลุ่ม 1	กลุ่ม 2	กลุ่ม 3	กลุ่ม 4	กลุ่ม 5
ตับ	15.47 ± 0.74	15.23 ± 0.61	14.77 ± 0.67	13.64 ± 0.21	13.82 ± 0.74
ลำไส้เล็ก	10.06 ± 0.48	10.33 ± 0.39	10.49 ± 0.54	10.06 ± 0.47	10.03 ± 0.54
หัวใจ	1.65 ± 0.10	1.64 ± 0.10	1.61 ± 0.04	1.55 ± 0.07	1.51 ± 0.07
ไต	3.40 ± 0.17	3.25 ± 0.12	3.53 ± 0.11	3.02 ± 0.15	3.26 ± 0.16
ม้าม	1.06 ± 0.19	0.98 ± 0.11	1.13 ± 0.17	1.02 ± 0.04	1.00 ± 0.14
ลำไส้ใหญ่	1.70 ± 0.16	1.72 ± 0.30	1.96 ± 0.32	1.77 ± 0.19	1.58 ± 0.33

หมายเหตุ แสดงค่าเป็น Mean±SD

ตารางที่ 62 จำนวนเฉลี่ยของ ACF ต่อพื้นที่ (ตารางเซนติเมตร) ในลำไส้ใหญ่ของหนูทดลองในกลุ่มที่ได้รับ DMH ในระยะเวลา 5 เดือน

กลุ่มที่	G3	G4	G5
จำนวนเฉลี่ยของ ACF ต่อพื้นที่ (ซม^2)	116.00 ± 48.50	79.30 ± 29.30	82.40 ± 39.80

หมายเหตุ แสดงค่าเป็น Mean±SD

ตารางที่ 63 จำนวนเฉลี่ยของ ACF ต่อพื้นที่ (ตารางเซนติเมตร) ในส่วนต่างๆ ของลำไส้ใหญ่ของหนูทดลองในกลุ่มที่ได้รับ DMH ในระยะเวลา 5 เดือน

ส่วนของลำไส้ใหญ่	จำนวนเฉลี่ยของ ACF ต่อพื้นที่ (ซม^2)		
	กลุ่ม 3	กลุ่ม 4	กลุ่ม 5
ส่วนต้น	3.90 ± 5.30	0.00	0.00
ส่วนกลาง	38.00 ± 27.50	21.00 ± 18.50	28.40 ± 29.80
ส่วนปลาย	73.40 ± 27.00	57.80 ± 26.40	53.50 ± 22.80

หมายเหตุ แสดงค่าเป็น Mean±SD

ตารางที่ 64 จำนวน ACF ต่อพื้นที่ (ตารางเซนติเมตร) ในลำไส้ใหญ่ส่วนปลายของหนูทดลองในกลุ่มที่ได้รับ DMH ในระยะเวลา 5 เดือน แบ่งตามประเภทของ ACF (1 ถึง ≥ 5crypt/ACF)

ประเภทของ ACF	จำนวน ACF ต่อพื้นที่ (ซม^2)		
	กลุ่ม 3	กลุ่ม 4	กลุ่ม 5
1 crypt/ACF	6.49 ± 6.27	6.06 ± 7.69	5.84 ± 6.96
2 crypts/ACF	12.01 ± 8.70	12.34 ± 10.48	11.26 ± 7.19
3 crypts/ACF	9.74 ± 6.49	7.58 ± 6.49	9.52 ± 7.47
4 crypts/ACF	7.47 ± 7.14	3.68 ± 1.73	5.41 ± 4.89
≥ 5 crypts/ACF	37.66 ± 10.12	28.79 ± 15.79	21.43 ± 9.03

หมายเหตุ แสดงค่าเป็น Mean±SD

ตารางที่ 65 พื้นที่เฉลี่ยของ ACF ที่แสดงขนาดของ ACF ประเภทที่มีจำนวน crypt ใน ACF ≥ 5 crypts ในลำไส้ใหญ่ส่วนปลายของหนูทดลองในกลุ่มที่ได้รับ DMH ในระยะเวลา 5 เดือน

	พื้นที่เฉลี่ยของ ACF ($มม^2$)		
	กลุ่ม 3	กลุ่ม 4	กลุ่ม 5
ลำไส้ใหญ่ส่วนปลาย	0.14 ± 0.06	0.11 ± 0.05	0.14 ± 0.06

หมายเหตุ แสดงค่าเป็น Mean±SD

ตารางที่ 66 การย้อมติด surface markers (ร้อยละของเซลล์) ของในเลือดของหนูทดลองทั้ง 5 กลุ่ม ที่ได้จากการเจาะเลือดที่หัวใจ เมื่อสิ้นสุดการทดลองที่ระยะเวลา 5 เดือน

กลุ่มการทดลองที่	Surface marker (%)					
	CD2	CD3	CD4	CD8a	CD11a	CD25
1	63.59 ± 7.96	63.59 ± 7.96	44.45 ± 4.90	40.73 ± 8.98	96.37 ± 1.60	21.63 ± 3.38
2	66.71 ± 17.18	66.71 ± 17.18	50.66 ± 24.36	25.25 ± 13.75	83.58 ± 34.14	18.33 ± 12.89
3	61.93 ± 6.69	61.93 ± 6.69	40.83 ± 8.20	28.60 ± 6.46	98.32 ± 0.51	23.18 ± 3.66
4	76.09 ± 12.86	76.09 ± 12.86	54.47 ± 14.40	25.72 ± 4.88	98.74 ± 0.85	32.31 ± 3.84
5	66.16 ± 24.21	66.16 ± 24.21	41.30 ± 26.49	30.25 ± 18.75	94.35 ± 7.06	21.86 ± 6.82

กลุ่มการทดลองที่	Surface marker				
	CD45	CD54	CD80	CD86	RT1B
1	99.84 ± 0.18	74.98 ± 7.17	23.55 ± 8.24	20.08 ± 5.01	22.48 ± 5.87
2	85.53 ± 22.24	68.32 ± 11.10	10.28 ± 5.74	29.13 ± 32.16	27.58 ± 15.57
3	99.66 ± 0.27	77.53 ± 2.99	20.98 ± 3.81	21.31 ± 5.39	26.09 ± 3.13
4	99.74 ± 0.41	77.65 ± 9.21	17.15 ± 9.06	14.67 ± 5.23	20.76 ± 2.18
5	98.98 ± 0.75	79.35 ± 6.89	19.85 ± 5.51	24.50 ± 11.13	35.28 ± 23.20

หมายเหตุ แสดงค่าเป็น Mean±SD

3. การทดสอบฤทธิ์ของซิริซินในการยับยั้งการดูดซึมโคเลสเตอรอล

ตารางที่ 67 ผลของซิริซิน 50μg/ml ต่อการนำเข้าของโคเลสเตอรอล ในเซลล์เพาะเลี้ยง Caco-2

Sample	Cholesterol uptake (% control)						Mean	SD.	SEM
(50 ug/ml)	1	2	3	4	5	6			
PBS	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00
EZE	50.07	57.47	60.64	56.56	67.49	61.09	58.89	5.78	2.36
BSA	84.19	76.73	129.86	110.79	104.34	89.20	99.18	19.65	8.02
sericin A	65.67	86.08	80.28	84.84	83.45	88.42	81.46	8.20	3.35
sericin B	82.16	99.42	74.03	92.17	71.78	62.62	80.36	13.67	5.58
sericin C	86.86	96.55	106.95	114.88	75.65	83.62	94.08	14.87	6.07

หมายเหตุ EZE = ezetimibe 100 μM

ตารางที่ 68 ผลของซิริซิน 100 μg/ml ต่อการนำเข้าของโคเลสเตอรอล ในเซลล์เพาะเลี้ยง Caco-2

Sample	Cholesterol uptake (% control)					Mean	SD.	SEM
(100 ug/ml)	1	2	3	4	5			
PBS	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00
EZE			50.21	83.05		83.05	23.22	16.42
BSA			93.37	117.92		117.92	17.36	12.27
sericin A	56.14	119.34	74.30	109.48	92.58	90.37	25.69	11.49
sericin B	63.16	101.23		125.43	98.89	97.18	25.66	12.83
sericin C	66.08	124.28	113.19	134.12	121.58	111.85	26.65	11.92

หมายเหตุ EZE = ezetimibe 100 μM

ตารางที่ 69 ผลของซิริซิน 500 μg/ml ต่อการนำเข้าของโคเลสเตอรอล ในเซลล์เพาะเลี้ยง Caco-2

Sample	Cholesterol uptake (% control)						Mean	SD.	SEM
(500 ug/ml)	1	2	3	4	5	6			
PBS	100.00	100.00	100.00	100.00	100.00	100.00	100.00	0.00	0.00
EZE	60.64	56.56	67.49	61.09	50.07	57.47	64.29	4.51	2.26
BSA	121.25	106.65	103.78	119.01	77.50	98.32	111.40	8.74	4.37
sericin A	107.77	90.54	84.90	88.34	107.92	105.74	86.62	10.19	5.10
sericin B	113.28	97.54	101.87	95.46		84.47	98.66	7.96	3.98
sericin C	109.23	91.40	102.57	73.74		97.99	102.57	9.01	7.76

หมายเหตุ EZE = ezetimibe 100 μM

ตารางที่ 70 ผลของซิริซิน 1000μg/ml ต่อการนำเข้าของโคเลสเตอรอล ในเซลล์เพาะเลี้ยง Caco-2

Sample	Cholesterol uptake (% control)				Mean	SD.	SEM
(1000ug/ml)	1	2	3	4			
PBS	100.00	100.00	100.00	100.00	100.00	0.00	0.00
EZE			83.05	66.39	74.72	11.78	8.33
BSA	111.81	114.93	110.66	110.66	110.66	2.02	1.01
sericin A	149.25	122.92	126.67	133.84	130.26	11.64	5.82
sericin B	128.78	115.68	122.62	108.03	115.33	8.94	4.47
sericin C	122.73	129.98	142.22	129.20	135.71	8.14	4.07

หมายเหตุ EZE = ezetimibe 100 μM

ตารางที่ 71 น้ำหนักของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วงระยะเวลา 5 เดือน

ชนิดของอาหาร	น้ำหนักตัว (กรัม)					
	เริ่มต้น	เดือนที่ 1	เดือนที่ 2	เดือนที่ 3	เดือนที่ 4	เดือนที่ 5
Control diet	189.67±9.61	237.38±7.09	352.96±18.12	42588±26.39	496.83±27.72	502.75±27.71
Sericin diet	194.17±27.00	234.67±27.58	336.92±50.88	411.17±33.21	465.67±41.41	483.85±41.41
High cholesterol diet	175.33±23.21	345.83±17.74*	433.50±24.31*	487.67±24.95*	510.83±27.29	514.83±26.61
High cholesterol+ sericin diet	210.83±4.45*#	337.33±31.05*	414.67±17.48*	477.17±20.34*	491.50±19.67	519.50±17.92

หมายเหตุ แสดงค่าเป็น Mean±SD

*$p<0.05$ เปรียบเทียบกับ control diet

#$p<0.05$ เปรียบเทียบระหว่างอาหารประเภทเดียวกันที่มีหรือไม่มีซิริซิน

ตารางที่ 72 การเปลี่ยนแปลงของน้ำหนักของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในแต่ละช่วงระยะเวลา 5 เดือน

ชนิดของอาหาร	น้ำหนักตัวที่เปลี่ยนแปลง (กรัม)				
	เดือนที่ 1	เดือนที่ 2	เดือนที่ 3	เดือนที่ 4	เดือนที่ 5
Control diet	47.71±11.19	163.29±9.47	236.21±18.59	307.17±19.99	313.08±23.08
Sericin diet	40.50±8.12	142.75±30.60	217.00±17.04	271.50±25.52*#	289.68±19.14
High cholesterol diet	170.50±16.65*	258.17±35.70*	312.33±32.64*	335.50±33.36	339.50±31.79
High cholesterol+ sericin diet	126.50±30.26*#	203.83±14.82*#	263.33±18.12*#	280.67±19.72*#	308.67±16.91

หมายเหตุ แสดงค่าเป็น Mean±SD

*$p<0.05$ เปรียบเทียบกับ control diet

#$p<0.05$ เปรียบเทียบระหว่างอาหารประเภทเดียวกันที่มีหรือไม่มีซิริซิน

ตารางที่ 73 ปริมาณอาหารที่กินของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วงระยะเวลา 5 เดือน

ชนิดของอาหาร	ปริมาณอาหาร (กรัม)				
	เดือนที่1	เดือนที่ 2	เดือนที่ 3	เดือนที่ 4	เดือนที่ 5
Control diet	10.73±2.58	17.90±2.00	19.37±0.95	21.59±2.67	18.21±0.92
Sericin diet	12.52±2.38	18.88±2.26	20.10±1.61	22.21±1.64	19.37±2.67
High cholesterol diet	16.43±0.27*	20.42±0.43*	17.55±0.65*	28.03±0.54*	30.00±0.00*
High cholesterol + sericin diet	15.25±0.83*#	19.92±0.20*#	23.09±0.40*#	27.13±0.05*#	30.00±0.00*

หมายเหตุ แสดงค่าเป็น Mean±SD

*$p<0.05$ เปรียบเทียบกับ control diet

# $p<0.05$ เปรียบเทียบระหว่างอาหารประเภทเดียวกันที่มีหรือไม่มีซิริซิน

ตารางที่ 74 ระดับของ total cholesterol ในเลือดของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วงระยะเวลา 5 เดือน

ชนิดของอาหาร	Total cholesterol (mg/dl)				
	เริ่มต้น	เดือนที่ 2	เดือนที่ 3	เดือนที่ 4	เดือนที่ 5
Control diet	86.33±3.06	117.00±9.54	105.50±1.99	104.12±1.48	89.24±11.98
Sericin diet	85.67±3.79	114.00±13.11	138.47±3.35*#	116.22±3.20*#	82.80±5.60
High cholesterol diet	120.67±8.19*	134.83±18.71	128.56±10.40*	152.37±21.96*	156.50±24.36*
High cholesterol + sericin diet	104.17±11.02*#	118.51±63.95#	123.32±8.64*	141.21±13.13*	152.00±15.40*

หมายเหตุ แสดงค่าเป็น Mean±SD

*$p<0.05$ เปรียบเทียบกับ control diet

# $p<0.05$ เปรียบเทียบระหว่างอาหารประเภทเดียวกันที่มีหรือไม่มีซิริซิน

ตารางที่ 75 ระดับของ High density lipoprotein (HDL) ในเลือดของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วงระยะเวลา 4 เดือน

ชนิดของอาหาร	HDL (mg/dl)				
	เริ่มต้น	เดือนที่ 2	เดือนที่ 3	เดือนที่ 4	เดือนที่ 5
Control diet	61.33±2.89	47.00±3.46	45.75±4.08	38.86±1.47	17.13±1.41
Sericin diet	65.67±4.16	38.67±3.06*#	51.39±11.34	35.47±2.80*#	18.85±3.47
High cholesterol diet	39.95±6.68*	17.23±2.53*	24.69±1.99*	50.74±18.23	27.34±12.80
High cholesterol + sericin diet	65.00±4.90#	19.46±1.63*#	21.81±1.97*#	52.21±11.69	32.35±5.79*

หมายเหตุ แสดงค่าเป็น Mean±SD

*$p<0.05$ เปรียบเทียบกับ control diet

# $p<0.05$ เปรียบเทียบระหว่างอาหารประเภทเดียวกันที่มีหรือไม่มีซิริซิน

ตารางที่ 76 ระดับของ Triglyceride ในเลือดของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วงระยะเวลา 5 เดือน

ชนิดของอาหาร	HDL (mg/dl)				
	เริ่มต้น	เดือนที่ 2	เดือนที่ 3	เดือนที่ 4	เดือนที่ 5
Control diet	108.33±9.61	105.33±29.14	106.86±2.02	122.68±23.11	75.27±16.26
Sericin diet	82.33±12.50*#	65.00±19.16	86.08±0.34*#	88.69±8.28*#	64.04±14.20
High cholesterol diet	44.17±9.50*	208.34±50.26*	95.29±10.98	93.64±23.97*	75.33±14.83
High cholesterol + sericin diet	82.50±15.86*#	221.91±18.16*	72.36±10.10*#	103.56±22.13*	65.33±12.13

หมายเหตุ แสดงค่าเป็น Mean±SD

*$p<0.05$ เปรียบเทียบกับ control diet

# $p<0.05$ เปรียบเทียบระหว่างอาหารประเภทเดียวกันที่มีหรือไม่มีซิริซิน

ตารางที่ 77 ระดับของ total cholesterol ในเลือดของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วง14 วัน

Treatments	Serum total cholesterol (mg/dl)				
	0	4 days	8 days	11 days	14 days
High-cholesterol	80.23±12.35	108.35±17.91#	151.99±6.24#	164.62±20.46#	177.68±20.05#
+Sericin 10 mg/kg	98.20±9.77	116.76±6.31	127.60±6.71**	144.04±5.83	139.25±2.67**
+Sericin 100 mg/kg	92.67±8.70	112.82±9.29	131.30±8.32**	143.73±9.92	131.58±6.90**
+Sericin 1000 mg/kg	81.87±11.74	120.84±11.70	124.79±12.78**	133.33±10.17*	130.92±6.42**

หมายเหตุ #เปรียบเทียบระหว่างค่าในและวันกับวันที่ 0 (baseline)

*เปรียบเทียบระหว่างกลุ่มทดลองกับกลุ่ม High-cholesterol ($p<0.05$)

**เปรียบเทียบระหว่างกลุ่มทดลองกับกลุ่ม High-cholesterol ($p<0.01$)

ตารางที่ 78 ระดับของ non-HDL cholesterol ในเลือดของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วง 14 วัน

Treatments	Serum non-HDL (mg/dl)				
	0	4 days	8 days	11 days	14 days
High-cholesterol	45.83±18.14	74.53±18.05#	121.15±7.48#	136.78±17.46#	143.66±13.72#
+Sericin 10 mg/kg	64.03±6.27	76.01±4.40	100.87±5.33**	113.32±6.74*	103.29±2.07**
+Sericin 100 mg/kg	56.00±10.65	71.28±8.84	103.20±6.32**	107.93±8.91*	91.97±7.74**
+Sericin 1000 mg/kg	41.43±5.85	77.79±11.81	99.38±11.07**	98.37±10.13**	93.18±7.49**

หมายเหตุ #เปรียบเทียบระหว่างค่าในและวันกับวันที่ 0 (baseline)

*เปรียบเทียบระหว่างกลุ่มทดลองกับกลุ่ม High-cholesterol ($p<0.05$)

**เปรียบเทียบระหว่างกลุ่มทดลองกับกลุ่ม High-cholesterol ($p<0.01$)

ตารางที่ 79 ระดับของ triglyceride ในเลือดของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วง14 วัน

Treatments	Serum triglyceride (mg/dl)				
	0	4 days	8 days	11 days	14 days
High-cholesterol	71.92±10.82	105.76±29.63#	112.64±39.10	93.81±23.93	127.98±39.44#
+Sericin 10 mg/kg	91.55±11.25	140.27±8.52*	129.31±31.79	100.93±8.67	122.26±18.57
+Sericin 100 mg/kg	53.94±18.37	86.06±27.82	100.47±31.18	143.29±29.64*	126.73±27.96
+Sericin 1000 mg/kg	44.80±12.73	96.67±24.12	91.91±15.66	125.40±29.61	142.66±7.89

หมายเหตุ #เปรียบเทียบระหว่างค่าในและวันกับวันที่ 0 (baseline)

*เปรียบเทียบระหว่างกลุ่มทดลองกับกลุ่ม High-cholesterol ($p<0.05$)

ตารางที่ 80 ระดับของ triglyceride ในเลือดของหนูทดลองที่ได้รับอาหารชนิดต่างๆ ในช่วง14 วัน

Treatments	Serum HDL (mg/dl)				
	0	4 days	8 days	11 days	14 days
High-cholesterol	34.40±6.90	33.82±0.97	30.84±2.57	27.85±8.96	34.02±6.83
+Sericin 10 mg/kg	34.17±4.34	40.76±3.62**	26.73±1.82*	30.71±2.31	35.97±1.12
+Sericin 100 mg/kg	36.97±2.44	41.54±2.87**	28.10±2.44	35.81±2.28	39.61±2.76
+Sericin 1000 mg/kg	40.44±7.74	43.06±1.01**	25.40±1.95**	34.96±1.88	37.74±2.05

หมายเหตุ *เปรียบเทียบระหว่างกลุ่มทดลองกับกลุ่ม High-cholesterol ($p<0.05$)

**เปรียบเทียบระหว่างกลุ่มทดลองกับกลุ่ม High-cholesterol ($p<0.01$)

4. การพัฒนาอาหารเสริมซิริซิน

4.1. การวิเคราะห์หาปริมาณ % Drug content

ตารางที่ 81 ตารางแสดง % drug content

ตำรับ	LOT	Sericin B ใน เม็ดยา ตามทฤษฎี (mg)	Absorbance 275 nm	Sericin B (mg/ml)	Sericin B ที่มีอยู่จริงในเม็ดยา (mg)	% drug content	Mean	SD
A1	1	350.0	0.5520	0.70	351.71	100.49	100.55	0.46
		350.0	0.5550	0.71	353.62	101.03		
		350.0	0.5500	0.70	350.44	100.13		
		350.0	0.5623	0.72	358.28	102.37		
		350.0	0.5630	0.72	358.71	102.49		
	2	350.0	0.5943	0.76	378.63	108.18	108.24	0.52
		350.0	0.5977	0.76	380.75	108.79		
		350.0	0.5920	0.75	377.15	107.76		
		350.0	0.6013	0.77	383.08	109.45		
		350.0	0.5947	0.76	378.84	108.24		
	3	350.0	0.6187	0.79	394.10	112.60	110.30	2.17
		350.0	0.5950	0.76	379.05	108.30		
		350.0	0.6043	0.77	384.99	110.00		
		350.0	0.6200	0.79	394.95	112.84		
		350.0	0.5937	0.76	378.21	108.06		
A2	1	350.0	0.5830	0.74	371.42	106.12	106.65	1.13
		350.0	0.5817	0.74	370.58	105.88		
		350.0	0.5930	0.76	377.78	107.94		
		350.0	0.5973	0.76	380.54	108.73		
		350.0	0.6017	0.77	383.29	109.51		
	2	350.0	0.6060	0.77	386.05	110.30	109.47	0.72
		350.0	0.5990	0.76	381.60	109.03		
		350.0	0.5993	0.76	381.81	109.09		
		350.0	0.6083	0.78	387.53	110.72		
		350.0	0.6070	0.77	386.68	110.48		
	3	350.0	0.6057	0.77	385.84	110.24	110.54	0.30
		350.0	0.6090	0.78	387.96	110.84		
		350.0	0.6073	0.77	386.90	110.54		
		350.0	0.5943	0.76	378.63	108.18		
		350.0	0.6077	0.77	387.11	110.60		

ตารางที่ 81 ตารางแสดง % drug content (ต่อ)

ตำรับ	LOT	sericin B ในเม็ดยา ตามทฤษฎี (mg)	Absorbance 275 nm	sericin B (mg/ml)	sericin B ที่มีอยู่จริงในเม็ดยา (mg)	% drug content	Mean	SD
A3	1	350.0	0.5823	0.74	371.00	106.00	103.56	4.18
		350.0	0.5423	0.69	345.56	98.73		
		350.0	0.5820	0.74	370.79	105.94		
		350.0	0.6053	0.77	385.62	110.18		
		350.0	0.6053	0.77	385.62	110.18		
	2	350.0	0.5703	0.73	363.37	103.82	106.73	2.88
		350.0	0.5867	0.75	373.75	106.79		
		350.0	0.6020	0.77	383.51	109.57		
		350.0	0.6003	0.76	382.45	109.27		
		350.0	0.6080	0.77	387.32	110.66		
	3	350.0	0.6077	0.77	387.11	110.60	108.30	2.24
		350.0	0.5943	0.76	378.63	108.18		
		350.0	0.5830	0.74	371.42	106.12		
		350.0	0.5877	0.75	374.39	106.97		
		350.0	0.5860	0.75	373.33	106.67		
B2	1	350.0	0.5517	0.70	351.50	100.43	99.74	0.89
		350.0	0.5423	0.69	345.56	98.73		
		350.0	0.5497	0.70	350.23	100.06		
		350.0	0.5640	0.72	359.34	102.67		
		350.0	0.5927	0.76	377.57	107.88		
	2	350.0	0.5673	0.72	361.46	103.27	104.28	1.60
		350.0	0.5830	0.74	371.42	106.12		
		350.0	0.5682	0.72	362.03	103.44		
		350.0	0.5493	0.70	350.01	100.00		
		350.0	0.5930	0.76	377.78	107.94		
	3	350.0	0.5363	0.68	341.75	97.64	101.30	5.61
		350.0	0.5920	0.75	377.15	107.76		
		350.0	0.5410	0.69	344.72	98.49		
		350.0	0.6090	0.78	387.96	110.84		
		350.0	0.6067	0.77	386.47	110.42		

4.2. การพัฒนาตำรับยาเม็ด sericin B เบื้องต้นด้วยเทคนิค Wet granulation

พัฒนาตำรับยาเม็ด Sericin B Protein โดยการเปลี่ยนแปลงปัจจัยในกระบวนการผลิตดังตารางข้างล่างและมีวิธีการเตรียมดังนี้

1. ชั่ง explotab 1.5%, Avicel PH 101 และ Cericin B แล้วให้เข้ากันโดยใช้วิธีผสมแบบ Geometric dilution (เริ่มผสมจากสารที่มีปริมาณน้อยไปหาสารที่มีปริมาณมาก) โดยทำการ ผสมสารในโกร่ง

2. เมื่อผสมเสร็จแล้วให้ spray น้ำปริมาณ ~ 4-8 ml (สำหรับเตรียมทำยาอม 6 เม็ด) ให้เป็นก้อนหมาด

3. นำไปผ่านแร่ง No. 14 แล้วอบแห้งที่ 60 °C นาน 4 ชั่วโมง เมื่อแกรนูลแห้งจากนั้นจึงนำไปผ่านแร่ง No.16

4. ชั่งน้ำหนักของแกรนูลที่ได้ จากนั้นเติม explotab อีก 1.5% และ Aerosil 200 0.2% (โดยคำนวณจากน้ำหนักแกรนูลที่ได้หลังผ่านการอบ) โดยทำการผสมแบบ Geometric dilution ในถุง

5. เมื่อผสมเข้ากันดีแล้วจากนั้นจึงนำสารมาชั่งก่อนไปทำการตอกเม็ดด้วยเครื่อง Hydrolic pressure (ใช้แรงตอก 1 ตัน ค้างนาน 1 นาที)

6. ทำการเปลี่ยนแปลงปัจจัยตามตารางข้างล่างกำหนด

ตารางที่ 82 แสดงส่วนประกอบของตำรับเมื่อเปลี่ยนแปลงปริมาณ Diluent ,ชนิด Diluent กับ % Explotab

ตำรับ	ปัจจัยที่เปลี่ยนแปลง	Ingredient						แรงตอก	Result	
		Sericin B (mg)	Avicel PH101 (mg)	Lactose (mg)	Explotab (%)	Aerosil 200 (%)	Isopropanol : น้ำ		ความหนา (mm)	แตกตัวในน้ำ (นาที)
A 1	Avicel PH 101	500	100	-	3%	0.2%	0 : 100	1 ตัน hold 1 นาที	0.408	> 30
A 2		500	150	-	3%	0.2%	0 : 100		0.415	> 30
A 3		500	200	-	3%	0.2%	0 : 100		0.465	> 30
A 4	Lactose	500	-	100	3%	0.2%	0 : 100		0.415	> 30
A 5		500	-	200	3%	0.2%	0 : 100		0.482	> 30
A 6		500	-	300	3%	0.2%	0 : 100		0.551	> 30
A 7	Explotab (%)	500	-	200	4%	0.2%	0 : 100		0.503	> 30
A 8		500	-	200	5%	0.2%	0 : 100		0.504	> 30
A 9		500	-	200	6%	0.2%	0 : 100		0.504	> 30

ตารางที่ 83 แสดงส่วนประกอบของตำรับเมื่อเปลี่ยนแปลงปริมาณ Diluent ,ชนิด Diluent กับ % Explotab

ตำรับ	ปัจจัยที่เปลี่ยนแปลง	Ingredient						แรงตอก	Result	
		Sericin B (mg)	Avicel PH101 (mg)	Corn starch(mg)	Explotab (%)	Aerosil 200 (%)	Isopropanol : น้ำ		ความหนา (mm)	แตกตัวในน้ำ (นาที)
B1	Avicel PH 101	400	200	-	3%	0.2%	0 : 100	1 ตัน hold 1 นาที	0.398	> 30
B2		400	300	-	3%	0.2%	0 : 100		0.449	> 30
B3		400	400	-	3%	0.2%	0 : 100		0.514	> 30
B4	Explotab (%)	400	300	-	4%	0.2%	0 : 100		0.444	> 30
B5		400	300	-	5%	0.2%	0 : 100		0.445	> 30
B6		400	300	-	6%	0.2%	0 : 100		0.429	> 30
B7	Avicel PH 101 และ Corn starch	400	-	300	3%	0.2%	0 : 100	1 ตัน hold 30 วินาที	0.456	> 30
B8		400	150	150	3%	0.2%	0 : 100		0.456	> 30
B9		400	-	400	3%	0.2%	0 : 100		0.535	> 30
B10		400	200	200	3%	0.2%	0 : 100		0.535	> 30
B11	Explotab (%) และ Corn starch	400	-	300	6%	0.2%	0 : 100	1 ตัน hold 30 วินาที	0.492	> 30
B12		400	-	300	12%	0.2%	0 : 100		0.505	> 30
B13		400	-	300	15%	0.2%	0 : 100		0.509	> 30
B14		400	-	400	6%	0.2%	0 : 100	1 ตัน hold 1 นาที	0.569	> 30
B15		400	-	400	9%	0.2%	0 : 100		0.552	> 30
B16		400	-	400	12%	0.2%	0 : 100		0.549	> 30

ตารางที่ 84 แสดงส่วนประกอบของตำรับเมื่อเปลี่ยนแปลงปริมาณ Sericin กับ Diluent

ตำรับ	ปัจจัยที่เปลี่ยนแปลง	Ingredient						แรงตอก	Result	
		Sericin B (mg)	Avicel PH101 (mg)	Corn starch (mg)	Explotab (%)	Aerosil 200 (%)	Isopropanol : น้ำ		ความหนา (mm)	แตกตัวในน้ำ (นาที)
C1	Sericin B และ Corn starch	350	-	350	9%	0.2%	0 : 100	1 ตัน hold 30 วินาที	0.479	> 30
C2		350	-	450	9%	0.2%	0 : 100	1 ตัน hold 1 นาที	0.547	> 30
C3		300	-	400	9%	0.2%	0 : 100	1 ตัน hold 30 วินาที	0.477	22
C4		300	-	500	9%	0.2%	0 : 100	1 ตัน hold 1 นาที	0.553	> 30

ตารางที่ 85 แสดงส่วนประกอบของตำรับเมื่อเปลี่ยนแปลงอัตราส่วน Isopropanol: น้ำ

ตำรับ	ปัจจัยที่เปลี่ยนแปลง	Ingredient						แรงตอก	Result	
		Sericin B (mg)	Avicel PH101 (mg)	Corn starch(mg)	Explotab (%)	Aerosil 200 (%)	Isopropanol : น้ำ		ความหนา (mm)	แตกตัวในน้ำ (นาที)
D1	Isopropanol : น้ำ	300	-	400	9%	0.2%	25 : 75	1 ตัน hold 30 วินาที	0.547	27
D2		300	-	400	9%	0.2%	50 : 50		0.569	10
D3		300	-	400	9%	0.2%	75 : 25		0.549	9
D4		300	-	400	9%	0.2%	100 : 0		0.552	> 30 ไม่เป็นแกรนูล

ตารางที่ 86 แสดงส่วนประกอบของตำรับเมื่อเปลี่ยนแปลงปริมาณ Sericin B กับ อัตราส่วน Isopropanol: น้ำ

ตำรับ	ปัจจัยที่เปลี่ยนแปลง	Ingredient					แรงตอก	Result	
		Sericin B (mg)	Corn starch(mg)	Explotab (%)	Aerosil 200 (%)	Isopropanol : น้ำ		ความหนา (mm)	แตกตัวในน้ำ (นาที)
E1	Sericin B และ Isopropanol : น้ำ	350	400	9%	0.2%	50 : 50	1 ตัน hold 30 วินาที	0.513	15
E2		350	400	9%	0.2%	75 : 25		0.511	15
E3		400	400	9%	0.2%	50 : 50	1 ตัน hold 1 นาที	0.528	23
E4		400	400	9%	0.2%	75 : 25		0.520	19

หมายเหตุ เม็ดยาตำรับที่เปลี่ยนแปลง Sericin B และ Isopropanol: น้ำ มีรอยแตกบริเวณขอบ

ตารางที่ 87 แสดงส่วนประกอบของตำรับเมื่อเปลี่ยนแปลงปริมาณ Diluent กับ % Explotab

ตำรับ	ปัจจัยที่เปลี่ยนแปลง	Ingredient						แรงตอก	Result	
		Sericin B (mg)	Avicel PH101 (mg)	Corn starch(mg)	Explotab (%)	Aerosil 200 (%)	Isopropanol : น้ำ		ความหนา (mm)	แตกตัวในน้ำ (นาที)
F1	Avicel PH 101 และ Corn starch	350	100	300	9%	0.2%	50 : 50	1 ตัน hold 30 วินาที	0.516	12
F2		350	200	200	9%	0.2%	50 : 50		0.501	12
F3		350	300	100	9%	0.2%	50 : 50		0.537	7
F4		350	400	0	9%	0.2%	50 : 50		0.533	10
F5	Explotab (%)	350	300	100	0%	0.2%	50 : 50	1 ตัน hold 30 วินาที	0.485	6
F6		350	300	100	3%	0.2%	50 : 50		0.507	7
F7		350	300	100	6%	0.2%	50 : 50		0.530	4
F8	Avicel PH 101 และ Corn starch	350	262.5	87.5	0%	0.2%	50 : 50	1 ตัน hold 30 วินาที	0.464	8
F9		350	225	75	0%	0.2%	50 : 50		0.407	19

หมายเหตุ เม็ดยาตำรับที่เปลี่ยนแปลง Avicel PH 101 และ Corn starch มีรอยแตกบริเวณขอบ